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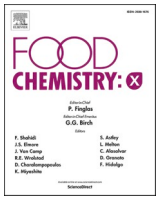

Improving chestnut physicochemical properties through fermentation – Development of chestnut Amazake

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ABSTRACT

The increased awareness of population regarding the impact of consumption habits is leading to interest in new, innovative, diversified and health promoting foods. In this work, two new amazake fermented products were developed with chestnut (*Castanea sativa* Mill.), using rice or chestnut koji as source of glycolytic enzymes. The analysis of the amazakes evolution showed improvements in chestnuts physicochemical characteristics. The fermented products presented higher values of soluble protein, sugars, starches, antioxidant capacity, and similar values of ascorbic acid for chestnut koji amazake. The adhesiveness increased, which is related to the enhanced concentrations of sugars and starches. The evolution into less structured products was observed in the firmness followed by a consistent decrease of the viscoelastic moduli.

The developed chestnut amazakes can represent a suitable alternative to traditional amazake, creating an opportunity for valorisation of chestnut industrial by-products, as new, tasty, and nutritive fermented products with potential functional characteristics.

1. Introduction

The increasing awareness of consumption habits impact on the human health and on the environment is inspiring consumers to adopt more plant-based diets, where animal products are only consumed occasionally (Aschemann-Witzel, Gantriis, Fraga, & Perez-Cueto, 2021). This creates opportunities in the market for innovative, diverse, and healthy foods, aligned with the new trends (Aschemann-Witzel et al., 2021). Fermented foods, either using fermentative or enzymatic hydrolysis processes (Marco et al., 2021) are one of the products that can be developed, increasing nutritional value and health benefits (Birch & Bonwick, 2019; Granato et al., 2020).

Chestnut (*Castanea sativa* Mill.) fruit is an important amylaceous nut crop with high nutritional value. With origin in the eastern Mediterranean region, it is probably-one of the oldest foods consumed by the ancient European population (De Vasconcelos, Bennett, Rosa, & Ferreira-Cardoso, 2010; Pinto et al., 2020).

In Portugal, a major producer of chestnut, the most important production areas are located in Trás-os-Montes. This activity has a strong connection to rural populations and contributes to the agricultural exports and economy of this region (Borges, Gonçalves, de Carvalho, Correia, & Silva, 2008; Ferreira-Cardoso, Sequeira, Torres-Pereira, Rodrigues, & Gomes, 1998). The ability to supply the market is constrained by factors such as the low fruit homogeneity, product seasonality and reduced preservation time. Due to their perishable nature, Portuguese chestnuts are mainly sold as a fresh product during harvesting season, or as frozen product throughout the year, both in national and international markets. As consequence, high amounts of low-value by-products, such as fragmented and low calibre fruits, are generated (Pinto et al., 2020),. These by-products have a high potential for the valorisation through circular economy approaches and for the creation of added value products. Chestnut should be a part of a balanced diet, due to their numerous health benefits (De Vasconcelos et al., 2010). Usually it is consumed during autumn, as an alternative to rice, pasta or potatoes, alone or as a side dish, in soups, and stews, after being boiled, mashed or roasted, to increase nutrient accessibility and improve digestibility and flavour. In bakery and confectionery, chestnut is used to replace wheat flour (De Vasconcelos et al., 2010; Gonçalves

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et al., 2010).

Chestnut composition can present great variations according to the cultivar (Borges et al., 2008), edaphoclimatic conditions and cultivation inputs (De Vasconcelos et al., 2010). These amylaceous fruits differ from fresh fruits due to their low water (45.4–53.3 g/100 g fresh matter) and high starch (38.6–64.9 g/100 g dry matter) content, and from oleaginous fruits due to their low fat (1.6–3.1 g/100 g dm) content (Borges et al., 2008; De Vasconcelos, Bennett, Rosa, & Cardoso, 2007). In addition, the high content in soluble fermentable fibres (starches) of chestnut can stimulate good intestinal microbiota and short-chain fatty-acids production, contributing to decrease digestion and sugar absorption rates, reducing fat and cholesterol absorption, reinforcing immune system and promoting satiety and regulation of bowel transit (Cruz, Abraão, Lemos, & Nunes, 2013; De Vasconcelos et al., 2010). Chestnut is also rich in calcium, iron, magnesium, potassium, phosphorous, selenium, amino acids, and ascorbic and folic acids. Moreover, it contains phytochemicals as lutein and zeaxanthin and several polyphenolic compounds important for cell protection and antioxidant activity (Barreira, Casal, Ferreira, Oliveira, & Pereira, 2009; De Vasconcelos, Bennett, Rosa, & Ferreira-Cardoso, 2009).

The interest for new forms of consumption is increasing, in response to the growth of specific markets to meet dietary needs, such as gluten-free and nutritionally enriched products (Aschemann-Witzel et al., 2021). The nutritional composition of chestnuts, rich in fibres and carbohydrates, makes them suitable alternatives to rice in the production of functional fermented foods and beverages, such as sake, mirin and amazake.

