Wood Chemistry

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What's good in the wood: unveiling chemical shifts induced by *Cryptotermes brevis* digestion

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Abstract: The West Indian drywood termite, *Cryptotermes brevis*, causes large economic impacts by consuming wooden structures. The complexity of the lignocellulosic matrix presents many challenges to termites, who have evolved methods to tolerate recalcitrant components. Chemical variations among wood species and the ability of *C. brevis* to consume them remains understudied. This work compared the chemical composition of hoop pine (*Araucaria cunninghamii*) and shining gum (*Eucalyptus nitens*) before (whole wood) and after ingestion (frass) by *C. brevis*. Holocellulose content in hoop pine declined from 58.7 % of the original wood to 10.9 % in frass, while shining gum holocellulose content decreased from 55.7 % to 12.5 % in the frass. Reductions in hemicellulose were 62 % and 38 %, respectively, while >90 % reductions in α-cellulose were observed within either wood source. Klason lignin levels were similar in wood and frass for both diets. The total extractives did not differ between wood and frass, but specific compounds varied independently. The results suggest the *C. brevis* gut and/or its microbiome primarily utilised carbohydrate components while essentially disregarding lignin and extractives. The results improve the understanding of how wood sources affect termite activity and may advise future material choices for improved pest management.

Keywords: termite; timber; biochemistry; cellulose; lignin; secondary metabolites

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1 Introduction

Wood is the primary external nutrient source for drywood termites, consisting of cellulose and hemicellulose (carbohydrate polymers), lignin (a phenolic polymer), various secondary metabolites, and minor components (Filipiak 2018). However, this diet contains very low levels of proteins and other biologically essential elements, such as nitrogen (Scharf 2020). Termites are among the most important groups of timber pests worldwide, causing extensive damage to timber-in-service and other wooden articles (Ewart and Cookson 2014). Termites primarily derive energy from cellulose and hemicellulose, but lignin also plays a critical role in their diet, as lignin-deficiencies can reduce termite survival (Katsumata et al. 2007; Tarmadi et al. 2017).

Wood is naturally well-defended against termites and other xylophagous insects due to high cellulose crystallinity, lignification, and toxic secondary metabolites. Cellulose and hemicellulose, with β-glycosidic bonds, resist natural degradation and require specialised enzymes for metabolism by termites (Tokuda et al. 2002). Lignin and phenolic monomers provide wood with physical and chemical protection against termite digestion (Ke et al. 2011). Wood typically contains 0.03–0.10 % nitrogen, with C:N ratios ranging from 350:1 to 1250:1 in some conifers (Veerkamp et al. 1997). This low nitrogen content limits amino acid biosynthesis, slowing the growth of wood-feeding insects and microbes (Scharf 2020).

While the complexity of the lignocellulosic matrix poses a major utilisation challenge, termites must also deal with secondary metabolites in wood, which can be toxic, repellent, or even lethal (Bhatla and Lal 2023; Rech-Cainelli et al. 2015; Santos et al. 2017). These compounds significantly enhance the natural durability of wood against termite attack (Kirker et al. 2013, 2024). Extractives may be stable or degrade quickly through auto-oxidation or microbial/insect detoxification. Some studies have found no direct correlation between chemical constituents and termite feeding (Cosme Jr et al. 2020), suggesting other factors also impact susceptibility. Feeding performance also cannot be fully attributed to physical properties, such as density (Haigh et al. 2023), highlighting the complex interactions leading to natural resistance to termites.

Termite guts are uniquely adapted to efficiently extract nutrients from lignocellulose, overcoming the challenges of a wood-based diet. The hardened mouthparts of drywood termites break down wood fibres, increasing the surface area exposed to digestion before the food reaches the gut (Cribb et al. 2008). Drywood termites are protist-dependent and rely on obligate symbioses with co-evolved bacteria and protists in their gut to digest and sustain themselves on these recalcitrant materials (Peterson and Scharf 2016). Symbiotes mainly contribute enzymes that hydrolyse monosaccharides and other energy sources, including xylan and carboxymethylcellulose (Saadeddin 2014; Tsegaye et al. 2019). Gut microbes also maintain appropriate environmental conditions within the gut. For example, some archaeal organisms reduce carbon in the hypoxic regions to permit homoacetogenic spirochetes to fix nitrogen (Brune and Ohkuma 2011). This process is vital to maintain amino acid production in nitrogen-poor wood, though other studies have demonstrated the ability of certain termite species to partially utilise atmospheric nitrogen (Mullins et al. 2022). Some termite gut microbes are transient from the environment, but all protozoa and many bacteria/archaea are true symbiotes (Boucias et al. 2013). The gut anatomy supports efficient nutrient acquisition and protective functions, including maintaining microbial habitats, detoxification, antioxidation, and forming a peritrophic matrix (Scharf and Tartar 2008).

