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# Nutritional, functional and rheological properties of bunya nut flour as a versatile gluten-free option

Jaqueline Moura Nadolny [a,b], Bernadine M. Flanagan [b], Heather M. Shewan [a,*], Heather E. Smyth [b], Odette Best [c], Jason R. Stokes [a]

[a] School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia
[b] Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD 4072, Australia
[c] School of Nursing and Midwifery, University of Southern Queensland, Ipswich, QLD 4305, Australia

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ABSTRACT

Bunya nuts are a starch-rich food that has been consumed by Indigenous communities for thousands of years. In addition to consuming fresh nuts, they were commonly processed into flour. However, their properties as a gluten-free flour are still unknown. In this study, we investigated the nutritional, functional and rheological properties of bunya flour prepared using different methods, as well as the isolated starch, and compared to wheat, rice and chestnut flours. Overall, raw bunya nut flour showed higher fibre (8.4 %d.b.), lower protein (4.7 %d.b.) and higher fat content (4.4 %d.b.) when compared to rice and wheat flours. Its proximate composition was similar to chestnut flour. By altering the preparation method for bunya nut flour we showed that its functional properties can be altered, for example, raw and whole bunya flours showed good foam and emulsion capacity and stability, whereas roasted bunya flour had poor emulsion activity and stability but a high water absorption capacity. Raw and fermented flours showed high peak viscosity when heated in water. Bunya flours, except for the roasted one, showed a slow rate of starch hydrolysis and high resistant starch content compared to wheat and rice flours, especially considering the role of the inner coating when not removed from the nut. Bunya nut flour is a versatile option and can overcome issues found for gluten-free flours such as poor nutritional quality and sensory properties of the resultant products.

## 1. Introduction

Bunya nuts (*Araucaria bidwillii*) are native to South-east Queensland, Australia, and have been largely consumed by Indigenous peoples and nations for thousands of years (Swan, 2017). They come from the same family as *pinhão* and *piñones*, from Brazil and Chile, respectively, and their composition is very similar, as shown in a recent study (Moura Nadolny et al., 2023). The nut is comprised of a hard husk, an inner coating, a kernel and an embryo. It grows inside a cone produced by a 60-meter-tall Araucaria tree and each cone can hold approximately 100 nuts. The husk is the only part that is not edible (Moura Nadolny et al., 2023). Common methods of preparation of bunya nut kernels include roasting, boiling in water, fermenting in running water or grinding into a flour. Bunya nuts are rich in starch (66.8 g/100 g dried sample) and dietary fibre (8.3 g/100 g dried sample) and are naturally free of gluten (Moura Nadolny et al., 2023). Due to their high moisture content and water activity, they are perishable if left at room temperature. Therefore, one of the most common traditional (Swan, 2017) and current uses is to dry and grind the nuts into a flour, especially because they are seasonal.

Flour produced from food staples such as maize, rice and wheat is very common in our diets (Dereje et al., 2020). Also, interest in gluten-free products is growing as the number of people with gluten sensitivity increases (Santos et al., 2021). Problems related to gluten-free products include nutritional deficiencies, poor sensory quality, especially regarding texture and flavour, high starch hydrolysis and storage problems as mould growing and staling if not kept in the freezer. Research on the nutritional, functional and sensory properties of gluten-

*Abbreviations:* WAC, water absorption capacity; OAC, oil absorption capacity; FC, foam capacity; FS, foam stability; EA, emulsion activity; ES, emulsion stability; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; PCA, principal component analysis; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols.

* Corresponding author.
*E-mail address:* h.shewan@uq.edu.au (H.M. Shewan).

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free flours, as well as how processing or addition of other components influence these properties is becoming more and more important (Capriles et al., 2021). Also, the fact that bunya nuts are not a cereal, tuber or legume, could impart unique starch and flour properties that could be suitable for different food applications.

There is currently no scientific literature on the properties of bunya nut flour and starch according to the Web of Science™. Studies on the properties of the flour for the other two species of Araucaria nuts are limited. One study investigated the influence of extrusion cooking on the nutritional and sensory properties of *pinhão* flour extrudates (Zortéa-Guidolin et al., 2017). The extrudates showed good expansion and textural properties, as well as good acceptance among consumers. They also had an increased slowly digestible starch content compared to the raw material before extrusion. Another study analysed the chemical composition and gelatinisation properties of both fresh and frozen raw and cooked *pinhão* flour dried at different temperatures (Capella, 2008). The main findings include that *pinhão* flours are a good source of fibre and that the retrogradation of the raw flour was much lower than the cooked flour. There were no significant changes regarding macronutrients. Furthermore, a study showed that the skin surrounding *pinhão* (inner coating) have the potential to inhibit salivary and pancreatic α-amylase due to its high tannin content (Silva et al., 2014), which could slow down the starch hydrolysis. Regarding aroma and flavour characteristics, Moura Nadolny et al. (2022) showed that depending on how they are prepared and in comparison to chestnuts, bunya nuts may show a range of different aromas and flavours.

Since starch is the major component, it is expected that the properties of the flour will largely depend on the properties of the starch. For this reason, the starch was isolated and its main characteristics were also analysed to improve understanding of how the starch influences the properties of each type of bunya nut flour.

The aim of this study is to investigate the physico-chemical, functional, pasting and nutritional properties of raw and whole (not removing the inner coating) bunya nut flour as well as processed bunya nut flours (from roasted, boiled and fermented nuts) to provide insight into potential applications for these flours. We also compare these properties with those from commercial wheat, rice and chestnut flours. Wheat flour is the most common flour used for bakery products, while rice flour is the most commonly used gluten-free flour because of its low price and bland flavour. We hypothesise that bunya nut flour will be a suitable gluten-free alternative to wheat flour and that by altering the pre-treatment of the flour, physical properties can be varied for a range of baking applications. Also, the whole flour may provide lower digestibility due to the presence of the inner coating, which contains high phenolic and fibre content. The inner coating is the equivalent of the skin of other nuts, which have also demonstrated a range of health benefits (Gonçalves et al., 2023).

As a result of this study, we expect to expand the utilisation of bunya nut flour to add value through improved health, sensory, storage and textural aspects of foods, when incorporated into wheat or rice flour, or used it on its own. Also, the findings might benefit researchers studying not only the species in Brazil (*Araucaria angustifolia* – *pinhão*) and Chile (*Araucaria araucana* – *pinones*), due to similarities in composition and possibly starch characteristics, but also studies looking at the properties of other types of flours and starches.

## 2. Methods

### 2.1. Wheat, rice and chestnut flours

Wheat flour was obtained from Coles, QLD, Australia. Rice flour was purchased online from Wholesalers Gluten-free, Australia and chestnut flour from Naturitas, Australia.

### 2.2. Bunya nut flour preparation and starch isolation

Bunya nuts were purchased from Blackbutt, South-East Queensland, Australia, sealed in polyethylene bags under vacuum (each bag containing approximately 400 g) and stored (−18 °C) until required. The nuts were then taken out of the freezer and left at room temperature for 1 h. Roasted nuts were prepared by baking for 20 min at 120 °C. Fermented nuts were prepared by soaking 600 g of bunya nuts in 2 L of distilled water for 9 days, changing the water every 2 days, similarly to the natural fermentation of chestnuts (Neri et al., 2010). The nuts were cut in half using an equipment specific for cutting bunya nuts, which includes a wood support and a knife attached to a lever. The kernels were removed and dried in an air-drying oven (Anko 3-in-1 Air Fryer Oven, QLD, Australia) at 50 °C until constant weight before grinding using a coffee grinder (Anko Coffee Grinder, QLD, Australia). The whole flour was prepared similarly to the raw flour, with the inner coating being kept and ground with the kernel instead of being separated during dehulling.

The method used for starch isolation was developed in this work specifically for bunya nuts using as a basis the methods developed by Bello-Perez et al. (2006) and Henriquez et al. (2008). The bunya nut samples were left at room temperature for 1 h after removal from the freezer. The external husk of the nut was cut and removed, as well as the inner coating and embryo. The kernel was ground in distilled water (1 kg/1.5 L) in a Thermomix® TM6 (Vorwerk Elektrowerke GmbH & Co. KG, Germany) for 5 min at speed 5. The homogenate was sieved (53 μm) and the process was repeated once. The water containing the starch was left in a fridge (4 °C) for 1 h and the precipitated starch was washed with distilled water followed by drying in a vacuum oven (50 °C) until constant weight (moisture = 13 %). The yield was calculated as the amount of starch isolated divided by the weight of the nut. Fig. S1, in the supplementary material, shows the starch isolation steps.