Koji-amazake is a sweet, viscous, non-alcoholic fermented product traditional from Japan and originally prepared through combination of polished rice, rice-koji and water, followed by incubation at 50–55 °C (Oguro, Nishiwaki, Shinada, Kobayashi, & Kurahashi, 2017). The rice-koji is produced through solid-state fermentation of the koji mold spores (tane-koji) inoculated on whole grain, generally steamed rice. Koji mold is composed by *Aspergillus* sp., usually *A. oryzae*. During the amazake production, the enzymes produced by the fungus mycelium in the rice-koji, such as amylases, are responsible for the hydrolysis of the starch in the grains mainly into free glucose, but also in other sugars such as trehalose, kojibiose, maltose, nigerose, isomaltose and maltotriose (Kurahashi, 2021; Oguro, Nakamura, & Kurahashi, 2019), generating a naturally sweetened product. Besides the sugars coming from the grain hydrolysis, koji-amazake is rich in many other compounds that are produced by *A. oryzae* during the koji production process, such as complex B vitamins, (including thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), and biotin (B7)), organic acids such as citric acid, succinic acid, malic acid and pyruvic acid (Oguro et al., 2017), and lipids, mainly derived from the *A. oryzae* cell membrane, such as N-20-hydroxyoctadecanoyl-l-O-D-galactopyranosyl-9-methyl-4,8-sphingadienine and N-20-hydroxyoctadecanoyl-l-O-D-glucopyranosyl-9-methyl-4,8-sphingadienine (Hamajima et al., 2016). Furthermore, the fermentation by koji-rice also originates amino acids and compounds with antioxidant activity such as tocotrienol (Wada, Sakamoto, & Matsugo, 2018), ergothioneine, choline and betaine (Kurahashi, 2021; Oguro et al., 2017). Many physiological functions and health benefits have been associated with koji-amazake consumption, such as, blood pressure lowering, anti-obesity, liver-protection and anti-amnesic (Kurahashi, 2021; Saigusa & Ohba, 2007).

Currently, there is an increasing trend in drinking amazake, and various types of non-traditional amazake beverages are commercially available on the market, including brown rice and oat amazake. Following this trend, this work aimed to test the possibility of using chestnut instead of rice, as raw material to develop amazake type fermented foods. Here, we take advantage of the rich nutritional composition of chestnut and the benefits resulting from koji mold, to produce food products with potential health benefits and improved physicochemical and sensorial properties. Two types of koji were prepared in order to compare their performance, the traditional rice-koji and the alternative chestnut-koji. The determination of physicochemical characteristics (chemical composition, texture, and linear viscoelastic behaviour) was used to monitor and compare the evolution of these amazakes during their fermentation/hydrolysis process. The new amazake fermented product could contribute to add value to the low-priced by-products of chestnut agro-food industry. while promoting a more healthy, diversified and nutritive diet near the consumers.

2. Materials and methods

2.1. Microorganisms

The inoculum of *Aspergillus oryzae* (Vision Brewing Co., Nedlands, Australia) was prepared by inoculating a spore suspension in PDA (Potato Dextrose Agar) medium. Plates were incubated at 28 °C until sporulation.

2.2. Koji

Blanched rice was washed and hydrated overnight in demineralized water at room temperature (25 °C). After draining, the rice was steam cooked using a Yämmi 2 (Food Processor, Continente, Portugal) for 20 min at 100 °C and cooled to room temperature.

Frozen chestnuts (Castanhas de Trás-os-Montes – Continente) were defrosted at room temperature, crushed into fragments from 1 to 5 mm using Yämmi 2, for 3 cycles of 2 s at speed 10. Crushed chestnuts were steam cooked for 20 min at 100 °C using Yämmi 2 and cooled to room temperature.

Koji was prepared according to Santos, Mansidão, Mota, Raymundo, and Prista (2021). Briefly, the rice and chestnuts were distributed in perforated aluminium trays lined with cheesecloth, sprinkled with *A. oryzae* dry spores, and covered with cheesecloth. Incubation occurred at room temperature under static conditions, with manual agitation and spraying with sterile demineralized water three times a day, until a strong growth was observed at the grains surface.

2.3. Amazake production

Frozen chestnuts (Castanhas de Trás-os-Montes – Continente) used for amazake production were prepared as described in section 2.2, steam cooked for 25 min at 100 °C using Yämmi 2 and cooled to room temperature.

Two amazakes were developed either using rice (CHR) or chestnut (CH) koji as source of enzymes produced by *A. oryzae*. Chestnuts were used as the main raw material for both amazake types. Amazake production was performed with a modified version adapted from Oguro et al. (2017). Briefly, steam cooked chestnuts were mixed with 15 % (w/w) of rice or chestnut koji and incubated for 24 h at 50 °C. Four replicates were produced for each type of amazake (CH and CHR). For each amazake, aliquots of 45 g were periodically collected during the 24 h fermentation.

