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Performance of technological and microbiological quality characteristics of germinated rice: A comparative study of cooking techniques

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ABSTRACT

Germination is a process used to improve the nutritional and sensory quality of brown rice, but technological and microbiological characteristics under different cooking techniques and storage conditions are neglected. In this scenario, this study aimed to evaluate the effect of rice types (germinated, parboiled and polished) on the physical, chemical and microbiological characteristics in terms of different cooking rice preparation methods and storage conditions. In general, germination did not affect crystallinity and DSC parameters but was effective in reducing the cooking time (31–22 min) using a conventional pan. A resistant starch content was observed after 30 days under freezing and increasing pronouncedly if samples were cooked in an electric rice cooker (100 %), highlighting the prebiotic aspect. After cooking (t = 0 h), germination induced the highest values of hardness (19.7 N) and chewiness (24.6 N) in germinated brown rice prepared by electric cooker. The freeze-thaw cycling (t = 30 days) reduced hardness (19.2 −12.7 N) and chewiness (17.1–5.3 N) compared to commercial and non-germinated samples ($p < 0.05$). Solely germinated rice stored at room temperature (25 °C) showed *B. cereus* growing. It is concluded that germination has the potential to become an innovative process to improve gamma-aminobutyric acid (GABA), changing the carbohydrate chemistry and texture, adding value to brown rice. In contrast, germination can favor the *B. cereus* growing under specific conditions and refrigeration or freezing immediately cooking consist in safe procedures to prevent outbreaks related to germinated rice-based product consumption.

1. Introduction

Rice (*Oryza sativa* L.) is a staple food mostly produced and consumed in Asian countries (FAO, 2023). These grains are commonly consumed after polishing, as this process reduces the cooking time and improves sensory characteristics such as taste, appearance, and texture (Saleh et al., 2019). However, brown rice (BR) has superior nutritional quality to polished rice (PR) and constitutes an improved source of vitamins, minerals, fiber, γ-oryzanol, and phytochemical components that can benefit human health (Mir et al., 2020).

Germination is a process used to improve the bioactive compounds in BR such as phenolic acids, flavonoids, γ-oryzanol, γ-tocotrienol and γ-aminobutyric acid (GABA), with different physiological benefits reported in the literature, e.g., anti-obesity, hypotensive, anti-depression-like, hypocholesterolemic, learning and memory deficits, antidiabetic, and anticancer activity (Cho & Lim, 2016). In addition to all the

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nutritional benefits, germination also improves sensory characteristics of brown rice especially reducing the hardness and the cooking time (Chao et al., 2021). These modifications in sensory characteristics are due to the increase of α-amylase enzymatic activation during the germination process that degrades the starch chains causing alterations in molecular and crystalline structures (Wang et al., 2020; You et al., 2016).

Rice is traditionally cooked by boiling in conventional pan, but today's fast-paced changed the lifestyle, affected eating habits and including new ways to cook rice such as the use of microwave and the electric rice cookers (Thuengtung et al., 2019; Zhao et al., 2024). In the last decade, consumers are more sensitive to information about the level of food processing from media reports, dietary guidelines and public policies, and voluntary on-pack labels (Mintel, 2023). In addition, consumers are adopting new financial strategies to cutbacks and trade down to cheaper options due to the global rise in food prices. The main strategies adopted by consumers to save money in 2023 were cooking at home more often instead of eating out and also buying products in smaller quantities (EUROMONITOR, 2023). In this scenario, domestic cooking practices include the preparation, portioning by servings and subsequent freezing that consisting of an example of convenience and minimally processed food along with extended shelf life (Mintel, 2024).

However, the technological, microbiological and virulent aspects of frozen domestic preparations are often neglected. *B. cereus* can contaminate a wide food category list including cereals (mainly rice), cereal-derived ingredients, brown and polished rice and ready-to-eat rice dishes as risottos and fried rice (Kramer & Gilbert, 1989). *Bacillus cereus* is a gram-positive aerobic or facultative anaerobic spore-forming bacterium that is an important food safety concern (Drobniewski, 1993). The presence of *B. cereus* is different according to the type of food, cereals, especially rice that consists of the most contaminated group. Additionally, some *B. cereus* strains are heat resistant and can multiply under low temperature conditions (4 °C) (Rahnama et al., 2023; Rodrigo et al., 2021). Because of this change in consumption, it is necessary to investigate the microbiology of this food, which is often stored in the refrigerator or freezer.

According to the International Food Safety Authorities Network (INFOSAN), in 46 evaluated incidents, *B. cereus* was the one microorganism involved as biological hazard (WHO, 2024). In Brazil, during the period from 2014 to 2023, the highest incidence of food outbreaks occurred in homes and *B. cereus* was the fifth etiological agent (Brasil, 2024). Considering this scenario, it is envisaged that there are underreported cases of *B. cereus* on processed cereals particularly in rice.