### 2.3. Proximate composition

Measurements were performed in triplicate and included fat by Soxhlet extraction (AOAC, 2002), protein by Foss Kjeltec (Kjeldahl method – AOAC procedure 2055, Chang and Zhang (2017)), ash content using a muffle furnace (AOAC method 923.03) and total starch (McCleary et al. (1997). Dietary fibre analysis was performed using the method of Lee et al. (1992). The amylose-amylopectin ratio of the isolated starch was determined using the iodine binding method of Knutson (2000).

### 2.4. Scanning electron microscopy

The microstructure of the samples was analysed using a scanning electron microscope (JSM-5000 NEOSCOPE; JEOL, Tokyo, Japan) operating at an accelerating voltage of 10 kV at 800 x resolution for raw and roasted flours and 1300 x resolution for fermented and whole flours. Dried bunya nut flours and starch were added to adhesive tape and attached to the microscope (SEM) holder and coated with gold.

### 2.5. Particle size distribution

The particle-size distribution of the flour was measured using a laser diffraction particle size analyser (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, U.K.). The particle size distribution was calculated automatically by the Mastersizer 3000 software using Mie scattering theory and a refractive index of 1.53.

### 2.6. Functional properties

The functional properties were measured for the four bunya nut flours in comparison to wheat, chestnut and rice flours. Water and oil absorption, emulsion activity and stability and foam capacity and stability were investigated for the flours.

### 2.7. Water and oil absorption capacity

The water absorption capacity (WAC) of the flours was determined by the method of Sosulski et al. (1976) slightly modified. Flour (1 g) was mixed with distilled water (20 mL) and allow to stand at ambient temperature for 30 min, then centrifuged for 30 min at 4000 rpm. Water absorption was examined as grams of water bound per gram of flour. The oil absorption capacity was also determined by the method of Sosulski et al. (1976). Flour (1 g) was mixed with 10 mL sunflower oil (10 mL) (Sp. Gravity: 0.921) and allowed to stand at ambient temperature for 30 min, then centrifuged for 20 min at 4000 rpm. Oil absorption was examined as grams of oil bound per gram of flour.

#### 2.7.1. Emulsion activity and stability

Emulsifying properties (EA, ES) were determined according to the method given by Naczk et al. (1985). Flour sample (3.5 g) was homogenized for 30 s in water (50 mL) using a T 25 digital ultra-turrax (IKA) at 5000 RPM. Sunflower oil (25 ml) was added, and the mixture was homogenized again for 30 s. Then, more sunflower oil (25 mL) was added, and the mixture homogenized for 90 s. The emulsion was divided evenly into two 50 ml centrifuge tubes and centrifuged at 3000 RPM for 5 min. Emulsifying activity (EA) was calculated by dividing the volume of the emulsified layer by the volume of emulsion before centrifugation $\times$100. The emulsion stability (ES) was determined using the samples prepared for measurement of emulsifying activity. They were heated for 15 min at 85 °C, cooled and centrifuged again 3000 RPM for 5 min. The emulsion stability was expressed as the % of emulsifying activity remaining after heating.

#### 2.7.2. Foam capacity and stability

The capacity (FC) and stability (FS) of foams were determined by the method of Lin et al. (1974). A 3 % (w/v) dispersion of flour sample (100 mL) in distilled water was homogenized using a T 25 digital ultra-turrax (IKA), at 4000 RPM, for 1 min. The blend was immediately transferred into a graduated cylinder. The volume was recorded before and after whipping and measured as the % of volume increase due to whipping. The foaming capacity was expressed as the % of volume increase (Eq. (1)). Foam stability was measured as the percentage of foam volume left after 60 min (Eq. (2)).

$$\text{Foam capacity} = \frac{\text{Foam volume}}{100\,\text{mL}} \times 100 \tag{1}$$

$$\text{Foam stability} = \frac{\text{Foam volume after 60 minutes}}{\text{Foam volume}} \tag{2}$$

### 2.8. Pasting properties

Pasting properties of the bunya nut flour were studied by using a Modular Compact Rheometer (MCR502 – Anton Paar, Graz, Austria), equipped with a starch cell attached to the pressure cell. Apparent viscosity profiles were recorded using flour suspensions (15 % w/w). Apparent viscosity is calculated automatically by the software (Rheoplus) using conversion factors determined by the manufacturer (Matignon et al., 2014). The temperature–time conditions included a heating step, from 50 to 95 °C (after an equilibration time of 1 min at 50 °C), a holding phase at 95 °C for 5 min, a cooling step from 95 to 50 °C at 6 °C/min and a holding phase at 50 °C for 2 min. The total process takes around 25 min.

### 2.9. Rheological properties

Dynamic oscillatory measurements were performed on a MCR502 rheometer (Anton Paar, Graz, Austria) with the pastes from the end of the pasting measurements after cooling to 25 °C. Measurements were carried out at 25 °C using a 35 mm parallel plate system at a gap of 1 mm. The sample was loaded between the parallel plates and the excess was trimmed off. After resting for 5 min to relax stresses before starting the test, the data was collected within the linear viscoelastic region (LVR), as determined by preliminary amplitude sweep tests performed in the range of 0.1 and 100 Pa shear stress, at a constant frequency of 1 Hz. Small amplitude oscillatory shear frequency sweep tests were performed over the range 0.1 and 100 Hz at 1 Pa shear stress within the LVR. From each trial, storage modulus ($G'$, Pa), loss modulus ($G''$, Pa) and the ratio between them (tan $\delta$) were analysed.

### 2.10. Digestibility

The *in vitro* digestion method was performed as described by Minekus et al. (2014) with slight modifications. The digestion buffers of the simulated phases were prepared and the pH was adjusted to make salivary, gastric and intestinal fluids (referred as SSF, SGF, and SIF) reach pH values of 7.0, 3.0 and 7.0, respectively. The digestion solution was incubated in a water bath at 37 °C and constantly mixed at 200 rpm. All fluids were pre-warmed to 37 °C. Salivary buffer (2 mL) containing α-amylase (0.15 unit per mg starch) was added into 80 mg starch (dry weight basis) and kept for 2 min, followed by the simulated gastric fluids (2 mL) containing pepsin (8 unit per mg starch) for 30 min. The "0h" aliquots were collected before the addition of simulated intestinal fluids (4 mL) with pancreatin based on amylase activity (1.6 unit per mg starch). The aliquots (50 μL) were collected over eight hours and were added to a $Na_2CO_3$ (0.5 M) solution (450 μL) to stop amylase hydrolysis, followed by centrifugation (2000g, 10 min). The released reducing sugar was measured using a 4-hydroxybenzoic acid hydrazide (PAHBAH) method for the calculation of the digested starch (%), as described previously (Zou, Sissons, Warren, Gidley, & Gilbert, 2016). The rapid digestible starch (RDS) was determined as the amount of reducing sugar released within 20 min of *in vitro* digestion. The slowly digestible starch (SDS) between 20 and 120 min and the resistant starch (RS) was calculated as the total starch minus the amount of reducing sugar released within 120 min of *in vitro* digestion.

## 3. Results

### 3.1. Flour preparation

The different types of flours from bunya nuts were prepared (Fig. 1) and differences on their appearance such as colour and particle size can be perceived. Also, even simple processing methodologies such as roasting or the use of the inner coating in the bunya flour results in very different flour appearance. On top of the figure are the commercial flours and at the bottom, the different bunya flours.

### 3.2. Proximate composition

Moisture, total starch content, total dietary fibre content, protein, fat and ash were determined and compared among the different types of bunya nut flours and also with the commercial flours (Table 1). All the flours showed a moisture content below 14 %. According to Tegge (1987), flour specifications usually limit the flour moisture to 14 % or less due to problems with storage stability and the production of off odours and flavours in flours that have a higher value. Therefore, the low moisture content of the flours suggest they would be suitable for long storage.

Ash content is related to the inorganic and mineral residue after the combustion of the flour. Flours that are less purified and contain bran may have higher amounts of ash content because there is a greater portion of minerals contained in the bran (Czaja et al., 2020). However, it may cause issues in the production of breads due to the fact that these ingredients may weaken the dough structure (Czaja et al., 2020).