The West Indian drywood termite (*Cryptotermes brevis* Walker) (Blattodea: Kalotermitidae) is considered the most destructive drywood termite species in the world (Scheffrahn et al. 2009), with anthropogenic timber structures, including wooden homes and furniture, providing an ideal nesting environment (Ghaly and Edwards 2011). Effective management of *C. brevis* depends on understanding how these termites degrade wood to sustain their colonies. These termites spend their entire life cycle within wood (Grace et al. 2009). Pseudergates (false workers) are the primary wood consumers, causing most of the damage due to their numerical dominance and feeding activity (Cosme Jr et al. 2018). Here, the ability of *C. brevis* pseudergates to utilise the lignocellulose components of two Australian native wood species, hoop pine (*Araucaria cunninghamii* Mudie) and shining gum (*Eucalyptus nitens* Deane and Maiden) was assessed. The differences in cellulose, lignin, and secondary metabolite contents were examined between intact wood and faecal pellets (frass) produced following ingestion. Further investigations identified the specific extractives encountered, and potential approaches used by the termites to manage these compounds are proposed. The work aimed to identify the differences in these wood components between intact and digested wood, and between the two diets, to det`ermine key components of their metabolism and present a novel insight in termite digestion.

2 Materials and methods

2.1 Termite and frass collection

Colonies of *C. brevis* were collected from an infestation in Maryborough, Queensland. Infested wooden materials were transported to the laboratory at the Ecosciences Precinct, Brisbane, where termites were manually extracted from the timber with a chisel and fine paintbrush, placed on filter paper and acclimated in a controlled environment (25 °C, 75 % relative humidity (RH)) before use in the trial. Colonies of fifty pseudergates (at least second instar) were established and placed onto either hoop pine or shining gum on a Petri dish within a 400 mL plastic container for the duration of the trial. The wood consisted of two veneers (5 cm × 2 cm × 0.5 cm), glued along the long axis using PVA glue to a consistent angle to form a V-shape (after Cosme Jr et al. (2018)). The wood of each species was taken from a single parent board to control for the variability in wood properties across timbers. These two wood species were shown to produce similar feeding and survival rates under laboratory conditions (Haigh et al. 2023) and so were selected as model species.

Five replicate colonies were established for each wood species and incubated at 25 °C and approximately 80 % (±5 %) RH. Frass produced in the first two weeks was removed and discarded to exclude frass derived from the filter paper. The termites were incubated for eight months to allow a sufficient volume of frass to accumulate for analysis. Previous tests suggested that chemical changes with age were not significant over that time period (Haigh et al. 2024). Frass from each replicate dish was placed into separate 20 mL glass vials, collecting all frass present to account for chemical variability within colonies. Five sub-samples of the parent wood boards used to feed the termites were ground to a fine sawdust (Knifetec 1095) of around 2 mm length, comparable to the size of termite frass (Bobadila et al. 2020). The wood and frass were dried overnight at 103 °C and weighed.

2.2 Extractive content

Wood secondary metabolites (extractives) were extracted from 200 mg of wood or frass per replicate via Soxhlet extraction using an acetone: toluene: ethanol mixture (ATE, 4:1:1 ratio), which extracted the compounds from both wood species effectively in pilot trials. Samples were sealed in porous fibre filter bags (F57-200: ANKOM, New York, USA)

and submerged in boiling ATE in a flask for 6 h. The bags were manually rotated every hour to ensure soaking consistency. Upon completion, the bulk of the solvent was evaporated, and the remaining extractive solutions were collected in pre-weighed 2 mL glass vials and evaporated to dryness under nitrogen gas before the mass of the extractives was recorded.

ATE was added to each dried sample to minimise the variances in extraction efficiency by bringing them all to the same extractive concentration (6.5 mg/mL). The samples were then analysed by gas-chromatography-mass spectrometry (GC-MS) (Agilent 6890 and Agilent 5975, respectively), along with an ATE blank control. GC-MS conditions were optimised based on Haigh et al. (2024), injecting 3 µL of sample with the following conditions: inlet temperature 250 °C, carrier gas helium at 15 cm s^{-1}, split ratio 13:1, transfer-line temperature 280 °C, initial temperature 40 °C, rate 1: 40 °C min^{-1} to 200 °C, rate 2: 3 °C min^{-1} to 300 °C, final time 11 min. The MS was held at 280 °C in the ion source, with a scan rate of 2.40 scans s^{-1}. The resulting peaks were assessed across samples, and identities were tentatively assigned using the National Institute of Standards and Technology (NIST) mass spectral library (Miller 2005), removing all peaks that were found in the blanks. Relative percentage areas of the assigned peaks were calculated from the chromatograms to allow comparisons between the samples.