2.4. Aspergillus oryzae viability in kojis

The viability of *A. oryzae* in rice and chestnut kojis was assessed initially and during the amazakes production process from collected samples. Sequential dilutions from 10^{-9} to 10^{0} were inoculated in PDA medium and incubated at 28 °C. The observed colonies were counted and expressed in terms of CFU (colony forming units) per 1 g of fresh matter. Plates were inoculated in duplicates for each amazake replicate.

2.5. Chemical composition analysis

A fraction of each sample (200 mg) was diluted at 1:10 (w/v) in demineralised water, vortexed for 30 s., centrifuged at 10.000 g for 5 min and the supernatant collected. The supernatant was used to

determine soluble protein through Bradford method (Bradford, 1976), and total reducing sugars and starch using DNS (3,5-Dinitrosalicylic acid) method adapted from (Garriga, Almaraz, & Marchiaro, 2017). Each sample was incubated with and without a solution of 20 mg of α-amylase from porcine pancreas (Type VI-B, ≥5 Units/mg solid, Sigma-Aldrich) in 50 mL of PBS (pH 6.9). The samples were measured at 540 nm and the difference between total (with α-amylase) and free (without α-amylase) reducing sugar concentrations corresponded to the digestible starch, after multiplication by a 0.9 factor. All the results of soluble protein, total reducing sugars and digestible starch were determined and presented in g/100 g of fresh and dry matter.

For dry matter determination, approximately 10 g of each fresh amazake sample was freeze-dried (CoolSafe Scanvac, Labogene, Denmark). Dry matter and water content were calculated considering fresh and dry weight and results presented in g/100 g of fresh matter.

The ascorbic acid content was determined by a titration AOAC method optimized by Oliveira, Godoy, and Prado (2010) using 2,6-dichlorophenol-indophenol (DCPIP) as an indicator. The results were expressed in mg of citric acid equivalents per 100 g of fresh and dry matter.

Titratable acidity was determined by titration with 0.1 N NaOH and controlled using a pH electrode and phenolphthalein as indicator. Values were expressed in citric acid equivalents (Bureau, Boas, Giovinazzo, Jaillais, & Page, 2020) and presented in g/100 g of fresh matter.

For total phenolic compounds concentration, the previous methanol extracts of amazake were used following an adaption of Waterhouse (2002) method, with gallic acid as standard. The results were presented in mg of gallic acid equivalents per g of fresh matter.

2.6. Invitro antioxidant activity evaluation

The antioxidant capacity was determined by two adapted methods, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Brand-Williams, Cuvelier, & Berset, 1995) and ferric reduction antioxidant power (FRAP) (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Hawkins Byrne, 2006). Sample extraction was performed at 1:10 (w/v) in methanol, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solutions were used for calibration. FRAP and DPPH results were expressed in mg of TROLOX equivalents per g of fresh matter.

2.7. Texture measurements – Firmness and adhesiveness

The texture analysis of amazakes was performed using a TA.XTplus Texture Analyser (Stable Micro-Systems, Godalming, UK) in a temperature-controlled room at 20 ± 1 °C. Texture results were expressed in terms of firmness and adhesiveness obtained by texture profile analysis (TPA or 'two bites' test), in penetration tests, using a cylindrical probe of 11 mm diameter (TA-11). The specific test conditions were: 8 mm penetration, 2 mm/s pre- and posttest speed, 1 mm/s test speed, 5 s of waiting time between two cycles, and with a load cell of 5 kg. Amazake samples were analysed in containers (7 mm diameter and 5 cm height), performing at least three replicates per sample.

Firmness (N) is the maximum force recorded in texturogram (force versus time) in first penetration cycle and represents the force required to compress the material between the molars. Adhesiveness (-N.s.) is the negative area of the texturogram, and represents the work required to remove the probe from the material and related with the force required to remove food sticking to mouth during chewing (Bourne, 2002).

2.8. Small amplitude oscillatory measurements

The study of the viscoelastic linear behaviour of amazakes was performed by dynamic small-amplitude oscillatory shear (SAOS) measurements in a controlled stress rheometer: Thermo Scientific HAAKE MARS III (ThermoFisher Scientific, Karlsruhe, Germany), with an UTC – Peltier system for temperature control. A serrated parallel plate sensor system (PP20-20 mm diameter) was used to overcome the slip effect, with a 1.5 mm gap, previously optimized for this material. For each sample, stress sweep tests (variation of the sinusoidal stress applied to a constant frequency value) were applied to define the viscoelastic linear region – the range of stress in which the viscoelastic moduli (G', storage modulus and G'', loss modulus) are independent of the applied stress. The mechanical spectrum of each amazake was obtained by frequency sweep tests: variations of G' and G'' over a range of different frequencies at a constant stress, within the linear viscoelastic region. The samples of amazake were placed on the measuring device, covered with paraffin oil to avoid evaporation, and stabilized for 5 min at 20 °C. Frequency sweep tests were conducted, after sample stabilization, by ranging the frequency between 0.001 and 100.0 Hz at 20 °C with a constant shear stress inside the linear viscoelastic region (previously determined by a stress sweep test: $f = 10.0$ Hz and $\tau = 0.1$–100 Pa). All the measurements were performed at least in triplicate. The results of G' and G'' were graphically presented in Pa.