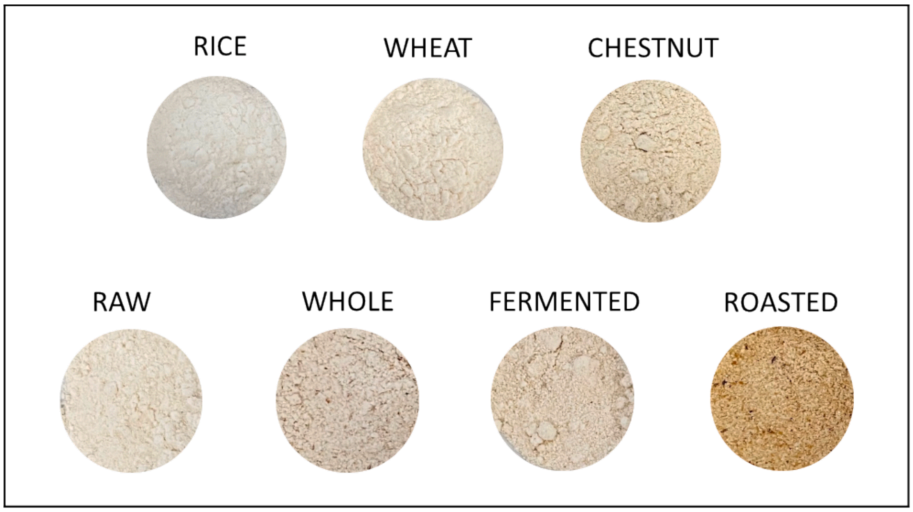


**Fig. 1.** The different flours investigated in this study. On top the commercial flours. At the bottom, the raw and processed bunya nut flours.

**Table 1**
Proximate composition of commercial and bunya nut flours.

| | Moisture (%) | Protein | Fat | Ash | Carbohydrate | Total starch | Dietary fibre |
|---|---|---|---|---|---|---|---|
| *Commercial flours* | | | | | | | |
| Wheat | 12.5 ± 0.1[d] | 13 ± 1[a] | 1.4 ± 0.5[c] | 0.80 ± 0.05[bc] | 72.3 | 75 ± 2[a] | 3.6 ± 0.2[e] |
| Rice | 11.6 ± 0.1[e] | 7.9 ± 0.7[b] | 1.4 ± 0.2[c] | 0.34 ± 0.05[c] | 78.76 | 73 ± 3[a] | 2.2 ± 0.3[f] |
| Chestnut | 9.6 ± 0.2[f] | 7.0 ± 0.5[b] | 4.9 ± 0.2[a] | 2.13 ± 0.07[a] | 76.37 | 70.0 ± 0.1[c] | 9.0 ± 0.1[cd] |
| *Bunya nut flours* | | | | | | | |
| Raw | 13.6 ± 0.3[b] | 4.7 ± 0.5[cd] | 4.4 ± 0.8[ab] | 2.1 ± 0.6[a] | 75.2 | 77 ± 5[a] | 8.4 ± 0.6[d] |
| Whole | 12.9 ± 0.3[c] | 4.6 ± 0.03[cd] | 5.1 ± 0.2[a] | 2.1 ± 0.2[a] | 75.3 | 62 ± 4[b] | 9.78 ± 0.08[b] |
| Fermented | 9.7 ± 0.3[f] | 3.6 ± 0.5[d] | 3.4 ± 0.2[b] | 1.2 ± 0.1[b] | 82.1 | 55.6 ± 0.8[b] | 9.4 ± 0.2[c] |
| Roasted | 16.2 ± 0.4[a] | 5.2 ± 0.5[c] | 5.6 ± 0.4[a] | 2.01 ± 0.06[a] | 70.99 | 61 ± 8[bc] | 14.4 ± 0.4[a] |

Values are shown in %d.b. (except for moisture).

Refined flours, such as wheat (0.5–2.5 % ashes – Czaja et al. (2020)) have a lower ash content due to the removal of bran layers. Whole flours have more of the bran so their ash content is also higher (Cardoso et al., 2019). Except for the fermented bunya, wheat and rice flours, all the others had a higher and similar ash content of approximately 2 %. Bunya flour contains non-gluten proteins (also high in mineral content) since it is not refined, which explains why it showed a higher ash content than wheat and rice. The chestnut flour must have gone through a similar process and had a similar value. Also, bunya nuts, when dried, may have the inner coating attached to the kernel. The decrease in the ash content for the fermented flour might have happened during soaking due to leaching of soluble salts (Adebo et al., 2022).

Total starch content for bunya nut flours was in the range of 55.6–77 %. Raw bunya flour had the highest starch content, while fermented flour has the lowest. Fermentation can cause decrease of starch due to starch hydrolysis by enzymes present in the nut or metabolic activity of microorganisms causing the degradation to simple sugars (Adebo et al., 2022). In comparison to pinhão flour, Zortéa-Guidolin et al. (2017) found a value for total starch equal to 76.89 ± 1.06 g/100 g d.b., the same as raw bunya flour. In terms of protein, bunya nut flours had the lowest values, in the range of 3.6–5.2 %, while wheat flour had the highest (13 %). The lowest value was for the fermented flour (3.6 %), due to potential degradation or leaching of proteins to the water during soaking (Adebo et al., 2022).

Regarding the fat content of bunya nut flours, they ranged from 3.4 % for fermented to 5.6 % for roasted. Fermentation may cause an increase in lipase activity and metabolism of lipids, decreasing the fat content. Even though bunya nut flour showed low fat content, roasting may cause heat-induced breakdown of the bonds that exist between the fat and matrix of the nut, resulting in release of the oil present inside this matrix (Oboh et al., 2010). Also, when the inner coating is ground with the kernel, the fat content increases due to the fat contained in the coating (Heshe et al., 2016). Although the fat content of flours is related to flavour retention, high fat content can make the flour susceptible to lipidoxidation, which can cause off-flavours (Wei et al., 2021). The higher fat content of the whole and roasted flours is still much lower than other gluten-free flours, especially nut flours such as coconut (25 %) (Raczyk et al. (2021) or almond (43 %) (Almeida et al., 2019; Bakare et al., 2016) which could be an advantage in terms of rancidity. Also, the phenolic compounds present in the inner coating of the whole flour, besides having antibacterial effect and protecting against microorganisms, it might assist in retarding lipid oxidation during storage (Shahidi & Ambigaipalan, 2015).

The dietary fibre content of bunya nut flours was higher than for wheat and rice flours. The recommended amount of dietary fibre is 25 g daily and there is a need to increase the consumption of dietary fibre among the population (Lecumberri et al., 2007), which makes bunya nut flour a suitable ingredient for this purpose. Roasting can produce flours with highest amounts of fibre. This can happen due a range of factors, such as reaggregation of starch granules or interactions between starch and proteins, both resulting in higher particle size (Torbica et al., 2023; Yang et al., 2023), which agrees with Fig. 3. Also, the inner coating retained in the whole flour provided extra fibre (16 %) to the whole flour even though this part of the nut is present in low amounts.

The starch was isolated from the bunya nuts as described in the methods section and the yield was 31 % from the kernel, which is similar to values found for piñones (35.9 %), isolated using the same size of sieve, but different solvent (sodium hydroxide) (Henriquez et al., 2008).

In comparison to pinhão (44.1 %), where a bigger size sieve was used (150 μm) and water was used as the solvent (Daudt et al., 2014), it was lower.

In terms of composition, the isolated starch from the kernel of the bunya nut contained 91 % total starch, 1.88 % protein, 0.02 % fat and 9.53 % moisture. The isolation of starch performed by Bello-Perez et al. (2006) yielded 86 % of total starch (purity) using water as the solvent whereas the isolation performed by Zortéa-Guidolin et al. (2017) ranged from 92.0 to 96.6 % using other solvents such as toluene and sodium chloride. This is an advantage regarding bunya nuts, since water can be used to isolate the starch from bunya nuts with high purity and without the hazards that may be caused by other solvents.

The apparent amylose content of the bunya nut starch was 31.37 ± 2 %, which is similar to the values found by Cordenunsi et al. (2004) for pinhão starch (29.6 ± 2.5 %). Apparent amylose content varies depending on the starch source, but the values are very similar, which is expected within starches of similar botanical sources, especially considering error values during analysis.