2.3 Cellulose content

All wood and frass samples were first extracted in ATE as described above before being assessed for holocellulose, α-cellulose and hemicellulose content following a modified Jayme-Wise method (Gaudinski et al. 2005). Control samples of purified microcrystalline cellulose (Sigma-Aldrich, Missouri, USA) and bagasse lignin (Machay bagasse, Soda lignin, Queensland University of Technology) were included as positive controls of wood components (bagasse lignin contains both guaiacyl and syringyl units (Sun et al. 2003), similar to wood lignins). All samples were dried at 103 °C overnight and weighed before further processing. Sugars were extracted by placing samples in excess boiling deionised (DI) water until no more colour formed in the solution (around 2 h). Samples were then placed in a solution of DI water (15 mL), sodium chlorite (200 mg) and glacial acetic acid (90 µL) and sonicated at 70 °C for 3 h. The same quantities of sodium chlorite and acetic acid were added two more times with the sonication repeated, then the samples were left in solution overnight. The solution was removed, and samples were sonicated in DI water for thirty minutes. The rinse was repeated three times before the samples were oven-dried at 103 °C and weighed. This mass gave the total holocellulose content in the samples.

The samples were then extracted in 100 mL of sodium hydroxide solution (17 % w/v) at room temperature for 1 h, rinsed in DI water with sonication for thirty minutes and then rinsed with an acetic acid: water mixture (1:10 ratio) with sonication for thirty minutes before a final repeat of the water rinse. The samples were oven-dried at 103 °C overnight and weighed to provide a measure of α-cellulose. Due to particulate matter in the solutions after sonication, all samples were centrifuged at 4,000 rpm (Eppendorf 5810 R) for 5 min after each rinse before the fluid was removed and the samples resuspended. Hemicellulose was calculated as the mass of holocellulose minus the mass of α-cellulose in the samples.

Cellulosic component losses were assessed by calculating termite digestibility coefficients (TDCs). Mass values were standardised as milligrams of components per gram of dry starting material, and TDCs were determined for each cellulose component (x) using the following formula:

$$\frac{mass\ of\ x\ in\ wood - mass\ of\ x\ in\ frass}{mass\ of\ x\ in\ wood} \times 100$$

2.4 Lignin content

The acid-insoluble portion of lignin (Klason lignin) was measured following standard ASTM D-1106-96 (ASTM 2024; Liyanage and Pieris 2015), with slight modifications. Extractive-free samples, along with cellulose and lignin controls, were placed in concentrated H_2SO_4 (72 %) at room temperature for 2 h. The solution was then diluted with DI water to 3 % H_2SO_4 and autoclaved at 121 °C and 103.4 kPa for thirty minutes. The material was vacuum-filtered through a sintered glass funnel (Sigma-Aldrich, Missouri, USA). The precipitate was dried at 103 °C overnight and then weighed to give the mass of Klason lignin in the samples. The acid-soluble portion of lignin in the samples was also estimated by measuring the UV absorbance of the unprecipitated solution at 205 nm on a UV–Vis spectrophotometer (Beckman-Coulter DU 800) using an absorptivity value of 110 L/g/cm.

2.5 Polyphenols and flavanols

In addition to total extractives, further investigations were conducted to examine specific secondary metabolites in the samples, such as total polyphenols and flavanols. Soluble polyphenols were extracted from 50 mg of wood or frass

samples twice in methanol: water solution (8:2, v/v) with sonication for 30 min, followed by agitation for another 30 min 1 mL of each supernatant was removed, pooled, and dried under nitrogen gas before the phenolic residue was redissolved in methanol (250 µL) (after Hayes et al. (2014)).

Total polyphenols were quantified by an adapted Folin-Ciocalteu method (Pizzo et al. 2011; Singleton and Rossi 1965). The extract (20 µL) was diluted with ultra-pure water (80 µL), sodium carbonate (400 µL, 75 g/L) and Folin-Ciocalteu's phenol reagent (2N, 500 µL, Sigma, diluted 10-fold in ultra-pure water) and incubated at 40 °C for 5 min. Absorbance was measured at 735 nm (Beckman-Coulter DU 800) and expressed in mg equivalent of gallic acid per gram dry mass based on a calibration curve created with aqueous gallic acid (Sigma, 0–100 µg/mL).