2.9. Statistical analysis

The mean and standard deviation of the experimental data and statistical analysis were conducted by GraphPad Prism software (version 5.0). One-way analysis of variance (ANOVA) was performed and when significant differences were found between treatments, post hoc analysis using Tukey's test was conducted ($\alpha = 0.05$).

3. Results and discussion

3.1. Chemical composition and antioxidant activity of amazakes

The nutritional composition was determined for the two amazakes produced using rice koji (CH) or chestnut koji (CHR) and compared to commercial Clearspring brown rice amazake (RC) (Table 1). The obtained values for crude protein range between 7.60 and 7.89 g/100 g of product dry weight and show non-significant differences ($p > 0.05$) between the two chestnut amazakes produced and the commercial rice product. These are also in agreement with published reference values for the raw materials (Borges et al., 2008; De Vasconcelos et al., 2007; Gonçalves et al., 2010).

Reducing sugars and digestible starch increased significantly after 24 h of process, for both CH and CHR. CH amazake presented values significantly lower ($p < 0.05$) of total free reducing sugars than CHR, from which 26 and 27 %, respectively, correspond to starch. This difference was expected since *A. oryzae* spores presented higher initial viability in rice (4.7×10^9 CFU/g) than in chestnut (7.3×10^6 CFU/g) koji, which may directly translate in higher amylolytic and proteolytic activities. In comparison to the RC, with 32 g of carbohydrates per 100 g of fresh weight, with around 40 % corresponding to starch, CH and CHR present lower sugar concentrations. However, higher values should be expected since quantification of reducing sugars excludes sucrose, the main sugar present in most Portuguese cultivars of chestnuts (Barreira, Pereira, Oliveira, & Ferreira, 2010).

The increase in reducing sugars during CH and CHR amazake production (Fig. 1A), is also accompanied by variations in digestible starch (Fig. 1B), which increased for CH during the first 18 h and CHR during the 24 h, resulting in significant ($p < 0.05$) higher starch for CHR in the final product (Fig. 1B). This may be related to the breakdown of molecular bonds, from hydrolytic activity of *A. oryzae* enzymes, decreasing the degree of structuring and increasing accessibility to starch (Elkhalifa, Schiffler, & Bernhard, 2004). The α-amylase can decompose and liquefy starch in smaller units, which can then be converted into glucose. The action of proteases can also have an important role, promoting protein hydrolysis into peptides, helping the α-amylase action (Okuda, Iizuka, Xu, & Wang, 2019), while contributing to the development of the flavour and aroma of the products (Furukawa, 2012).

Table 1
Physicochemical properties and antioxidant activity of cooked chestnut, commercial rice amazake (RC), and chestnut koji (CH) and rice koji (CHR) chestnut amazakes.

		Cooked Chestnut	RC	CH	CHR
Water content (g) [(a)**]		57.55 ± 0.001[a]	58.83[a]	55.05 ± 1.38[ab]	51.02 ± 0.59[b]
Dry matter (g) [(a)**]		42.45 ± 0.001[a]	41.17 [a]	44.95 ± 1.38[ab]	48.98 ± 0.59[b]
Crude protein (g) [(a)*]		6.28 ± 1.12 [(1)]	7.89 ± 0.09[a]	7.67 ± 0.20[a]	7.60 ± 0.16[a]
Soluble protein (g) [(a)**]		0.50 ± 0.13[a]	-	1.19 ± 0.04[b]	1.35 ± 0.07[b]
Soluble protein (g) [(a)*]		1.17 ± 0.31[a]	-	2.64 ± 0.08[b]	2.75 ± 0.15[b]
Total reducing sugars (g) [(a)**]		0.77 ± 0.13[a]	-	6.69 ± 1.47[b]	14.09 ± 1.72[c]
Total reducing sugars (g) [(a)*]		1.81 ± 0.30[a]	-	14.88 ± 3.29[b]	28.77 ± 3.52[c]
Digestible starch (g) [(a)**]		0.60 ± 0.10[a]	-	1.74 ± 0.07[b]	3.83 ± 0.13[c]
Digestible starch (g) [(a)*]		1.28 ± 0.08[a]	-	3.88 ± 0.16[b]	7.82 ± 0.27[c]
Ascorbic acid (mg $_{CAE}$) [(a)**]		3.30 ± 0.25 [(3)]	3.38 ± 0.04[a]	5.87 ± 0.27[b]	3.87 ± 0.43[a]
Ascorbic acid (mg $_{CAE}$) [(a)*]		7.83 ± 0.59 [(3)]	8.22 ± 0.16[a]	13.07 ± 0.70[b]	7.90 ± 1.01[a]
Titratable acidity (g) [(a)**]		-	0.46 ± 0.18[a]	0.60 ± 0.10[a]	0.55 ± 0.03[a]
Antioxidant capacity (mg $_{TRE}$/g) **	DPPH	1.02 ± 0.02 [(2)]	2.87 ± 0.25[a]	8.72 ± 0.67[b]	8.78 ± 1.20[b]
	FRAP	4.38 ± 0.30 [(2)]	1.78 ± 0.07[a]	13.14 ± 2.23[b]	12.43 ± 2.55[b]
Total phenolic compounds (mg $_{GAE}$/g) **		6.95 ± 1.17 [(1)]	6.56 ± 0.11[a]	6.28 ± 0.44[a]	5.13 ± 0.84[a]