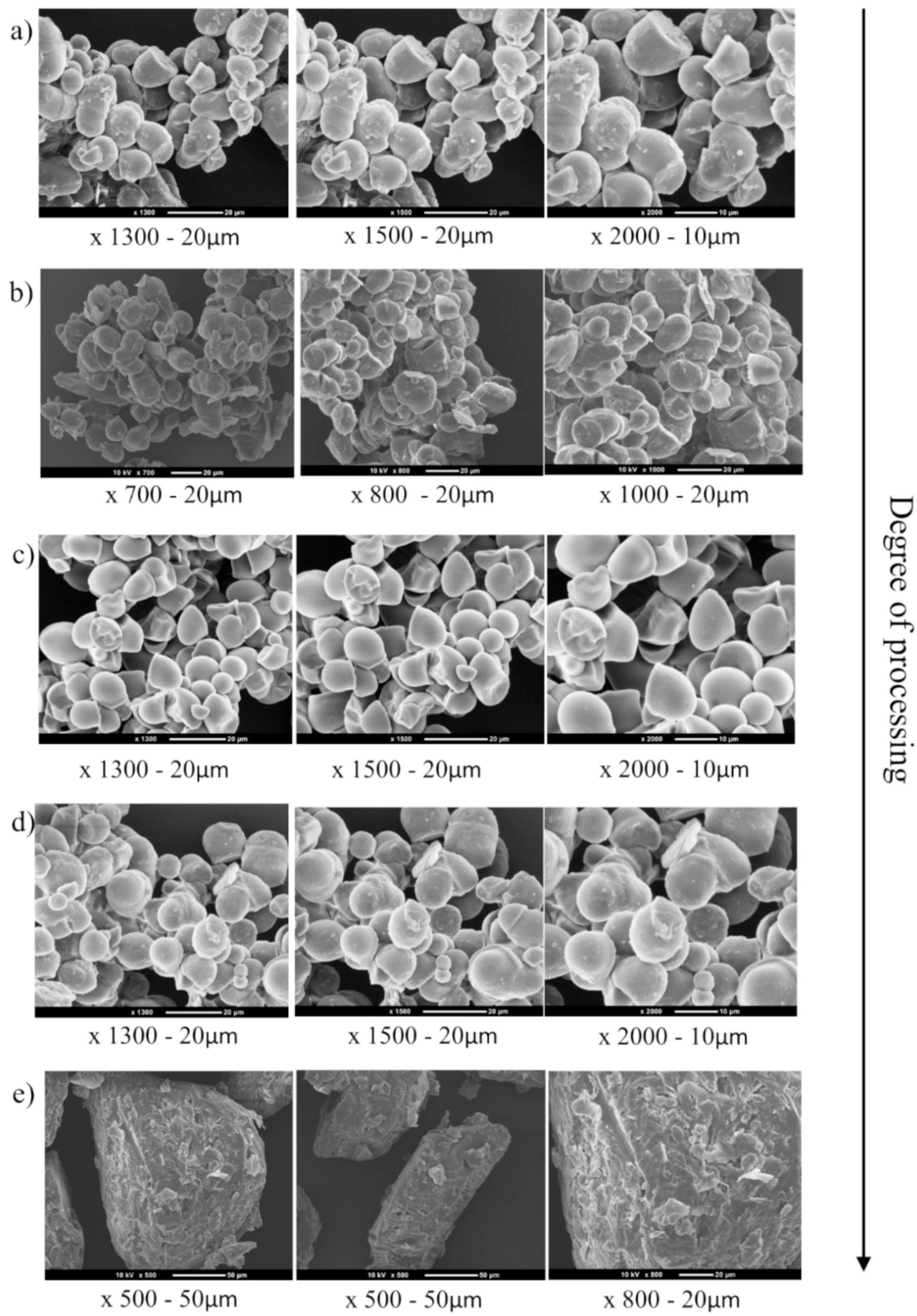


**Fig. 2.** Scanning Electron Microscopy images of the bunya nut flours and the isolated starch from the untreated flour with the inner coating (a), without the inner coating (b) and the isolate starch from the nut (c) to the processed flours by fermentation (d) and roasting (e). The magnification and size of the scale are shown below each figure.

### 3.3. Scanning electron microscopy

Starch morphology varies among plant species due to their biological origin (Singh et al., 2003). Rice granules, for example, are polygonal and are the smallest known to exist in cereal grains (2–7 μm) (Wani et al., 2012), whereas wheat starch granules are bigger and more round or lenticular (Zhou et al., 2013). Microscopy observations of bunya nut isolated starches suggest that the native granules are similar to cassava starch granules, smooth and round with a flat base (Fig. 2). Size values for bunya nut starch are also similar to cassava (around 10–15 μm). *Pinhão* starch is also similar to bunya starch in shape and size (Zortéa-Guidolin et al., 2017). The fermented flour in comparison to the raw and whole flours shows a cleaner granule, which suggests that some of the compounds present around the granules, such as fat and protein, as well as broken granules, might have been leached to the water during soaking. Also, microscopic images of the roasted flour confirm the gelatinisation of the starch. The images confirm that each flour morphology is different and suggest that each of them might be suitable for specific uses. For instance, the clear granule from the fermented flour and the isolated starch might be useful for thickeners such as in porridges or soups due to the absence of fat and protein molecules that could impede starch swelling.

### 3.4. Particle size

The particle size distribution for each commercial flour in comparison to raw bunya flour is shown in Fig. 3a and for each processed bunya flour in comparison to the raw bunya flour is shown in Fig. 3b. The raw

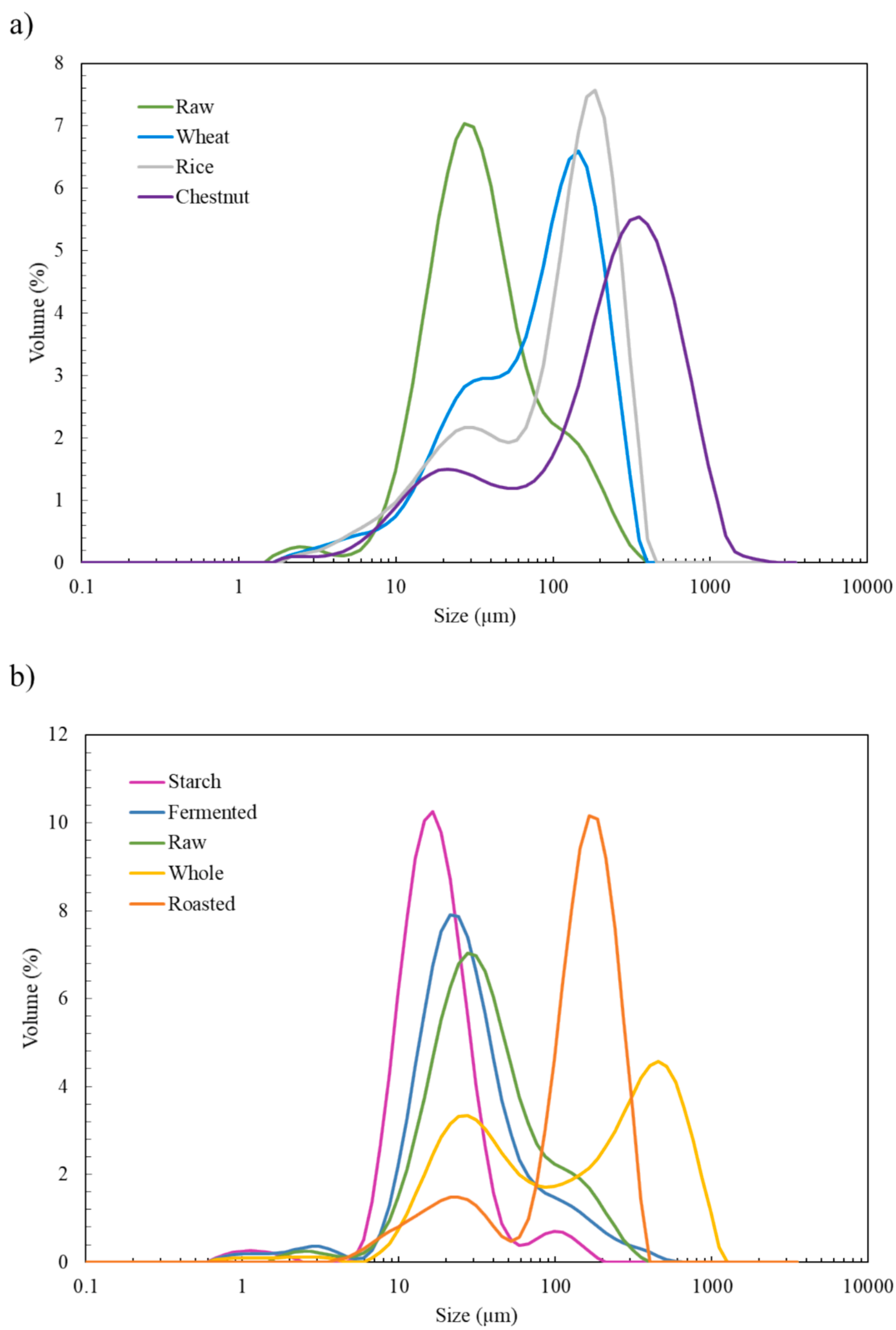


**Fig. 3.** Particle size distribution for commercial flours and bunya nut flours in μm.

and fermented flours have particle sizes in agreement with specifications for commercial wheat flour, where most of the particles are smaller than 212 μm (The Codex Alimentarius, 2009). Regarding legislation for flours, there is no globally recognized terminology for dry-milled bunya nut products in terms of nomenclature. For maize, for example, the commonly accepted terms are coarse meal (1190–730 μm), medium meal (730–420 μm), and fine meal (420–212 μm) (Gwirtz & Garcia-Casal, 2014).

