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Proceedings of the tropical fruits in human nutrition and health conference 2008

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The Conference was sponsored by the OECD Co-operative Research Programme on Biological Resource Management for Sustainable Agricultural Systems, whose financial support made it possible for most of the invited speakers to participate in the conference.

On 26 March 2009, the Department of Primary Industries and Fisheries was amalgamated with other government departments to form the Department of Employment, Economic Development and Innovation.

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Foreword

Horticultural industries are Queensland's second largest primary producer, with a total gross value of more than $1.9 billion in 2008. Queensland's diverse geography and climate supports the production of more than 120 horticultural products. With a growing worldwide demand for quality, nutritious food Queensland Primary Industries and Fisheries continues to focus its activities on accelerating growth within the sector.

In many ways, Queensland's tropical fruit industry also has a major role to play in the successful implementation of our Q2 vision, which highlights five ambitions for the state: *strong; green; smart; healthy;* and *fair*.

In particular, the health and wellbeing of our population is a priority. As partners to the industry we aim to help the Queensland horticulture market address the continued global trend towards healthier foods. Consumer research shows that people are increasingly concerned about their diet and the role food can play in staying healthy.

We want Queenslanders to look to naturally healthy foods to maintain their wellbeing rather than rely on a diet rich in fortified processed foods and nutritional supplements. We actively support major programs to ensure our young people have greater opportunities to boost their daily nutritional requirements through increased fruit and vegetable intake. Most important, we want our tropical fruits to be a mainstay of daily eating habits.

Progress in global supply chains is also resulting in more widespread distribution of a greater range of tropical fruits. Although the central role of fruits and vegetables as a core component of a healthy diet is well established, the specific benefits associated with tropical fruit are not well defined.

This conference brought together eminent scientists to promote international understanding and cooperation relating to tropical fruits, and the role they can play in health and nutrition. I hope the networks developed through the *Tropical fruits in human nutrition and health conference 2008* will raise the awareness of the need for more research in this area and help identify gaps in knowledge so we can develop and market tropical fruits that have even greater potential health benefits. These Conference Proceedings should provide a valuable resource for policy makers and inform public debate in the high priority areas of functional foods, nutrition, health and wellbeing.

Tim Mulherin

Minister for Primary Industries, Fisheries and Rural and Regional Communities

Tropical fruits in human nutrition and health conference 2008—an overview

Tropical fruit as excellent resources for nutrigenomics

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The biodiversity of tropical vegetation is well-characterised by the wide range of plants that serve as fruit and vegetables for human consumption. The domestication of most tropical fruit started quite a long time ago, and has now reached the super-domestication level. However, there are a vast number of plants whose fruit could be valuable for human consumption, but whose domestication is still far from reaching the economical by feasible stage (Vaughan et al., 2007). A good example of this is the current effort in Queensland to economically produce achachairu, a plum-sized, orange-coloured, valuable, marketable fruit from South America. Lemons, oranges, bananas, avocados, kiwis, mangoes, lychees, papayas and pineapples are well-established in modern agriculture, while others still need both genetic improvement and new technologies before they can be economically cultivated on a large scale (such as ablu, black sapote, carambola, durian, guava, jaboticaba, jackfruit, longan, mangosteen, passionfruit, persimmon, pitaya, rambutan or star apple). All of these, regardless of their production scale, are well-known for their high vitamin C, A or B levels, with increased amounts of essential micronutrients, elements such as iron, manganese, zinc or calcium (www.nal.usda.gov/fnic/foodcomp).

For this simple reason, tropical fruit have an important role in human nutrition, as they are rich sources of vitamins, minerals, essential amino acids and several other secondary metabolites. Throughout the last century, nutritional science focused on discovering the vitamins and minerals and defining their use in preventing deficiency diseases. Scorbut and beriberi are well-documented diseases caused by vitamin deficiency. When exploration of the New World began, sailors often suffered from vitamin deficiencies. They realised quite quickly that they could fight this by eating fresh vegetables and fruit.

Today in the developed world nutrition-related health problems have shifted to over-nutrition, obesity and new type diabetes, forcing an urgent paradigm change in modern medicine and nutritional science. To prevent the development of disease, nutrition research is investigating how nutrition can be optimised to maintain homeostasis at a cellular, tissue, organ and the whole body levels. This requires an understanding of how nutrients act at the molecular level. It also involves the investigations of nutrient-related interactions at the gene, protein and metabolic levels.

This is how and why nutrigenomics was born. This fascinating technological advance will definitely improve our understanding of nutrition. There is great potential for the technologies to be used as new tools for nutrition science. Recently the genome sequence of papaya has been published (Ming et al., 2008), opening up new horizons for use of tropical fruit in nutrigenomics. This new field of research studies the molecular relationship between nutrition and the responses of genes, with the aim of extrapolating how such changes affect human health (Chavez and Munoz de Chavez, 2003).

Nutrigenomics focuses on the effect of nutrients on the genome, proteome and metabolome. It applies the new sciences, such as genomics, proteomics, transcriptomics and metabolomics, to human nutrition in order to understand the relationship between health and nutrition (Müller and Kersten, 2003). Current statistics underlay the increasing role of tropical fruit in human nutrition: it is sufficient to mention the banana and its role in feeding the world (www.biobanana.com). The genetic improvement of bananas is quite well advanced using conventional breeding with modern biotechnology, which also includes genetic engineering.

The biofortification of bananas by increasing their β-carotene, α-tocopherol and iron content will be beneficial mostly for regions of the world where bananas are the major staple food source (Khanna et al., 2009). There is also a well-established technology for the use of bananas as edible vaccine delivery system. Mark Davey presented an ex-ante analysis of the impact of vitamin A-rich bananas on the burden of illness and mortality related to vitamin A deficiency in terms of disability-adjusted life years, as a fast and cost-effective strategy (Davey et al., 2009). The sequencing of the genomes of tropical fruit such as papaya gave a boost to their further genetic improvement (Ming, 2009).

The challenges facing tropical fruit researchers are enormous, as they have to elaborate processing technologies that preserve valuable nutritional components to achieve fast trade without post-harvest losses, and to keep the fruit fresh as long as possible. The first task is variety development, involving selection and breeding, which will hopefully soon reach the stage for genetic improvement technology. Collection and selection are important in the step-by-step approach presented in a display by Yan Diczbalis at the *Tropical fruits in human nutrition and health conference*, held on Queensland's Gold Coast (Fig.1).

Figure 1. Tropical fruit display at the *Tropical fruit in human nutrition and human health conference* held in Queensland, presented by Yan Diczbalis (author's photo library).

Although, tropical fruit are widely available, their nutritional values must be emphasised via teaching courses and education of the public, as well as marketing strategies (Fig.2).

Figure 2. Roadside market in Natal, Republic of South Africa (author's photo library).

Both are very important elements in the public acceptance of new products. A successful program was presented involving a 'super fruit' strategy by Julian Melletin, while Sara Jaeger (2009) discussed the consumer drivers for fruit products. The tropical richness in fruits is well recognised by the public and governments, as shown by the research support provided, from the farm to the fork. The papers presented in this volume underline the region's scientific and economic interests. The high quality of research in Australia and New Zealand on tropical fruit and their nutritional value in relation to human health is exemplary.

The revolution in molecular biology and its technological arsenal have given us an enormous chance to produce healthy food, which will overcome both malnutrition and over-nutrition.

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Tropical fruits, nutrition and population health

Biofortification of bananas

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Keywords: bioavailability, micronutrients, transgenic bananas, biofortification

Abstract

Bananas are one of the world's most important fruit crops, a significant component of diets in the wet tropics and sub-tropics and, in some instances, the staple food. Uganda is one such country where bananas are the staple food with the East African Highland banana, a cooking banana, the primary starch source. As with many starchy, staple foods, these bananas are low in pro-vitamin A, iron and protein and, as a result, banana-based diets are low in these nutrients. In Uganda, this inadequate nutrition manifests as high levels of vitamin A deficiency (VAD), iron deficiency anaemia (IDA) and stunting in children. This combination of micro- and macro-nutrient deficiencies with bananas as the staple food is not restricted to Uganda, but also occurs in the highlands of Kenya and Tanzania as well as Burundi, Rwanda and eastern areas of the Democratic Republic of Congo. Genetic improvement of bananas has been a major challenge because cultivated bananas are essentially sterile and conventional breeding, while possible, has delivered only a few acceptable, improved cultivars to replace the selections now grown in nearly all wet tropical and sub-tropical environments. The most logical and achievable strategy for nutrient enhancement of these bananas is through genetic modification. The Queensland University of Technology (QUT), Australia and the National Agricultural Research Organisation (NARO), Uganda commenced a project in 2005 to enhance the micronutrient content of East African Highland bananas (EAHB) under the Bill and Melinda Gates Foundation's Grand Challenges in Global Health program. This paper briefly reviews the available information on the nutritional status of bananas and highlights some of the strategies being used by the QUT program for biofortification of bananas to improve the nutritional status in Uganda and surrounding countries.

Introduction

Bananas are cultivated in more than 120 countries in the tropics and sub-tropics with an annual production around 81 million tonnes (FAOSTAT, 2008). Despite being a fruit crop, banana is fourth on the list of the developing world's most important crops, after rice, wheat and maize (Samson, 1992). Approximately 87% of all the bananas grown worldwide are produced by small-scale farmers for local consumption as a food security crop, and for local markets. They provide a major staple food crop for millions of people and play an important role in the social fabric of many rural communities. In many African countries, bananas are particularly important because they produce a good quality food year-round and are adaptable to a wide range of cropping systems. East Africa is the largest banana-producing and consuming region in Africa, with Uganda being the world's second leading producer after India, with a total annual production of about 10.5 million tonnes. In addition, Ugandans consume, on average, greater than 220 kg of bananas per person per year, which is the highest in the world. Uganda has a population in excess of 25 million, more than 80% of whom live in rural areas. The country also carries a heavy public health burden with high levels of infectious diseases, primarily HIV and malaria, and very high levels of malnutrition, a consequence of heavy dependence on nutritionally poor banana-based diets. This inadequate nutrition manifests as high levels of VAD, IDA and stunting in children.

The East African highlands constitute a secondary centre of banana diversity with more than 80 locally evolved cultivars. These cultivars, collectively called the East African Highland Bananas (*Musa* spp., group AAA-EAHB), include both cooking and brewing types. Although the cooking banana is the primary staple food in the region and is the primary starch source, the sweet dessert banana, Sukali Ndizi, is preferred by young children and as a weaning food for infants.

Enhancing the nutritional composition of the EAHB and dessert bananas through genetic improvement is a major challenge, because most cultivated bananas are essentially sterile and, as such, are not amenable to conventional breeding. Genetic engineering offers many opportunities for biofortifying these cultivars through manipulation of metabolic pathways involved in synthesis of provitamin A and iron. Biofortification is the process of producing food crops that are rich in bioavailable micronutrients and may involve adding a nutrient that does not originally exist in that crop, increasing the content of an existing nutrient, or making an existing nutrient more bioavailable.

In this paper we discuss the malnutrition issues in Uganda, the nutritional inadequacies of their staple crop and the strategies for biofortifying the Ugandan bananas through optimisation of bioavailable nutrients in transgenic bananas, a program funded by the Bill and Melinda Gates Foundation under the auspices of the Grand Challenges in Global Health program.

Results and discussion

Uganda: The challenge of micronutrient malnutrition

Vitamin and mineral deficiencies remain a significant public health problem in many parts of the world, particularly in developing countries where deficiencies of vitamin A, iron, iodine and other micronutrients led to adverse health consequences. Ugandans also have a high disease burden associated with malnutrition resulting from inadequate dietary intake of nutrients. The most recent data on the nutritional status of the Ugandan population is provided by the Uganda Demographic and Health Survey published by the Uganda Bureau of Statistics (UDHS 2006, UDHS, 2008). The study found that 87.5% of women of child-bearing age are iron deficient, as are a staggering 97.6% of children aged from 6 months to 5 years. Iron deficiency and IDA is highest in the young children, with children living in rural areas at a higher risk compared with their urban counterparts.

Regional variation is also observed, with the north, east, and east-central regions having a higher prevalence of iron deficiency and IDA compared with other regions. The study also determined that, while iron deficiency is the major cause of anaemia in women and children in Uganda, infection appears to act in synergy with iron deficiency, particularly in children, with the result that there is a higher proportion of children with iron deficiency and moderate or severe anaemia compared with those with iron deficiency and mild anaemia or iron deficiency without anaemia. The Ugandan National Anaemia Policy stipulates that where the prevalence of anaemia is over 40%, infants and children over 6 months of age should get iron supplementation appropriate for their age. However, the UDHS 2006 study revealed that only 5.5% of children aged 6–59 months received iron supplements in the week preceding the survey. This indicates that that operation of the National Anaemia Policy has not been effective in reaching infants and young children.

VAD is regarded as an issue of public health importance if 15% or more of a defined population has a plasma retinol concentration less than 0.7 µmol/L (Sommer and Davidson, 2002). The UDHS 2006 study used retinol binding protein (RBP) in dried blood spots (DBS) as a surrogate marker of serum retinol for the assessment of VAD with less than 17.325 µg/ml of RBP as an indicator of deficiency. The prevalence of VAD was 20% in children from 6–59 months old and 19% in women of reproductive age. Since vitamin A is also required for the absorption of dietary iron, VAD is also often accompanied by IDA (Haas et al., 2005).

In Uganda, the proportion of energy and protein per capita obtained from plant products is 94% and 85%, respectively (FAOSTAT, 2008), reflecting that plant products form the major part of the diet of

an average Ugandan. Children are fed bananas (locally known as *matoke*) from about 6 months. The diet therefore predisposes the population to VAD, iron deficiency and anaemia. Kiwanuka et al. (1999) documented low dietary intake and poor bioavailability of iron due to the high proportion of plant foods in the diet in pregnant women attending antenatal clinic at Mbarara University Teaching Hospital. Bachou (2002) indicated that level of malnutrition in Uganda is substantial, with nearly four in ten Ugandan children under five years of age (38%) stunted (short for their age), 6% wasted (thin for their height), and 16% underweight. Regional variation exists in nutritional status of children, with stunting levels highest in southwest and northern regions. Wasting is highest in southwest and east-central regions. The percentage of underweight children is highest in southwest, east-central, and northern regions.

Banana: the staple food in Uganda

In the mid-altitude zones of sub-Saharan East Africa, 35% of the world's bananas are produced, providing 25% of the carbohydrate requirement for ~70 million people living in that region (INIBAP, 1994). Cooking bananas are subsistence crops in large parts of sub-Saharan East Africa, including areas where micronutrient deficiency has been identified as a problem. In Uganda, for example, cooking bananas form the major part of the diet almost everywhere. They are consumed in a multitude of ways, including roasted, baked, fried, boiled, steamed, dried, pureed, or eaten raw. Few staple crops offer such versatility. African domestic banana production is based on a vast array of banana cultivars and landraces which include dessert, cooking, roasting and beer bananas, based on use of their end products (Acland, 1971).

Dessert bananas are consumed raw when ripe and sweet. However, there are two types of cooking bananas. The first type is cooked when the fruits are green and can be allowed to ripen and then eaten as dessert bananas (Simmonds, 1966). The second type of cooking banana is unpalatable raw even when the fruits are ripe and therefore requires cooking before being consumed. These are the true plantains (Swennen and Vuylsteke, 1987). Beer bananas have bitter pulp and are astringent and cannot be eaten raw or cooked; beer is made from this type. In Uganda and Sudan, banana beer is also distilled to produce banana alcohol, or '*waragi*'. Cooking and processing has contradictory effects on carotenoid levels. Processed foods may have higher levels of bioavailable carotenoids because of the loosening of the food matrix, allowing them to be more easily absorbed (Englberger et al., 2003). On the other hand, cooking, especially at relatively high temperatures and for long periods of time, destroys carotenoids, and converts *trans* isomers into *cis* isomers, which have lower vitamin A activity (Booth et al., 1992).

Nutritional value of bananas

Bananas are a cheap energy source. On a fresh weight basis, bananas contain 27% carbohydrate, 1.0% protein and 0.3% fat providing 116 kcal of energy per 100 g of flesh (roughly equivalent to the flesh provided by one banana). There is a great deal of diversity present within this genus and there are banana varieties with naturally orange-coloured fruit flesh, indicating that these varieties could be an important source of dietary provitamin A carotenoids (pVACs), especially in countries such as Micronesia where these Fe'i type banana cultivars are grown (Englberger et al., 2003). One orange fleshed Micronesian cultivar contains up to 1554 µg β-carotene equivalents per 100 g of ripe fruit pulp (Englberger et al., 2006b). This group of bananas is, however, mostly confined to the Pacific. Carotenoid content in general is highly variable between genotypes and cultivars with plantains having a greater amount than other bananas.

Analysis of carotenoid composition of banana reveals that majority of the pVACs found in banana are *trans*-α-carotene (t-AC) and *trans*-β-carotene (t-BC), with a small amount of *cis*-β-carotene (c-BC) and some lutein (Davey et al., 2006; Wall, 2006; Englberger et al., 2006a,b; Khanna et al., unpublished data). The proportion of the different pVACs present varies significantly according to the genotype and in the Micronesian varieties, the proportion of t-BC was found to be higher than t-AC,

varying from 54 to 90% of the total pVAC (t-BC + t-AC) content whereas in all other banana cultivars analysed, the t-AC content is always higher than the t-BC (Davey et al., 2006; Khanna et al., unpublished). These variations can have important consequences for the overall nutritional content of the fruit of the different genotypes because t-AC has only 50% of the provitamin A activity of t-BC.

Flesh colour is a reliable indicator of carotenoid content, which varies from creamy white (low β-carotene) through yellow to orange (high β-carotene). For instance, the white fleshed Williams (Cavendish AAA) cultivar (sampled from South Johnstone DPI&F fields, Australia) contains an average of 119 µg β-carotene equivalents and the lady finger (AAB) contains an average of 178 µg β-carotene equivalents (Englberger et al., 2006; Khanna et al., unpublished data). Plantains contain 203–695 µg of β-carotene equivalents per 100 g fresh weight of the pulp (Honfo et al., 2007). Mpologoma (AAA) and Mbwazirume (AAA), two of the East African Highland cooking bananas, contain an average of 146 and 191 µg β-carotene equivalents respectively per 100 g of fruit pulp (Fungo 2008), which equals 12 and 16 µg retinol activity equivalents (RAE) respectively. Considering that the dietary reference intake (DRI) for vitamin A is 700 µg RAE for adult females and 900 µg RAE for adult males, even consumption of 1 kg of bananas per day would meet less than 20% of the DRI (even with the assumption of 100% bioavailability). Estimating from the colour of the fruit flesh, it is likely that the other East Africa Highland banana cultivars would also contain similar low levels of β-carotene equivalents.

Apart from pVACs, Cavendish bananas contain 0.1–0.27 mg of α-tocopherol (vitamin E) per 100 g (Holland et al., 1991; Khanna et al., unpublished data). On a diet consisting solely of banana, 10 kg would need to be consumed to meet the DRI. Vitamin C content of bananas varies widely in the range of 2.5–17.5 mg ascorbic acid per 100 g of fruit pulp, depending on the cultivar (Wall, 2006). With DRI for vitamin C being 75–90 mg/day, some of the cultivars may be considered as a reasonable source of vitamin C. The thiamine, vitamin B6 and folate levels are low in most banana cultivars (Englberger et al., 2006a).

A range of mineral concentrations have been reported for different banana genotypes and cultivars (Hardisson et al., 2001; Wall, 2006). Cavendish bananas have a low iron content; 0.26–0.45 mg (depending on from where the sample was taken) per 100 g of fruit flesh. Some of the EAHB analysed also revealed iron in the range of 0.2–0.4 mg per 100 g of fruit flesh, depending on the cultivar tested (Fungo, 2008). The DRI for iron is 18 mg for adult females and 10 mg for males and even consuming 1 kg banana per day (children consume much less), anaemic women and children in sub-Saharan Africa will not be able to meet their iron requirements if the existing banana cultivars continue to be used as the staple food.

Strategies for combating micronutrient malnutrition (MNM)

It is evident that vitamin A and iron deficiency are the main public health problems in Uganda and probably other East African countries. There are various strategies that can be used for addressing these deficiencies including: (i) food aid; (ii) artificial dietary supplements; (iii) changed cropping systems; and (iv) improved food quantity and quality in current systems.

Clearly, food aid is not a sustainable option in either the medium or long term. Artificial dietary supplements have been effective in addressing iodine deficiency disorders through the use of iodised salt. This is also being attempted for vitamin A deficiency through the distribution of capsules. However, there are continuing problems with effective distribution of vitamin A capsules and the ability to sustain this approach over the long term is questionable. The delivery of adequate micronutrients through a daily normal diet is certainly the most attractive strategy, either by changing current cropping systems or by improving the quantity and quality of the current cropping systems.

Changing cropping systems through altered crop selection can theoretically result in quite dramatic changes in the delivery of adequate and nutritionally balanced diets, but there are significant barriers to the wide adoption of this strategy in developing countries. The major barrier is farmer/consumer resistance to the proposed change, based on food preferences, knowledge of cropping systems and individual crops, and cultural and social parameters (McIntyre et al., 2001). In many instances,

including with bananas, this preference extends beyond the crop species to the individual cultivar or landrace.

The most appropriate strategy to address nutrient deficiencies in subsistence/small farmer systems is to improve the quantity and quality of currently accepted crops and cropping systems. There is already evidence that micronutrient content can potentially be increased through conventional plant breeding (Bouis, 2003), although there are few examples of this strategy in staple crops in developing countries. The development of 'golden rice' has demonstrated that there is an alternative strategy to biofortify staple crops, through molecular breeding (Ye et al., 2000; Paine et al., 2005). This is particularly significant for bananas, for which conventional breeding programs have been of limited use.

Banana improvement

The nutritional composition of the banana fruit can be enhanced through genetic improvement of the existing and consumer/farmer-accepted banana cultivars. However, vast majority of banana cultivars and landraces grown today are essentially sterile or have extremely low fertility. This has had major implications for banana improvement. First, other than somatic mutations, bananas cultivated today are almost certainly similar to the original selections and have probably not undergone any genetic improvement over thousands of years. Second, this sterility or low fertility has severely limited the ability to genetically improve bananas through conventional breeding. The consequence of these factors is that, in the foreseeable future, it will be impossible to improve specific traits in currently accepted banana cultivars and landraces through conventional breeding.

In contrast, the genetic modification of bananas has moved rapidly. In less than a decade, transformation of bananas has become routine, at least in a few centres around the world. The first reports of banana transformation were in 1995 (May et al., 1995; Sagi et al., 1995). One of these techniques proved extremely difficult to replicate in other locations and the other was limited to a single cultivar, Bluggoe, which was of limited production interest. The development of a protocol for generating embryogenic cell suspensions from immature male banana flowers (Cote et al., 1996) was a major improvement and this led to the transformation of the major commercial cultivar, the Cavendish. Our laboratory firstly reported the transformation of Cavendish by microprojectile bombardment (Becker et al., 2000) and then by *Agrobacterium tumefaciens* mediated transformation (Khanna et al., 2004). The protocols of Becker et al. (2000) and Khanna et al. (2004) have been applied to a range of different cultivars and genotypes. The technology to transform bananas and express new transgenes in bananas is now well established. It is clear that this is the only strategy currently available to genetically improve existing banana cultivars.

Biofortification through genetic manipulation

Genetic improvement of the nutritional quality of food crops, also known as biofortification, is a promising strategy to combat malnutrition in developing countries. Over the past decade, there has been rapid progress in the understanding of biochemical synthesis for various nutrients, particularly micronutrients in plants. This has led to the development of transgenic strategies for enhancing the micronutrient content of plants through modification of the existing biosynthesis pathways or the addition of new biosynthetic pathways. Examples of enhanced nutrient content or composition include 'golden rice' (Paine et al., 2005,) with increased pro-vitamin A carotenoid content and high-iron rice (Haas et al., 2005). These products have a significant promise in improving intakes of key nutrients in at-risk populations (Meenakashi et al., 2007). This modification is targeted particularly at southern and southeast Asia where rice, rather than bananas, is the primary staple food and where VAD also is a major public health issue.

Strategies for enhancing pro-vitamin A carotenoids

Carotenoids are lipophilic isoprenoid compounds synthesised by all photosynthetic organisms (including plants, algae, and cyanobacteria) but also by some non-photosynthetic bacteria and fungi. Most carotenoids are located, together with chlorophylls, in functional pigment-binding protein structures embedded in photosynthetic (thylakoid) membranes. Geranylgeranyl diphosphate is the substrate for all carotenoid production. It is modified by phytoene synthase (*psy*) to generate phytoene. Subsequently, two desaturases transform phytoene first to phytofluene and then via ζ-carotene to neurosporin, which spontaneously changes into lycopene. Lycopene isomerase and lycopene β-cyclase produce the final product β-carotene (Ye et al., 2000), which after ingestion is split into two molecules of retinol (vitamin A) in human mucosal cells. The identification of the carotenoid biosynthesis genes in plants and other organisms has opened the door to the biotechnological overproduction of carotenoids of nutritional interest in crops. The genes encoding these enzymes have been isolated from a variety of bacteria, fungi and plants, and functionally characterised (for review see Hirschberg, 2001). Here, we will review some of the metabolic engineering approaches carried out in crop plants to increase the levels of nutritionally relevant carotenoids. Most of work has been done in rice, but important accomplishments have taken place in other crops.

The enzyme that catalyses the first committed step in the carotenoid biosynthesis pathway, *psy*, was the first target for the biotechnological manipulation of the carotenoid composition. The production of β-carotene (pro-vitamin A) in carotenoid-free rice endosperm (Ye et al., 2000) represented a breakthrough not only in the biotechnology of carotenoids but also in the public awareness of the potential of this approach to improve food nutritional value. The daffodil (*Narcissus pseudonarcissus*) genes encoding *psy* and *lcyB* (under the control of the endosperm-specific glutelin promoter) and the bacterial desaturase encoded by the *crtI* gene fused to a plastid-targeting sequence (constitutively expressed) were transferred to Japonica rice plants. The resultant transgenic rice grains, named 'golden rice', showed a distinctive yellow colour following accumulation of carotenoids. The total carotenoid amount of 1.6 μg g^{-1} dry weight was achieved in heterozygous plants. However, the highest increase in carotenoid levels was achieved when the daffodil *psy* was substituted by the maize *psy* and the carotenoid content of the rice grain reached up to 37 μg g^{-1} dry weight (Paine et al., 2005). These results highlighted the importance of the step catalysed by *psy* in the control of carotenoid biosynthesis.

In canola (*Brassica napus*) seeds, expression of a bacterial *crtB* gene extended with a plastid-targeting sequence under a seed-specific promoter increased the total carotenoid content of mature seeds up to 50-fold (Shewmaker et al., 1999). However, approaches expressing the bacterial *crtB* gene could only lead to a four-fold increase in the amount of total carotenoids in tomato fruit (Fraser et al., 2002). The carotenoid content of potatoes was increased by targeted overexpression of the bacterial *crtB* gene in tubers of *Solanum tuberosum*, a species with low carotenoid levels and *S. phureja*, a carotenoid-accumulating species (Ducreux et al., 2005). When the patatin promoter and a plastid-targeting sequence were used to specifically increase *psy* activity in the plastids of developing tubers, *S. tuberosum* and *S. phureja* lines accumulated around seven-fold and three-fold higher carotenoid levels, respectively, than untransformed lines. The carotenoid profile also changed dramatically in transgenic *S. tuberosum* tubers, which were highly enriched in lutein (19-fold increase) and β-carotene (more than 10 μg g^{-1} dry weight). In addition, higher α-tocopherol levels were also observed in some of these lines.

Transgenic approaches that have been effective in modifying carotenoid content in plants to enhance their nutritional value also include modification of the carotenoid pathway by shifting to another carotenoid product in tomato (Römer et al., 2000). New genetic and genomic approaches are now in progress to identify regulatory factors that might significantly contribute to improve the nutritional value of plant-derived foods by increasing their carotenoid levels.

Strategies for enhancing iron

Iron is present in sufficient amounts in most soils, but it is not always accessible to plants because it is either chemically bound or is present in unavailable forms. Furthermore, unlike pro-vitamin A, iron cannot be produced by the plant. Thus, the approach to increasing the iron content of the plant is relatively more complex. Transgenic approaches to biofortification rely on improving mobilisation of iron from the soil, uptake from the rhizosphere, translocation through the aerial tissues and accumulation of Fe in a bioavailable form in edible tissues. Iron uptake, transport, and storage are tightly regulated to prevent both iron deficiency and toxicity, thus ensuring optimal plant development.

Plant iron storage takes place in the apoplasmic space, in the vacuoles and in the ferritins (Briat and Lobreaux, 1998). Plant ferritins are located in the plastids and each ferritin particle can store up to 4500 iron atoms (Harrison and Arosio, 1996). Accumulation of iron in the various plant tissues during growth and development is a dynamic process resulting from an integrated regulation of genes encoding proteins for iron transport and storage. These processes depend on the plant genotype and are greatly influenced by environmental cues.

Leaves are a major organ for iron accumulation in plants, and 80% of this storage is in the chloroplasts. In response to deficiencies, plants use different strategies to acquire iron from the soil. In strategy I, plants such as *Arabidopsis thaliana*, iron deficiency induces synthesis of FRO2, a ferric-chelate reductase (Robinson et al., 1999). This leads to generation of ferrous iron, which is taken up across the root plasma membrane by specific transporter(s). IRT1 is the major root plasma membrane iron transporter (Eide et al., 1996; Vert et al., 2002). Regulation of the IRT1/FRO2 high-affinity iron uptake system in the root requires iron and an unknown shoot-borne signal (Vert et al., 2002).

Expression of the soybean ferritin gene under the control of an endosperm specific promoter in rice increases the Fe content in the endosperm by 30% (Goto et al., 1998). Intriguingly, ferric reductase activity was up-regulated in response to the expression of soybean ferritin in transgenic tobacco (Van Wuytswinkel et al., 1998), suggesting that ferritin expression 'tricks' the plant into behaving as if it were growing under iron-limiting conditions. *A. thaliana* plants expressing FRO2 under the control of the CaMV 35S promoter grew better on low iron, compared with control plants, further reinforcing the concept that reduction of ferric iron to ferrous iron is one of the rate limiting steps in iron uptake (Connolly et al., 2003).

As in case of FRO2, constitutive expression of IRT1 in *A. thaliana* resulted in accumulation of the IRT1 protein in the roots only when iron was limiting (Connolly et al., 2002). *A. thaliana* plants that expressed IRT1 also accumulated higher levels of zinc than wild-type plants but FRO2 expressing plants did not (Connolly et al., 2003). Seeds from transgenic barley expressing *Arabidopsis* zinc transporter (AtZIP1) showed higher Fe and Zn content than the controls, although seeds from transgenic lines were significantly smaller than those from non-transformed plants (Ramesh et al., 2004). Apparently, processes controlling iron movement within the plant are responsible for iron accumulation within the grain. Douchkov et al. (2004) expressed a nicotianamine synthase gene from *A. thaliana* in transgenic tobacco plants. The presence of extra copies of the nicotianamine synthase gene co-segregated with up to 10-fold elevated levels of nicotianamine compared with wild type. The increased nicotianamine level led to a significantly increased iron level in leaves of adult plants, and an improvement of the iron use efficiency in adult plants grown under iron limitation.

Grand Challenges in Global Health initiative: Banana Biofortification program at QUT

The Grand Challenges in Global Health initiative is a partnership dedicated to supporting scientific and technical research to solve critical health problems in the developing world. In 2003, the Bill and Melinda Gates Foundation announced 14 grand challenges and one of the grand challenges was to create a full range of optimal bioavailable nutrients in a single staple plant species. As part of

this program, QUT and NARO commenced a banana biofortification project in 2005 to enhance the micronutrient content of EAHB.

The ultimate aim of this project is to address the chronic micronutrient malnutrition in Uganda through transgenic biofortification of a major staple food, EAHB. This aim will be fulfilled through the generation of farmer and consumer-acceptable edible bananas with significantly increased fruit levels of pro-vitamin A and iron. The strategy has been to develop the technology in Australia using Cavendish as the model and progressively transfer the technology to Uganda. The products released in Uganda will be generated in Uganda by NARO. The technical strategy has been to use the phytoene synthase as the main gene for improving the pVAC content of banana. For iron enhancement, different genes including ferritin, IRT1 and FRO2 are being used in isolation or in combination, to generate transgenic lines with high iron content. QUT has generated a wide range of single trait transgenic Cavendish and lady finger lines with various combinations of promoters and transgenes and these first generation lines were planted in Australia's first GM banana field trial in December 2008. NARO has developed a reproducible EAHB transformation and regeneration system, which was considered a major hurdle, and now has transgenic plants in the glasshouse. Transgenic EAHB and Sukali Ndizi field trials are expected to commence in Uganda by July 2009.

Conclusions

Poor nutrition is a major global health problem. A promising long-term solution is to genetically modify staple crops to contain high levels of essential nutrients. Biofortified bananas will offer one such cost-effective intervention that will reach the small farming populations which comprise majority of the malnourished in East African countries. These genetically improved and nutritionally enhanced bananas are posed to be a powerful, low-cost and sustainable malnutrition alleviation strategy targeting the rural areas where the most vulnerable populations live.

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HarvestPlus agenda in relation to tropical fruits

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Keywords: banana, plantain, *Musa*, pro-vitamin A, biofortification, Africa, nutrition, breeding

Abstract

HarvestPlus seeks to reduce micronutrient malnutrition among the poor by breeding staple food crops that are rich in micronutrients through a process called biofortification. HarvestPlus has targeted banana and plantain (*Musa* spp.) to deliver higher levels of pro-vitamin A carotenoids (pVAC) to those communities in sub-Saharan Africa with a higher prevalence of vitamin A deficiency. Iron and zinc have also been considered but, to date, the natural genetic variation discovered for Fe and Zn in *Musa* is below 50% of the target increment so the major focus is on improving pVAC concentration. The initial plant breeding strategy has emphasised a fast-track approach that selects pVAC-dense genotypes from existing material that is locally adapted. Working with the International Institute for Tropical Agriculture (IITA), Bioversity International and the National Agricultural Research System (NARS) partners associated with these institutions, considerable testing of the nutrition and agronomic characteristics under relevant conditions in African target countries has taken place. The breeding strategy entails combining best pVAC sources with African adapted elite varieties that carry the productivity, disease and virus resistance, and sensory traits. Rapid generation advance and propagation techniques are used and further developed and the development of molecular markers for micronutrients and those facilitating breeding has increased breeding effectiveness. Plant breeding progress has led to improved sampling procedures and from the screening process, genotypic variation to reach the target of an extra 20 micrograms/gram pVAC has been discovered in *Musa* varieties from the Asian and Pacific regions while progenitors have been identified by IITA, Bioversity and NARS partners. Once pVAC-dense varieties have been fully developed, the *Musa* deployment strategy will capitalise on existing Consultative Group on International Agricultural Research (CGIAR), NARS and Non-governmental Organisation (NGO) partnerships in germplasm development, training/capacity building and germplasm deployment in Africa.

Introduction

The goal of HarvestPlus is to improve the health of poor people by breeding staple food crops that are rich in micronutrients, a process referred to as biofortification. Micronutrient malnutrition, primarily the result of diets poor in bioavailable vitamins and minerals, affects more than half of the world's population, especially women and pre-school children. The biofortification strategy seeks to take advantage of the consistent daily consumption of large amounts of food staples, thereby providing a nutritional boost that was previously not available.

The International Network for Improvement of Banana and Plantain (INIBAP) estimates that 85% of all bananas are grown in smallholdings and eaten locally, more often than not as a staple food. In some areas close to 1 kg of cooking banana are eaten every day.

To reach the Millennium Development Goal's target of halving the proportion of undernourished people by 2015, new technologies and approaches are needed to help address the problem. HarvestPlus seeks to bring the latest advances in agriculture and nutrition science to bear on the persistent problem of micronutrient malnutrition. Improving the nutritional content of banana is one area being explored.

Materials and method

Breeding bananas and plantains (*Musa*) is complex as commercial varieties are sterile triploids (3X), which are developed from crossing fertile diploids (2X) and tetraploids (4X). Among the fertile groups, a high degree of cross-incompatibility can exist. Further, the *Musa* crop cycle is long and consequently, the initial biofortification strategy emphasises a fast-track approach (selected from existing material) that entails evaluating what appears to be existing high pVAC *Musa* that are locally adapted in Africa. HarvestPlus will test the nutrition and agronomic characteristics under relevant conditions in African target countries and deploy material to farmers along with crop management recommendations.

The breeding strategy entails combining best pVAC sources with African adapted elite varieties that carry the productivity, disease and virus resistance, and sensory traits. Rapid generation advance and propagation techniques are used and further developed, and the development of molecular markers for micronutrients and those facilitating breeding has increased breeding effectiveness in HarvestPlus II. Proof-of-concept research using Near Infrared Spectrocopy (NIRS) for pVAC in *Musa* is part of the 2008 agenda.

Results and discussion

Measuring potential impact

A review of consumption patterns of *Musa* was commissioned for several sub-Saharan African countries, supplemented by semi-quantitative surveys of consumption in peri-urban areas. This information was then used to compute the Disability-Adjusted Life Years (DALYs) that could be saved from a biofortified *Musa*.

Nutrition

An initial targeting exercise using estimates of banana/plantain consumption and retinol equivalency determined that an additional increment of 20 micrograms/gram provitamin A would need to be added to a baseline of 16 micrograms/gram provitamin A in banana/ plantain. Based on an intake of 300 g of banana/plantain (fresh weight) per day for adult women or 150 g/day for children 3–5 years of age, a 50% loss of provitamin A following processing and cooking, and a provitamin A retinol equivalency of 12:1, this target increment of 20 µg/g fresh weight would provide approximately one half of the current estimated average requirement for total dietary vitamin A (Food and Nutrition Board/Institute of Medicine, 2002).

To our knowledge, studies of the bioavailability of provitamin A from banana have not yet been conducted. It is of interest to determine the nutrient bioavailability from banana, particularly for provitamin A. Conventional varieties rich in provitamin A vary a lot in their ratio of α-carotene to β-carotene. In a selection of banana cultivars from Australia, Englberger and colleagues reported ranges of β-carotene content from 0.5 to 14.1 µg g^{-1} fresh weight and of α-carotene content from 0.6 to 10.6 µg g^{-1}; the ratios of β-carotene to α-carotene ranged from 0.5 to 4.8 (Englberger et al., 2006). This is of interest for the bioefficacy of such bananas to contribute to improved vitamin A status because it is currently believed that the conversion of α-carotene to the active form of vitamin A in humans is only half as efficient as it is for β-carotene. That is, while it is generally accepted that 12 µg β-carotene in a typical diet are required to produce 1 µg of retinol and this ratio would be 24:1 for α-carotene (Food and Nutrition Board, Institute of Medicine, 2002). Nonetheless, these conversion rates would need to be established specifically for Musa and in the context of the diets of potential target populations.

Plant breeding

Sampling protocols for *Musa* have been developed and published (Davey et al., 2007; Stangoulis and Sison, 2008). Further, the long crop cycle along with the extra resources required to establish

nurseries due to the large size of the plant and quarantine restrictions, complicated screening. More than three hundred genotypes have been assayed by IITA, Bioversity and NARS partners, and more than five hundred core collection accessions have been pre-screened/described for pVAC indicative pulp color. Maximum values for pVAC discovered in African varieties and final products developed at IITA are close to the target increment and are subject to further validation in genome × environment (G × E) trials. Variation to reach the target for pVAC has been discovered in *Musa* varieties from the Asian and Pacific regions. HarvestPlus research revealed differences in the carotenoids profiles. The variation discovered to date for Fe and Zn in *Musa* is below 50% of the target increment.

Progenitors have been identified by IITA, Bioversity and NARS partners. Diploid populations for genetic analysis of minerals have been developed from more than twenty crosses. First generation crosses for high pVAC have been conducted.

Adapted genotypes have been evaluated for use as parents and/or to fast-track in multi-location trials in Nigeria and Cameroon along with local checks. The G × E effect has been evaluated from more than thirty on-station and on-farm trials in Nigeria and Cameroon and results from the various experiments and partners are currently compiled. Preliminary results reveal stability for pVAC. Preliminary results on agronomic performance and micronutrient content of a high-micronutrient cultivar in high-density systems with different fertiliser applications are also available.

Dissemination activities

The Musa deployment strategy capitalises on existing CG, NARS and NGO partnerships in germplasm development, training/capacity building and germplasm deployment in Africa. Multilateral cooperation includes projects such as: Building Impact Pathways for Improving Livelihoods in *Musa*-based Systems in Central Africa (Bioversity/INIBAP—IPGRI (International Plant Genetic Resources Institute) led); Sustainable and Profitable Banana-based Systems for the African Great Lakes Region (IITA led); Enhancing the resilience of agro-ecosystems in Central Africa: a strategy to revitalise agriculture through the integration of natural resource management coupled with resilient germplasm and marketing approaches (Tropical Soil Biology and Fertility Institute of CIAT–led), and supported by strategic research and capacity building by the Katholieke Universiteit Leuven and the Université Catholique de Louvain-la-Neuve. Further, lead centres already operate regional and national multiplication centres and have experience in successful large scale rollout in Africa.

In the context of the Rwanda crises, the Belgium Development Cooperation Agency (BTC/CTB) project multiplied 2.5 million plants of 24 varieties that reached 0.5 million people. On a country specific basis, additional partnerships will be formed in deployment strategies based on stakeholder meetings and gap analysis. Furthermore, strategies will be harmonised with deployment efforts for other biofortified crops in the same country.

Future activities

Measuring potential impact

DALY analysis will be conducted to determine potential cost-effectiveness, which in turn will determine target countries.

Nutrition

The true retinol equivalency will need to be determined to better evaluate the feasibility of this crop in meeting target levels, and hence to improve vitamin A status. Ideally, a human study to estimate the retinol equivalency of pVAC rich banana/plantain from a test meal using the modified ‘area under the curve’ method, as used for maize and cassava, will be undertaken.

Plant breeding

Additional varieties will be assessed for pVAC, and the variation in micronutrients, anti-nutrients and fruit quality traits evaluated. Furthermore, different sampling and screening procedures will be compared for a set of representative genotypes.

Additional parents will be identified and the inheritance pattern for minerals established. Guidance on more efficient breeding and selection methods for *Musa* will be documented. Additional crosses for high pVAC will be conducted and a seedling nursery will be established from 2007 crosses following embryo rescue.

Effects of environment and influence of harvest cycle on micronutrient variability will be assessed from more than ten multi-location trials in Nigeria and Cameroon, and at least one candidate variety will be planted on-farm for evaluation in Nigeria. The effects of fertiliser input in high-density planting and associated technologies on micronutrient variability will be evaluated on-farm in Cameroon.

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Fruit intake for healthy body size in the Pacific region

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Keywords: Pacific Islands, obesity, overweight, fruit intake

Abstract

Studies of predominantly Asian and Pacific Islander populations, conducted in Hawaii and several Pacific islands, have suggested that a pattern of dietary intake higher in fruit intake may protect against overweight and obesity. Among adult hotel workers in Hawaii, an extra daily serving of fruit was associated with 0.3 kg/m^2 lower body mass index (BMI), adjusting for age, sex, ethnicity, place of birth, acculturation, meat intake, and sweet drink intake. Fruit juice availability in vending machines was associated with another 0.3 kg/m^2 lower BMI. In a different study of adult diet in Hawaii a cold food eating pattern (with predominant foods being breakfast cereal, fruit and fruit juice) was associated with a 0.1 kg/m^2 lower BMI. Children in the northern Pacific were also found to consume less than the recommended amount of fruit, while rates of overweight and obesity are increasing. In Hawaii, adolescent girls ate less than two servings of fruit per day, on average. In the Commonwealth of the Mariana Islands, children ate less than one serve of fruit per day (on average), while overweight and obesity (measured by BMI for age and sex) was 25% among 2–3 year olds and 45% among 7–10 year olds. We have used these dietary intake studies to enhance the Cancer Research Center of the University of Hawaii's (CRCH) food composition database with foods and recipes of the Pacific region. Research data may be analysed at CRCH by contract. The database contains 146 nutrients and food components per 100 grams, as well as flavonoids, isoflavonoids, conjugated linoleic acid, and glycemic load. A user friendly subset of the database is available on the publically available Pacific Tracker, on the Cancer Research Center website, and on the College of Tropical Agriculture Hawaii Foods website.

Introduction

Overweight and obesity are significant public health problems in the Pacific Region, as in the rest of the world (Davis et al., 2004). Definitions of overweight and obesity differ among populations (especially among Asian and Pacific Islander populations); though it is clear that excess body weight and fat pose health risks. Changed dietary patterns, toward energy-dense lower fiber foods, have been implicated as contributory to overweight and obesity (Astrup et al., 2008). Increasing fruit consumption holds promise for managing body fat and weight, and protecting health in the Pacific Region.

Because fruits are low in fat and high in water and fiber, incorporating them into the diet can reduce energy density, increase satiety and decrease food and energy intake (Rolls et al., 2004). Ways in which fruit consumption could decrease weight are through their lower energy density (kcal or kJ/g), higher fiber content, and relatively low glycemic index (time to convert the carbohydrate in them to blood glucose) as compared to many other food choices. 'Eat more fruit' is a positive behavioral and public health message that promotes eating food, whereas a nutrient-based message is more difficult to interpret back into food intake guidelines, and may lead consumers to seek nutrients from supplements.

Findings from studies outside of the Pacific

Whole fruit has been found to be more satiating than puree, which is more satiating than (fiber free) fruit juice (Haber et al., 1977; Bolton et al., 1981). Increasing fruits and vegetables in the diet, without decreasing fat content, has resulted in a spontaneous overall decrease in energy intake in clinical trials (Bell et al., 1998), though still not with sufficient consistency to confirm the relationship. Adding advice to reduce fat and energy intake to a whole fruit diet has improved success (Rolls et al., 2004).

Most studies have not separated the influence of fruit and vegetable intake on health. In a review of the relationship between fruit and vegetable consumption and body weight (Tohill et al., 2004), only two of 17 studies in children (one prospective cohort and one cross-sectional) explored the association between fruit and vegetable intake and body weight as a primary objective. One of those, a 3-year prospective cohort (Field et al., 2003), found no association between change in age and sex-specific *z* scores of body mass index (weight (kg)/height $(m)^2$, BMI) and total fruit and vegetable intake, fruit intake (without juice) and fruit juice intake in either boys or girls. In boys, but not girls, lower vegetable intake was associated with overweight, but not after adjustment for energy intake.

The other study, by Lin and Morrison (2002), found that boys at the greater than the 85^{th} percentile of BMI for age ate fewer total vegetables, less fruit and fewer white potatoes than did healthy weight boys using US National Continuing Survey of Food Intake of Individuals (CSFII, cross-sectional) data; and, similarly, less fruit (including fruit juice) was consumed by girls greater than the 85^{th} percentile of BMI for age compared to healthy weight girls (controlling for age, gender and race/ethnicity). No significant differences were observed in vegetable or white potato consumption between the three weight-status groups. A limitation in both of these studies is that anthropometric measurements were self-reported. Six studies looked at the cross-sectional association between overweight and fruit juice and fruit drink consumption in children, with inconsistent results.

Eight of the 16 studies in adults reviewed by Tohill et al. (2004), showed a significant association (seven negative, one positive) between higher intakes of fruits or vegetables and lower body weight status, as did the study by He et al. (2004), which also showed relationship of fruit and vegetable intake to decreased risk of weight gain in women in a prospective design. The category of exposure (total fruits and vegetables, vegetables only, or fruit only) did not modify the direction of the association. Higher variety of fruits and vegetables in the diet was associated with lower body fat. Method of fruit preparation, physical form of fruit (purees vs. whole fruit) and food intake patterns varied in the studies, as did adjustment for demographic factors and total energy intake. Since these factors may influence results, they should be considered in future studies on fruit intake and body weight.

Environmental approaches to manage obesity, such as increasing availability of fruit in the environment, may be needed for sustainable maintenance of healthy body size at the population level.

Materials and methods

The objectives of this paper are:

1. to present data related to fruit-rich dietary patterns and body size among children and adults of the northern Pacific.
2. to describe specific recipes, tools and, databases that we have developed and are developing to evaluate dietary intake of Pacific populations.
3. to describe future research directions.

Results

Fruit intake among adults of the Pacific region

In a cross-sectional study among 514 multi-ethnic adults (35–85 years) in Hawaii, a cold food eating pattern was identified and adjusted for energy intake level using factor analysis of food groups that were derived from the Multiethnic Cohort Study's calibrated food frequency questionnaire (Stram et al, 2000). The cold food eating pattern was correlated ($r = –0.13$, $p = 0.003$) with a lower BMI as compared to other eating patterns (Maskarinec et al., 2000). Other eating patterns were described as 'meat', 'vegetable', and 'bean'. The meat pattern was positively associated with BMI ($r = –0.169$, $p = 0.0001$), whereas vegetable and bean patterns were also negatively associated with BMI ($r = –0.076$, $p = 0.084$ and $r = –0.132$, $p = 003$, respectively). The ethnic mix of the population in Hawaii (Chinese, Japanese, native Hawaiian, white and other) makes the food environment especially rich. The directions of association were the same in each ethnic group.

Fruit intake was studied in the hotel worksite environment in the Work, Weight and Wellness (3W) study (Vogt T PI) at baseline (Williams et al., 2007). The 3W study examined the impact of a worksite lifestyle and weight management program on BMI, weight, health behaviours, worker productivity, and worker attitudes about weight, nutrition and physical activity among 4530 employees of participating hotels in Hawaii. Intake of sweet drinks and meats were positively associated with BMI while intake of fruit was negatively associated with BMI in this group, controlling for age, sex, race/ethnicity, immigration and acculturation (Novotny et al., 2008). Lower fruit intake and vending fruit juice availability in the environment were associated with a higher BMI (Oshiro et al., 2008). Table 1 summarises the association of fruit consumption and BMI of adults in the Pacific.

Table 1. Fruit consumption is negatively associated with BMI in cross-sectional (adjusted) studies of adults in Hawaii

Authors, Year	Study population	Fruit measure (tool), analysis	(Adjusted) Result
Maskarinec, G et al., 2000	35–85 years Hawaii (n = 514)	Cold food eating pattern (tool) factor analysis	(energy) r = –0.13, p=0.003
Novotny, R et al., 2008	hotel workers Hawaii (n = 4530)	Servings of fruit consumed in a day (FFQ) Multiple regression	(Age, gender, race/ethnicity, place of birth, acculturation, sweet drink and meat intake) b = –0.21, SE = 0.09, p = 0.02
Oshiro, C et al., 2008	hotel workers Hawaii (n = 4536)	Servings of fruit consumed in a day (FFQ) Multiple regression Fruit juice in vending machine (FFQ) Multiple regression	(age, gender, race/ethnicity, education, cafeteria set menu, cafeteria hours) b = –0.39, SE = 0.19, p = 0.05 (age gender, race/ethnicity, education, cafeteria set menu, cafeteria hours, fruit intake) b = –0.29, SE = 0.13, p = 0.03

Fruit intake among children of the Pacific region

High-school (n = 590) and middle-school students (n = 649) on Guam participated in the 1999 Safe and Drug Free Schools and Communities Youth Risk Behavior Surveillance Study (CDC, 1999). Baseline data collected by Leon Guerrero and Workman (2002) showed that 27% of Guam's adolescents were considered overweight or obese. Males were more likely to be overweight or obese (31%) compared with females (23%). Only 25% of adolescents consumed any fruit or

vegetables. Fruit and vegetable intake did not differ by gender, ethnicity, age, school grade, BMI or physical activity level.

Servings of fruit/day and BMI are described by Paulino et al. (2008) among children, 2– 10 years of age in the Commonwealth of the Northern Mariana Islands (CNMI). Fruit intake recommendations, for this age group, range from 2–2.7 servings/day, based on energy levels. In comparison with these recommendations, the 2–3 year-olds consumed 0.9 servings/day and were 12% overweight and, 13% obese; the 4–6 year olds consumed 0.6 servings/day and were 13% overweight and 13% obese; and the 7–10 year old groups consumed 0.9 servings/day and were 18% overweight and 27% obese.

In the Female Adolescent Maturation (FAM) Study in Hawaii, we assessed nutrient and food intake of girls, aged 9 to 14 years (Daida et al., 2006). Compared with the US recommendations of 2–4 servings per day, fruit intake was low in Asians (1.3 ± 1.2) and whites (1.7 ± 1.2 servings/day). This intake was composed of 0.6 ± 1.0 servings of citrus, melon and berries, and 0.8 ± 0.7 other fruits (Lee et al., 2007). Eighty percent of girls did not meet fruit intake recommendations. Two years later, at ages 11–16 years, 84% of girls did not meet recommended levels of intake (Lee et. al., 2007). Table 2 summarises cross-sectional, unadjusted studies describing fruit consumption and BMI of children in the Pacific.

Table 2. Fruit consumption and BMI of children in the Pacific- cross-sectional (unadjusted) descriptive studies

Author, Year	Study population	Fruit measure	Fruit intake	Overweight or obese[1]
Leon Guerrero and Workman, 2003	high school and middle school students Guam (n = 1203)	number of times fruit eaten in past seven days	15.2% ate five pieces a day 24.7% consumed fruits or vegetables	27%
Lee, SK et al., 2007	9–14 years 11–16 years Hawaii (n = 150)	Servings per day	1.3 ± 1.2 servings/day 1.3 ± 1.2 servings/day	29%
Paulino, Y et al., 2008	2–3 years 4–6 years 7–10 years CNMI (n = 420)	Servings per day	0.9 servings/day 0.6 servings/day 0.9 servings/day	25% 26% 45%

[1]Centers for Disease Control > 85th percentile BMI for age and sex

We recently received a grant to: 1) develop and evaluate the impact of the PacDASH intervention (based on DASH eating principles, which include increased fruit intake) for preventing weight gain in overweight children of the Pacific region and 2) describe environmental, social, economic, and cultural factors associated with body size and composition of children of the Pacific region, for whom there are few national data (Novotny R, PI). A quick survey of 5–9 year old children in Hawaii (Novotny R, personal communication) was conducted to assess types of fruits consumed by children of this age group. In response to the question 'what fruits do you eat?', 77 children responded: apple, banana, peach, orange, grapefruit, grapes, cherries, watermelon, melon, strawberry, mango, nectarine, fuji apple, lychee, kiwi, dragon fruit, papaya, and fruit cocktail. Also reported were: broccoli, corn, carrots, pizza, spaghetti and saimin (noodles with soup)!

Supermarket survey in Hawaii

In a survey of 221 customers in four Hawaii supermarkets (Oshiro et al., 2004), 75% had fruits and vegetables in their shopping bags. The top 10 fruits were: banana, apple, orange, papaya, grape, grapefruit, tangerine, avocado, cantaloupe, and honeydew melon. When asked, 'What would you like to know about fruits and vegetables?' customers were most interested in information related to health, freshness and quality and origin of fruits and vegetables (e.g. local origin).

Tools to further research on fruit intake and BMI in the Pacific region

Food composition data

Under the direction of Dr. Suzanne Murphy, the Nutrition Shared Support Resource at the Cancer Research Center of Hawai'i (CRCH, Murphy, 2002) develops and manages a food composition database that houses 2400 foods and recipes, including local foods and ethnic dishes. The database contains 146 nutrients and food components per 100 grams, as well as flavonoids, isoflavonoids, conjugated linoleic acid, and glycemic load. Research data may be analysed at CRCH by contract. A user-friendly subset of the database is available on the publicly available Pacific Tracker, on the University of Hawaii's CRCH website (http://pactrac.crch.hawaii.edu/) and on the College of Tropical Agriculture Hawaii Foods website (http://hawaiifoods.hawaii.edu).

The Pacific Tracker (PacTrac) Dietary Intake Assessment Tool

The PacTrac was developed from the USDA Interactive Healthy Eating Index (www.usda.gov/cnpp) in a previous grant (Novotny R., P.I., Murphy et al., 2006) The resultant PacTrac dietary assessment system can be used to analyse local diets, assess dietary adequacy and excess, and provide nutrition education. The food composition database for PacTrac was developed by starting with the CRCH food composition database (Murphy, 2002). Appropriate food lists were obtained by gathering foods and recipes from 24-hour recalls administered by collaborators in the Healthy Living in the Pacific Islands (HLPI) Initiative from American Samoa, Commonwealth of the Northern Mariana Islands, Federated States of Micronesia, Hawaii, Guam, Palau, and the Republic of the Marshall Islands (Novotny R, PI). Additional nutrient composition data were obtained from a variety of published references. PacTrac was tested in qualitative interviews of mothers and children and in surveys comparing dietary data entered with PacTrac to the same data entered utilizing more traditional methodology (Martin et al., 2006)

Hawaii Foods website

The Hawaii Foods Website (www.hawaiifoods.hawaii.edu, University of Hawai'i, 2007) is a public information website that was created to assist individuals in making better food choices and improve their diet. Nutrient information on foods commonly consumed in Hawai'i is available in this user-friendly resource. It is made available through the collaborative efforts of the University of Hawaii's College of Tropical Agriculture and Human Resources (CTAHR) and Cancer Research Center of Hawaii.

A DASH of Aloha, Healthy Hawai'i Cuisine and Lifestyle book

The *DASH of Aloha* book was created by the Kapi'olani Community College, of the University of Hawaii (Kapi'olani Community College, 2007). A team of experts in diet, nutrition, and healthcare introduce a unique and delicious way to eat well by tailoring the Dietary Approaches to Stop Hypertension (DASH) dietary principles to the island palate and lifestyle (NIH/NHLBI, 2006) Recipes include local farm products highlighting Hawaii's agriculture. Education is provided on how to utilise the DASH eating plan for heart and kidney health.

Discussion

Increasing fruit intake in the diet shows promise to prevent weight gain, maintain weight and possibly to decrease weight. Our studies in Hawaii suggest that, among adults, an extra serving of fruit is associated with a BMI that is about a quarter of a unit lower. Our studies in the northern Pacific show children consuming about one serving of fruit per day, and more than a quarter of children are overweight and obese.

Further study is warranted since not all studies have shown consistent results, and most study designs have been cross-sectional. For example, Utter et al., (2007) described the relationship of fruit and vegetables and obesity in a New Zealand population of high school students (n = 3490) who were predominantly of Pacific Island ethnicity and where 57% were overweight or obese. Eating more servings of fruits and vegetables was associated with a lower BMI among students who were not trying to change their weight. However, students who were trying to lose weight had the opposite effect, where consumption of more fruit and vegetables/day was associated with a higher BMI. Thus, weight control behaviors should be considered when analysing cross-sectional research relationships of fruit and vegetable intake and BMI.

In conclusion, more study is needed that separates fruits from vegetables, identifies specific fruits and examines the form of fruit consumed and its preparation method. Studies should consider and adjust for possible weight loss behavior in interpreting relationships of food intake and weight status of study subjects. Studies should use longitudinal and intervention designs, and environmental approaches to further understand the potential of fruit in determining healthy body size in the Pacific.

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Cultivar selection can have significant implications for our health

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Abstract

It is well recognised that the nutrient composition of fruits and vegetables is variable, affected by a range of factors, particularly growing conditions (climate, soil), maturity and cultivar. However, the extent of variation across cultivars is not generally recognised, and rarely considered by health professionals who base their advice to the public on food composition tables that present 'representative' values. Further, food producers have generally given more priority in cultivar selection and breeding to characteristics such as yield and post-harvest handling than to nutrient composition. In this paper we present recent estimates of nutrient composition across a range of cultivars of foods commonly grown and eaten in the Pacific Islands, including banana, breadfruit, giant swamp taro and pandanus. The impact of cultivar selection is most starkly shown by β-carotene in bananas, which ranged from 38 to 8508 µg/100 g between cultivars. These are used to illustrate the potential impact of cultivar differences on human health. The selection and promotion of nutrient-rich cultivars appears to have benefits for addressing nutritional deficiencies as well as contributing to chronic disease prevention. Brief case studies from the Federated States of Micronesia (FSM) and Solomon Islands are presented to demonstrate the contemporary importance of promoting nutrient-rich cultivars in Pacific Island countries.

Introduction

Most consumers select their fruits and vegetables from a limited range of cultivars. In Australia and most industrialised countries, supermarkets might offer choices across a few different tomatoes, bananas and other foods. While the range of cultivars found in home gardens and markets in many developing countries might be broader, in both situations there is generally little recognition of nutritional differences between cultivars.

The inherent variability of nutrient composition differences across cultivars is well established, as described in the following quote from Rand et al. in 1987: 'many … sources of nutrient variation are inherent in the foods themselves. These include geographical region of production, cultivar/species, changes in fortification levels, and agricultural practices in general'. What is missing, however, is information on nutritional composition across the range of cultivars (Greenfield and Southgate, 2003).

In this paper we use several widely-used food composition sources to illustrate the limitations of the food composition data currently available, and present some recently published estimates of nutrient composition for several Pacific Island foods to demonstrate the extent of differences across cultivars. These differences could have a significant impact on our health, and do in many populations. This is illustrated with reference to changing dietary patterns and food selection in the Federated States of Micronesia (FSM). We finish by describing various current initiatives that are using cultivar differences to address current and emerging health problems in some Pacific Island countries.

What do we know about composition differences across tropical fruit cultivars?

The limitations of food composition databases for considering the health implications of cultivar differences can be seen by looking at the level of detail for selected foods in databases relevant to the Pacific region.

Over the last couple of decades, much effort has gone into food composition analyses of Pacific Island foods and updating food composition tables. The advances are easily seen by comparing the sizes of the published tables. The food composition tables published by the South Pacific Commission in 1983 comprised a total of 33 pages (Fiji National Food and Nutrition Committee and Fiji School of Medicine, 1983). An improved set of tables was provided in 1994 (Dignan et al., 1994). The most recent version published by Food and Agriculture Organisation (FAO) in 2004 was 135 pages (Dignan et al, 2004). But even in the most recent version the amount of information on fruits is limited. A total of 87 fruits are listed, including all of the fresh, canned, and dried fruit items. There are only four banana values presented in the fruit section—for 'Australian', 'common varieties', 'PNG', 'Samoan'. This is a small sample of the range of bananas consumed. There were also five banana values presented in the starchy staples section, but this is also an under-representation when the number of banana cultivars consumed in the Pacific as a staple is considered. Similarly, the range of pandanus consumed is not well represented with only two values, for pandanus fruit and pandanus paste.

There are similar trends and outcomes in the Australian food composition databases. In 1983 there were 87 food codes for 'fruits'; by 1995 this had expanded to 232 codes for 'fruit products and dishes' (Cook, Rutishauser and Allsop, 2001). The 2006 version has 83 items for processed fruits and 76 for unprocessed. In this there are only two codes for bananas: 'Cavendish', and 'ladyfinger' (Food Standards Australia New Zealand, 2008). The extent of information in the USDA National Nutrient Database is much more extensive, but still limited once you look at particular fruits and vegetables. A search of the online database for 'banana' identifies 53 items, with 3 items under 'fruit'; 'bananas, dehydrated, or banana powder'; 'bananas, raw'; and 'fruit salad, (pineapple and papaya and banana and guava), tropical, canned, heavy syrup, solids and liquids' (United States Department of Agriculture, 2008). These are not particularly informative if looking for guidance on cultivar differences. The remainder of the banana items are for processed foods such as infant foods and muffins.

There is a growing focus on cultivar differences in the scientific literature, but this is not yet reflected in the databases used by health professionals, those working in agriculture and related areas. The outcome is that there is a limited practical understanding of extent of variation in nutrient composition across cultivars. The flow-on effects are that:

- the food guides and dietary advice that comes from organisations such as the Australian National Health and Medical Research Council and other authoritative bodies does not reflect the potential health significance of cultivar differences
- little attention has been given to cultivar selection and breeding in food production.

But this is starting to change, as shown by the range of papers presented at this conference.

Recent composition estimates for selected Pacific Island foods

In 1998, Englberger and co-workers began a series of studies in FSM to identify cultivars of a range of foods commonly used by previous generations and to assess their nutritional composition. Our initial interest was on carotenoid content because of the need for alternative food sources to address the vitamin A deficiency (VAD) that has become widespread in parts of Micronesia in recent decades. The work was later expanded to include other nutrients, providing a strong illustration of the diversity in nutrient profile across cultivars of a single food.

Bananas became a core focus of the research because of their importance as a staple food in FSM, their yellow and orange flesh coloration which is a characteristic of carotenoid content and the

customary use of selected bananas in infant feeding. But food habits have changed and it was found that the community's knowledge about banana cultivars, their location and features were often quite limited. Ethnographic methods using key informant interviews were used to list cultivars, their names and locations, and to gather information on their production, acquisition, consumption and cultural acceptability. This involved interviewing men and women, farmers, elder people and government officers from agriculture, education and health.

Samples were located where possible, and taken for analysis to a range of accredited laboratories that collaborated with the work over time, including in Fiji; Switzerland; Adelaide, Australia; and Honolulu, Hawaii, Atlanta, Georgia, San Francisco, California and Washington DC in the United States. The logistics were frequently difficult, involving rare bananas, mostly hand-carried to the laboratories, mostly as frozen samples.