(a) Per 100 g, *dm, dry matter, **fm, fresh matter. TRE, Trolox equivalents. GAE, Gallic acid equivalents. CAE, Citric acid equivalents. FRAP, Ferric Reducing Antioxidant Power, DPPH, 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity. Two fermentation replicates analysed at least in duplicate for each condition. Values with the same letter are not significantly different according to a Tukey test (α = 0.05). (1) (Gonçalves et al., 2010), (2) (Suna, Avşar, Koçer, & Çopur, 2021), (3) (Barros, Nunes, Gonçalves, Bennett, & Silva, 2011).

In this case, considering that a temperature of 50 °C was used in amazake production, the action of amylolytic enzymes is favoured over proteolytic enzymes (Okuda et al., 2019). This fact may explain the observed continuous increase in soluble protein throughout the process (Fig. 1C). This possibly resulted from the protein release from the loosening of the starch net, and/or from the lower proteolytic activity. More, although significant differences ($p < 0.05$) were observed between CH and CHR, the final products presented similar values of protein (Table 1).

For the total titratable acidity, low values with no significant variations ($p > 0.05$) were observed along the chestnut amazakes evolution and between CH, CHR and RC amazakes.

Concerning antioxidant compounds, the quantified values of ascorbic acid for CH were significantly higher ($p < 0.05$) than those observed for RC and CHR, which presented similar results (Table 1). According to the literature, raw chestnuts lose around 37 % of their ascorbic acid content upon boiling, reducing from around 5.29 ± 0.38 to 3.30 ± 0.25 mg ACE/100 g fresh weight (Barros et al., 2011). In the present work, steamed chestnuts were used, and no reduction in this compound was observed for CH amazake. The obtained lower value of ascorbic acid in CHR as compared to raw chestnuts might be a consequence of the rice koji incorporation. Indeed, rice amazake also presented lower levels of ascorbic acid. The determination of the antioxidant capacity of the amazakes resulted in similar profiles for both methods used, DPPH and FRAP (Fig. 2A and B). A strong correlation was determined between

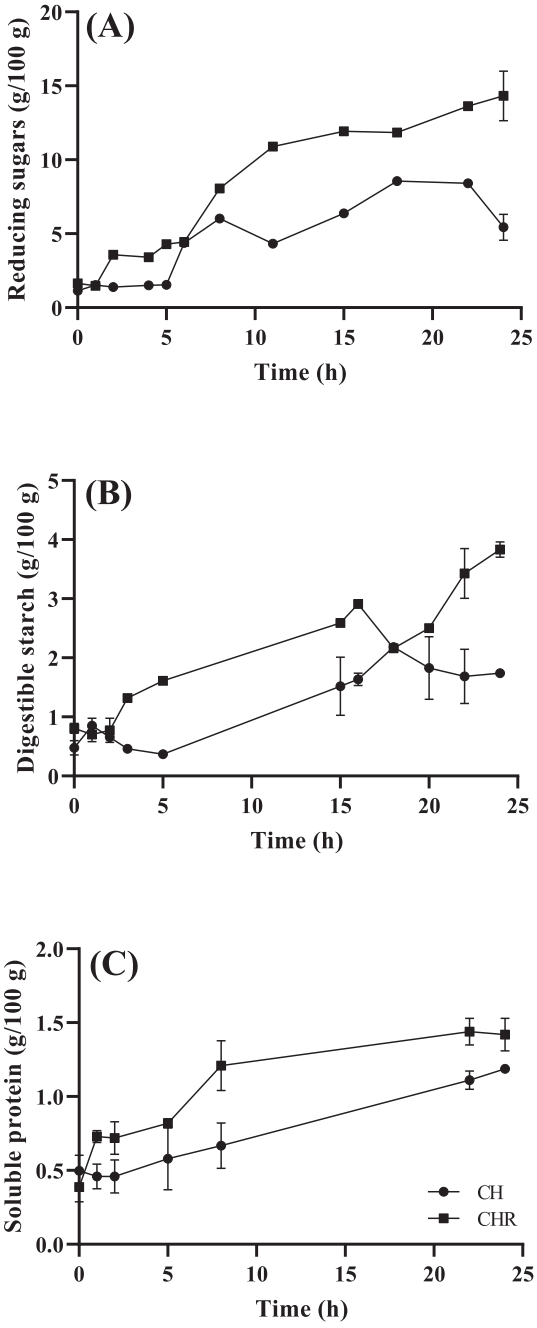

Fig. 1. Reducing sugars (A), digestible starch (B), and soluble protein (C) evolution, along the chestnut koji (CH) and rice koji (CHR) chestnut amazakes production process. Four fermentation replicates performed for each condition. Results presented as mean ± standard deviation.

antioxidant capacity obtained from the two methods with a coefficient (R^2) of 0.939 (Appendix 1). Significantly lower values ($p < 0.05$) were obtained for the RC amazake in comparison to CH and CRH, which may be related with the lower antioxidant capacity of rice in comparison to chestnut (Suna et al., 2021). An increase in the values, although not significant ($p > 0.05$), is visible for CH from 8 to 24 h. However, no significant differences were observed between CH and CHR final products.