Because the inner coating pieces were blended with the flour, the particle size distribution for the whole flour showed some particles above this value. After roasting, the roasted flour became very difficult to grind due to its hardness and bigger granules could be seen and felt when handling it. This is likely to be due to starch gelatinisation during roasting and the subsequent retrogradation. This flour, in consequence, showed bigger particle sizes, being considered more a fine meal (420–212 μm) than a flour. More detailed information on the size of each flour and starch can be found in the supplementary material (Tables S1 and S2).

### 3.5. Functional properties

The results for the functional properties are presented in Table 2. Functional properties relate to the interaction between composition, structure, molecular conformation and physico-chemical properties of food components. It is useful information for industry since it helps to predict how new ingredients will behave in specific systems, for example how to mix flours to obtain a more nutritious food (Chandra et al., 2015).

Water absorption capacity (WAC) is the ability of a product to associate with water under conditions where water is limited and is an important functional property required in food formulations, especially in baking applications such as dough handling (Iwe et al., 2016). Higher WAC, higher the water quantity that can be added during food preparation, improving handling characteristics. Higher water absorption of flour helps to maintain the freshness of bread and cakes (Falade & Okafor, 2014). Roasted bunya nuts produced a flour with higher water absorption. These results are in accordance with Sindhu et al. (2019), where hydrothermally treated starch samples increased their water absorption capacity by 3-fold while oil absorption capacity decreased. However, all bunya flours showed more water absorption capacity than wheat flour, which suggests that these flours could be used as ingredients in bakery products with improved handling characteristics, such as the ones explained above.

Oil absorption capacity is related to mouthfeel, flavour retention (Iwe et al., 2016) and storage of foods, since it promotes flavour binding and prevents oxidative rancidity (Cruz-Solorio et al., 2018). The whole bunya nut flour showed the highest oil absorption, while the raw and fermented flours showed similar values when compared to wheat flour, another reason for these flours to be considered suitable for the food industry.

Good foam capacity and stability are attributes for flours intended for producing a variety of baked products such as breads and cookies and also act as functional agents in other food formulations. Foams are used to improve the texture, consistency and appearance of foods. Foam stability is also important regarding the ability to maintain the whip volume as long as possible in some bakery formulations (Lin et al., 1974). Bunya flour showed the highest foam capacity compared to the commercial flours. Protein content is one of the main factors that cause an increase in foam capacity in flours (Ohizua et al., 2017) and the foaming capacity of wheat flour is generally regarded in the literature to be due to its high protein content (Akubor et al., 2000). However, the rice flour has a higher protein content when compared to the raw bunya flour. A potential explanation could be the solubility of the rice flour, which is very low and could have decreased the protein's ability to cause foaming (Mune & Sogi, 2016). Another reason could be that the starch of bunya flour is also responsible for the foaming, potentially more than the protein in this case. This was found by observing and measuring the foam capacity of the isolated starch. In terms of stability, raw bunya flour had the highest stability while rice flour showed almost no stability. For chestnut flour, both the capacity and the stability were slightly lower than bunya flour, with a loss of foam of around 12 % after one hour.

Regarding the different processed bunya nut flours, the reduced foaming capacity of roasted bunya nut flour in comparison to the raw flours may be related to protein denaturation and gelatinisation of starch upon heating. Fermented bunya nut flour had almost zero foam capacity. This was also reported for cowpea powder (Okaka & Potter, 1979) and faba bean flour (Chandra-Hioe et al., 2016). Fermentation converts starch and sugar into alcohol and carbon dioxide, which leads to lower pH. Lower pH may expose hydrophobic groups and inhibit the migration of protein to the air–water interface (Çabuk et al., 2018). Also, the protein content of the fermented flour was lower than the raw. In terms of foam stability, after one hour, the whole bunya nut flour samples kept the same volume of foam and the raw bunya nut flour decreased only 8 %.

Emulsifying activity (EA) is defined as the ability of the flour to emulsify oil (Kaur & Singh, 2005). The raw, fermented and whole bunya flours showed a higher emulsifying activity and stability when compared to rice flour and similar to wheat. The isolated bunya starch showed almost no emulsification activity, which is common in native starches due to their hydrophilicity. Roasted samples showed a very small emulsifying activity, mainly due to protein denaturation during roasting. The water solubility index of the samples was measured using the method described in the supplementary document and the results are shown in Table S3.

**Table 2**
Functional properties of bunya nut raw flour compared to commercial flours.

| Commercial flours | Wheat | Rice | Chestnut |
|---|---|---|---|
| WAC (g/g) | $1.00 \pm 0.02^{f}$ | $2.03 \pm 0.08^{c}$ | $2.2 \pm 0.1^{c}$ |
| OAC (g/g) | $0.99 \pm 0.07^{b}$ | $1.6 \pm 0.1^{a}$ | $1.6 \pm 0.1^{a}$ |
| FC (%) | $11 \pm 1^{b}$ | $5 \pm 1^{d}$ | $8.2 \pm 0.3^{c}$ |
| FS (%) | $58 \pm 6^{d}$ | $0 \pm 0^{e}$ | $88 \pm 6^{b}$ |
| EA (%) | $48 \pm 4^{bc}$ | $11 \pm 1^{d}$ | $56 \pm 2^{a}$ |
| ES (%) | $19 \pm 1^{d}$ | $0 \pm 0^{f}$ | $44 \pm 1^{b}$ |

| Bunya flours | Raw | Roasted | Fermented | Whole |
|---|---|---|---|---|
| WAC (g/g) | $1.28 \pm 0.03^{e}$ | $2.56 \pm 0.1^{b}$ | $1.30 \pm 0.03^{e}$ | $1.66 \pm 0.05^{d}$ |
| OAC (g/g) | $0.99 \pm 0.03^{b}$ | $0.88 \pm 0.07^{b}$ | $0.93 \pm 0.04^{b}$ | $1.41 \pm 0.04^{a}$ |
| FC (%) | $12.8 \pm 0.8^{a}$ | $1.7 \pm 0.3^{e}$ | $0 \pm 0^{e}$ | $6.2 \pm 0.3^{d}$ |
| FS (%) | $93 \pm 3^{ab}$ | $69 \pm 5^{c}$ | $0 \pm 0^{e}$ | $100 \pm 0^{a}$ |
| EA (%) | $52 \pm 2^{ab}$ | $7 \pm 1^{d}$ | $45 \pm 1^{c}$ | $48 \pm 1^{bc}$ |
| ES (%) | $51 \pm 1^{a}$ | $5 \pm 1^{e}$ | $26 \pm 2^{c}$ | $44 \pm 2^{b}$ |

WAC: water absorption capacity; OAC: oil absorption capacity; FC: foam capacity; FS: foam stability; EA: emulsion activity; ES: emulsion stability.

### 3.6. Pasting analysis

The results for the pasting properties of bunya nut flours at 15 % concentration in water are presented in Fig. 4. Pasting properties measure the behaviour of the flour when heated up in excess water. They affect texture and digestibility of foods and are key steps to better predicting some properties of starch-based products (Copeland et al., 2009; Iwe et al., 2016).

Pasting temperature is an indication of the minimum temperature required for the granules to start swelling. It also relates to the water-binding capacity, since the flour might be able to absorb more water before its starch granules start to swell to allow maximum swelling (Iwe et al., 2016). The higher protein content of wheat, rice and chestnut flours could be responsible for their pasting temperature being higher

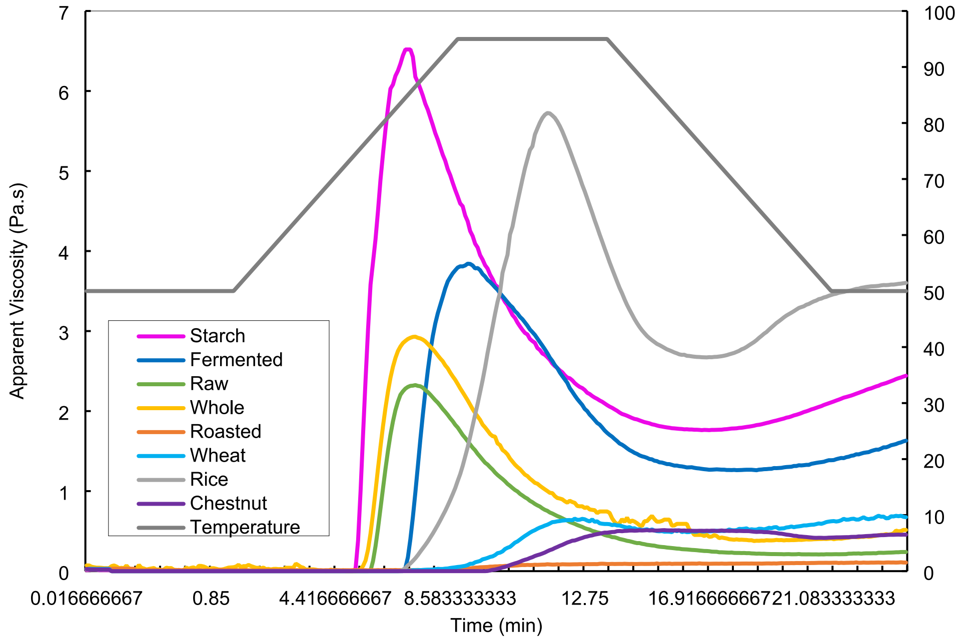


**Fig. 4.** Pasting properties of bunya nut flours and the isolated starch in comparison to commercial flours.

than bunya raw flour, since proteins may restrict starch swelling (Devi et al., 2020). Fat content also causes restricted swelling and this can be seen for chestnut flour, for example, which had the higher pasting temperature potentially due to the formation of lipid amylose-complexes during thermomechanical processing method (Panyoo & Emmambux, 2017). Bunya nut starch had the lowest pasting temperature due to fact that the isolated starch has very little fat and protein. Following this same trend, the fermented flour was expected to be between the raw and the starch. However, the pasting temperature of the fermented was higher. This could have happened due to the lower amount of starch and higher amount of fibre in the fermented sample.