The outcomes are reported in various publications. In 2003 and 2006 we reported on the carotenoid content of 17 banana cultivars, showing a range of estimates from 38 to 8508 µg β-carotene per 100 g (Englberger, Darnton-Hill et al., 2003; Englberger, Schierle et al., 2006). β-carotene is converted to vitamin A in the body and is a major source of vitamin A activity for most people. For comparison, the variety Cavendish, the banana most commonly marketed, consumed and cited in food composition tables, has a β-carotene content of approximately 21 µg per 100 g. Whereas the Cavendish will not be a major food source of vitamin A precursors, several of the Micronesian bananas will fully meet vitamin A requirements due to the amounts commonly eaten in the community.

Other foods commonly consumed in the Pacific, including breadfruit, giant swamp taro, and pandanus were also assessed, identifying cultivars of each that could potentially make an important contribution to daily vitamin A requirements (Englberger, Aalbersberg et al., 2003a; Englberger, Aalbersberg et al., 2003b, Englberger, Schierle et al., 2008).

Analyses for other vitamins and minerals have also shown high levels of riboflavin, niacin, α-tocopherol in some bananas. With normal patterns of consumption, these bananas could meet a high proportion of requirements for these nutrients (Englberger, Aalbersberg et al., 2003a; Englberger, Schierle et al., 2006). This would not be expected based on understanding from our usual food composition tables.

The University of Auckland, in collaboration with the Island Food Community of Pohnpei (IFCP), is now carrying out a study on content of resistant starch in five cultivars of Pohnpei bananas, still in the green mature stage, the stage when they are commonly consumed. Preliminary findings indicate that there are cultivar differences. Foods rich in resistant starch may help protect against colon cancer and diabetes, thus certain cultivars of green banana may offer potential benefits in respect to their resistant starch content.

In summary, this work has shown significant variation in nutrient composition across cultivars of common Pacific Island foods, and the values in food composition tables frequently do not represent the potential contribution of some cultivars for meeting nutrient requirements. This has led to inappropriate nutrition messages in some locations, and suggests an untapped potential for production and promotion. These points are well illustrated by the history of dietary change and health promotion in the FSM.

Implications—case study: Federated States of Micronesia (FSM)

The FSM comprises four states (Pohnpei, Chuuk, Yap and Kosrae) and 607 islands (atolls and volcanic). The culturally diverse population is approximately 107 000 and local food production is mainly subsistence. The traditional diet comprises starchy staple foods (especially banana, giant swamp taro, breadfruit, yam, cassava and pandanus), seafood, fruits and sugarcane (chewed as a snack). The traditional diet contains few vegetables and the green leafy vegetables widely consumed elsewhere are largely unacceptable to the population. In 2002, we reviewed a broad range of published and unpublished literature to understand influences on dietary change in FSM and the implications for health and agriculture initiatives (Englberger, Marks and Fitzgerald, 2003).

Historical records suggest that through until the 1940s the major health problems were due to infectious diseases and intestinal parasites. There is little evidence in these records of malnutrition, diabetes, hypertension and other chronic diseases.

Following World War II, FSM became a US trust territory of the United Nations, leading to the introduction of US food and health programs. In the 1960s a US Department of Agriculture (USDA) supplementary feeding program commenced, introducing rice and tinned food on a large scale. Other nutrition programs followed their introduction in the US (e.g. Special Supplementary Food Program for Women, Infants and Children—WIC; Expanded Food and Nutrition Program—EFNEP; needy, elderly and disaster relief). These were often not adapted to the local diet, cultural preferences or other local needs. By 1985, the school lunch program was being provided to one third of the total population.

These food aid programs have shaped food habits. In children there was a shift away from traditional diets, including a shift to breast milk substitutes and bottle feeding of infants and changes from previously used infant foods. Some of the adverse outcomes were recognised early, with some reversal of infant feeding practices resulting from breastfeeding promotions in the 1990s. Amongst adults, there was increasing use of and reliance on rice, sugar and flour. Recent surveys show the extent of the changes and current reliance on imported foods with the main protein sources in the diet now being local fish, and imported chicken, imported fatty meats and canned fish. Overall there appears to have been a loss of confidence in the value of local foods, with the range and proportion of local foods in the diet declining significantly, and a loss of traditional knowledge. As shown earlier, many of the staple foods consumed in the traditional diet were 'nutrient rich' cultivars. This was especially the case for the Karat banana, which had been commonly used as an infant food.

The dietary changes have been accompanied by a change in the health profile, most dramatically illustrated by VAD. Serious problems of VAD were first recorded in Chuuk in 1988 and further studies showed that there were high prevalence and serious problems of VAD disorders in all of FSM (Lloyd-Puryear et al., 1991; CDC 2001; Yamamura et al., 2004). Diet-related chronic diseases are now major health problems among adults, including obesity, diabetes and hypertension. This shift away from traditional diets and associated changes in disease profile has also been seen in many other Pacific Island countries, though perhaps not to the same extent.

The United Nations Children's Fund (UNICEF) led efforts in FSM from the mid-1990s to eliminate the VAD amongst children. Their projects over 15 years used the strategies found to be successful elsewhere, principally promoting production and promotion of green leafy vegetables. As noted earlier, the green leafy vegetables widely consumed elsewhere are largely unacceptable to the FSM population and the programs had little impact on the levels of deficiency. It is tragic to recognise now that there are a range of highly acceptable and carotenoid-rich foods locally available that could have been promoted over this time. This oversight is largely the outcome of the low level of knowledge about the extent of diversity of nutrient composition across cultivars, since the values in food composition tables for banana, giant swamp taro, breadfruit and pandanus and the other foods described earlier do not identify these foods as potentially significant sources of carotenoids.

Recent initiatives in FSM

After the first analyses of the Karat banana in 1998, a campaign for promoting Karat was carried out. A visible impact was seen. Prior to the campaign Karat was not sold in the local markets, whereas after the campaign it started appearing in the markets and is now sold regularly. Another focus was on the Daiwang cultivar, which was reported to be mainly used for feeding pigs and was never sold in local markets. However, after it was found to be carotenoid-rich and promoted, it was also found in the markets.

On October 16, 2003, coinciding with the commemoration of World Food Day, a group of interested individuals met for the foundation meeting of the Island Food Community of Pohnpei. In early 2004, IFCP became a legally chartered FSM non-governmental organisation.

Using a participatory, inter-agency, community-based, and research-based approach, IFCP organised a broad social marketing campaign, aimed at increasing local food production and consumption. Social marketing is the application of marketing principles to the design and management of social programs (Griffiths, 1994). Many methods and materials were used. One of the campaign aims was to convey the message that the local yellow- and orange-fleshed cultivars of banana, breadfruit, giant swamp taro and pandanus have rich nutrient content and health benefits. Another aim was to share the 'CHEEF' benefits of local foods: Culture, Health, Environment, Economic and Food security.

A specific focus was made on promoting Karat, through workshops, videos, t-shirts, billboards, displays, newsletters, recipes, and through a proclamation by the Governor declaring it as the State Banana of Pohnpei. Karat bumper stickers, postcards, telephone cards, song and even national postal stamps were developed and distributed. At the same time, the 'Let's Go Local' slogan, coined in the 1980s by a Pohnpei leader, was revived in order to promote all locally grown foods, and was repeated through t-shirts, video, song, and an email network.

Posters were developed to present photographs of the carotenoid-rich cultivars, nutrient content and health messages. These were widely distributed and hung in local markets, shops, offices, schools, health clinics, libraries, post offices and banks. A youth group of high school students, known as the 'Let's Go Local High School Club' was formed and started teaching about local foods, using the posters as a teaching tool.

Promotional pens and pencils with yellow and orange coloration shared messages to encourage planting and eating 'yellow varieties'. Face-to-face discussions, workshops, displays, school talks, cooking competitions, student essay and art competitions and farmers' crops competitions focusing on yellow-fleshed cultivars were also organised.

A DVD film entitled 'Going Yellow' was developed, which presented a humorous family drama as well as scientific findings on Karat and its rich nutrient content. The film has been shown many times on television and was also made available through video shops.

Other mass media was used regularly. More than 100 articles, most with either an accompanying photograph or recipe, were published in the local newspaper. A website, www.islandfood.org, was established. Press releases were regularly prepared and broadcast by two local radio stations. A drama club and breastfeeding club were formed.

A genebank field collection, focusing on banana, giant swamp taro and pandanus cultivars was established, aiming for conservation, research and development of planting materials. A focus was also made on small-scale processing of local food, in order to extend its shelf life and provide convenience. A program was established for local construction and distribution of charcoal ovens, which are environmentally friendly, energy-efficient, income-saving, and convenient in comparison to the traditional earth oven and which also provide a healthy way of cooking.

In 2005, Pohnpei was involved as a case study in a global health project, led by the Centre for Indigenous Peoples' Nutrition and Environment (CINE), based at McGill University, Canada. Pohnpeians learned that all over the world people are experiencing similar problems relating to the shift towards processed foods and neglect of traditional food systems, followed by health problems, and they gained strength and confidence in working together towards common goals. Preliminary findings showed household dietary improvements, improvement in attitudes toward local food and a great increase in use of local food in traditional gatherings. Another impact of the project was the spread of 'Go Local' to several other communities interested in carrying out the project.

Although IFCP has focused its work in Pohnpei, its messages have been spreading to the other FSM states and Pacific Island countries. Yap and Kosrae have invited IFCP to facilitate 'Go Local' workshops in order to help them start their own campaigns, and Chuuk has also shown interest in starting a 'Go Local' campaign.

The Republic of the Marshall Islands and Republic of Kiribati requested assistance in assessing their many pandanus cultivars for β-carotene content. Colourful posters were developed, printed, and distributed, and planting programs for rare cultivars were organised. Regional organisations have indicated interest in establishing 'Go Local' workshops throughout the Pacific and have asked IFCP to share about their program at regional workshops.

Although there is still a long way to go in Pohnpei, a strong movement has started towards shifting back more to local foods and conserving rare cultivars, such as Karat and other yellow/orange-fleshed bananas. As is being said more and more often, let's go local!

Solomon Islands case

In recent decades, there have also been great dietary changes in the Solomon Islands, with growing dependence on and esteem for imported low-nutrient foods such as rice, instant noodles, refined wheat products and sugar. Studies indicate suboptimal vitamin A status and consumption in population sub-groups (Schaumberg, Linehan et al., 1995; Brimblecombe, 2000), and while the VAD rates are much lower than those identified in FSM, they are of public health significance (Paterson and Crossland 1998). There have also been large increases in rates of diabetes, cardiovascular and renal diseases and certain cancers (UNICEF, 2005). With these health problems and the increasing pressures on subsistence farming in Solomon Islands (Bourke, McGregor et al.; Jackson, Tutua et al., 2006; Jackson, Jansen et al., 2008), food and nutrition security needed to be addressed urgently.

In response to these issues, a collaborative project was begun in 2007 to promote carotenoid-rich bananas and sweet potato varieties (i.e. orange-fleshed sweet potato (OFSP)), built on the work of Lois Englberger and the Island Food Community of Pohnpei and Graham Lyons of the University of Adelaide. Initially we worked with Solomon Islands colleagues and then villagers in rural areas to investigate the social aspects of OFSP, carotenoid-rich bananas and other micronutrient-rich foods, how they fit into the traditional food system and what factors may be important for promoting them. As in FSM, the project took an inter-agency participatory approach, involving agencies including Australian Centre for International Agricultural Research (ACIAR), HarvestPlus, International Potato Centre (CIP), Secretariat of the Pacific Community (SPC), Kastom Gaden Association (KGA), Solomon Islands Ministry of Agriculture and Livestock, Island Food Community of Pohnpei, Queensland Department of Primary Industries and Fisheries (DPI&F), and Makira Ulawa Province as well as community groups.

In the first phase on Makira, which is known for its banana diversity, some 50 varieties of sweet potato and 60 varieties of banana were investigated. Names of the varieties were explored, as well as plant characteristics, beliefs, practices, traditional knowledge, and factors relating to production, marketing, consumption and acceptability, all of which impact on the potential for promoting these crops. Research methods included ethnography, key informant interviews, informal focus group discussions, free listing, pile sorting, photography, market survey and literature review.

Despite significant logistic problems, more than 20 OFSP and 16 banana varieties were collected and sent to Fiji and Australia for analysis. The data are now being interpreted and will be published soon. Findings are similar to those from Pohnpei—Solomon Islands already have banana and sweet potato varieties rich in carotenoids and other micronutrients. Promising sweet potato varieties (with β-carotene levels over 100 mg/kg dry weight, and which are highly regarded by local consumers for their insect/pathogen resistance, yield, flavour, texture and storage ability) were identified and are being multiplied by Kastom Gaden Association. In addition, financial support has been provided to key agriculture and education officers on Makira, who are involved in sweet potato field

trials, banana and giant swamp taro seed gardens, and training programs for women. Rare high-carotenoid banana germplasm was collected and transferred to Fiji for tissue culture from which they can be returned in the event of variety loss from events such as cyclones.

Community workshops, talks and distribution of promotional material are essential, as local knowledge of the health benefits of micronutrient-rich local foods is not widespread at present. Seven workshops promoting OFSPs, high-carotenoid bananas and nutritious local foods in general, were held on Makira in October 2007, including several workshops on the Makira Weathercoast, a remote area with significant nutrition, food security, livelihood and transport issues. There was great interest among local people in this activity and more than 700 people attended the workshops (Englberger, Lyons et al. 2007). In 2008, five additional workshops were facilitated in villages in the north of Malaita, the most densely populated province of the Solomon Islands, where there are early signs of growing nutrition problems. In these workshops, we used some of the materials from the IFCP and also trialled draft posters to promote carotenoid-rich foods from the Solomon Islands, which were prepared following the 2007 community research. National radio broadcasts were made to further promote the messages about the health benefits of local over imported foods. Again we worked collaboratively with Solomon Islands partners and there was great interest from people in the nutrition and agriculture messages and consequently strong engagement.

Similar workshops were facilitated in 2008 in Papua New Guinea in the Lae area, in Morobe Province, where young children were identified as having the highest risk of VAD in PNG. These workshops were held in collaboration with The National Agricultural Research Institute (NARI), Department of Health and other organisations. In addition to the survey and social marketing components of the program, imports of OFSP varieties with valuable traits from CIP Peru (via SPC, Fiji) and Indonesia (via DPI&F—now QPIF—Australia) are in progress. Once they clear quarantine, these imported varieties will be tested at several sites in the Solomon Islands and PNG.

Conclusions

Where Pacific communities have become dependent on cash incomes and imported foods, they rely on a severely reduced variety of foods and consequently become less food secure, which debilitates ‘the very fabric of society’ (Pollock nd). As in other countries where diets and health status have undergone similar transitions (e.g. see Raschke and Cheema 2008), reinvigorating confidence in local foods, through promotion of their nutritional value and facilitating their acquisition and production are important strategies for improving health and wellbeing in Pacific Island communities. Expanding nutrient composition data to reflect important cultivar differences is vital to this ongoing work.

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Health effects of fruit

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Keywords: fruit, cardiovascular disease, type 2 diabetes, cancer, tropical fruit

Abstract

Much of the epidemiology relating fruit to health outcomes is a combination with vegetables, but there are a small number of studies that separate them and show that fruit alone is as powerful as or more powerful than vegetables in some areas. There is only limited information available specifically on tropical fruits.

Cardiovascular disease

In relation to cardiovascular disease, Law et al. (1998) did a meta analysis of ischemic heart disease and found that comparing the 90th centile of intake to the 10th centile of intake, which corresponded to a four-fold increase in fruit intake and a two-fold increase in vegetable intake, there was a 12–19% reduction in risk for all measures: fruit, vegetables, carotenoids, vitamin C, fruit fibre and vegetable fibre, and serum concentration of carotenoids and vitamin C, adjusted for other risk factors.

Dauchet reviewed the area in 2006 and found in nine studies that consisted of 91 379 men, 129 701 women, and 5007 Coronary Heart Disease (CHD) events, the risk of CHD was decreased by 4% [Relative Risk (RR) (95% Confidence Interval (CI)): 0.96 (0.93–0.99), P = 0.0027] for each additional portion per day of fruit and vegetable intake and by 7% [0.93 (0.89–0.96), P < 0.0001] for fruit intake. Pereira analysed 10 cohort studies that contained 5249 incident total coronary cases and 2011 coronary deaths that occurred among 91 058 men and 245 186 women. After adjustment for demographics, body mass index, and lifestyle factors, each 10-g/d increment of energy-adjusted cereal, fruit, and vegetable fibre intake RRs corresponding to 10-g/d increments were 0.90 (95% CI, 0.77–1.07), 0.84 (95% CI, 0.70–0.99), and 1.00 (95% CI, 0.88–1.13), respectively, for all coronary events and 0.75 (95% CI, 0.63–0.91), 0.70 (95% CI, 0.55–0.89), and 1.00 (95% CI, 0.82–1.23), respectively, for deaths. Results were similar for men and women. In the Japan Public Health Centre-based prospective study, 77 891 men and women aged 45–74 years underwent 459 320 person-years of follow-up until the end of 2002. 3230 cancer cases and 1386 CVD cases were identified. Higher consumption of fruit, but not vegetables, was associated with significantly lower risk of CVD: multivariate hazard ratios for the highest versus lowest quartiles of intake were 0.81 (95% confidence interval (CI): 0.67, 0.97; trend p = 0.01) for fruit and 0.97 (95% CI: 0.82, 1.15; trend p = 0.66) for vegetables (Takachi, 2008).

Dauchet et al. (2005) analysed strokes and found that in seven studies with 90 513 men, 141 536 women, and 2955 strokes, the risk of stroke was decreased by 11% (RR 95% CI: 0.89 [0.85 to 0.93]) for each additional portion per day of fruit, by 5% (RR: 0.95 [0.92 to 0.97]) for fruit and vegetables, and by 3% (RR: 0.97 [0.92 to 1.02]; NS) for vegetables. The association between fruit or fruit and vegetables and stroke was linear, suggesting a dose-response relationship.

Intake of fruit may reduce the risk of neovascular macular degeneration. Participants who consumed 3 or more servings per day of fruits had a pooled multivariate relative risk of 0.64 (95% confidence interval, 0.44–0.93; P value for trend = .004) compared with those who consumed less than 1.5 servings per day. The results were similar in women and men. However, intakes of vegetables, anti-oxidant vitamins, or carotenoids were not strongly related to either early or neovascular ARM (Cho, 2004).

Type 2 diabetes

There is a modest amount of evidence that fruit consumption may protect against developing type 2 diabetes mellitus (DM) and when positive this is usually in association with vegetables and not alone. In the Finnish Mobile Health clinic study with 4304 men and women, 383 cases of type 2 DM occurred over 23 years (Montonen, 2005). There was a 31% reduction in the group with the highest consumption of fruit and berries. In the Nurses Health study (Bazzano, 2008) whole fruit consumption was associated with a lower hazard of diabetes (0.82 [0.72–0.94]).

There are four studies where fruit provides protection along with vegetables (Gittelsohn, 1998; Montonen, 2005; Ford, 2001; Sargeant, 2001) and nine studies where it does not (Williams, 1999; Liu, 2004; Feskens, 1995; Colditz, 1992; Woo, 2003; Hodge, 2007; Lundgren, 1989; Meyer, 2000; Gulliford, 2001). In the EPIC study (European Prospective Investigation of Cancer), being in the highest quintile of fruit intake lowers HbA1c by 0.1% (self-reported diabetics were excluded) while green leafy vegetables reduced HbA1c by 0.17% (Sargeant, 2001).

In the same study there was a 62% reduction in incidence of DM in the top quintile of plasma vitamin C (Harding, 2008), suggesting fruit is important in this group at reducing risk, although the risk reduction was not as great as with plasma vitamin C (RR 0.78 ,95% confidence interval, 0.60–1.00). Using another marker of both fruit and vegetable intake, plasma carotenoids, in a prospective study over 9 years with 1389 healthy older volunteers (aged 59–71), there was a 84% reduction in impaired glucose tolerance or type 2 diabetes in the highest quartile of plasma carotenoid (Arkbaly, 2008). Montonen et al. (2004) reported after 23 years of follow-up, in a cohort of 4303 participants free of diabetes at baseline, that β-cryptoxanthin intake was significantly associated with a reduced risk of type 2 diabetes (relative risk 0.58 [95% CI 0.44–0.78]). Other longitudinal results are more conflicting. A nested case-control study (470 case and 470 control subjects) conducted on middle-aged and older U.S. women (aged > 45 years) by Wang et al. (2006) did not confirm the prospective association between different baseline plasma carotenoids compounds (lycopene, α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin) and risk of type 2 diabetes. Similar negative results were found by Reuanaen et al (1998).

Cancer

Fruit appears to have a distinctive role in protecting from lung cancer and possibly upper aero digestive tract cancer. The data in relation to the lower digestive tract is more variable and there is no association with breast (Smith-Warner et al., 2001), ovarian (Koushik et al., 2005) prostate (Koushik et al., 2005) or bladder (Larsson et al., 2008) cancers.

In the EPIC study, total fruit intake was associated with a 40% reduction in the incidence of lung cancer (0.46–0.78, p< 0.01). Similarly, for upper aero-digestive cancers total fruit led to a 40% reduction in risk (0.38–0.97 p = 0.041) (Linseisen et al., 2007). In the NIH AARP Diet and Health study, higher consumption of several botanical subgroups was significantly inversely associated with risk, but only in men. For example, the relative risks of lung cancer among men in the highest versus lowest quintiles of intake of rosaceae (apples, peach, nectarines, plums, pears and strawberries), convolvulaceae (e.g. sweetpotato), and umbelliferae (e.g. carrot) were 0.82 (95% confidence interval (CI): 0.73, 0.91), 0.86 (95% CI: 0.75, 0.96), and 0.86 (95% CI: 0.78, 0.96), respectively (Wright et al., 2008). In the same cohort with 787 cases of head and neck cancer, fruits (fifth vs. first quintile: 0.87, 0.68–1.11), rosaceae (apples, peach, nectarines, plums, pears and strawberries, 0.60, 0.49–0.73), and solanaceae (peppers and tomatoes, 0.82, 0.69–0.98) were associated with decreased risk. In a pooled analysis of 8 prospective lung cancer studies with 3206 cases (Smith-Warner et al., 2003) a 6–23% reduction in lung cancer risk was observed for quintiles 2 through 5 vs. the lowest quintile of consumption for total fruits (RR = 0.77; 95% CI = 0.67–0.87 for quintile 5; p-value, test for trend < 0.001).

For oesophageal cancer in the Shanghai cohort study fruit, including oranges/ tangerines were protective (Fan et al., 2008). In Japan (Bae et al., 2008) there was a 28% reduction in risk of stomach cancer associated with high intake of citrus fruits (summary OR = 0.72; 95% CI = 0.64–0.81; P value < 0.0001).

In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (Millen et al., 2007), there was a 25% reduction per 4.5 serves fruit/day for colon cancer and colon adenomas (but not rectal cancer). In a Japanese study (Nomura et al., 2008), there was a 20% reduction in colorectal cancer in men only (0.64–0.99). In the EPIC study, no association between fruit and vegetables and colorectal cancer (CRC) was found (Gonzalez et al., 2006). In the NIH study, men with high scores on the fruit and vegetable pattern were at decreased risk of colorectal cancer [relative risk (RR) for quintile (Q) 5 versus Q1: 0.81; 95% CI: 0.70, 0.93; P for trend = 0.004] (Flood et al., 2008). In the Pooling project of 14 cohort studies, the association of CRC with fruit was weak and non-significant (RR 0.94 p = 0.28) (Koushik et al., 2007). Intakes of vitamin A, carotenoids and vitamin C from fruit and vegetables are associated with protection from renal cancer in men only (Lee et al., 2006).

Interestingly, with pancreatic cancer there was an increased risk with higher intakes of total sugars, fructose, and sucrose, and the association with fructose was significant when the highest and lowest quartiles were compared (relative risk: 1.35; 95% CI: 1.02, 1.80; P for trend = 0.046). A significant association was found with fruit and juices intake (1.37; 1.02, 1.84; P for trend = 0.04) but not with soda intake in the 162 150 participants in the Hawaii-Los Angeles Multiethnic Cohort Study (Nothlings et al., 2007).

In the Japan Public Health Centre-based prospective study noted above, consumption of fruit or vegetables was not associated with decreased risk of total cancer: corresponding hazard ratios were 1.02 (95% CI: 0.90, 1.14; trend p = 0.95) for fruit and 0.94 (95% CI: 0.84, 1.05; trend p = 0.16) for vegetables (Takachi et al., 2008).

Total mortality

Fruit intake as part of a healthy lifestyle can reduce total mortality. In the EPIC-Norfolk cohort 20 244 men and women aged 45–79 years with no known cardiovascular disease or cancer were followed up to 2006 (follow up of 9–13 years). Participants scored one point for each health behaviour: current non-smoking, not physically inactive, moderate alcohol intake (1–14 units a week) and plasma vitamin C > 50 mmol/l indicating fruit and vegetable intake of at least five servings a day, for a total score ranging from zero to four. After an average 11-year follow-up, all-cause mortality (1987 deaths) for men and women who had three, two, one, and zero compared to four health behaviours were respectively, 1.39 (1.21–1.60), 1.95 (1.70–2.25), 2.52 (2.13–3.00), and 4.04 (2.95–5.54) p < 0.001 trend. The trends were strongest for cardiovascular causes. The mortality risk for those with four compared to zero health behaviours was equivalent to being 14 years younger in chronological age (Khaw et al., 2008).

Role of tropical fruit

Major components

The major components in fruits in general and tropical fruits in particular with health attributes include fibre, vitamins (particularly vitamin C), potassium, carotenoids and polyphenols. Sapodilla, kumquat, durian and avocado were found to have the highest amounts of fibre. Kumquat and sapodilla, at 6.4 g and 5.4 g of fibre per serving, respectively, both provide more than 20% of the daily reference value for fibre, and therefore are excellent sources of fibre. In comparison, an apple contains about 2.5 g/100 g or about 3 g for an average apple.

Vitamin C content is one of the major attributes of all fruits tropical and non-tropical and Acerola juice, which contains 3872 mg per serving, and raw acerola cherries, which contain 822 mg per serving, are extraordinarily high in vitamin C. Guava, 188 mg per serving, is also an excellent source, followed by passion fruit juice (74 mg/serving), longan (70 mg/serving), and lychee (70 mg/serving) which are all comparable to an orange. In comparison, apple contains about 8 mg per fruit and a banana 17 mg. The vitamin C content of many fruit is higher when it is slightly immature and declines as the fruit hits peak ripeness.

For a few fruits, such as the jujube fruit, the vitamin C content does the opposite—it rises with increased ripeness. Vitamin C content also decreases with storage. Far more important is the variety which can have a 10-fold difference in vitamin C content. Folate is another important nutrient that protects against DNA damage, neural tube defects and some childhood leukaemias. Avocado, durian, and guava are the tropical fruits with the most folate. With 61 µg/serving, 44 µg/serving and 40 µg/serving, respectively, they are considered 'good' sources of folate.

Data on the folate content of some fruits are non-existent. In comparison, an orange contains about 18 ug/100 g or 20–24 ug/serve. Potassium is associated with a lower blood pressure and a lower rate of strokes and can counteract some of the harmful effects of sodium. Passion fruit juice, durian, plantain, guava and avocado contained the most potassium. Passion fruit juice, in particular, is an excellent source of potassium, containing almost 700 mg of potassium (20% of the daily reference value) per serving. Banana still provides a reasonable amount at 480 mg/serve.

- One of the best tropical fruits is Acerola cherry or Barbados cherry (*Malpighia glabra, Malpighia emarginata*) which is extraordinarily high in vitamin C and is also a rich source of vitamin A, iron, and folate. The fruit juice has also been found to contain carotenoids, such as β-carotene.
- Avocado (*Persea americana*) is especially high in protein, fibre, niacin, thiamin, riboflavin, folic acid and zinc. Avocado contains α and β-carotene and lutein/zeathanthin, but not lycopene.
- Durian (*Durio zibethinus*) is high in fibre, folate and potassium. No carotenoid or phenolic data is available.
- Guava (*Psidium guajava*) is a source of fibre, vitamin C, folate and potassium, and is also high in lycopene and β-carotene. Guava also contains ellagic acid, gallic acid conjugates and quercetin glycosides.
- Kiwi is a great source of vitamin C.
- Mango is high in fibre, very high in β-carotene (similar to apricots and cantaloupe), high in vitamin C.
- Papaya is high in calcium (for a fruit), folic acid, vitamin C, fibre, and carotenoid.
- Mangosteen is rich in polyphenols, as is snake fruit.
- Banana contains potassium (483 mg/serve), fibre (3 g/140 g banana), B vitamins and a variable amount of vitamin C (17 mg/serve).

Fruits contain a wide variety of phenolic compounds that contribute to their anti-oxidant actions. Murcia et al. (2001) showed the order of efficiency, as anti-oxidant scavengers, between tropical fruits to be: passion fruit > lime > passiflora > kumquat > avocado > pineapple > physalis > papaya > carambola > mango > banana. Luximon-Ramma et al. (2003) showed that the anti-oxidant activities (FRAP) of the fruits tested ranged (from 0.3 to 34 µmol TE g^{-1} FW and from 2.86 to 323 µmol Fe^{2+} g^{-1} DW) and total phenolics in the fruits ranged from 118 to 5638 µg g^{-1} FW and from 0.66 to 31.5 mg g^{-1} DW). The highest anti-oxidant capacities were observed in red and yellow *Psidium cattleianum Sabine* 'Chinese guava', sweet and acid *Averrhoa carambola* L. 'starfruit', *Syzygium cumini* L. *Skeels* 'jamblon', and white *Psidium guajava* L. 'guava'.

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Nutritional bioactives and functional foods

Mango processing with particular consideration of carotene retention and pectin recovery

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Keywords: Cultivar, fruit processing, *Mangifera indica* L., maturity, pectin, post-harvest ripening; pro-vitamin A

Abstract

Plantation of perennial fruit trees could be an ecologically important alternative to erosion-prone annual crop and vegetable production systems on sloping sites in the mountainous regions of Southeast Asia. In view of market liberalisation and rising competition, the role of fruit processing in terms of a value-adding increase in productivity is no longer limited to the reduction of post-harvest losses. As an exemplar for mango fruits (*Mangifera indica* L.), grown in Northern Thailand, the potential of cultivars for product differentiation through the promotion of niche products with special high-quality attributes has been explored. Retention of pro-vitamin A in mango processing was investigated for various process parameters in purée production as well as in solar and conventional drying. Sulphitation, which is under critical consideration, has been avoided in fruit drying by early inactivation of browning enzymes through increased drying temperatures. Solar drying was associated with higher β-carotene losses compared to high-temperature short-time processes. Apart from thermally induced degradation, light-induced isomerisation to 9-*cis*-β-carotene occurred. With the focus being on β-carotene degradation and *trans*-*cis*-isomerisation, the effects of thermal processing steps on the nutritive value of foods were systematically studied. Because β-carotene stability greatly depended on the physical state of the plant matrix, the chromoplast characteristics of mango mesocarp was elucidated by ultra-structural studies. Since mango peels represent ~15–18% of the total fruit weight, the disposal of waste is a challenging problem in increasing fruit processing. Therefore, the potential of mango as a source of high-quality pectin was assessed. Besides yields and composition, the gelling properties and molecular sizes of pectins extracted from mango peels of different fruit ripeness were characterised and the starch contents quantified. It has been demonstrated that mango may serve for the diversified manufacture of solid and fluid products rich in β-carotene and techno-functional food ingredients.

Introduction

Since the prohibition of opium production, the Thai Government, supported by international donors, has put an enormous effort into replacing poppy by sustainable crops (Highland Research and Development Institute, 2007), thus reducing deforestation, erosion and loss of soil fertility in the mountainous regions of northern Thailand. Significant productivity growth has been achieved through diversification into fruits, vegetables, and flowers (Rerkasem, 2005). Compared to annual vegetable production systems, plantation of perennial fruit trees has been considered particularly suitable for the sloping hillsides in terms of erosion control and drought tolerance. Besides the lower consumption of irrigation water, fruit trees represent less labour-intensive sources of cash income for the farmers, concomitantly influencing land use strategies in terms of long-term land ownership (Sruamsiri and Neidhart, 2007). Whereas inaccessible border regions may benefit from recent regional free trade agreements through facilitated cross-border trade, market liberalisation has overall increased the competition from across the border, where opium replacement crops are partly grown on a large scale (Rerkasem, 2005). Thus, further product differentiation promoting niche products with special high-quality attributes is coming to the fore, both on the fresh produce and processing level.

On the way towards sustainable production, our research aimed at developing adjusted strategies for small-scale and industrial fruit processing in northern Thailand. Mango was chosen as an exemplar because of its dual role as a pro-vitamin A source and cash crop with high export potential both as fresh and processed fruit, (Neidhart et al., 2007). Although this fruit crop is naturally adapted to the tropical lowlands, the northern part of Thailand includes significant producing areas, providing ~21% of the total Thai mango fruit production (Sruamsiri and Neidhart, 2007). Vitamin A deficiency, being the leading cause of preventable blindness in children, has been known as a public health problem in more than half of all countries, especially in Africa and Southeast Asia, due to maternal mortality and significantly increased morbidity and mortality for common childhood infections (WHO, 2008). The major risk groups are young children (< 5 years) and pregnant women in low-income regions.

Generally, the production of high-quality food equally depends on the selection of suitable raw material and the appropriate processing technology. After considerable accumulation of starch during fruit growth, the climacteric mango fruit is usually harvested at the mature-green stage and subjected to post-harvest ripening until appropriate ripeness for processing. Although mostly eaten ripe as a dessert fruit, mango fruits have been utilised for long time at nearly every stage of growth. Varieties preferred mature-green are distinguished from others by their sweet, non-starchy, non-astringent flavour at this stage of development (Bally, 2006). Depending on the cultivar, maturity, and regional preferences, the fruit is processed on different scales into pickles, pulps, jams, and chutneys, and is frozen or dried. Mango processing is thus much more diversified compared to other major fruit crops like apples and citrus fruits, which are mainly processed into juices. Processing requires sliceable fruits for canning and drying and soft, full-ripe fruits for pulping. As Thai mango cultivars have scarcely been considered, their quality profiles were studied and processing options were aimed at appropriate utilisation of carotene-rich cultivars.

In industrialised mango processing, productivity could be further increased by the transformation of waste into by-products. Compared to fruits of temperate zones, considerably higher ratios of byproducts arise from mango processing due to higher amounts of inedible waste material such as peels (15–18% of total fruit weight) and seeds (13–29%) (Vásquez-Caicedo et al., 2002). Increasing mango processing in most producing countries has recently boosted the worldwide interest in value-adding utilisation of industrial mango residues. Mango has been deemed a promising, but not yet exploited alternative pectin source (Endress et al., 2006). To identify possible resource inherent obstacles to the exploitation of mango by-products the suitability of different types of mango residues for pectin recovery by conventional hot-acid extraction was comprehensively explored.

Thus, mango utilisation has been reviewed below from a holistic point of view. For major cultivars grown in northern Thailand, plant physiological and technological prerequisites for optimum product qualities have been evaluated as regards possible options and constraints for integrated mango processing.

Mango products of high vitamin A value

Selection of mango fruits rich in β-carotene

Biosynthesis of carotenoids is one of the major biochemical changes during post-harvest ripening of mango fruits, where the bright colours of those pigments act as attractants. Due to small levels of all-*trans*- and *cis*-β-cryptoxanthin, only β-carotene is of pro-vitamin A relevance in mango (Mercadante and Rodriguez-Amaya, 1998). However, β-carotene is among the predominant carotenoids, together with violaxanthin dibutyrate and *cis*-violaxanthin dibutyrate, as shown first for ripe mesocarp of cv. 'Kent' (Pott et al., 2003a). Butyric acid has been known as one of numerous volatile compounds that are responsible for the inherent aroma profile of mango, but the acylation of plant xanthophylls with butyric acid is an unusual attribute of the mango carotenoid pattern. For example, the xanthophyll ester profiles of apricots comprised saturated and unsaturated fatty acids, those of pumpkins exclusively saturated ones, but always involving acyl chains ≥ C12 (Kurz et al., 2008). Similarly, palmitic (C16:0), palmitoleic (C16:1, ω-7) and linolenic acid (C18:3, ω-3) were

identified as the principal fatty acids of the total lipids in the ripe 'Kent' mesocarp (Pott et al., 2003a). As revealed by carotenoid quantification after saponification of xanthophylls esters, alterations in carotenoids during post-harvest ripening of mangoes were limited to quantitative changes, whereas the carotenoid pattern depended on the cultivar and geographic (i.e. climatic) effects (Mercadante and Rodriguez-Amaya, 1998). All-*trans*-β-carotene, violaxanthin and 9-*cis*-violaxanthin (Figure 1) represent the major carotenoids of saponified mango mesocarp samples (Mercadante and Rodriguez-Amaya, 1998; Pott et al., 2003). Violaxanthin is the main precursor of abscisic acid (ABA) via 9-*cis*-violaxanthin and subsequently *cis*-xanthoxin. It is a C15 apocarotenoid that acts as plant growth regulator and increases during various forms of environmental stress and seed dehydration (Taylor and Ramsay, 2005).

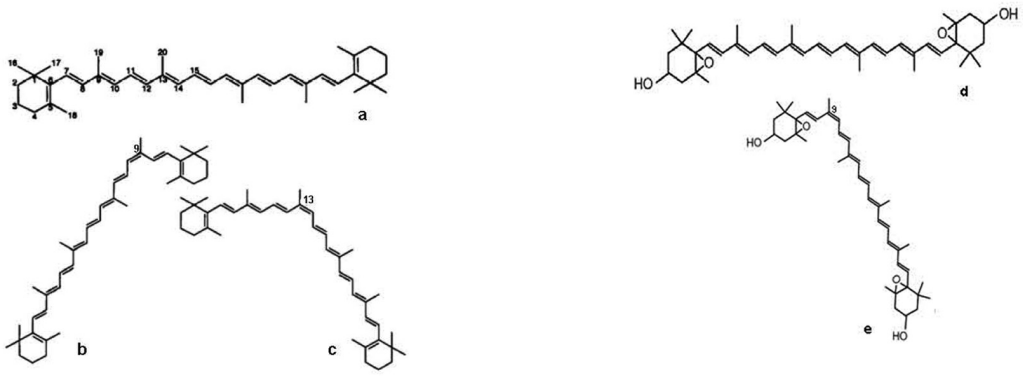


Figure 1: Selected carotenoids, including the major carotenoids of mango fruits after saponification.
a, all-*trans*-β-carotene; b, 9-*cis*-β-carotene; c, 13-*cis*-β-carotene; d, violaxanthin; e, 9-*cis*-violaxanthin.

As lipophilic substances, carotenoids are synthesised in plastids (chloroplasts and chromoplasts) from C_5-isoprene units. They are derived from the isoprenoid pathway where all-*trans*-β-carotene finally results from direct cyclisation of all-*trans*-lycopene catalysed by the enzyme β-cyclase (Taylor and Ramsay, 2005). The carotenoid-biosynthetic enzymes are organised in multi-enzyme complexes within in the plastid membrane. Membrane-bound processes include the rate-limiting condensation of two geranylgeranyl diphosphate molecules to phytoene by phytoene synthase, which is mostly expressed in an organ and/or plastid type-specific manner. The sequestration of carotenoids within the cell is another form of regulation. In chloroplasts, end-product carotenoids are bound to pigment-protein complexes in the thylakoid membranes, where they exert the dual function as accessory pigments in light-harvesting antennae by transferring energy to the photosystem reaction centres and as quenchers of triplet-excited states in chlorophyll molecules, the latter to protect the organisms against photodynamic destruction.

During fruit ripening, the photosynthetic chloroplasts develop into chromoplasts by disintegration of the thylakoid membranes and the development of new pigment-bearing structures, while chlorophylls degrade and carotenoids accumulate. Changes in the plastid number and the thylakoid membrane organisation modulate the phospholipid environment inside the chromoplasts, thus regulating carotenoid accumulation in addition to the formation of chromoplast-specific proteins. Esterification of xanthophylls by fatty acids concurrently increases, thus improving lipophilicity for subsequent sequestration. In this way, the prevailing use of a short-chain fatty acid like butyric acid in mango for this purpose allows accumulation of violaxanthin at notably high levels.

No *cis*-β-carotene isomers are produced in the isoprenoid pathway. Nevertheless, similar to chlorophyll-containing tissues (Schieber and Carle, 2005) and other fruit species including peaches (Kurz et al., 2008), but unlike carrot (Marx et al., 2003), naturally occurring *cis*-β-carotene isomers (Figure 1) were detected in all of the numerous mango cultivars studied at levels depending on the total β-carotene content (Vásquez-Caicedo et al., 2005). The percentages of *cis*-isomers (14–40%) were almost constant during ripening, but specific for each cultivar. Unlike the predominance

of the 9-*cis* configuration in case of the violaxanthin *cis*-isomers, 9-*cis*- and 13-*cis*-β-carotene occurred at almost equal portions (Vásquez-Caicedo et al., 2005), but 13-*cis*-β-carotene portions of 24–27% and only traces of the 9-*cis* isomer were also found (Pott et al., 2003b). Besides the maturity-depending total β-carotene content, the relative amounts of the respective isomers thus define the vitamin A values of fresh mango fruits. The carotene stereoisomers display different physicochemical and biological properties, including differences in anti-oxidant capacity and bioavailability (Schieber and Carle, 2005). Compared with the thermodynamically more stable all-*trans*-β-carotene, relative pro-vitamin A activities of 9-*cis*- and 13-*cis*-β-carotene are only 38 and 53%, respectively (Castenmiller and West, 1998).

The recent raise of the bioequivalence ratio of all-*trans*-β-carotene to retinol from 6 to 12 µg (i.e. the amount of all-*trans*-β-carotene corresponding to 1 µg of retinol, is still up for debate) (Thurnham, 2007). However, according to the stable-isotope-based studies reviewed by Thurnham, the bioefficacy is generally poor, whereas optimal bioconversion is promoted by small, regular supplies of β-carotene-containing foods. To be consistent with the majority of studies performed on mango so far, the vitamin A values reported below are based on the 6:1 bioconversion rate and the above-mentioned relative activities of the *cis*-isomers.

The natural occurrence of *cis*-β-carotene isomers, being more soluble in nonpolar solvents than the all-*trans* form, suggested ultra-structural studies of the chromoplast type in mango fruits by means of transmission electron microscopy (Vásquez-Caicedo et al., 2006). Chromoplasts are generally classified as globular, tubular, reticulo-tubular, membranous, and crystalline types, which imply differences in the physical properties and bioavailability of the deposited carotenoids. The latter are associated with specific proteins as small plastoglobuli in the most frequent, often xanthophyll-containing globular chromoplasts or, for example in pepper, as fibrils in tubular chromoplasts. All-*trans*-lycopene in tomato and all-*trans*-β-carotene in carrot are sequestered as crystals that are assumed to markedly enhance the stability of the all-*trans* configuration.

As revealed by our study (Vásquez-Caicedo et al., 2006), numerous plastoglobuli of varying size and electron density were typical for the mango chromoplasts. They comprised the main part of the carotenoids, thus supporting the partial dissolution of the pigments in lipid droplets that facilitates *trans*-*cis* isomerisation. In this way, concurrent accumulation of β-carotene and xanthophyll esters may be enabled to high levels. Because various pigment-bearing tubular membrane structures were also observed, mango chromoplasts were assigned to the globular and reticulo-tubular types. Moreover, the large portions of naturally occurring *cis*-β-carotene isomers in mango fruits contrasted with the predominance of the all-*trans* isomer characteristic of carrots used as control. For the latter, the crystalline type of chromoplasts was confirmed by our study. In mango chromoplasts, *trans*-*cis* isomerisation might be light-mediated (e.g. when the fruit is exposed to sunlight on the tree). However, the constant portions of *cis*-β-carotene isomers found throughout fruit ripening (Vásquez-Caicedo et al., 2005) suggested other factors.

Among the fruit batches of the nine Thai mango cultivars subjected to the same post-harvest ripening conditions (29 ± 2°C, 90–95% relative humidity (RH); Vásquez-Caicedo et al., 2005), only those of ‘Maha Chanok’, ‘Kaew’, ‘Nam Dokmai #4’, and ‘Chok Anan’ developed bright yellow-orange mesocarp colourations, resulting in total *β*-carotene contents of 65–113 µg/g of mesocarp dry weight (DW) and vitamin A values of 892–1573 retinol equivalents (RE)/100 g of DW at full ripeness (i.e. at a sugar/acid ratio (TSS/TA) of ~50). The high pro-vitamin A value of ‘Nam Dokmai #4’ fruits (1573 ± 140 RE/100 g of DW) was attributed to a notably high total β-carotene content (113 ± 9 µg/g of DW) that was achieved despite the presence of a considerable portion of *cis*-isomers (~30%).

By contrast, full-ripe ‘Kaew’ fruits displayed a similar pro-vitamin A value (1246 ± 2 RE/100 g of DW) that was less affected by the *cis*-isomers, constituting only 14–18% of the total β-carotene content throughout ripening. Contrarily, the fruits of the cultivars ‘Mon Duen Gao’, ‘Rad’, ‘Kiew Sawoei’, ‘Okrong Kiew’, and ‘Okrong Thong’, which developed rather poor mesocarp colourations, reached

total β-carotene contents and pro-vitamin A values of only 10–22 µg/g and 136–298 RE/100 g of DW, respectively, at comparable sugar/acid ratios. In this way, cultivars rich in β-carotene were distinguished from others with poor β-carotene production. On the average, the former group reached ~5 times higher pro-vitamin A values compared with the latter. It should be noted that the second group included typical green-eaten cultivars, like 'Kiew Sawoei', that are usually consumed at the mature-green or half-ripe stage when β-carotene biosynthesis has not yet reached the levels mentioned above.

During post-harvest ripening, the all-*trans*-β-carotene contents of mango mesocarp rose exponentially at cultivar-specific rates affected by exogenous factors (Vásquez-Caicedo et al., 2005). Because all-*trans*-β-carotene is one of the major pigments defining the mesocarp colour of mango fruits, these relationships were further explored with respect to a potential use in fruit selection for processing. For this purpose, the kinetics of each key quality parameter undergoing post-harvest ripening changes was studied by regression analysis to evaluate the interdependencies among the quality parameters by comparison of the regression models. As shown for the nine cultivars studied, mesocarp colour, expressed by the hue angle (*H*°), followed cultivar-specific power law functions with increasing all-*trans*-β-carotene contents (Vásquez-Caicedo et al., 2005). Since the relative amounts of *cis*-β-carotene isomers were almost constant throughout ripening, post-harvest ripening processes may be performed until a specified cultivar-specific mesocarp hue is reached. This is an easily accessible nutritive indicator to ensure desired pro-vitamin A values of fruits to be processed.

From a processing point of view, however, firmness and the sugar/acid ratio are crucial fruit properties that need to be specified besides mesocarp colour, especially as to size-reducing operations and sensory product quality. Therefore, the post-harvest ripening behaviour of the mango cultivars was additionally studied in terms of the development of major processing properties (Vásquez-Caicedo et al., 2004). Although the cultivars differed in their rates of sugar accumulation (total soluble solids, TSS) and acid degradation (titratable acids, TA) during post-harvest ripening (29 ± 2 C, 90–95% RH), the close connection between carbohydrate and acid metabolism was reflected by an empirical power law that described the cultivar-independent decline of acidity with rising TSS/TA ratio (R^2 = 0.96, n = 51 records).

Thus, the pH value initially rose markedly until pH 3.9 at a TSS/TA ratio of ~33, followed by a weaker increase (Figure 2) when acid degradation continued at almost constant TSS levels. According to Figure 2, the pH limit for thermal preservation of pulps and nectars at temperatures below 100°C (pH 4.5) was naturally reached at a TSS/TA ratio of ~81; at a TSS/TA ratio of ~50, considered as typical consumption ripeness for cultivars used full-ripe, the fruits presented at approx. pH 4.1. However, only the cultivars rich in β-carotene developed the yellow-orange mesocarp colour desired for mango nectars until this ripeness stage, as discussed above. For the specification of suitable cultivars and ripeness stages for processing, ripeness-based quality profiles of each cultivar were expressed by cultivar-dependent power laws correlating increasing TSS/TA with declining mesocarp firmness and its hue angle, respectively (Vásquez-Caicedo et al., 2004).

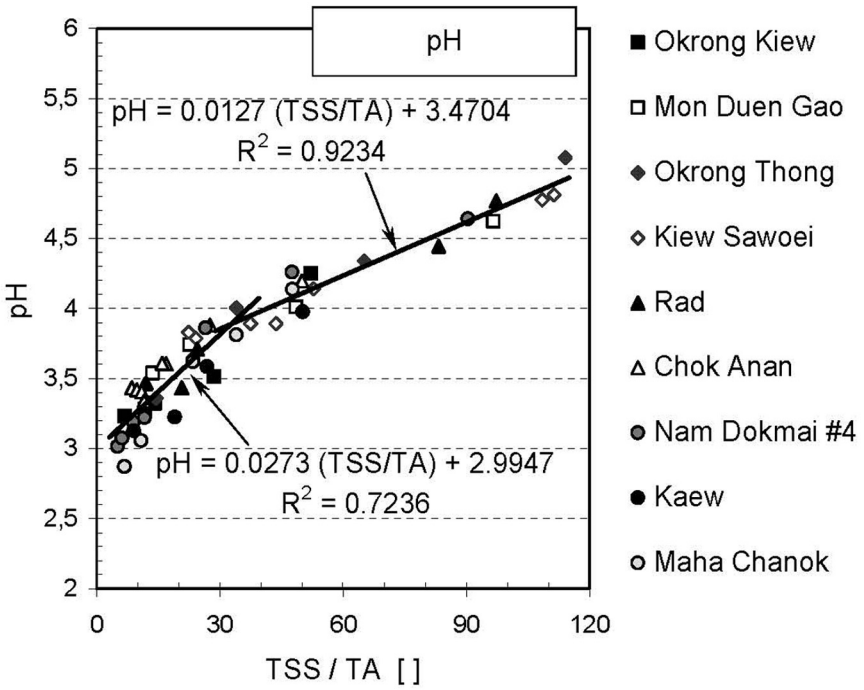


Figure 2: Post-harvest ripening of mango fruits (29 ± 2°C, 90–95% RH): general pH changes with increasing sugar/acid ratio (TSS/TA). According to Vásquez-Caicedo et al. (2004), adapted.

As an indicator of desirable taste, the TSS/TA ratio is widely applied by processors as the ripeness indicator and for fruit product standardisation. However, since adequate texture and colour are equally important, a ripeness indicator, which reflects the cultivar-specific post-harvest ripening profiles outlined above, was of interest, especially for ripeness-based fruit sorting prior to processing. Because of the high correlation of near-infrared (NIR) spectral data of whole mango fruits with post-harvest ripening time (R^2 = 0.96–0.97; n = 3 fruit batches), non-destructive FT-NIR spectroscopy proved suitable in principle for the monitoring of post-harvest ripening (Mahayothee et al., 2004). This was provided that an absolute property, which indicates the ripeness-dependent quality profiles irrespective of the time scales of individual ripening processes, can be used for calibration. For the Thai mangoes studied, peel colour was, overall, considered to be an unreliable ripeness indicator. Peel colour hardly changed during ripening of some cultivars as it did not reflect the pronounced alterations in mesocarp colour (Vásquez-Caicedo et al., 2004). Moreover, peel colour and fruit firmness were found to be preferentially affected by calcium carbide (CaC_2) that has widely been used as ripening accelerator (Mahayothee et al., 2007a), being a low-investment alternative to ethylene treatments. However, due to the unbalanced acceleration of softening at the expense of fruit quality, the use of CaC_2 appears to be inadvisable. By contrast, the cultivar-dependent power law relationships between mesocarp colour (hue angle) and the TSS/TA ratio (Vásquez-Caicedo et al., 2004) were robust to such exogenous factors affecting ripening (Mahayothee et al., 2007a).

To deduce a meaningful ripeness indicator that combines relevant fruit properties, it was therefore sufficient to consider the TSS/TA ratio and mesocarp firmness without the colour (Neidhart et al., 2007). For mango fruits, we developed a relevant post-harvest ripeness index (RPI) and introduced it in our studies for the precise specification of ripeness stages (Mahayothee et al., 2007b; Vásquez-Caicedo et al., 2005). As a key feature of this index, linearity of its change (decline) with ripening time was aimed to facilitate the prediction of ripening times needed until the desired fruit quality is reached, as illustrated by Sirisakulwat et al. (in press). In this way, the RPI is considered to be a useful indicator during controlled post-harvest ripening to provide fresh mango fruits of both appropriate processing properties and high pro-vitamin A value (Neidhart et al., 2007).

As the nature of the structures where carotenoids are deposited in the chromoplasts and the physical state of the pigments were shown to be crucial for the stability of the all-*trans* configuration already in the fresh plant material (Vásquez-Caicedo et al., 2006), carotenoid stability during processing may considerably vary among plant foodstuffs depending on their chromoplast types. This is discussed below for mango.

Production of non-sulphited mangoes

In most mango-producing regions, especially Thailand, fruit drying is of great economic importance (Sruamsiri and Neidhart, 2007). Particularly for small scale processing of seasonal surplus fruits, drying offers chances to generate regional added value. It uses highly flexible production lines that can be adapted to a broad range of fruit species, which are mostly only available during short harvest periods. Moreover, dehydrated mango bars and slices may efficiently improve the pro-vitamin A status of the population year-round. However, alternative technologies not requiring treatments like sulphitation, blanching or osmotic dehydration need to be devised. Sulphitation especially has still been widely applied in mango drying practice to protect the fruits from browning and microbial spoilage, but has been under critical consideration with respect to the allergen labelling of foodstuffs implemented by European Union Member States in November 2004 (Directive 2003/89/EC). Accordingly, sulphur dioxide and sulphites at concentrations exceeding 10 mg/kg or 10 mg/l, expressed as SO_2, have to appear on the label. Precise process control is thus required to meet trade specifications as regards residual sulphite limits.

High-temperature drying, as part of a holistic process concept, was shown to be suitable for the manufacture of non-sulphited dried mango slices (Pott et al., 2005). Besides necessary firmness for cutting, enzymatic and non-enzymatic browning can also be controlled to some extent by the selection of cultivar and ripeness stage. Since the activities of browning enzymes peroxidase (POD) but mainly polyphenoloxidase (PPO), greatly increase in the mesocarp during post-harvest ripening (Vásquez-Caicedo et al., 2004), fruits may be preferably processed after limited ripening until a specified processing maturity to confine native PPO activities. The application of sufficiently high drying temperature (~80 C) contributes to colour retention by early enzyme deactivation. Moreover, concomitant occurrence of low pH values and limited contents of reducing sugars (Spreer et al., 2007), which are the crucial reactants of the Maillard reaction, are beneficial to reduce non-enzymatic browning during storage. Since the Maillard reaction mostly occurs during storage at unfavourable conditions, respective packaging and storage prerequisites should complete the high-temperature drying concept to achieve optimum colour retention, as discussed by Pott et al. (2005). Finally, reduction of drying times by drying until a water activity just below the critical value for microbiological stability (0.6) further contributes to improved colour retention by suppressing the Maillard reaction during drying.

As a particular economic benefit, productivity of fruit drying is increased by shorter process times resulting from shortened drying processes and the absence of pre-treatments that are no longer needed. This concept was verified for three mango cultivars, subjected to various ripening conditions by applying suitable ranges of processing ripeness defined by RPI levels that were specific for each cultivar (Mahayothee et al., 2007b)., Using this approach 100 g of sulphite-free dried mango slices of superior sensory product quality from ‘Kaew’ and ‘Chok Anan’ fruits resulted in all-*trans*-β-carotene contents of 29–54 and 20–23 µg/g and corresponding vitamin A values of 483–900 and 333–383 RE/100 g respectively. These levels would provide sufficient amounts of pro-vitamin A for adults according to the respective estimated mean requirements (females, 270 RE/day; males, 300 RE/day) and recommended safe intakes (females, 500 RE/day; males, 600 RE/day) of vitamin A (FAO and WHO, 2002).

Due to the low moisture content of dried fruit products, the dried mango slices were thus good pro-vitamin A sources despite β-carotene degradation and isomerisation caused by processing (Pott et al., 2003b). Irrespective of the types of processes studied, drying resulted in complete degradation

of the xanthophyll esters, which might therefore be taken as marker of unprocessed mango products (Pott et al., 2003a). β-Carotene was partly degraded, whereas isomerization depended on the drying process (Pott et al., 2003b). Solar drying in a sunlit tunnel dryer was shown to cause higher *β*-carotene losses than high-temperature short-time processes performed at 75°C in the dark in an over-flow dryer. As lower drying temperatures were reached in solar drying (62°C maximum), comparatively long drying times were necessary, thus causing prolonged exposure of the fruit slices to heat and light.

Thermally induced degradation, including the marked formation of the 13-*cis* isomer, was observed for all processes. Additionally, light-mediated isomerisation to 9-*cis*-β-carotene considerably reduced the vitamin A value of solar-dried mango slices. Despite similar portions of *cis*-β-carotene isomers in the fresh fruit (19–27%), their relative amounts increased to 51–64% after solar drying, but only to ~37% after drying in the over-flow dryer. However, solar drying of carotene-rich raw material still yielded mango products of high pro-vitamin A values, as shown by samples of 'Nam Dokmai' and 'Kaew' fruits with 425 and 1011 RE/100 g of dried product (Pott et al., 2003b). The safe intakes of vitamin A recommended for children below 7 years (500 RE/day) and pregnant women (800 RE/day) (FAO and WHO, 2002) would be met by a daily consumption of 50–118 and 79–188 g of those products.

β-Carotene retention in fluid mango products

Our study of β-carotene stability during mango processing was completed by including the manufacture of fluid mango products as a complementary process to fruit drying. Fluid mango products, like purées and nectars, are mostly produced on much larger scales, thus usually involving continuous process lines. Owing to severe cell rupture during pulping, the carotenoids are more prone to oxidative degradation and isomerization than in solid mango products. This is due to the enhanced exposure to oxygen and endogenous oxidative enzymes without the protective plant matrix. Because of the enhanced release of carotenoids by tissue disintegration, bioavailability may be significantly improved, but the effect might be overcompensated by oxidative degradation and isomerisation, as reviewed by Vásquez-Caicedo et al. (2007a). As pulping requires soft texture, the fruits should be processed fully ripe, that is, after maximum β-carotene accumulation. Modern industrial year-round mango drink and nectar production is generally from purée intermediates produced during peak harvest seasons. The fruit component in the final nectar may have been subjected to up to four thermal treatments required for steam peeling, thermal deactivation of native browning enzymes prior to pulp liquefaction, and pasteurisation of purée and nectar (Vásquez-Caicedo et al., 2007a).

Due to adverse flow properties of the often highly viscous mango pulp (Dube et al., 2004), the pulp may be subjected to controlled pectin degradation prior to thermal preservation to standardise its viscosity for technological and sensory reasons (pulp liquefaction). Since the activities of oxidative enzymes (PPO and POD) are particularly high in fully ripe fruits (Vásquez-Caicedo et al., 2004), their immediate deactivation after pulping is crucial to avoid rapid browning due to polyphenol oxidation. This also serves to minimise carotenoid oxidation in the presence of free radicals. Furthermore, electrophilic compounds like quinones are among the factors affecting carotenoid isomerisation (Schieber and Carle, 2005).

Although heating is usually performed continuously according to the high-temperature short-time principle to minimise damage, thermal β-carotene stability was assumed to be less in mango purée than in carrot juice because of its partial dissolution in oil droplets in the globular mango chromoplasts and its predominantly crystalline nature in carrot chromoplasts (Vásquez-Caicedo et al., 2006). The well-studied isomerisation of carrot juices has recently been reviewed by Schieber and Carle (2005, 2008), documenting the notably high stability of crystalline β-carotene in usual juice production processes that may involve pasteurization at ~95°C after acidification or sterilization at 121˚C (F = 5–20 min). Formation of 13-*cis*-β-carotene up to proportions of > 5% (max. 10%) only

resulted from extensive blanching of whole carrots using conditions that are usually not applied (90–100˚C, 30–60 min). This was due to enhanced dissolution of crystalline β-carotene in cellular lipid droplets (Marx et al., 2003).

In accord with this, the experimental addition of grape seed oil to carrot mash enhanced *trans*-*cis* isomerisation both in unheated and heat-preserved juices. In addition to the predominant carotene crystals, plastoglobuli, being much smaller than those of mango chromoplasts, were already detected in the chromoplasts of fresh carrots by transmission electron microscopy (Vásquez-Caicedo et al., 2006). They might carry undetectable traces of *cis*-isomers. Without the addition of oil, extensive sterilisation of carrot juice at 121 and 130°C with *F* values ≥ 40 and ≥ 20 min, respectively, was required to induce the formation of the 9-*cis* isomer besides 13-*cis*-β-carotene in the juices (Marx et al., 2003).

For comparison, experimental pasteurisation of mango purée between 85 and 93°C for up to 16 min of exposure time, equivalent to heating at 93.3°C for 0.34–14.91 min ($P^{z=8.9°C}_{Tref=93.3°C}$), caused significant *trans*-*cis* isomerisation. This was shown by a 1.18–1.95 fold increase in 13-*cis*-β-carotene depending on the thermal load (Vásquez-Caicedo et al., 2007a). Formation of the 13-*cis* isomer was chiefly induced above 90°C, but the initially high rates of its formation declined with rising holding time. At $P^{z=8.9°C}_{Tref=93.3°C}$ values ≥ 0.88 min, the relative amounts of 9-*cis*-β-carotene also rose increasingly, though overall were less than the 13-*cis* isomer. However, despite significant degradation and isomerisation, total β-carotene retention was ≥ 93%, corresponding to maximum vitamin A loss of 15.4%. This worst case, mimicked by holding times of 16 min, was expected for small-scale batch production based on bottle pasteurisation, which requires longer heating than continuous high-temperature short-time processes. Whereas carotenoid quantification was limited to the β-carotene isomers in this study, increasing carotenoid isomerisation during pasteurisation with rising thermal load was also shown by characteristic changes in the UV/Vis spectra of ethanolic carotenoid extracts obtained from mango purée. This was revealed in an increasing hypsochromic wavelength shift and an additional maximum between 360 and 380 nm. Despite complete PPO deactivation, detrimental colour changes were increasingly observed, especially in terms of declining colour intensity (chroma).

Pilot-plant production of mango nectar, which was based on four thermal treatments as mentioned above and included continuous heating of all fluid products, resulted in a final retention of 83% of the vitamin A value of the fruit dry matter (Vásquez-Caicedo et al., 2007a). The higher loss of 17%, contrasting with losses of only 7.5–10% in the thermally comparable pasteurisation processes described above, was attributed to the combined effects of light, oxygen, heat, and loss of cell compartmentalisation during mango nectar production. The application of several heating steps at pasteurisation temperatures in modern industrial production of mango purée and nectar, where faster processing should limit oxidative degradation even more compared to the semi-continuous pilot plant process, can thus be deemed to have little harmful effect on vitamin A retention. On the other hand, they hardly reduce the allergenic potency of mango fruits caused by some heat-stable allergenic proteins (Dube et al., 2004).

Vitamin A losses of hot-filled mango purées during storage were shown to be chiefly caused by oxidative degradation of β-carotene, irrespective of different exposure to oxygen and light (Vásquez-Caicedo et al., 2007b). Additional isomerisation was limited to photoisomerisation under the experimental exposure to light, as revealed by the relative increase in 9-*cis*-β-carotene, which was accompanied by a decline in the 13-*cis* isomer portion. However, oxygen removal and headspace minimisation were most crucial for pro-vitamin A retention.