Regarding the total phenolic compounds (Fig. 2C, Table 1), the observed differences between RC and CH and CHR final products were not significant ($p > 0.05$). Contrary to antioxidant capacity, the values in the same range for RC can indicate the high presence of flavonols in CH and CHR, not accounted through this method. Mustafa et al. (2021)

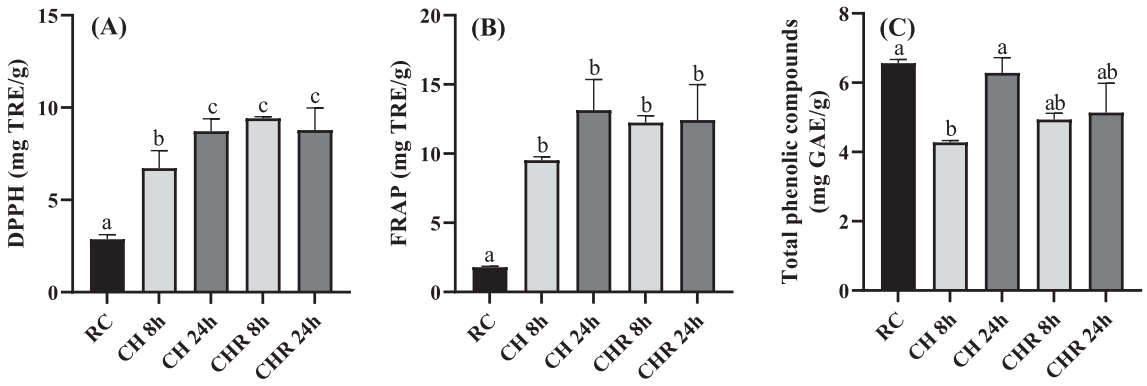

Fig. 2. Antioxidant capacity determined through DPPH (A) and FRAP (B), and total phenolic compounds, for commercial rice amazake (RC) and chestnut koji (CH) and rice koji (CHR) chestnut amazakes, at 8 and 24 h of the process. Two fermentation replicates performed in duplicate for each condition. Results presented as mean ± standard deviation. Values with the same letter are not significantly different according to a Tukey test (α = 0.05).

determined the amount of these compounds in chestnut, observing their increase after cooking. In opposition, Gonçalves et al. (2010) did not observe significant variation upon cooking of the chestnuts. However, it was described that the total phenolic composition of chestnut depends on the cultivar and may increase, decrease, or remain unchanged after cooking (Barros et al., 2011). In comparison to other products from chestnut, as chestnut pickles (Suna et al., 2021), higher values of phenolic compounds are observed for the amazakes. For example, CH presents 139.71 mg GAE/100 g of dm, while for the pickles the highest value observed is 126.11 mg GAE/100 g of dm.

From the analysis of steamed chestnut and the nutritional compositions of chestnuts described in the literature (Barros et al., 2011; Borges et al., 2008; De Vasconcelos et al., 2007; Mustafa et al., 2021; Suna et al., 2021), it was possible to compare these raw materials with the developed amazakes (Table 1). Similar values of water content and dry matter were observed between CH and chestnuts, with crude protein for both CH and CHR slightly higher than the value described for chestnuts. In comparison to the steam cooked raw material, the amazakes presented higher concentrations of reducing sugars, digestible starch, and soluble protein. A great improvement was observed in terms of antioxidant potential (DPPH and FRAP), and the values of total phenolic compounds and ascorbic acid in CH were similar to those described in the literature for chestnut (Borges et al., 2008; De Vasconcelos et al., 2007; Gonçalves et al., 2010). From these results it can be stated that the hydrolytic process promoted by koji contributes to improve the chemical composition of chestnut, by enhancing bioavailability of nutrients, without degradation of components due to the processing.

3.2. Texture measurements – Firmness and adhesiveness

The evolution of texture in terms of firmness and adhesiveness, for the chestnut amazakes (Fig. 3) and the comparison between the final products and commercial amazake (Appendix 2) are presented.

The data in Fig. 3A showed a significant ($p < 0.05$) decrease in CH firmness from 31.81 ± 2.44 N at the beginning to 15.01 ± 0.55 N after 8 h of hydrolysis, followed by stabilization. As for CHR, variations in firmness are only observed after 8 h, decreasing from 32.38 ± 2.38 to 14.13 ± 2.71 N. Despite the evolution observed along the process, no significant differences ($p > 0.05$) were observed between the initial and final firmness for both products. In comparison to these results, the firmness for RC was significantly lower, 0.477 ± 0.05 N, which can be a consequence of the higher water content and lower dry matter (Bourne, 2002), resulting in a product with low consistency.