Contrary to wheat and chestnut flours, the rice flour shows high peak viscosity and breakdown viscosity, which has been previously related to the ease of the starch granules swelling and disintegrating under shear and heat (Waziiroh et al., 2021). Rice flour also showed high final viscosity. Native rice starches usually have between 6–10 % amylose, which means the amylopectin content is high in these flours. Amylopectin is responsible for the increase in viscosity, which explains why this flour had a higher peak and final viscosity compared to the others (Ronie & Hasmadi, 2022). The higher peak viscosities of the isolated bunya nut starch and fermented bunya nut flour indicate the ability of the starch to swell and is also related to the low fat and protein content of these flours, since these components restrict the swelling, as discussed above. The fermented flour, in comparison to the raw, shows a higher peak. This could have happened due to leaching of soluble matter during soaking. High peak viscosities can be an advantage for stiff dough products, for example, as well as products that need high viscosities such as porridges. According to Bakare et al. (2016), higher values for peak viscosity corresponded to greater resistance of starch to be digested by amylase and, consequently, might lead to a lower glycemic index.

Except for the roasted, all bunya flours showed very low setback and final viscosities, which means it was difficult for the starch molecules to reassociate. Because the isolated starch showed slightly higher setback and final viscosities followed by the fermented flour, we believe the low final viscosities of the bunya flours are mainly due to the fact that they are higher in lipids than wheat, for example, impeding the rearrangement of the starch. Costa et al. (2013) and Zortéa-Guidolin et al. (2017) found similar values for peak and breakdown viscosities of pinhão starch compared to bunya. However, the setback and final viscosities for bunya were much lower even though their starch granules have similar structure and size, which could be explained by differences in lipid, protein and amylose content (Wang et al., 2015).

Although many studies mention the direct relationship between the paste viscosity during the cooling cycle (setback viscosity) and tendency to staling due to amylose retrogradation, the staling process also depends on the role of amylopectin, which happens hours or days later (Copeland et al., 2009). As shown in Fig. 4, bunya nut flour does not increase its viscosity at the end of the measurement, which would suggest that short term (2 h) staling of baked goods made with this flour could be less. However, to assess the long-term staling, the gels from pasting were stored for 24 h and rheological measurements were applied to them.

### 3.7. Rheological properties

The viscoelastic properties of the gels from the pasting measurements were measured by dynamic, small deformation tests to be able to assess the consistency of these gels. All gels had G′ higher than G″, which means the elastic properties were dominant. The storage modulus G′ and loss tangent (tan δ) were used to compare samples. A higher G′ and a lower tan δ indicate a more elastic and solid-like material (Peressini & Sensidoni, 2009). Loss tangent (tan δ) is related to gel strength and it is the relationship between viscosity and elasticity (G″/G′), with rigid materials having lower values. All the gels showed tan δ between 0.101 and 0.268, which is a characteristic of weak gels.

Regarding the processed bunya flours (Fig. 5), roasted and whole flours had the highest values for G′ and the lowest for tan δ, which means the gels formed with the roasted flour and the whole flour were more rigid. Roasting and cooling cause crosslinking between starch components and result in a harder gel due to increased binding (Gu et al., 2022). For the whole flour, the increase in G′ with the higher fibre content agrees with a study performed with pectin gels enriched with dietary fibre by Figueroa and Genovese (2018). This could have happened because the fibre acts as a filler, increasing the density of the network and reinforcing the structure of the gel. The lower starch concentration in the fermented flour could explain that. Among the commercial flours and bunya raw flour (Fig. 6), G′ and tan δ values follow the same trend, with bunya raw flour and chestnut having higher G′ and lower tan δ than wheat and, especially, rice flour. A reason could be the increased swelling power (Fig. 4) of the bunya nut and chestnut flours

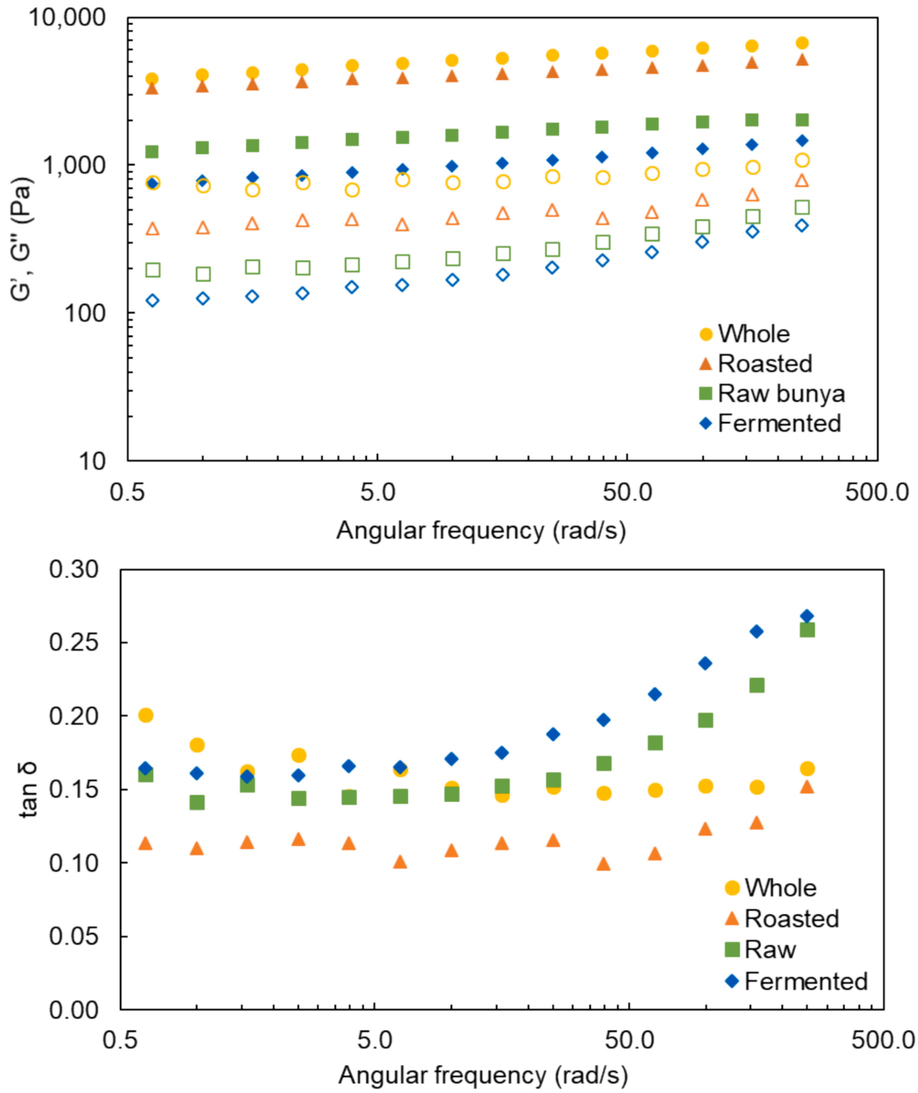


**Fig. 5.** Frequency sweep for each type of bunya flour gel (15 % flour in water) at 25 °C and shear stress = 1 Pa. Storage and loss moduli for bunya nut flour gels on the top and loss tangent for bunya nut flour gels at the bottom. Open symbols in the top graph represent loss modulus G″ and filled symbols storage modulus G′.

(Takahashi & Seib, 2005), which is dependent on the type of starch.