Flavanols (specifically flavan-3-ols and condensed tannins) were estimated by a colourimetric method using 4-dimethylamino-cinnamaldehyde (DMACA – Merck) (Pizzo et al. 2011; Treutter 1989). The phenolic extracts (50 µL) were mixed with methanol (930 µL) and DMACA solution (20 µL, 100 mg DMACA in 10 mL of 1.5 M methanolic sulfuric acid) and incubated at room temperature for 2 h. Absorbance was measured at 630 nm, and flavanol content was expressed in mg equivalent of catechin per gram dry mass based on a calibration curve created with aqueous catechin (Merck, 0–50 µg/mL).

2.6 Analysis

Proportions were arcsine squared transformed prior to analysis. All data sets were assessed for normality using the Shapiro-Wilk measure, with all data having approximate normal distributions. Differences between wood reference samples and frass were measured by one-sample t-tests for the holocellulose and lignin analyses, and chi-squared test for the α-cellulose and hemicellulose data. Students' *t*-tests and Kruskal–Wallis tests were used to assess polyphenol and individual extractive content differences between samples. Correlations between extractive contents and total polyphenols were assessed using Pearson's product-moment correlation. The above analyses were performed in SPSS (V. 29.0). The multivariate data attained from the identified peaks in the extractive analyses were square root transformed and visualised using non-metric multidimensional scaling (nMDS) ordination and analysed using Analysis of Similarity (ANOSIM) and a measure of multivariate dispersion (MVDISP). The compounds contributing most to dissimilarity between groups were identified using similarity percentages (SIMPER) analysis. The nMDS, ANOSIM, MVDISP and SIMPER analyses were performed using PRIMER-e (V 7.0.23) software.

3 Results

Termites fed on hoop pine produced an average of 282 ± 21 mg of frass per test unit over the 8-month feeding period. One shining gum replicate produced <10 mg of frass, so was excluded; the remaining four replicates averaged 269 ± 21 mg of frass per test unit.

3.1 Extractives

There were no significant differences in total extractive contents between hoop pine wood and frass (4.5 ± 0.5 % and 3.2 ± 0.3 %, respectively; $t = 2.17$, $p = 0.061$), shining gum wood and frass (5.3 ± 0.2 % and 4.5 ± 0.9 %, respectively; $t = 1.19$, $p = 0.274$) or between the two diets when comparing wood ($t = 1.54$, $p = 0.163$) or frass ($t = 1.56$, $p = 0.163$) contents.

GC-MS analysis identified 27 peaks after excluding contaminants and those found in the blanks (see Supplementary Table S1). Of those, 13 were observed across all treatments, 6 were present only in shining gum-related samples, and the remainder were variable between wood and frass. Distinct separations were observed among all groups (Figure 1A, ANOSIM: $R = 0.86$, $p < 0.001$), however, hoop pine wood exhibited higher multivariate dispersion values (1.6–3.2 times

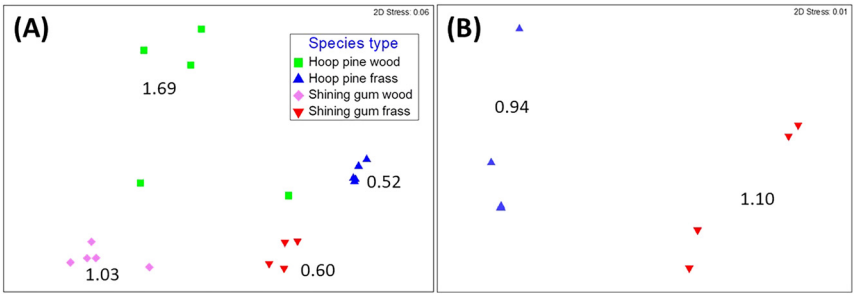

Figure 1: nMDS ordinations of *Cryptotermes brevis* frass samples from: (A) colonies fed on hoop pine or shining gum diets, compared to undigested wood and (B) separate ordination comparing only hoop pine and shining gum frass data. Each data point represents an individual sample. Data were square root transformed and compared based on Bray-Curtis similarity, with two-dimensional stress displayed. Values of multivariate dispersion shown with each group.

Table 1: SIMPER analysis of relative peak areas of compounds identified in extractives from GC-MS analysis of *Cryptotermes brevis* frass from colonies fed on hoop pine or shining gum diets, compared to the corresponding non-consumed wood.