In terms of adhesiveness (Fig. 3B), for CH this parameter increases mostly between 8 and 15 h, while for CHR the increase is more linear, especially from 8 h onward. The final chestnut amazakes, CH and CHR, presented significant differences in adhesiveness with values of 7.16 ± 1.06 and 13.24 ± 0.69 -N.s. Also, the commercial rice amazake (RC), presented a significantly lower value, 1.45 ± 0.14 -N.s, than CH and CHR final products. The most significant increase in adhesiveness was observed for CHR (Fig. 3B and Appendix 2).

Although firmness only started to decrease after 8 h for CHR, afterwards this amazake presented the most pronounced reduction, which is coherent with the theory of a higher enzymatic activity for the product. This observation associated with an accentuated increase in adhesiveness, may be explained by a higher degradation of structural components from the koji enzymes (Oguro et al., 2019). The higher activity of

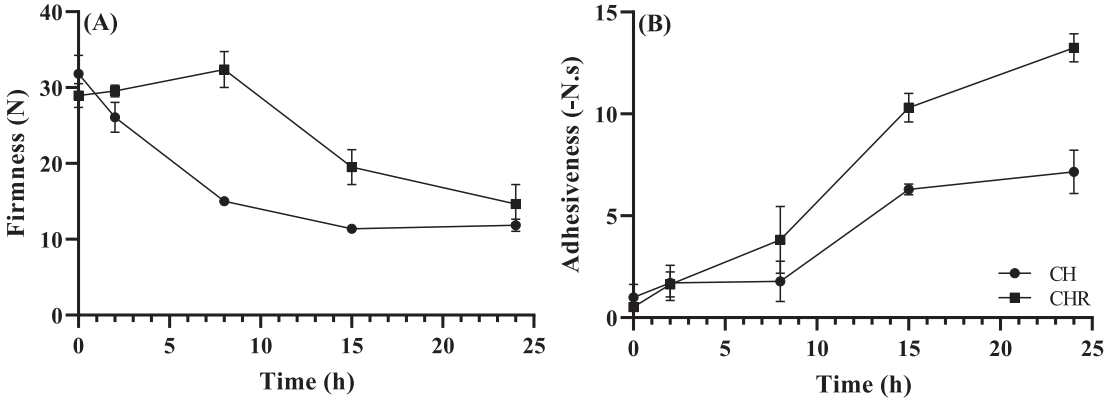

Fig. 3. Texture parameters evolution along the chestnut koji (CH) and rice koji (CHR) chestnut amazakes production process. (A) Firmness, (B) Adhesiveness. Two fermentation replicates performed at least in triplicate for each condition. Results presented as mean ± standard deviation.

hydrolytic enzymes, and higher carbohydrates and protein in the rice (Oguro et al., 2019) added to CHR, possibly resulted in a product initially more structured in terms of starch and glucose, consequently impacting the texture parameters. Also, the higher adhesiveness for CHR might be explained by the increase of reducing sugars, and digestible starch concentrations, which were more significant than in CH.

3.3. Small amplitude oscillatory measurements

In addition to the texture profile analysis, the linear viscoelastic behaviour was evaluated, through frequency sweep tests for the different amazakes (Fig. 4), allowing to obtain information about their structure evolution.

From all mechanical spectra, it is observed that the elastic component (G') is always higher than G'' in the entire range of frequencies studied, with similar distances between both moduli. For all products, evolution of G' and G'' is slightly dependent in the frequency variations, which is suggestive of weak gels like behaviour (Ross-Murphy, 1994).

A decrease in the storage modulus (G') and loss modulus (G'') was observed, from the initial to the final products, for both chestnut amazakes (CH and CHR), indicating a reduction in the degree of structure (Clark, 1991; Doucet, Gauthier, & Foegeding, 2001). The CHR presented higher values of G' and G'' than CH, which should be related with the development of a stronger structural system. This behaviour should be related with the higher content of starch prevenient from the rice koji in CHR. The results are in concordance with the firmness (Fig. 3, section 3.2), suggesting significant variations in the degree of CHR structure from the initial to final material. For the final chestnut amazake products, no significant differences ($p > 0.05$) in the degree of structure were observed between CH and CHR, suggesting that koji plays a key role in the amazake process, as the source of hydrolytic enzymes (Oguro et al., 2019). During the enzymatic process, the raw materials inter molecular bonds break, interfering in the structural characteristics (Clark, 1991; Doucet et al., 2001), and resulting in products with reduced viscoelastic functions. The results obtained can be supported by the supposedly higher enzymatic activity present in CHR, which also presents the higher variation in the degree of structure from the initial to the final product.

The lower values of G' and G'' observed for the commercial rice amazake (RC), are also in accordance with the results obtained for firmness (section 3.2).