Based on both pasting and rheological properties, we observe that, although the raw, roasted and whole bunya nut flours show a low setback viscosity, the stored gels had a higher G′ and lower tan δ, which means they formed more rigid gels. The starch in the roasted bunya flour had been already gelatinised during roasting, which is why the pasting profile for this flour shows a flat curve compared to the raw bunya flour. The pasting curve shows the gelatinisation and breakdown of starch granules, which had already happened during the preparation. On the other hand, rice flour had the highest setback viscosity and the lowest G′. As mentioned above, amylose plays a role in retrogradation only for the short timeframe while amylopectin continues to retrograde after hours or even days. Therefore, to assess the retrogradation behaviour of flours and starches, pasting cannot be the only parameter analysed. Rheology of the final gels must be performed to elucidate the time-dependant effects driven by amylose and amylopectin regarding staling.

### 3.8. Digestibility

The digestibility of starch depends on the size and morphology of the granule, amylose/amylopectin ratio, the presence of amylose–lipid complexes, and the method of processing (Zortéa-Guidolin et al., 2017). The *in vitro* hydrolysis curves for each of the flours are shown in Fig. 7. All the flours showed different behaviour. Raw bunya nut flour had a much lower digestion rate than wheat and rice flours. The raw bunya flour hydrolysis curve was similar to the chestnut curve. The results for the digestibility of chestnut flour agree with a study performed with different chestnut cultivars, where the author found a hydrolysis curve with the same characteristics of this study (Hao et al., 2018). Bunya nut and chestnut starch granules are bigger and smoother than rice starch granules, for example. Big granules may strengthen the structure by absorbing water, which could have happened in this case. Regarding the different processed flours, the roasted flour curve had the highest values and had an initial rapid rate, reaching a constant rate at around 80 min. This can be attributed to the gelatinisation of the starch during roasting, which causes disruption of the granules, facilitating the access of the enzyme. This was also stated by Wang et al. (2017) in a study on the starch digestibility of cooked rice.

We hypothesise that the flour digestibility results can be applied to final products made with the flours, such as breads. A study performed in *pinhão* nuts found that the glycemic index of cooked *pinhão* was 23 % lower than for white bread and that the nut can be considered a food with low GI (Cordenunsi et al., 2004).

Rapidly digestible starch (RDS) is rapidly digested and absorbed, increasing the blood glucose faster than the other two. Resistant starch (RS) is digested only at the end of the intestinal tract. Slowly digestible

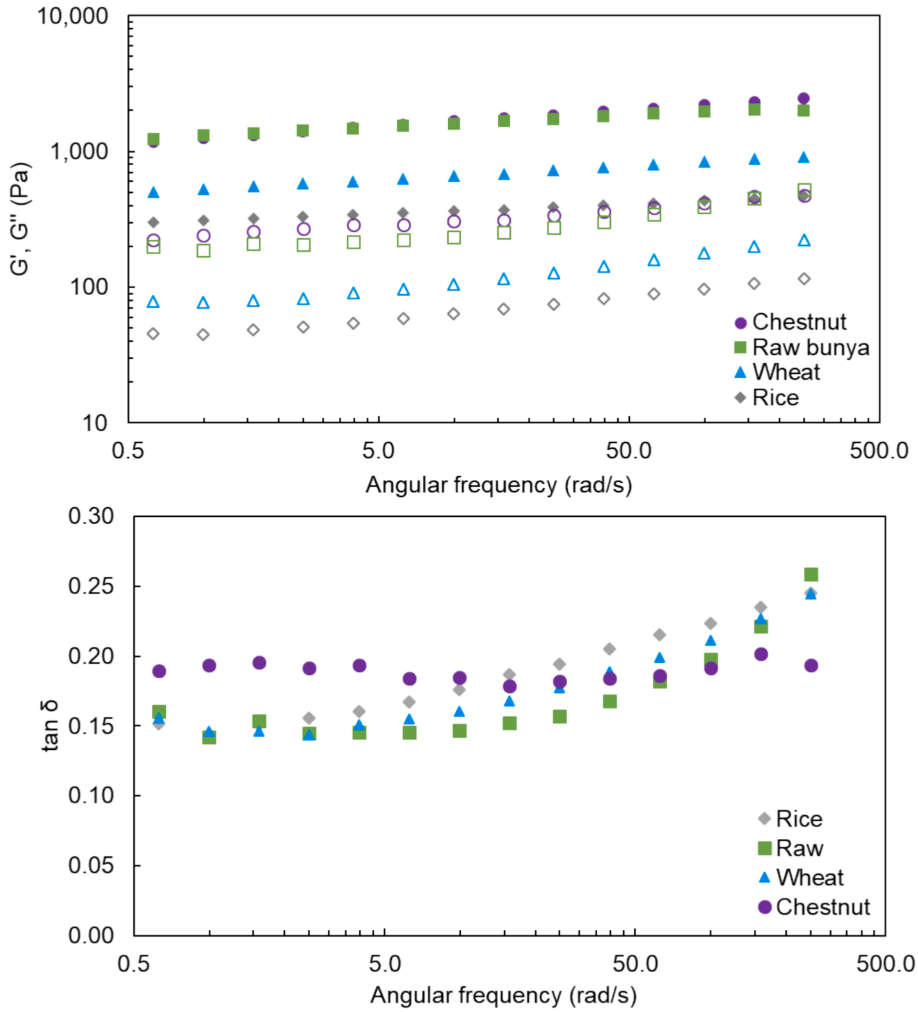


**Fig. 6.** Frequency sweep for each type of commercial and raw bunya flour gel (15 % flour in water) at 25 °C and shear stress = 1 Pa. Storage and loss moduli for the flour gels on the top and loss tangent for the flour gels at the bottom. Open symbols in the top graph represent loss modulus G″ and filled symbols storage modulus G′.

starch (SDS) provides a slow and long release of glucose, which is also beneficial (Zhang & Hamaker, 2009). The starch fraction that is resistant to enzyme digestion, known as resistant starch, is divided into five types: physically inaccessible starch entrapped in a cellular matrix (type 1), native non-gelatinised starch (type 2), retrograded starch (type 3), chemically modified starch (Conforti & Lupano, 2008) and self-assembled starch V-type complexes (Gutiérrez & Tovar, 2021). The amount of residual starch after 2 h of ileal digestion followed the order: Fermented > Whole > Chestnut > Raw > Wheat > Rice > Roasted. Studies performed with pinhão have shown the antioxidant potential of the inner coating (Koehnlein et al., 2012; Santos et al., 2018) and how it can act as an inhibitor of salivary and pancreatic α-amylase due to their tannin content (Silva et al., 2014). These past results suggest that the low digestibility and large amount of residual starch could be due to the inner coating of the whole bunya flour acting as an enzyme inhibitor, interfering in the *in vitro* digestion. In terms of SDS, rice and bunya nut raw flours had the highest values and for RDS, the roasted flour showed the highest value, as shown in the hydrolysis curves in Fig. 7.

The amount of resistant starch might also explain the differences found in the storage modulus. According to Fig. 5 and Fig. 8, for the commercial flours and raw bunya flour, the higher the storage modulus (G′), as the resistant starch may act as a filler in the structure, similarly to the fibre, as mentioned above. For the processed flours, although the roasted one had a low resistant starch content, it showed the higher storage modulus (G′). A study showed that the addition of roasted pea flour in comparison to raw pea flour increased the storage modulus of model doughs. A modification of the proteins contained in the flour due to roasting, as well as moisture redistribution, might strengthen the gel network (Kotsiou et al., 2021). A PCA biplot with a brief summary of the results, including the water solubility index, is shown in the supplementary material (Fig. S2).

## 4. Conclusions

In this study we have provided an understanding of the composition and properties of four types of bunya flours, as well as the isolated starch, and three common commercial flours, and outlined the effect these differences may have in food and nutritional applications. We have shown that among the types studied there is variation not only in the macronutrient composition and functional properties but also starch gelatinisation behaviour and digestion rates. We also show that, within the bunya flours, a range of functional properties, starch gelatinisation behaviour and digestion rates are observed dependent on the treatment of the nuts prior to flour production.