Recovery of pectin from processing residues

As gelling and stabilising agents, pectins are commonly used in the food industry, whereas their bioactivities are of dietary and pharmacological interest (Endress et al., 2006). Compared to the major sources of materials used for pectin production, residues from mango processing originate

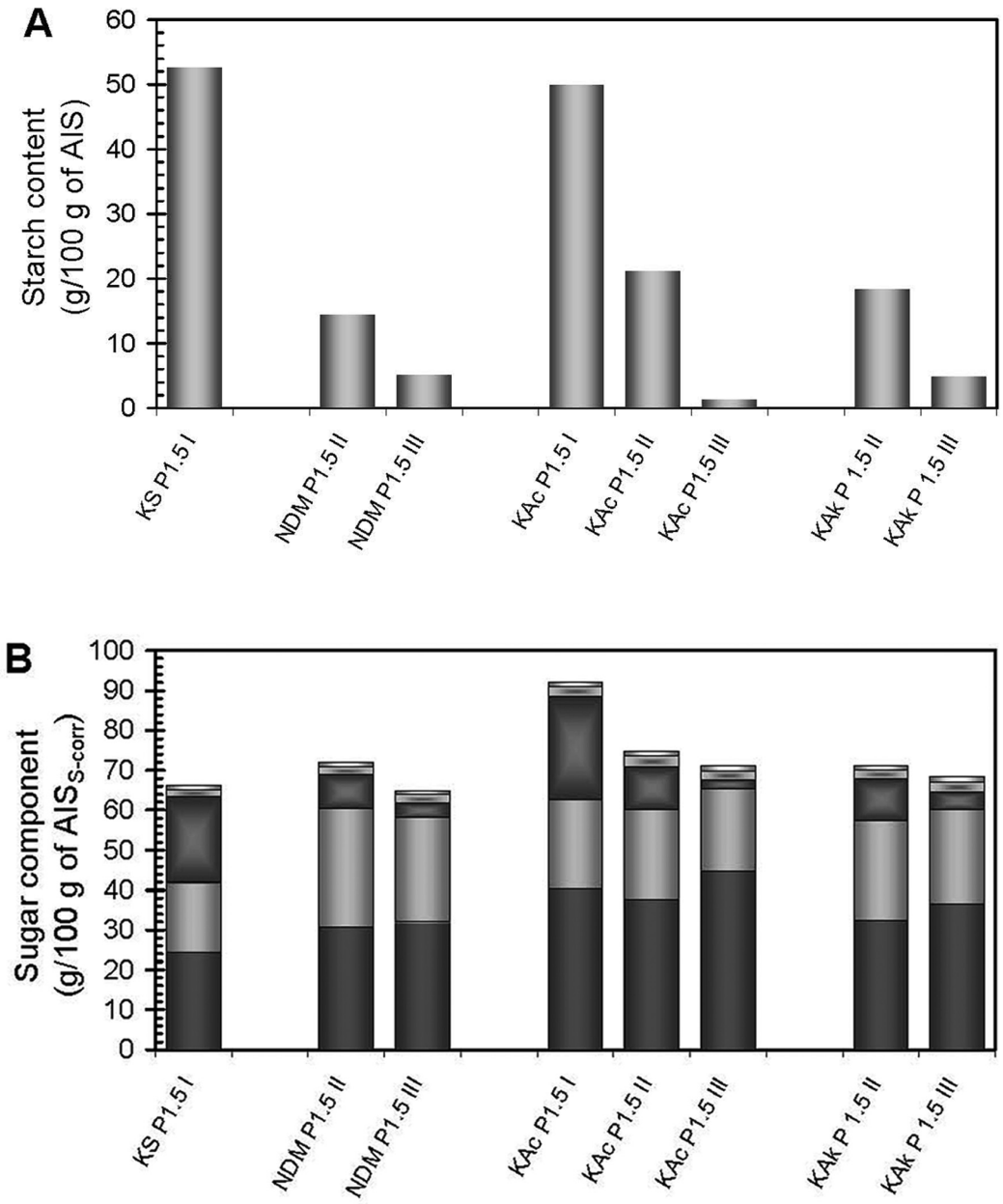
from quite heterogeneous raw material in terms of cultivars, maturity, and available amounts. As part of the cell wall polysaccharides, pectic substances influence the strength, porosity and adhesion of the cell walls during the development of higher plants (Endress et al., 2006). Softening of the fruit during post-harvest ripening results in solubilisation of pectic polymers and degradation of hemicelluloses and starch to various extents, depending on the cultivar and the part of the fruit (Sirisakulwat et al., 2008; Neidhart et al., in press). Therefore, yields and quality of the obtainable crude pectic polymers may vary much more than in case of the established pectin sources. Our study was based on the evaluation of 78 mango pectins, which were recovered from the dried peels and mesocarp of four cultivars at different fruit ripeness as well as from industrial peel waste and the by-products of pulp production.

The impact of fruit ripeness on yield and quality of pectins from mango peels has comprehensively been addressed by Sirisakulwat et al. (2008) for various cultivars. Pectins from peels and mesocarp have recently been compared by Neidhart et al. (in press), demonstrating the broad range of recoverable pectins with respect to yield, molecular structure, and functional properties. As confirmed by our studies, mango has been deemed a promising source of rapid-set to ultra-rapid set high-methoxyl pectins. In this respect, they are similar to apple and citrus pectins, which may be modified after their recovery to produce a broad range of tailor-made commercial pectins with different degrees of methylation and setting properties (Endress et al., 2006). However, high yields up to 42%, as obtained from peels of mature-green fruits, were largely attributed to co-extracted starch (Sirisakulwat et al., 2008).

Since native starch did not always degrade completely until processing of the fruit, co-extraction of starch may be significant (Figure 3A). Despite the decline of the starch content, the co-existence of amyloplast and chromoplast plastid types at different ripeness stages, as revealed by transmission electron microscopy, indicated a dynamic interconversion of the plastid structures in mango mesocarp tissue throughout post-harvest ripening (Vásquez-Caicedo et al., 2006). Similar to pectin recovery from early harvested apples (Endress et al., 2006), additional enzymatic treatments for the artificial removal of starch might thus be necessary. Without such treatments, comparison of pectin yields is only acceptable on a starch-adjusted basis.

Unlike the crude pectin yields, these starch-adjusted net pectin yields were rather constant throughout ripening, amounting to 11–21% for peel pectins (Sirisakulwat et al., 2008) and ≤ 9.3% for pulp pectins (Neidhart et al., in press), consistent with earlier reports. In terms of yield and average key properties, mango peel pectins came close to apple pectins (Sirisakulwat et al., 2008). However, one of the key problems that may limit the broad exploitation of mango peels results from the great variability of the galacturonic acid content, which is the key component constituting the partially methylated galacturonan main chains.

According to the legal specification of pectins (Endress et al., 2006), a galacturonic acid content ≥ 65% on an ash- and moisture-free basis is required, but not always met by mango pectins (Neidhart et al., in press). Whereas the broad utilisation of mango pulp residues may particularly be limited by rather low net pectin yields (Neidhart et al., in press), the gelling and thickening capacities of mango peel pectins were shown to be restricted by a characteristic, almost monodisperse fraction with a peak molecular weight of 16 000–19 000 and the occurrence of expanded galactans (Figure 3B) (Sirisakulwat et al., 2008). Mango by-products thus still appear to be a less readily exploitable source. Nevertheless, their potential for techno-functional food ingredients has become evident.



Figures 3A and 3B: Impact of fruit ripeness on pectins from mango peels of 'Kiew Sawoei' (KS), 'Nam Dokmai #4' (NDM), 'Kaew Chuk' (KAc), and 'Kaew Khiew' (KAk) (hot-acid extraction, pH 1.5): Figure 3A. Co-extracted starch in crude pectins (AIS) and figure 3B contents of major sugar residues on a starch-free basis (AIS_{S-corr}). GalUA, galacturonic acid (titrimetic; M_r = 176.13 g/mol); Gal, galactose; Glc (non-starch), glucose (without glucose of starch); Ara, arabinose; Rha, rhamnose; ripeness stages, I (mature-green), II (after major softening), III (full-ripe). According to Sirisakulwat et al. (2008), adapted.

Conclusion

Interactions between raw material quality, post-harvest handling, and final process technologies as well as resulting ways to optimise product quality have exemplarily been demonstrated for the mango fruit. By means of the options suggested for small- or large-scale processing, the vitamin A value of the mango fruit can largely be retained. The potential and present limits of integrated mango processing in combination with pectin recovery from by-products have been identified.

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Healthful and nutritional components in select Florida tropical fruits

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Keywords: tropical fruit, healthful, nutritional, anti-oxidant, phenolic, citrus

Abstract

Fourteen tropical fruits from south Florida (red guava, white guava, carambola, red pitaya (red dragon), white pitaya (white dragon), mamey, sapodilla, lychee, longan, green mango, ripe mango, green papaya and ripe papaya) were evaluated for phenolic compounds, anti-oxidant activity, ascorbic acid (vitamin C), total fibre and pectin. ORAC (oxygen radical absorbance capacity) and DPPH (1,1-diphenyl-2-picrylhydrazyl, radical scavenging activity) assays were used to determine anti-oxidant activity. The total soluble phenolics (TSP), ORAC, and DPPH ranged from 205.4 to 2316.7 µg gallic acid equivalent/g puree, 0.03 to 16.7 µmole Trolox equivalent/g puree and 2.1 to 620.2 µg gallic acid equivalent/g puree, respectively. Phenolic components included ellagic acid, quercetin glycosides, gallic acid conjugates, catechin, catechin conjugates, hydroxycinnamates, flavone glycoside, kaempferol, mangiferin, and gallotannins. Total ascorbic acid (TAA), total dietary fibre (TDF) and pectin ranged from 13.6 to 159.6 mg/100 g, 0.88 to 7.25 g/100 g and 0.2 to 1.04 g/100 g, respectively. For tangerine lines, TAA ranged from 14.9–331.1 mg/100g, α-carotene 3–206 µg/100g, β-carotene 3–256 µg/100g and β-cryptoxanthin 0.1–70 µg/100g. The anti-oxidant activities, TSP, TAA, TDF and pectin appeared to be influenced by cultivar (papaya, guava and dragon fruit) and ripening stage (papaya and/or mango). Some of the above tropical fruits as well as tangerine hybrids were analysed for carotenoid content. Citrus peel was studied for healthful compounds including polymethoxylated flavones (PMFs) that have anti-cancer, anti-inflammatory and cardio-protective activities. Data demonstrate the potential benefits of several of these fruits as well as citrus peel for human health.

Introduction

Limited information is available on the nutritional value of tropical and subtropical fruits, especially the more exotic species. The USDA-ARS Citrus and Subtropical Products Laboratory, Winter Haven recently did a study on select tropical fruits from South Florida that were analysed for components that could be beneficial to human health (Mahattanatawee et al., 2006). Studies were also conducted on phenolic compounds in citrus peel, particularly the polymethoxylated flavones (PMFs) that have cardio-protective, anti-inflammatory and anti-cancer properties (Morin et al., 2008).

All fruits and vegetables provide anti-oxidants in the form of vitamins and other compounds including vitamin C, E, A and polyphenols. Some of these healthful compounds come in the form of pigments such as anthocyanins and carotenoids (Ness et al., 1997; Eastwood, 1999). Phenolic compounds account for a major portion of the anti-oxidants in many plants (Duthie and Crozier, 2000). Ascorbic acid is also abundant in many fruits and is thought to play a role in disease prevention due to its ability to scavenge free radicals in biological systems (Block, 1991). Carotenoids, such as α- and β-carotene, are precursors to vitamin A, whereas lycopene and β-cryptoxanthin are not, but are considered anti-oxidants (Breithaupt et al., 2007). Complex carbohydrates also play an important role in a healthy diet. For this reason fruits and vegetables are essential to the human diet, not only providing anti-oxidants for protection against cellular damage caused by exposure to high levels of free radicals (Ames et al., 1993), but also aiding digestion (Weisburger et al., 1993).

The objective of this study was to obtain nutritional information for Florida-grown tropical fruits (red guava, white guava, carambola, red dragonfruit, white dragonfruit, mamey sapote, sapodilla, lychee, longan, mango and papaya) in terms of anti-oxidant activity, total soluble phenolics (TSP), total ascorbic acid (TAA), total dietary fibre (TDF) and pectin. Since different ethnic groups prefer different maturity stages of some fruits, like mango and papaya, both ripe and green stages were assayed. In addition, qualitative analyses by HPLC-PDA-MS of the phenolic constituents in selected tropical fruit were conducted (Mahattanatawee et al., 2006). Finally, carotenoid content of some of these fruits, TAA and carotenoids of tangerine breeding lines were explored and an overview of PMFs in citrus peel has been presented.

Materials and methods

Fruit

Fourteen different tropical fruits from South Florida including red guava (*Psidium guajava* L., 'Sardina'), white guava (*Psidium guajava* L., Thai cultivar), carambola (*Averrhoa carambola* L., 'Arkin'), red pitaya (red dragon fruit, *Hylocereus* sp., *'Red Jaina'*), white pitaya (white dragon fruit, *Hylocereus* sp., '*David Bowie*'), mamey sapote (*Pouteria sapota*, **'***Pantin*'), sapodilla (*Achras* (*manilkara*) *zapota*, **'***Brown Sugar*'), lychee (*Litchi chinensis*, '*Mauritius*'), longan (*Dimocarpus longana*, '*Kohala*'), green and ripe mango (*Mangifera indica*, '*Keitt*'), green papaya (*Carica papaya*, 'Exp. 15', a variety that is produced for the green papaya market), and ripe papaya (*Carica papaya*, **'**Red Lady', a variety that is produced for the ripe papaya market) were obtained from Florida tropical fruit growers. A composite of at least 10 fruit were combined per each of three replicate samples (Mahattanatawee et al., 2006).

Qualitative analysis of phenols in fruit pulp

The phenolic compounds in the current study were analysed in the fruit pulp with a Waters (Milford, MA) Alliance HPLC, equipped with a Waters 996 PDA detector and a Waters/Micromass ZQ single-quadrupole MS. Separation of the phenols was accomplished on a 250 x 4.6 mm i.d. RP-Amide C16 (Supelco) column, with multistep linear water/acetonitrile/2% formic acid gradients at flow rates of 0.75 ml/min (Mahattanatawee et al., 2006).

Determination of TAA

The TAA was assayed as previously described with some modification (Nisperos-Carriedo et al., 1992). The fruit puree (25 g) was blended with 25 ml 0.05 N H_3PO_4 for 3 min. The ascorbic acid and dehydroascorbic acid were determined by using HPLC with an organic acid column (OA-1000, 9 µm, 300 mm x 6.5 mm, Alltech Associates Inc.). (Mahattanatawee et al., 2006).

Sample preparation for pectin content

The sample preparation method was according to Theander (1995) with some modification. Triplicate 500 mg dry fruit puree samples were transferred into 50 ml polypropylene centrifuge tubes (Beckman Instruments, Inc.). Acetate buffer (5 ml) was added at pH 5.0 along with 40 µl α-amylase (heat stable α-amylase from *Bacillus amyloliquefaciens*, Sigma-Aldrich, Inc.). The solution was mixed, placed in a boiling water bath for 1 h, and cooled to 40°C. A 500 µl amyloglucosidase solution (from *Aspergillus niger*,) was added and tubes were then incubated overnight in a 60°C water bath equipped with shaker (shaker water bath Gallenkamp model BKS-350) (Mahattanatawee et al., 2006).

Galacturonic acid determination

The determination of galacturonic acid in the hydrolysed samples was optimised from the original method of Scott (1979) by Luzio (2004) using a microplate reader (Power Wave 340 microplate reader with KC4 version 3.01 software, BioTek Industries) (Mahattanatawee et al., 2006).

Total dietary fibre assay

The assay was based on the method published in the 16th Edition of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1995) using the total dietary fibre assay kit from Sigma-Aldrich (TDF 100A) (Mahattanatawee et al., 2006).

Carotenoid analysis

Three fruit per each of three replicates for tropical fruits and juice from a blend of 30 fruits/tangerine sample was homogenised using a blender. The homogenate was centrifuged, the pellet collected then dissolved in acetone, and the solution injected onto a HPLC (20 µl loop), equipped with a YMC Carotenoid column from Waters. Flow rate was 1 ml/min, at 30°C. The mobile phase was 40% H_2O, 81% methanol (MeOH), 15% tert-butylmethyl ether (MTBE) to 4% H_2O, 6% MeOH and 90% MTBE. Compounds were detected using a PDA detector scanning 200–700 nm.

Statistical analysis

Correlations and 95% confidence intervals were determined using Excel (Microsoft Inc).

Results and discussion

Total phenolic, ascorbic acid and anti-oxidant activity

The selected Florida tropical fruits had widely different levels of TSP, TAA and anti-oxidant activity. The TSP, TAA, ORAC, and DPPH ranged from 205.4 to 2316.7 µg gallic acid equivalent/g puree, 7.5 to 188.8 mg/100 g puree, less than 0.1 to 16.7 µmol Trolox equivalent/g puree, and 2.1 to 620.2 µg gallic acid equivalent/g puree, respectively (Table 1) (Mahattanatawee et al., 2006).

Table 1. Total phenolic, total ascorbic acid, anti-oxidant activity by ORAC and DPPH for selected Florida-grown tropical fruits.

Fruit	Total phenolic	Total ascorbic acid	ORAC	DPPH
	µg GA/g puree	mg/100 g puree	µM TE/g puree	µg GA/g puree
Red guava	2316.7 ± 167.6	122.3 ± 15.0	16.7 ± 0.6	609.3 ± 31.9
Carambola	2207.7 ± 156.7	16.9 ± 1.6	12.9 ± 1.0	620.2 ± 40.9
White guava	1589.3 ± 75.4	201. ± 17.4	9.9 ± 0.7	298.6 ± 22.6
Red dragon	1075.8 ± 71.7	55.8 ± 2.0	7.6 ± 0.1	134.1 ± 30.1
Mamey sapote	1010.5 ± 40.2	7.5 ± 2.1	6.6 ± 0.3	247.1 ± 18.3
Lychee	770.1 ± 30.1	8.1 ± 1.5	5.4 ± 0.2	103.8 ± 13.8
White dragon	523.4 ± 33.6	13.0 ± 1.5	3.0 ± 0.2	34.7 ± 7.3
Ripe mango	508.9 ± 29.4	92.8 ± 2.5	2.2 ± 0.1	123.7 ± 12.3
Green mango	505.8 ± 51.8	29.8 ± 7.4	1.5 ± 0.2	167.5 ± 13.4

Fruit	Total phenolic	Total ascorbic acid	ORAC	DPPH
	µg GA/g puree	mg/100 g puree	µM TE/g puree	µg GA/g puree
Sapodilla	501.8 ± 39.3	11.9 ± 1.8	1.4 ± 0.1	2.1 ± 0.2
Longan	481.9 ± 37.4	14.0 ± 0.5	3.3 ± 0.1	69.6 ± 19.7
Ripe papaya (cv. Red lady)	442.2 ± 29.7	153.8 ± 12.1	5.3 ± 0.3	65.1 ± 15.8
Green papaya (cv. Red lady)	311.1 ± 18.9	56.7 ± 3.5	2.6 ± 0.2	29.7 ± 5.4
Green papaya (cv. Exp. 15)	205.4 ± 35.8	57.2 ± 1.3	< 0.1	10.4 ± 1.6

Data for total phenolic, ascorbic acid, ORAC and DPPH are + 95% confidence interval. Mahattanatawee et al., 2006.

Carambola and red guava had the highest anti-oxidant activity of the selected Florida fruit while sapodilla and green papaya 'Exp. 15' had the lowest (Table 1). Both ripe papaya and mango exhibited higher anti-oxidant activity (ORAC for both fruit, DPPH for papaya only) and TSP compared to their green counterparts, perhaps due to the increase in TAA and carotenoids as the fruits ripened. Likewise, TAA was higher in ripe mango and papaya than in the respective green fruits (Mahattanatawee al., 2006). Red guava was found to have ellagic acid conjugates, flavone glycosides, and gallic acid conjugates; carambola had catechin, proanthocyanidin dimmer and trimer conjugates; red/white dragonfruit had hydroycinnamates; mamey sapote had flavone glycosides; lychee had flavone (quercetin and kaempferol) glycosides; green/ripe mango had mangiferin, gallotannins (tetramers to nonamers); sapodilla had catechin conjugates; longan, ellagic acid conjugates, flavone (quercetin and kaempferol); and green/ripe papaya had catechin conjugates (Mahattanatawee et al., 2006).

Comparison of anti-oxidant data to literature values

Since ORAC has been extensively used to evaluate anti-oxidant activity of fruits and vegetables, the data from this study were compared to published ORAC values that used fluorescein (Wu et al., 2004) as the fluorescent probe rather than β-phycoerythrin, which gave lower values in earlier ORAC assays (Ou et al., 2001). Guava, carambola, red dragon fruit, mamey sapote, lychee and ripe papaya ORAC values were higher than or similar to published ORAC values for other common fruits and vegetables. The TSP values for guava and carambola also compared favorably with other fruits and vegetables (Mahattanatawee et al., 2006).

Total dietary fibre and pectin

The TDF and pectin ranged from 0.9 to 7.2 g/100 g and 0.2 to 1.04 g/100 g, respectively (Table 2). Red guava, as with anti-oxidant activity, was highest in TDF and pectin followed by mamey, sapodilla and white guava for TDF, while white guava exhibited the similar pectin levels as in mamey sapote. Green papaya and green mango had more TDF than their ripe counterparts, although ripe mango had slightly more pectin than green mango. The variety of papaya grown for the green (unripe) market, that is preferred by certain ethnic groups ('Exp. 15'), had higher levels of TDF and pectin than the green or ripe stage for the papaya variety produced for the ripe market ('Red Lady'). Red dragon fruit exhibited higher TDF and pectin than did white dragon fruit (Mahattanatawee et al., 2006).

Table 2. Total dietary fibre (TDF) and pectin for selected Florida-grown tropical fruits.

	TDF	pectin
Fruit	**g/100 g fruit**	**g/100 g fruit**
Guava (red)	7.2 ± 0.0	1.04 ± 0.02
Mamey sapote	6.1 ± 0.0	0.77 ± 0.02
Sapodilla	4.4 ± 0.1	0.35 ± 0.01
Guava (white)	4.0 ± 0.1	0.77 ± 0.01
Dragon (red)	3.2 ± 0.1	0.27 ± 0.01
Papaya (green, 'Exp.15')	2.1 ± 0.0	0.60 ± 0.02
Papaya (green, 'Red Lady')	1.8 ± 0.0	0.51 ± 0.01
Mango (green)	1.6 ± 0.0	0.48 ± 0.01
Lychee	1.6 ± 0.0	0.48 ± 0.01
Papaya (ripe, 'Red Lady')	1.5 ± 0.1	0.49 ± 0.01
Mango (ripe)	1.4 ± 0.0	0.51 ± 0.01
Carambola	1.3 ± 0.0	0.27 ± 0.01
Dragon (white)	1.1 ± 0.0	0.12 ± 0.00
Longan	0.9 ± 0.0	0.20 ± 0.00

Data for TDF and pectin are ± 95% confidence interval.
Mahattanatawee et al., 2006.

Comparison of fibre data with literature values

Tropical fruit TDF and pectin values of some tropical fruits were higher than reported values for many other common fruits. Red guava and mamey sapote had TDF values that were higher than many common fruit literature values except raspberries and cherries. Red dragon fruit, white guava and sapodilla all had higher TDF values than most common fruits with the exception of the above and blackberries (Matattanatawee et al., 2006).

Comparison of total ascorbic acid and carotenoids for tangerine lines

The preliminary data for TAA ascorbic acid in 89 University of Florida tangerine breeding lines showed a wide range from 14.9 to 320.1 g/100 g. Carotenoids ranged from 3 to 206 µg/100 g for α-carotene, 3 to 256 for β-carotene, 0.1 to 70 for β-cryptoxanthin and only trace amounts of lycopene.

Comparison of carotenoids for tropical fruits

Most of the edible portion of the tropical fruits in this study, except for tangerines, were low in carotenoids, but high in anthocyanins (phenolic compounds). However, ripe papaya, mamey sapote, sapodilla and ripe mango all had substantial levels of carotenoids ranging from 2.2 to 17 g/100 g α-carotene, 12.3 to 445 g/100 g β-carotene, and 1.8 to 34 g/100g β-cryptoxanthin. The edible part of lychee fruit also had 7 g/100 g β-cryptoxanthin. Tangerine fruit are higher than most other fruit for carotenoids.

Work on PMFs

PMFs are highly concentrated in orange peel oil nonvolatile residues, which are also rich in waxes and phytosterols. Methods were developed to recover highly purified PMFs from these residues as well as rapid FTIR methods for analysis (Manthey, 2006). This work also resulted in a patent for PMFs that are active in reducing and preventing cardiovascular diseases including hesperetin from oranges, nobiletin from tangerines (Whitman et al., 2005; Guthrie et al., 2006; Roza et al., 2007; Morin et al., 2008) that are active as anti-inflammatories (Manthey and Bendele, 2008) as well as tangeretin and nobilitin from tangerine, which inhibit cytokine production (anti-tumor) (Manthey et al., 2001; Morley et al., 2007).

Conclusion

Overall, the data from this study indicate that consumption of Florida tropical fruit varieties may deliver healthful benefits by supplying natural anti-oxidants and dietary fibre that are protective against cellular damage, while improving digestion and maintaining blood sugar levels. Guava would appear to be an especially wholesome fruit. Carambola seems also to have healthful benefits, being very high in anti-oxidants and as well as mamey sapote, being high in TDF and compared to most fruits. Red dragon fruit and sapodilla were likewise elevated in TDF, and red dragon fruit was also high in anti-oxidant activity compared to other fruits and vegetables. Citrus fruit, including the peel, offer healthful benefits including anti-oxidant, anti-inflammatory, and cardio-protective activities especially from the peel for PMFs, and vitamin C and carotenoids (pro-vitamin A).

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By-products from mango (*Mangifera indica* L.) processing as a source of functional compounds

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Keywords: Mango, processing, functional compounds, by-products, pectin, polyphenolics

Abstract

Mangoes (*Mangifera indica* L.) are popular exotic fruits that are both consumed fresh and processed into a large number of products (e.g. nectar, purée, chutney, slices, fruit bars, and leather). The by-products originating from mango processing, mainly peels and kernels, are a promising source of high-value components such as peel pectin and seed fat. Numerous studies carried out during the past decade have indicated that the peels and seeds also contain large amounts of polyphenolics, in particular xanthone and flavonol glycosides, hydrolysable tannins, phenolic acids, and alk(en)ylresorcinols. These phenolic compounds possess various biological activities (e.g. anti-oxidant, anti-inflammatory, and antimicrobial properties, and could therefore be used as functional food ingredients and natural food additives). This review summarises our investigations on the characterisation of polyphenols from mango fruits and by-products.

Introduction

Exotic fruits have experienced increasing popularity over the past decades because of their attractive appearance and their delicious taste and aroma. Mangoes (*Mangifera indica* L.) are among the most important tropical fruits and are often referred to as the 'king of fruits' (Tharanathan et al., 2006). They belong to the Anacardiacea family (order Sapindales), which includes more than 70 genera. Other anacardiaceous plants used as foods are cashew nuts (*Anacardium occidentale* L.), pistachio (*Pistacia vera* L.), pepper tree (*Schinus terebinthifolius*), and jocote (*Spondias purpurea*). Some members of this family like poison ivy (*Toxicodendron radicans*) indigenous to the United States and found throughout the North American continent are also known to cause contact dermatitis. Others are exploited as a source of tanning agents (e.g. *Schinopsis* species ('quebracho')).

Between 1961 and 2004, global mango production has increased from approximately 11 million tonnes to more than 26 million tonnes (Figure 1). India, China, Thailand, Mexico, and Pakistan are the most important producing countries, with India being the undisputed leading state. Other countries with significant mango production are the Philippines, Brazil, and Indonesia. However, in contrast to the aforementioned countries, their production does not exceed one million tonnes (FAOSTAT, 2005).

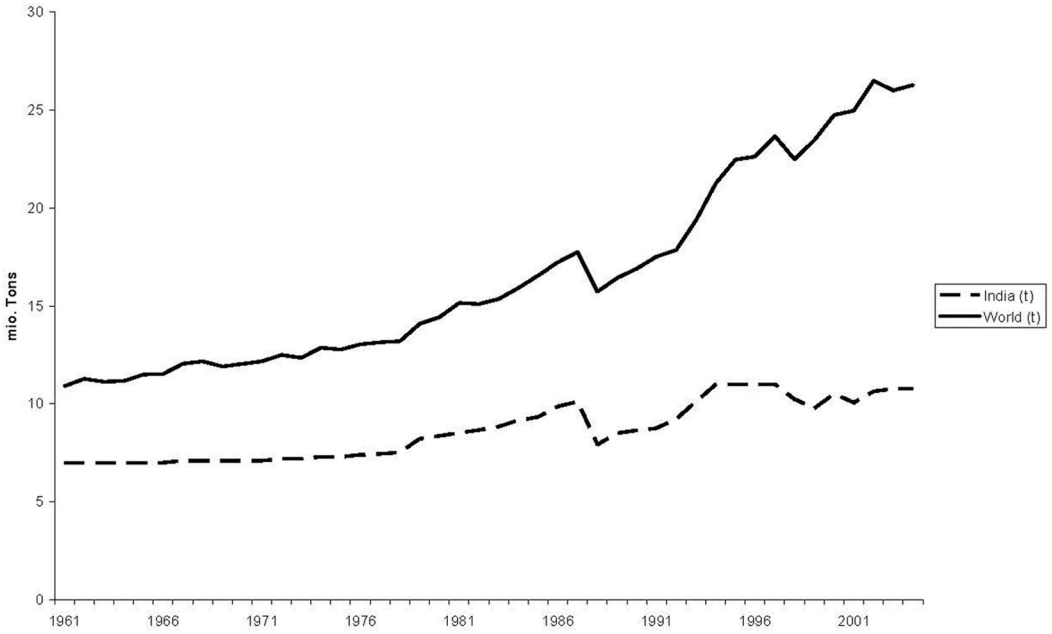

Figure 1. Mango production between 1961 and 2004 (India: dashed line; world: solid line).

Mangoes are consumed as fresh fruits and processed into a large number of products. Canned mango slices, purée, nectar, frozen mango products and ready-to-serve beverages are usually produced from ripe fruits, whereas unripe mangoes are processed into products like powders and chutneys (Ramteke et al., 1999). Apart from their appealing sensory properties, mangoes are valued for their high vitamin C and provitamin A contents. In fact, mangoes greatly contribute to the pro-vitamin A intake in tropical and subtropical regions.

As a consequence of increasing demand and growing production figures, large quantities of by-products emerge from mango processing, especially peels and kernels. Depending on the size of the kernels and the technology used for peeling, these by-products may amount to 60% of the total fruit weight. So far, their utilisation has been a neglected field, which is surprising because both peels and seeds are sources of high-value components. The peels contain pectin which has interesting technological and nutritional properties. However, owing to their high water content, the peels are prone to rapid microbial decay and need to be dried for stabilisation, which is associated with high costs and therefore represents an economically limiting factor.

Mango kernels are utilised as a source of seed fat, which has organoleptic properties similar to cocoa butter and is used as a cocoa butter substitute. However, compared with the amounts of kernels emerging from mango processing, the percentage of utilised by-products appears to be very low. A rapidly increasing number of studies on secondary plant metabolites, in particular polyphenolic compounds, published during the past decade shed a new light on mango by-products. It is expected that these investigations are instrumental in facilitating the utilisation of by-products from mango and other tropical fruits.

Phenolic compounds

Phenolic compounds, frequently also referred to as polyphenols or polyphenolics, are secondary metabolites which occur virtually ubiquitously in the plant kingdom. They are an extremely diverse class of compounds which can broadly be classified into phenolic acids and flavonoids. The phenolic acids can be subdivided into hydroxybenzoic acids (C_6-C_1), which include, among others, *p*-hydroxybenzoic, gallic, protocatechuic and salicylic acids, and hydroxycinnamic acids (C_6-C_3). Well-known members of the latter subclass are caffeic, ferulic, and *p*-coumaric acids.

The coumarins are lactones of *o*-hydroxycinnamic acids. Flavonoids share a C_6-C_3-C_6 backbone and represent a large subclass, with approximately 9000 compounds identified until 2004 (Williams and Grayer, 2004). They are classified into flavonols, flavan-3-ols, flavones, flavanones, and anthocyanins. The latter are beautifully colored plant pigments which render flowers and other plant parts red, orange, or blue.

Apart from modifications to the basic C_6-C_3-C_6 skeleton, the structural diversity of flavonoids is also due to their conjugation to sugars at different sites of the molecules and acylation of these saccharide moieties with aliphatic (e.g. acetic and oxalic acids) or phenolic acids (e.g. hydroxycinnamates). Other subclasses of phenolics include anthraquinones and stilbenes with a C_6-C_2-C_6 structure and xanthones with a C_6-C_1-C_6 backbone. The term 'tannins' includes both condensed (procyanidins) and hydrolysable tannins (gallotannins, ellagitannins) and refers to the capability of some phenolic compounds to precipitate ('tan') proteins. The structures of selected phenolic compounds are presented in Figure 2.

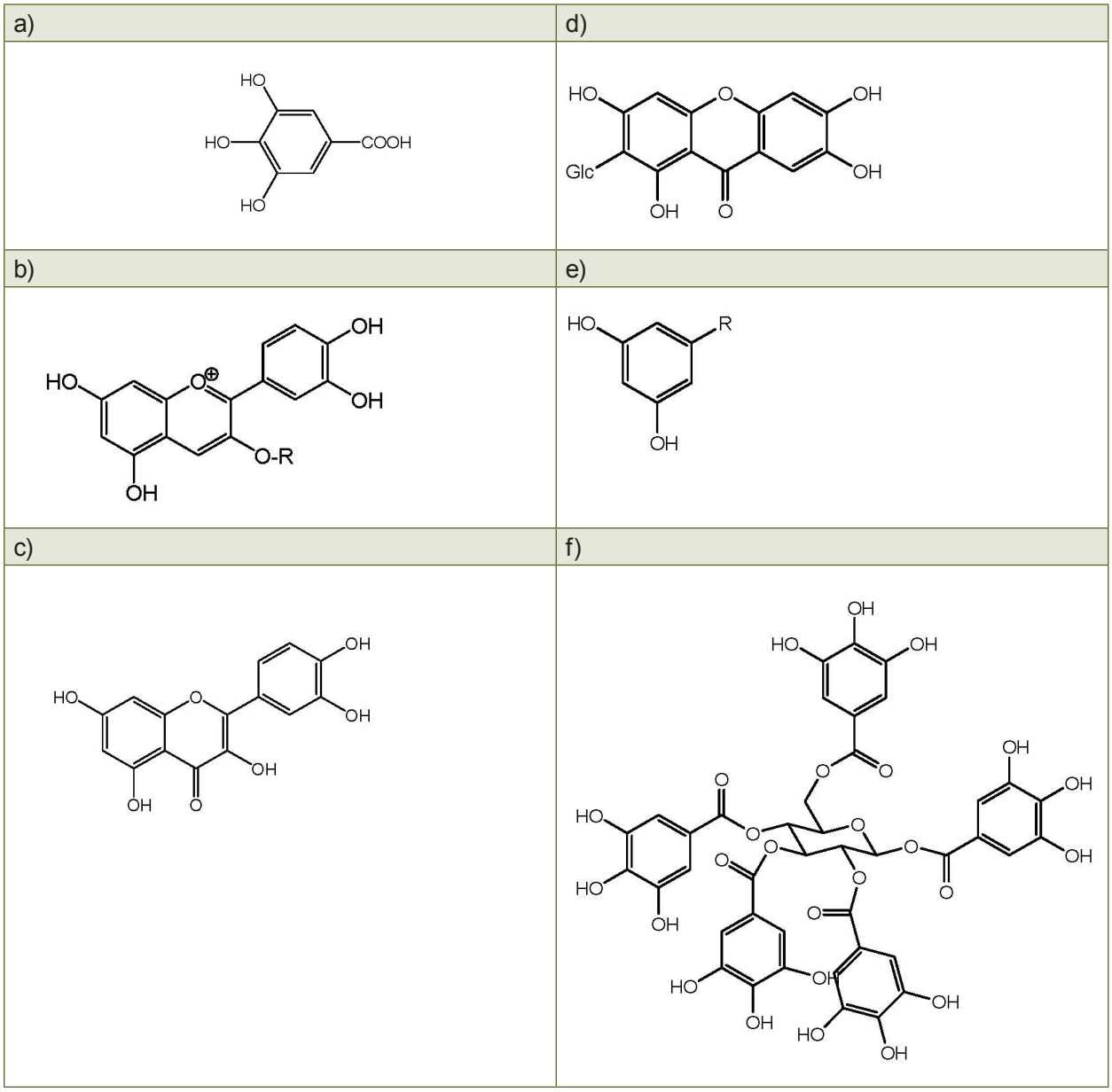

Figure 2: Structures of selected phenolic compounds. a) Gallic acid (a hydroxybenzoic acid), b) cyanidin (an anthocyanidin), c) quercetin (a flavonol), d) mangiferin (a xanthone), e) general structure of 5–alk(en)ylresorcinols, f) penta-*O*-galloylglucose (a hydrolyzable tannin).

Until the end of the 20th century, little was known about polyphenols in mango, which is remarkable in view of the popularity and economic importance of this fruit. Early reports date back to the late 1960s and early 1970s and were based on relatively simple analytical techniques such as paper chromatography and thin-layer chromatography. However, although these studies could not provide a complete structural elucidation, they proved to be extremely valuable for subsequent investigations because they revealed the presence of flavonols, xanthones, gallic acid derivatives and gallotannins, and other phenolic compounds in mango fruits, leaves, and bark.

The lack of information on the profile of mango polyphenols is surprising also for another reason. Extracts of various parts of the mango plant have been reported to possess bioactivities (e.g. antiviral, antibacterial, analgesic, anti-inflammatory, and immunomodulatory activities and have traditionally been used for the treatment of various ailments) (Barreto et al., 2008). Likewise, aqueous stem bark extracts referred to as 'Vimang' have been used in Cuba as a remedy for various diseases. Vimang displays strong anti-oxidant activity (Martínez et al., 2000) and has been demonstrated to contain mangiferin as the predominant polyphenol (Núñez Sellés et al., 2002). Considering these effects and the properties described for polyphenolics it becomes evident that mangoes can be regarded as a rich source of phenolic compounds, as very recently reviewed by Masibo and He (2008).

This contribution summarises our investigations during the past decade on the characterisation of polyphenols in mango fruits and by-products using sophisticated analytical methods, in particular high-performance liquid chromatography with diode array and mass spectrometric detection. We also report our very recent studies on the functional properties of phenolic compounds isolated from mango peels and seeds.

Characterisation of flavonoids and xanthones in mango peels

In view of the apparent lack of information on mango phenolic compounds, the focus of our investigations was the characterisation of the polyphenol fraction in mango fruits and products. Preliminary studies on a commercial mango purée concentrate revealed the presence of several quercetin (Q) glycosides such as Q-3-galactoside, Q-3-glucoside, Q-3-rhamnoside and Q-3-arabinoside, and of three kaempferol glycosides. The flavonol glycosides eluted in an 'organ pipe profile' that had so far not been described in mango. In addition to the flavonoids, a gallotannin and free gallic acid as well as mangiferin were detected (Schieber et al., 2000). Since no information on the raw material and technology used for the production of the purée concentrate was available, no conclusions could be drawn at that time as to the origin of the phenolic compounds found. However, it was reasonable to assume that the polyphenols at least in part originate from the peels because mango purée is produced both from peeled and unpeeled fruits and total polyphenols are higher in the peel than in the flesh at all stages of mango fruit development (Lakshminarayana et al., 1979).

In continuation of these studies and as an integral part of our efforts to develop processes for the utilisation of by-products of fruit and vegetable processing as a source of valuable compounds (Schieber et al., 2001), subsequent investigations were targeted towards the comprehensive characterisation and quantification of polyphenols in mango peels and kernels. In peel samples of the mango cultivar 'Tommy Atkins' a profile of flavonol glycosides was found which was very similar to that previously described in the commercial purée concentrate (Schieber et al., 2003). Therefore, this characteristic 'organ pipe profile' may even be used for authentication purposes. Furthermore, four xanthone *C*-glycosides (mangiferin, isomangiferin and their respective gallates) were identified based on their fragmentation in MS^n experiments.

In contrast to *O*-glycosides, *C*-glycosides do not generate abundant aglycone ions but show a loss of 120 amu caused by fragmentation of the sugar moiety. This study corroborated our assumption that the polyphenols found in the purée concentrate originated from the peels. In this study first evidence was also obtained for the presence of a rhamnetin glycoside, which had so far not been reported in mango peels. Rhamnetin can easily been distinguished from its isomer isorhamnetin by means of

mass spectrometry, as previously shown by Justesen (2001) and successfully applied by our group for the detection of isorhamnetin glycosides in several apple cultivars (Schieber et al., 2002).

To further evaluate the potential of mango peels as a source of valuable components, a screening of 14 economically important cultivars with respect to their polyphenol and pectin contents was performed (Berardini et al., 2005a). While total amounts of flavonol and xanthone glycosides of up to 4.8 g/kg were found in the peels, only minute levels were detected in the flesh, which provided final proof that phenolics found in mango purées originate from the peels. These investigations also confirmed the characteristic elution profile of flavonol glycosides reported in the above-mentioned studies.

The contents and degrees of esterification of pectins extracted from the lyophilised peels ranged from 12.2 to 21.2% and from 56.3 to 65.6%, respectively, suggesting mango peels also as a promising source of high-quality pectin. In order to confirm the identity of the flavonol previously characterised as a rhamnetin glycoside (Schieber et al., 2003), it was isolated by preparative and analytical HPLC, which revealed the presence even of two rhamnetin derivatives. Their structure was unambiguously elucidated using nuclear magnetic resonance (NMR) spectroscopy and established as rhamnetin 3-*O*-β-galactopyranoside and 3-*O*-β-glucopyranoside, respectively.

Interestingly, apart from the known cyanidin 3-*O*-galactoside, an unknown anthocyanin hexoside so far not reported in mango was found in red-colored cultivars. Subsequent investigations using NMR spectroscopy and mass spectrometry demonstrated the presence of a new anthocyanin methoxylated in position 7 of the flavonoid A-ring, 7-*O*-methylcyanidin 3-*O*-β-D-galactopyranoside (Berardini et al., 2005b). From a chemotaxonomical point of view it is interesting to note that in mangoes both 7-methoxylated flavonol glycosides and anthocyanins are found.

Phenolic lipids in mango peels

Phenolic lipids, sometimes also referred to as 'long-chain phenols', are amphiphilic compounds that have in common a non-isoprenoid side chain attached to a hydroxybenzene ring. In some plants of the Anacardiaceae family such as poison oak and poison ivy, the aromatic moiety of the phenolic lipids consists of a catechol. These so-called urushiols, or alkylcatechols, cause allergic dermatitis, which may affect humans of all races and skin color. The phenolic lipids present in mango belong to the cardol type, which is characterised by a resorcinol (*m*-1,3-dihydroxybenzene) ring with the side chain attached to the 5-position. These alkylresorcinols (ARs) have recently attracted intense interest due to their numerous biological activities (Kozubek and Tyman, 1999).

It is well known that mangoes may also cause allergic contact dermatitis, and the compound considered responsible for these reactions was identified as 5-2(*Z*)-heptadecenylresorcinol. Mango pickers are affected most, especially after contact with the sap after the fruits have been removed from the trees. More recent reports suggest that also contact with the flesh may lead to dermatitis (Weinstein et al., 2004).

In view of the interesting biological activities of ARs and because phytochemical investigations based on LC-MS were lacking, we conducted a study on the characterisation of ARs in the peels of mangoes of the cultivar 'Tommy Atkins'. For this purpose, a rapid and very efficient method for the purification of the peel extracts using solid-phase extraction on polyamide was developed. This method allowed the detection even of several minor compounds, the presence of which in mango had so far not been described (Knödler et al., 2007). Among the 15 compounds analysed, 3 major and 12 minor C_{15}-, C_{17}-, and C_{19}-substituted resorcinols and related components showing various degrees of unsaturation were characterised by their specific fragmentation patterns in collision-induced dissociation experiments.

This study marked the first report on the occurrence of mono-, di-, and tri-unsaturated C_{15}-homologues, saturated and triunsaturated C_{17}-homologues, and mono- and diunsaturated C_{19}-homologues in mango peels. The major ARs found in the peels were heptadecadienylresorcinol,

pentadecylresorcinol, and heptadecenylresorcinol. In addition to the above-mentioned minor ARs, several hydroxylated C_{15}- and C_{17}-homologues so far also not described in mango peels were detected. The methods for the extraction, purification and characterisation of ARs developed in this study provide the basis for more comprehensive investigations into the presence of these compounds in mango products and their stability during processing.

In continuation of the study by Knödler et al. (2007), we aimed at the characterisation of the anti-inflammatory properties of the ARs isolated from mango peels. Bioassay-guided extraction and fractionation of the peels revealed the presence of the novel 5-(11'*Z*-heptadecenyl)-resorcinol and the known (8'*Z*,11'*Z*-heptadecadienyl)-resorcinol previously not described in mango. The structures of both compounds were determined by extensive NMR and mass spectrometric studies, employing also GC-MS methods after derivatisation of the isolated monounsaturated compound with dimethyl disulfide to determine the position of the double bond. Both ARs exhibited potent cyclooxygenase (COX)-1 and COX-2 inhibitory activity.

The IC_{50} values ranged from 1.9 to 3.5 µM and from 3.5 to 4.4 µM and thus came close to those of reference drugs such as indomethacin and NS-398. The results obtained in this study also suggest that COX inhibitory activity increases with the degree of unsaturation in the AR side chain. Leukotriene formation catalysed by 5-lipoxygenase was only slightly inhibited (Knödler et al., 2008).

Hydrolysable tannins in mango peels and seeds

Hydrolysable tannins are characterised by a glucose core esterified with either gallic acid ('gallotannins') or ellagic acid ('ellagitannins'). The hydroxyl groups of gallic acid in gallotannins can be linked to gallic acid residues, resulting in compounds of varying substitution degrees. In mangoes, gallotannins have been found both in the peels, kernels, stem bark, leaves, and pulp, but information on their structures were rather limited. Using LC-MS, we detected 18 gallotannins in the peels, 21 in the kernels, and 8 in the pulp of mangoes of the cultivar 'Tommy Atkins'. Photometric quantification using the rhodanine assay revealed that the kernels were the most abundant source of gallotannins (15.5 mg/g dry matter), whereas only 1.4 mg/g and 0.2 mg/g were found in the peels and pulp, respectively (Berardini et al., 2004). Interestingly, the elution profile of the gallotannin fraction extracted from the kernels was very similar to that reported by Kabuki et al. (2000), who demonstrated that mango kernel extracts exhibit antibacterial activity. The inhibitory effects were more pronounced against gram-positive than gram-negative bacteria. The authors assumed that the active component was 'a type of polyphenol' but could not draw conclusions as to the exact chemical nature of the antimicrobial principles. The apparent similarity of the elution profile obtained in our study (Berardini et al., 2004) provided strong evidence that the antibacterial activity reported by Kabuki and colleagues (2000) was due to hydrolysable tannins. Our very recent studies confirmed that gallotannins isolated from mango kernels, especially penta-, hexa- and heptagalloylglucose, exhibit strong activity against various food spoiling bacteria, whereas the growth of the probiotic bacteria investigated was not inhibited.

In conclusion, our own investigations and the results reported by others demonstrate that mangoes are an immensely rich source of phenolic compounds, especially flavonol and xanthone glycosides, hydrolysable tannins, and phenolic lipids. These components possess interesting biological activities, such as antioxidative, antimicrobial and anti-inflammatory properties. Therefore, they are promising candidates for use as functional food ingredients and natural food additives and might even be considered as therapeutic agents in medicinal applications. As far as food science is concerned, studies on their safety and efficacy should be carried out prior to applying them as food ingredients. In this context, their stability and potential interactions with other food constituents such as proteins need to be addressed, especially since phenolic compounds may undergo oxidation to quinones, which are known to readily react with nucleophilic groups (e.g. thiol and amino functions).

From this review it also becomes evident that mango, a well known and economically important fruit, has so far been underestimated, and underexploited, with respect to its phytochemical potential.

Research into the profile of secondary plant metabolites of many other tropical fruits is still in its infancy. Advances in food and natural product chemistry and the fact that sophisticated analytical methods like LC-MS are nowadays used virtually as a matter of routine will greatly enhance our knowledge about the composition of tropical fruits. As most 'exotic' fruits are grown in the tropical and subtropical zones, collaboration with research institutions in these countries is strongly encouraged.

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Bioavailability and sensory evaluation

Prevention of obesity and type 2 diabetes associated with metabolic syndrome using some plant-based food factors

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Keywords: obesity, metabolic syndrome, adipocyte, anthocyanin, ginger

Abstract

Adipocyte dysfunction is strongly associated with the development of obesity and insulin resistance. It is accepted that the regulation of adipocytokine expression is one of the most important targets for preventing obesity and amelioration of insulin sensitivity. There are many kinds of fruits and vegetables cultivated in tropical areas, and these crops include various phytochemicals which could be useful for development of functional foods. In this review, we shows that anthocyanins and ginger-derived components have a potent anti-obesity and and anti-diabetic effect in a mouse model and adipocytes. Dietary cyanidin-3-glucoside (C3G) prevented obesity in mice. The downregulation of fat synthesis-related enzyme expression by C3G caused anti-obesity of C3G. C3G and cyanidin (Cy) significantly moudulated adipocytokine expression in human adipocytes using DNA micrroarray analysis. These anthocyanins upregulated adiponectin via a peroxisome proliferator-activated receptor γ (PPARγ) independent mechanism. Dietary C3G clearly ameliorated hyperglycemia and increased insulin sensitivity in diabetic mice. The anti-diabetic mechanism of dietary C3G in the type 2 diabetic model involves elevation of Glut4 expression, which contributes to the suppression of retinol binding protein 4 in white adipose tissue. This decreases glucose output into the blood and increases insulin sensitivity. Ginger is widely used as a spice and herbal medicine. The main ginger-derived components are 6-shogaol (6S) and 6-gingerol (6G). Both 6S and 6G significantly inhibited the tumor necrosis factor-α (TNF-α) mediated downregulation of the adiponectin expression in 3T3-L1 adipocytes. 6S functions as a PPARγ agonist with its inhibitory mechanism due to the PPARγ transactivation. 6G is not a PPARγ agonist, but it is an effective inhibitor of TNF-α induced c-Jun-NH_2-terminal kinase signaling activation and thus, its inhibitory mechanism is due to this inhibitory effect. These findings provide a biochemical basis for the use of tropical crops, which might have important implications in the prevention and treatment of metabolic syndorome via the improvement of adipocyte dysfunction.

Introduction

The 'metabolic syndrome' is characterised by a group of metabolic risk factors in one person. Obesity is increasing throughout the country, and abdominal obesity is one of the central causal components in metabolic syndrome. It is strongly associated with insulin resistance including hardening of the arteries and an increased risk for cardiovascular disease (Kahn and Jeffrey, 2000).

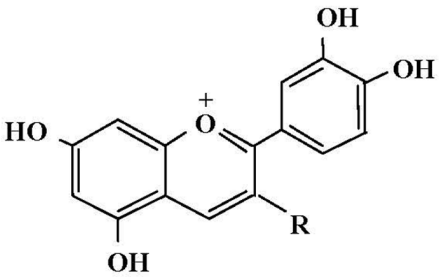
Adipocytes are the primary site of energy storage and accumulate triacylglycerol during nutritional excess. Adipocytes synthesise and secrete biologically active molecules called adipocytokines (Matsuzawa, 2005). Dysregulation of adipocytokine production is strongly associated with metabolic syndrome and amelioration of adipocyte dysfunction including adipocytokine expression is one of the important targets for prevention and therapies of metabolic syndrome.

There are many kinds of fruits and vegetables cultivated in tropical areas, and these crops include various phytochemicals which could be useful for development of functional foods. Food factors, including tropical fruits, are expected to be beneficial for prevention of adipocyte dysfunction.
In this paper, we review anti-obesity and anti-diabetic effects of anthocyanins, and amelioration of adipocyte function by ginger-derived components.

Results and discussion

Anthocyanin prevents obesity in mice.

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom. They are widely distributed in the human diet through crops, beans, fruits, vegetables and red wine (Harborne and Grayer, 1988), suggesting that we ingest significant amounts of anthocyanins from plant-based daily diets. In general, anthocyanin pigments are stable under acidic conditions, but are unstable and rapidly broken down under neutral conditions (Brouillard, 1988). Therefore, anthocyanins have not been recognised as a physiological functional food factor (Brouillard, 1988). However, we demonstrated that cyanidin-3-glucoside (C3G) (Figure 1), which is a typical anthocyanin, had anti-oxidative and anti-inflammatory activities based on *in vitro* and *in vivo* studies (Tsuda et al., 1998; Tsuda et al., 1999; Tsuda et al., 2002A; Tsuda et al., 2002B). These findings suggest that C3G has beneficial effects beyond its anti-oxidant activity.



R = -*o*-β-D-glucose; cyanidin-3-glucoside (C3G)
R = OH; cyanidin (Cy)

Figure 1. Chemical structure of cyanidin-3-glucoside (C3G) and cyanidin (Cy). This figure is reprinted from (Tsuda, 2008) with permission from the American Chemical Society.

Our study was designed to examine the preventive effect of anthocyanin-rich food on the development of obesity and hyperglycemia induced by feeding a high-fat (HF) diet. 'Purple corn color' (PCC) is made from purple corn (*Zea mays* L.). In Japan, about 50 000 kg per year of PCC (containing a large amount of C3G) is used for coloring foods, such as soft drinks, confections and other foods. We used this C3G-enriched food color for our experiments.

The body weight of the HF group was significantly higher than that of the control, PCC, and HF + PCC groups between the fifth to twelfth week of feeding. On the other hand, the body weight of the control and HF + PCC groups did not differ throughout the experimental period (Tsuda et al., 2003). The weights of all adipose tissues were significantly greater in the HF group than the control group. However, dietary PCC clearly suppressed HF-diet induced increases in tissue weight deposits (Figure 2). The data indicate that dietary PCC has a significant anti-obesity effect (Tsuda et al., 2003). The dietary PCC resulted in a significant decrease in the fat synthesis enzymes and transcription factors expression. This can potentially cause body fat and liver lipid accumulation (Tsuda et al., 2003).

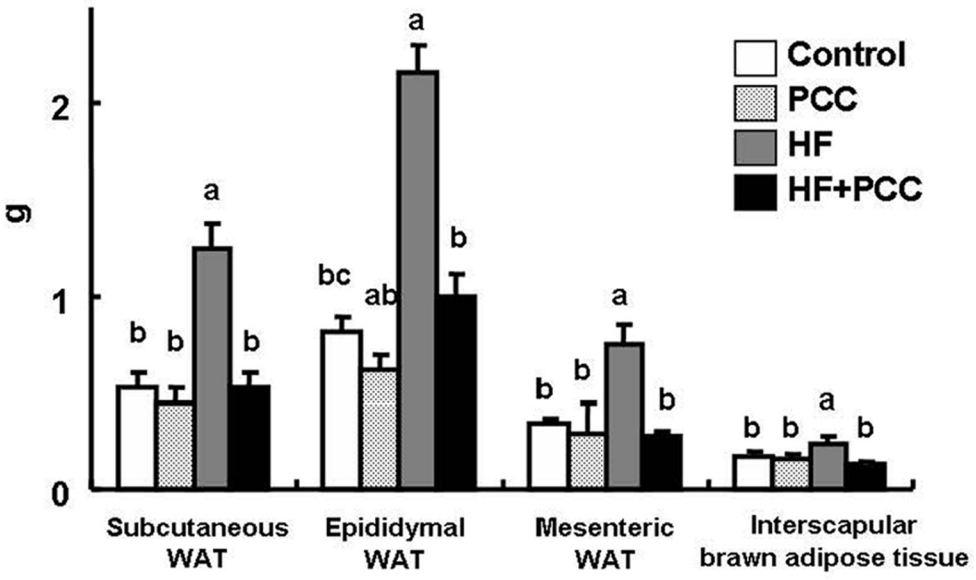


Figure 2. Adipose tissue weight of mice fed the control, PCC, HF or HF + PCC diets during 12-week period. Values are means ± SEM, n = 6. Means without a common letter differ, P < 0.05. This figure is reprinted from (Tsuda, T., 2008) with permission of American Chemical Society.

Regulation of adipocytokine gene expression by anthocyanins

DNA microarray technology will help us discover how anthocyanins regulate the genes responsible for the preventing of obesity and ameliorating insulin sensitivity. In this part, microarray profiling of gene expression in human adipocytes was carried out to determine the effect of anthocyanins.

Human adipocytes were treated with C3G or cyanidin (Cy) for 24 hours. Hierarchical clustering display of data based on the significant genes showed that the genes were grouped into nine clusters each containing from 19 to 234 genes (Tsuda et al., 2006). The array data identified the significantly up-regulated or down-regulated adipocytokines. Adiponectin, (one of the most important adipocytokines) was upregulated, and plasminogen activator inhibitor-1 (PAI-1) and IL-6 were downregulated (Tsuda et al., 2006). The gene expression level obtained using the quantitative real-time PCR was consistent with that obtained using microarray analysis (Tsuda et al., 2006).

Adiponectin and anthocyanin

Adiponectin is one of the most important adipocytokines, and is specifically and highly expressed in adipocytes. The plasma adiponectin concentration and mRNA expression level are decreased in the obese and insulin resistant states. Amelioration of adiponectin expression is one of the crucial targets for prevention and treatment of metabolic syndrome. The gene expression of adiponectin is regulated by peroxisome proliferator-activated receptor γ (PPARγ)) (Maeda et al., 2001, Iwaki et al., 2002). PPARγ is a ligand-activated transcription factor and a member of the nuclear hormone receptor superfamily that functions as a heterodimer with a retinoid X receptor. Agonists, such as thiazolidinediones, induce activation of PPARγ, which causes adipocyte differentiation and plays an important role in insulin sensitivity. Anthocyanins may act as PPARγ ligands resulting in increased adiponectin gene expression. To test this mechanism, the PPARγ ligand activity of anthocyanins was assayed. These anthocyanins did not induce luciferase activity even if their concentration was increased to 100 μM (Tsuda et al., 2004; Tsuda, 2008). These data indicate that anthocyanins induce the adiponectin gene expression without stimulating PPARγ ligand activity, i.e., induction is via a PPARγ independent mechanism.

Anthocyanins ameliorate hyperglycemia and increase insulin sensitivity via downregulation of retinol binding protein 4 expression.

Our prior studies showed that C3G influences adipocytokine expression and regulates those responsible for amelioration of insulin resistance in type 2 diabetes. We examined anti-diabetic effects using type 2 diabetic model (KK-A^y) mice (Sasaki et al., 2007).

The serum glucose concentration was significantly suppressed in the C3G group compared to that in the control group during weeks 3 and 5 (Figure 3a). The result of the insulin tolerance test clearly showed that dietary C3G ameliorates insulin resistance (Figure 3b).

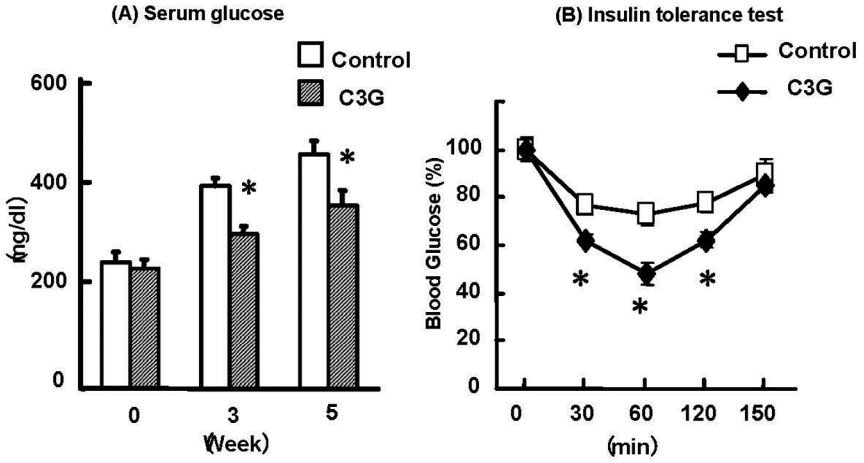


Figure 3. Serum glucose concentration (A) and Insulin tolerance test (B) in KK-A^y mice fed the control or C3G diet for 4 week. Values are the means ± S.E. (n = 6).

*Significantly different at $P < 0.05$ compared to the control in each period. This figure is reprinted from (Sasaki et al., 2007) with permission from Elsevier.

In our previous study, the administration of C3G was found to upregulate adiponectin expression in adipocytes (Tsuda, et al. 2006). We expected that the amelioration of hyperglycemia and insulin sensitivity would be due to upregulation of adiponectin. Contrary to our expectations, there was no significant difference in the gene expression and serum protein level of adiponectin between the groups. The gene expression level of the adiponectin receptors, AdipoR$_1$ and R$_2$, in both the skeletal muscle and liver was also not affected by dietary C3G.

Obesity is associated with macrophage infiltration into adipose tissue and the activation of inflammatory pathway caused with the development of insulin resistance (Weisberg et al., 2003; Xu et al., 2003). The gene expression level of tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) in the white adipose tissue was significantly decreased in the C3G group compared with the control group.

We have a discrepancy between our prior study using adipocytes and this study using type 2 diabetes model mice concerning adiponectin expression, suggesting that the anti-diabetic effect of C3G is through another mechanism. It has been observed that some polyphenols inhibit α-glucosidase activity. However, this effect of C3G and Cy occurred at an extremely low level, and the amelioration of insulin resistance by dietary C3G was not due to inhibition of α-glucosidase activity (Iwai et al., 2006; Matsui et al., 2001).

Dietary C3G modulates glucose transporter 4 (Glut4) and Retinol binding protein 4 (RBP4) expression.

Yang et al. showed that retinol binding protein 4 (RBP4) is an adipocytokine, and its expression and secretion in adipose tissue is closely associated with glucose uptake and insulin sensitivity (Yang et al., 2005). In the obese or diabetic state, the expression of the glucose transporter 4 (Glut4) is reduced in adipocytes, and the reduction is accompanied by an increase in RBP4 expression and secretion into the blood. This increase causes impairment of insulin signaling in the skeletal muscle and stimulates glucose production in the liver. These changes lead to a high glucose concentration

in the blood. Therefore, dysregulation of the adipocyte Glut4-RBP4 system is strongly associated with type 2 diabetes, and lowering RBP4 is a new important target molecule for the prevention and therapy of type 2 diabetes.

Recent studies also demonstrate association of RBP4 with insulin resistance and association of single nucleotide polymorphisms in the RBP4 gene with type 2 diabetes in human subjects (Cho et al., 2006; Craig et al., 2006; Gavi et al., 2007; Graham et al., 2006; Munkhtulga et al., 2007). However, some reports found no correlation of RBP4 level with obesity (Janke et al., 2006; Takashima et al., 2006).

In our study, the gene expression level of Glut4 in white adipose tissue was significantly higher in the C3G group. Also, protein expression was significantly enhanced in the C3G group in both whole cell lysate and plasma membrane. The gene expression level of RBP4 in white adipose tissue was significantly suppressed in the C3G group, but in the liver, it did not differ between groups. Serum RBP4 concentration was significantly reduced in the C3G group, indicating that this significant reduction is associated with down-regulation of RBP4 in white adipose tissue. Glucose-6-phosphatase is one of the rate-limiting gluconeogenic enzymes, and the gene expression level of glucose-6-phosphatase was significantly suppressed in the C3G group by the administration of C3G.

Anti-diabetic mechanism for C3G

The anti-diabetic mechanism of dietary C3G in the type 2 diabetic model involves elevation of Glut4 expression, which contributes to the suppression of RBP4 in white adipose tissue. This decreases glucose output into the blood and increases insulin sensitivity. A decrease in the MCP-1 expression mediated by C3G can contribute to inhibition of down-regulation of Glut4 expression (Sartipy and Loskutoff, 2003). Another possible mechanism for regulation of the Glut4 expression or translocation to the plasma membrane is related to the PPARγ or AMP activated protein kinase (AMPK) (Yamaguchi et al., 2005). Interestingly, although the data are not shown, AMPK activation has been induced by administration of C3G or Cy in adipocytes (Tsuda et al., 2004, Tsuda 2008). C3G may stimulate AMPK activation and enhance Glut4 expression in adipose tissue (Sasaki et al., 2007) (Figure 4).

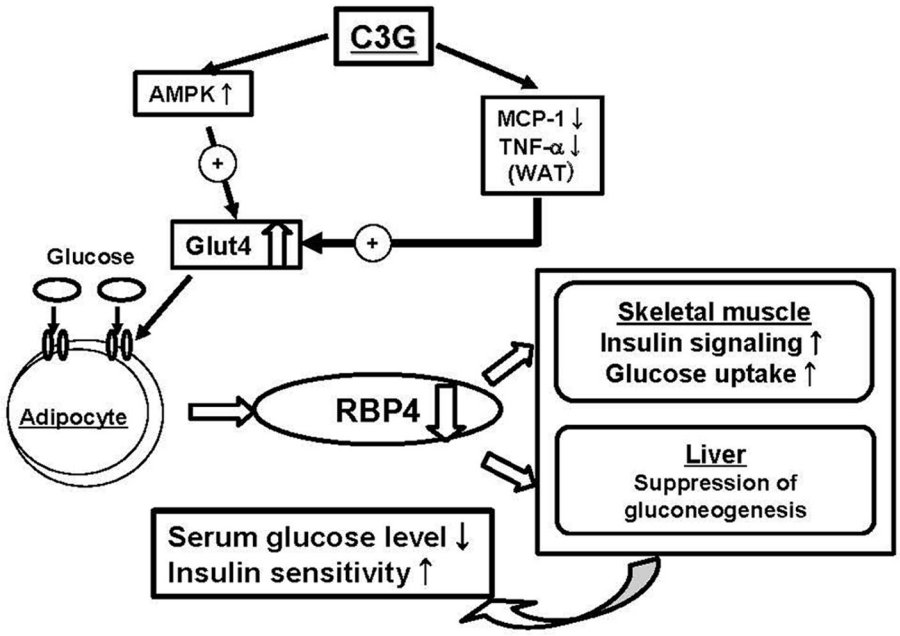


Figure 4. Proposed scheme for amelioration of hyperglycemia and insulin sensitivity by the C3G. This figure is reprinted from (Sasaki et al., 2007) with permission from Elsevier.