Compound (potential role)	Mean % abundance	Mean % abundance	Mean % abundance	Mean % abundance	Contribution to dissimilarity (%)	Contribution to dissimilarity (%)	Contribution to dissimilarity (%)
	Hoop pine wood	Hoop pine frass	Shining gum wood	Shining gum frass	Hoop pine wood - frass	Shining gum wood - frass	Hoop pine frass - shining gum frass
τ-Sitosterol (lipid metabolism)	18.5 ± 4.1	0.0	27.5 ± 4.3	14.3 ± 1.9	14.3	4.2	14.7
Stigmast-4-en-3-one (cholesterol metabolism)	0.0	0.0	0.0	13.0 ± 0.9	0.0	10.4	14.1
Dioncophyllin A (unknown)	5.8 ± 4.3	23.4 ± 5.4	19.2 ± 3.1	9.2 ± 1.0	12.1	3.8	6.6
Cholest-4-en-3-one (cholesterol metabolism)	0.0	0.0	0.0	3.6 ± 0.2	0.0	5.5	7.5
3-Hydroxy-7-methoxy-3-phenyl-4-chromanone (antifungal defence)	0.0	0.7 ± 0.7	0.0	3.6 ± 2.1	<4.1	3.9	5.3
Vanillin (lignin breakdown product)	5.8 ± 3.2	0.0	10.5 ± 3.1	0.0	6.6	9.0	0.0
Bibenzyl (antifungal/ breakdown product)	0.0	5.6 ± 0.9	0.0	2.4 ± 0.8	8.0	4.2	3.5
Syringaldehyde (toxic breakdown product)	5.4 ± 4.0	0.0	3.9 ± 0.4	1.9 ± 1.2	4.9	<3.0	4.7

The average abundance (±SE) of eight compounds that contributed most (>43 %) to the dissimilarity between groups and their contributing percentages are displayed.

greater, Figure 1A) compared to other groups. Extractive profiles in wood differed significantly from those in frass for both hoop pine (R = 0.77, p = 0.008) and shining gum (R = 1.0, p = 0.008). Termite frass from hoop pine-fed and shining gum-fed groups also differed significantly (R = 1.0, p = 0.008, Figure 1B).

SIMPER analysis identified the compounds contributing to the distinctions between the groups (Table 1). Many straight-chain alkanes identified were present at higher proportions in the frass of either wood species than the corresponding whole wood (see Supplementary Table S1). Compounds present only in the wood but absent in the frass, such as vanillin, significantly influenced the differences. Likewise, compounds only found in the frass and not the wood contributed similarly, for example, 3-hydroxy-7-methoxy-3-phenyl-4-chromanone and bibenzyl. Cholest-4-en-3-one was detected only in the shining gum frass and strongly distinguished this group. Hoop pine frass showed greater dissimilarity due to the absence of compounds like syringaldehyde, which was present in all other treatments. Dioncophyllin A was present in all treatments and also contributed to dissimilarities, with higher abundance in hoop pine frass compared to wood, while shining gum wood had higher levels than its frass. Additionally, τ-sitosterol and stigmast-4-en-3-one were present in shining gum frass but absent in hoop pine frass, further showing the distinctions between frass groups.

3.2 Cellulose

Frass contained significantly less holocellulose (hoop pine: 58.7 % in wood vs. 10.9 ± 1.4 % in frass; t = 25.84, p < 0.001, shining gum: 55.7 % in wood vs. 16.5 ± 2.21 % in frass; t = 14.76, p < 0.001). The relative proportions of α-cellulose and hemicellulose differed between wood and frass in both hoop pine (χ^2_1 = 11.5, p = 0.0007) and shining gum (χ^2_1 = 17.3, p < 0.001). α-cellulose had the most substantial reduction between wood and frass (Figure 2). No significant differences in the cellulose proportions were observed between wood species (χ^2_1 = 0.12, p = 0.75) or frass samples (χ^2_1 = 1.3, p = 0.25). TDC values confirmed the higher digestibility of α-cellulose compared to hemicellulose in both woods, with approximately 1.4–2.4 times higher TDC values. Only hemicellulose TDCs differed between the wood species (t = 2.46, p = 0.043) with less degradation in shining gum (Table 2). Control samples validated method effectiveness, as >96 % of purified cellulose was recovered, and a negligible quantity (2.1 %) of lignin was calculated as cellulose.