In terms of practical applications, the physicochemical (particle size < 212 µm and moisture content < 14 %) and functional properties of the bunya nut flour compared to wheat flour suggest that bunya nut flour is suitable for different food applications, especially baked goods. More specifically, raw bunya flour is suitable for dough handling and retention of flavour since its water and oil absorption capacities are similar to wheat flour. Although its foam capacity was also similar to wheat flour, it showed a more stable foam, which could be useful for the formulation

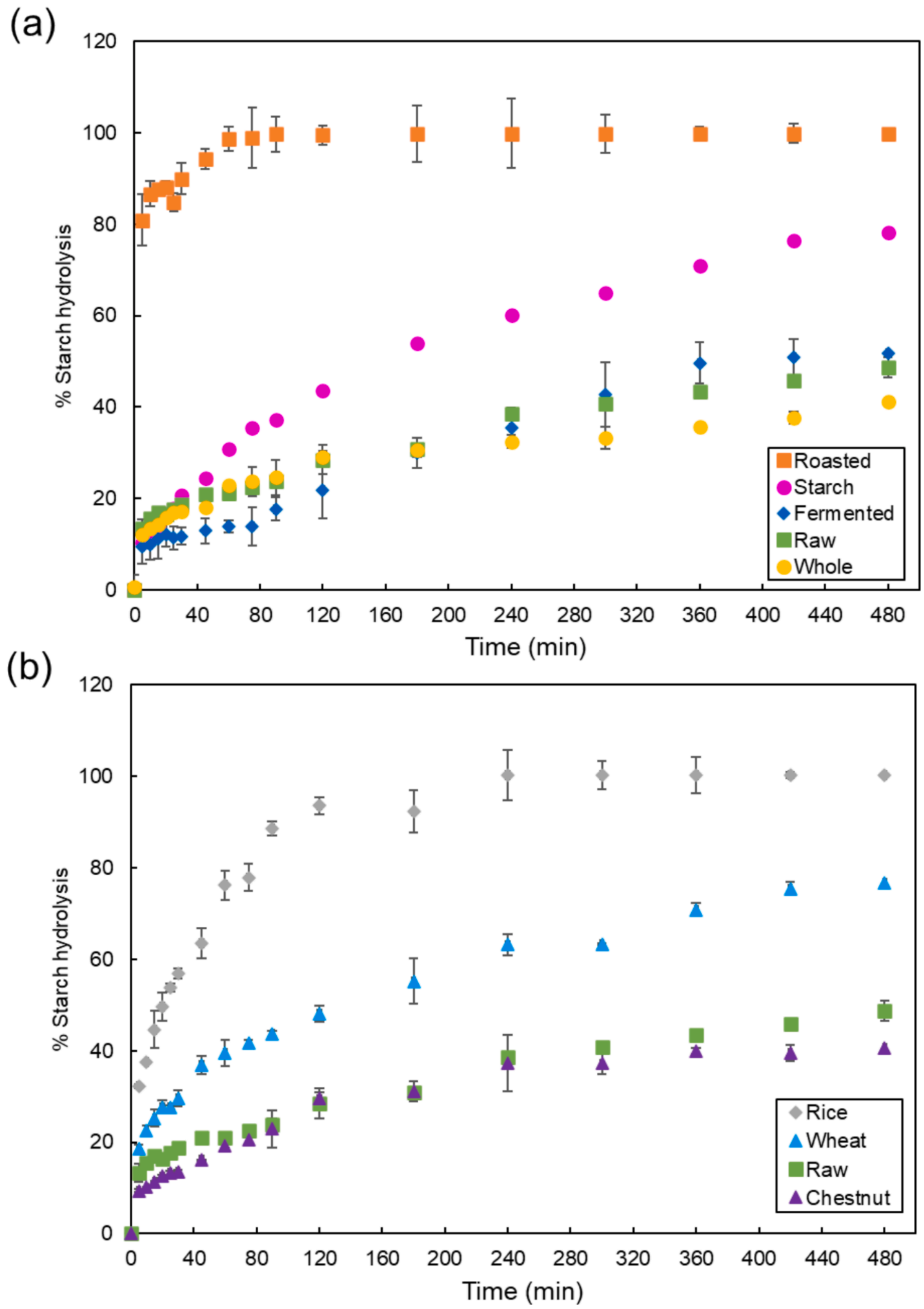


**Fig. 7.** *in vitro* starch hydrolysis (%) curves for rice, wheat, chestnut and bunya nut flours.

of muffins and cookies, for example. The raw bunya flour also showed good emulsion activity and stability when compared to wheat flour. Fermentation removed surface proteins and ashes, which led to an increase in the swelling ability of this flour and a decrease in the emulsion capacity. This flour could substitute other flours in porridge such as rice flour (Sri Lanka) and maize flour (Mexico, East Africa) porridges. The roasted bunya flour gelatinised during the thermal treatment, resulting in faster digestibility. However, its increased water absorption makes this flour a good choice for breads. The use of the inner coating, which is commonly discarded, not only for bunya nuts but also other types of nuts, increased the fibre content leading to a slower starch hydrolysis and a higher storage modulus due to the fibre acting as a filler in the matrix. This flour could be used as an alternative source of fibre and antioxidants without greatly changing the characteristics of the raw flour, especially because many commonly consumed gluten-free flours have fast starch hydrolysis. The gelation ability and the high peak viscosity of the isolated starch during heating could be relevant for the development of thickener in food products. Regarding pasting properties, the raw bunya flour showed almost no short-term retrogradation, which is different from other commercial flours, including other gluten-free flours. Also, pasting parameters should not be the only result evaluated to assess retrogradation of flour and starch. Other methods, such as rheology, might be an important complement to that.

By investigating the properties of the processed flours, we have shown the potential it has in terms of nutritional, functional and rheological aspects. In addition, gluten-free flours have three main issues in the food industry: poor flavour, texture and nutritional quality. The use of alternative flour sources, such as bunya nuts, overcomes some of these challenges (Moura Nadolny et al., 2022) while providing new opportunities. Importantly, we have demonstrated that flour properties can be

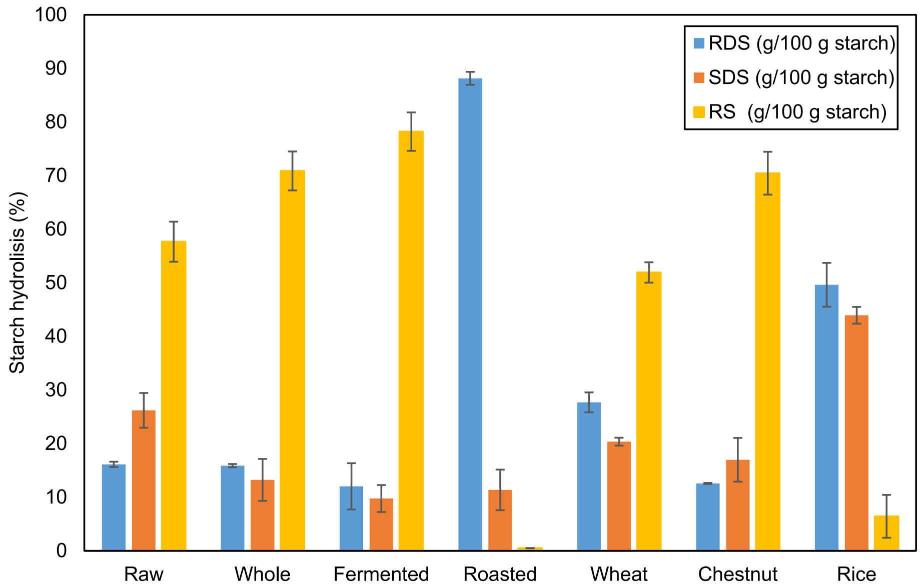


**Fig. 8.** Percentages of rapidly digestible (RDS) slowly digestible (SDS) and resistant (RS) starch fractions in the seven flours analysed.

manipulated to enhance or degrade specific functional properties by selecting the right pre-treatment. This finding is of value to bunya flours and a range of other gluten free replacements for wheat flour that do not commonly demonstrate the same functional properties but are used in the same manner as wheat, for example in breadmaking. This also gives the opportunity to use bunya flour for a much wider array of applications than that offered by standard gluten-free flours. Also, bunya nuts are native to Australia and, together with the other two species, is a native and Indigenous food, which improves its value as the world is shifting towards a more sustainable and local food supply chain.

Future studies may include performing enzymatic essays to test the inhibitory effect of the inner coating of bunya flour on alpha amylase, as well as looking at whether the processing of the nut into flour could result in phenolics *versus* starch complexes, which has the potential to act as a type V resistant starch (Gutiérrez & Tovar, 2021). Besides being gluten-free, because bunya nuts are from the same family and have similar composition to *pinhão*, they might be considered as low in FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols), as the species is mentioned in a range of “low fodmap foods” tables in Brazil (Brazilian Gastroenterology Federation). Based on the digestibility of the bunya flour showed here, future studies could be conducted to evaluate their ability to be commercialised as a low fodmap source of food. Also, this study shows that complementing rice flour or wheat flour with bunya nut flour could be an option. Further evaluation using a combination of these flours could be an opportunity to show whether it would enhance pasting and functional properties, well as improve health benefits and sensory attributes of products, all of which is desirable.

## CRediT authorship contribution statement

**Jaqueline Moura Nadolny:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bernadine M. Flanagan:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Heather M. Shewan:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Conceptualization. **Heather E. Smyth:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization. **Odette Best:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Jason R. Stokes:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jaqueline Moura Nadolny reports financial support was provided by Australian Research Council (ARC) Industrial Transformation Training Centre for Uniquely Australian Foods (IC180100045). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.115627.

## Data availability

Data will be made available on request.

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