Ginger-derived components inhibit downregulation of adiponectin expression via different mechanisms in adipocytes. Inflammatory molecules including TNF-α) and MCP-1 are expressed and upregulated in the adipose tissue of the obese state and in type 2 diabetes (Hotamisligil et al., 1993, Kamei et al., 2006, Kanda et al., 2006). These inflammatory adipocytokines induce the down-regulation of adiponectin (Suganami et al., 2005, 2007). Therefore, anti-inflammatory compounds may ameliorate adipocyte dysfunction associated with metabolic syndrome by inhibiting the down-regulation of adiponectin expression.

Ginger, which is the rhizome of the plant Zingiber officinale Roscoe, is widely used as a spice and herbal medicine. The main ginger-derived components are gingerol and shogaol (Figure 5a). Ginger is not a fruit, but there are many types of ginger cultivated in tropical areas. In this part, we demonstrate that both of the ginger-derived components (6-shogaol; 6S, and 6-gingerol; 6G) can significantly inhibit the down-regulation of adiponectin expression that is induced by TNF-α in 3T3-L1 adipocytes. With regard to the molecular action and mechanism, the present study clarifies that both 6S and 6G function by inhibiting the down-regulation of adiponectin, although they do so via different molecular mechanisms (Isa et al., 2008).

6S and 6G inhibit downregulation of adiponectin.

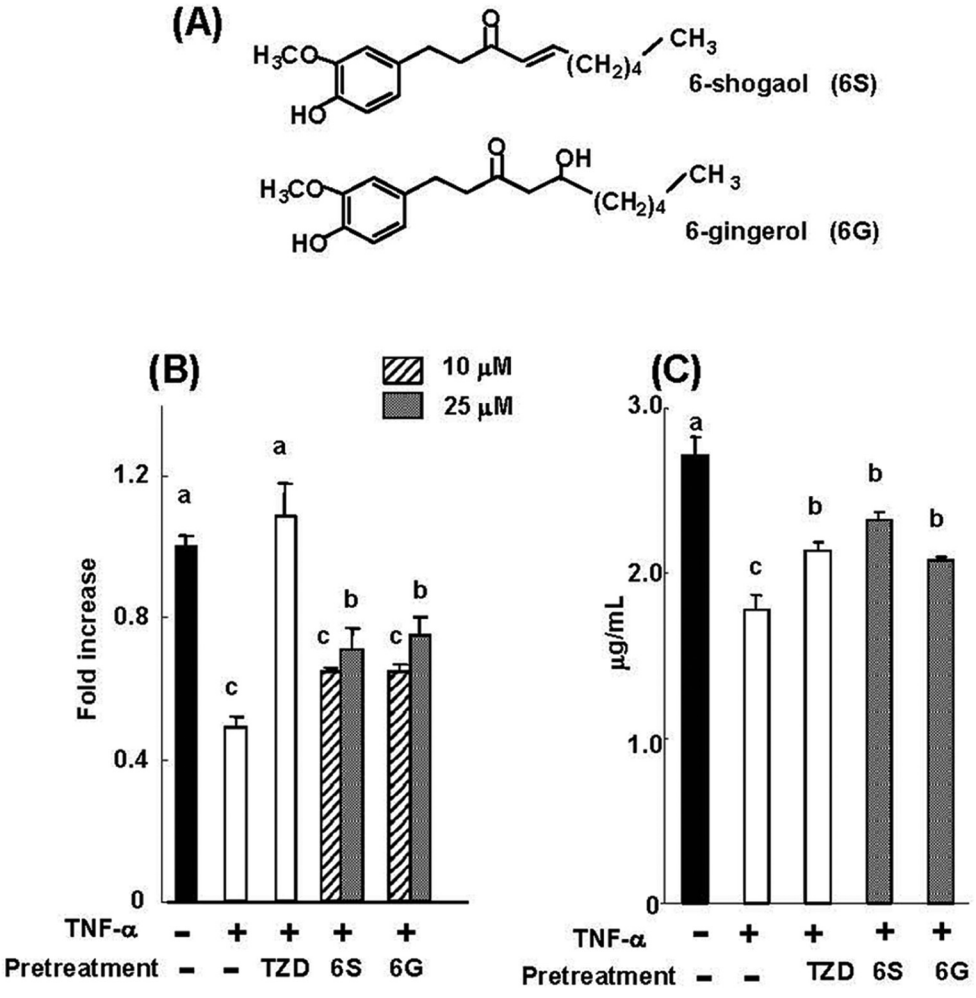


Figure 5. (A) Chemical structure of the ginger-derived components. (B, C) Inhibitory effect of ginger-derived components on the TNF-α mediated down-regulation of adiponectin (B) and the secreted adiponectin concentration in media (C) in 3T3-L1 adipocytes. Values are means ± SEM, n = 3–6. Values without a common letter are significantly different at $p < 0.05$. This figure is reprinted from (Isa et al., 2008) with permission from Elsevier.

TNF-α mediated down-regulation of adiponectin was significantly inhibited by pretreatment with 6S and 6G (Figure 5b). Zingerone, which has no carbon side chains, 10-shogaol and 10-gingerol, which

have longer alkyl carbon chains than 6S and 6G had no significant effects. The TNF-α mediated decrease in the secreted adiponectin in media was also inhibited by 6S and 6G pretreatment (Figure 5c).

6S increases the PPARγ transcriptional activity

As one of the possible mechanisms for the inhibition of TNF-α induced down-regulation of adiponectin, we examined whether 6S and 6G were able to increase PPARγ transcriptional activity. 6S is a significant agonist for PPARγ compared to the control. However, there was no significant activation of PPARγ observed after the administration of 6G (Figure 6). The administration of 6S resulted in a significant up-regulation of adiponectin and aP2 expression (Figures 7a and b). Pretreatment with a specific PPARγ antagonist (GW9662) significantly diminished the 6S-induced up-regulation of adiponectin and aP2, with both of the gene expression levels for the antagonist and the 6S treatment group found to be the same as that seen for the control. These results indicate that the administration of 6S inhibited the TNF-α induced down-regulation of adiponectin by increasing PPARγ transcriptional activity. However, the mechanism for the inhibitory effect by 6G is different.

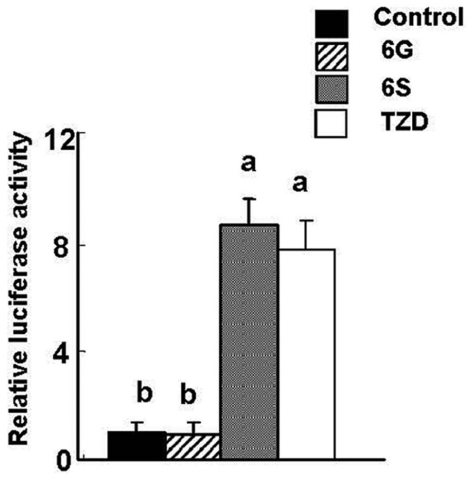


Figure 6. The PPARγ transcriptional activity for 6G, 6S and a positive control (TZD). Values are means ± SEM, n = 4. Values without a common letter are significantly different at $p < 0.05$. This figure is reprinted from (Isa et al., 2008) with permission from Elsevier.

6G modulates MAPKinase (MAPK) signaling

The other possible mechanism by which 6G can inhibit the TNF-α mediated down-regulation of adiponectin is by modulating the MAPK signaling pathway that is activated by the stimulation of TNF-α, and which inhibits suppression of adiponectin expression. A recent report has demonstrated that the TNF-α mediated adiponectin mRNA suppression was inhibited by pretreatment with a specific c-Jun-NH2-terminal kinase (JNK) inhibitor (SP600125), while inhibitors of other MAPKs had no effect (Kim et al., 2005). We also confirmed that various MAPK inhibitors can modulate the TNF-α mediated down-regulation of adiponectin. Our data indicated that the TNF-α mediated down-regulation of adiponectin was inhibited only by pretreatment with a JNK inhibitor (Figure 7c). Thus, inhibition of TNF-α mediated JNK activation might be a potential target for attenuating the down-regulation of adiponectin, and 6G inhibits the down-regulation of adiponectin via this mechanism. Therefore, we evaluated the effect of 6G pretreatment on TNF-α induced JNK activation. 6G significantly inhibited the phosphorylation of JNK (Figures 8a and b). In addition, 6G pretreatment also significantly inhibited the activation of SEK1/MKK4, which is one of the upstream kinases of JNK (Figure 8c). The TNF-α mediated activation of the JNK signaling pathway is accompanied by an increase in the phosphorylation of transcription factors, such as ATF-2 or c-Jun. ATF-2 and c-Jun were activated by the treatment of TNF-α, however, these activations were also inhibited by the pre-administration of 6G (Figure 8d). Until now, it is unknown whether the activation of these

transcription factors is involved with down-regulation of adiponectin, however, we showed that the inhibition of the JNK signaling pathway by the treatment of 6G is accompanied by a decrease in the TNF-α mediated activation of these transcriptional factors.

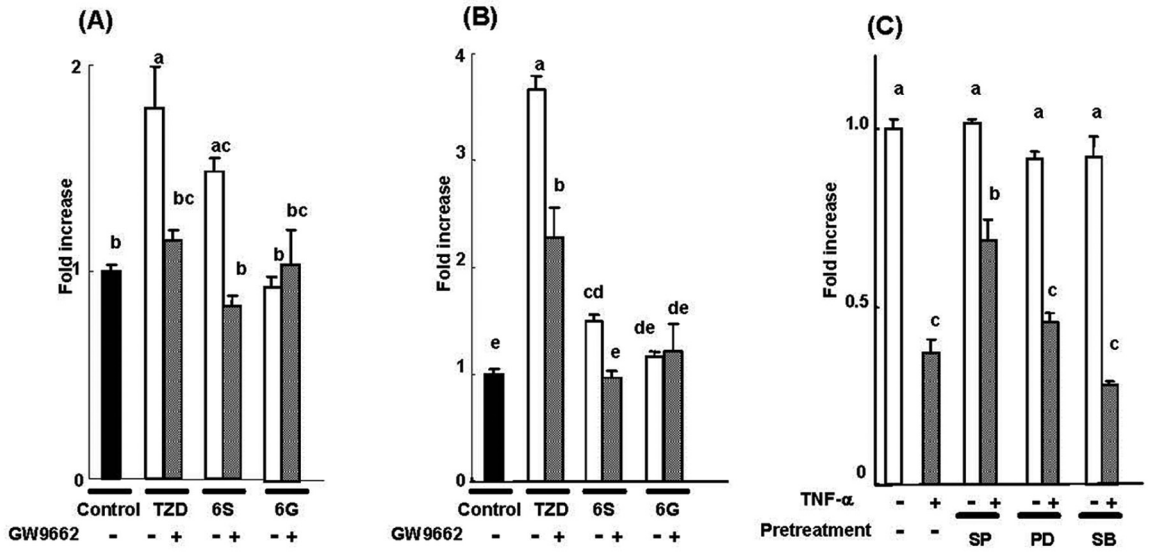


Figure 7. (A); (B) The gene expression level of adiponectin (A) and aP2 (B) in 3T3-L1 adipocytes treated with 6S, 6G (25 μM) or TZD (10 μM) with or without the PPARγ antagonist GW9662 (20 μM). (C) The effect of various MAPK inhibitors (SP600125; 20 μM, PD98059; 10 μM, SB203580; 5 μM) on the TNF-α mediated down-regulation of adiponectin in 3T3-L1 adipocytes. Values are means ± SEM, n = 3–6. Values without a common letter are significantly different at $p < 0.05$. This figure is reprinted from (Isa et al., 2008) with permission from Elsevier.

It is also possible that 6S may modulate the JNK signaling pathway and similar to 6G, inhibit the down-regulation of adiponectin. Interestingly, 6S pretreatment did not inhibit TNF-α-induced JNK activation in adipocytes (Figure 8e).

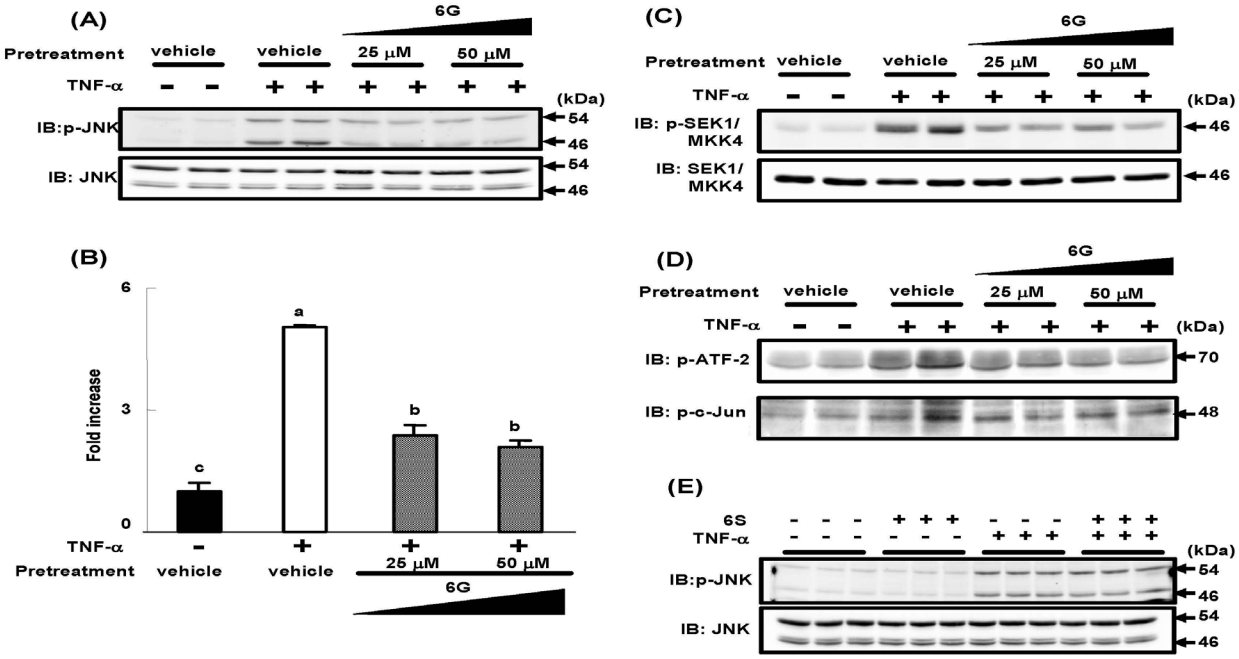


Figure 8. Immunoblot analysis of the various JNK signaling proteins in 3T3-L1 adipocytes pretreated with 6G (A, B, C, D) or 6S (E) before treatment with TNF-α. (B), The quantification of the phospho-JNK protein intensity was expressed relative to the control (= 1.0) after normalisation using the total JNK protein intensity. Values are means ± SEM, n = 4. Values without a common letter are significantly different at $p < 0.05$. This figure is reprinted from (Isa et al., 2008) with permission from Elsevier.

These results indicate that: 1) the main mechanism for inhibiting TNF-α mediated down-regulation of adiponectin by 6G is due to inhibition of the JNK signaling pathway, and 2) 6S also significantly inhibits the down-regulation of adiponectin, does not inhibit the JNK signaling pathway, but instead acts as a functional PPARγ agonist (Isa et al., 2008).

Conclusion

These studies indicate plant-based food factors in tropical fruits and vegetables have a unique potential that can be used to prevent or treat the metabolic syndrome. Anthocyanins are effective anti-obese and anti-diabetic food factors via improvement of metabolic changes or adipocyte function. Ginger-derived components (6S and 6G) can significantly inhibit TNF-α mediated downregulation of adiponectin in adipocytes, although they do so via different mechanisms. These findings provide a biochemical basis for the use of tropical crop-derived food factors, which might have important implications for the prevention of diabetes via the improvement of adipocyte dysfunction. We need to develop functional foods from tropical crops and contribute to human health.

Acknowledgments

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Fruit phytochemicals: the relationship between molecular structure, bioavailability and human health

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Keywords: bioavailability; phytochemicals; anthocyanins; carotenoids; ellagitannins; flavonoids

Abstract

Plant foods, fruits and vegetables, contain many different types of phytochemicals that are believed to provide health benefits for consumers. Fruit phytochemicals are grouped together by molecular structure into chemical classes such as carotenoids, glucosinolates, terpenoids, and phenolics (phenolic acids, flavonoids, stilbenes and lignans) etc. The flavonoid group is further divided into flavonols, flavones, flavanones, and anthocyanins. Many fruit phytochemicals have potent bioactivity due to their molecular structure; however, for health benefits to occur, these compounds (or related metabolites) must have a degree of bioavailability and be able to interact with the fundamental biochemistry of complex organisms such as animals and humans. The degree of bioavailability for each phytochemical is determined by the specific chemical properties and these vary both between phytochemical classes and between compounds within each class. We will discuss the relationships between molecular structure and bioavailability and the implications for possible health benefits. Variations in bioavailability among phytochemicals will be discussed and contrasted, using anthocyanins as a focus. The chemistry of anthocyanins is complex and despite substantial claims, the health benefits have been questioned because of reported low levels of bioavailability. In most cases we have a very limited understanding of phytochemical bioavailability, but the currently available information shows that there are large variations between compounds, suggesting that maximising the consumption of a variety of different phytochemicals with complementary chemistries will help to maximise the health benefits of fruits.

Introduction

Fruit and vegetable consumption and health

There is robust evidence that increased consumption of fruits and vegetables promotes good health and provides protection against degenerative diseases, such as cardiovascular disease (CVD), some cancers, and the onset of dementia (Joshipura, et al., 2001; Knekt, et al., 2002; Ness and Powles, 1997; Steinmetz and Potter, 1996; Youdim and Joseph, 2001). This has led to the recommendation that individuals consume more than 400 g (or 5 servings) of fruit and vegetables per day (Anon, 2003).

Fruits and vegetables contain a diverse range of phytochemicals that may act individually or in concert to produce disease protective effects. Some of the phytochemicals thought to be associated with health benefits are carotenoids, polyphenolics, other anti-oxidants, vitamins, folate, calcium, selenium, potassium, iron and dietary fibre. Even though it has long been recognised that increased fruit and vegetable consumption reduces the risk of chronic disease, it is not known definitively which component (or components) of fruits and vegetables are the main contributors to the health effect. Despite substantial *in vitro* evidence for health benefit effects for a range of phytochemicals contained in fruits and vegetables, it has often been difficult to demonstrate health benefits in human studies.

The role of bioavailability for the expression of health benefits

For a phytochemical to exert a beneficial effect on an organism, a degree of bioavailability must exist. Bioavailability is defined in various ways, for example for nutrients where the route of administration is nearly always oral, the term 'bioavailability' describes the quantity or fraction of the ingested dose that is absorbed (Heaney, 2001). The most usual definition of bioavailability is the proportion of the compound that is digested, absorbed and metabolised through normal pathways and is often just characterised as plasma concentration (Mason, 2000) or urinary excretion. However, the term 'bioavailability' can also be defined as the ability of a phytochemical to interact with an organism (cells, tissue or organ) to elicit a biological response. This means an understanding of the bioavailability processes leading to the health benefit is important for organs distant from the gastrointestinal tract (GIT), as well as for organs and tissues which are part of the GIT.

Selected phytochemicals

The phytochemicals present in fruits and vegetables are classified according to chemical structure. For the purposes of this discussion four compounds, from different chemical classes with different molecular structures will be considered. These are as follows (see Figure 1 for chemical structures).

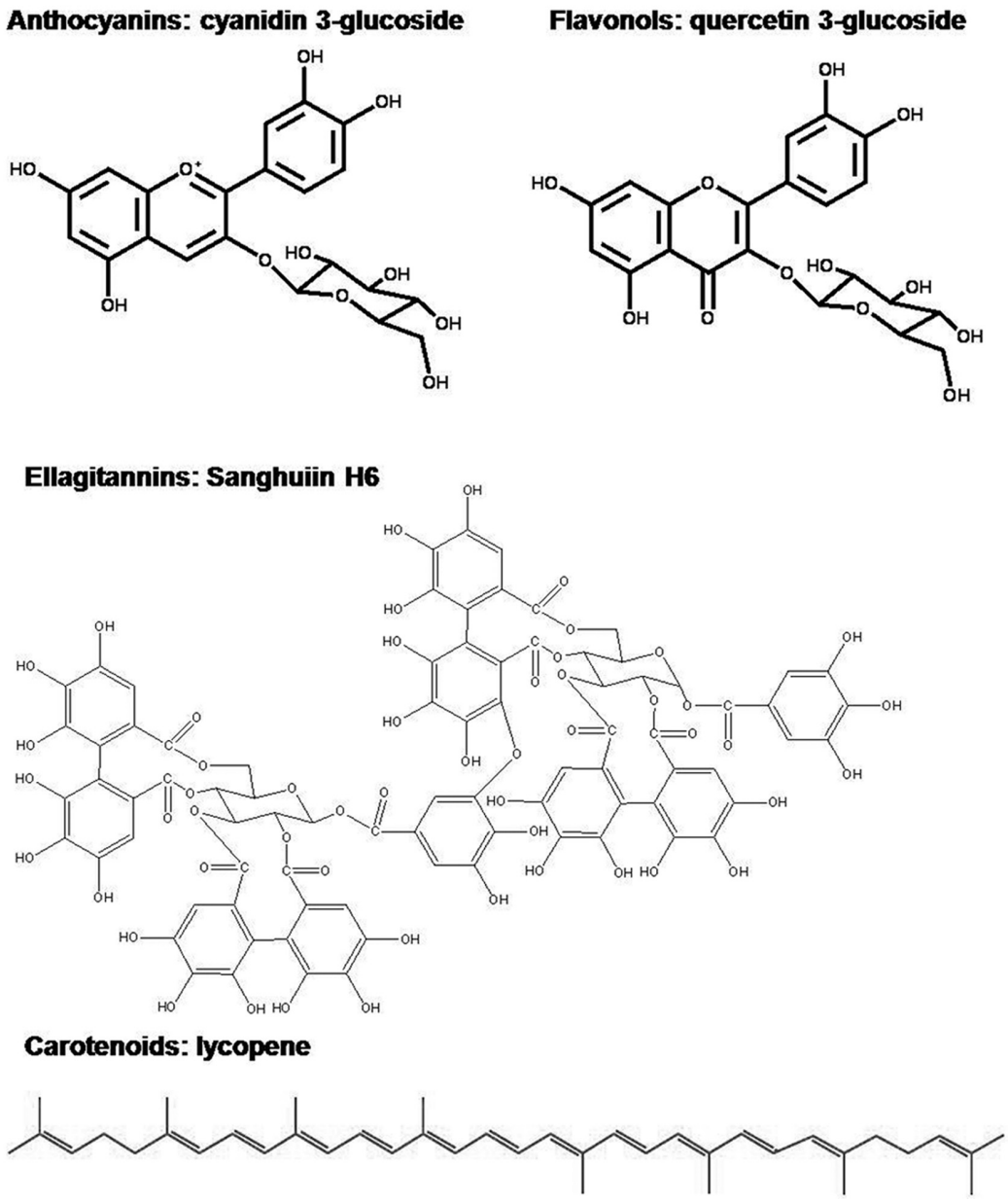

Figure 1. The structures of phytochemicals discussed.

Cyanidin 3-glucoside (anthocyanin): Anthocyanins contain the characteristic C6-C3-C6 ring structure of all flavonoids. They exist almost exclusively in nature as glycosides with six major variations of the phenolic (aglycone) moiety. Anthocyanins are water soluble and can adopt a number of structural conformations depending on pH. They are most stable at acidic pH and less stable at neutral pH. Anthocyanins are highly coloured compounds and provide many of the orange, red and blue colours present in plants.

Quercetin 3-glucoside (flavonol): Flavonols are also a sub-class of the flavonoids and differ from anthocyanins by having a higher oxidation status of the C3 ring. Flavonols are polyphenolic, water-soluble compounds, and have (especially quercetin) long been associated with cancer chemoprevention properties.

Sanghuiin H6 (ellagitannin): Ellagitannins are high-molecular weight, water-soluble polyphenolic compounds that are classified as hydrolysable tannins because of their ability to bind to and precipitate protein, which imparts an astringent taste to ellagitannin-containing foods. Ellagitannins are polymers of hexahydroxydiphenic acid (HHDP), gallic acid and a polyol, either glucose or quinic acid (Cerda et al., 2005; Clifford and Scalbert, 2000; Gupta, et al., 1982; Hager et al., 2008; Koponen et al., 2007; Vrhovsek et al., 2006). They have limited distribution and occur in plants foods such as pomegranates, strawberries, raspberries, blackberries, muscadine grapes, persimmons, walnuts and hazelnuts, and in oak-aged wines (Cerda et al., 2005; Clifford and Scalbert, 2000; Koponen et al., 2007; Wada and Ou, 2002).

Lycopene (carotenoid): Carotenoids are fat-soluble compounds that are derived from the isoprenoid biosynthetic pathway. They are highly coloured because of conjugation of the multiple double bonds along the hydrocarbon chain. Numerous molecular variations occur, all containing a central hydrocarbon backbone with various cyclised end groups. Some carotenoids can be converted to vitamin A *in vivo* and therefore have pro-vitamin activity.

The molecular structure and chemical properties of phytochemicals vary by chemical class and among individual compounds within each class. This diversity of structure is responsible not only for the differing biological activity, but also for biochemical properties such as bioavailability. In this paper the diversity in bioavailability will be discussed by considering firstly the known features of anthocyanin bioavailability and then further comparison with three phytochemicals from the different chemical classes described above.

Materials and methods

Phytochemical analysis

Phytochemical components were measured in various fruits according to the method described by Connor and colleagues (Connor, et al., 2005). Briefly this involved extraction of fresh (or frozen) fruit with ethanol/water/formic acid (80:20:1) and analysis by reversed-phase high performance liquid chromatography (RP-HPLC). For most of the samples, separation was achieved using a Synergi 4µ Hydro 250 × 4.6 mm column (Phenomenex, Auckland NZ) with a binary gradient using acetonitrile and 5% formic acid in water. Components were detected using a photodiode array detector set at 280, 310, 370 or 520 nm. Component concentrations were calculated by reference to external calibration curves of authentic standards. When standards were not available, concentrations were calculated with reference to the calibration of an appropriate standard as equivalents. For example, all anthocyanin concentrations were calculated as cyanidin 3-glucoside (Extrasynthese, France) equivalents.

Bioavailability studies

In vitro bioabsorption studies using Ussing chambers were carried out as described previously (Matuschek et al., 2006; Walton et al., 2006b). Bioavailability studies with blackcurrant anthocyanins in rat and pig were performed as described (Walton et al., 2008; Walton et al., 2006a). Human bioavailability and metabolism studies with berryfruit anthocyanins were preformed as described (Cooney et al., 2004; McGhie et al., 2003).

Results and discussion

Health benefits of anthocyanins

Anthocyanin compounds are commonly consumed because they occur in a wide variety of foods and often at significant concentrations. Initially consumption was estimated to be as high as 180–255 mg/d in the United States (Kuhnau 1976); which is much higher than most other flavonoids. More recent studies have reported the average anthocyanin consumption to be in the order of 82 mg/d in Finland and 12.5 mg/d in the United States (Wu, et al., 2006). However, some fruit, in particular berryfruit, are very rich sources of dietary anthocyanins (Siriwoharn et al., 2004; Wada and Ou, 2002; Wu et al., 2004a; Wu and Prior, 2005) and can contribute 10s to 100s of mg of anthocyanins in a single serving.

The biological activities of anthocyanins have been recently reviewed (Galvano, et al., 2004; Kong, et al., 2003) and besides anti-oxidant capacity (Matsumoto, et al., 2002; Stintzing, et al., 2002; Wang, et al., 1997), anthocyanins have a number of therapeutic properties. These include cancer chemoprevention (Chen et al., 2006; Hou, 2003; Zhang et al., 2005), anti-inflammatory effects (Kelley et al., 2006; Tall et al., 2004; Wang et al., 1999), vasoprotective effects (Matsumoto et al., 2004; Shin et al., 2006), and benefits to vision (Lee et al., 2005; Matsumoto et al., 2005; Matsumoto et al., 2003; Nakaishi et al., 2000). Recently, epidemiological studies have reported associations between anthocyanin consumption and cardiovascular disease (Mink et al., 2007).

Anthocyanin bioavailability

Numerous studies have been published on the bioavailability of anthocyanins and the results have been reviewed (McGhie and Walton, 2007). Most studies have utilised animals or humans, and measured bioavailability as concentrations of anthocyanins appearing either in plasma or urine. It has been consistently reported that the original anthocyanins ingested appear in plasma and are excreted in urine, indicating that one pathway for absorption does not involve metabolism. However, metabolism of anthocyanins does occur, with glucuronide and methyl conjugates being reported (Cooney et al., 2004).

It appears that the degree of absorption and type of metabolism differ between anthocyanins depending on both the glycoside moiety and the nature of the phenolic aglycone (Ichiyanagi et al., 2006; McGhie et al., 2003; Wu et al., 2004b). Bioavailability based on plasma concentrations and amounts excreted in urine are consistently reported to be low, at < 0.1% of the ingested dose and approximately ten-fold less than other flavonoids (Manach et al., 2005). However, distribution within plasma (e.g. plasma protein binding) and to tissues and organs has not been investigated in depth, so it is not yet clear if this apparent low level of bioavailability is real, or if other factors are involved.

Our group has used Ussing chambers and animals as experimental systems to explore the dynamics of anthocyanin bioabsorption. Using Ussing chambers, it appears that absorption varies between tissues of the mouse GIT and is higher in the jejunum than in the ileum or colon, although stomach tissue was not evaluated in this study (Matuschek et al., 2006). Furthermore, using rats we showed that the amount of anthocyanin present in GIT tissue relative to the corresponding luminal contents was highest for the jejunum (Walton, et al., 2008). Both these results suggest that there is a specific active transport mechanism for anthocyanins present in the jejunum but absent in other areas of the GIT.

We and others have examined the impact of food components on anthocyanin bioavailability and shown that a viscous food matrix such as oat or wheat meal appears to have little overall effect on the total amount of anthocyanins absorbed, but increases the time to maximum concentration (C_{max}) (Walton et al., 2008; Walton et al., 2006a).

General view of anthocyanin bioavailability

Figure 2 is a representation of the bioavailability of anthocyanins based on our current understanding. Anthocyanin glycosides can be rapidly absorbed from the stomach after ingestion by a process that may involve bilitranslocase, and they enter the systemic circulation after passing through the liver. A portion of the anthocyanin is metabolised by methylation and glucuronidation reactions, and some of the metabolites are transported to the intestine in the bile. Anthocyanin glycosides that are not absorbed from the stomach move into the small intestine where, because of the higher pH, they convert to a combination of hemiketal, chalcone, and quinonoidal forms. Further absorption appears to take place in the jejunum. The transport mechanism involved has not been identified, but if similar to flavonols, may involve hydrolysis of the glycosides by various hydrolases and absorption of the phenolic aglycone. Absorbed anthocyanins enter the systemic circulation after passage though the liver and may be metabolised. Anthocyanins that reach the colon are exposed to a substantial microbial population and may be degraded to sugar and phenolic components, with the phenolic components further degraded by disruption of the C-ring, to yield phenolic acids and aldehydes. These products, derived from the ingested anthocyanins, may contribute to the health effect of anthocyanins either directly in the GIT or after absorption from the colon.

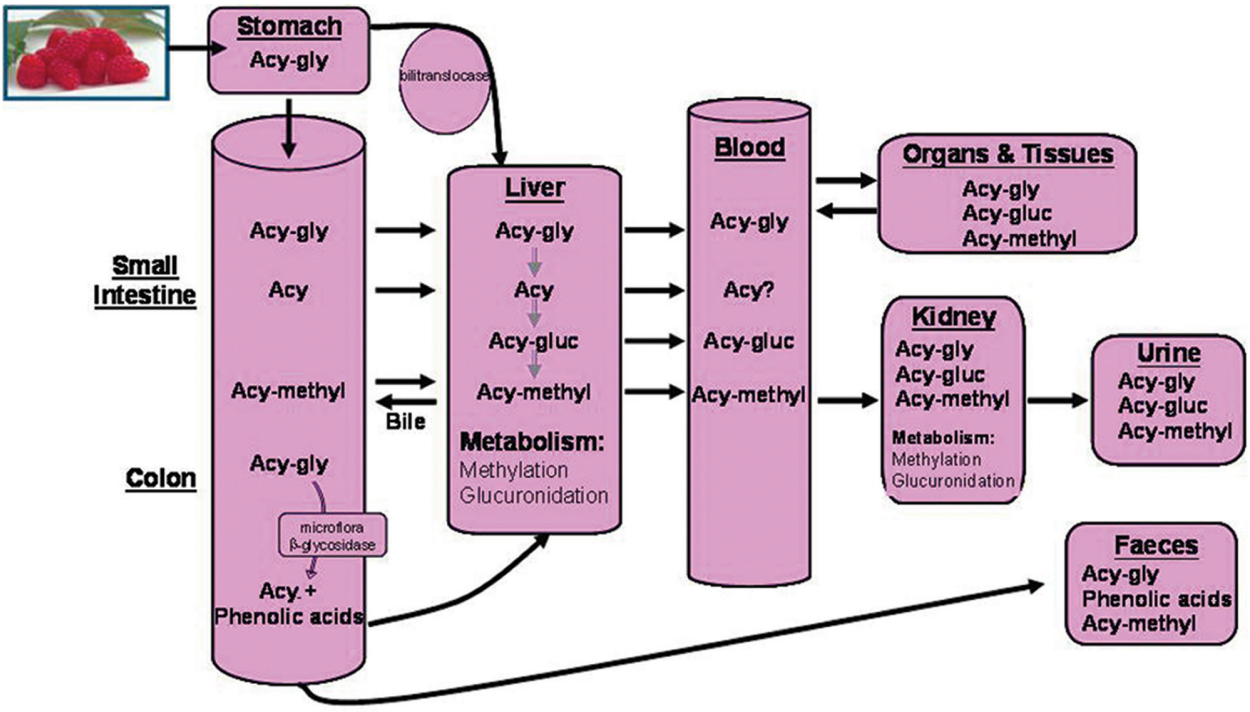

Figure 2. The pathways of absorption, metabolism and excretion for anthocyanins.

Much of the detail is missing about how anthocyanins are absorbed, and how the variation of molecular structures consumed in food, and the forms generated *in vivo* contribute to the health benefits.

Comparison with other phytochemicals

As comparatively little is known about the tissue distribution of most phytochemicals, the comparison of bioavailability for anthocyanins, flavonols, ellagitannins and carotenoids is based on the processes in the GIT that lead to appearance of the phytochemicals in the circulatory blood system. The essential elements of the bioabsorption of flavonols, ellagitannins, and carotenoids are summarised in Figure 3 and discussed below.

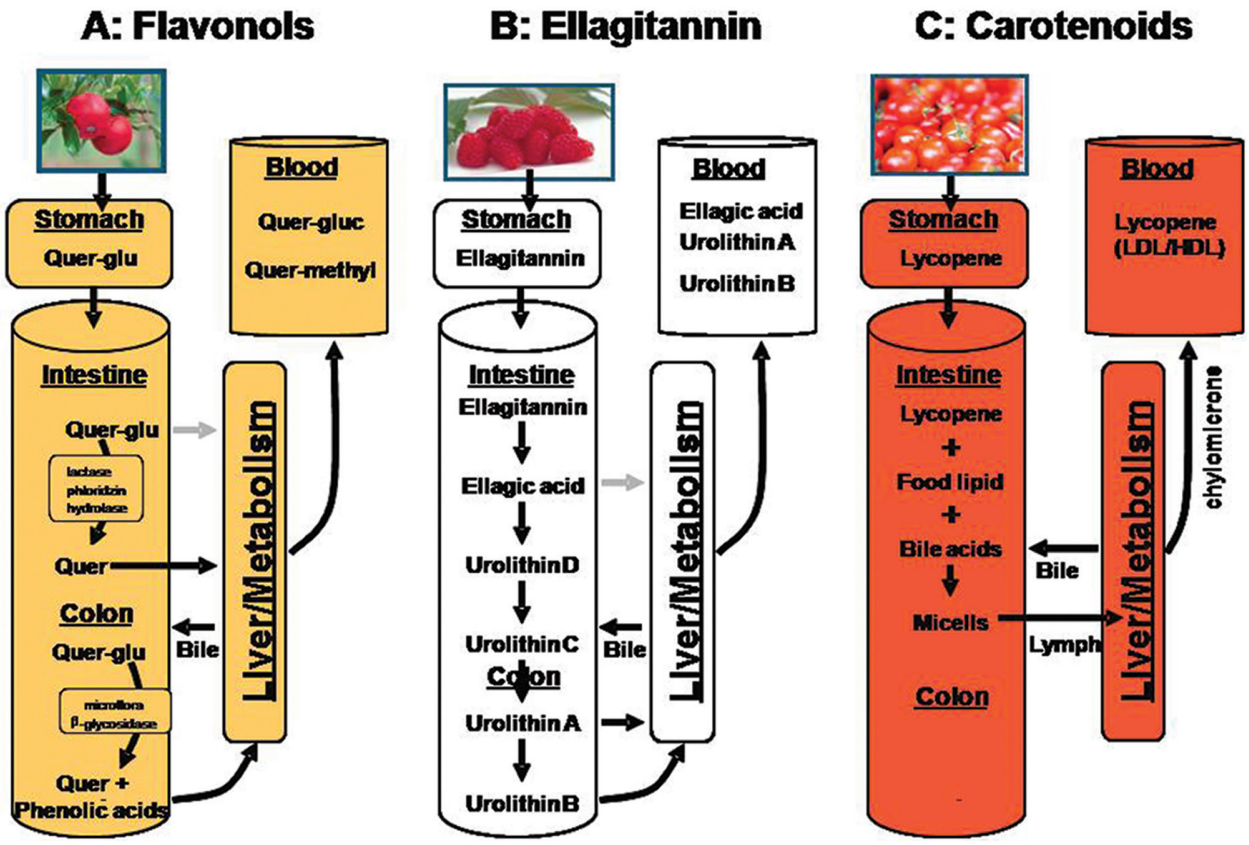

Figure 3. Diagram showing the differences in absorption and metabolism for three phytochemicals with different molecular structures from the gastrointestinal tract.

Quercetin 3-glucoside (flavonol): Originally it was thought that quercetin glucosides could be directly transported into enterocytes by sodium-dependent glucose transporters. While this may still occur to some degree, it now appears that the major route for the absorption of quercetin glycosides starts with deglycosylation by lactase phloridzin hydrolase (LPH) in the lumen or by β-glucosidases in the enterocyte, followed by metabolism of the aglycone to generate quercetin glucuronide and sulphate conjugates. The quercetin metabolites are then transported by the portal vein to the liver and then into systemic circulation. Unlike anthocyanins, the original quercetin glycosides consumed do not appear in the blood. The degree of bioavailability is determined by the deglycosylase activity. For example, quercetin rutinoside (rutin), is only slowly deglycosylated and has much lower bioavailability than the quercetin glucosides, which are readily deglycosylated by LPH.

Sanghuiin H6 (ellagitannin): The majority of studies have shown that ellagitannins are not absorbed directly from the GIT, presumably because of high polarity and large molecular size. However, ellagitannins are chemically degraded in the stomach and small intestine to ellagic acid and a portion of this is absorbed and can be detected in both plasma and urine. The remaining ellagic acid is further metabolised by gut microflora into dibenzopyranones (urolithin A, B, C and D). Initially urolithins A and B were identified in plasma and urine as metabolites of ellagitannin consumption (Cerda et al., 2005). More recent research, using pig as a model, has shown that urolithin D (a polar tetrahydroxy dibenzopyranone) is produced by bacterial metabolism in the jejunum and is then converted to urolithin C, A, and B through a series of bacterial mediated dehydroxylation steps during transit through the gut. As each hydroxyl moiety is removed the molecule becomes less polar and absorption into intestinal tissue and plasma, and urine concentrations increase (Espin, et al., 2007). Bioavailability of ellagitannin metabolites is critically dependent on gut microbial metabolism, which has been shown to vary between species and individuals similarly to the lignans and isoflavones (Atkinson et al., 2008; Cerda et al., 2005).

Lycopene (carotenoid): Carotenoid bioavailability has been extensively studied and reviewed (Rao and Rao, 2007; Yeum and Russell, 2002). Release from foods is a critical factor contributing to bioavailability of carotenoids and consequently bioavailability is often greater from processed than fresh foods. Released carotenoid is incorporated into micelles with fatty acids, bile acids, and phospholipids. The composition of the micelles can also affect bioavailability. Carotenoid-containing micelles passively diffuse into the intestinal mucosa, where they are converted into chylomicrons and secreted into the lymph, and then into the blood stream. The critical factors determining the bioavailability of carotenoids are the release from food and incorporation into lipid micelles, both factors that are relevant to lipid-soluble phytochemicals but play no role in the bioavailability of water-soluble compounds.

Concluding comments

Plants contain a very large number of compounds with different molecular structures which provide a large variation in biochemical and biological properties. In addition to the large number of different phytochemical components that may be present in any particular plant food, each phytochemical can have numerous separate bioactivities. For example, many phytochemicals have powerful anti-oxidant activity, but flavonoids also modulate intra-cell signalling processes, and carotenoids enhance gap junction communication, as two examples of non-anti-oxidant bioactivity. The mechanisms and processes of bioavailability are very complex. We are currently beginning to gain an understanding of the bioavailability of a few selected phytochemicals. We still need to understand the detailed processes for these compounds as well as understanding how these processes are influenced by the presence of additional phytochemicals and other components from foods. Bioavailability has a central and critical role in the expression of health benefits following consumption. Greater understanding of bioavailability will help increase the potential for consumers to gain even more health benefits from phytochemical-containing foods than is currently the case.

Acknowledgments

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Methods to determine the bioavailability of phytochemicals

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Keywords: bioavailability, bioaccessibility, phytochemicals, polyphenols

Abstract

The FDA has defined bioavailability as the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action. Food components must be bioavailable in some form to exert biological effects. Phytochemicals, especially polyphenols, have been shown to be strong anti-oxidants, and may exert a wide range of health benefits through anti-oxidant or other mechanisms. However, without knowing the rate and extent of their absorption, metabolism and tissue or cell distribution, the role of polyphenols in disease prevention will be a 'black-box'. Methods for determining the bioavailability and/or bioaccessibility (amount of an ingested nutrient/food component that is available for absorption in the gut after digestion) of phytochemicals involve human and animal studies (*in vivo*) as well as simulated experiments performed in a laboratory (*in vitro*). Several simulated digestion and absorption *in vitro* methods, and *in vivo* human studies/clinical trials for determining absorption and bioavailability of phytochemicals from plant food are presented and critically assessed.

Introduction

Bioavailability is defined as 'the rate and extent to which the active substances or therapeutic moieties contained in a drug are absorbed and become available at the site of action' (Shi and Le Maguer, 2000). This definition also applies to active substances (nutrients) present in foods. Food components like phytochemicals (e.g. polyphenols, carotenoids, phytosterols, and xanthophylls) must be bioavailable in some form to exert biological effects. In particular, polyphenols have been shown to be strong anti-oxidants, and may exert a wide range of health benefits through their anti-oxidant properties or other mechanisms (Hertog et al., 1996; Geleijinse et al., 2002; Lambert et al., 2005; Vita, 2005). Therefore, knowledge of the absorption, distribution, metabolism and tissue or cell distribution is a prerequisite for assessing the role of phytochemicals/polyphenols in disease prevention. Methods for determining the absorption and bioavailability of phytochemicals may involve human and animal *in vivo* studies and/or simulated experiments performed *in vitro* (Figure 1).

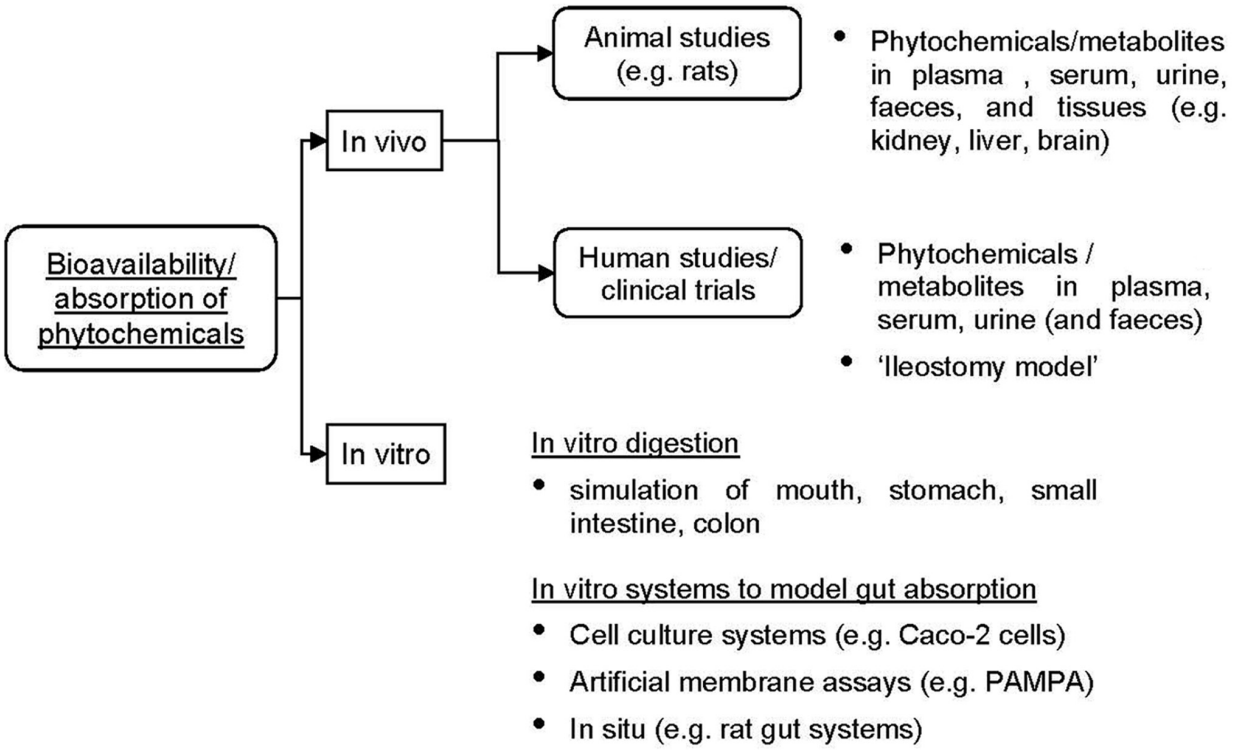

Figure 1. Methods to determine bioavailability of phytochemicals.

Bioavailability of phytochemicals is a complex and comprehensive topic and only certain aspects will be covered here. Therefore, appropriate *in vitro* and *in vivo* methods were selected based on available studies from our own research. The selected studies are summarised including necessary Figures and Tables to highlight the main results. Both approaches, *in vitro* and *in vivo* methods, are completed by a critical assessment of the presented methods.

In vitro methods

In vitro methods are rapid, cost-effective, safe and do not have the ethical restrictions of *in vivo* methods. In vitro methods can be applied to either simulate the digestion and absorption process or only the digestion process (bioaccessibility of nutrients/phytochemicals). Bioaccessibility is defined as the fraction released from a food matrix and available for intestinal absorption, and is typically used for in vitro procedures (Hedren et al., 2002). Gastro-intestinal models have been used to study the release and in vitro absorption of several phytochemicals (e.g. phenolic compounds and carotenoids) from raw and processed fruits and vegetables. In general, these in vitro models are set up to mimic the physiological conditions in the mouth, stomach, and small intestine during mastication, digestion, and absorption (Parada and Aguilera, 2007). A number of in vitro assays can be used to modulate gut absorption. There are: in vitro cell culture systems (e.g. Caco-2 cell cultures), artificial membrane permeability assays (e.g. Parallel Artificial Membrane Permeation Assay (PAMPA)), and in situ/ex vivo models (e.g. rat gut systems) (Kohlmann, 2006; Mertsch, 2006). *In situ* models will not be covered in this paper.

Caco-2 cells

The Caco-2 cell assay is a popular model for intestinal absorption studies (Hidalgo et al., 1989). The cell line is derived from a moderately well-differentiated human colon adenocarcinoma having both enterocytic and colonocytic characteristics. Caco-2 cells are able to spontaneously undergo morphological and biochemical enterocytic differentiation in conventional culture conditions.

The cells differentiate structurally and functionally into a cell monolayer with polarization, apical brush border microvilli, and tight intercellular junctions. The assay requires that drug/nutrient/phytochemical absorption rates be determined 21 days after the Caco-2 cell seeding to allow for monolayer formation and cell differentiation.

Artificial membrane permeation assay

Kansy et al. (1998) introduced the PAMPA. The permeability of compounds (e.g. drugs) is measured through a membrane formed by a mixture of lecithin and an inert organic solvent on a filter support. PAMPA shows trends in the ability of molecules to permeate membranes by transcellular passive diffusion. Its simplicity, low cost, high throughput, and applicability within a wide pH range make it attractive, especially in modern drug discovery. We are currently using the system to study the permeability of phenolic acids (e.g. chlorogenic acid and caffeic acid) and other polyphenols (e.g. resveratrol) within the CSIRO Food Futures Flagship Research Program on 'Designed Biomaterials for Biofunctionality'.

Examples of in vitro digestion and absorption with Caco-2 cells

In vitro studies within our own research program are used as examples for the understanding of bioaccessibility (digestion of processed carrots) and absorption (anthocyanins/Caco-2 cell model) of phytochemicals and are presented below.

The objective of the 'carrot study' was to develop an *in vitro* digestion procedure and couple this with a cell culture model to assess the bioaccessibility and *in vitro* absorption of carotenoids from carrots processed to different stages. Carrot purees were prepared using different heat and shear processing and digested in a procedure that simulates the digestive processes occurring in the stomach and small intestine (Figure 2). The release of carotenoids was measured as a function of digestion time, under the simulated gastric and intestinal conditions (Figure 3). To determine the gut absorption characteristics of the carotenoids in the digesta material, a Caco-2 cell culture model was established to measure the transfer rate of carotenoids through the cell (Figure 2).

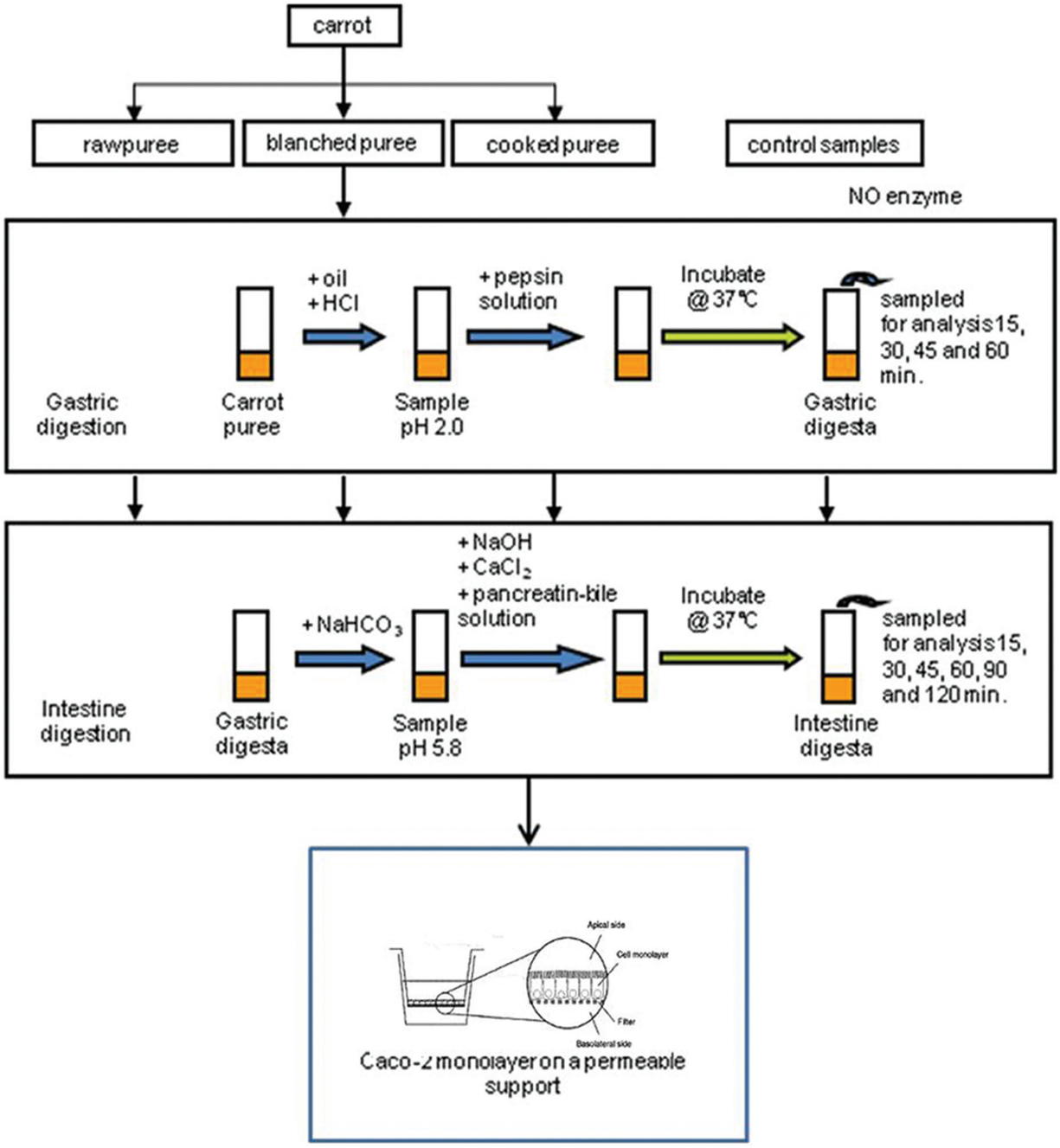

Figure 2. Integrated *in vitro* digestion and Caco-2 cell absorption model.

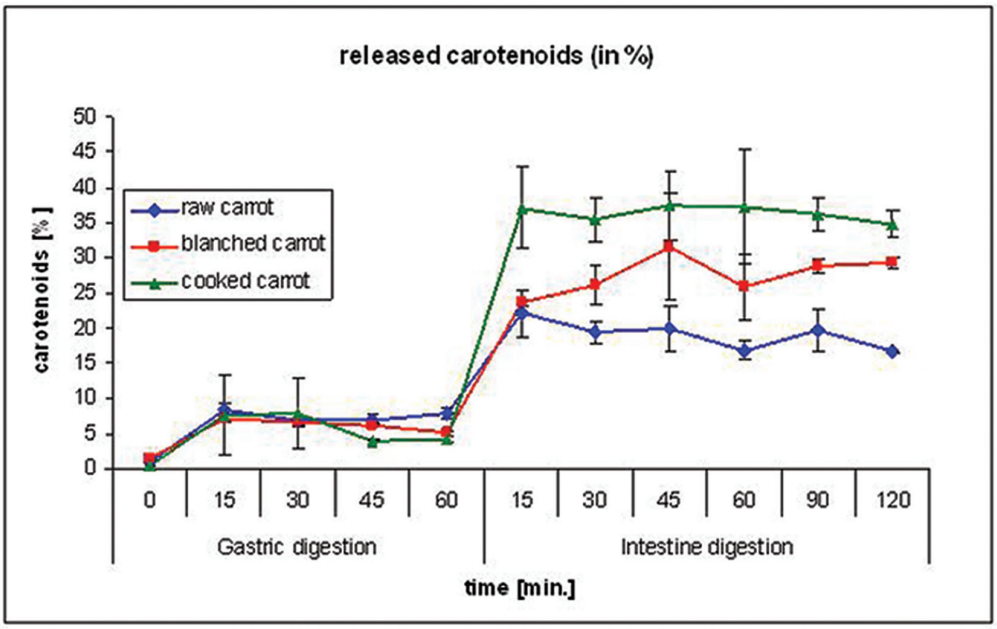

Figure 3. Release kinetics (%) of carotenoids (α- and β-carotene) during the digestion process; values are means ± SD (two trials); digesta samples were analysed by HPLC-PDA and LC-MS.

The results showed that there were no major differences in released carotenoids from processed carrots during the gastric digestion phase whereas, processing treatment had a clear impact on the release of these phytonutrients during intestinal digestion. The highest release of carotenoids was found from the cooked carrot puree (~38%), followed by blanched (~32%) and raw (~22%) carrot puree. These findings highlight the importance of understanding plant cell wall microstructure during processing steps to achieve enhanced bioavailability of phytonutrients. Absorption experiments with Caco-2 cells and β-carotene as well as whole carrot digesta are in progress.

In the second example, the absorption of blackcurrant anthocyanins was investigated by monolayers of human intestinal epithelial Caco-2 cells mounted in Ussing type chambers (Steinert et al., 2008). Anthocyanins have been reported to have multiple biological properties imparting benefits to human health. In order to understand their role in human nutrition, it is important to determine biokinetic data such as bioavailability. The purpose of the present study was to focus on the potential absorption of anthocyanins from blackcurrant (*Ribes nigrum L.*). Caco-2 monolayers were used as an *in vitro* model of the absorptive intestinal epithelium. Caco-2 cells were grown on permeable filters mounted into Ussing-type chambers. The monolayer integrity was monitored by measuring the trans-epithelial electrical resistance (TEER). Luminal to serosal transport of anthocyanins was examined by determining anthocyanin disappearance from the luminal solution of Ussing chambers (referred to as transport chambers) compared with that of Ussing chambers which were blocked by impervious inserts (controls). The results showed that anthocyanins disappeared from the luminal side, not due to anthocyanin degradation processes, but rather, at least in part, to metabolism within the cells (Figure 4). The net disappearance of anthocyanins (after correction for anthocyanins degradation) from the luminal solution was calculated ($max_{t\ 20\ min}$ ~ 11% for total anthocyanins) and labelled as 'absorption efficiency' of the cells (Figure 5).

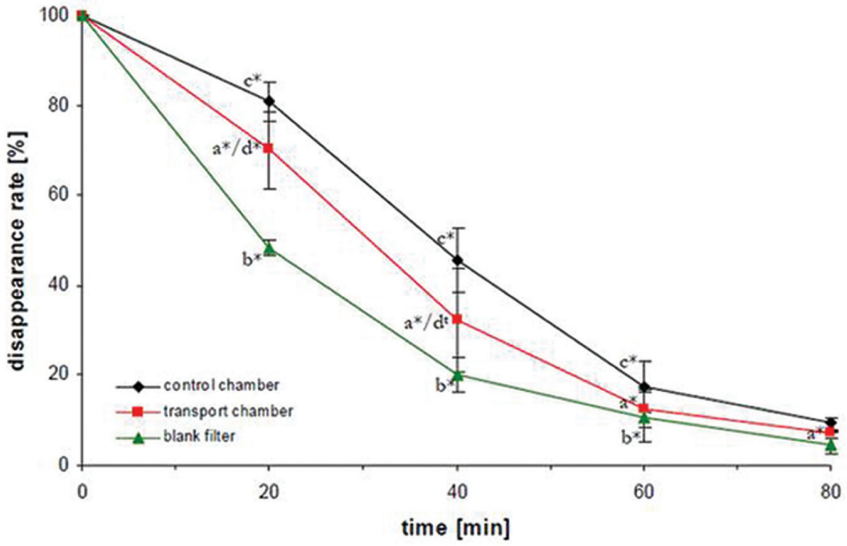

Figure 4. The disappearance rate of total anthocyanins [%] in luminal (gut lumen) solution of control chambers, transport chambers and blank filters. Values are the means ± SD (n = 5). Different lower case letters indicate significance (P<0.05) for treatments: *a* indicates differences over time in transport chambers, *b* differences over time in blank filters, *c* differences over time in control chambers, *d* differences between control chamber and transport chamber; anthocyanins were analysed by HPLC-PDA; *p < 0.05, ^{t}p < 0.07. Adapted from: Steinert, 2007, Diploma Thesis, Jena University, Jena Germany.

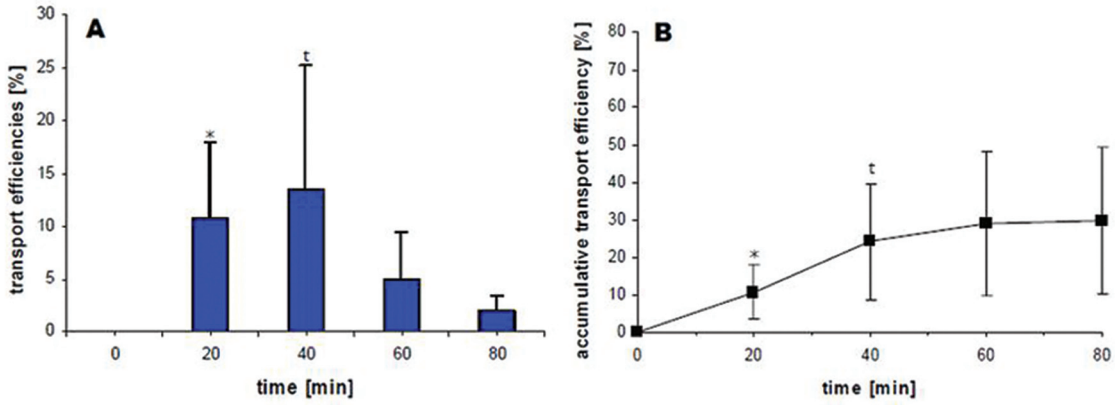

Figure 5. Absolute (A) and accumulative (B) transport efficiencies of total anthocyanins over time. Values are the means ± SD (n = 5). A: *p < 0.05, ^{t}p < 0.07 indicates differences to time point 0 (no absorption). B: *P < 0.05, ^{t}P < 0.07 indicates differences to prior sample time; anthocyanins were analysed by HPLC-PDA. Adapted from: Steinert, 2007, Diploma Thesis, Jena University, Jena Germany.

It is likely, that anthocyanins were transported via specific pathways across the apical side (gut lumen side) into the cell and at least partly metabolised within the cell. This apical transport might occur to a much larger extent than the further translocation across the basolateral membrane ('blood vessel side'). Thus, cell metabolism and translocation across the basolateral membrane may be the key determinants of anthocyanin absorption and bioavailability.

Although *in vitro* methods and models are rapid, safe, and do not have the ethical restrictions of *in vivo* methods, several drawbacks and limitations of these methods should be critically considered. To date, no *in vitro* model is capable of covering all aspects of *in vivo* digestion and absorption, distribution, metabolism and elimination. Caco-2 cell cultures, which represent a popular model for *in vitro* absorption study, display heterogeneity from batch to batch and several authors described changes in morphology, proliferation and permeability characteristics with increased passage number of cells (Walter and Kissel, 1995; Mertsch, 2006). The presence of villi and crypts in

human gut with a higher surface area and a different cellular composition compared to a Caco-2 cell monolayer might lead to higher permeability *in vivo*. Further limitations of *in vitro* cellular layers in comparison to *in vivo* conditions are: absence of mucus, different expression of enzymes and transporters. Furthermore, artificial membrane assays like PAMPA are purely artificial methods. They do not resemble real lipid bi-layer structures as barriers for permeation.

In vivo methods

Most commonly, *in vivo* bioavailability studies imply the ingestion of a certain dose of a nutrient, phytochemical or drug and following changes of its concentration in the blood plasma or serum compared with time (e.g. postprandial period). The bioavailability is best described by the following three parameters (Frick et al., 2006):

- area under the systemic concentration-time curve (AUC)
- the maximal plasma concentration (C_{max})
- the time to reach C_{max}, (t_{max}).

The AUC is a measure of the absorption intensity, whereas C_{max} and t_{max} give an idea of the rate of absorption.

Besides the main bioavailability measured in plasma/serum (AUC, C_{max}, and t_{max}), the urinary excretion, or relative urinary excretion, is an important and commonly used parameter to measure the extent of phytochemical absorption (Manach et al., 2005). Figure 6 shows a typical study design (schematic) for these kinds of studies.

Typical enrolment criteria for subjects are: non-smoking individuals between 18 and 45 years, normal for weight and BMI, (clinically) healthy, not using any medication, certain dietary restrictions such as no alcohol or the avoidance of food containing specific phytochemicals, and no hypersensitivities.

The absorbed and bioavailable phytochemicals, including their measurable metabolites, are normally analysed by HPLC coupled with photodiode array detection (PDA)/-fluorescence, and mass spectrometry (MS). Furthermore, several anti-oxidant assays (see selection below) are available and can be used to evaluate the impact of absorbed phytochemicals and metabolites on plasma/serum anti-oxidant status. Commonly used anti-oxidant assays (selection) are:

ORAC (Oxygen Radical Absorbance Capacity): hydrophilic and lipophilic AOC, readily automated, high throughput assay

TEAC (Trolox Equivalent Anti-oxidant Capacity): hydrophilic and lipophilic AOC, automated and adapted to microplates, high throughput assay

PCL (Photochemiluminescence): hydrophilic and lipophilic AOC, very sensitive (nanomolar range)

FRAP (Ferric Reducing Anti-oxidant Power): simple, speedy, inexpensive, robust, high throughput assay

FC (Folin-Ciocalteu) or **TP** (Total Phenolics): hydrophilic AOC, simple, inexpensive, high throughput assay.