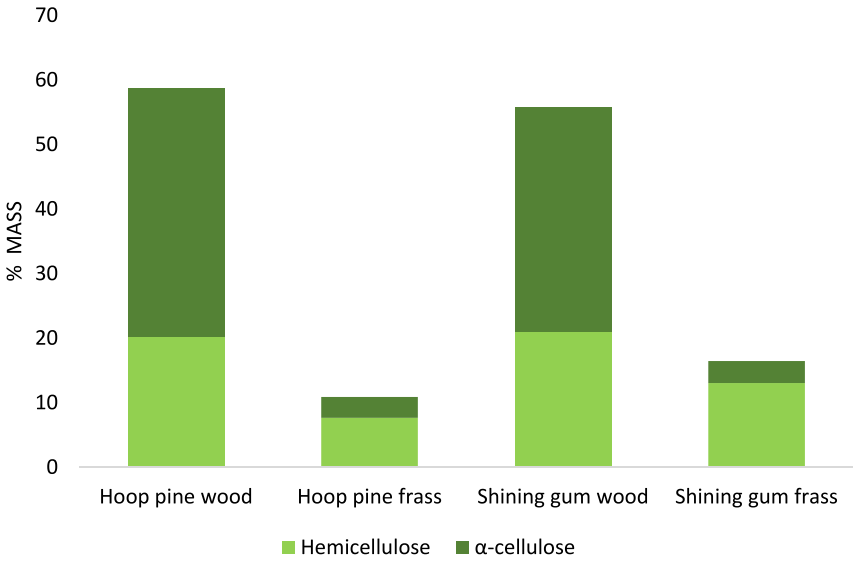

Figure 2: Hemicellulose and α-cellulose contents (as % mass) of *Cryptotermes brevis* frass from colonies fed a hoop pine or shining gum diet versus levels of non-consumed wood. Holocellulose content is shown by the combined bar value.

Table 2: Termite digestibility coefficients (±SE) for the carbohydrate components of two wood species consumed by *Cryptotermes brevis*.

Wood species	Termite digestibility coefficient		
	Holocellulose	Hemicellulose	α-Cellulose
Hoop pine	81.4 ± 2.3	62.2 ± 3.9 a	91.6 ± 3.3
Shining gum	70.5 ± 4.0	37.7 ± 10.2 b	90.2 ± 2.7

Values derived using component masses per gram of dry mass of the entire sample. Values with different letters within a column indicate significant differences between groups.

3.3 Lignin

Termite frass had significantly higher lignin levels than the corresponding wood samples for both hoop pine (t = 26.04, p < 0.001) and shining gum (t = 11.07, p = 0.002). Hoop pine wood contained roughly twice as much lignin as the shining gum wood, and these differences followed with the frass (t = 5.72, p < 0.001). Frass and wood samples had negligible acid-soluble lignin values, like the blank sample tested (data not shown). This supported the use of Klason lignin, the most abundant lignin type in biomass (Goff et al. 2012), as the most appropriate metric for these comparisons.

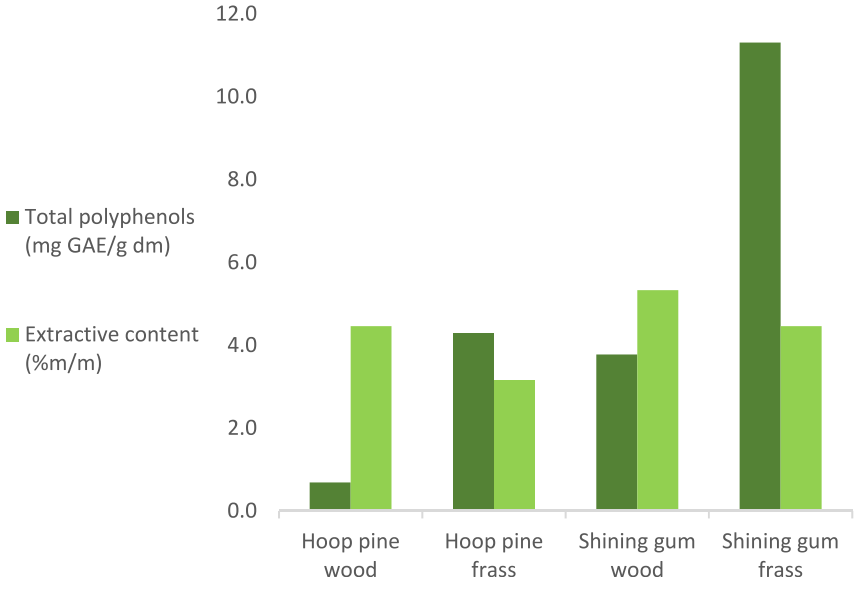

Figure 3: Mean extractive contents and total polyphenols (as gallic acid equivalents or GAE, mg per gram of dry mass) of *Cryptotermes brevis* frass from colonies fed a hoop pine or shining gum diet compared to the corresponding wood.