The evolution of G' at 1 Hz (Table 2) allowed to obtain a more detailed analysis about the variation of viscoelastic behaviour. As expected, CHR amazake presented significantly higher initial values of G', associated to a more structured raw material. Variations in the structure of CHR occur only after 8 h, with G' following a similar profile (Appendix 3) to the obtained results for firmness (Fig. 3, section 3.2). After 8 h, the viscoelastic moduli for CHR decreased significantly, presenting

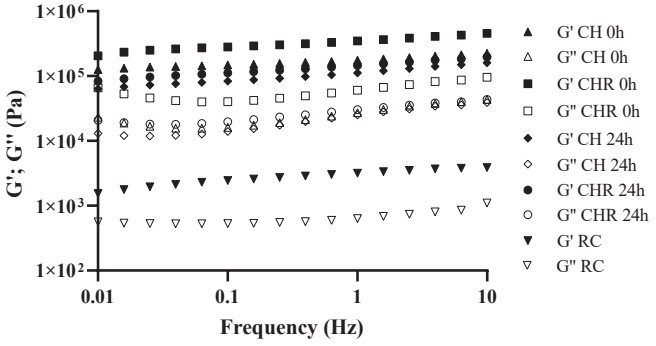

Fig. 4. Viscoelastic functions (G' and G'' moduli) with frequency variation, evolution from 0 to 24 h for chestnut koji (CH) and rice koji (CHR) chestnut amazakes and comparison to commercial rice amazake (RC). Mechanical spectrums presented selected from data obtained from two fermentation replicates performed at least in triplicate for each condition.

Table 2
Viscoelastic modulus (G') at 1 Hz frequency. Evolution from 0 to 24 h for chestnut koji (CH) and rice koji (CHR) chestnut amazakes and comparison to commercial rice amazake (RC).

Amazake		G' (MPa)
Commercial (RC)	Control	0.0029 ± 0.0003^{a}
Chestnut koji (CH)	0 h	0.19 ± 0.03^{bg}
	8 h	0.15 ± 0.01^{bdg}
	15 h	0.12 ± 0.004^{bf}
	24 h	0.11 ± 0.01^{cdf}
Rice koji (CHR)	0 h	0.33 ± 0.02^{e}
	8 h	0.35 ± 0.06^{e}
	15 h	0.22 ± 0.02^{g}
	24 h	0.14 ± 0.02^{bc}

Two fermentation replicates were performed at least in triplicate for each condition. Values with the same letter are not significantly different according to a Tukey test ($\alpha = 0.05$).

similar values to CH amazake after 24 h. Regarding the evolution of G' (1 Hz) for CH, only initial and final products presented significant differences. Otherwise, the obtained G' for commercial rice amazake was significantly lower ($p < 0.001$) from both final products of chestnut amazake with values of 10^3-fold lower. This behaviour reflects a much less structured system in the commercial product.

The higher starch content in CHR amazake, in comparison to CH may explain its greater impact in decomposition at the end of the fermentation. The process behind may be mainly dependent on amylolytic enzymes. For instance, α-amylase liquefies starch to produce dextrins, and α-glucosidase hydrolyses dextrins into glucose (Kurahashi, 2021; Wong, 1995). In fact, the process temperature favours the activity of this class of enzymes (Oguro et al., 2019). Regarding rice amazake, the activity of proteolytic enzymes has also been referred (Kurahashi, 2021). Although further studies would be required to clarify if proteases intervein in the process (Kurahashi, 2021), we can hypothesize that their action could contribute to increase digestible starch availability for follow-up hydrolysis.

In CH amazake, the lower starch content available due to the use of chestnut koji, may have contributed to the observed slower process, presenting less variability in the G' values along time. Although similar values of antioxidant capacity were obtained for CH and CHR, the higher content of ascorbic acid in CH may have influenced the faster structural stabilization of the product (Cort, 1982). This observation is also supported by firmness and adhesiveness apparent tendency to stabilise for CH amazake after 15 h.

4. Conclusions

In this study-two types of chestnut amazake were produced (with rice or chestnut koji) and compared to a commercial brown rice amazake as control.

The analysis of the products in terms of chemical composition, texture and linear viscoelastic behaviour allowed to monitor the hydrolytic effect of koji, providing information in the physicochemical variations occurring to chestnut during the process.

From the comparisons between literature data and this work was possible to observe the impact of raw materials composition in the products characteristics. The commercial rice amazake (RC) presents higher sugar content, but significantly lower values were observed in terms of antioxidant capacity in comparison to CH and CHR. The suitability of chestnut as an alternative to rice in the production of amazake products, with possible lower glycaemic index, is evident from the results. Also, the processing of chestnut through an ancient hydrolytic process using koji can result in a fermented product with higher bioavailability and improved organoleptic properties, more nutritious and with higher antioxidant capacity than the original raw material. A simple sensory evaluation performed in our lab revealed that CH and

AHR presented a creamier, softer and more pleasant texture and more balanced flavours and sweetness than CR amazake, being preferred by tasters.

The work developed using frozen chestnuts presents an opportunity for the valorisation of chestnut industrial by-products (fragmented and low calibre fruits), through the creation of new fermented foods that are tasty, nutritive, and present potential functional properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100597.

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