Examples of in vivo *bioavailability of phytochemicals/polyphenols*

The following are examples of absorption and bioavailability of red grape anthocyanins, plant anti-oxidants, and apple polyphenols obtained from human studies (within our own research) together with a critical assessment of the methods.

In the first example, pharmacokinetic parameters and the bioavailability of dietary anthocyanins consumed in red wine (containing 11.4% ethanol, v/v) and red grape juice were compared in nine healthy volunteers (Frank et al., 2003). Individuals were given a single oral dose of either 400 mL of red wine (279.6 mg total anthocyanins) or 400 mL of red grape juice (283.5 mg total anthocyanins). Within 7 hours of consumption, the urinary excretion of total anthocyanins was only 0.23 and 0.18% of the administered dose, respectively (Table 1). Pharmacokinetic parameters derived from plasma and urine concentrations exhibited higher variability after ingestion of red grape juice (Tables 1 and 2).

Table 1. Summary of urinary pharmacokinetic/excretion parameters of total anthocyanins following consumption of a single oral dose of anthocyanins as red grape juice or red wine by nine healthy subjects. Adapted from Frank et al., 2003.

	R_{max} (µg/h)[a]	t_{max},R (h)[b]	Ae(0–7) (µg)[a]	Xe(0–7) (%)[a]
Red grape juice	241.4 (82.3)	0.5 (0.5–1.5)	653.6 (66.6)	0.23 (66.6)
Red wine	137.6 (29.2)	1.5 (1.5–2.5)	491.0 (19.8)	0.18 (19.8)

[a] geometric means (with geometric coefficients of variation (%)); [b] Median (range); total anthocyanins (by HPLC-PDA): sum of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside; Ae(0–7): total amount of anthocyanins recovered in urine from time 0 up to 7h; Xe(0–7): relative amount of anthocyanins excreted in urine expressed as percentage of dose within 7h.

Table 2. Summary of plasma pharmacokinetic parameters of total anthocyanins following consumption of a single oral dose of anthocyanins as red grape juice or red wine by nine healthy subjects. Adapted from Frank et al., 2003.

	C_{max} (ng/mL)[a]	t_{max} (h)[b]	AUC (ng*h*mL^{-1})[a]
Red grape juice	100.1 (64.2)	0.5 (0.5–1.0)	168.4 (42.3)
Red wine	42.9 (16.0)	1.5 (1.0–1.5)	100.8 (14.4)

[a] geometric means (with geometric coefficients of variation (%)); [b] Median (range); total anthocyanins (by HPLC-PDA): sum of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside.

Compared to red grape juice, the relative bioavailability of anthocyanins from red wine was calculated to be 65.7, 61.3, 61.9, 291.5, 57.1, and 76.3% for the glucosides of cyanidin, delphinidin, malvidin, peonidin, petunidin, and its sum (total anthocyanins), respectively. Urinary excretion of anthocyanins was fast, and the excretion rates exhibited mono-exponential characteristics with time after ingestion of both red grape juice and red wine. There is no indication for a bioavailability enhancing effect of ethanol on red grape anthocyanins.

The effect of a single oral dose of a beverage rich in polyphenols and ascorbic acid/vitamin C (study beverage, SB) on the anti-oxidant status (FRAP and PCL assay), as well as on levels of ascorbic acid and uric acid (an important endogenous anti-oxidant which contributes substantially to the *in vivo* anti-oxidant activity), was investigated in the second study with six healthy human subjects (Netzel et al., 2007). The SB intake (780 mg total phenolics and 97.6 mg ascorbic acid per subject) resulted in a statistically significant ($P < 0.05$) 8.9 fold increase in ten hour exposure to ascorbic acid in blood plasma as compared to water (control) (Figure 7 and Table 3). A tendency for higher antioxidative exposure in plasma as assayed by FRAP and PCL was observed without reaching statistical significance (Figure 8 and Table 3). Uric acid in plasma and urine was not affected by the ingestion of SB or water. Compared to the ingestion of water, SB consumption resulted in a significantly increased urinary excretion of ascorbic acid (+330 %) and total anti-oxidants estimated by the PCL (+ 43%) and FRAP (+ 29%) assay, within 24 hours (Table 4). In summary, the study

demonstrated that after consumption of a beverage rich in polyphenols and ascorbic acid, these compounds are bioavailable for humans and could be active as anti-oxidants *in vivo*.

Randomised cross-over design by multiple administrations

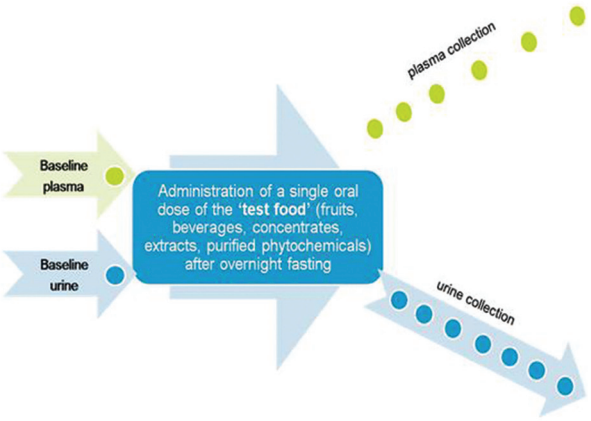

Figure 6. Typical study design (schematic).

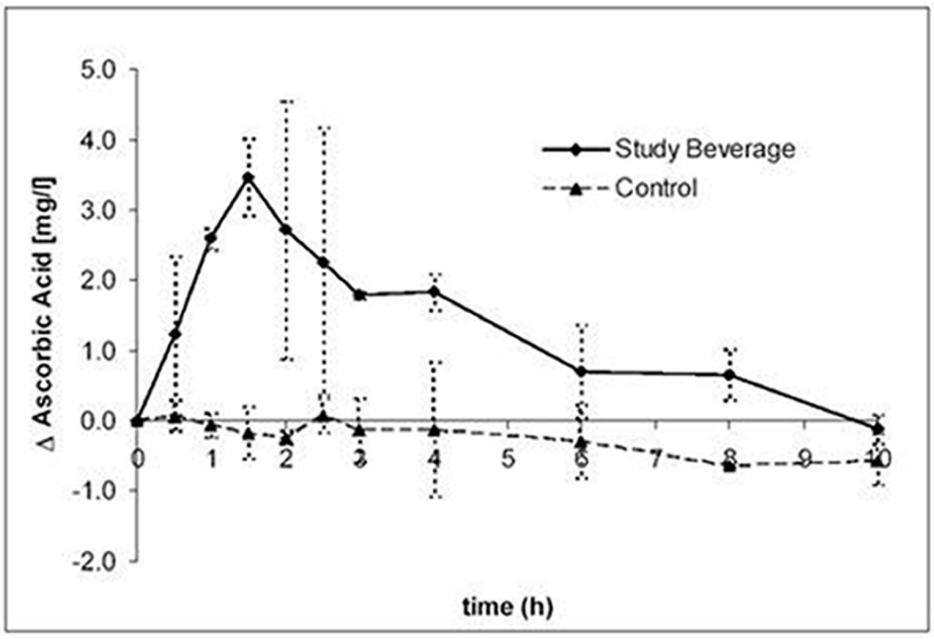

Figure 7. The effect of drinking 400 mL of SB or water on ascorbic acid concentrations in human plasma (mean ± SD, n = 6). Adapted from Netzel et al., 2007.

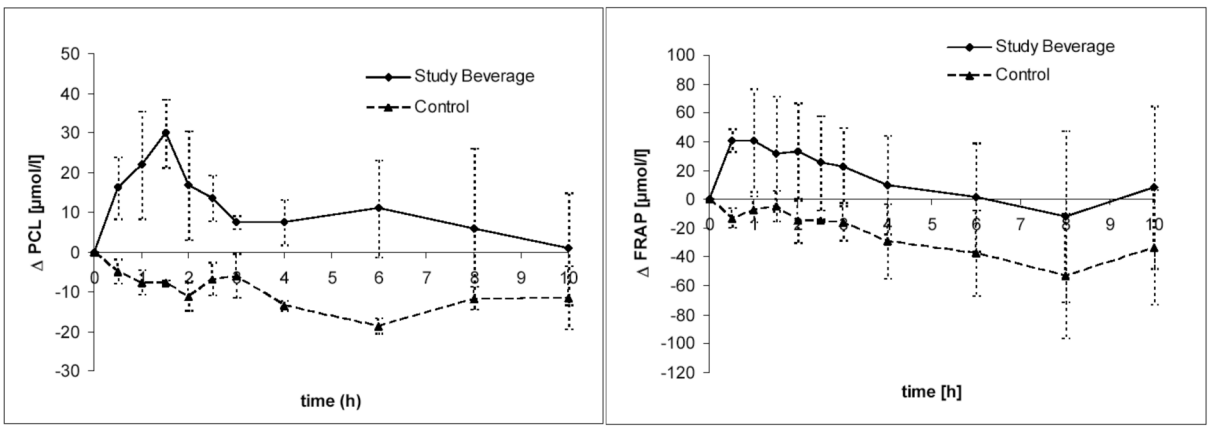

Figure 8. The effect of drinking 400 mL of SB or water on the anti-oxidant potential of human plasma as determined

by the changes in PCL and FRAP values (mean ± SD, n=6). FRAP values are expressed as µmol Fe^{2+} and PCL values as µmol Trolox equivalents. Adapted from: Netzel et al., 2007.

Table 3. Plasma AUC[a] of FRAP, PCL, and ascorbic acid following ingestion of a single oral dose of 400 mL SB or 400 mL water (control) to six healthy subjects. Adapted from: Netzel et al., 2007.

Parameter/substances	Control	SB
PCL (µmol*h/l)	0.63 ± 1.07	121 ± 117
FRAP (µmol*h/l)	44.6 ± 103	228 ± 373
Ascorbic acid (mg*h/l)	1.80 ± 1.96	15.9 ± 12.6*

[a]Area Under the response Curve that is above baseline (the concentration at dose time t = 0 h); data represent mean ± SD; *P < 0.05, SB versus Control (ANOVA); FRAP values are expressed as µmol Fe^{2+}, PCL values are expressed as µmol Trolox equivalents; ascorbic acid was analysed by HPLC-PDA

Table 4. Summary of urinary excretion data (anti-oxidant activity [PCL and FRAP], ascorbic acid, and uric acid) following ingestion of a single oral dose of 400 mL SB or 400 mL water (control) to six healthy subjects. Adapted from: Netzel et al., 2007.

Parameter/substances	Control	SB
PCL (mmol/24 h)	1.71 ± 0.14	2.44 ± 0.51*
FRAP (mmol/24 h)	6.15 ± 0.73	7.91 ± 0.71*
Ascorbic acid (mg/24 h)	11.1 ± 3.1	47.8 ± 24.3*
Uric acid (mg/24 h)	306 ± 108	305 ± 144

[a]Data represents mean ± SD; *P < 0.05, SB versus Control (ANOVA); FRAP values are expressed as mmol Fe^{2+}, PCL values are expressed as mmol Trolox equivalents; ascorbic acid and uric acid were analysed by HPLC-PDA

Table 5. Urinary excretion (n = 8 healthy subjects) of polyphenols and metabolites. Adapted from: Netzel et al., 2005.

identified compounds	control treatment (24 h-urine)	apple juice treatment (24 h-urine)
phloretin	nd	0.31 ± 0.15 mg[1]
phloretin-monoglucuronides	nd	1.72 ± 0.71 mg[1]
		→ 4.4 % compared to the ingested dose[2]
hippuric acid	232 ± 143 mg	719 ± 203 mg[1,*]

data: mean ± SD; nd: not detectable; [1]identified/confirmed by HPLC-PDA-ESI-MS/MS; [2]calculated as the ratio of amounts excreted (within 24 h) to amounts/dose ingested (as phloretin); *P < 0.05

Finally, the urinary excretion of apple juice polyphenols as well as their potential metabolites, was investigated in eight healthy subjects (Netzel et al., 2005). After a single oral dose of 700 mL of apple juice (containing 418 mg of polyphenols), apple polyphenols were recovered predominantly as metabolites in the volunteers' urine. Furthermore, compared to the ingestion of water (control treatment), apple juice consumption resulted in a significantly increased urinary excretion of hippuric acid (potential colon metabolite) within 24 hours (Table 5). This study showed that the metabolites (glucuronides and hippuric acid), and not the native apple polyphenols, may be responsible for at least some of the health effects attributed to apple/apple juice consumption.

Human studies involving clinical trials are still the 'gold standard' to assess the bioavailability of a drug, nutrient or phytochemical. However, there are also some drawbacks and limitations which should be considered. High inter-individual variations were observed in the present studies as well as those reported in the literature (Manach et al., 2005). Therefore, a large number of subjects are often needed to reach statistical significance ($P \leq 0.05$) which makes these studies cost-intensive and time consuming. A reason for this extensive variability between subjects could be that some individuals might have different levels of metabolizing enzymes or transporters which affect the efficiency of phytochemical absorption and metabolism and therefore their concentrations in plasma/serum and urine. Compared to *in vitro* studies, *in vivo* studies are subject to ethical restrictions which limit their application spectrum. Variability in the physiological state of the subjects cannot be excluded despite strict enrolment criteria. Furthermore, possible interactions of phytochemicals with other components in the diet or test meal cannot be excluded.

Future directions

Future work regarding the absorption and bioavailability of phytochemicals, *in vitro* and *in vivo*, should focus on the research directions listed below to get a better understanding of how these compounds can interact with chronic disease processes and exert their potential health benefits *in vivo*: (1) Improvement of cost effective *in vitro* methods for the reliable determination of bioavailability of genuine compounds as well as of their potential *in vivo* metabolites, and (2) Focus on the role of gut bacteria in metabolism and bioavailability of phytochemicals. The consumption of dietary phytochemicals could lead to the formation of specific metabolites in the human gut (especially colon), and it is possible that these metabolites offer protective effects against diseases attributed to phytochemical consumption. Therefore, future research should focus on the health effects of these metabolites using cell and animal models as well as their relevance to humans (clinical trials). *In vivo* methods are essential and will continue to be used as confirmatory tests to validate results of *in vitro* studies and to analyse some physiological conditions hard to reproduce in the laboratory (e.g. liver metabolism).

Summary

Bioavailability of phytochemicals is a complex and comprehensive topic and only certain aspects were covered here. Therefore appropriate *in vitro* and *in vivo* methods were selected based on available examples (studies) from current and/or completed research projects. The selected studies were summarised to highlight the main results. For both *in vitro* and *in vivo* methods, a critical assessment of the presented methods has been made. The bioaccessibility of carotenoids (*in vitro* digestion model) from processed carrots as well as the absorption of blackcurrant anthocyanins (Caco-2 cell model) were presented as examples for *in vitro* methods.

Although *in vitro* methods and models are rapid, safe, and do not have the ethical restrictions of *in vivo* methods, several drawbacks and limitations of these methods should be critically considered: e.g. to date, no *in vitro* model is capable of covering all aspects of *in vivo* digestion and absorption, distribution, metabolism and elimination; and Caco-2 cell cultures, which represent a popular model for *in vitro* absorption, display heterogeneity from batch to batch and several authors describe changes in morphology, proliferation and permeability characteristics with increased passage number of cells.

Human studies on the bioavailability of red grape anthocyanins from red grape juice and red wine, plant anti-oxidants from an anti-oxidant-rich beverage, and apple polyphenols from apple juice were presented as examples for *in vivo* methods. Human studies, particularly clinical trials, are still the 'gold standard' to assess the bioavailability of a drug, nutrient or phytochemical. But there are also some drawbacks and limitations which should be considered: e.g. high inter-individual variations were observed in the present studies as well as reported in the literature. Therefore, often a large number of subjects are needed to reach statistical significance ($P \leq 0.05$) which makes these studies

cost-intensive and time consuming; and compared to *in vitro* studies, all *in vivo* studies are subject to ethical restrictions which limit their application spectrum.

Future work should focus on the improvement of *in vitro* methods for the reliable determination of bioavailability of genuine compounds and of their potential *in vivo* metabolites as well as on the role of gut bacteria in metabolism and bioavailability of phytochemicals. It is possible that these gut metabolites offer protective effects against diseases attributed to phytochemical consumption.

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Consumer motivation for purchase and consumption of fruit products

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Abstract

Across the world, people are being encouraged to eat fruit, and in many countries public health campaigns advocate increased consumption. This essay provides a consumer-focused perspective on fruit consumption—giving consideration to both the barriers to consumption, as well as those factors that encourage consumption of fruit: taste/flavour, appearance, price, convenience, health benefits, product knowledge and quality. While the extant knowledge on consumer perceptions of fruits that are produced and sold globally in large quantities is sizable, similar knowledge for tropical fruits is much more limited. Hence, to provide a context for understanding consumer drivers for tropical fruit products, consideration is given to differences between traditional and tropical fruits and how these might affect consumer perceptions and choices. Inter-twined with the discussion of consumer drivers, research methodology is touched upon. Researchers in the primary industries are increasingly finding themselves involved in projects which require knowledge of consumer behaviour and market opportunities, as well as their product-specific expertise. Research on consumer behaviour is a central focus of disciplines including: sensory science, marketing, psychology, economics and anthropology. Each of these disciplines has their own collection of methodologies that provide valuable insights into consumer behaviour.

Introduction

Exotic appearances and flavours, often in combination with bioactive compounds such as carotenoids and polyphenols (e.g. Leong and Shui, 2002; Gouado et al., 2007; Haruenkit et al., 2007; Robles-Sanchez et al., 2007; Stangeland et al., 2007; Guo et al., 2008) that confer health benefits, mean that tropical fruits should have high consumer appeal. However, it is also easy to think of reasons why consumers might not choose tropical fruit, and in this essay, both factors that draw consumers towards tropical fruits, and those that may act as barriers to purchase and consumption are considered.

While there is a growing body of literature that considers the sensory properties of a wide range of tropical fruits and how product formulations can be optimised for consumer acceptability (e.g. John and Narasimham, 1993; Imungi and Choge, 1996; Man and Sin, 1997; Badrie et al., 1998; Abdullah and Cheng, 2001; Gujral and Khanna, 2002; Jaya and Das, 2003; Templeton et al., 2003; Lutchmedial et al., 2004; Perez-Lopez et al., 2006; de Sousa et al., 2007; Ramasaroop and Saulo, 2007; Laboissière et al., 2007) the extant literature on why consumers do or do not buy and eat tropical fruit is more limited, although not altogether non-existent (for some examples, see Deliza et al., 1999; Deliza et al., 2003; Deliza et al., 2005; Campbell et al., 2006; Poelman et al., 2008).

Fortunately, there is a sizable body of knowledge pertaining to mainstream fruits including apples, bananas, pears, oranges and kiwi. This can provide a useful starting point for obtaining a consumer-focused perspective on tropical fruits, and I will be drawing extensively upon it, including work from my own group at HortResearch in New Zealand. However, I wish to acknowledge from the outset, that the existing knowledge is limited in pertaining mostly to consumers from Western countries—not that this is a limitation unique to this literature, rather it is a feature of a majority of published scientific writing (e.g. McCrae, 2000; Steenkamp and Burgess, 2002; Langer et al., 2004; Salager-Meyer, 2008).

Factors that influence consumers' decisions to buy and eat fruit

Sensory characteristics

The critical importance of flavour in consumer choice of foods is broadly recognised (e.g. Steptoe et al., 1995; de Graaf, 2007; Tuorila, 2007). It is not hard to understand why. Take a moment to consider what you eat each day. I wager that the vast majority are foods that you like, and that you like more than moderately.

It is probably fair to say that, in the case of mainstream fruits, we have a good sense of what sensory characteristics people tend to like: good taste and good texture. For example, for pears, we know that there is a general preference among New Zealand consumers for juicy and sweet fruit (Jaeger, Lund et al., 2003). These key characteristics of ripeness are also demanded in apples, although with more subtle complexity. New Zealand consumers seem to prefer apples that are high in sweetness and acidity, in combination with a crisp and juicy texture (Harker et al., 2003). Kiwifruit is a crop we have studied extensively in New Zealand (e.g. Jaeger, Rossiter et al., 2003; Wismer et al., 2005; Harker et al., 2007), and as we find for apples and pears, and other fresh fruits (e.g. Crisosto et al., 2002; Crisosto et al., 2007; Saftnera et al., 2008), that consumer liking increases with sweetness.

Recent research by Harker et al. (2009) evaluated consumer liking for kiwifruit flavour that was predicted using the soluble solids content of ripe fruit (rSSC). The analysis examined the combined results from five studies that had been conducted over a seven-year period and involved more than 700 consumers. As has been previously reported (e.g. Jordan et al., 2000), Harker et al. (2009) found that dry matter content (DM) of kiwifruit at harvest was a good predictor of the rSSC of ripe fruit at the point of consumption. More importantly, Harker et al. (2009) demonstrated that most consumers provided their highest liking scores after tasting the sample that had the highest rSSC of the fruit presented to them.

As an aside, I note that while such knowledge is valuable for industry, it does not provide a clear indication of the economic value that could be associated with producing fruit that consumers really like. Complementary information on how liking is linked with purchase behaviours is needed. Key questions are whether consumers are willing to pay a premium for fruit they like a lot and whether such fruit, if available on the market would be purchased in greater quantity. To obtain such insights, research methodologies from economics and marketing are needed, for example experimental auctions (e.g. Jaeger et al., 2004; Kassadjian et al., 2005; Jaeger and Harker, 2005) and conjoint analysis (e.g. Jaeger, 2000; Jaeger and Rose, 2008).

Taste and flavour are, however, not the only sensory characteristics that are important to consumers. Appearance is also a key quality factor, as we have learnt through our work with fruits such as pears, avocado and kiwifruit. I'll use an example from pears to show how insights about consumer preferences for the appearance of fruit can be obtained. Gamble et al. (2006) used the methodology of choice-based conjoint analysis to assess Australian and New Zealand consumer preference for appearance in pears differing in shape, colour and russet. Twenty-seven images were created using software so that a standard pear could have different colours (green, yellow, and red), shapes (round, elongate-concave, and intermediate-straight), and different levels of blush (none, slight, full coverage) (Figure 1). When asked to indicate their most preferred fruit, preference was greatest for green and yellow colours with intermediate-straight or elongate-concave shapes.

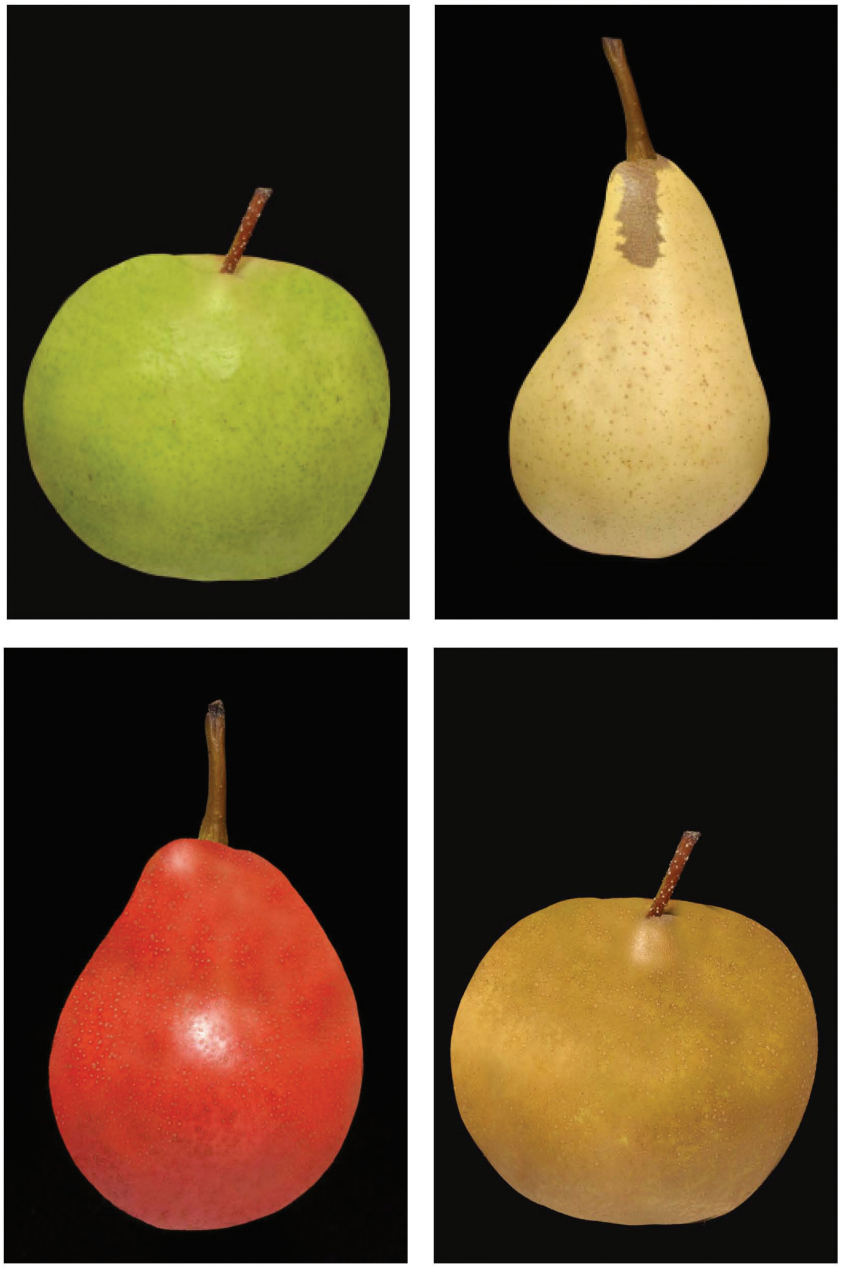

Figure 1. Examples of fruit images used by Gamble et al. (2006).

In the study by Gamble et al. (2006), pears with red colour and round shape were rarely chosen as 'most preferred' and we suggested that to some extent consumers were responding in terms of familiarity with existing pears. This insight, which pointed to a latent preference for pears with a shape and colour that was more, rather than less familiar, may be noteworthy in relation to tropical fruits. 'Fear' or 'avoidance' of the unfamiliar has previously been reported in the food domain, and food psychologists use a personality trait to measure this phenomenon. It is called food neophobia (Pliner and Hobden, 1992), and the extent to which a person is neophobic or neophilic affect the way that he/she interacts with new foods. For example, someone who is neophobic would response positively to statements such as: 'I don't trust new foods', 'If I don't know what is in a food, I won't try it', 'Ethnic food looks too weird to eat' and 'I am afraid to eat things I have never had before'. Tentatively, people who are neophobic may be less inclined to try tropical fruits because the fruits are novel. On the other hand, there is also a personality trait called 'variety seeking' (van Trijp and Steenkamp, 1992). Someone who scores high on a variety-seeking questionnaire would respond positively to statements such as: "I think it is fun to try out food items one is not familiar with', 'When

I eat out, I like to try the most unusual items, even if I'm not sure I would like them' and 'I am curious about food products I'm not familiar with'. Perhaps it could be expected that people who are high in variety seeking may approach tropical fruits more readily. These would be interesting questions to address, and to our knowledge, no one has done so to date.

Price

If faced with two identical product offerings, at different prices, economic wisdom has it that we choose the lowest priced. However, it is rare that we as consumers are faced with two identical products at different prices. Rather, price and many other product attributes tend to vary across the range of products we have to choose from within a product category. It is in this context that the role of price becomes interesting, and necessary to understand. Ultimately, what we seek to understand is how consumers trade price against other factors when making purchase decisions.

The following example, which was previously reported by Jaeger (2006), illustrates how consumers are forced to trade several factors against each other every time decisions about food are made. Specifically, the example looks at the price consumers are willing to pay for mangoes where a desired product feature—increased shelf-life—has been brought about by genetic modification (GM). As the storage life of a normal mango is only a few days after purchase, ways of bringing about improvements are sought, and they may include the use of genetic modification. To investigate trade-offs between price, convenience (i.e. longer storage life) and production technology (i.e. the use of genetic modification) we implemented a choice protocol (Table 1).

First, participants were asked if given a choice: would they buy a GM or GM-free mango. The fruits retailed at the same price. For insight into how price was traded against the other two non-sensory factors, participants were presented with a second choice question. The exact nature of this question depended on whether a participant had initially chosen the GM or the GM-free mango. Those that chose the GM-free fruit were asked whether they would be willing to purchase the GM fruit if it was sold at a 10% discount. Conversely, those who initially chose the GM fruit were asked whether they would buy the GM fruit when it was sold at a 10% premium.

Data were collected from two consumer samples (A and B). In Sample A, 22% of those consumers who had initially chosen the GM-free fruit opted for the GM fruit when it was sold at a 10% discount. Conversely, just under half (45%) of those participants who had chosen the GM fruit initially were willing to pay a 10% premium for the benefit of longer storage life. Thus, consumers in Sample A appeared price sensitive. In Sample B, however, it appeared that technology and convenience considerations were more important than price. Among those who initially had chosen the GM fruit, only a small minority (5%) showed price sensitivity and chose to buy the discounted GM fruit. Similarly, the vast majority (83%) of those who had chosen the GM fruit initially did so again when it was more expensive. In terms of the trade-offs between price, convenience and technology, this example illustrates that price is more important to some people than to others. For some people, however, price is less important than concerns over the use of GM technology in food production.

Table 1. Price sensitivity of two consumer samples to genetically modified mangos.

Choice profile	Consumer Sample A (n = 264)	Consumer Sample B (n = 187)
Discount 1. GM-free mango @ price P 2. GM @ 10% discount	22%	5%
Premium 1. GM mango @ price P 2. GM @ 10% premium	45%	83%
Price sensitivity	More sensitive	Less sensitive

Source: Jaeger (2006).

In thinking further about the role of price as a determinant for consumer choice of fruit, especially tropical fruit, I suggest that insights can also be gained by taking into account the differing role that mainstream and tropical fruit occupy in consumers' minds. Harker et al. (2005) reported how New Zealand consumers classify fruits such as apples and bananas as 'everyday fruit.' Tropical fruit, on the other hand, fit within a category labelled 'special fruit,' which are used and eaten differently. In relation to purchase and the price consumers are willing to pay, it may be that once the decision to purchase one or more 'special' tropical fruits is made—for example pineapple, lychee and coconut as 'extras' for a fresh fruit platter for a special function—price sensitivity may be relatively low because the decision that these special fruits are needed has been made. In this light, more understanding is required of price levels that deter purchase.

While we are not aware of much, if any, research that tries to determine price thresholds, or more generally the amount of money consumers are willing to pay for tropical fruit, it is possible to elicit such information from consumers. For readers considering doing so, it is useful to bring to attention the fact that there is mounting evidence in the literature of an inconsistency between what individuals say they will do when asked a hypothetical question and what they actually do. This is particularly noticeable when questions pertain to how much money one is prepared to pay to buy a certain product. Among others, Fox et al. (1998), Balistreri et al. (2001), List and Shogren (1998) and Lusk et al. (2007) reported that individuals tend to respond strategically to price questions including overstating instead of understating their actual willingness-to-pay (WTP) in hypothetical circumstances.

This effect, known as hypothetical bias, is more explicitly defined as 'the difference between hypothetical and actual statements of value, where actual statements of value are obtained from experiments with real economic commitments' (List and Gallet, 2001). For a person taking part in a study like the above, there is little risk attached to saying that s/he is willing to pay a certain amount of money for a mango fruit. Because of hypothetical bias, the validity of the findings of such research may, therefore, be questionable. Conjoint analysis, discrete choice modelling and experimental markets are alternative approaches to be considered. A distinguishing feature of such research is that research participants can be asked to use their own money to purchase the focal products, adding realism and commitment to the research on behalf of the participants.

Convenience

At HortResearch, we have over a number of years been interested in the role of convenience as a consumer driver for fruit products. This has been paralleled by broader research on what food-related convenience means to people (e.g. Candel, 2001; Swoboda and Morschett, 2002; McCullogh et al., 2003; Jaeger and Meiselman, 2004; Jaeger and Cardello, 2007), and there is a high level of convergence between findings. In the case of fruit, consumers identify several factors as contributing to convenience (Jaeger, 2003) including: 1) that they are consistently available in many outlets and for most of the year; 2) that they keep well and do not bruise easily; 3) that they are not messy to eat and that they can be eaten without the need for utensils; and 4) that they are suitable for a variety of uses including breakfast, as a snack or in desserts.

Fruit like apples and bananas 'perform' extremely well on these criteria. Conversely, fruit such as peaches, melon and mango 'under-perform' on one or more of these criteria (e.g. Jaeger et al., 2005). I would expect that many other tropical fruits also score lower on one or more of the convenience dimensions listed above. Therefore, it is interesting to speculate on what the implications of this reduced level of convenience may be. An immediate suggestion may be that tropical fruits would perform better in the marketplace if they were offered in more convenient formats, for example as fresh-cut. We have recently collected some data that suggest this may not always be the case and that fresh-cut formats may not be preferred, even though they offer increased levels of convenience. Jaeger and Rose (2008) used stated-choice experiments to study New Zealand consumer choices among apples, bananas, oranges and kiwifruits. High quality colour pictures of fruit—either whole or pre-cut and boxed (Figure 2) were presented together with information about the fruits' country of origin, brand, price, length of storage since harvest and whether or not they were grown organically. In accordance with the principles of choice modelling (and conjoint analysis), this information was varied systematically and enabled investigation of the relative importance of these product features for consumer choice, determined through advanced logistic regression (e.g. Hensher et al., 2005; Hair et al., 2006).

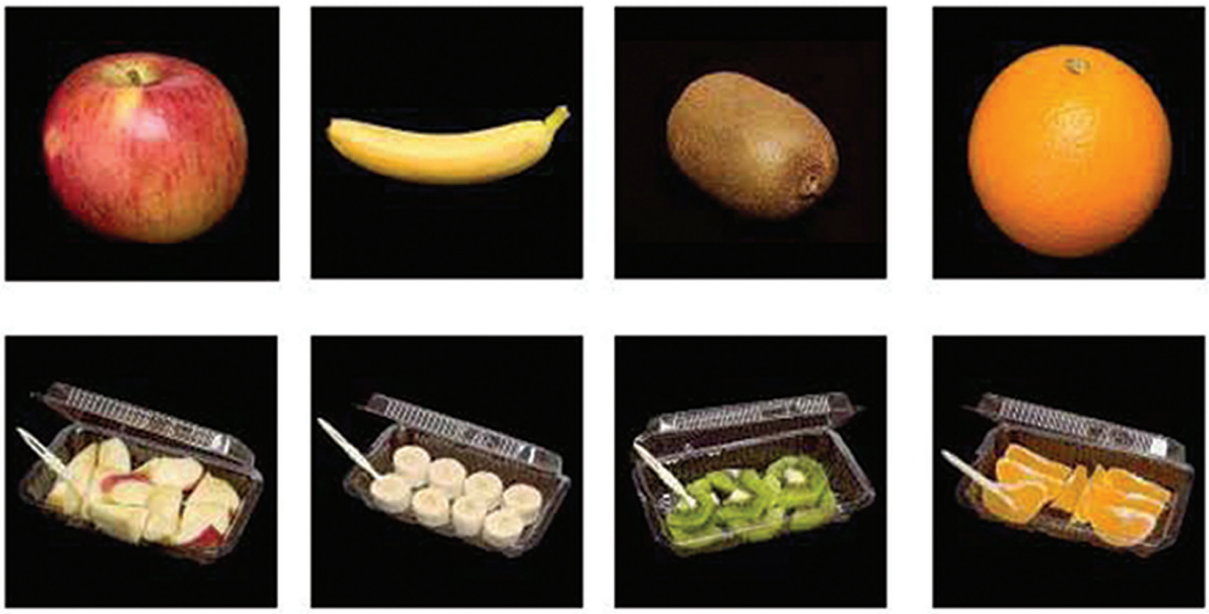

Figure 2. Examples of fruit images used by Jaeger and Rose (2008).

Jaeger and Rose (2008) reported that the parameter estimate for PREP (i.e. whether a fruit was whole or cut and pre-packed) was negative, meaning that, on average, fresh-cut fruit was less likely to get chosen than whole fruit. A possible explanation for this result is related to the price of fresh-cut fruit relative to whole fruit. To capture market place behaviour, in that study we added a significant price premium to the fresh-cut option. However, it is possible that this price premium

was not a major determinant of the negative utility associated with the fresh-cut option. Owen et al. (2002) reported that the choice of fresh fruit and vegetable purchases is often not determined by the price of an individual item, and that what matters more is the overall amount of money spent on this product category.

Another possible explanation is that consumers have some concerns about the food safety of fresh cut products. In focus groups with other fresh-cut fruit products (Gamble et al., unpublished), some people have said that they feel that fresh-cut fruit is no longer pure, that it has been tampered with, and that you never know about the hygiene of the facility/person who processed the fruit. However, analysis of the Jaeger and Rose (2008) data also revealed significant heterogeneity in the utility (or degree of lack of preference) associated with fresh-cut fruit. Specifically, some people viewed such fruit very negatively while others associated a small amount of positive utility with cut and packaged fruit. This is an important finding and one that mirrors common intuition. As consumers we are all different and all have different preference and food choice patterns. Markets can capitalise on that by supplying a range of products.

While the overall acceptance of fresh-cut fruit was low in the Jaeger and Rose (2008) study, some interesting insights on factors that moderated the level of acceptance of fresh-cut were also uncovered. For example, for fresh-cut eating occasions where participants were asked to imagine that they took their time to eat slowly and linger over the food compared with eating quickly, fresh-cut was evaluated more positively, perhaps because the multiple small pieces of fruit facilitated a slow/lingering way of eating. The location of a fresh fruit eating situation was also found to moderate the value placed on fresh-cut fruit. For eating situations taking place outside the home and together with other people, the fresh-cut option was relatively less preferred. While this may seem counter intuitive from a convenience perspective, fresh-cut is in a format that more readily can be shared with other people and some participants may not enjoy doing so. Having said that, we have other findings pointing to the ability to share fresh-cut fruit as a positive feature (Jaeger, 2000).

It is unlikely that there will be a simple answer to the question of whether fresh-cut is a desirable feature, and I wish to emphasise that we have probably as much evidence pointing to positive attitudes, as that pointing to negative attitudes in relation to fresh-cut. I'll share a final example in this matter that I consider instructive in again highlighting the complexity of individuals' food choice decisions, and the multitude of factors that impact upon them. The example is drawn from Bava (2006) who conducted an anthropological field study to explore food provisioning decisions among a small group of busy New Zealand women. Several of the participants talked about purchases of exotic fruits, which were found to inject some excitement into what was otherwise considered to be a mundane selection of apples, bananas, oranges, etc. One participant, Seline, who lived alone, was conscious of the need to consume fresh fruit and vegetables but found that it was difficult to motivate herself when the selection was considered boring. Rather, by indulging in luxury items she felt she could become more motivated to eat fresh fruit:

> *I don't shop at* [my local store] *… the food is second rate and the fresh is not really fresh. If I'm in a really extravagant mood I'll go to [the city supermarket] because they have really exotic vegetables and fruit.*

Here the participant was willing to travel 20 minutes to a store in the city offering a more 'exciting' selection of fresh produce. There is an additional component of behaviour that is also relevant here. Seline enjoyed purchasing fresh-cut packs of fruit salad, with a selection of honeydew melon, rock melon, watermelon and pineapple. In addition to being exotic, this fruit salad enabled Seline to have a variety of tropical fruits at one time. Seline felt that not only would it be difficult to consume an entire honeydew melon on her own before it spoiled, the size of most tropical fruit meant that it would be even less likely that she would consume a variety of these before they spoiled. Furthermore, eating the same fruit each day over the course of a week would essentially see her in the same predicament she was trying to avoid through purchasing tropical fruits—becoming bored with the lack of variety. The fresh-packed tropical fruit salads thereby provided convenience and variety to an otherwise mundane selection of fresh produce.

Product knowledge

As previously touched upon, quality of fruit is a very important determinant of choice. Over the last century, if not longer, post-harvest biologists and technologists have developed and established harvest and storage protocols that allow the fruit industry to maintain the quality of highly perishable produce over prolonged periods of time (e.g. Kader, 1997; Kupferman, 1997). This is clearly evident in the supermarkets today, where apples harvested up to 10 months ago can be taken from storage and sold. Many consumers appear to be unaware that this is the case, and I note, as an aside, that when they are given information about long storage periods, reactions can be negative. Lund et al. (2006) showed this clearly. Experimental markets were used to elicit the monetary value consumers place on 'Granny Smith' apples that had been stored for ~2 months ('New apples') compared with ~8 months ('Old apples').

The consumers were informed of the different storage durations, and were given the opportunity to purchase apples using their own money. Midway through the experimental market, the consumers were allowed to taste the apples. While the 'New apples' had been stored for a shorter duration, they were of poorer texture than the 'Old apples' – this was confirmed by penetrometer tests, which gave mean values of 68 and 84 N, respectively. 'New apples' were preferred over 'Old apples' by 90% of consumers, but this dropped to 60% after tasting and opinions on monetary value became polarised. One participant stated: 'I don't want to feed my children apples that are 8 months old—I want them to have fresh healthy apples!'. The Lund et al. (2006) study was a powerful demonstration of the emotional aspects that consumers also rely on when making purchase and consumption decisions. In regard to levels of product knowledge, that study exemplified how disconnected from seasonal cycles at least some consumers have become. This could be even more pronounced for tropical fruits, which for consumers in western markets are grown in distant countries.

Other 'dimensions' of product knowledge (or lack hereof) may also act as significant barriers to purchase and consumption of tropical fruit. We gained some insight to this a number of years ago in the case of persimmon. Full details are given in Harker et al. (2005) who report on a series of focus groups conducted on the topic of consumer perceptions and attitudes to novel fruit. At the time of the study, persimmon was the third largest tree fruit crop in New Zealand, with most production aimed at Asian markets. There was significant local market supply, and it was frequently available in supermarkets. The conversations among participants in regard to persimmon broadly covered the following issues:

> *I know that fruit. I see it every time I go shopping. It is that bright orange fruit that looks so nice. Does anyone know how to use it or what it tastes like? We just go past it in the supermarket because no one has told us what to do with it!*

Similar themes have emerged from other research on consumer perceptions of novel fruit (Jaeger and Harker, 2005), where kiwano (horned melon) and pomello (large thick-skinned citrus) were categorised using labels such as 'novel' and 'have not tried' and feelings of uncertainty of how to prepare and eat the fruit. If tropical fruit is to gain market share, consumers must be educated about them.

Wellbeing and health

As a product category, fruit is demanded particularly for its positive health benefits, which have been convincingly demonstrated (e.g. Block et al., 1992; Steinmetz and Potter, 1996; Hung et al., 2004; Pomerlau et al., 2008; Veer et al., 2008), and in many countries public health promotions and interventions aim to increase fruit consumption (e.g. Perry et al., 1998; Baranowski et al., 2000). However, many people fail to meet guidelines for consumption, and in New Zealand; for example, recent data show that only half of adults consume two or more servings of fruit each day (National Heart Foundation, 2008). The reason for this is unlikely to be that people are not aware of the positive diet-health link.

For fruit, it seems clear that many people seek out fruit, particularly because they recognise their advantages in that regard. Research that has been used to establish these insights include Means-End Theory and laddering methodology (e.g. Valli et al., 1999; Bredahl, 1999; Roininen et al., 2000; Baker et al., 2004). And what emerges from this approach of asking people questions about what is important to them and why is that: 1) Good personal health leads to a longer life; and 2) Fitness and well-being means you can enjoy life and do things you want to. In addition to uncovering why good personal health is important, laddering interviewing can also provide insight to what product characteristics people perceive as enabling them to achieve and maintain good health. As a basic example consider apples, where this link is: juicy and sweet tasting apple => apple tastes good and is liked => eat fresh fruit => get a balanced diet rich in vitamins => remain healthy => long life (Jaeger and MacFie, 2000).

An important question is how people may be better helped to include more fruit in their diet and one strong development in that regard is that of functional foods. This is also relevant in the case of tropical fruits, which, as already noted, tend to be rich in a range of bioactive compounds. If the fruit are not consumed fresh or in an unprocessed state, functional compounds may be extracted and used as ingredients in functional foods. There is a sizable literature on consumer drivers for functional foods providing health benefits. For the purpose of this essay, I think it is most valuable to consider some of the challenges associated with the development of functional foods, while also providing a few insights on how consumers perceive such products. I sometimes sense that there is a belief that most people will place the healthy properties of functional foods high above other factors, including taste and price. I am sceptical about this notion and I think it is wishful thinking that fruits/products with inferior sensory quality will be chosen by consumers because of other favourable characteristics such as health or functional benefits.

There are several reports in the extant literature (e.g. Verbeke, 2006; Krystallis et al., 2008) documenting how consumers are not willing to compromise on sensory quality when it comes to functional foods. They want proven health benefits *and* good taste. Unfortunately, functional ingredients can lack palatability. As an example, let us consider polyphenols, which are often found in fruits, and which have documented anti-oxidant activity (e.g. Bravo, 1998; Gardner et al., 2000; Lee et al. 2003; Arts and Hollman, 2005; Shukitt-Hale et al., 2005; Mullen et al., 2007). However, one of their key defining sensory characteristics is bitterness, which is an attribute that is typically associated with diminished acceptability (e.g. Stein et al., 2003; Lesschaeve and Noble, 2005). Unless such notes can be masked, high acceptance ratings may be hard to achieve. This will likely have consequences for market performance.

In addition to optimisation of product sensory characteristics, the provision of health benefit information can contribute to more positive evaluation of some products, particularly in relation to purchase probability (e.g. Tuorila and Cardello, 2002; Luckow et al., 2006). It seems important to ensure that consumers can see a direct personal health outcome and that they understand what the functional benefit means for them (e.g. Tuorila and Cardello, 2002; Krystallis et al., 2008). More broadly, willingness to buy functional foods is dependent on many factors, including the taste of the product, consumers' knowledge and trust in health-related information, their motivation to engage in health preservation behaviours, how healthy the product is perceived to be, how it is priced and the particular demographics of the consumer (e.g. Krystallis et al., 2008). Verbeke (2006) reported that belief in the health benefits from functional foods was the strongest determinant of willingness to compromise on taste. However, he also highlighted the declining willingness to compromise on taste. Krystallis et al. (2008) concur; if functional foods do not taste palatable, consumers will not buy them.

Quality of fruit in the home

The final factor that I am able to cover in this essay relates to the quality of fruit in the home. The primary reason for considering it is that we know very little about it. Yet, sensory properties of fruit are paramount not only in purchase decisions, but also in consumption decisions. Intuitively, it seems like a good idea to ask questions like: What happens to fruit once it has been purchased? What do consumers do with it and how does that influence quality? To gain some insights we conducted an explorative study with New Zealand consumers (Amos, 2005) [see also Campbell et al., in press]. The aim was to learn more about the extent that fruit deteriorates in consumers' homes and also whether there were any consumer behaviours or attitudes that extenuated or mitigates the effects of fruit deterioration. Using interviews conducted in participants' homes, we found that consumers were experiencing problems with fruit deteriorating in their homes. There was significant wastage of fruit with almost a third of households indicating they threw fruit away once a week. This level of frequency of disposal of fresh fruit that was considered beyond optimal eating quality was confirmed in a subsequent study with ~500 New Zealand consumers (Jaeger and Zhu, 2007).

In the study by Amos (2005), consumers had strategies to minimise deterioration and wastage, by shopping more than once a week, or if shopping only once a week, by selecting fruit that would ripen to the ready-to-eat stage at different times during the week. More than this, almost all consumers identified alternative ways of using fruit once it had deteriorated to the point that they would no longer consider eating it as fresh. Generally this involved cooking the fruit or using it as an ingredient in a cake. These strategies for avoiding wastage point to the high value or utility they place on fruit and fruit consumption. But they also place a question mark over what is done with tropical fruit that deteriorates past a point where it will not be consumed fresh. Do consumers have similar knowledge of alternative uses as they appear to have for mainstream fruit? If not, how may this influence growth of the tropical fruit sector?

A further reason for including the Amos (2005) study in this essay is that we obtained information from a group of 235 New Zealand consumers about the fresh fruit they had in their homes (Table 2). I thought it interesting to include here because it is the only data we currently have that relate to incidence of tropical fruit in consumers' homes. The data are encouraging in that consumers' fruit bowls contained a broad variety of fruit, including tropical fruit. While not represented in large quantities, the fact that tropical fruit were represented suggests there is scope for adding a broader variety of tropical fruits. That is, I think a very positive message on which to end this essay.

Table 2. Types of fresh fruit in the home of New Zealand consumers.

Fruit	Purchasers (n = 132)	Non-purchasers (n = 102)
Apple	78.0	88.2
Banana	82.6	87.2
Cherry	3.8	1.0
Feijoa	5.3	6.9
Grapefruit	12.9	18.6
Grapes	18.2	25.5
Green Kiwifruit	35.6	37.2
Gold Kiwifruit	14.4	22.5
Lemon	43.9	45.1
Lime	4.5	4.9

Fruit	Purchasers (n = 132)	Non-purchasers (n = 102)
Mango	3.0	2.9
Mandarin	49.2	48.0
Melon	7.6	4.9
Nectarine	10.6	12.8
Orange	45.4	52.0
Passionfruit	2.3	2.0
Pear	25.8	24.5
Peach	6.1	4.9
Pineapple	3.8	3.9
Plum	3.0	2.9
Other	2.3	2.0

Note. Data were collected from two groups of consumers. Those who were responsible for fresh fruit purchases in their households ('purchasers') and those who were not ('non-purchasers'). Data are expressed as percentages of the total of each group.

Source: Amos (2005).

Conclusion

In this essay I have attempted to discuss consumer drivers for fruits by covering some of the factors that influence (positively and/or negatively) consumers' purchase and consumption decisions. Where possible I have targeted the discussion towards tropical fruits, drawing on the knowledge we currently have for mainstream fruits. There are many other factors than those covered in this essay that are likely to influence consumer decisions around fruit. Sustainability issues seem particularly important to mention. I have not covered them here because there is no available information. This is a factor that only very recently has gained significant attention, but for tropical fruits, many of which are likely to be grown in countries with significant distances to export markets, it may become very important in the future. In this regard it may be instructive for readers to look at the New Zealand winery Grove Mill, which produces premium quality wines with minimal environmental impact, and which is the first winery to be certified for it sustainable practices (www.grovemill.co.nz).

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Tropical flavours to tempt consumers

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Abstract

Tropical fruits are visually and texturally unique and exhibit a broad range of odours and flavours from delicate and sweet to pungent and obtrusive. The nutritional and health benefits from tropical fruits are thought to be significant and are recognised by consumers. Nevertheless, if the taste of the fruit is bland or unpalatable, the consumer will not eat it despite the apparent health benefits in doing so. Understanding and optimising a balance between desirable flavours and bioactivity in tropical fruits is vital to ensure consumer acceptability and success of commercial products in a competitive fruit marketplace. Techniques we can use to assess the flavour and acceptability of tropical fruit include consumer assessments, sensory evaluation and compositional flavour analysis methodologies. Recent work by our group at Department of Primary Industries & Fisheries (DPI&F) Innovative Food Technologies has involved consumer studies assessing teenager's preferences for some exotic tropical fruits, and sensory and volatile flavour studies of mango as part of the DPI&F tropical fruit genomics initiative. Our results demonstrate the importance of flavour acceptability to the consumer and highlight the need to study flavour in more detail to understand its compositional and genetic basis in tropical fruits.

Introduction

Flavour is one of the most important criteria in consumer acceptability of food, including fruit. For the consumer, if the taste of a fruit is bland or unpalatable, they will not eat it, despite the apparent health benefits that could be obtained from doing so (Krystallis et al., 2008). Pleasurable eating experiences from fruit have the potential to create a 'halo effect', which can reinforce and boost consumers' perceptions of health benefit attained. Flavour of fruit comes from a balance between sugars, acids and volatile aroma compounds. It is the volatile aroma compounds in fruit that gives rise to the distinctive flavour of a particular fruit.

The volatile compounds produced in plant foods, including tropical fruit, represent less than 1% of the total fruit composition and comprise a broad range of chemical compounds with varying sensory potencies and concentrations. Only a small sub-set of these compounds are detectable to the human olfactory system. In fruits, compounds such as monoterpenes, norisoprenoids, furans and lactones, short-chain branched fatty acids, esters and alcohols of those acids, and sulphur and nitrogen-containing compounds have all been identified as important to the flavour of different fruits (Winterhalter, 1991). Many of the important flavour-contributing volatiles found in fruits are metabolites of bioactive compounds and may indeed be sensory cues for health and nutritional value (Goff and Klee, 2006). Our knowledge of the unique flavour nuances of exotic tropical fruits, and the consumer preferences for those fruits in Australia, is limited.

The techniques that can be applied to understand and measure flavour quality in tropical fruits include consumer evaluation, sensory studies using trained panellists and compositional flavour analyses. Our group at DPI&F Innovative Food Technologies in Hamilton, Queensland, have recently

conducted two projects related to tropical fruit quality. The first study involved consumer evaluation of exotic tropical fruits with teenagers to understand preferences for different fruits. The second study involved a detailed sensory and compositional flavour analysis of mango. These two studies are the topic of the present paper.

A consumer study with teenagers for exotic tropical fruit preferences

The projected value for exotic tropical and sub-tropical fruits such as longan, rambutan, mangosteen and durian in Queensland by 2010 is $25M (O'Connor and Diczbalis, 2003). Consumer demand for an exotic 'tropical fruit category' anchored with some stable baseline tropical fruits (e.g. mango) has been clearly identified and is expected to drive market growth rather than individual exotic fruit crops on their own (Horsburgh and Noller, 2005). The increasing importance of consumer perceptions as a strong market driver is well-recognised. However, documented information on consumer behaviour with respect to exotic tropical fruit consumption is fairly limited.

A recent consumer study showed that drivers for familiar fruits were that they were 'well-liked' and 'easily available in the shops', whereas unfamiliar fruits were considered to be 'expensive' (Jaeger et al., 2006). Consumer drivers of exotic tropical fruit are likely to be the attraction to novel and exciting eating experiences. Another report stated that 'consumers are interested by tropical fruit but not necessarily to take it home' (Horsburgh and Noller, 2005). Barriers to tropical fruit consumption could include lack of knowledge of taste and use of the products, the preparation time required and an association between 'exotic' and 'luxury' which result in only small volumes being purchased for special occasions.

This preliminary investigation aimed to gain a first insight into the experience and preferences of teenage consumers for a selection of four exotic tropical fruits with the dual benefit of promoting the consumption of exotic tropical fruits among a group of young people in Brisbane.

Material and methods

A total of 60 year-nine (~15 year old) students (38% male and 62% female) took part in the tasting during a workshop at the 'revolutionary science day' on the 9 May 2008 at Toowong College, Indooroopilly. With the exception of one participant, all students indicated they consumed fruits weekly and 77% daily. The questionnaire involved a section on background information, including fruit consumption habits and knowledge of tropical exotic fruits. The tasting section questionnaire involved ranking the preference order based on four fruits presented and describing specifically liked and disliked characteristics about each fruit.

The exotic tropical fruits included in the tasting were mangosteen, red dragon fruit (pitaya), carambola (star fruit) and persimmon which were purchased locally from Clayfield Fresh Market in Brisbane during the same week in May. The fruits were presented in a ready-to-eat format in cups labelled with three-digit codes as shown in Figure 1. Mangosteens were peeled and quartered, persimmons were sliced in segments, carambolas were sliced laterally in star-shaped pieces and dragon fruits were peeled and cut in wedges. An analysis of variance (ANOVA) was performed on the ranking data to assess any significant difference between the fruits in terms of preference order.

Figure 1. Photo of four tropical fruits presented to teenage consumers for evaluation, mangosteen, carambola, persimmon and dragon fruit (left to right).

Results and discussion

A summary of the percentage of teenage participants who had previously tasted a range of tropical fruits at least once is shown in Table 1. Not surprisingly, almost all the teenage consumers had tasted pineapple, banana and mango. Interestingly, papaya had only been tasted by about one third of respondents despite its year-round availability in supermarkets and green grocers in the Brisbane area. Lychee, however, had been tasted by 83% of the teenagers despite lychee's restricted seasonal availability and relatively high price.

Table 1. Percentage of participants who had previously tasted various tropical fruits.

Fruit	%	Fruit	%
pineapple [c]	97	star apple [r]	23
banana [c]	97	durian [r]	15
mango [c]	92	rambutan [r]	10
lychee [r]	83	jackfruit [r]	8
passion fruit [c]	77	mangosteen [r]	3
carambola/star fruit [r]	47	soursop [r]	3
guava [r]	47	black sapote [r]	3
custard apple [r]	42	breadfruit [r]	2
dragon fruit/pitaya [r]	37	abiu [r]	2
papaya/ pawpaw [c]	33	rollinia [r]	0
persimmon [r]	30		

[c] fruits classified as 'common' tropical fruits in the questionnaire.

[r] fruits classified as 'rare' tropical fruits in the questionnaire.

In terms of variety of fruits previously tasted, 23% of the students had tasted more than five of the 'rare' tropical fruits (i.e. other than pineapple, banana, mango, passionfruit and papaya). Most students (49%) indicated they ate 'rare' fruits rarely or never, or a monthly (31%). It is possible that most students had previously tried some of these exotic fruits only once.

None of the students indicated they had tasted rollinia previously and only one student indicated they had previously tasted either breadfruit or abiu. It is possible that many of the names of the exotic fruits were not recognised by the students. Students were given an exercise to match images of tropical exotic fruit to the fruit name. Most students performed well; however, only a small sub-set of the rare fruits were included in this exercise (Table 2).

Table 2. Percent of students who associated the fruit picture to its correct name.

Fruit	%
custard apple	85
carambola/star fruit	82
pitaya/dragon fruit	79
persimmon	54
rambutan	47
mangosteen	44

In the preference ranking exercise, the mangosteen was selected by 41% of the teenagers as their favourite fruit and was significantly preferred over dragon fruit and carambola (Table 3). Although the appearance and colour of the dragon fruit and carambola appealed to the students, the lack of flavour or unappealing 'sour' flavour may have resulted in an overall low ranking for these fruits. The comments for the appearance of the mangosteen segments were typically negative; however, the sweet 'tropical' flavour appealed to the students resulting in this fruit being the overall favourite.

Table 3. Results of the fruit preference ranking exercise (% of students).

	mangosteen	persimmon	dragon fruit	carambola
overall ranking*	A	AB	B	B
favourite fruit	41	25	18	16
2nd preferred fruit	21	28	30	21
3rd preferred fruit	25	25	21	30
least preferred fruit	13	23	31	33

* different letters indicate a significant difference in ranking by ANOVA

This workshop was designed for the target audience and facilitated the promotion of the tropical exotic fruit category to a group of Brisbane teenagers. Teenagers, and by extension children, can be considered significant players in purchase decisions made for the family and it can be useful to understand their preferences and requirements for tropical exotic fruits.

Mango flavour

Mango (*Mangifera indica*) fruit is one of the most popular tropical fruits among Australian consumers. The Australian National Mango Breeding Program has been linked to a QDPI&F mango fruit genomics research initiative which aims to profile the genetic and phenomic basis of fruit quality characteristics (Dietzgen et al., 2007). This research will provide marker-assisted breeding systems to fast-track plant breeding efforts to deliver mango varieties with improved horticultural and health properties, while retaining and enhancing consumer preferred flavour characteristics. The work conducted at Innovative Food Technologies involved profiling and understanding the flavours of parent and hybrid mango varieties in the breeding program and determining key compounds responsible for desirable flavour characteristics.

Material and methods

Two seasons (2006 and 2007) of mangoes including a number of varieties (Kensington Pride, Irwin, R2E2, Nam Doc Mai, Indian H-10, Calypso, and four other selections) were quantitatively assessed using sensory descriptive analysis techniques. A panel of ten trained tasters developed a defined attribute list for appearance, aroma, flavour, texture and aftertaste, together with a set of sensory reference standards. Five replicate mango samples of each variety were assessed using the defined attributes rating on line scales under controlled conditions.

Samples of mango were stored at –80°C for chemical analyses. Composite samples of ripe mangoes from more than 400 hybrid varieties from three breeding populations in Mareeba (North Queensland) were also stored for analysis during 2007 and 2008. Volatile chemical analysis was conducted on all samples using headspace solid-phase microextraction and gas chromatography mass spectrometry (GCMS).

Results and discussion

The results from sensory evaluation demonstrated a diversity of flavour types between different cultivars (Figure 2). Kensington Pride had high scores for terpene, fermented, buttery, tropical and floral aroma, while the Irwin variety had more cut grass and musty/dank odours. The Indian variety was perceived as having high green banana, cut grass and melon aromas. The Nam Doc Mai had some musty/dank aroma but was overall fairly low in aroma. The R2E2 variety has some fermented and buttery aromas, but was also high also melon aroma. The selections, hybrids of Kensington Pride and Irwin, had some similarities to both parents ranging in aroma between terpene, musty/dank and cut grass.

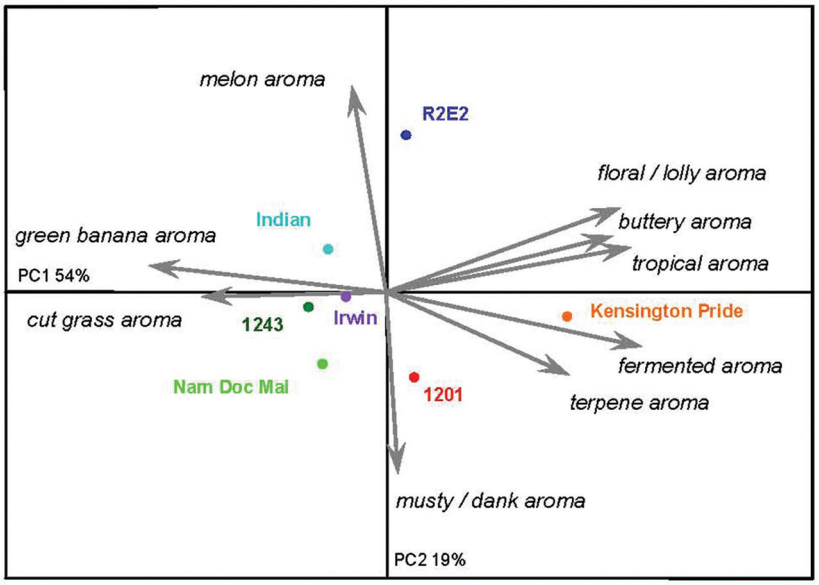

Figure 2. Principal component analysis bi-plot showing mean results of aroma sensory scores for 2006 season mango fruit (n = 5) (PC1 versus PC2).

The volatile profiles were combined with the sensory data and explored using Principal Component Analysis (PCA) to identify relationships between sensory attributes and volatile aroma compounds. In Figure 3, the chemical data is plotted with the sensory data as vectors. Vectors that are closely plotted with one another demonstrate a high correlation between those variables and may indicate a cause-and-effect relationship between chemical compounds and sensory attributes perceived. The Kensington Pride variety that had high scores for terpene aroma also had high levels of α-terpinolene, limonene and α-phellandrene. This aroma is distinctive for this variety and is considered to be a desirable flavour to target in the breeding program. It is likely that these compounds, particularly α-terpinolene, play a role in the perception of this important flavour attribute.

In the top right of the PCA plot (Figure 2), the cut grass aroma correlates well with hexanol and cis-3-hexanol. Both of these have strong grassy aromas as neat compounds. The Indian variety, with higher levels of melon aroma also had high levels of β-ocimene, furanone, alloocimene and carophyllene.

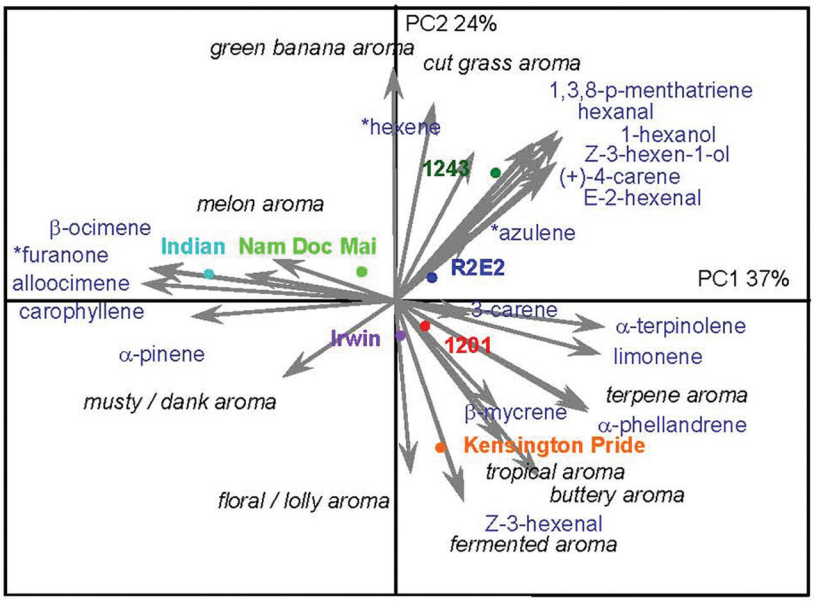

Figure 3. PCA bi-plot of sensory and volatile results for 2006 season mango fruit (n = 5) (PC1 versus PC2).