3.4 Polyphenols

Total polyphenols and flavanols were quantified using gallic acid and catechin calibration curves (R^2 = 0.962 and 0.999, respectively). Shining gum wood had significantly higher total polyphenol contents than hoop pine wood (t = 4.27, p = 0.003), a pattern mirrored in the frass samples (t = 11.96, p < 0.001) (Figure 3). Since there were similar levels of total extractives (see above), a larger proportion of the extractives of the shining gum were polyphenols compared with hoop pine. Lower total polyphenol levels were detected in wood compared to frass for hoop pine (t = 6.88, p < 0.001) and shining gum (t = 9.21, p < 0.001). Total polyphenols and the overall extractive contents were poorly correlated (r = −0.0016, p = 0.99). Flavanol levels in all wood and frass samples were below the detection limit (<1 µg/mL, as catechin equivalents).

4 Discussion

The results presented here confirmed that cellulose served as a primary energy source for *C. brevis*, with high TDC values for both wood species (Goodenough and Goodenough 1993; Leopold 1952). Notably, the ten-fold decrease in α-cellulose content highlighted its efficient utilisation by termites and aligned with recent findings (Alves et al. 2024). α-cellulose is a simpler glucose polymer than hemicelluloses, though the higher crystallinity of the structures often make them difficult to degrade. Despite this, the hemicelluloses were less degraded by the termites (with lower TDC values), likely due to the complex branching structure of these carbohydrates. For example, xylan breakdown requires specialised cleaving enzymes provided by bacterial symbiotes such as *Pseudomonas* species (Schäfer et al. 1996), which were detected in termite guts fed on hoop pine and shining gum (Haigh et al. unpublished data). This differentiation in digestion efficiency underscores the pivotal role of gut symbiotes, including spirochetes (Tokuda 2021) and devescovinid flagellates (Desai et al. 2010), in facilitating the breakdown of cellulose and hemicellulose. It may also provide a potential mechanism for understanding natural resistance of certain wood species to termite attack due to variances in lignocellulose structure. Although α-cellulose is more readily digested, both carbohydrates provide energy to the termites, evidenced by the common mutualisms shared with organisms that breakdown hemicelluloses within their guts (Mauldin 1977; Ni and Tokuda 2013; Paul and Varma 1993; Schäfer et al. 1996).

Lignin was more concentrated in *C. brevis* frass than in the wood of both species, with over 90 % of the original lignin recovered in the frass, likely due to an inability to metabolise lignin. Leopold (1952) demonstrated that *C. brevis* selectively digested carbohydrates in Douglas-fir wood, leaving most of the lignin relatively unchanged. This supports the findings here that lignin becomes relatively concentrated in frass as cellulose is removed through digestion. Katsumata et al. (2007) also reported lignin concentration in termite frass, suggesting structural modifications in aliphatic and phenolic hydroxyl groups rather than lignin decomposition. Hoop pine wood contained more lignin than shining gum, consistent with the general trend of softwoods having more lignin than hardwoods (Tarasov et al. 2018). This pattern was reflected in the frass, with hoop pine-derived frass containing significantly more lignin than frass from shining gum-fed termites. Cookson (1987) demonstrated that lignin degradation varied across termite species and wood types, with *Nasutitermes exitiosus* degrading up to 6.5 % of hardwood lignin, while *Coptotermes acinaciformis* and *Mastotermes darwiniensis* exhibited lower capacities. These findings support the hypothesis that *C. brevis* accesses the polysaccharides as primary energy sources while avoiding lignin. Many brown-rot decay fungi also modify but do not utilise lignin, reflecting the recalcitrance of this polymer (Zabel and Morrell 2020).

Reductions in some secondary metabolite levels during digestion of both wood species highlighted the role of gut microorganisms in utilising beneficial compounds, such as sitosterols (Ciufo et al. 2011; Subekti et al. 2017), or detoxifying potentially harmful ones, like syringaldehyde (Ibrahim et al. 2012), a lignin hydrolysate (Lee et al. 2012). Shining gum wood contained a broader range of extractives compared to hoop pine, consistent with other heartwoods (Schultz and Nicholas 2000) and hardwood species (Telmo and Lousada 2011). The hoop pine samples were taken from sapwood, complicating direct comparisons of extractive content between the two wood types. Termites fed shining gum had a lower diversity of gut bacterial symbiotes (27) than those fed on hoop pine (40) under the same conditions (Haigh et al., in prep.). The shining gum wood also had a lower overall recovery percentage (77.0 %, when combining the mass recovered from all investigations) than the hoop pine (98.4 %), potentially attributable to the higher prevalence of resins or other compounds not quantified in the samples.