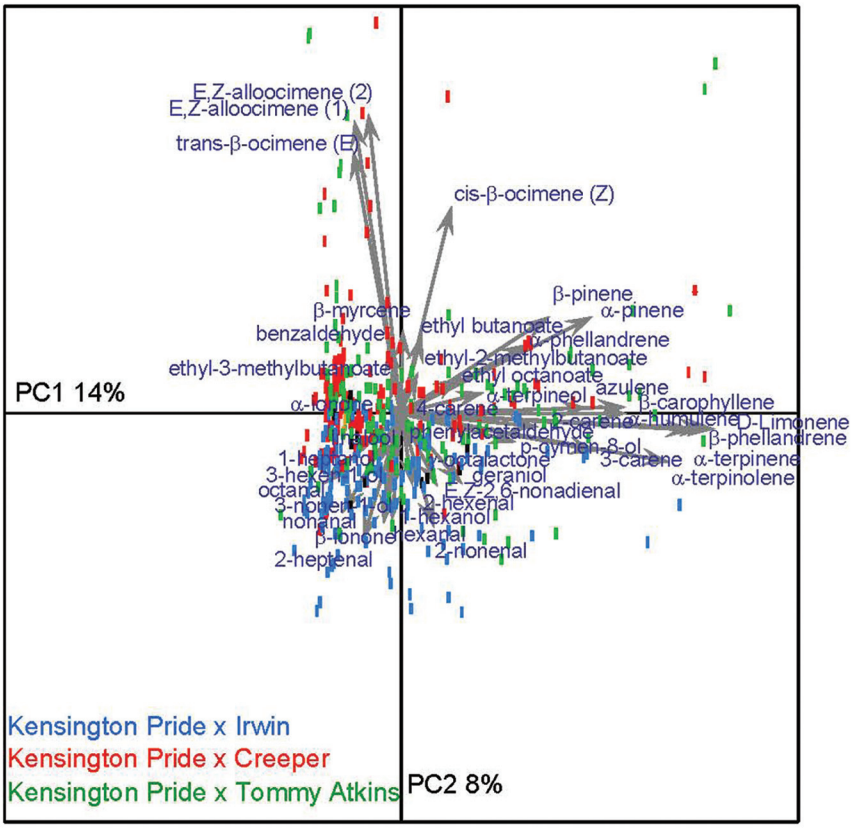

Figure 4. Summary of volatile compound results for three hybrid populations of mango.

The compounds identified as important to the sensory attributes of these varieties were targeted for quantitative method development so that a rapid and routine GCMS analysis could be applied to the varieties of mango in the hybrid populations. These populations included Kensington Pride cross Irwin, Kensington Pride cross Tommy Atkins and Kensington Pride cross Creeper (a dwarf variety). More than 400 varieties have been analysed to date. A PCA plot of the results from the chemical analyses of the hybrids is shown in Figure 4.

According to the PC analysis, the three populations were fairly overlapped in terms of volatile composition and did not neatly separate into clusters. This is not surprising considering the common parent (Kensington Pride) to all the hybrids. The Irwin hybrids were slightly separated as they cluster towards the bottom of the plot (Figure 4) and were distinguished as having low levels of ocimene and alloocimene and higher levels of aldehydes. The most important separation of the samples, which is shown across the PC1 axis, was due to the difference in concentration of terpenes, specifically, α-terpinolene, phellandrene, limonene and carophyllene. Most of the hybrids from all the populations had relatively low levels of terpenes (left half of the plot) while only a few hybrids had moderate and very high levels of terpenes (on the right half of the plot). It is the samples on the right side of the plot which, according to our sensory investigations, are also likely to have high levels of the desirable *terpene* aroma due to the higher concentration of α-terpinolene in these samples.

The flavour data collected from these populations is currently being linked to the database of expressed genes and associated DNA markers to identify genes that relate to α-terpinolene production and the biosynthesis of other volatile aroma compounds in mango fruit.

Conclusion

Without a good eating experience, health and nutritional value are not, on their own, good enough reasons for a consumer to actually eat a particular fruit. Increasing 'functionality' of fruits beyond 'normal' levels could have negative consequences on organoleptic attributes and may result in a decrease in consumption probability despite persuasive claims about health (Krystallis et al., 2008). Understanding and optimising a balance between desirable flavours and bioactivity in tropical fruits is therefore vital to ensure consumer acceptability and success of commercial products in a competitive fruit marketplace. These two studies demonstrate the broad range of techniques that can be applied to understand the flavour quality of tropical and exotic fruits and ensure consumer satisfaction.

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Fruit marketing and health

Influence of treatments on retaining nutritional compounds in fresh-cut fruit

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Keywords: fresh-cut, fruit, health, retain, polyphenols, quality

Abstract

According to the World Health Organization, the prevalence of overweight and obese people is increasing, especially in children, to levels previously unheard of. This epidemic which is linked to lifestyle, could herald a real public health crisis. Young adults face increased risk of heart disease at 30 or 35 years, and a reduction in life expectancy. Therefore, prevention of obesity right from infancy by healthy eating and physical activity is recommended. Fruits are an important part of a healthy diet and provide vitamins, polyphenols, minerals and fiber. In spite of this, the consumption of fruits and vegetables by children and teens continues to decline. A way to increase this consumption is to offer fruit as a ready-to-eat 'fresh-cut' product. Demand for fresh-cut fruits is increasing globally. However, wounding of fruit tissues promotes the production of ethylene, which hastens senescence and induces a number of physiological disorders that need to be minimised to obtain fresh-like, quality products. The content of potentially bioactive compounds in fresh-cut fruit can also be affected by factors such as genotype, pre-harvest factors, maturity stage, and processing operations such as cutting, washing, packaging and storage. Current treatments to retain the nutritional quality in fresh-cut fruit include storage under low temperature, controlled atmospheres (CA) or under modified atmosphere packaging (MAP), chemical preservatives and heat treatment. The effect of these treatments for reducing nutritional losses in fresh-cut fruit will be discussed.

Introduction

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health such as the occurrence of chronic diseases, including diabetes, cardiovascular diseases and cancer. Childhood obesity is associated with a higher chance of premature death and disability in adulthood. The WHO further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. According to WHO, the fundamental cause of obesity and overweight is an energy imbalance between calories consumed on one hand, and calories expended on the other.

The strategy to counter obesity (WHO, 2006) calls upon all stakeholders, public and private, to take action at global, regional and local levels. This is especially important for the most vulnerable in society—the poor and children—who have limited choices about the food they eat and the environments in which they live. Initiatives by the food industry to reduce the fat, sugar and salt content of processed foods, reduction of portion sizes, to increase introduction of innovative, healthy, and nutritious choices, and to review current marketing practices could accelerate health gains worldwide. For example, in Spain the Health Minister has published the guide 'To prevent childhood obesity'. In this guide they reported children do not like to peel fruit and they prefer fruit such as bananas which are easy to peel and with a pleasant, sweet taste. However, children should consume all kinds of fruit, as there are certain vitamins and minerals that are found in some and not others. They recommend making fruit presentation more palatable: Fruit on a stick inserted as a brochette, with squares of cut apple slices mixed with strawberry slices, include a colored straw in a juice, etc. An ideal fruit presentation would be as fresh-cut fruit. Having ready-to-eat fresh-cut fruits

and vegetables at home is likely to encourage children and adults to eat these more healthy snacks than many processed snack foods. Companies and the scientific community should participate in this strategy researching new varieties of fruit and vegetables rich on vitamins and new presentation of these products as fresh-cut. The aim of the present work was to review the main strategies used for keeping the potentially bioactive compounds in fresh-cut fruit.

Fresh-cut produce

Fresh-cut produce is defined as any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or pre-packed to offer consumers high nutrition, convenience and value while still maintaining freshness (IFPA, 2007).

However, wounding of fruit tissues induces a number of physiological disorders that need to be minimised to obtain fresh-like quality product (Soliva-Fortuny and Martín-Belloso, 2003). Anti-oxidant constituents are susceptible to degradation when exposed to oxygen or light as when the interior of the fruit is revealed by cutting (Klein, 1987). Oxidation also occurs on exposure to acidic pH or halides, such as hypochlorite used for sanitation (Wright and Kader, 1997a). Wounding also promotes the production of wound ethylene (Watada et al., 1990), which hastens senescence, including the oxidation of fatty acids by lipoxygenase, during which carotenoids may be degraded by co-oxidation (Thompson et al., 1987). Fresh-cut fruits are still under study because of the difficulties in preserving their fresh-like quality during prolonged periods (Soliva-Fortuny and Martín-Belloso, 2003).

Gil et al. (2006) determined that fresh-cut fruits maintained their nutritional quality, including vitamin C, carotenoids, and phenolics, in comparison with the whole fruit when both are held for up to 9 days at 5°C. These authors found that, in general, fresh-cut fruits visually spoil before any significant nutrient loss occurs. The post-cutting life based on visual appearance was shorter than 6 days for fresh-cut kiwifruit and shorter than 9 days for fresh-cut pineapple, cantaloupe, and strawberry. On the other hand, fresh-cut watermelon and mango pieces were still marketable after 9 days at 5°C. Losses in vitamin C after 6 days at 5°C were ≤ 5% in mango, strawberry, and watermelon pieces, 10% in pineapple pieces, 12% in kiwifruit slices, and 25% in cantaloupe cubes.

No losses in carotenoids were found in kiwifruit slices and watermelon cubes, whereas losses in pineapples were the highest at 25% followed by 10–15% in cantaloupe, mango, and strawberry pieces after 6 days at 5°C. No significant losses in total phenolics were found in any of the fresh-cut fruit products tested after 6 days at 5°C.

Total carotenoid contents decreased in cantaloupe cubes and kiwifruit slices, but increased in mango and watermelon cubes in response to light exposure during storage at 5°C for up to 9 days. In general, fresh-cut fruits generally have a more complicated physiology than fresh-cut vegetables. In addition, stage of ripeness may alter the physiological response to cutting.

The potentially bioactive compounds in fresh-cut fruit can also be affected by factors such as environmental and pre-harvest factors, genotype and processing operations.

Factor impact on bioactive compound in fresh-cut product

Environmental and pre-harvest factors

These factors have a strong impact on quality of intact product and consequently, on nutritional quality of fresh-cut product. Environmental factors including temperature, light intensity, light quality, altitude, soil, pH, soil type, fertilisers, production practices (organic versus conventional and field versus green house), irrigation, pests, and pollution, fruit or vegetable size affect the concentration of ascorbic acid, β-carotene, and/or folic acid in harvested fresh produce. A very good review has been

reported by Lester (2006) in this topic. Other factors influencing nutrient content such as maturity at harvest, harvesting method, and post-harvest handling (Kader, 1988; Lee and Kader, 2002) have been also reviewed.

Genotype

Breeding programs for fruit has been aimed at improving the yield and fruit size, the resistance to diseases and pests, the adaptation to particular growing systems and the harvesting speeds (i.e. reducing harvesting costs). Recently, research has been also focused on the quality of fruit (sensorial and nutritional). Nutritional quality is strongly affected by the type of fruit, the species and the variety within species (Capocasa et al., 2008). Perkins-Veazie et al. (2001) reported the lycopene content in fresh-cut watermelon can vary among cultivars. Perkins-Veazie and Collins (2004) found 'Summer Flavor 800' (seeded) watermelons were significantly higher in lycopene content than 'Sugar Shack' (seedless) regardless of storage interval up to 10 days at 2°C. Another example is the cultivar differences between 'Champaka' and 'Premium Select' pineapple (Marrero and Kader, 2006). A higher level of total ascorbic acid (TAA), β-carotene, total phenolics and anti-oxidant activity is present in 'Premium Select' pineapple compared to 'Champaka'. Breeding programs are constantly creating new cultivars with improved quality. Melon seed companies are also obtaining long and medium life melon cultivars which have a higher sugar content and firmer pulp. Silveira et al. (2007) found a firmer pulp in long life 'Galia' melon than in conventional ones. However, the conventional 'Galia' melon had a higher content in total vitamin C, followed by the medium and long life cultivars (Figure 1). These differences among cultivar were maintained during storage.

To select the correct cultivar with maximum nutritional proprieties will be especially critical with the product destined for the fresh-cut market.

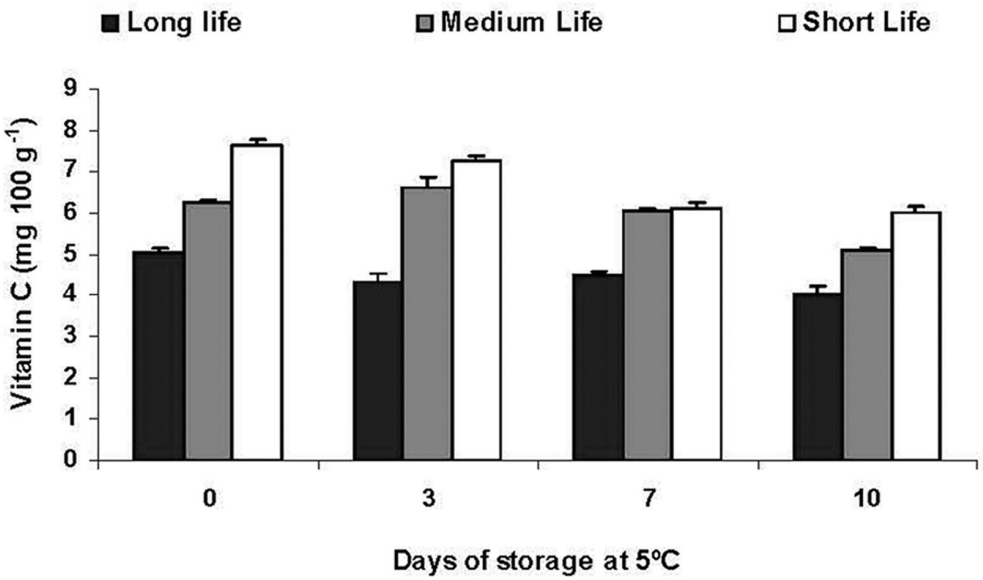

Figure 1. Levels of vitamin C in long-life ('Cyro'), medium ('Solarking') or short-life ('Galápago') fresh-cut 'Galia' melon stored for 10 days at 5˚C in air.

Processing operations—cutting and piece size

The physical damage or wounding caused by preparation of fresh-cut product increases respiration and ethylene production within minutes. This is associated with increases in rates of other biochemical reactions responsible for changes in color (including browning), flavor, texture, and nutritional quality (sugar, acid, vitamin content). This phenomenon has been observed for lettuce, melon, watermelon, tomato, celery, cabbage, etc. (Varoquaux and Wiley, 1994, Cantwell and Suslow, 2002, Gómez and Artés, 2005, Aguayo et al., 2004, Martínez et al., 2005).

The degree of processing also significantly affects the wounding response and is a major factor influencing shelf life and quality. The response of tissue to wounding usually increases as the severity of the injury increases. Another example is in fresh-cut lettuce; it has been found that the smaller the size the bigger the respiration rate and ethylene production, which stimulates the biosynthesis of enzymes, such as phenylalanine ammonia-lyase (PAL), which is related with senescence (Martínez et al., 2005). Also, the fact of presenting a great exposed surface area induces a higher dehydration, and consequently a high weight loss. At the same time, damaged tissues usually synthesise different metabolites (as lignin and cumarins) in order to heal the induced wounds and as a way of defense from the pathogens present. Lee and Kader (2002) reported that the ascorbic acid contents in most vegetables decrease when brushing, trimming and cutting occurs. However, Silveira et al. (2007) did find small differences among kind of cut (trapesoidal, slices and cylinder) in fresh-cut 'Galia' melon (Figure 2). Loss of vitamin C is also accelerated by water loss (Nunes et al., 1998) and its relative stability or degradation is highly dependent on temperature and commodity type (Klein, 1987).

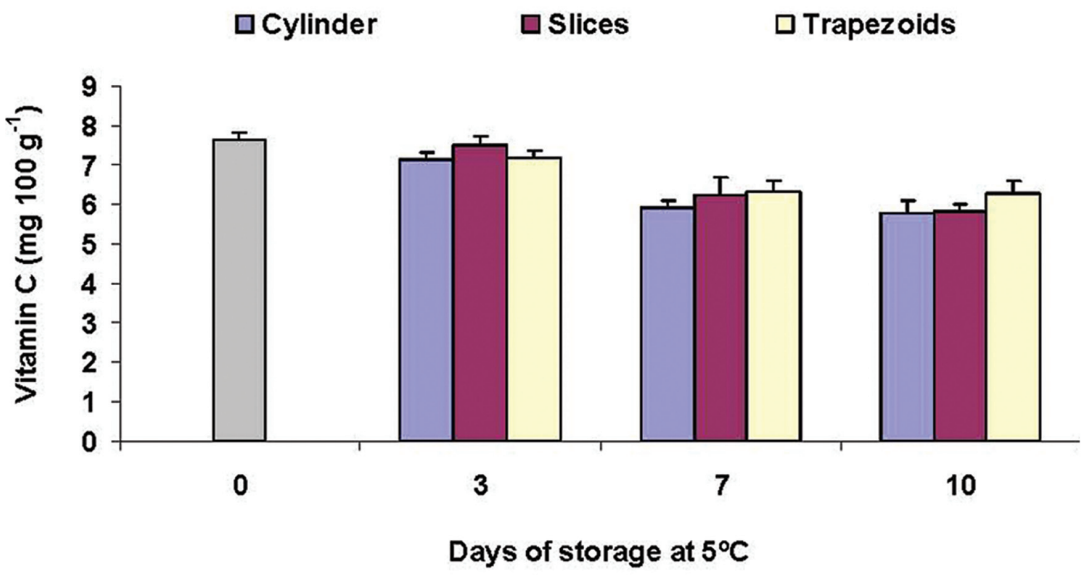

Figure 2. Vitamin C according the kind of cutting (cylinders, slices or trapesoids pieces) in fresh-cut 'Galia' ('Galápago') melon stored for 10 d at 5˚C in air.

Vitamins A and C and phenolic compounds could be inactivated as a result of wounding via exposure of interior tissues to light and air, enzymatic or chemical degradation in disrupted tissue, reaction with the products of lipid oxidation, wound ethylene, or exposure to chlorine used for sanitation (Wright and Kader, 1997b). In contrast, it has been also demonstrated that there is an increase in anti-oxidant capacity after wounding. It depends on the type of fruit or vegetable tissue. In a study of zucchini, white and red cabbage, iceberg lettuce, celery, carrot, parsnips, red radish, sweet potato and potatoes, phenolic changes ranged from a 26% decrease to an increase up to 191%, while anti-oxidant capacity changes ranged from a 51 % decrease to an increase up to 442% (Reyes et al., 2007).

One of the main causes of quality losses in fresh-cut products and the major challenge in fresh cut fruits is enzymatic browning (Weller et al., 1997, Toivonen and Delaquis, 2006). The presence of browning is basically due to the oxidation of phenolic compounds catalysed by the polyphenol oxidase enzyme (PPO), originating colourless quinones that later on polymerise forming melanins. These substances show brown, reddish or black coloration (Castañer et al., 1996). Many fruits (i.e. apple, peach, and pear) and some vegetables (i.e. potato, artichoke) have high levels of preformed phenolic compounds. Following cutting, very rapid surface browning takes place. This disorder has great importance for its high visual impact that decreases the sensorial acceptance by consumers and the marketability, as well as reducing the nutritional value of the products (Artés et al., 1999). In tissue with initial low levels of preformed phenolic compounds (e.g. celery, lettuce) browning results from the induced synthesis and subsequent accumulation of phenolic compounds (Castañer et al., 1996). At the same time, wounding induces synthesis of enzymes involved in browning reactions or substrate biosynthesis. Thus, relative oxidase activities and substrate concentrations can affect browning intensity in diverse tissues and crops. In the biosynthesis of the phenolic compounds, the PAL enzyme plays an important role, whose activity is notably increased by the presence of ethylene. This kind of alteration appears very often in fresh-cut products such as apple, pear, peach, potato, mushroom or lettuce.

Processing operations—washing treatments

There are many sanitisers and strategies in fresh-cut product for keeping an optimal microbial quality. Results on efficacy are well reported for conventional and new sanitisers agents such as chlorine compounds, organic acid formulations (such as peracetic acid, citric and ascorbic acids), chlorine dioxide, hydrogen peroxide, electrolysed water, steamer jet injections and essential oils (eugenol from clove, and thymol from thyme and oregano). The use of non-ionising, germicidal and artificial ultraviolet light (UV) at a wavelength of 190–280 nm (UV-C) is also effective for surface decontamination of fresh-cut products. Ozone (O_3) also acts as a strong oxidising agent, being very effective in destroying microorganisms.

In spite of many studies and even a recent review on the microbial effect of these sanitisers (Artés et al., 2009), there are not many reports about the effect of these sanitisers techniques on the nutritional compound of fresh-cut product.

For instance, Aguayo et al. (2008a) found the washing with neutral electrolysed water for one minute vs chlorine (150 ppm) reduced the anti-oxidant level of fresh-cut vegetables, in particular, the ones with low levels such as Red Chard (Figure 3). Silveira et al. (2009) found washing fresh-cut 'Galia' melon in chlorine (150 ppm) or peroxyacetic acid (68 ppm) reduced the vitamin C compared to the ozonated washing (0.4 ppm). In addition, Zhang et al. (2005) found that the vitamin C contents of fresh-cut celery treated with ozonated water (0.03 ppm) were significantly higher than that of non-treated material. It is possible that the O_3 had a negative effect on the activity of the major enzymes involved in the vitamin C degradation, ascorbate oxidase and ascorbate peroxidase. Aguayo et al. (2006) reported higher contents in ascorbic acid in tomatoes slices and whole tomatoes stored in ozone-enriched air applied cyclically (4 ± 0.5 μLL^{-1} O_3 for 30 minutes every 3 hours). Perez et al. (1999) attributed this effect to the activation of an antioxidative system that promotes the biosynthesis of vitamin C from the carbohydrate pool. The ascorbate function as an anti-oxidant is ensured by action of the ascorbate-glutathione cycle, which provides an efficient mechanism for recycling oxidised ascorbate and keeping the ascorbic acid (AA) reservoir in its reduced form (Foyer, 1993).

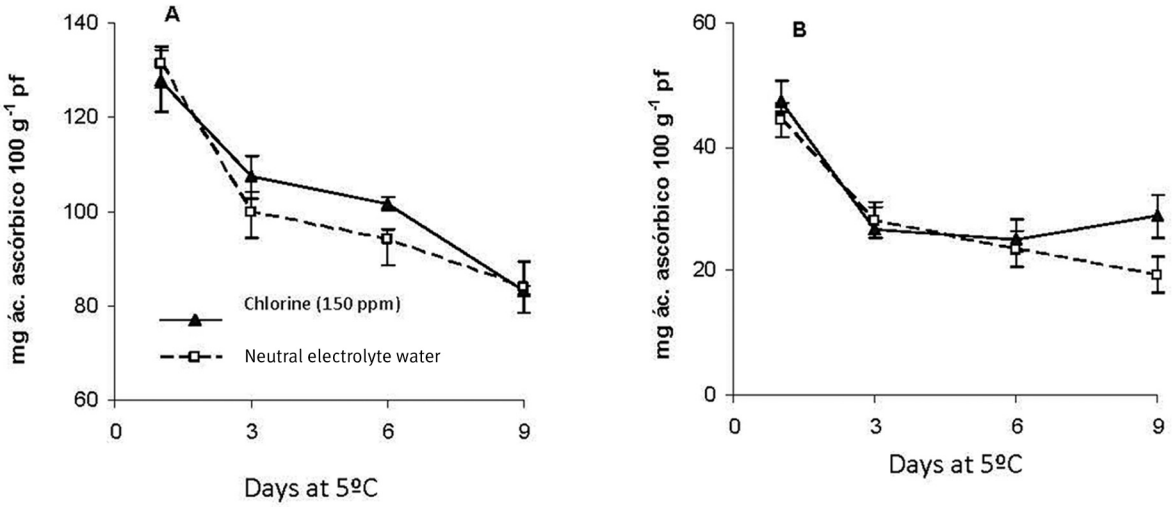

Figure 3. DPPH (mg AA 100 g-1) in Tatsoi (A) and Red Chard (B) washed using chlorine (150 ppm) or neutral electrolyse water and stored 9 days at 5˚C in MAP.

Main techniques to preserve nutritional quality

In order to achieve fresh-cut fruit and vegetables with fresh-like quality, safety and high nutritional value the industry needs to introduce or combine new techniques. The current trend is to fit a system that delivers very high quality products, nutritionally healthier and free from additives (Artés et al., 2009). The major preservation techniques applied to prevent or delay decreases in total quality are control of temperature (chilling storage) and modified atmosphere packaging (MAP), edible coatings, combined with chemical coadjutants, application of heat treatments (Leistner and Gould, 2000) and even the use of the ethylene inhibitor 1-MCP. One of the main factors to focus on is the control of the wound response which is the key to providing a fresh-cut product of good nutritional, sensorial and microbial quality. The importance of this factor has been explained in the processing operations (cutting and piece size).

Temperature

Storage temperature after cutting will certainly influence the effect of wounding on product quality (Toivonen and DeEll, 2002). This is a critical parameter to achieve maximum shelf life of fresh-cut products. To ensure high-quality products, it is recommended that fresh-cut products be kept at temperatures just above freezing; nevertheless, temperature needs to be adequately chosen in order to avoid damage such as chilling injury in sensitive commodities. For instance, chilling injury can decrease the pigment content of flesh in uncut watermelons, but no symptoms of chilling injury were found on the fresh-cut watermelon flesh stored at 2ºC.

Marrero and Kader (2006) found the main factor that affected the quality of fresh-cut pineapple was temperature. The post-cutting life of pulp pieces varied from 4 days at 10ºC to over 2 weeks at 0°C. Artés et al. (1999) determined that total quality of packaged tomato slices was better maintained at 2°C than at 10°C. This was associated with the fact that ethylene and respiration production rates were increased by cutting at the higher storage temperature as opposed to the lower storage temperature. The prediction of respiration rate is a very important factor to determine the losses in nutritional compounds. Table 1 illustrates how sugar levels decrease in fresh-cut ‘Cantaloupe’ melon when it was stored at 5°C versus 0°C. Low temperatures helped to keep the sugar content. A higher respiration rate was obtained at 5°C indicating a more active metabolism and usually a faster deterioration rate and decrease in nutritional compounds (Aguayo et al., 2004).

Table 1. Sugars content (g L-1) in fresh-cut Cantaloupe melon stored in air up to 10 days at 0 or 5˚C.

Time	Temperature	Fructose	Glucose	Sucrose
0 days		16.3 a	15.4 a	68.9 a
10 days	0˚C	15.3 a	14.6 a	62.9 b
	5˚C	15.3 a	9.4 b	57.7 c

Controlled and modified atmosphere (CA and MAP)

The principle behind CA and MAP technologies is to reduce the rate of respiration, reduce microbial growth, and retard enzymatic spoilage by changing the gaseous environment surrounding the food product. This is achieved by reducing the concentration of oxygen (O_2), which is required in respiration, or by adding an inhibitory gas such as carbon dioxide (CO_2). The balance between O_2 and CO_2 is critical, and an optimal ratio is required for each specific product (Kader et al., 1989).

The major difference between CA and MA storage is in the degree of control of the gaseous composition of the storage atmosphere. The CA implies a higher degree of control than MA in maintaining specific levels of O_2, CO_2, and other gases.

There are some post-harvest treatments which undoubtedly improve food quality by inhibiting the action of oxidative enzymes and slowing down deleterious processes. Storage of fresh fruits and vegetables within the optimum range of low O_2 and/or elevated CO_2 atmospheres for each commodity reduces their respiration and C_2H_4 production rates (Kader, 1986). Optimum CA retards loss of chlorophyll, biosynthesis of carotenoids and anthocyanins, and biosynthesis and oxidation of phenolic compounds. In general, CA influences flavour quality by reducing loss of acidity, starch to sugar conversion, and biosynthesis of aroma volatiles, especially esters. Retention of ascorbic acid and other vitamins results in better nutritional quality, including anti-oxidant activity, of fruits and vegetables when kept in their optimum CA (Kader, 1986). However, little information is available on the effectiveness of CA or MAP on the nutrient retention during storage.

In rocket salad stored up to 14 days at 5˚C, moderate levels of O_2 as 5%O_2 + 5% CO_2 followed by 5%O_2 + 10% CO_2 resulted in a higher total vitamin C levels than samples stored in air reached in 10% of CO_2 or air (Martínez-Sánchez et al., 2008). However, in fresh-cut products, high CO_2 concentration in the storage atmosphere has been described to cause degradation of vitamin C. Thus, concentration of 5, 10, or 20% CO_2 caused degradation of vitamin C in fresh-cut kiwifruit slices (Agar et al, 1999). Enhanced losses of vitamin C in response to CO_2 higher than 10% may be due to the stimulating effects on oxidation of AA and/or inhibition of dehydroascorbic acid (DHA) reduction to AA (Agar et al., 1999).

Wright and Kader (1997a) found no significant losses of vitamin C occurred during the post cutting life of fresh-cut strawberries and persimmons for 8 days in CA (2% O_2, air + 12% CO_2, or 2% O_2 + 12% CO_2) at 0°C. In studies of cut broccoli florets and intact heads of broccoli CA/MAP resulted in greater AA retention and shelf-life extension in contrast to air-stored samples (Barth et al., 1993; Paradis et al., 1996). Retention of AA was found in fresh-cut lettuce packaged with nitrogen (Barry-Ryan and O'Beirne, 1999). They suggest that high levels of CO_2 (30–40%) increased AA losses by conversion into DHA due to availability of oxygen in lettuce (Barry-Ryan and O'Beirne, 1999). The reduction of AA and the relative increase in DHA could be an indication that high CO_2 stimulates the oxidation of AA, probably by ascorbate peroxidase as in the case of strawberries (Agar et al., 1997) and of spinach (Gil et al., 1999). Mehlhorn (1990) demonstrated an increase in ascorbate peroxidase activity in response to ethylene. High CO_2 at injurious concentrations for the commodity may reduce AA by increasing ethylene production and therefore the activity of ascorbate peroxidase. Ascorbate oxidase from green zucchini fruit, which catalyses the oxidation of AA to DHA, has been found to be

unstable and to lose activity below pH 4 (Maccarrone et al., 1993). This could partially explain the lower DHA content of the strawberries (pH 3.4–3.7) and the higher DHA content of the persimmons (pH 5.4–6.0) (Wright and Kader, 1997a) as well as the tendency of some vegetables at pH near to neutral to lose AA during storage (Gil et al., 1998). In the same way, high O_2 concentrations (> 60%) reduce the vitamin C and anti-oxidant capacity as Odriozola-Serrano et al. (2008) found in fresh-cut tomato.

In conclusion, the loss of vitamin C after harvest can be reduced by storing fruits and vegetables in atmospheres of reduced O_2 and/or up to 10% CO_2 as Lee and Kader (2002) have reported. For instance, Odriozola-Serrano et al. (2008) found the combination of 5% O_2 + 5% CO_2 kept vitamin C levels in slices of tomato. CA conditions do not have a beneficial effect on vitamin C if high CO_2 (> 5%) concentrations are involved, although the concentrations above which CO_2 affects the loss of AA must be estimated for each commodity (Kader et al., 1989).

Reduced O_2 or elevated CO_2 are generally considered to reduce the loss of provitamin A, but also to inhibit the biosynthesis of carotenoids (Kader et al, 1989). Reducing O_2 to lower concentrations enhanced the retention of carotene in carrots (Weichmann, 1986). The carotene content of leeks was found to be higher after storage in 1% O_2 + 10% CO_2 than after storage in air (Weichmann, 1986). Wright and Kader (1997b) found a CA of CO_2 > 12%, increased β-carotene degradation in fresh-sliced stored peaches. Petrel et al. (1998) found no changes on the carotenoid content of ready to eat oranges after 11 days at 4°C in MAP (19% O_2 + 5% CO_2 and 3% O_2 + 25% CO_2). Finally, Odriozola-Serrano et al. (2008) found high O_2 levels (80, 60 and 21%) kept the lycopene levels in fresh-cut tomatoes.

CA and MAP also can directly influence the phenolic composition as reflected in the changes observed in anthocyanins. CO_2-enriched atmospheres (> 20%) used to reduce decay and extend the post-harvest life of strawberries induced a remarkable decrease in anthocyanin content of internal tissues compared with the external ones (Gil et al., 1997). Holcroft and Kader (1999) related the decrease in strawberry colour under CO_2 atmosphere with a decrease of important enzyme activity involved in the biosynthesis of anthocyanins, PAL and glucosyltransferase. A moderated CO_2 atmosphere (10%) prolongs the storage life and maintains quality and adequate red colour intensity of pomegranate arils (Holcroft et al., 1998). However, the arils of pomegranates stored in air were deeper red than were those of the initial controls and of those stored in a CO_2 enriched atmosphere.

MAP can also have a positive effect on phenolic-related quality, as in the case of the prevention of browning of minimally processed lettuce (Gil et al., 1998). In addition, modified atmosphere packaging of minimally processed red lettuce (2–3% O_2 + 12–14% CO_2) decreased the content of flavonol and anthocyanins of pigmented lettuce tissues when compared to air storage (Gil et al., 1998). However, CAs of 21% O_2 + 10% CO_2; 5%O_2 + 5% CO_2 or 5%O_2 + 10%CO_2 reduce the decrease of total phenolic compared to air, 13% vs 31% in rocket stored up to 14 days at 5ºC (Martínez-Sánchez et al., 2008). Odriozola-Serrano et al. (2008) also found the CA (5% O_2 + 5% CO_2) kept the phenolic compounds in slices of tomato. They supported low level of O_2 keeps the phenolic compounds meanwhile high level of O_2 (> 60%) reduce the content.

Edible coatings

One possible ‘packaging’ method for extending the post-harvest storage of fresh-cut product is the use of edible coatings. These are thin layers of material that can be eaten by the consumer as part of the whole food product. Coatings have the potential to reduce moisture loss, restrict oxygen entrance, lower respiration, retard ethylene production, seal in flavour volatiles and carry additives, such as anti-oxidants, that retard discoloration and microbial growth (Baldwin et al., 1995). This technology is still emerging, but there have been several successful developments using an array of materials, including lipids, polysaccharides, and/or proteins as the base components in the coatings. Functional additives are expected to improve the benefits from using these coatings. Potential preservative additives that are being considered are benzoic acid, sodium benzoate, sorbic

acid, potassium sorbate, and propionic acid (Baldwin et al., 1995). Potential anti-oxidant additives include ascorbic acid, citric acid, phosphoric acid, and other compounds. A cellulose-based edible coating applied to cut apples and potatoes was most effective in controlling moisture loss when the formulation contained soy protein (Baldwin et al., 1996). The addition of ascorbic acid to the formulation delayed surface browning, while the addition of sodium benzoate or potassium sorbate helped to control microbial growth. Adjustment of pH to 2.5 resulted in optimal control of both browning and microbial growth (Baldwin et al., 1996).

Oms-Oliu et al. (2008) studied the effect of incorporation of N-acetylcysteine at 0.75% and glutathione at 0.75% into edible coating formulations based on alginate (2%), pectin (2%) or gellan (0.5%) in fresh-cut pears. They found the use of polysaccharide-based edible coating by themselves did not contribute to maintain or increase anti-oxidant capacity of fresh-cut pears. However, the incorporation of N-acetylcysteine and glutathione best maintained the anti-oxidant capacity, increased vitamin C and phenolic content of both uncoated and coated pear wedges. The incorporation of anti-oxidant agents into edible coating helped to prevent nutritional losses.

Heat treatments

The use of mild heat treatments has been found to have profound physiological effects on fresh-cut fruit products and they can be very useful as a quality preservation agent. Heat-shock is a high temperature short time (HTST) method which is usually implied a washing step at a temperature ranging 45–70ºC for a few minutes, usually less than 5 minutes (Loaiza-Velarde et al., 1997). A heat-shock treatment that reduces browning, keeping the phenolic compounds, in fresh-cut lettuce (e.g. 90 s at 45°C) may work by redirecting protein synthesis away from the production of wound-induced enzymes of phenolic metabolism, and toward the production of innocuous heat-shock proteins (Saltveit, 2000).

The mechanism of the response to heat treatments relates to their effect on physiological processes; heat treatment inhibits ethylene synthesis, tissue response to ethylene, and cell wall degradation associated with hydrolytic enzymes such as polygalacturonase and galactosidases (Lurie and Klein, 1990). While respiration is initially enhanced, it subsequently falls below the level found in nontreated whole products. These effects may explain why heat treatments inhibit cutting-induced changes in fruits and vegetables and help to keep the nutritional quality in fresh-cut product.

1-Methylcyclopropene

One way to inhibit the ethylene action is the use of 1-methylcyclopropene (1-MCP). Ripening and senescence of intact and fresh-cut fruit have been delayed by 1-MCP action (Jiang et al., 1999; Aguayo et al., 2006; Vilas-Boas and Kader, 2006). 1-MCP is effective in delaying further ripening of partially ripe fresh banana (Pelayo et al., 2003), so it has potential commercial value to slow the changes associated with loss of quality and to extend the shelf-life of fresh-cut fruit, as shown for fresh-cut banana (Vilas-Boas and Kader, 2006). In pineapple, 1-MCP decreased respiration, browning and hydrolysis of ascorbic acid (Buda and Joyce, 2003). Ergun et al. (2006) reported that slices made from 1-MCP-treated papayas had double the shelf-life of slices made from untreated papayas. In this case, 1-MCP contributed to keep the total quality (sensorial and nutritional) in fresh-cut papayas.

Conclusion

Obesity is increasing in the world. One way to reduce the high number of obese people consists in increasing the consumption of fruit and vegetables, in particular, as fresh-cut products which are ready-to-eat. However, fresh-cut product suffers a physical damage or wounding caused by preparation. They increase their respiration and ethylene production and biochemical reactions can decrease the nutritional quality. Appropriate cultivar selection, crop management, post-cutting treatments, and packaging can minimise these effects. However, the effect of these techniques

depends of each commodity and it should be studied for each one. Researchers should investigate and innovate new techniques and products to promote healthy life styles.

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Challenges in the supply chain to improve the sales of tropical fruits for their health benefits

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Keywords: supply chain, horticulture, health, consumers, claims

Abstract

Food Standards Australia and New Zealand (FSANZ) are introducing major changes to the regulation of food labelling and advertising in Australia and New Zealand. In particular, it is removing the blanket prohibition on health claims for foods and is developing new regulations to cover nutrition, health and related claims. The new regulations are promoted by FSANZ as a 'significant and positive change for industry, with a wide range of claims permitted', which 'will enable further innovation in the food industry and will give consumers more information to help them choose the foods that make up their diet'. While these new regulations represent a significant opportunity for tropical fruits, there are a number of challenges that face marketers who use health benefits to promote sales. First, the standards themselves provide a rigorous framework which must be successfully navigated before any claims can be made. Second, due to a daily onslaught of new health messages, the credibility threshold is rising and consumers are becoming increasingly discerning about what they will or won't believe. Third, a positive health attribute does not negate the need traditional consumer requirements such as taste, quality, consistency and convenience. Nevertheless, health benefits are a compelling and worthy tool for promoting tropical fruit consumption.

Introduction

The obvious health benefits to be gained from eating more tropical fruits should result in more sales. However, the issues governing consumer purchase are not that simplistic. This paper reviews some of the hurdles that must be overcome to convince consumers to buy more fruit.

Consumer drivers

The following trends are relevant and favourable to the fruit industry's aim to increase consumer's fruit consumption:

Income

Consumers have more disposable income. In general, they now spend about 12% of their income on food in the home compared with approximately 25% fifteen years ago. In developed countries consumers value other attributes more than low prices. For example, they want what is good for their health as well as food that is produced locally.

In the case of older consumers, there is a trend that is described by social researchers as 'blowing the kids' inheritance", whereby these consumers prioritise spending to meet their own needs rather than saving it to pass on to their children.

Education

Consumers, in particular women, are becoming more educated about the food they buy. This leads to a more discerning consumer who is adventurous and demanding and who asks questions about their food's provenance.

Age

The so-called 'baby boomers' who were born during the demographic post-World War II period, have now reached retirement age. These consumers tend to eat less, eat at home—often alone—and eat healthily.

The trend towards healthy living was triggered by baby boomers that have a near obsession to 'die young late in life'. According to Sydney-based market researcher, Liz Dangar, it is a trend that stems from an increased concern with 'looking and feeling good today' or 'being at our best' rather than being based on concerns about disease prevention. While heart health and cancer have been the primary health concerns in the past, the main health concerns today are obesity and weight management. Fruit consumption can benefit many people wanting to lose weight.

Good for the planet

Food and food production are now being viewed more holistically. Consumers are more discerning; of course, they generally select foods that are good for themselves or their families, but now the food products have to be environmentally and socially sustainable as well. All aspects of a food's credentials are being scrutinised.

Fair Trade

Among the questions consumers ask themselves, are:

- Have pesticides been used to produce the food?
- Has the food been genetically modified?
- Is the product over-packaged?
- Is the packaging recyclable or biodegradable?

Fresh and natural

Connected with consumers' concerns about their own health and that of the planet's, is the desire for fresh and natural food. They want unadulterated products that are as close to their original state as possible. Indications of this are the rise in popularity of growers' markets and the increase in organically-farmed food.

Upgrading the palette

Consumers are becoming more adventurous with their food choices. Higher education levels and increased disposable income, along with ethnic influences and an increased propensity to travel, are all leading to more sophisticated tastes and the desire to experiment.

Quality and taste

Quality and taste are still at the top of the consumer's list of required attributes. A pleasurable eating experience reinforces the perception that a piece of fruit is healthy.

'I'm an individual'

While trend spotting is about finding consistencies in consumer behaviour it's not about adopting a standardised approach. Contemporary consumers like to think of themselves as individuals. They want to feel that producers and retailers are going to special lengths to meet their personal needs even if those needs are the same as everyone else's.

'Don't bore me'

Consumers are fickle. They want constant change, new things and different experiences. Every decision a consumer makes is not necessarily rational or practical but may be based on how the decision makes them feel. Worldwide, researchers are already looking at the impact of food on mood, brain function and cognition.

A report for the Centre for Food and Health Studies in the UK outlined the 10 key trends for food, nutrition and health in 2008 (New Nutrition Business, 2008). Of the 10 trends, 7 were directly relevant to fruit and vegetables. The report stated: 'The trifecta of success is to have some real or perceived health benefit which can meet consumer needs for convenient healthy snacking and which also has the advantage of providing benefits in an all-natural form – fruit and vegetables are true super foods!'

Supply chain factors

The supply chain is defined as every step or relationship – from grower to retailer – that contributes to the satisfaction of the consumer. In addressing the supply chain, it can be useful to think in terms of the traditional '4 Ps' of marketing.

Product

- adoption of growing techniques/protocols
- achieving production volumes/diversity
- post-harvest protocols
- packaging
- labelling

Price

- price points
- demand and supply forecasting

Place

- length and integrity of supply chain
- monitoring and quality assurance
- distribution

Promotion

- target
- positioning
- brand/image
- advertising and public relations

Case study – Vital Vegetables

While not concerned with tropical fruit, a current Horticulture Australia Limited (HAL) project that epitomises the challenges of addressing all '4Ps' is the Vital Vegetables project. This is a collaborative project undertaken by Australian/New Zealand government and industry and research institutes that to date has been a $20 million, 5-year exercise with a product has yet to be launched.

Vital Vegetables is the development of fresh, flavoursome and functional vegetables with higher phytonutrient content. An example is 'Booster broccoli', which contains double the normal antioxidant content. (http://vitalvegetables.com.au)

HAL is investing so much in Vital Vegetables because Australia and NZ have high production costs and their growers are competing against cheaper imported products. If they can't compete on price, they need to compete on quality, value and other features and benefits. As health is a key influencer of purchasing behaviour, this research and development has been conducted to create a new 'functional produce' category.

Vital Vegetables is a comprehensive program with five subprograms covering all of the '4 Ps'. The subprograms are:

Evaluation of bioactives

Developing analytical tools and methodologies to screen germplasm, understand metabolic processes and identify new bioactive compounds.

Bioefficacy

Validating efficacy using biomarkers, cell culture and animal models, bioavailability and compound interaction and the digestibility of functional carbohydrates.

Product enhancement

Assessing new 'functional' cultivars screened across varied production and postharvest protocols as well as molecular approaches to control freshness.

Processing and food properties

Defining health and ingredient properties of vegetable polysaccharides and exploring extraction and preservation technologies for processed foods and supplements.

Market evaluation

Undertaking market and consumer analysis to determine the target markets, their needs and expectations as well understanding market competitors. This also includes developing commercialisation and business strategies, setting priorities and product testing programs.

A number of consumer studies were undertaken to provide assurance that Vital Vegetables would meet a demand and wouldn't cannibalise other vegetables or compromise their standing in the eyes of consumers.

Negotiations regarding commercialisation arrangements are underway with the imminent launch of the first Vital Vegetable, 'Booster Broccoli'. In this instance, six of the top fruit and vegetable marketers have formed a consortium to meet all the demands of the 4 Ps to ensure a successful launch.

Case study – lychees

An example of a smaller scaled project investigating supply chain issues is another jointly HAL-funded project being undertaken by Scott Ledger at the Queensland Department of Primary Industries and Fisheries, which identified that retail merchandising still remains a major constraint to improving fruit quality and consumer demand for lychees.

Seven supply chains were monitored for handling conditions and fruit quality, from packing on farms in North and South East Queensland, to a wholesale distribution centre in Brisbane. Each supply chain was mapped for the varieties Kwai May Pink and Bengal.

Temperature through the supply chain was found to be variable and often too high. The life of the Kwai May Pink variety in bulk packs was discovered to be short (less than 5 days). Kwai May Pink browned quickly when removed from bulk bags; the older the fruit, the quicker the browning.

A range of edible surface coatings and acid treatment were tested but none of the coatings improved the red life of lychee and the acid treatment proved incompatible with the coatings.

Trials to investigate a range of pre-packing options showed that the selection of the plastic film is important to minimise moisture loss, prevent fogging of the package, and allow sufficient gas permeability.

Although it will be challenging to achieve significant improvement in retail quality, the supply chain handling system needs to be re-engineered with retail-ready pre-packs as the core innovation.

Health claims

Despite people's desire for healthy foods and a multitude of health messages conveyed by marketers, there is considerable confusion amongst consumers about the exact health properties provided by individual foods.

In a detailed study of 367 products lead by Dr. Charlene Elliott from the Canadian Institute of Health Research, 89% of children's food products (specifically excluding confectionery, soft drinks and bakery items) provided poor nutritional quality, but 62% of them still made health claims. (Elliot, 2008)

Health claims are regulated in every country to a greater or lesser degree. Strict regulations exist in the USA and the European Union is looking at harmonising existing regulations.

In Australia there is the *Trade Practices Act* 1974 as a shared Food Standards Code with New Zealand. There is also a proposed new Standard 'Nutrition, Health and Related Claims'.

Regardless of the country, there are some general principles that need to be understood in order to legally communicate health benefits of any food product:

- Claims must be reasonable, i.e. not distorted, misleading or deceptive (It is possible to mislead and deceive by silence or half-truths)
- Claims must be true and capable of substantiation
- The touchstone is the overall impression of a reasonable person
- Messages should claim that no single food alone can markedly impact health.

Nutrition content claims

Nutrition (or nutrient) content claims are statements claiming the amount of a nutrient, energy or a biologically active substance that exists in a food product.

General level claims

A general level (functional) health claim can refer to the presence of a nutrient or substance in a food and to its effect on a health function. This form of claim cannot refer to a serious disease or condition or to an indicator of a serious disease (e.g. blood cholesterol). Under new regulations in Australia and New Zealand, the food will need to pre-qualify via a 'nutrient profiling calculator'.

High level claims

High-level (prophylactic) health claims are the ones that make reference to a serious disease or biomarker claiming a preventing or protecting capability. In Australia and New Zealand these are currently illegal but the new regulations will change that. Pre-approval by FSANZ will be required.

Therapeutic claims

Therapeutic refers to the treatment and cure of disease resulting from consuming certain foods, but therapeutic claims are not legal in most countries because only drugs can have therapeutic claims attached to their marketing.

Pre-approved claims

In Australia there will be seven pre-approved high level claims in the new regulations, one of which links fruit and vegetable consumption to reduction in the risk of heart disease.

In the USA, where similar regulations have been in place for some years, there are still only eleven high-level health claims approved by the Food and Drug Administration (FDA). Three of these relate to fruit and vegetables addressing heart disease and cancer. The fact that there are only eleven indicates the degree of difficulty in achieving a high-level health claim.

In Australia two high-level claims were examined but not substantiated for pre-approval:

- wholegrain cereals and coronary heart disease
- long-chain omega-3 fatty acids and cardiovascular disease.

In gaining substantiation for pre-approved health claims, critical elements include:

- clarity around the dietary element and health effect, i.e. the causal relationship
- totality of the data
- relevance to target group in Australia and New Zealand
- achievability in dietary context.

There are consequences in contravening these laws protecting health claims made by food products, as the recent ‘Ribena’ example demonstrated. The global food and drugs giant GlaxoSmithKline was forced to make public apologies in many countries and pay significant fines after two 14-year-olds from New Zealand found as part of a school experiment that its popular blackcurrant drink ‘Ribena’ contained almost no vitamin C, as had been advertised.

Case study – apples

Within the constraints of the law, however, there is still plenty of scope for getting the message across about the health benefits of your product. HAL recently produced an apple report, which collated and assessed all the existing evidence of the health benefits of eating apples.

This has been translated into a series of advertisements which remain within the current Australian regulations, but manage to communicate, in a compelling way, their benefits. What is required is some clever word play and confidence that consumers will know how to piece together the information to assume there are significant health benefits achieved by eating a particular food.

Health and cost benefits

The European Prospective Investigation into Cancer and Nutrition research project (EPIC), is designed to investigate the relationships between diet, nutritional status, lifestyle, environmental factors and the incidence of cancer. It has found that high levels of fruit and vegetables reduce the risk of dying early from any cause by 20 per cent. Researchers indicate that people could gain this benefit by eating just one extra portion of fruit or vegetables a day (http://epic.iarc.fr/).

In monetary terms, a business case funded by the Australian Commonwealth Government and documented in the report, 'Better Health: It’s Simple' Business Case for a National Fruit & Vegetable Campaign (Australian Fruit and Vegetable Coalition, 2003) found that one extra serve of fruit and vegetables per day would deliver a potential saving of $513 million per annum in relation to cancer and cardiovascular disease alone.

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Biodiscovery technologies

The genomics of fruit quality

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Keywords: translational genomics, bioinformatics, functional testing, candidate genes, transient assays, transgenic plants

Abstract

Consumers of whole foods, such as fruits, demand consistently high quality and the development of new varieties with enhanced health, convenience, novel taste, and a reduced impact on the environment. Conventional breeding of temperate fruit crops such as apples and kiwifruit, the focus of *HortResearch*, exploits both existing cultivars and the extensive germplasm collections of related species and novel accessions. Our genomics research is focused on the key producer and consumer traits. We achieve this by defining the biology of our key fruit traits and developing an understanding of the processes in model plants. Our translation genomics research then transfers this molecular information to our target crops. To do this, we have developed an extensive fruit expressed sequence tag database and are in the process, through collaboration, of developing a whole genome sequence for these crops. In our work on fruit colour, we have described both the metabolic and regulatory genes involved in anthocyanin accumulation, through sequencing and functional conservation with genes from model plants.

Introduction

Although different plants have very distinct forms, the basic molecular mechanisms necessary for plants to grow and reproduce are conserved between species. This is well illustrated by the ABC model of the flower development (Coen and Meyerowitz, 1991) that has proven able to be used to describe aspects of almost all flowering plants. It is this conservation of molecular function between plants that has allowed the dissection of complex traits in crop species. This is possible because orthologous genes that are described in model plants such as *Arabidopsis*, maize and *Antirrhinum* can also be identified and characterised in crop plants. In many cases, there is a high degree of conservation in function between the genes of model and crop plants. These similarities act to highlight the relatedness of function of very diverse plants. The advantage of exploiting model plants to dissect complex traits in crops is very clear.

Model plants usually have short generation times: an advantage possessed by the pea as a model system over the apple (Knight, 1799). More recently, good plant models also need to have an effective transformation system and extensive genomics resources such as is found in *Arabidopsis* and rice. In addition, the ability to generate and identify mutants is a desirable attribute of model plants, including the transposon-induced mutation of both *Antirrhinum* and maize. With the advent of high throughput sequencing methods and the increasing number of plant genomes that have been or are being sequenced (Zahn et al., 2008), it is now easier than ever to identify candidate genes from crop plants, using the knowledge gained from research on model plants. Here we show, with an example, how *translational genomics* has been used in our research to identify candidate genes, to demonstrate conservation of function, and to help understand the mechanism of anthocyanin biosynthesis in apple.

Materials and methods

Protein and DNA sequences, from genes of known function, were analysed using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) to search the apple sequence database (Newcomb et al., 2006).

Full length sequences were aligned and clustered using Clustal W (opening = 15, extension = 0.3) using Vector NTI 9.0 software. Conserved motifs were extracted and re-aligned as above. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar et al., 2004), using the minimum evolution phylogeny test and 1000 bootstrap replicates. Candidate genes were then cloned in front of the cauliflower mosaic virus 35S promoter into binary vectors and used in transient infiltration assays (Hellens et al., 2005). *Nicotiana tabacum* cv. Samsum plants were grown under glasshouse conditions and anthocyanin accumulation was assayed six days after infiltration. Apple transformation was performed according to Espley et al., (2007).

Results and discussion

Sequences with homology to the MYB class of transcription factors were identified in the apple EST database. As the region of homology between different plant species is mainly conserved in the R3R3 region, and the conservation is at the protein level, BLASTN searches were performed. In these searches, the translated query sequence is used to search a six frame translated nucleotide sequence database. Performing a search on amino acid sequences has proven to be an effective way of identifying homologous genes (genes that are related by descent from a common DNA ancestor). However, the list of gene candidates that are generated from such a search is an insufficient basis for predicting function.

In our search for MYB genes that regulate anthocyanin biosyntheses in apple, we started with the genes from a number of species where gene function has been determined. This included genes from *Arabidopsis* (Borevitz et al., 2000), grape (Kobayashi et al., 2004) and petunia (Quattrocchio et al., 1999).

Sequence alignment, using ClustalW, was then used to identify regions of homology between candidate genes from our apple EST database and the MYB genes that had previously been identified and characterised. For this class of gene, a region at the N-terminus of the protein, termed R2R3, was the most highly conserved and the region that was used for further analysis.

A phylogenetic tree (Figure 1) was constructed, based on alignment of the R2R3 region and manual alignment to address any inconsistencies that were introduced during the automated process. Bootstrap values (represented as numbers up to 100) are at the root of inferred division into (usually) two clades of genes. Bootstrap values provide a measure of confidence that the division of a given gene or gene clusters into separate clades is significant; values below 75 suggest less confidence and branches that have bootstrap values of less than 50 are poorly supported, and in such cases, derived clades from that root may be considered as one and the same.

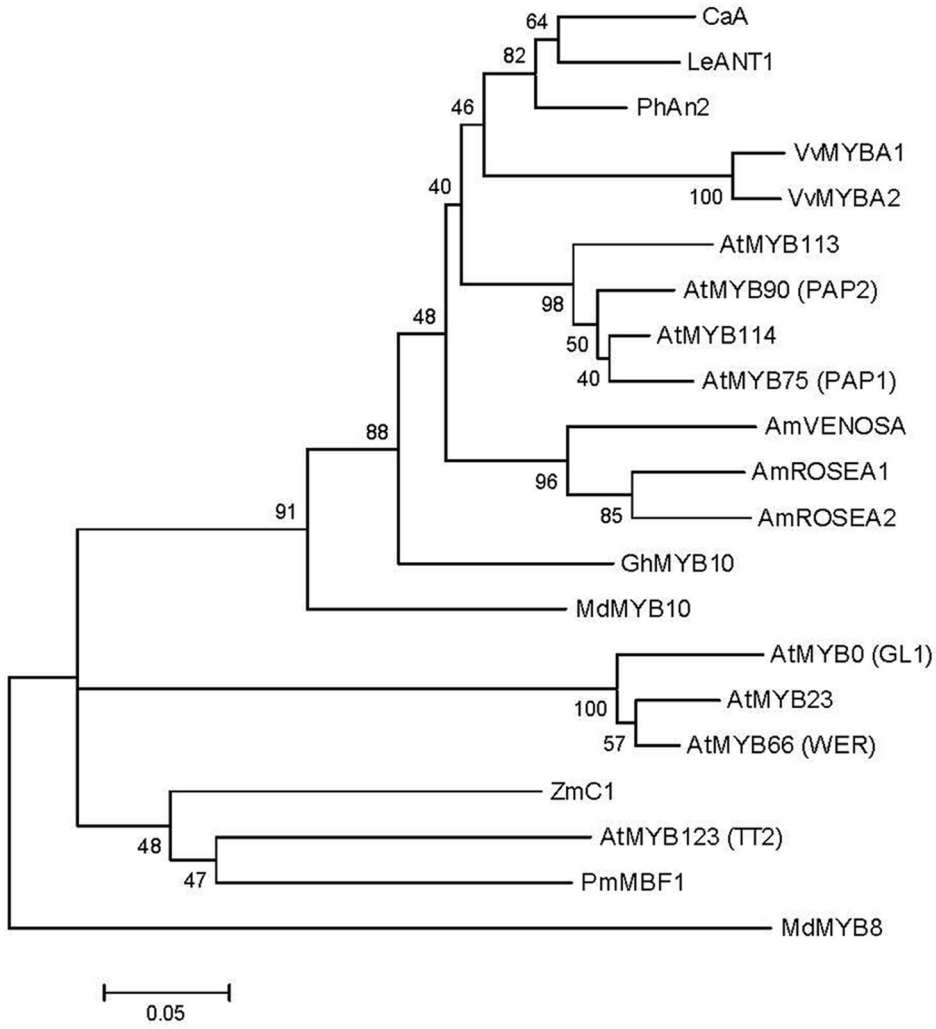

Figure 1. Phylogeny of MYB genes involved in regulation of anthocyanin biosynthesis. The alignment this tree is based on used amino acid sequences in the conserved R2R3 domains of: *Antirrhinum majus*: AmROSEA1, ABB83826; AmROSEA2, ABB83827; AmVENOSA, ABB83828. *Malus domestica* MdMYB10, DQ267896; MdMYB8, DQ267899. *Arabidopsis thaliana* AtPAP1, CAB09230; AtPAP2, NP176813; AtMYB113, NM105308; AtMYB114, NM105309; AtMYB66, NM121479; AtTT2, Q9FJA2; AtGL1, AAC97387. *Vitis vinifera* (Vv)VvMYBA1, AB242302; VvMYBA2, AB097924; *Capsicum annuum* CaA, CAE75745. *Petunia hybrida* PhAN2, AAF66727. *Solanum lycopersicum*, syn. *Lycopersicon esculentum* LeANT1, AAQ55181; *Gerbera hybrida* GhMYB10, CAD87010. *Picea mariana*: PmMBF1, AAA82943. *Zea mays* ZmC1, AAK81903.

In the case of the apple *MYB10* gene, the sequence was clearly part of the 'anthocyanin' clade that includes many of the well-characterised dicotyledonous genes known to regulate anthocyanin such as *AtMYB75* or *PAP1* (Borevitz et al., 2000) (Figure 2A, B), *AmROS1* and *2* (Schwinn et al., 2006) and *PhAN2* (Quattrocchio et al., 1999). The *MdMYB10* sequence was also distinct from other MYB genes involved in trichome development, such as *AtMYB66* and MYB genes known to regulate a related part of the phenylpropanoid pathway involved in biosynthesis of tannins, such as *AtMYB123* (*TT2*). This is supporting evidence that the candidate genes from apple may, indeed, be orthologous and perform the same function as the PAP genes from *Arabidopsis*. However, further experimental evidence is required to support this hypothesis.

Transient expression of candidate genes in the leaves of plants such as *Nicotiana benthamiana* or *N. tabacum* can provide further evidence of gene function. For transcription factors, it is possible to identify a target promoter sequence and fuse this to a chemiluminescent reporter, such as luciferase (Hellens et al., 2005). However, this requires considerable knowledge of the transcription factor targets. For anthocyanin biosynthesis, there are a number of well-characterised genes that are

regulated by *AtMYB75* (*PAP1*) in Arabidopsis (Dare et al., 2008). Work on the anthocyanin pathway can also take advantage of the conservation that exists between the function of regulatory genes in heterologous systems. In *N. tabacum*, the expression of the appropriate MYB genes from a range of diverse species is able to regulate the endogenous anthocyanin pathway.

Genes were selected based on sequence similarity and clustered into phylogenetic clades as described above. The candidate MYB genes were infiltrated into the leaves of *N. tabacum*, (Figure 2C). Patches of high concentrations of anthocyanins accumulated in the region of agro-infiltration with MYB gene but not in controls. This accumulation took 4–6 days and was most striking when light levels were high. In some instances it was necessary to co-infiltrate a second transcription factor, bHLH, as both MYB and bHLH proteins are needed for anthocyanin regulation, although in some cases, the endogenous bHLH protein was able to interact with the MYB gene under investigation. It was also interesting to note that when this experiment was performed in *N. benthamiana*, a necrosis, rather than an anthocyanin accumulation, was observed.

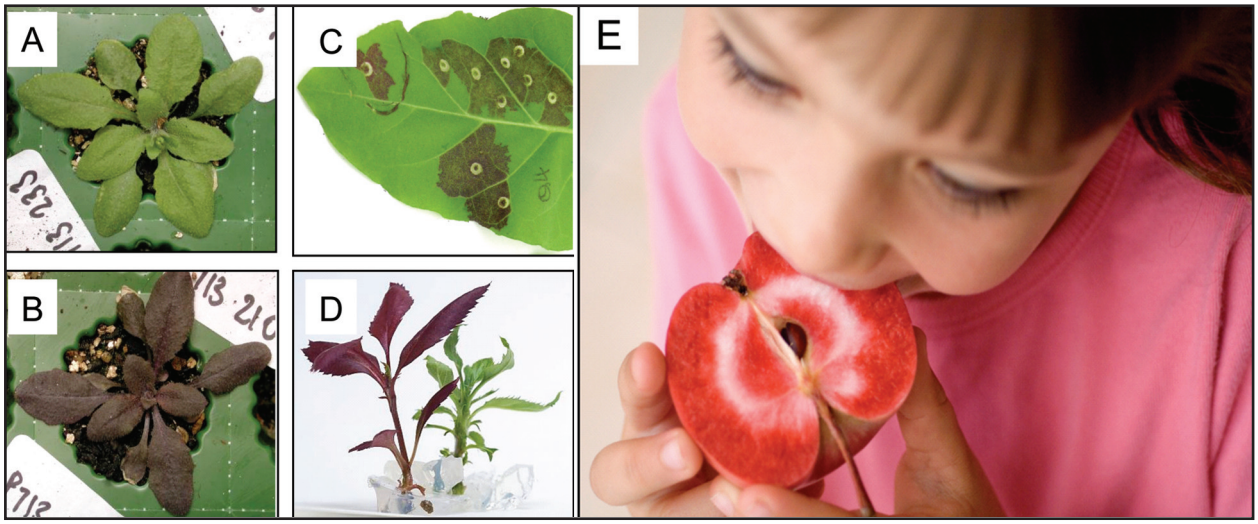

Figure 2. Translational genomics. Genes characterised in model plants, such as Arabidopsis (A+B). A. *Arabidopsis thaliana* ecotype 'Columbia'. B. *A. thaliana* transformed with 35S-AtMYB75, with ectopic accumulation of anthocyanin. C. transient agroinfiltration of candidate colour regulatory genes from apple into the leaves of *Nicotiana tabacum.* D. stable transformation of apple cv. 'Royal Gala' with candidate MYB gene. E, red-fleshed apple variety currently available in the *HortResearch* germplasm collection.

Evidence of anthocyanin regulation, based on transient infiltration, took only 4–6 days and based on this information, it was then possible to select candidate transcription factors to be stably transformed into both model and crop plants (Figure 2D). Pre-screening candidate genes with the transient infiltration systems increases the chance of generating the desired phenotype, which is a considerable advantage when the timeframe for transformation of woody perennials, such as apple, can be many months to years.

Once key genes have been identified and their function determined, it is then appropriate to consider how this information or knowledge can be exploited. Marker assisted selection (MAS) uses such gene knowledge to predict the phenotype of progeny, and is based on the inheritance of an allele of a gene. In the case of red-fleshed apples, the novel (mutant) version of the allele exists in *Malus* x *pumila* var. *Niedzwetzkyana* (Harris et al., 2002), and also in *Malus* x *domestica* 'Red Field' Open Pollinated ('Red Field'; Espley et al., 2007) (Figure 2E). This allelic information can also be used in the generation of cisgenic apple varieties. Cisgenic (as opposed to transgenic) is a novel term used when transformation experiments are performed with genes that have entirely the same or similar genetic origin as the plant species that is to be transformed (Schouten et al., 2006).