The observed variations in secondary metabolites altered through digestion suggest that *C. brevis* employs various physiological strategies to manage these compounds. Some metabolites, such as 2′,4′-dimethyl-2,4,6-trinitrodiphenylamine and 10,11-dihydro-10-hydroxy-2,3,6-trimethoxydibenz(b,f)oxepin in shining gum, and

hexadecanoic acid, (2,2-dimethyl-1,3-dioxolan-4-yl) methyl ester, and τ-sitosterol in hoop pine, were present only in the wood samples, suggesting they were metabolised or detoxified during digestion. Vanillin, also detected only in wood, is a product of lignin breakdown (Fache et al. 2016) and is metabolised anaerobically by gut symbiotes in related termite species (Harazono et al. 2003), suggesting that the *C. brevis* microbiome may utilise some lignin-related compounds. The lack of this compound in the frass affords further evidence that the lignin may be solubilised by the organisms within the termite gut, before re-polymerising and, therefore, not appearing reduced in the frass. This lignin avoidance mechanism has been demonstrated in fungi (McCarthy et al. 1986), and may be convergently established in termite digestion.

In contrast, bibenzyl was found exclusively in frass. Given its antifungal properties (Schultz et al. 1990), bibenzyl might be synthesised by termites or their symbiotes, although it could also result from the breakdown of larger aromatic compounds in the wood. Similarly, 3-hydroxy-7-methoxy-3-phenyl-4-chromanone, which is a homoisoflavone (Gao et al. 2015), was detected only in frass extracts. Flavonoids provide a range of phytochemical functions in plants, including antifungal defence (Galeotti et al. 2008).

Interestingly, some compounds were found in all treatments except hoop pine frass, including coniferyl alcohol, a building block for lignin (Hänninen et al. 2011). The differences in both lignin levels as well as monomer components in hoop pine may be producing different interactions with lignin than those fed on shining gum (Cookson 1987). Alternative lignin modification approaches may have led to diverse lignin breakdown products. Another compound also absent only from hoop pine frass samples was 2,2′-methylene bis 6-(1,1-dimethylethyl)-4-methylphenol, which has been suggested as a potential endocrine disruptor (Andres and Dulio 2024), though its biological function in relation to termites remains unclear.

The two sterol compounds solely identified in the frass of the termites fed on shining gum, cholest-4-en-3-one and stigmast-4-en-3-one, are likely involved in the bioconversion of steroids for cholesterol metabolism within the termite (Dockyu et al. 2018; Pham et al. 2021). Their presence may reflect a dietary response to the shining gum, enabling termites to sustain metabolic function when processing this hardwood. Like all arthropods, *C. brevis* cannot synthesise sterols (Jing and Behmer 2020), making their metabolic systems highly dependent on dietary sources.

Dioncophyllin-A, a naphthylisoquinoline alkaloid found in tropical rainforest plants such as lianas (Yücer et al. 2024), was detected in all samples. It was more abundant in the wood of the shining gum but was more concentrated in the frass from hoop pine. Information on the natural roles of this compound is limited, so understanding its biological relevance proves difficult. The straight-chain alkanes derived from the wood accumulated in relatively higher amounts in the frass, as noted in other saproxylic insect digestive processes, such as the release of micronutrients rather than sequestration within body tissues (Chen and Forschler 2016).

Total polyphenol levels were higher in shining gum wood and frass samples, despite having similar overall extractive contents to hoop pine. Relative polyphenol levels were poorly correlated with total extractives, indicating greater contributions from other secondary metabolites in hoop pine. The relative total polyphenol levels in both sets of frass samples were higher than the corresponding wood samples, suggesting that these compounds were concentrated as they passed through the termite gut without being metabolised. This further highlights the complexities associated with *C. brevis* lignocellulose digestion and the adaptations made to exploit this food source.

The inferences drawn in this work enhance the knowledge base surrounding lignocellulose digestion by *C. brevis*. This information may be applied to future pest management practices through selectively choosing materials less suitable for termite digestion due to altered lignocellulosic composition. Further, the analysis of poorly processed wood metabolites may present opportunities for developing novel termiticides or provide a model for additional innovations in this regard. Finally, investigations into the roles of symbiotic gut microorganisms can be improved with the knowledge surrounding the detoxification of dietary compounds attained here.

5 Conclusions

Drywood termites and their associated gut symbiotes are highly adapted to their challenging diets, utilising the polysaccharides available in wood while modifying extractives and largely disregarding lignin. The data reveal the ability of *C. brevis* to metabolise, alter, or discharge compounds associated with different woods, improving understanding of the natural durability of these timbers and how termites can manage a range of diets. The research provides opportunities for enhancing *C. brevis* management by utilising the comprehensive knowledge of termite wood digestion unveiled here.

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