Conclusion

In this paper we have described how we have taken information on the control of anthocyanin biosynthesis from the model plant Arabidopsis and used it to identify the orthologous gene in apple. We have then proven the function of this apple gene, identified the mutation that causes ectopic red trees and fruit, and used this knowledge both in the generation of red-fleshed apples through genetic engineering but also in developing a DNA marker for marker assisted breeding.

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Exploiting banana biodiversity to reduce vitamin A deficiency-related illness: a fast and cost-effective strategy

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Keywords: vitamin A deficiency, banana biodiversity, biofortification, *Musa*, eye health, micronutrients, African countries

Abstract

Bananas, including plantain and cooking bananas (*Musa* spp.) are a staple food for millions of people in Sub-Saharan Africa and Asia, with *per capita* consumption reaching levels as high as 137 kg/year in Uganda. However, while production is generally sufficient to support regional populations, vitamin A deficiency (VAD), particularly amongst children and pregnant women remains a significant public health problem. VAD leads to increased levels of child and maternal mortality as well as illnesses such as corneal scarring and blindness. The main source of dietary vitamin A (vit A) is from the consumption of plant-derived carotenoids. Recent results from a large scale screening of *Musa* show that there is a high degree of variability in the fruit carotenoids content of individual *Musa* genotypes, with values ranging from undetectable to as high as 11 340 µg/gdw (Davey et al., 2009b). This indicates that there is a sufficient degree of genetic diversity to be able to breed for enhanced (biofortified) fruit carotenoids content in this crop. However, *Musa* breeding is complicated by the relatively slow crop cycle, and the high degree of sterility of cultivars so that the direct introduction or 'fast-tracking' of existing carotenoid-rich cultivars can offer substantial savings in affected regions. Here we discuss the biodiversity in fruit carotenoids and vit A content across individual *Musa* cultivars and genome classes. We then evaluate the potential impact and cost-effectiveness of fast-tracking existing vitamin A-biofortified banana cultivars. The health effects, or the 'burden of illness and mortality' related to VAD was calculated in terms of disability-adjusted life years (DALYs), which is the number of years of healthy life lost due to VAD-related illnesses. Results indicate that fast-tracking can lead to a 9.6–17.1% reduction in the burden of illness due to VAD in three African study countries, Ghana, Rwanda and Uganda, and that it can be more cost-effective than other health-nutrition interventions.

Abbreviations

α-carotene, all-*trans*-α-carotene; β-carotene, all-*trans*-β-carotene; cis-carotene, 13-*cis*-β-carotene; pVACs, provitamin A carotenoids; BCE, β-carotene equivalents; DALYs, Disability-adjusted life years; DHA, Demographic and Health Surveys; EAR, estimated average requirements; RDA, recommended daily allowance; Vis/NIRS, Visible and Near Infrared Reflectance Spectroscopy; vit A, Vitamin A, retinol.

Introduction

It has been estimated that more than 850 million people are malnourished and that many more suffer from an insufficient intake of essential micronutrients. Most prominent are deficiencies in iron (Fe), zinc, and vitamin A (vit A, retinol). The scale of the problem is enormous with Fe-deficiency affecting 4–5 billion people and with around 20% of the world's population thought to be at risk from Zn-deficiency (Wuehler et al., 2005). Similarly, vit A deficiency (VAD) is a recognised public health problem in 118 countries, with close to 20 million pregnant women and 100–140 million pre-school children being vitamin A-deficient. Of these pre-school children, 250 000–500 000 become blind every year and ~50% die within 12 months of becoming blind (Faber and van Jaarsveld, 2007).

Combating micronutrient deficiencies

Typical approaches to combat micronutrients deficiencies include providing doses of micronutrients in the form of pills, capsules or syrups (supplementation) or the addition of micronutrients to processed foods (food fortification). Both of these approaches have been widely utilised but in general it has proven difficult to target vulnerable people on the margins of society (such as those living in rural areas), as these groups generally eat less processed foods. Additionally, supplementation and fortification programs require a developed infrastructure and financial commitments from year to year. Therefore, it is now generally accepted that diversifying the diet and increasing the micronutrients content of staple crops grown (biofortification) are more sustainable approaches to combating micronutrients deficiency. In particular, biofortified (nutritionally-enriched) crops are seen as a low-cost, sustainable way of reaching people with poor access to health-care systems and/or formal markets, and thus implicitly target low-income households. To address these issues, the Consultative Group for International Agricultural Research (CGIAR) formed 'HarvestPlus', an initiative aiming 'to reduce the effects of micronutrient malnutrition (especially vitamin A, Fe and Zn-deficiencies) by harnessing plant breeding to develop staple food crops that are rich in micronutrients' (i.e. via crop biofortification).

Biofortification in Musa

Bananas (including plantain and cooking bananas, *Musa* spp.) are the world's fourth most important food crop, with an annual production of about 100 Mt. They are also a staple food for millions of impoverished people across many regions of the developing world and thus are an important source of vitamins and micronutrients. Therefore biofortified *Musa* cultivars can have a significant impact on population health and wellbeing. Traditionally however, *Musa* breeding programs have focused on traits such as yield improvement and disease resistance and little attention has been paid to nutritional quality. This is in part because breeding for enhanced micronutrients content is technically demanding and expensive. Further, most cultivated bananas are sterile hybrids derived from inter- and intraspecific crosses between two diploid wild species, *Musa acuminata* and *M. balbisiana* (Simmonds and Shepherd, 1955). Together with the relatively slow crop-cycling period this means that conventional breeding programs are lengthy, complicated and expensive. An alternative approach is biofortification through the application of biotechnology. Here, crops are transformed with (trans)genes designed to lead to the accumulation of carotenoids. Biotechnology has had some high-profile successes, as evidenced by the 'Golden Rice' story from the lab of Ingo Potrykus (Beyer et al., 2002). Certainly, for staple crops in which the levels of the target traits are low, or non-existent (e.g. vitamin A content of rice endosperm) and where there is little trait diversity in the consumed tissues, then such approaches are probably the only realistic possibility for biofortification. The sterility problems with *Musa* hybrids means that there is a clear case for a biotechnological approach, although progress is currently hampered by the poor status of publicly-available nucleotide sequence information in *Musa*.

Existing Musa cultivars as a source of dietary vitamin A

Most dietary vitamin A is obtained from plants in the form of so-called pro-vitamin A carotenoids (pVACs). This is a group of about 50 naturally-occurring plant carotenoid species which are broken down in the body to yield vitamin A (Fraser and Bramley, 2004; Yeum and Russell, 2002). Naturally orange-coloured foods are often an indication of a high carotenoids content and orange-fleshed banana fruits have already been shown to contain high levels of pVACs (Davey et al., 2006; Englberger et al., 2003a). Indeed, recent publications have reported on the occurrence of orange-fleshed *Musa* cultivars with exceptionally high pVACs content (Englberger et al., 2003a; 2006). In other words suitable *Musa* cultivars, 'naturally-biofortified' for vitamin A are already available and could be directly introduced to complement or replace existing cultivars in effected regions. This is the 'fast-tracking' approach.

In this paper, we describe the results from the first, large-scale systematic survey of the fruit pulp micronutrient content in *Musa* spp. using standardised analytical tools and sampling procedures to assess the degree of biodiversity for *Musa* fruit vitamin A and carotenoid content. The aim of this work was to establish 'baseline' or 'reference' micronutrients values for a wide range of individual *Musa* cultivars and to determine the potential of new and existing *Musa* cultivars to contribute to an improved nutritional intake of populations in regions where this crop is an important staple. To do this we also report on a study to quantify the impact of the 'fast-tracking' of naturally vitamin A-biofortified *Musa* cultivars. In this approach it was first necessary to determine the current vitamin A health status of populations, from which we were able to compare the relative efficacy and cost-effectiveness of various possible health intervention strategies. Finally we also carried out an *ex ante* analysis of the potential impact of the direct introduction of existing, vitamin A-rich *Musa* cultivars on the incidence of VAD in peoples in afflicted (vitamin A deficient) regions. Overall, results are discussed within the framework of the development of strategies to improve the nutritional and health status of vulnerable groups within *Musa*-consuming population groups through alleviation of micronutrients deficiencies.

Results and discussion

Biodiversity in Musa fruit micronutrients content

Assessing the degree of biodiversity for vitamin A content requires an understanding of the degree of variability within and between cultivars, the factors influencing trait variability, as well as methods for accurate trait phenotyping. At the time that we started work on this project, essentially no systematic studies of fruit pulp nutrient content in *Musa* spp. had been carried out, so initial work focused on developing robust and rapid procedures to quantify fruit pulp carotenoids content and on understanding the sources of biological variation.

Carotenoids analysis

Existing protocols for the extraction and analysis of plant tissue carotenoids were considered too lengthy and complicated for implementation within a large scale screening program, so protocols for the high-resolution screening of the pVACs content of *Musa* fruit by HPLC were developed (Davey et al., 2006). Typical results for the C_{18} and C_{30} RP-HPLC analysis of banana pulp carotenoids content demonstrate that *Musa* fruit pulp has a relatively simple carotenoids profile, consisting primarily of only all-*trans* α-carotene (α-carotene), and all-*trans* β-carotene (β-carotene) (Figure 1).

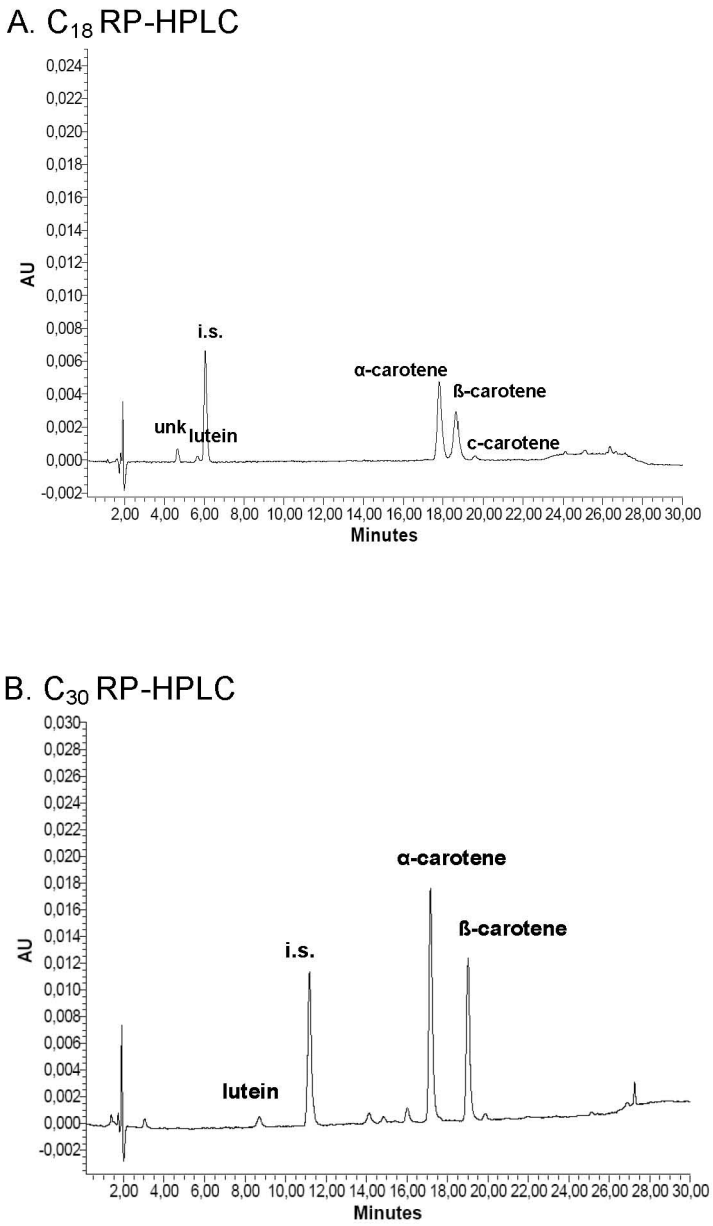

Figure 1. Typical HPLC chromatograms for the analysis of fruit pulp carotenoids from *Musa* cultivar Oonoonoo kenogoa.

α-carotene = all-*trans*-α-carotene, β-carotene = all-*trans*-β-carotene, c-carotene = 13-*cis*-β-carotene, i.s. = internal standard, *trans*-apo-8'-β-carotenal.

More recently we have also developed predictive models for the quantification of individual and total carotenoids in *Musa* pulp, based on visible/near infrared reflectance spectroscopy (Vis/NIRS) spectra. Results from the Vis/NIRS analysis of a diverse range of genotypes show that the procedure is suitable for the high throughput screening of *Musa* fruit pulp, with coefficients of cross-validation being as high as 0.89 for β-carotene content (Davey et al., 2009a). The advantages of Vis/NIRS are that it is very rapid, non-destructive so that the sample can be used for additional analyses if necessary. In addition, the operating costs are very low and no (toxic) chemicals and/or waste products are produced.

Sources of biological variability and sampling procedures

Optimised analytical protocols (Davey et al., 2006) were used to characterise the sources of carotenoids variation in *Musa* and to develop fruit sampling procedures (Davey et al., 2007). Surprisingly, results showed that even within a single fruit, statistically-significant differences in pVACs content are present. Substantial variation in the pVACs content of fruit obtained from a single hand, from individual hands within the same bunch and between plants of the same variety was also observed. Therefore to encompass the entire range of pVACs variability within a plant, sampling procedures were standardised as harvesting fruit from hands located at the top middle and bottom of the bunch and where possible from three individual bunches of the same variety harvested at the same time and maturity stage (Davey et al., 2007).

Genetic diversity in *Musa* fruit total pVACs content

Our standardised sampling and HPLC protocols were used to quantify individual and total carotenoids of fruit from 171 *Musa* cultivars covering essentially all genome groups (Davey et al., 2009b). The mean total pVACs content for the 171 genotypes analysed was 1617 µg/100gdw, (517 µg/100gfw), but values ranged from 0 (undetectable) to 11 337 µg/gdw (3457 µg/100gfw), with a mean variation in total pVACs content of +/- 20.5% per cultivar. Importantly, these results demonstrate that there is a sufficiently large degree of genetic variation within the *Musa* germplasm pool to potentially breed for this trait (Hulshof et al., 2007).

The results further show that there are no clear divisions between the mean total carotenoids content per genome group and that the genetic basis for high fruit pVACs content is widely distributed within the *Musa* germplasm pool (Figure 2).

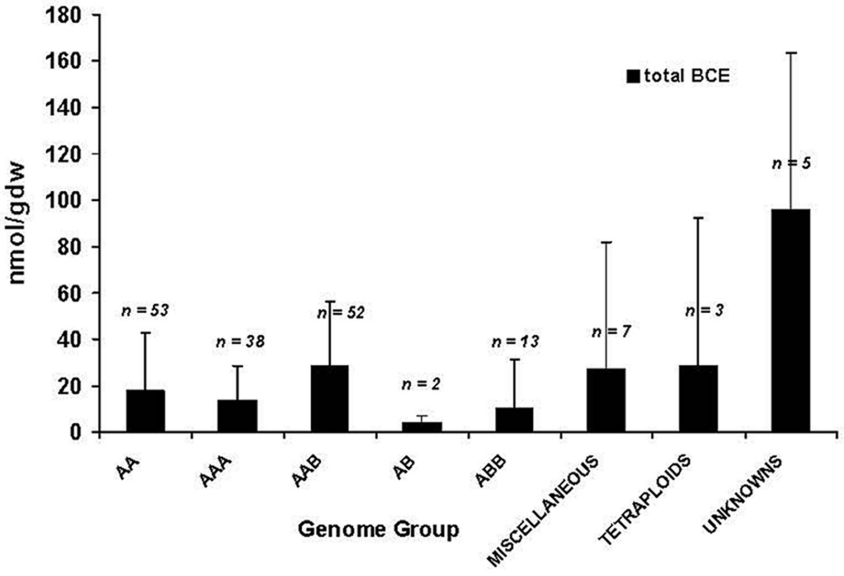

Figure 2. Mean fruit total all-*trans* β-carotene equivalents (BCE) content per genome group across the sample group of 171 *Musa* genotypes analysed. BCE's determined by C18 reverse phase-HPLC. 'Miscellaneous' refers to a selection of unusual genoptypes represented by only one variety. 'Tetraploids' represents a collection of 3 (different) tetraploid varieties.

The distribution of mean fruit pVACs concentrations per cultivar across the sample group is clearly statistically 'non-normal', with most cultivars (64%) having a total pVACs content of less than 1074 µg/100gdw, and the number of cultivars having a higher pVACs content decreasing exponentially (Figure 3).

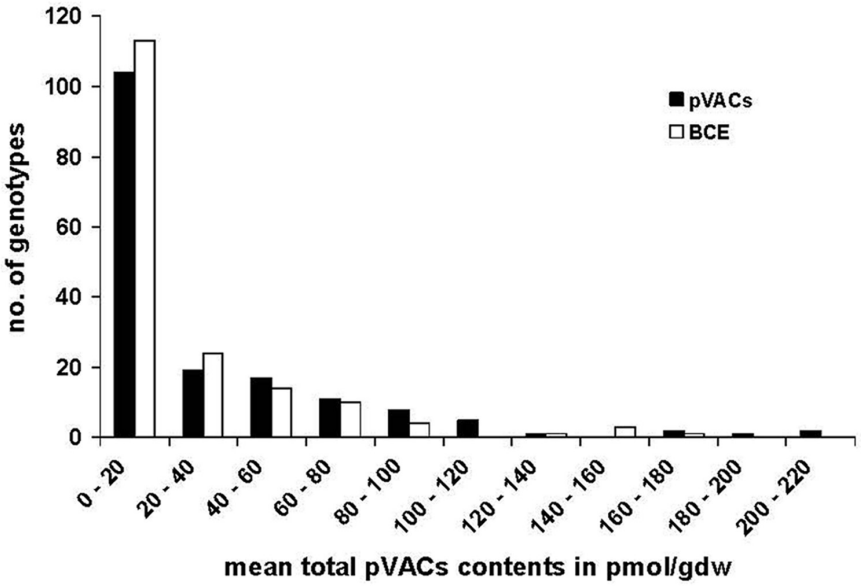

Figure 3. Distribution of fruit mean total pVACs and BCE content within the 171 *Musa* genotypes analysed

These results suggest therefore that the combination of alleles responsible for high fruit pVACs content are not widely-distributed within the *Musa* germplasm pool. Presumably this is due to the preferential selection by farmers for other traits such as disease resistance or white flesh (and thus low pVACs), or due to co-segregation of alleles for high pVACs with undesirable crop characteristics such as poor or abnormal agronomic performance. In this study, the cultivars with the highest fruit pVACs had values of between 6660–11 337 µg/100 gdw (2200–3457 µg /100 gfw).

Musa pVACs profiles

β-carotene is at least twice as efficient as a precursor of retinol than α-carotene and other pVACs. Therefore the net vitamin A nutritional value of a fruit depends not only on the concentration of the pVACs but also on the relative proportions of the individual pVACs present. In *Musa* fruit pulp, over 90% of the total carotenoids nearly always consisted of α- and β-carotene alone with the remaining < 10% of the peaks consisting of *cis* carotenoids (Davey et al., 2006; Davey et al., 2009b). Lutein which is also present has important health benefits but does not have provitamin A activity (Calvo, 2005). Interestingly the percent proportion of the individual pVACs appears to be stable and characteristic for each genotype. Although high β-carotene content is a preferred trait, only three genotypes (2%) had a pVACs profile in which the content of β-carotene was greater than 80%, and none of these also had a high total pVACs content. This contrasts with results reported by Englberger and colleagues on the analysis of several rare Micronesian *Musa* cultivars in which the cultivars with the highest pVACs content consistently also had high proportions (75–100%) of β-carotene (Englberger et al., 2003; 2006).

Musa vitamin A nutritional value

On the basis of the content of individual pVACs, the overall vitamin A nutritional value of fruit can be expressed in terms of β-carotene equivalents (BCE's) or retinol activity equivalents (RAEs) using a conversion factor of 12:1 (Yeum and Russell, 2002). These values can then be used to calculate vitamin A nutritional content and determine the contribution of fruit to the vitamin A recommended dietary intakes (RDAs). Within the HarvestPlus program, dietary target intakes are based on population-weighted Estimated Average Requirement (EAR) values (Tarasuk, 2006) rather than governmental RDAs. For children, the vitamin A EAR is 250 µg RAE/day, while for all other members of the population the EAR is 500 µg RAE/day. Assuming that *Musa* fruits are not

the only source of dietary vitamin A, target levels for biofortification are based on the biofortified crop providing 50% of the EAR. From this we can calculate that as little as 100 gfw of fruit pulp from cultivars with the highest pVACs content is sufficient to provide ~95% of the EAR for children and 47% of the EAR for adults. This demonstrates that there are existing edible and commercial *Musa* cultivars with RAEs high enough for 'fast-tracking' and to have a significant impact on population vitamin A health status at modest and realistic consumption levels.

Population vitamin A health in the target regions

Data used

As discussed by Garming and Ekesa (Garming and Ekesa, 2008), population and health status data were obtained from WHO statistics (WHO, 2006), as well as the most recent Demographic and Health Surveys (DHS), for Ghana (DHS, 2003), Rwanda (DHS, 2005) and Uganda (DHS, 2006). Data on banana production and consumption in these three countries were obtained from the FAO statistics division, (FAOSTAT, 2008) while additional detailed information for Uganda and Tanzania was obtained from a large-scale survey of banana farmers (Smale and Tushemereirwe, 2007). National production estimates for Ghana were provided by the Crops Research Institute, Ghana and earlier reports to HarvestPlus (Bioversity International, 2007). From these reports and a report from Uganda (Fungo, 2007) an estimate of the β-carotene and RAE intakes based on various local banana cultivars was made.

Current vitamin A intakes

A comparison of the estimated intakes and recommended daily allowances (RDAs) for the target groups shows that the average current vitamin A intake is 30–40% below the recommended levels and is lowest in Rwanda (Table 1). This is consistent with the observation that the prevalence of vitamin A deficiency (VAD) is higher in Rwanda than in Uganda and Ghana (Table1).

Table 1. Current intake (µg/day) of vitamin A in the study countries (based on DHS, 2003, 2005, 2006) and RDA for each target group.

Target group	RDA	Uganda		Rwanda		Ghana	
		µg/d	% RDA	µg/d	% RDA	µg/d	% RDA
Children under 5	350	225	64	210	60	229	65
Pregnant women	770	543	71	539	70	573	74
Lactating women	1300	907	70	903	69	937	72

Incidence of VAD-related illnesses

Three main population target groups for VAD were identified i) young children (under 5 years of age), ii) pregnant women and iii) breastfeeding women. The main VAD-related health impairments considered in this study are an increased mortality rate, corneal scarring and blindness, a higher incidence of measles amongst young children, and night-blindness (Table 2). The mortality rate due to VAD is assumed to be 3% (Stein et al., 2005). It is estimated that this could be reduced to 2% if these children received an adequate dietary vitamin A intake, but for the remaining children at risk, the situation is so severe that they could only be saved if they received therapeutic doses of vitamin A (Jones et al., 2003).

Table 2: Prevalence of vitamin A deficiency-related health impairments.

Target group	Uganda	Rwanda	Ghana
Prevalence of VAD			
Children under 5	27.9%[1, 2]	39.0%[3]	26.0%[6]
Night-blindness			
Children under 5	0.3%[4]	0.39%[4]	0.26%[7]
Pregnant women	5.0%[4]	3.0%[6]	2.0%[7]
Lactating women	5.0%[4, 9]	3.0%[6, 9]	2.0%[7, 9]
Corneal scarring			
Children under 5	0.14%[4]	0.20%[4]	0.13%[4]
Blindness			
Children under 5	0.07%[4]	0.10%[4]	0.07%[4]
Measles			
Children under 5	31.9%[5]	14.4%[4]	1.40%[7, 9]

[1]Fungo, 2007, [2]Uganda Bureau of Statistics, 2006, [3]WHO, 2006, [4]Maclaren and Frigg, 2001, [5]DHS, 2006, [6]DHS, 2005, [7]DHS, 2003, [8]Bioversity International, 2007[b], [9]Stein et al., 2005.

Assessing population health—the DALYs methodology

The current health status and the potential benefits arising from the introduction of vitamin A (naturally) biofortified *Musa* cultivars were calculated using the approach of Stein et al. (2005). Here the burden of disease is calculated using the 'disability life–adjusted years' (DALYs) method. This method generates a single index for population morbidity and mortality related to the incidence of a particular disease. For a detailed discussion of this methodology see (Meenakshi et al., 2007; Stein et al., 2005; Zimmermann and Qaim 2004).

Measuring the burden of illness using the Disability Adjusted Life Years (DALYs) approach

The overall burden of disease is calculated as the sum of the 'years of life lost' (YLL) and the sum of the 'years lived with a disability' (YLD). The severity of the disability is further weighted with a scale from 0 to 1 where 0 represent perfect health and 1 represents death. The DALYs lost are then calculated according to equation 1 (Zimmermann and Qaim 2004).

(Eq. 1)
$$DALYs_{lost} = \sum_j T_j M_j \left(\frac{1 - e^{-rL_j}}{r} \right) + \sum_i \sum_j T_j I_{ij} D_{ij} \left(\frac{1 - e^{-rd_{ij}}}{r} \right)$$

T^j = Number of people in target group j, M_j = Mortality rate associated with VAD, R = Discount rate for future life years, L_j = Average remaining life expectancy for target group, I_{ij} = Incidence rate of disease in target group j, D_{ij} = Disability weight for disease i in target group j, d_{ij} = Duration of disease i in target group j

Table 3. Annual burden of vitamin A-related illness expressed as DALYs lost for each target group and per person.

Target group	Uganda		Rwanda		Ghana	
	DALYs	DALYs/ person	DALYs	DALYs/ person	DALYs	DALY/ person
Children under 5	91 220	0.022	48 860	0.031	60 149	0.018
Mortality	89 466		48 063		59 017	
Night-blindness	115		60		84	
Corneal scarring	611		308		462	
Blindness	763		385		577	
Measles	264		45		9	
Pregnant women	1383	0.002	308	0.001	1,282	0.001
Lactating women	1657	0.002	369	0.001	1,536	0.001
Total	94 260		49 538		62 967	

The DALYs analysis for Uganda, Rwanda and Ghana shows that the group most affected by VAD is young children (Table 3), confirming earlier findings of Meenakshi and colleagues in Uganda (Meenakshi et al., 2007). It is also clear that the greatest contribution to VAD-related DALYs is child mortality, and that with 0.031 DALYs lost per child under 5, Rwanda has the highest burden of VAD-related illness, followed by Uganda, and then Ghana. VAD also clearly impacts pregnant and lactating women more in Uganda than in Ghana and Rwanda, but the higher incidence of measles in Uganda reflects the lower overall measles vaccination rate in this country compared to Ghana and Rwanda.

Quantifying the potential impact of biofortified *Musa*

The potential impact of an increased dietary vitamin A intake on the incidence of VAD-related disease depends on several factors including banana consumption levels, the relative vitamin A content of extant versus 'new' *Musa* cultivars, the extent to which the current banana cultivars will be replaced by new cultivars, the bioavailability of pVACs from banana foods, the losses of RAEs during post-harvest handling, storage and processing and the dose-response effect of an increased vitamin A intake on consumer health. Importantly, the nutrient dose-response is non-linear and individuals with a higher level of deficiency will display a relatively higher beneficial health effect—for discussion see (Garming and Ekesa 2008; Stein et al., 2005; Zimmermann and Qaim 2004).

High- and low-impact scenarios

Because a considerable degree of uncertainty exists for a number of the assumptions used in the analyses, both a high- and a low-impact scenario were developed. These were based on either more-or-less conservative estimates of consumption rates, the RAE levels of new cultivars and the extent of adoption of the new cultivars as detailed by Garming and Ekesa (Garming and Ekesa 2008). The per capita consumption of *Musa*-based foods was calculated from production data in each country, using a factor of 55% to account for weight losses associated with the peel and rachis. The share of banana used for brewing was excluded from the consumption data for children and women and in the high impact scenario children are assumed to consume the same amount as adults. This is a realistic assumption as bananas are the most common weaning foods and are also popular children's snacks within banana growing communities (Kikafunda et al., 1996). In the low-impact scenario it is assumed that children consume only 50% of the average production available for *per capita* consumption. From the results of the Bioversity screening projects, *Musa* cultivars with high pVACs (BCEs) content have been identified, and the potential for increasing the RAE content

was based on introducing cultivars that had the highest measured BCE/RAE levels relative to the local, most-highly consumed varieties—as summarised in Table 4.

Table 4. β-carotene levels and consumption rates of popular and biofortified *Musa* cultivars.

	Uganda	Rwanda	Ghana
Most popular Musa cultivar	Nakitembe[1]	Nakitembe	Apantu/Apem[2]
BCEs (µg/gfw)	382[2, 3]	382[2, 3]	1400/468[2]
Consumption per capita (gfw/day)	378[2, 3]	261[2, 3]	199[4]
BCEs in improved cultivar (µg/gfw)*	14 600[3]	14 600[3]	18 710[2]

*Based on the average levels measured in Papua New Guinean (PNG) cultivars for Uganda, and Rwanda, and the plantain cultivar 'Batard' for Ghana.
[1]Edmeades and Karamura 2007, [2]Bioversity International, 2007, [3]Fungo, 2007, [4]Bioversity International.

In accordance with generally accepted assumptions, a conversion factor of 12:1 for the absorption of vitamin A from ingested dietary β-carotene was used. This, however, is a rather conservative assumption and others have utilised conversion factors of 6:1. Indeed, the value may even be as low as 2:1 under some circumstances (Zimmermann and Qaim 2004). It is also known that the absorption of pVACs from food strongly depends on the individual's intake of fat at mealtimes, so that nutrition education and the dissemination of recipes for the efficient preparation of banana foods will contribute to improving vitamin A bioavailability. Based on preliminary results from processing studies, a conservative estimate of a 40% loss was used for both plantain bananas (in Ghana) and cooking bananas (in Uganda and Rwanda), (Bioversity International, 2007). It is, however, expected that further research can reduce such losses further. Based on the results from a Bioversity International survey on the adoption of new *Musa* cultivars in Uganda and Tanzania (Nowakunda and Tushemereirwe, 2004), a conservative adoption rate of 7.5% (lower impact) and 15% (higher impact) was chosen.

Impact results

The results of the *ex-ante* analysis show that the introduction of *Musa* cultivars with high BCE content can be expected to significantly alleviate the burden of VAD in all three study countries (Table 5). The extent of the impact ranges from 1.9%–17.2%, with the largest impact (in terms of DALYs lost) being expected to occur in Uganda. This reflects the greater dependence on banana as a staple food in this country, compared to Ghana and Rwanda. The highest DALYs reductions would accrue amongst children under 5, which again is the group most affected by VAD.

Table 5. Estimated percentage reduction in DALYs lost through *Musa* cultivars with improved content of β-carotene.

Target group	Uganda		Rwanda		Ghana	
	Greater-impact scenario	Lower-impact scenario	Greater-impact scenario	Lower-impact scenario	Greater-impact scenario	Lower-impact scenario
Children under 5	17.2%	2.8%	11.6%	1.9%	9.6%	2.2%
Pregnant women	9.6%	3.3%	6.5%	2.2%	4.6%	2.3%
Lactating women	5.5%	1.9%	3.8%	1.3%	2.6%	1.3%
Total	17.1%	2.8%	11.6%	1.9%	9.6%	2.2%

Importantly, the high impact scenario developed here is not an overly optimistic scenario, but rather illustrates the size of realistically-achievable reductions in DALYs lost through introduction and dissemination of high BCE *Musa* cultivars. In contrast, the low impact scenario can be considered

as a fairly pessimistic projection, but one which still demonstrates that under adverse conditions, a reduction of VAD related illness of about 2–3% can still be expected. This impact is similar to the predictions developed for vitamin A biofortification in cassava and maize in the lower-impact scenario of Meenakshi et al. (2007). A sensitivity analysis of the factors most relevant for a reduction in VAD is dominated by child consumption rates such that a lower infant consumption level erases the differences in impact between children and women.

Cost-effectiveness of fast-tracking biofortified vitamin A-rich *Musa* cultivars

The costs associated with a biofortification program include budgets for research and development, breeding, and dissemination (Meenakshi et al., 2007). However, for a 'fast-tracking' approach as proposed here, breeding costs are negligible since existing high RAEs *Musa* cultivars will be used. Using values based on actual Bioversity International budget outlays (Garming and Ekesa 2008), total costs are expected to range from 6.7 (Ghana) to 23 MUS$ in Uganda over a 15-year period. Of these costs, the highest expenditure is on dissemination interventions for the new varieties. It will also be essential to extend longer-term support to the target populations with nutrition education, and food preparing and processing training for up to 15 years to achieve the expected impacts. Other assumptions used in the calculation of costs are as detailed by Garming and Esaka (Garming and Ekesa 2008). The time horizon for calculating the benefits in terms of DALYs saved was assumed to be 30 years and using a discount rate of 3%, reflecting the assumption of time preference[1]. The costs per DALYs saved were then calculated by dividing the total intervention costs by the total value of DALYs saved.

Table 6. Costs per DALYs saved for two scenarios in Uganda, Rwanda and Ghana (in US$).

Scenario	Uganda	Rwanda	Ghana
Higher-impact scenario	87.2	68.7	69.6
Lower-impact scenario	512.8	405.2	289.7

The estimated costs per DALYs saved in each of the three countries is shown in Table 6. According to (Meenakshi et al., 2007), costs of less than 196$/DALY saved are considered to be highly cost-effective. In the lower-impact scenario developed here, the costs per DALYs saved are much higher than this, at 289$ in Ghana and 512$ in Uganda. However, in the high-impact scenario, the costs are significantly lower than this threshold, ranging between 68.7$–87.2$/DALY.

Conclusion

Food fortification and supplementation are two approaches that have been used to reduce VAD but which have generally proved difficult to implement in developing countries (West, 2000). For these reasons, breeding for enhanced nutritional content (biofortification) or the introduction of non-indigenous cultivars with high micronutrients content (fast-tracking), are now thought to be the most promising strategies.

Worldwide well over a thousand banana cultivars are recognised and results from our screening program show that there is substantial biodiversity in fruit vitamin A content. An impact analysis indicates that fast-tracking of naturally biofortified *Musa* cultivars has the potential to significantly reduce the burden of VAD-related diseases in countries where there is a high incidence and high consumption of bananas as a staple food, and can lead to a significant reduction in DALYs, particularly for children under 5. Further, in the 'higher-impact' scenario we show that fast-tracking is a highly cost-effective approach and is economically viable compared to alternative health interventions strategies. Of the factors influencing impact, the average consumption level of bananas by children is probably the most important, and results suggest that this should be a key focus of this

[1] The underlying assumption is that individuals, as well as society as a whole, prefer to enjoy an immediate benefit rather than one in the future. Therefore the value of saving one DALY now is higher than saving it next year instead.

type of strategy. However, before new *Musa* varieties can be introduced on a large scale, agronomic, post-harvest and processing trails will have to be completed. This work is necessarily complemented by educational programs on post-harvest/cooking methods, dietary diversity, dietary combinations and food safety with the major aim of enhancing the bioavailability of the vitamin A.

Models for the extent of impact and cost-effectiveness of fast-tracking were developed for Uganda, Rwanda and Ghana as these were the only countries were sufficient background data were available, but undoubtedly the conclusions can be applied to other lands in the region in which *Musa* is a staple crop. On-going programs to study the agronomic properties of promising cultivars, as well as the bioavailability of vitamin A from fruit and processed meals will help refine the accuracy of these analyses as will the identification of additional novel *Musa* varieties with improved nutritional content.

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Genomics, super-domestication and crops

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Keywords: food production, biotechnology, gene pool, *Musa*, plant genome evolution

Abstract

This report aims to discuss some of the challenges which agriculture must address during the third millennium in providing food to the global population. Agriculture has changed from being a subsistence activity, and economic drivers become critical to enable farmers to produce food: they can stop farming and use land for other uses or abandon it, they can increase volumes (for example by growing for biofuels or feeding to animals), or reduce costs. They can also grow higher value crops, including particularly tropical fruit crops. Consumers, with their wish to eat more meats and oils, and among the richer part of society eat healthy foods with high sustainability, also provide drivers for future agricultural crops and practices. Conservation, documentation and exploitation underpin research and development of new crops and varieties. I argue that farmers will be able to deliver appropriate crops to a growing population by exploitation of appropriate technology and use of the gene pool—the range of genes present in plants. Biotechnology and understanding the behaviour of the plant genome provides a range of tools and options that allow crop 'super-domestication'—the planning of requirements of new characters in our crops.

Farmers and food

In the 5000 years since farming started, the primary producers—farmers—have continuously delivered increasing quantities of food with better safety and quality. Over the millennia, they have chosen suitable species for cultivation, selected appropriate genotypes, and improved the genetic background of these cultivars, often in combination with improved methods and agronomy. During the 20th Century, and particularly in the second half, the rate of these improvements accelerated. The activities of farmers in producing better food had extensive socio-economic impacts in all countries. Whilst interventionist healthcare has had some role, in the Western world, the production of better food has been largely responsible for the increase in life expectancy from under 50 years to nearly 80 between 1901 and 2000.

The number of farmers, and the effort that societies have put into farming, has steadily declined over the period from the start of agriculture: hunter-gathers and early farmers spent their whole lives engaged in collecting food, while current Western societies involve some 2 to 5% of their population in agriculture, working for substantially shorter hours than their ancestors as agricultural labourers. The year 2008 marks a significant global event, when more than half of the world's population of 6.7 billion has become urban. There is little doubt that this drift to the cities will continue in the future, placing the burden of food production on ever fewer farmers. In addition, significant amounts of land worldwide will be taken out of production for both, social or societal reasons and because the land becomes unsuitable for cropping, increasing the requirement for efficient agriculture.

As agriculture changed from being a subsistence activity, shown globally in the urbanisation figures, economic drivers became critical to encourage farmers to produce food. Although crop sales-prices are often tempered with political decisions and influences, ranging from shared-effort in collective farms, to schemes for the distribution and cost of water, farmers have to make money if they are to survive. When costs of growing crops by farmers are greater than the value of the crops, land will be abandoned or changed to more profitable activities. As a consequence of reducing crop prices in Western Europe, the last decade has seen increasing amounts of land removed from agricultural production for leisure activities paid for by consumers such as golf courses or horses. Taxpayer

subsidies—for public land access and less intensive management practices—or regulations and laws preventing some practices also influence farmer behaviour. In some countries, no management regime may be able to produce food crops profitably, so land may be abandoned on a larger or smaller scale. The factors leading to abandonment vary widely—high labour costs or low labour availability, high input costs (water, fertiliser and crop protection chemicals), poor or stressed growing conditions, poor access to markets, for example, all being relative to the locally set agricultural prices, but all have the same consequence.

Farmers have alternatives to making money other than 1) lower cost production or 2) abandoning agriculture and finding alternative land uses: they can 3) increase the amount of product they sell, or 4) produce alternative and higher value products. Globally, people cannot eat more than a certain amount, so there is no opportunity to produce more food to be directly eaten by people. In the early 2000s, as many people were over-nourished as were under-nourished in the world, so although inequality and unevenness of food distribution remains a challenge, there is minimal opportunity for total market expansion by selling plant foods to humans. However, farmers can produce more feed, which is eaten by animals and their products—meat, milk or eggs—are used by humans. Meat production has increased at twice the rate of the increase in crop production in the last 40 years (FAOstat, 2008).

Furthermore, while cattle thrive on a cellulose (grass) diet using plants which humans cannot eat, both chicken and pig meat production have overtaken beef and milk production in recent decades, and bovine production has come to require cereal and legume-based feeds. Thus arable farmers have increased their market beyond the requirement of an increasing population. The market size can also be increased by using crops for new uses. Demand for biofuels has meant an expansion in the market for oil and starch crops; although the impact on prices and food availability is debated widely, biofuels have certainly increased the market size for farmers, like production of animal feeds. Withdrawal of subsidies for unplanted land in Europe and observations of planting of previously abandoned lands in the Americas could suggest that new demand and associated price increases is bringing extra land back into production.

A fourth alternative to making money for farmers is the production of different crops with higher value or lower production costs. This change in production can involve commodity crops and niche crops. Over recent years, changes in crop production have included the expansion of oil crops—oil palm, rapeseed or canola, and soybean—and the relative increase in fruits, with large increases relative to wheat and rice seen in tropical and sub-tropical fruits such as oranges, bananas and mangoes.

Feeding the world's human population has always been at a cost to the environment since 'wild' species have been collected and habitats altered. All forms of exploitation of the environment for food collection have degraded the local environment and destroyed biodiversity, whether hunter-gather or primitive (e.g. slash and burn), smallholder, or the larger scale agricultural practices as used over the last 200 years. Arguably, many of the threats to sustainable food production have been with us since the transition from hunter-gatherer to farming, and then become critical at multiple points during the advance of agriculture: human destruction of habitats, change in local and global climate, challenges from new crop diseases, weeds or insects, and changes in the crops and their distribution as required by the population. The unprecedented scale of food production shows that these challenges have been addressed, but arguably involve some unsustainable practices that affect the whole of the food chain. In many areas, salinity, erosion and changing rainfall patterns may also have removed land from production.

Scientists and food

Both technological and scientific developments have underpinned the increases in agricultural productivity which farmers have achieved. As pointed out (Heslop-Harrison, 2004), biotechnology provides a set of powerful tools and approaches to improve plants or animals, and to make or

modify products which can be focused to meet challenges of quality, quantity and sustainability. The application and exploitation of biotechnology will undoubtedly make a major contribution to increasing the quantity, improving the quality and ensuring the sustainability of agriculture in this millennium, but biotechnology must be developed in conjunction with the requirements of farmers and end users, as well as moral imperatives regarding the environment and health while issues of global access to appropriate technologies must be considered (Heslop-Harrison, 2004).

In the world, there are approximately 250 000 species of flowering plants. During the history of agriculture, about 200 of these species have been domesticated, when substantial genetic changes have been selected by people to make them more convenient and productive under farming conditions where most of the primary production is used by humans. What additional candidates are there for domestication as new crops? There are relatively few a priori reasons why a species might be selected: among the world's most widely cultivated crops, there are plants where seeds, tubers, stems, flowers, fruits or leaves are eaten.

Seed endosperms from wheat, rice and maize are the major crops, providing most of the calories that are eaten in the diets of the global population, and a situation which will not change, although requiring continuous work to maintain. However, the next 30 crop species include many different crop parts: a stem (sugarcane), roots and tubers (cassava and potato), seed cotyledons (soybean), leaves, bulbs, and numerous fruits from diverse monocots and eudicot families. Other properties of the crop genomes—such as whether they are diploid or polyploid, have high or low chromosome numbers, or large or small genome sizes—also have no particular correlation with the success of a crop in agriculture, perhaps a surprising conclusion given the fundamental nature of the genome and the consequences of it characteristics for selection and evolution.

As well as food security—generating plants which are stable in their yield – a major challenge for scientists involved in crop science is related to the global environment. Water availability for agriculture may be diminishing and irrigation regimes are rarely sustainable in the long-term, often exploiting non-renewable fresh water resources, or degrading land through increasing salinity. Agricultural productivity per unit area, if not absolute production, must continue to rise. The balance and desirability of alternative strategies ranging between large areas of lower intensity agriculture or smaller areas of highly intensive agriculture mitigated by 'preserved' habitats as refugia for native biodiversity and environments are essentially social and political decisions. Progress in the crop sciences can both increase the range of options available by providing crop varieties suitable for different agricultural systems, and can also define the medium-term outcomes of different strategies (see Heslop-Harrison, 2004).

The advent of a new millennium has marked a paradigm shift in plant genetics and evolutionary biology. With the complete DNA sequence of the genomes known in about six relatively diverse angiosperm plant species, we now have reference sequences for nearly all angiosperm genes. We can use these sequences as a basis to identify the allelic variants of these sequences in all other species, and to identify features of their expression. Increasingly, the functions of these genes, whether in single-gene traits or quantitative trait control, are known. In a comparative context, genomic sequences can be used to see how species and their genomes are related to each other, and how they have evolved. Thus, we can identify recent and common relatives of species from DNA sequence, including understanding the key events leading to evolution through hybridisation and polyploidy. We can look at how genes and genomes have changed in long timescales (millions of years associated with speciation), medium timescales associated with species selection or domestication, and short timescales of years associated with plant breeding. By knowing what has happened in the past, we can characterise the types of changes that are likely to be possible in the future. In the last decade, plant breeding has become an increasingly targeted and quantitative process, a process that can be defined as super-domestication (Vaughan et al. 2007).

Based on socio-economic factors, including changes in national and global trade patterns, recognition of requirements for sustainability, nutritional and health needs, and importantly

developing crops suitable for changed climates, targets can be set for new crop varieties and sometimes introduction of new crops. Appropriate technologies can then be applied to deliver these needs. Banana, the most widely grown tropical fruit, plays a critical role in the healthy nutrition and wellbeing of the world's population in both industrialised and developing countries. Current varieties are sub-optimal, as diseases become virulent, market needs change, yield needs improvement, and production practices are recognised as being unsustainable. Varieties are based on their genetic component, and the use of genetic information in a comparative context can play a major role in the development of new, super-domesticated, varieties.

Both for the farmers that grow them and for the consumers that eat them, tropical fruit crops often have higher value, make greater contributions to local economies and labour markets, and improve both the health and quality of life of consumers more than the commodity or calorie crops. Hence continuing work is required to ensure that they are not left behind in the research that underpins their future.

Breeding programs for tropical crops have to meet the same goals as those for major crops, with additional problems that little is known about breeding systems and genetics. These include abiotic stress—environmental resistances, and biotic stress—caused by organisms including insects and fungi, or by viruses. Post-harvest and quality products are also even more critical as often these characters mean tropical fruits cannot compete with established crops. Storage, seedlessness, sweetness and easy preparation are all addressed in other chapters in this volume; some of these are relevant in context of marketing in the west, others to use as a local subsistence or local market cash-crop. Plant breeders must work with the problems of these orphan crops: parent identification, propagation methods whether grafting or from seed, hybridisation which may be difficult for example.

Consumers and food

While many socio-economic and health indicators show slow changes, instability, and differences between east and west or industrial and developing countries, some key statistics related to food and farming show smooth but substantial changes. The drift to the cities over centuries has been discussed above, along with the dramatic increase in consumption of meat and plant-derived fats and oils over the last 50 years. More processed and refined products have also become an increasing proportion of the diet, features which are discussed in other papers in this volume. This change in crops has also had consequence for farming and its impact on the environment.

The struggle to obtain enough calories, and an appropriate balance of micronutrients, is of over-riding importance to the billion most poor people in the world, typically subsistence farmers with an income of less than one US$ per day. For many of the remaining people, obtaining and eating food is a key contributor to their quality of life, and a varied range of food crops contributes to this. Their choice is, though, influenced by marketing and the related availability, coming from both commercial organisations and as public health messages. Increasingly, the richest people consider issues related to the environment and farming in the food they eat, although their perceptions may be much manipulated by advertising and media presentation.

Meeting the challenges

Food and fruits face major challenges in the 21st Century, with complex relationships between the scientists and breeders who produce the crops which are grown by farmers and eaten by consumers. In the long term, the gene pool is the source of both new crops and new sources of genes for existing crops. Conservation, documentation and exploitation underpins research and development of new crops and varieties. Biotechnology and understanding the behaviour of the plant genome provides a range of tools and options that allow crop 'super-domestication'—the planning of requirements of new characters in our crops.

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Papaya genomics

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Keywords: *Carica papaya*, genome mapping and sequencing, expressed sequence tags, sex chromosomes, bacterial artificial chromosomes

Abstract

Papaya is a major fruit crop in tropical and subtropical regions worldwide. It has long been recognised as a nutritious and healthy fruit rich in vitamin A and C, ascorbic acid, potassium and fiber. Papaya has been recommended for prevention of vitamin A deficiency that causes childhood blindness in tropical and subtropical developing countries. Papaya has a small genome of 372 Mb, a short generation time, and evolutionarily primitive sex chromosomes. These characteristics make papaya an attractive system to study genome and sex chromosome evolution and serve as a model for tropical fruit tree genomics. Genomic resources such as high density genetic maps, a physical map, and an expressed sequence tag database have been developed. A draft genome of female papaya with XX chromosomes was assembled using whole genome shotgun sequences as tools for papaya improvement. Physical maps of the male specific region of the Y chromosome (MSY) and its X counterpart have been constructed and sequencing of sex specific regions on the X and Y chromosomes is near completion. The papaya draft genome and the MSY sequences will enhance our capacity to identify candidate genes for target traits of nutritional and medicinal value besides productivity, to provide genome-wide DNA markers for papaya breeding programs, and to explore the evolution of sex chromosomes and the origin of dioecy in the family of *Caricaceae*.

Introduction

Papaya (*Carica papaya* L.) is an important fruit crop in the tropics. It is primarily a fresh-market fruit and is also used in drinks, jams, and as a dried and crystallised fruit candy. Green fruit, leaves, and flowers can be cooked as a vegetable (Watson, 1997). Papaya fruit is rich in vitamins A and C and is a good source for the minerals K, Mg, and B. The content of one medium papaya exceeds the adult minimum daily requirements of vitamins A and C established by the U.S. Food and Nutrition Board (USDA, 2001). Papaya fruit is ranked number one in terms of potential health benefits among 34 common fruits based on the percentage of the United States Recommended Daily Allowance (USRDA) for vitamin A, vitamin C, potassium, folate, niacin, thiamine, riboflavin, iron, and calcium plus fiber (Liebman, 1992).

Papaya ranks higher than any other fruit on a per serving basis for several nutrients including carotenoids, ascorbic acid, potassium, and fibre. Among carotenoids, red-fleshed papaya contains primarily lycopene, followed by cryptoxanthin and other carotenes, while yellow-fleshed papaya contains cryptoxanthin as the dominant carotenoid followed by other carotenes with no lycopene (Yamamoto, 1964). For ascorbic acid, papaya contains 70–90 mg/100 g, higher than that of strawberry or mango (Beyers et al., 1979). Papaya consumption results in a higher β-cryptoxanthin concentration in plasma than tangerine, orange, or watermelon (Irwig et al., 2002). With such an impressive nutrient content and potential for positive health benefits, there is enormous potential for increasing demand of papaya products worldwide.

Papain (EC: 3.4.22.2), obtained from papaya latex, is the most widely studied and utilised member of a large family of cysteine proteases. Papain is used to develop selective inhibitors to the animal cysteine proteases that exhibit abnormal activity in a variety of diseases including: muscular dystrophy, osteoporosis, pulmonary emphysema, and tumor growth (Czaplewski et al., 1999).

Papain also has direct medical applications for wound debridement, the removal of necrotic tissue (Mekkes et al., 1997); external treatment of hard tissues, wart and scar tissue removal, acne treatment, depilation, skin cleansing treatment, and inclusion in toothpaste. It is used for treatment of Parkinsonism and for tetanus vaccines and immunoglobulin samples for intravenous injection (Brocklehurst et al., 1981).

Papain is also used in beer brewing for chill proofing (Kennedy and Pike, 1981). The enzyme is used in the production of specialised fish protein concentrate for use as a milk replacer when feeding calves and piglets. The enzyme is used to improve the protein dispersibility index of soya flour. It is used in the tenderisation of meat by its action on connective tissue and muscle protein. Beef has been routinely subjected to papain tenderisation (Brocklehurst et al., 1981). Papain is used in the preparation of fish protein concentrates from fish waste, the development of roast beef-like flavors by partial hydrolysis of proteins, and for production of dehydrated pulses and beans (Brocklehurst et al., 1981).

Besides its nutritional and medicinal properties, papaya has a number of characteristics that contribute to its being used as an experimental model for tree crops. Papaya has a short juvenile phase of 3 to 8 months and a short generation time of 9 to 15 months. Flowering and fruiting are continuous throughout the year with the production of one to three ripe fruit per week and hundreds of fruit over the life of the tree. It has an efficient breeding system, each fruit producing about 800 (hermaphrodite) or 1000 (female) seeds, and a single tree producing hundreds of fruit in its lifetime to provide an abundance of offspring for genetic studies. Hand pollination is easily done using pollen from male or hermaphrodite flowers on stigmata of female or hermaphrodite flowers. A clonal propagation system is well established, which allows testing of individual plants in multiple environments.

Papaya is among the limited number of plant species that are trioecious with three sex forms – female, male, and hermaphrodite. In any given breeding system of papaya, it is either dioecious with male and female or gynodioecious with hermaphrodite and female. Sex determination in papaya is controlled by primitive sex chromosomes with a small male specific region about 10–15% of the Y chromosome, showing typical characteristics of chromosome rearrangements and suppression of recombination around the sex determination genes (Liu et al., 2004; Yu et al., 2008a). There are two slightly different Y chromosomes, Y controlling male and Y^h controlling hermaphrodite (Ming et al., 2007). All combinations of the Y and/or Y^h chromosomes are lethal, resulting in no pairing and recombination between YY, YY^h, and Y^hY^h chromosomes. For this reason, there is no breeding system in papaya that produces all three sex types. Papaya offers a rare opportunity to use genomics for studying the evolution of sex chromosomes and the origin of dioecy in the family *Caricaceae*.

Results and discussion

Papaya genome

Papaya is an exceptionally promising system to explore tropical tree genomes. As a genomic model, papaya is appealing because it has a small genome of 372 Mb (Arumuganathan and Earle, 1991), diploid inheritance with 9 pairs of chromosomes, and a well-established transformation system (Fitch et al., 1992), in addition to the advantageous biological features mentioned above. Furthermore, it is a member of the order Brassicales and shares a common ancestor with *Arabidopsis* about 72 million years ago (mya) (Wikström et al., 2001). Genomic resources of papaya have been built up in the past decade, which led to the sequencing of the papaya genome.

Genetic mapping

A high-density genetic map is essential for the integration of genetic and physical maps and ultimately for assigning the sequence scaffolds to papaya chromosomes. It is the first step toward isolating and cloning genes of interest via map-based cloning approach. High density genetic maps

are also an important tool for genomic dissection of complex traits, comparative analysis of plant genomes, and marker-assisted selection.

Genetic mapping in papaya started 70 years ago with three morphological markers: sex form, flower colour, and stem colour (Hofmeyr, 1939). For the next fifty years, there had been no genetic mapping due to the lack of markers. When DNA markers were used for mapping in the 1980s, restriction fragment length polymorphism (RFLP) markers were first used in attempts to construct a genetic map without success for two reasons: 1. The Southern filters of papaya can only be used twice, not 20 times like for other plant species, which makes the RFLP marker system inefficient and costly for papaya. The reason for this unusual phenomenon is unknown. 2. The polymorphism rate among parental lines of papaya mapping population is very low due to the inbreeding nature of hermaphrodite papaya and narrow gene pool used in papaya breeding program (Kim et al., 2002). When randomly amplified polymorphic DNA (RAPD) markers emerged in the early 1990s, they were quickly adopted and the second map was constructed based on 62 RAPD markers (Sondur et al., 1996). The sex determination gene *Sex1* was mapped on linkage group 1 flanked by OPT12 and OPT1C approximately 7 cm away on each side. These two markers were further analysed and sequence-characterised amplified region (SCAR) markers were developed. It turned out that T1 is present in all papaya samples and was used as a positive control for PCR analysis of papaya samples, while T12 along with another SCAR marker W11 showed co-segregation with sex (Deputy et al., 2002).

In the turn of this century, high throughput amplified fragment length polymorphism (AFLP) markers made it possible to construct a high density genetic map of papaya in our pursuit of cloning the sex determination gene. We used Li-Cor sequencers to generate 1778 AFLP markers and the third map was constructed with 1501 markers, including 1498 AFLP markers, the papaya ringspot virus coat protein marker, morphological sex type, and fruit flesh colour (Ma et al. 2004). These markers were mapped into 12 linkage groups and covered a total length of 3294 cm, with an average distance of 2.2 cm between adjacent markers. The sex determination gene was mapped on linkage group 1 (LG 1), following the designation of Sondur et al. (1996), with 225 co-segregating AFLP markers, which accounted for 67% of the 334 markers on LG 1 and 16% of the markers of the entire genome. The large group of co-segregating markers provided strongly support to the hypothesis that recombination is suppressed around the sex determination locus in papaya (Storey, 1953).

Sequence-based DNA markers, such as microsatellite or simple sequence repeat (SSR), are highly informative markers for integration of genetic, physical, and cytomolecular maps. As part of the papaya genome sequencing project, we mined SSR markers from the whole genome shotgun sequence and the BAC end sequences of papaya. We surveyed over 11 000 SSR markers and mapped 713 markers, including 712 SSR markers and one morphological marker, using an F2 population derived from SunUp and AU9 (Chen et al., 2007). This fourth map consists of nine major linkage groups corresponding to the nine chromosomes and three minor linkage groups. Two of the three minor groups were merged to two major groups based on assembled genome sequence and molecular cytogenetic evidence (Ming et al., 2008). This sequence tagged map is being used for molecular cytogenetic mapping of papaya chromosomes.

Physical mapping

A papaya BAC library was constructed from hermaphrodite plants of the transgenic cultivar SunUp and consists of 39 168 clones from two separate ligation reactions (Ming et al., 2001). The average insert size was 132 kb (86 kb for 18,700 clones from ligation #1 and 174 kb for 20,468 clones from ligation #2). Two chloroplast probes, *ropB* and *trnK*, were hybridised separately to the library, yielding a total of 504 chloroplast clones or 1.3% of the library. A cotton rDNA probe hybridised to 61 BACs (0.16%). This library was estimated to provide 13.7x papaya genome equivalents, excluding the false positive (empty clones) and chloroplast clones. Eleven papaya cDNA and 10 *Arabidopsis* cDNA probes detected an average of 22.8 BACs per probe in the library.

The entire set of 39 168 BAC clones of the papaya BAC library was fingerprinted using the high-information-content fingerprinting system (Luo et al., 2003) to produce high quality fingerprints for physical map construction. One-fifth of these fingerprints were excluded due to empty insert clones, incomplete restriction enzyme digestion, highly repetitive sequences, or failure to size on the capillary sequencer. A total of 30 824 fingerprints, estimated as 11x genome equivalents, were used to construct a papaya physical map. After automated overlap evaluation and manual review, 26,466 papaya BAC clones were assigned to 963 contigs. This physical map was integrated with the genetic map and genome sequence using BAC end sequences and a recently constructed sequence-tagged high-density genetic map (Chen et al., 2007). The estimated genome coverage of the physical map is about 95.8%, while 72.4% of the genome was aligned to the genetic map (Ming et al., 2008).

In an exploratory experiment for *Brassica* physical mapping, Dr. Andrew Paterson at the University of Georgia tested 2277 OVERlapping oliGOnucleotide (overgo) probes, representing anchors to *Arabidopsis* and genetically mapped *Brassica* loci, against 36 864 papaya BACs. These overgos were applied in four multiplexed experiments involving 576 probes applied with triple redundancy (24 × 24 × 24). The overgo data produced have been incorporated into the current papaya physical map. These data provide a starting point for the comparative analysis of papaya and *Arabidopsis* genomes. A total of 1259 overgo probes and 16 single copy gene probes were anchored on the fingerprint contigs. Ten of the 16 single copy gene probes were anchored on single contigs. Among the 1259 overgo probes, 483 of them were anchored on single contigs (Q. Yu, A. Paterson, P. Moore, R. Ming, unpublished data).

Expressed sequence tag (EST) sequencing

Five papaya flower cDNA libraries were constructed, three from pre-meiosis (< 4 mm) flower buds (male, hermaphrodite, and female) and two from mature flower buds (hermaphrodite and female). ESTs from these five libraries were sequenced from the 5' end to produce 31 652 clean sequences with a minimum length of 200 nucleotides. The average read length of a clean sequence was 486 nucleotides with a minimum quality score of 20. The final clean sequences were used in clustering and assembly using a paracel transcript assembler. Contaminant sequences from *E. coli*, mitochondria, chloroplast, cloning vector, and RNA were filtered during the cleanup stage. Repeat sequences were masked and annotated. EST sequences were then clustered based on local similarity scores of pair wise comparison using 88% similarity over 100 nucleotides (nt). Clusters containing only one sequence were grouped as singletons. The EST clusters were assembled into contigs (contiguous sequence) by multiple-sequence alignment that generates a consensus sequence for each of the clusters, with criteria of 95% identity over 30 nt overlap. A unigene set of 8571 EST contigs and singletons was assembled. Blast analysis indicated that about 82% of the unigenes from these papaya libraries have homologous sequences in the protein database of *Arabidopsis* (Q. Yu, P. Moore, R. Ming, unpublished). In addition, a normalised and subtractive cDNA library was constructed using pooled RNA samples isolated from roots, leaves, seeds, Cali, three sex types of flowers, and three ripening stages of fruit. Over 50,000 EST sequences were generated from this library, yielding an additional 7791 unigenes with a total of 16 362 unigenes for genome annotation (Ming et al., 2008).

Papaya genome sequencing

The agricultural importance in the state of Hawaii and the unique biological feature of the primitive sex chromosome justified the formation of Hawaiian papaya genome sequencing consortium. The transgenic variety SunUp female genomic DNA was used for genome sequencing for its impact on the papaya industry and to avoid complications of genome assembly in the heterozygous male specific region of the Y chromosome. Moreover, SunUp was developed through transformation of Sunset that has undergone more than 25 generations of inbreeding, an ideal homozygous genotype for a genome sequencing project.

The genome of SunUp female was sequenced at 3X coverage using whole genome shotgun (WGS) approach with Sanger sequencers (Ming et al. 2008). It was assembled into contigs containing 278 Mb and scaffolds spanning 372 Mb including embedded gaps. The estimated residual heterozygosity of SunUp is 0.06%, confirming the highly inbred nature of this Solo variety. Of 16 362 unigenes derived from ESTs, 15 219 (92.5%) matched this assembly. Among 706 BAC end and WGS sequence-derived SSR markers on the genetic map, 652 (92.4%) could be used to anchor 167 Mb of contigs or 235 Mb of scaffolds to papaya linkage groups in the current genetic map. Papaya chromosomes contain heterochromatin knobs, concentrated in the centromeric and pericentromeric regions. The heterochromatic regions account for approximately 17% of the genome, representing about 30–35% of the genomic DNA due to their highly condensed nature. A large portion of the heterochromatic DNA was likely not covered by WGS sequence, as evident by the absence of centromere-specific repeats from the shotgun sequences. The 278 Mb of contig sequence was estimated to represent about 75% of the papaya genome and more than 90% of the euchromatic regions, which is in line with the 92.5% of the EST and 92.4% of genetic markers covered by the assembled genome.

The assembled genome was masked using a *de novo* papaya repeat database for genome annotation. Gene predictions were combined with spliced alignments of proteins and transcripts to produce a reference gene set of 28 629 gene models. Among the 21 784 (76.1%) genes with average length of 1057 bp sharing similarity to proteins in the non-redundant (NR) database from the National Center for Biotechnology Information (NCBI), 9760 (44.8%) of them supported by papaya unigenes. However, among 6845 genes with average length of 309 bp that had no hits to the none-redundant protein database in GenBank, only 515 (7.5%) were supported by papaya unigenes, implying that many predicted papaya-specific genes were false positive. If the 515 genes with unigene support represent 44.8% of the total, then 1150 predicted papaya-specific genes might be real, and the number of predicted genes in the assembled papaya genome would be 22 934. Considering that the assembled genome covers 92.1% of the unigenes and 92.4% of the mapped genetic markers, the number of predicted genes in the papaya genome could be 7.9% higher, or 24 746, about 20% less than *Arabidopsi*s (Arabidopsis Genome Initiative, 2000; Hanada et al, 2007), 34% less than rice (International Rice Genome Sequencing Project, 2005), 46% less than poplar (Tuskan et al., 2006), and 19% less than grape (Jaillon et al., 2007). This number is likely the upper limit for papaya genes, because EST-based unigenes and predicted genes from WGS sequence may each be fragmented and counted multiple times, as demonstrated by initially inflated gene numbers estimated from rice WGS draft sequences.

Papaya has an AT-rich genome, with an overall G+C content of 35.3%, nearly identical to that of *Arabidopsis* at 35%. Coding exons contain significantly higher G+C content, averaging 44.4%.

The papaya genome consists of about 52% repetitive sequences, including 43.4% of the papaya genome is homologous to identifiable transposable elements (TEs) and an additional 8.5% repetitive sequences that are currently unannotated but are likely to be novel TEs. Most of the >600 types of repeats in Repbase[12] are represented in papaya, with the dominant class being retrotransposons (40% of the genome) and some of the abundant types being *Ty3-gypsy* (27.8%) and *Ty1-copia* (5.5%) retrotransposons. An interesting feature of papaya is the relatively low abundance of known DNA transposons (0.20%) compared to other plant genomes. The papaya genome is dominated by papaya-specific TE families, accounting for 38% of the genome sequences.

Papaya is the 5th angiosperm genome to be sequenced and the first transgenic crop to be characterised at the whole genome level. It contributed to our understanding of genome evolution of angiosperm and many unique features of this intriguing tropical fruit. Major findings from the papaya genome sequence include:

- Papaya has fewer genes than *Arabidopsis*, with reductions in most gene families and biosynthetic pathways, making it an excellent system in which to study the function of complex biosynthetic pathways and networks.

- The lower gene number is largely because, unlike *Arabidopsis*, the papaya genome contains no recent genome-wide duplication, with fewer opportunities for subfunctionalisation, implying that papaya genes may be more representative of ancestral angiosperms than *Arabidopsis* genes. This lack of a genome-wide duplication event makes papaya a valuable outgroup for comparative genomics of the *Brassicaceae*.
- Under the assumption that a generalised angiosperm plant could potentially require only the types and minimal numbers of genes that are shared among divergent plant species, we estimate that a minimal angiosperm genome would contain about 13 311 genes.
- Papaya contains significantly fewer (only 25%) disease resistance gene analogs than *Arabidopsis*, suggesting that papaya may have evolved alternative defense mechanisms.
- Papaya also contains significantly fewer P450 genes than *Arabidopsis*, with some subfamilies expanded, some completely absent, and others novel to the papaya genome.
- Despite reduced gene numbers in most biosynthetic pathways, the number of predicted MADS-box family members is strikingly higher (171 versus. 78 in rice and 141 in *Arabidopsis*) in pawpaw than in other sequenced plant genomes.
- Pawpaw has fewer members of gene families involved in fruit ripening, with the exception of starch synthase, possibly reflecting a need for starch storage in the stem and during early fruit development.
- Tremendous amplification in pawpaw of genes related to volatile development implies strong natural selection for enhanced attractants that may be key to fruit (seed) dispersal by animals and aboriginal peoples.
- Papaya contains fewer circadian clock and light-signaling genes than either poplar or *Arabidopsis*, suggesting that papaya does not require the same level of control for daily and seasonal timing.
- Genome-wide searches for transgenic sequences revealed only three insertions, including a functional cassette with the intact PRSV coat protein gene, a fragment of the *nptII* gene, and a fragment of the *tetA* gene. None of the insertions disrupted functional genes.

Sex chromosomes

Sex determination in papaya is controlled primarily by genetic factors, although environmental conditions can trigger sex reversal, particularly in hermaphrodite and male plants. Hermaphrodites and males are heterogametic, whereas females are homogametic. Seeds from self-pollinated hermaphrodite trees and the occasional male flowers with recovered carpels always segregate into hermaphrodite to female, or male to female, at the ratio 2:1, not the typical Mendelian segregation ratio of 3:1, because the combination of homozygous male or hermaphrodite sex determination factors is lethal as postulated previously (Storey, 1938; Hofmeyr, 1938). Seeds from female trees segregate hermaphrodite to female or male to female at the ratio of 1:1, depending on the pollen source.

Advances in genomics over the past two decades led to the development of DNA markers linked to papaya sex determination. Four SCAR markers were developed that have proven reliable and accurate for predicting sex types (Parasnis et al., 2000; Deputy et al., 2002; Urasaki et al., 2002). However, the moderate cost for sexing seedlings and the lack of automation for transplanting selected individuals make it impractical to test and then transplant hectares of seedlings in a brief time for commercial production. Understanding the fundamental mechanism and identification of the sex determination genes could provide the ultimate approach to solving the problems of segregation of sex types in papaya.

Identification of primitive sex chromosomes in papaya

The segregation of sex types in any mapping population make it the focal point to map the sex determination genes in genetic mapping projects of papaya, because of the biological significance of sex determination and the potential implication on papaya production. The first and second linkage maps of papaya placed sex determination genes on a linkage group (Hofmeyr, 1939; Sondur et al., 1996). The ability to map the sex determination genes in papaya was the primary reason for our working hypothesis that there were no sex chromosomes in papaya when we initiated a project to clone the sex determination gene using map-based cloning approach, because classical heteromorphic sex chromosomes do not recombine across majority of the chromosomes. A high density linkage map of the papaya genome was constructed to further characterise the papaya sex determination locus using 1498 AFLP markers, the PRSV coat protein marker, morphological sex type, and fruit flesh colour (Ma et al. 2004). The sex determination locus was mapped to the middle of a large linkage group (LG1) having a large cluster of 225 sex co-segregating markers. This map clearly demonstrated severe suppression of recombination at or around the sex determination locus as proposed previously (Storey 1953).

The sex determination locus was fine-mapped using 4,380 informative chromosomes (two each from 2190 female and hermaphrodite plants of three F_2 and one F_3 populations) and six DNA markers. Despite the large populations screened, not a single recombination event was detected (Liu et al. 2004). The non-recombining (NR) region was physically mapped using our 13.7x BAC library to produce a 2.5 Mb physical map containing 57% of the random sex co-segregating markers developed from co-segregating AFLP markers. Random subclones from non-redundant BACs on the physical map were sequenced to assess the genomic features of the NR region. Sequencing results revealed that the NR region has lower gene density and higher percentage of repetitive sequences. Coupled with the suppression of recombination in a 4–5 Mb region and high degree of sequence divergence between homologous chromosomes in this region, the sequence feature of this NR region is compatible with features of primitive sex chromosomes as envisioned by evolutionary biologists (Charlesworth and Charlesworth, 1978; Charlesworth, 1991). It was concluded that sex determination in papaya was controlled by a pair of primitive sex chromosomes and that the NR region is the male specific region of the Y chromosome (MSY) (Liu et al., 2004).

The cytologically homomorphic sex chromosomes of papaya offer an explanation for the earlier observation of precocious separation of a pair of chromosomes at anaphase I of meiosis of pollen mother cells (Storey, 1953). The small MSY in the middle of the Y chromosome was not pairing with its X chromosome counterparts. A weak attraction of this pair of chromosomes would make their separation and migration towards each pole of the dividing cell easier and earlier. Two slightly different Y chromosomes exist in papaya; one controlling males, designated as Y, and the other controlling hermaphrodites, designated as Y^h (Ming et al., 2007). The lethal effect of any combination of the Y and Y^h chromosomes is likely due to the loss of function of essential regulatory genes on the Y and Y^h chromosomes.

Molecular cytogenetics of sex chromosomes

The MSY of the Y^h chromosome was mapped to near the middle of the genetic linkage group (Ma et al., 2004) and because most papaya chromosomes are metacentric, we postulated that the MSY might be in the vicinity of the centromere. To physically locate the MSY on the Y^h chromosome, two MSY BACs, 54H01 and 76M08, were hybridised on interphase, prometaphase, metaphase, and anaphase chromosomes using florescent in situ hybridisation (FISH) (Yu et al., 2007). Both BACs located on or near the centromere. BAC 54H01 hybridised strongly on the Y^h chromosome and weakly on the X chromosome. BAC 76M08 hybridised only on the Y^h chromosomes but not on the X chromosome, suggesting more extensive sequence divergence between the X and Y^h chromosomes in this region. More detailed FISH analysis revealed five Knobs in the MSY, including four MSY-specific Knobs (Zhang et al., 2008). The centromere of the Y chromosome appeared to be embedded in the MSY.

Physical mapping of the MSY and its X counterpart

Physical mapping of the MSY was initiated from the male specific marker W11 (Deputy et al., 2002; Liu et al., 2004). Screening the 13x hermaphrodite BAC library with the sex-linked SCAR marker W11 produced four positive BACs. A contig map was constructed by cloning the BAC ends and hybridising the ends to the four positive BACs. The two outermost ends were used to screen the BAC library to identify and confirm two groups of positive BACs. One large BAC contig spanning 990 kb was constructed by this stepwise chromosome walking process. In addition, 42 AFLP derived SCAR markers were hybridised to the BAC library and generated four more contig maps. After exhausting the genomic resources available at the time, the first MSY physical map was constructed spanning 2.5 Mb and consisting of two major and three smaller contigs containing four SCAR, 82 *Carica papaya* BAC end (cpbe), and 24 *Carica papaya* sex-specific markers (cpsm) around the sex determination locus.

The second phase of the MSY physical mapping began with fingerprinting all 39 168 clones of the papaya hermaphrodite BAC library (see physical mapping section above). Previously identified MSY BACs were confirmed by FISH. The positive BAC clones were used to detect contigs from the genome wide physical map. Chromosome walking extended the contigs. The relative positions of a set of MSY BACs were verified by fiber FISH and pachytene FISH mapping. Sex co-segregating SSR markers from genetic mapping were used for physical mapping. These SSR markers frequently fell in the already established contigs, but occasionally an SSR marker would provide a new starting point on the MSY or on the corresponding region of the X chromosome. To date, about 8 Mb of the MSY region and 5 Mb of its X counterpart have been mapped with one gap each remaining in the MSY and X physical map. The gap on the MSY was filled on the X physical map, while the gap on the X was filled on the MSY, suggesting that the physical maps of MSY and X counterpart are near completion. The 3 Mb extra sequence of the MSY are largely accounted by the four MSY-specific knobs due to accumulation of transposable elements and perhaps local duplication (Q. Yu, P. Moore, J. Jiang, A. Paterson, R. Ming, unpublished data).

Sequencing of X- and Y-BACs

We have sequenced seven MSY and two X BACs to examine the genomic features of the MSY region (Yu et al. 2007, 2008a). None of these BACs contained known centromere-specific sequences, but they each contained abundant gypsy retroelements and several copia elements, which are features typical of the pericentromeric regions of plant chromosomes. Expression analysis revealed no genes in five of the seven BACs, thus demonstrating the extreme gene paucity in the MSY region. Without papaya specific repeat database, only 20% of the sequences of the MSY were classified as repetitive, but when papaya specific repeat database became available from the papaya genome sequencing project, the content of repetitive sequences jumped to 85% of the MSY and 58% of the X sequences comparing with 52% average of the papaya genome, demonstrating the rapid accumulation of repetitive sequences in the MSY (Ming et al., 2008).

Direct comparison of homologous X and Y^h BAC sequences provided quantitative data for documenting the process of the Y chromosome degeneration and for estimating the time of divergence between the X and Y chromosomes. Two pairs of X and Y^h BACs were sequenced and direct alignment of their sequences revealed three inversion events on the MSY (Yu et al., 2008a). Further analysis of the aligned sequences of the two X and Y^h BAC pairs showed 9.6% to 35.2% DNA sequence expansion on the MSY BACs. Gene expression analyses indicated seven genes on the two X-BACs and four genes on the two Y-BACs. All four genes on the Y BACs had their X counterparts. One of the three unmatched genes appeared to have been either deleted or translocated to another part of the MSY or to autosomes, since this gene located within the matched regions of the X and Y BACs. The other two unmatched genes located in the unaligned region of the X BACs. The time of divergence between the X-Y^h gene pairs was estimated to be between 0.5 to 2.2 mya, supporting the concept of recent origin of the sex chromosomes in papaya (Yu et al., 2008a).

Sequencing of Y^h and Y BACs

A pair of dioecious X- and Y-specific BACs was sequenced and their sequences were compared with corresponding gynodioecious X- and Y^h-specific BACs (Yu et al., 2008b). Numerous chromosomal rearrangements were detected between the X- and Y-specific BACs, including inversions, deletions, insertions, and duplications. DNA sequence expansion was documented on the Y BAC as happened on the homologous Y^h BAC. Dioecious X and gynodioecious X^h-specific BACs were virtually identical sharing 99.97% sequence identity with only seven single nucleotide polymorphism and five single nucleotide indels. The Y- and Y^h-specific BACs shared high degree (98.6%) of DNA sequence identity, while the X and Y BACs shared about 84.4% sequence identity. Local chromosomal rearrangements between Y and Y^h BACs were detected, as the consequence of suppression of recombination in the male specific region and the isolation of Y and Y^h chromosomes enforced by the lethal effect. Analysis of sequence divergence between three dioecious X and Y gene pairs resulted in the estimated ages of divergence from 0.6 to 2.5 million years, reinforcing the hypothesis of a recent origin of the papaya sex chromosomes. The estimated age of divergence between Y and Y^h chromosomes was approximately 73,000 years, prior to the origin of agriculture about 1000 years ago (Gupta, 2004). The hermaphrodite Y^h chromosome is likely evolved from an ancestral Y chromosome naturally, and did not result from human selection as once suggested.

Implications for evolution, medicine and agriculture

Much genetic diversity and evolutionary innovation are in the tropics, yet tropical plants remain under-explored in their basic biology and their enormous potential for novel discovery. The genomic resources of papaya are valuable for studying unique biological features of this triecious species and for improving the quality and productivity of this nutritious tropical fruit. The lack of recent genome duplication makes papaya a valuable resource for studying Brassicales genome evolution, perhaps clarifying what appears to have been a tumultuous evolutionary history in *Arabidopsis*. For example, fewer circadian clock and light signaling genes suggest that papaya may not require the same level of control for daily and seasonal timing to its tropical environment as temperate plants do. Alternatively, papaya might have developed alternative ways of integrating timing information that are specific to day neutral plants, such as strict adherence to the diel light/dark changes. Likewise, the dramatically fewer disease resistance genes and more transcription factors suggest novel defense and regulatory mechanisms that could be uncovered in papaya.

The draft sequence of the papaya genome will help explore its medicinal and nutritional applications and enhance the value of this tropical fruit tree species to benefit both farmers and consumers. Papaya leaves, flowers, fruits, and seed extracts have been used in folk medicine and modern medicine. Papain is used to develop selective inhibitors to the animal cysteine proteases that exhibit abnormal activity in a variety of diseases, including muscular dystrophy, osteoporosis, pulmonary emphysema, and tumor growth. Nutritionally, papain is used in beer brewing for chill proofing and in meat tenderisation. Eight candidate papain genes were annotated in this draft genome, emphasising that papaya is specialised to produce this protease.

Superficially, papaya seems to parallel many mammals in association between the presence of sex chromosomes and lack of genome duplication. However, papaya sex chromosomes are recently evolved (Yu et al., 2208a.b) and likely to have influenced only a short period of its evolutionary history.

Sequencing of the male specific region of the Y chromosome (MSY) and its X chromosome counterparts will have profound applications in papaya production. Hermaphrodites are preferred in most production regions of the world for their higher productivity since every hermaphrodite tree will produce fruit, whereas using female trees for fruit production involves the loss of 6–10% of field space for growing male trees to pollinate the females. The lack of true breeding hermaphrodite varieties results in reduced productivity due to sex segregation among the seedlings. Planting of multiple seeds or seedlings per hill is practiced to assure that there are no more than 3% female

trees in the field. The multiple plants in a hill must be grown for 4–6 months until sexes can be determined. The undesirable sex and redundant plants are then removed to keep one hermaphrodite plant for fruit production. This practice is inefficient of time, labor, water, and nutrients, and also results in delayed production due to competition among the seedlings for the several months during their early stage of development. Identification of sex determination genes in papaya will lead to the engineering of true breeding hermaphrodite varieties without the Y chromosome to improve papaya production.

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Discovery of bioactives in tropical fruits

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Abstract

High throughput platforms for the identification of bioactives have been widely used for many years by large pharmaceutical companies to identify potential leads for new therapeutics. More recently, high throughput instruments have been applied to nutraceutical research. Luminescence and fluorescence-based methods allow the identification of bioactives that act via a diverse array of mechanisms including the modulation of transcription factors, ion channels, receptors and transporters. We have identified that signature molecules found in tropical fruit, such as mangiferin in mangoes, are modulators of specific transcription factors. Fractions from mangoes also exhibit bioactivity for transcription factors as identified by luminescence-based gene reporter assays. Specific fractions inhibiting the proliferation of breast cancer cells have been identified using microplate-based assays. The ability of high throughput-based methods to assess cellular signals such as calcium using fluorometric imaging plate reader (FLIPR) microplate readers, and the advent of high content screening methodologies for the simultaneous assessment of a variety of cellular processes, should greatly accelerate the identification and characterisation of bioactives in tropical fruits.

Introduction

Studies of fruits and vegetables have identified an array of bioactive compounds with demonstrated and potential health benefits (Prasain and Barnes, 2007). The potential nutritional benefits of foods are a key factor in purchasing choices made by consumers. Despite increasing numbers of studies focused on the identification and characterisation of bioactives in tropical fruits, the field is still in its infancy compared to more temperate crops such as blueberries, cranberries and green tea. In the more temperate crops both extraction sub-fractions and relevant signature molecules such as anthocyanins, quercetin, and (-)-epigalloocatechin-3-gallate have been investigated in the context of a variety of processes including tumorigenesis, cell growth and apoptosis (Ju et al., 2005; Seeram, 2008; Seeram et al., 2006; Zhang et al., 2006).

This paper provides a review of some of the high throughout approaches that are used and/or have the potential to accelerate the characterisation and identification of bioactives in tropical fruits. The paper also provides some methods and results of recent work looking at a class of transcription factors in the context of mango relevant compounds.

The microplate, high throughput screening and the advent of cell-based assays

The advent of the 96 well microplate and the subsequent development of 384 and 1536 well microplate formats have been major developments in the field of biomolecular screening and drug discovery more generally (Pereira and Williams, 2007). Although initially the focus of pharmaceutical and drug discovery researchers, the microplate has now become an integral tool in biomedical research. Early researchers using 96-well microplates could not have envisaged the impact of robotics or the diversity of assays that have been developed because of the technology.

Microplate assays, once the focus of assays involving isolated enzymes, increasingly involve cell based assessment. Cell based assays in the context of biomolecular screening often involve

absorbance, fluorescence or luminescence as the method of end point detection. Below, we describe and give examples of assays using each of these end point methods and use them to highlight the increasing complexity of cell based assays in biomolecular screening and their applicability to the study of bioactives in tropical fruits.

Absorbance endpoint assays and the assessment of cellular proliferation

Absorbance microplate readers are widely available in most biomedical and biochemical research laboratories. Their uses are diverse, ranging from the measurement of protein content of cell lysates, enzyme-linked immunosorbent assays (ELISAs) for the measurement of cytokine levels in biological samples, as well as cell based assays including those assessing cellular proliferation. Microplate based proliferation absorbance assays are arguably the simplest form of cell based assay, and often involve indirect measures of cell numbers, such as those based on the activity of mitochondrial enzymes. A specific example are tetrazolium dyes such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS), which when reduced in viable cells can be assessed using an absorbance microplate reader (Berridge et al., 2005).

Limits of absorbance-based cellular proliferation assays in the context of biomolecular screening include interference by extracts that contain components with spectral properties that overlap with the reagents used to assess the number of viable cells. Although most absorbance proliferation assays are assessed at high wavelengths, appropriate controls are a requirement and include assessing the effects of extracts on the proliferation assay when they are added just prior to the addition of assay reagents, ensuring insufficient time for them to alter the cell cycle and proliferation. Such controls will help determine if an extract has components that absorb at wavelengths that interfere with the assay, have an immediate effect on the reaction cycle used in the assay, or induce immediate cellular toxicity through cell lysis. Extracts with such properties can then be assessed using methods that are more appropriate. Secondary assays could include alternative proliferation assays or cytotoxity assays such as lactate dehydrogenase release (Smith et al., 2004). Absorbance based cellular proliferation assays are now widely used in nutraceutical research.

Luminescence endpoint assays and the assessment of the transcription factor modulation

The production of light via luminescence is a powerful tool for biomolecular screening. There are now a wide array of microplate based luminescence instruments, some of which are also capable absorbance and fluorescence detection. Luminescence is highly sensitive and assays are as diverse as cellular proliferation and the measurement of intracellular calcium (Cobbold and Rink, 1987). One of the most powerful and widely used assays based on luminescence is transactivation assays to indentify and characterise modulators of transcription factors (Li et al., 2006). Transcription factors are key regulators of gene transcription and are common targets for pharmaceuticals. Transcription factors with significance in disease include the oestrogen receptor and peroxisome proliferator-activated receptors (Grun and Blumberg, 2006).

Transactivation assays for biomolecular screening often use cell lines that do not endogenously express the transcription factor of interest such as the cos-7 and CHO cell lines. Assays usually involve the exogenous overexpression of the transcription factor of interest e.g. oestrogen receptor α (ERα) as well as a sequences containing the appropriate promotor sequence (e.g. oestrogen response element (ERE) for ERα studies) and a cDNA of the luciferase enzyme. The cell based assay then involves assessment of the ability of extracts or bioactive signature molecules to increase luciferase levels directly or to modulate the ability of activators of the transcription factor (e.g. oestradiol for ERα) to act on their targets. Assay kits to measure luciferase levels via luminescence are widely available; many of which are directly produced for microplate based assays (Li et al., 2006). Such assays are increasingly been used in nutraceutical research.

Fluorescence endpoint assays and the assessment of intracellular calcium signaling and high content screening

Fluorescence has been used in a variety of biochemical assays as a method of endpoint detection, including assays for enzyme activity and cell based assays such as those to assess proliferation. Although the increased selectivity of fluorescence assays is a potential advantage over absorbance assays, fluorescence based assays also allow cell based assays to be performed that are not possible using absorbance. Two specific examples discussed here relate to the measurement of intracellular calcium and a technique known as high content screening.

Changes in intracellular-free calcium are a key signal in many cellular processes. The first fluorescence based probes for the measurement of intracellular-calcium were developed by the research group of Roger Tsien in 1982 (Cobbold and Rink, 1987), who in 2008, shared the Nobel Prize for chemistry for his work on green fluorescence protein. These probes are similar to calcium chelators in structure but with the addition of fluorophores, which bestow the ability to undergo changes in fluorescence properties (intensity and in some cases spectral properties). Due to their high polarity, they are usually used in an ester form, which intracellular esterases cleave, causing the calcium sensitive moiety to be trapped in the cytoplasm where it can be used to report changes in intracellular free calcium via fluorescence detection (Cobbold and Rink, 1987).

The assessment of intracellular free calcium was recognised immediately as a powerful tool in research to improve the understanding of how the calcium signal regulated a variety of cellular processes and to allow, with fluorescence microscopy, researchers to see how increases in calcium are often not homogenous through the cytoplasm (Cobbold and Rink, 1987). However, it was not until the advent of specialised fluorescence microplate readers capable of assessing fluorescence in all wells of a microplate, that the potential of these probes for high throughput screening was fully realised (Monteith and Bird, 2005). The ability of many G-protein coupled receptors to elevate intracellular free calcium, allowed such instruments to have immediate application in high throughput screening drug discovery programs to design and identify novel modulators of specific G-protein coupled receptors, one of the most common target for therapeutics (Hodder et al., 2004). These instruments have also been used to characterise the effects of ion channel modulators such as capsaicin the 'hot' active component of chilli peppers (Jerman et al., 2000); the future will see the application of this technology to identify and characterise other bioactives in foods, including tropical fruits.

One area of great interest in biomolecular screening is a new technique known as high content screening (Korn and Krausz, 2007; Rausch, 2006). The assays are high content because they supply a large amount of information regarding various cell functions. Put simply, high content screening often uses fluorescence detection, however, it is more analogous to a fluorescence microscope than a fluorescence microplate reader (Abraham et al., 2004). The instrumentation collects images from individual or multiple regions of a well, often at multiple excitation and emission wavelengths (multiplexing) (Abraham et al., 2004). This allows numerous cellular processes, such as cell morphology, the cellular cytoskeleton, apoptosis, mitochondrial potential, receptor internalisation to be assessed in the same well of a microplate (Abraham et al., 2004). This is a powerful tool for biomolecular screening and is used to indentify drug leads from combinatorial chemistry libraries and natural product extracts as well as identify new drug targets using inhibitory RNA screens (Korn and Krausz, 2007; Rausch, 2006). The use of high content screening is also now extending beyond the classic cell lines used in screening to stem cells (Richards et al., 2006). The near future should see this technology increasingly applied to nutraceutical research, where it will greatly accelerate the discovery of new bioactives from edible fruits.

Below, we describe an experiment assessing the effects of mango signature molecules on the activity of peroxisome proliferator-activated receptors as an example of the application of microplate cell based assays in the study of tropical fruit bioactives. Similar studies are currently being performed in our laboratories on tropical fruit extracts and on other transcription factors.

Materials and methods

Quercetin, mangiferin, dimethyl sulfoxide (DMSO) and fatty acid free bovine serum albumin (BSA) were obtained from Sigma Aldrich (Sydney, Australia). Norathyriol was a kind gift from Dr Chun-Nam Lin (Kaoshsiung Medical University). Dulbecco's Modified Eagle's Medium (DMEM) and foetal bovine serum were purchased from JRH (Sydney, Australia). GW7647 and GW0742 were obtained from Calbiochem (EMD Biosciences, Inc., San Diego, CA). Ciglitazone was purchased from Sapphire Biosciences (Sydney, Australia). LipofectAMINE 2000 reagent was purchased from Promega (Sydney, Australia) Stock solutions for quercetin, mangiferin, and norathyriol were prepared on the day of the experiment.

Human PPARα and PPARβ, in the plasmid pcDNA3.1, were kindly provided by A/Professor Jeffrey M. Peters (Pennsylvania State University). Human PPARγ plasmid pcDNA3.1hPPARγ was a kind gift of Professor John Prins (University of Queensland). The plasmid pTK3XPPREluc containing three copies of a PPRE cloned upstream of a luciferase reporter was a kind gift of Prof. Ron Evans (Howard Hughes Medical Institute).

Transactivation assays

Cos-7 cells were cultured in growth media consisting of DMEM with 10% foetal bovine serum, L-glutamine (4 mM), penicillin G (100 units/mL), and streptomycin sulfate (100 µg/mL). Cells were plated at 1.2×10^4 cells/well into a 96-well plate and were allowed to adhere for 24 h prior to transient transfection using LipofectAMINE 2000 reagent (0.8 µL/well). Transient transfections were conducted under serum- and antibiotic-free conditions according to the manufacturer's directions.

Transactivation assays were performed on cells transfected with transfection control plasmid pSV-b-Gal (200 ng), pTK3XPPREluc (250 ng), and 100 ng of the appropriate PPAR isoform plasmid (PPARγ, PPARα, or PPARβ). After transfection (5 hours), media was replaced with serum- and phenol red-free medium supplemented with 1.5% BSA and appropriate agent(s) for 19 hours. Cells were then lysed with 1× luciferase lysis buffer (Promega) for PPRE-luciferase activity or with 1× reporter lysis buffer (50 µL) (Promega) for β-galactosidase (β-gal) assay. Luciferase expression was measured using a luciferase enzyme activity assays (Bright-Glo Luciferase Assay System (Promega)). Luminescence was measured using either a NOVOstar or FLUORstar fluorescence microplate reader (BMG Labtechnologies, Offenburg, Germany). β-gal assay buffer contained, 200 mM sodium phosphate buffer, pH 7.3, 2 mM $MgCl_2$, 100 mM β-mercaptoethanol, 1.33 mg/mL *o*-nitrophenyl β-galactopyranoside. Plates were incubated for 2 hours (37 °C). Absorbance at 415 nm was assessed using using a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Reagents Park, Australia).

Statistical analysis

Statistical analysis, for the generation of dose−response curves, and for IC_{50} values was performed using Prism V4.03 (GraphPad software Inc).

Results and discussion

Microplate based luminescence transactivation studies were used to assess potential PPAR isoform modulation properties of molecules associated with mangoes, namely quercetin, magiferin and its aglycone derivative norathryiol. Assays were validated through the ability of the isoform selective PPAR activators to induce luciferase expression against the appropriate PPAR isoform. Maximal concentrations were selected for PPARα (GW7647, 1 µM), PPARβ (GW0742, 1 µM) and PPARγ (ciglitazone, 100 µM). Mangiferin, quercetin and norathyriol did not activate any of the PPAR isoforms. However, quercetin and norathyriol inhibited the ability of PPAR specific ligands to activate their target receptor. This inhibition was determined by the generation of dose response curves (100 nM – 1 mM) for quercetin, mangiferin and norathryiol in the presence of the appropriate PPAR activator, allowing calculation of an IC_{50} value for each agent (see table 1).

There results demonstrate the potential use of luminescence detection cell based assays for the assessment of the the bioactive properties of signature molecules in a microplate environment. They also demonstrate the potential importance of metabolic pathways in the mode of action of some bioactives. More recent studies from our laboratory have focused on extracts from tropical fruits. Microplate based assays will allow extensive numbers of sub-fractions to be assessed while still exploiting the relevance of cell based assays.

The next 5 years should see an expansion in the number and complexity of assays used to assess the bioactives in tropical fruits. The use of microplate cell based assays will greatly increase the productivity of such studies.

Table 1: Assessment of the inhibitory activity of quercetin, norathyriol and mangiferin on ligand-induced transactivation of PPARα, PPARβ and PPARγ.

	IC_{50} (μM)		
	PPARγ	**PPARα**	**PPARβ**
Quercetin	56.3	59.6	76.9
Norathyriol	153.5	92.8	102.4
Mangiferin	> 1000	> 1000	> 1000

Quercetin and norathyriol inhibit the ability of PPAR ligands to transactivate their PPAR isoform target. Mangiferin is not effective in modulating the activity of PPARs. Adapted from published studies (Wilkinson et al., 2008).

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Poster presentations

Papaya (*Carica papaya*) as a source of glucotropaeolin and its active derivative, benzyl-isothiocyanate

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Abstract

Papaya, otherwise known as papaya, is one of few tropical fruit species to contain a glucosinolate. Papaya specifically contains the glucosinolate, 'glucotropaeolin', which hydrolyses to form benzyl-isothiocyanate, a compound found to be a significant inducer of chemoprotective phase 2 enzymes, have anti-proliferative action against cancer cell growth, and have anthelmintic action. The only other significant horticultural source of glucotropaeolin appears to be the culinary herb, garden cress (*Lepidium sativum*). Although both papaya and garden cress were confirmed to be significant sources of glucotropaeolin, the proportion of the active derivative, benzyl-isothiocyanate, formed during hydrolysis of garden cress seed was quite low (~10%), with phenyl-acetonitrile (75%) and benzyl-thiocyanate (15%) constituting the main hydrolysis products. By contrast, hydrolysis of papaya seed extract yielded 100% benzyl-isothiocyanate. This appeared to be related to the absence of nitrile-forming activity in papaya, in direct contrast to garden cress. Within the papaya itself, glucotropaeolin levels were highest in the latex, tissues containing latex such as new leaves, and the seed itself. Flesh of both ripe and immature papaya fruit contained considerably lower concentrations, but as glucotropaeolin imparts an unpalatable 'nasturtium' flavour to fruit, attempts to increase glucotropaeolin levels in the edible fraction either by breeding or selection would be inadvisable from a culinary perspective. Rather, glucotropaeolin could be more efficiently extracted from non-edible sources such as latex, young leaves, or seeds.

Introduction

Papaya (*Carica papaya*) is a tropical fruit normally eaten for its sweet, distinctively flavoured flesh. Otherwise known as papaya, it is one of few tropical fruit species to contain a glucosinolate, a class of compounds that are generally associated with vegetables and herbs of the brassica family (Fenwick et al., 1983). Papaya specifically contains the glucosinolate, 'glucotropaeolin' (Gmelin and Kjaer, 1970), which hydrolyses to form benzyl-isothiocyanate, a compound that has been found to be a significant inducer of chemoprotective phase 2 enzymes (Tawfiq et al, 1995; Zhang and Talalay, 1998; Zhang, 2000; Ye and Zhang, 2001), have anti-proliferative action against cancer cell growth (Cortesi et al., 1998; Nastruzzi et al., 2000), and have anthelmintic action (Kermanshai et al., 2001). The only other significant horticultural source of glucotropaeolin appears to be the culinary herb, garden cress (*Lepidium sativum*) (Ettlinger and Hodgkins, 1956).

The current study presents information on the comparative level of glucotropaeolin in papaya and garden cress seed extracts, and assesses the relative extent to which benzyl-isothiocyanate is formed as a hydrolysis product. The concentration of glucotropaeolin in edible and non-edible papaya tissues of different maturity is also presented.

Materials and methods

Comparison of garden cress and papaya seed extracts

Glucotropaeolin analysis

Garden cress seed (Yates) was purchased commercially and papaya seed was extracted from an unnamed variety of fruit purchased from a local retail outlet. The gelatinous sarcotesta was removed from the papaya seedcoat and the seed allowed to dry under ambient conditions for 14 days.

Approximately 0.4 g seed was weighed and boiled in 10 ml deionised water for 5 minutes to inactivate myrosinase-induced degradation of glucosinolates. Seed was homogenised with an Ultra-Turrax for two minutes and centrifuged for 15 minutes at 13 000 rpm. The supernatant was collected and filtered through Whatman's No.1 filter paper. The filtrate was made up to 20 ml with distilled water and refiltered through a 0.2 µm syringe filter. Supernatants were analysed for glucosinolates by HPLC using a Prevail C18 column as described by West et al. (2002). Quantification of glucotropaeolin was initially based on commercially available high-purity (99.3%) sinigrin, and converted to glucotropaeolin based on the relative peak areas obtained with an equimolar concentration of sinigrin and glucotropaeolin (i.e. 1 µmol sinigrin equated to 0.86 µmol glucotropaeolin).

Hydrolysis products and nitrile-forming activity

Benzyl-isothiocyanate and other hydrolysis products (phenyl-acetonitrile and benzyl-thiocyanate) were measured in papaya and garden cress seeds which had been crushed and allowed to autohydrolyse for 1 hour at 20ºC. Hydrolysis products were measured by GC-FID using the method of Matusheski et al. (2004).

Nitrile-forming activity (or ESP activity) was assayed by incubating an exogenous source of epiprogoitrin with purified myrosinase enzyme in the presence of the seed extract and measuring the subsequent ratio of epithionitrile to simple-nitriles formed (Matusheski et al., 2004).

Glucotropaeolin concentration in different papaya tissues

Glucotropaeolin levels were quantified in fresh seed (sarcotesta attached) and a number of mature and immature tissues of papaya using the same procedure used for the dry seed extracts above. Papaya tissues included skin, placenta, and flesh, of immature and ripe fruit, as well as young and old leaves, and fruit latex (from the immature fruit). All tissues were obtained from the same tree.

Results

Garden cress seeds had approximately double the level of glucotropaeolin of papaya seeds (Figure 1). However, the proportion of benzyl-isothiocyanate formed during hydrolysis was considerably less in garden cress. While glucotropaeolin derived from papaya hydrolysed to form predominantly benzyl-isothiocyanate, garden cress produced largely phenyl-acetonitrile (75%) and benzyl-thiocyanate (15%), with only 10% benzyl-isothiocyanate. Garden cress also exhibited significant epithionitrile-forming activity, with an ESP activity (epithionitrile: simple nitrile ratio) of 13.8. ESP activity was totally absent in papaya.

Amongst different papaya tissues, glucotropaeolin was highest in latex, followed by newly expanding leaves, skin of immature green fruit, and fresh seed tissue (Figure 2). In contrast, glucotropaeolin levels were low in both ripe and immature flesh, and undetectable in ripe placental tissue.

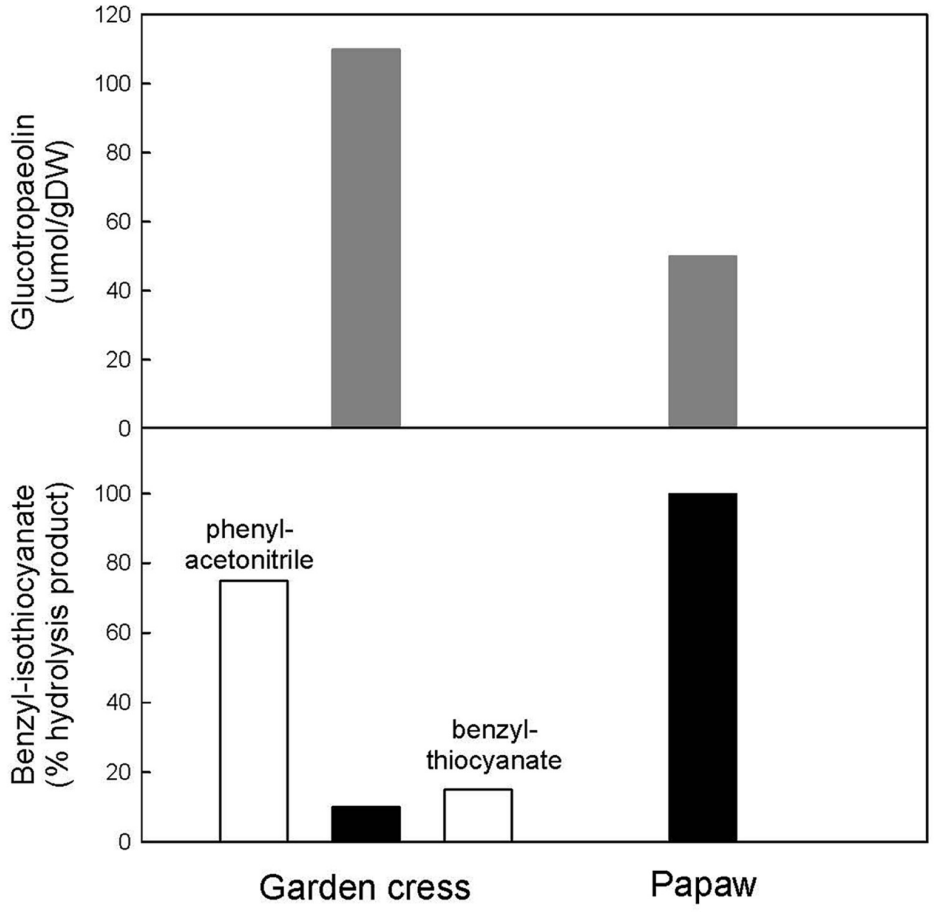

Figure 1. Glucotropaeolin concentrations and percentage hydrolysis to benzyl-isothiocyanate (solid bars) in dry seed extracts of garden cress and papaya. While papaya glucotropaeolin was fully hydrolysed to benzyl-isothiocyanate, the main hydrolysis products of garden cress were phenyl-acetonitrile and benzyl-thiocyanate.

Discussion

Although much interest has been focused on garden cress as a significant source of glucotropaeolin, it would appear that papaya is a better source of the bioactive hydrolysis product, benzyl-isothiocyanate. This stems primarily from the observation that benzyl-isothiocyanate accounts for only 10% of the hydrolysis products formed in garden cress, as opposed to nearly 100% in papaya. Consequently, benzyl-isothiocyanate concentration in dried garden cress and papaya seed (containing 110 µmol/g and 50 µmol/g glucotropaeolin, respectively, in dry seed) would equate to approximately 11 and 50 µmol/g benzyl-isothiocyanate, respectively. It should be noted that our assessment of fresh, undried papaya seed, with the gelatinous sarcotesta still attached, gave a lower glucotropaeolin concentration of 7 µmol/gFW (Figure 2), similar to that obtained (12.7 µmol/gFW) for fresh papaya seed by Nakamura et al. (2007).

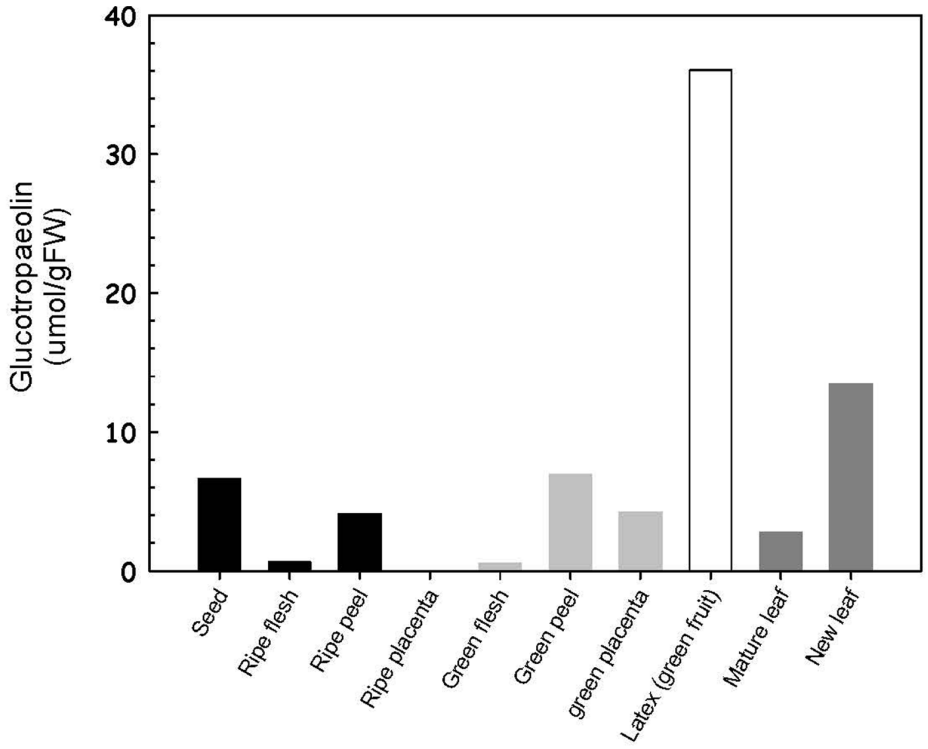

Figure 2. Glucotropaeolin concentrations in fresh papaya tissue. Concentration was highest in latex, newly-expanding leaves, green peel and seeds (gelatinous sarcotesta attached) and lowest in edible flesh (ripe or immature) and ripe placental tissue.

The present study indicates for the first time that papaya lacks ESP activity, or the ability to produce nitriles from glucosinolates. In contrast, garden cress produced predominantly phenyl-acetonitrile following hydrolysis of glucotropaeolin, an hydrolysis product with little or no bioactivity in regard to phase 2 enzyme induction, anti-proliferation of cancer cells, or anthelmintic action. Nitrile formation in garden cress appears to be due to the presence of a thiocyanate-forming protein (TFP), which catalyses thiocyanate and simple nitrile formation from glucotropaeolin (Burow et al., 2007). Based on the evidence of the present paper, papaya does not have an active TFP.

In fresh papaya tissue, glucotropaeolin was highest in latex, followed by newly-expanding leaf, green peel and seed, and lowest in the edible flesh fraction (mature or immature) and ripe placenta. It would appear that seeds and tissues with a high content of latex have highest glucotropaeolin concentration. This is in agreement with Tang (1973), who identified the locality of glucotropaeolin as being principally in the latex and seed (but absent in the sarcotesta). As the edible flesh fraction of papaya contains little latex, it is not surprising that it also contains a low concentration of glucotropaeolin.

While it is tempting to increase the potential health benefit of papaya by breeding papaya fruit high in glucotropaeolin, it is known that glucotropaeolin imparts an unpalatable ‘nasturtium’ or ‘garden cress’ flavour to papaya fruit, which contrasts greatly with the sweet papaya flavour. Although lines of papaya fruit can vary in their degree of ‘nasturtium’ flavour, it tends to be disliked by consumers and would therefore be inadvisable from a culinary perspective. Rather, glucotropaeolin could be more efficiently extracted from non-edible sources such as latex, young leaves, or seeds.

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The health benefits of tropical fruit grown in Queensland, Australia

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Abstract

A review of the literature for 28 tropical fruits currently grown in Queensland was undertaken to understand what is known on their health benefits and indicate gaps for future research. The documented traditional uses of the edible portion of these fruits showed extensive applications for many different health purposes. Tropical fruits are a valuable source of vitamin C and all of the fruits, except for mangosteen, had vitamin C levels per serve greater than 10% of the recommended dietary intake. Some of the fruits were also reported to contain significant amounts of vitamin A, B-group vitamins and fibre. As sources of anti-oxidant activity, guava and carambola were found to have consistently high values relative to other tropical fruit. As well as the in vitro anti-oxidant data, guava, banana, durian, papaya and persimmon have also been shown to increase *in vivo* anti-oxidant capacity in animals or humans. Studies investigating the health benefits of individual fruit consumption were also found for 15 of the fruits. The majority of the literature was concentrated on bananas and plantains, with anti-cancer activity and promotion of gastrointestinal tract health being the major projected benefits. Other benefits reported were the ability to improve lipid profiles and have a protective effect on the liver. However, the majority of studies have been done using rodent models and there is a need for more human clinical studies to properly test the health benefits of these tropical fruits.

Introduction

The increasing epidemiological evidence for the health benefits of fruit and vegetable consumption is attracting great interest. There is burgeoning research into unearthing the mechanisms behind these observed benefits. However, knowledge on the health properties of tropical fruit products appears to lag behind that of temperate crops such as apples, pears, plums and berry fruit as well as crops grown in warm dry climates such as citrus, olives and pomegranate. This is despite most tropical fruits being recognised for health or medicinal properties by populations indigenous to the areas where they are customarily grown. Some of this traditional knowledge has stimulated health research. This combined with nutritional compositional analysis, is indicating which properties of tropical fruits can contribute to the health and wellbeing of consumers.

Results and discussion

Traditional uses

Traditional medicines have mostly utilised the edible and non-edible parts of plants, such as the leaves, sap, skin, seeds, roots and bark, either alone or in combinations. These traditional medicinal uses are being supported by new and more rigorous science, including in vitro assays, *in vivo* and epidemiological studies. There are much higher levels of most phytochemicals in the non-edible parts of plants when compared to the edible portions. However, this review has focused specifically on the edible portion of tropical fruits excluding other parts of the plant except where they are used together with the edible portion. The edible portions of 21 fruits were found to have documented use in traditional medicine and this is summarised in Table 1.

Table 1. Traditional medicinal uses of some tropical fruits.

Fruit	Traditional medicinal use	Geographical origins
Abiu (*Pouteria caimito*)	Used for coughs, bronchitis and other pulmonary complaints (Morton, 1987).	South America
Banana and Plantain (*Musa* spp.)	Green banana has been used in Asian communities to treat intestinal disorders (Rabbani et al., 2004).	Southeast Asia
Breadfruit (*Artocarpus altilis*)	In the West Indies the crushed pulp is applied as a poultice to tumours (Morton, 1987).	Polynesia
Carambola or starfruit (*Averrhoa carambola*)	The fresh fruit, dried fruit and juice have all been used by the peoples of India, Brazil and China to treat a variety of conditions including bleeding, fevers, diarrhea, eye afflictions, kidney and bladder upsets and vomiting (Morton, 1987).	Sri Lanka
Custard/sugar apple (*Annona reticulata/ Annona squamosa*)	*Annona reticulata* used as a poultice for boils, abscesses and ulcers (Morton, 1987). Crushed ripe fruit of *Annona squamosa* used on tumours (Morton, 1987).	Tropical America
Durian (*Durio zibethinus*)	Eaten as a vermifuge (Morton, 1987).	Southeast Asia
Guava (*Psidium guajava*)	In Mexico the fruit is combined with leaves, shoots and bark as a decoction for treating obstetric problems, hypoglycaemia, skin disorders, dental caries, wounds, dehydration and respiratory disturbances and a poultice for treating fever (Gutierrez et al., 2008). In Brazil, the ripe fruit together with flowers and leaves are used as a decoction in treating anorexia, cholera, diarrhoea, dysentery and other digestive problems, inflamed mucosa, laryngitis, sore throat and oral ulcers and skin problems (Gutierrez et al., 2008). In Africa the fruit is used to treat dysentery, in a decoction with leaves and bark or just the fruit peel (Mueller and Mechler, 2005). In Sudan, a decoction of the fruit peel has been drunk to relieve coughing (Mueller and Mechler, 2005). In China, guava has been used as an adjuvant to treat non insulin dependent diabetes mellitus (Mueller and Mechler, 2005).	Tropical America
Jackfruit (*Artocarpus heterophyllus*)	The Chinese have used jackfruit as a general tonic and to overcome the effects of alcohol (Morton, 1987).	India

Fruit	Traditional medicinal use	Geographical origins
Lime (*Citrus latifolia, Citrus aurantifolia, Citrus hystrix*)	*Citrus aurantifolia*, the pickled fruit is used as poultice for headache and neuralgia (Morton, 1987). In India the pickled fruit is used to combat indigestion. The fruit juice is used as a treatment for mosquito bites, vermifuge, antiseptic for wounds and ulcers, tonic, diuretic, bleeding, heart palpitations, cough, rheumatism and arthritis, hair loss, halitosis (Morton, 1987; Ticktin and Dalle, 2005). *Citrus latifolia* juice has been used to treat injuries from stinging corals (Morton, 1987). *Citrus hystrix* fruit has been used as an antiflatulent. *Citrus latifolia* and *Citrus aurantifolia* have been prescribed by midwives in Honduras (Ticktin and Dalle, 2005). All limes have been used as preventatives and remedies for scurvy due to their vitamin C content (Morton, 1987).	Southeast Asia
Longan (*Dimocarpus longan*)	The fresh fruit has been used as a general tonic and specifically for insomnia, heart palpitations, forgetfulness, dizziness, anxiety (Bensky et al., 2004), stomach complaints (Morton, 1987), fever (Morton, 1987), as a vermifuge (Morton, 1987) and as a sexual tonic (Chye, 2006). The dried flesh has been used to treat insomnia, amnesia and other mental disorders (Morton, 1987; World Health Organization Regional Office for the Western Pacific, 1990). Longan is found in several herbal mixtures including one used to treat chronic fatigue in South Korea (Shin et al., 2004). Others may assist with pregnancy (Dagar, 1989; Ling et al., 1996), weight management (Wijaya et al., 1995) and learning and memory (Nishizawa et al., 1991).	Burma/Southern China
Lychee (*Litchi chinensis*)	Lychees have been used to relieve coughing, tumors and to reduce enlarged glands (Morton, 1987). Termed a 'heating' food in traditional Chinese medicine (Huang and Wu, 2002).	Southern China
Mamey sapote (*Pouteria sapota*)	Used to treat gastric ulcers and dysentery (Morton, 1987).	Tropical America
Mangosteen (*Garcinia Mangostana*)	Mainly the rind (pericarp) is used as a remedy for diarrhoea, dysentery and controlling fever.	Southeast Asia

Fruit	Traditional medicinal use	Geographical origins
Papaya (*Carica papaya*)	Unripe fruits high in the enzyme papain are used to tenderise meat. It is used as a treatment for tropical ulcers and as a wound dressing in the West Indies (Hewitt et al., 2000; van Wyk and Wink, 2004). In Uganda, the fruit, together with root and leaf, is used in the treatment of gynaecological disorders (Kamatenesi-Mugisha et al., 2007). In Asia the fruit has been used as a contraceptive (Anonymous, 1994).	Tropical America
Passionfruit (*Passiflora edulis*)	Juice used as a digestive stimulant and to treat gastric cancer (Morton, 1987).	Tropical South America
Persimmon (*Diospyros kaki*)	Used in eastern India, to cure diarrhoea, fever and ulcers (Sinha and Bansal, 2008).	China/Japan
Pineapple (*Ananas comosus*)	Pineapple juice is taken as a diuretic, to ease labour, as a gargle for sore throats and as a remedy for nausea. Unripe pineapple is irritating to the throat and mucosa. Pineapple is used as a vermifuge; it is a strong purgative and is used as an abortifacient and as a treatment for sexually transmitted diseases. Generally it is employed as a digestive, for its anti-inflammatory action after surgery and to reduce swellings in cases of physical injuries (Morton, 1987).	Southern Brazil and Paraguay
Pommelo (*Citrus grandis*)	The juice is used as a vermifuge (Morton, 1987)	Southeast Asia
Rambutan (*Nephelium lappaceum*)	Unripe fruit is used as a vermifuge, anti-pyretic and an aid in digestion and to relieve diarrhoea and dysentery (Morton, 1987).	Malaysia, Southeast Asia
Rollinia/Biriba (*Rollinia* spp)	Used as an anti-pyretic, a restorative and general tonic and due to its vitamin C content as a prevention and treatment for scurvy (Morton, 1987).	Tropical America
Soursop (*Annona muricata*)	In Nigeria the fresh fruit juice is used as an anti-pyretic and in Dominica the fruit is eaten to induce lactation (Ross, 2003). In Trinidad and Tobago soursop is used to provide useful quantities of a range of electrolytes to combat dehydration caused by acute diarrhoea (Enweani et al., 2004).	Tropical America
Star apple (*Chrysophyllum caimito*)	Has been used in the Philippines to treat animal wounds (Palacpac-Alo, 1990). The ripe fruit is eaten to sooth mucosal inflammation in laryngitis and pneumonia, given as a treatment for diabetes mellitus and prepared as a gargle to relieve angina. Slightly unripe fruits are eaten to overcome intestinal upsets in Venezuela. In excess, however, they have been known to cause constipation. The pulverised seed is taken as a general tonic, diuretic and anti-pyretic (Morton, 1987).	Tropical America

Nutrient profile

In evaluating the nutrient profile of the tropical fruits we examined their fibre, vitamin and mineral composition. Where data was available, the United States Department of Agriculture Nutrient database (USDA, 2008) was used otherwise sources are as cited. Almost all tropical fruits are good sources of vitamin C, only mangosteen (*Garcinia Mangostana)* had less than 10% RDI*. Yellow-orange and red coloured fruits are usually good sources of vitamin A, mango (*Mangifera indica*), papaya (*Carica papaya*), persimmon (*Diospyros kaki*) and plantain (*Musa* spp.) range from 5–7% RDI per 100g. Folate is usually associated with green leafy vegetables but some tropical fruits are also a source. Some tropical fruits are also good sources of fibre. The best sources of specific nutrients have been highlighted for vitamin C content (Table 2.1), vitamin A content (Table 2.2) folate content (Table 2.3), B-group vitamins (Table 2.4) and fibre (Table 2.5). The Tables were constructed using an inclusion criteria of ≥ 10% of daily intake (DI*), for fibre and ≥ 10% recommended dietary intake (RDI*) per 100g, for vitamins and minerals, as described in the Australian and New Zealand Food Standards (ANZFS, 2008).

Table 2.1 Vitamin C sources from tropical fruits.

Fruit	Vitamin C %RDI* per 100 g	Fruit	Vitamin C %RDI* per 100 g
Abiu (Morton, 1987)	109	Mamey Sapote (Morton, 1987)	22–49
Avocado	22	Mango	62
Banana	19	Papaya	155
Black sapote (Morton, 1987)	480	Passionfruit (Morton, 1987)	67
Breadfruit	64	Persimmon	17
Carambola	76	Pineapple	106
Custard Apple	77–94	Pitaya (red fleshed) (*Hylocereus costaricensis*) (Mahattanatawee et al., 2006)	140
Durian	44	Pommelo	150
Guava	545	Rambutan (*Nephelium lappaceum*) (Morton, 1987; USDA, 2008)	11–67
Jaboticaba (*Myrciaria cauliflora*) (Morton, 1987)	57	Rollinia (Morton, 1987)	73
Jackfruit	18–22	Sapodilla (*Manilkara zapota*)	33
Lime	65	Soursop	46
Longan	210	Star apple (*Chrysophyllum caimito*) (Morton, 1987)	7.5–38
Lychee	180		

Table 2.2 Vitamin A sources from tropical fruits.

Fruit	Vitamin A %RDI* per 100 g
Jakfruit (Chandrika et al., 2005)	19
Mamey sapote (Alia-Tejacal et al., 2007)	20

Table 2.3 Folate sources from tropical fruits

Fruit	Folate %RDI* per 100 g
Avocado	41
Plantain	11
Papaya	19

*DI = daily intake, DI for fibre = 30 g, *RDI = recommended daily intake, RDI for vitamin C = 40 mg, vitamin A = 750 mg, vitamin B = 10mg, folate = 750 mg, niacin = 10 mg

Table 2.4. Relative anti-oxidant capacity of selected fruits.

Fruit	µmol Trolox Equivalents/100 g (Mahattanatawee et al., 2006)
Guava	1670 (red-fleshed)
Carambola	1290
Pitaya	760 (red-fleshed)
Mamey sapote	660
Lychee	540
Papaya	530
Longan	330
Mango	220
Sapodilla	140
Blueberries#	6552 (USDA, 2007)

Included to indicate the relative anti-oxidant capacity of tropical fruits.

Table 2.5 Fibre sources from tropical fruits.

Fruit	Fibre %DI* per 100g
Avocado	22
Guava	18
Mamey Sapote	10
Passionfruit	35
Sapodilla	18

Anti-oxidant capacity

In vitro assays

There have been several studies which have assessed the in vitro anti-oxidant capacity of a range of tropical fruits (Guo et al., 2003; Mahattanatawee et al., 2006; Surinrut et al., 2005; USDA, 2007). The data from one of these is presented in Table 3. Variability in the assays used and the results reported across the different studies makes it difficult to clearly rank the 28 fruits for anti-oxidant content. However, two fruits have shown consistent high performance amongst the tropical fruits. Guava (*Psidium guajava*) was the highest ranked tropical fruit in five different studies, across three of the commonly used assays, oxygen radical absorbance capacity (ORAC), (Mahattanatawee et al., 2006; USDA, 2007) ferric reducing anti-oxidant power (FRAP) (Guo et al., 2003) (Patthamakanokporn et al., 2008) and 2,2-diphenyl-l-picrylhydrazyl (DPPH) (Lim et al., 2007). Carambola was the highest ranked tropical fruit in one study (Mahattanatawee et al., 2006) (DPPH) and second in three studies (Lim et al., 2007; Mahattanatawee et al., 2006; Surinrut et al., 2005) (ORAC, DPPH). Furthermore, our research has shown guava and carambola (*Averrhoa carambola*) to be the two best performing tropical fruits in the Trolox equivalent anti-oxidant capacity (TEAC) assay (unpublished data). No published values of anti-oxidant activity, by ORAC, FRAP, TEAC or DPPH methods, were found for abiu (*Pouteria caimito*), black sapote (*Diospyros digyna*), breadfruit (*Artocarpus altilis*) or rollinia (*Rollinia deliciosa*).

In vivo assays

There have been several *in vivo* studies investigating the effects of tropical fruit consumption on plasma lipoprotein and anti-oxidant capacity of organs. In humans a meal of bananas (*Musa* spp.) was shown to significantly decrease plasma and lipoprotein lipid peroxidation (Yin et al., 2008). Guava consumption over a 4 week period increased serum anti-oxidant status in male adults (Rahmat et al., 2006). Durian (*Durio zibethinus*) inhibited decreases in plasma anti-oxidant activity in rats on cholesterol-rich diets (Haruenkit et al., 2007; Leontowicz et al., 2008; Leontowicz et al., 2007b). Rats fed red-fleshed papaya (*Carica papaya*) showed higher anti-oxidant activity in the heart muscles compared to animals not fed papaya (Chandrika et al., 2003). Papaya juice fed to rats, lowered lipid peroxidation and increased the total anti-oxidant capacity of blood (Mehdipour et al., 2006). Increased anti-oxidant capacity was seen in both the liver and skin of rats that were fed persimmon powder (Hosotani et al., 2005) Health benefits of consumption

Avocado

Diets supplemented with avocado (*Persea Americana*) protected against chemically-induced liver damage (Kawagishi et al., 2001a) and improved wound healing (Nayak et al., 2008). In an animal study, ingestion of avocados significantly improved plasma lipid concentrations, lowered triglycerides and increased HDL-cholesterol. This improved anti-atherogenic properties (Perez Mendez and Garcia Hernandez, 2007). However, no changes to plasma lipids were observed in human subjects fed avocado (Pieterse et al., 2005).

Banana

Banana consumption has shown good protective activity against mutagens in mice (Edenharder et al., 1998). Plantains have a large body of research supporting the health benefits of their consumption. It is proposed that consuming plantains, amongst other fruits and vegetables, may be responsible for the lower incidence of colorectal cancer in Polynesians living in New Zealand (Botting et al., 1999). Anti-carcinogenic activity was seen in mice, where 30% of animals receiving dietary banana survived versus none of the non-banana group following carcinoma cell injection (Guha et al., 2003). Plantains were shown to remedy persistent diarrhoea (Rabbani et al., 2004; Rabbani et al., 2001), inhibit chemically induced rises in blood-pressure of rats (Osim and Ibu, 1991) and have anti-ulcer effects in a variety of animal models (Costa et al., 1997; Mohan Kumar et al., 2006; Sanyal et al., 1963).

Carambola

Carambola pomace, when included in hamster diets as a source of dietary fibre, showed potential benefits to gut health (Chau and Chen, 2006; Chau et al., 2005a). However, consumption of carambola and carambola juice has resulted in neural and renal damage in people with kidney problems (Fang et al., 2008; Wang et al., 2006). Both the fruit and juice also inhibit liver enzyme activity, which can alter prescription drug pharmacokinetics (Hidaka et al., 2004; Hosoi et al., 2008).

Custard/sugar apple

One study has investigated the effects of feeding sugar apple (*Annona squamosa*) pulp to both normal and diabetic rabbits. Total cholesterol and triglycerides levels were reduced and HDL-cholesterol was increased. Consumption decreased specific enzymes that indicate improved liver function and protection of the liver and heart. There was also an increase in the glucose tolerance of the diabetic rabbits, with a lowering of glycohemoglobin, glucose and protein in urine (Gupta et al., 2005).

Durian

In rats fed a high cholesterol diet, durian showed inhibited rises in plasma lipids and glucose levels (Haruenkit et al., 2007; Leontowicz et al., 2008; Leontowicz et al., 2007b).

Guava

The influence of guava on glycemic response in humans is unclear with both hypoglycemic (Cheng and Yang, 1983) and hyperglycemic effects (Rai et al., 2007) reported. In studies where guava has been included as a dietary intervention, for selected hypertensive patients, there has been a significant lowering of total cholesterol, triglycerides and blood pressure and an increased HDL-cholesterol (Singh and Rastogi, 1997; Singh et al., 1993; Singh et al., 1992). A recent study in young men showed increases in HDL-cholesterol from guava consumption over a 4-week period (Rahmat et al., 2006).

Lychee

Freeze-dried lychee (*Litchi chinensis*) added to the diet of male rats has shown protective effects against chemically-induced liver damage (Kawagishi et al., 2001b).

Mango

Diets supplemented with mango showed promotion of liver health, inhibition of the effects of mutagens on bone marrow and the kidneys (Anilakumar et al., 2003) and in colonic damage (Boateng et al., 2007).

Mangosteen

Dietary mangosteen minimised the effects of a high cholesterol diet, in rats, with significantly lower total cholesterol, LDL-cholesterol and triglycerides (Leontowicz et al., 2006; Leontowicz et al., 2007a).

Papaya

Rats fed a diet containing 100g per day papaya (with guava, vegetables and mustard oil), following a high fat diet, had significant reductions in blood lipids and lipid peroxides but significant rises in plasma levels of Vitamins A, C, E and carotene. There was reduced mortality, smaller aortic plaques and less and smaller coronary artery plaques when compared to animals fed a low fat diet, (Singh et al., 1995). Fermented papaya preparation (FPP) fed to hypertensive rats was shown to have reduced cerebral oxidative stress (Aruoma et al., 2006). FPP also had beneficial effects on DNA damage and redox state in elderly humans (Marotta et al., 2006), improved memory in amnesiac mice (Imao et al., 2001) and lowered lipid peroxidation in rats (Mehdipour et al., 2006).

Passionfruit

The seed from passionfruit (*Passiflora edulis*) has shown promise as a source of dietary fibre that may improve intestinal health (Chau et al., 2005b) and lower plasma cholesterol levels (Chau and Huang, 2005). Passionfruit pulp contains alkaloids that may be responsible for the relaxing effect felt after consumption (Lutomski et al., 1975).

Persimmon

Frequent intake of persimmon was found to be associated with lower concentrations of a marker for age-related oxidative stress in Japanese people over the age of 70 (Kuriyama et al., 2006). Consumption of whole dried persimmon (Gorinstein et al., 2000), as well as dried pulp and peel (Gorinstein et al., 1998), showed protective effects against a high cholesterol diet, with inhibition of rises in total cholesterol, LDL-cholesterol and triglycerides (Gorinstein et al., 1998; Gorinstein et al., 2000). Persimmon peel powder lowered food intake, blood glucose, triglycerides and total cholesterol whilst restoring HDL-cholesterol levels in diabetic rats (Lee et al., 2006).

Pineapple

In rats, pineapple (*Ananas comosus*) juice improved the metabolism and clearance of blood lipoprotein particles (Daher et al., 2005). Human studies demonstrated that short term pineapple consumption had no deleterious effects on the function of the gastro-intestinal tract of healthy individuals (Burroughs and Calloway, 1968). There is evidence that pineapple can act as an anti-allergen (Braun et al., 2005). Bromelain increases absorption of the flavonoid quercetin and inhibited histamine production and release (Center, 2008).

Pommelo

Pommelo (*Citrus grandis*) fruit and juice are known to increase absorption of certain prescription drugs, which can lead to increased therapeutic and/or side effects (Farkas and Greenblatt, 2008; Grenier et al., 2006; Guo et al., 2007).

Soursop

Soursop (*Annona muricata*) contains alkaloids that may be responsible for the relaxing effect felt after consumption (Bourne and Egbe, 1979; Hasrat et al., 1997).

Conclusion

Most health benefits have been inferred from nutrient composition. Some tropical fruits are outstanding sources of phytochemicals such as vitamins, fibre and micro-nutrients including anti-oxidants. The majority of studies have been in vitro studies on various extracts from the fruit rather than consumption studies on whole fruit. Very few human clinical studies have been undertaken with the majority of *in vivo* studies having used rodent models. More human studies will improve the ability to market tropical fruit for their health properties.

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