In the literature, there is a lack of information concerning effects of combining the type of cooking with storage time and temperature, rice varieties, processing conditions, and cooking methods that affect the structure of starch and the microbiological security. From this perspective, this study aimed to investigate the effect of different cooking methods and storage conditions on the physical properties, carbohydrate profile, texture parameters and *Bacillus cereus* contamination of rice cooked in conventional pans, microwave, and electric rice cookers using different rice types (non-germinated brown rice, germinated brown rice, commercial parboiled brown rice, and commercial polished rice).

2. Material and methods

2.1. Material

BRS Catiana (*Oryza sativa* L.), a genetic breeding irrigated cultivar, was donated by Brazeiro Sementes® (Uruguaiana-RS, Brazil) and two commercial rice samples were used: commercial brown rice (CBR) and commercial polished rice (CPR).

2.2. Germination process of brown rice

Germination was performed according to the methodology described by Oliveira et al. (2023), with some modifications (4 h of soaking and 16 h of germination). The BRS Catiana rice were separated into non-germinated brown rice (NGBR) and germinated brown rice (GBR).

2.3. Proximate composition

The proximate composition was determined according to the AOAC standard methods: (i) protein content: method n. 46–13 (AOAC, 2000); (ii) fat content: method n. 945.38 (AOAC, 2000); (iii) total dietary fiber content: method n. 985.29 (AOAC, 2000); (iv) ash content: method n. 923.03 (AOAC, 2000); (v) the moisture content was determined by oven-drying the samples to constant weight at 105 °C; (vi) the energy value was determined according to RDC no. 360 of December 23, 2003 (ANVISA, 2003), and the carbohydrate content was calculated by difference.

2.4. Physical properties

2.4.1. Pasting properties

The pasting properties were determined using a series 4 Rapid Visco Analyzer (RVA) (Newport Scientific Pty Ltd., Warriewood, Australia) according to method 76–21.01 (AACC, 2010).

2.4.2. X-ray diffraction pattern

The crystalline structure was analyzed using a D2 Phaser X-ray diffractometer (Bruker, Rheinfelden, Germany) operating at a Cu-K wavelength of 0.154 nm, 30 kV, and 10 mA. Samples were scanned in duplicate from 2° to 32° (2θ) at a rate of 0.05°/min, with a step size of 0.02° and a divergence slit width of 0.6 mm, equipped with a Lynxeye detector (Bruker, Rheinfelden, Germany). The runs are recorded and diffractograms were generated by Diffrac. Eva v. 3.2 software (Bruker, Rheinfelden, Germany).

2.4.3. Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) analyses were carried out using a Q200 DSC device (TA Instruments, New Castle, USA), according to Bernardo et al. (2018). The dry basis samples (~2 mg) were accurately weighed and transferred to hermetic aluminum pans containing deionized water (2:1). Then, the pans were sealed and conditioned for 18 h at room temperature prior to the experiments. Scanning was performed from 5 to 120 °C at a rate of 10 °C/min. An empty pan was used as reference. The DSC data were analyzed to calculate the onset (T_o), peak (T_p), conclusion (T_c) temperatures as well as enthalpy of gelatinization (ΔH).

2.4.4. Color measurement

CIELab measurements of rice grains were measured (10 replicates) using a Color Quest XE (Hunter Lab, Reston, USA) in reflectance mode. The color parameters were expressed in terms of L^*, a^*, and b^*, with the whiteness index (WI) was calculated according to Equation (1):

$$W.I. = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (1)$$

2.5. Bioactive compounds

The contents of *γ-aminobutyric acid* (GABA), ferulic acid, sinapic acid, and *p*-coumaric acid were determined according to the methodology described by Oliveira et al. (2022).

2.6. Cooking stage

The samples (NGBR, GBR, CBR and CPR) were cooking carried out the Ranghino test (Juliano, B. O., & Bechtel, 1985) by three different cooking procedures: conventional pan (cp), domestic microwave (m), and electric rice cooker (erc). For the conventional pan, 100 mL of deionized water was boiled (99 °C) in a 250 mL glass beaker containing 5 g of rice. For the microwave oven, a proper plastic pan (Plasvale®, 13.5 × 24.0 × 21.0 cm, Gaspar-SC, Brazil) containing 300 mL of pre--heated (99 °C) deionized water setted 15 g of rice and was then placed into the microwave oven at level 1 (620 W) (Consul®, model CMA20BBBNA, Joinville-SC, Brazil). The same water:rice ratio (20:1) was used for preparation using the electric rice cooker (400 W) (Mondial® Bianca Rice, model NPE-05–5X, Sorocaba-SP, Brazil). The cooking time measurement started immediately after the rice was placed in the boiling water. Ten grains of rice were removed and pressed between two clean glass plates to assess their degree of cooking. The final cooking time was established when at least 90 % of the grains no longer had an opaque core or an uncooked center. The optimal cooking time (t_c) was achieved when the sample was maintained for 2 min longer to ensure that the core of all grains was completely gelatinized. The water uptake ratio was determined according to the methodology described by Juliano and Bechtel (1985).

2.7. Carbohydrate characteristics

The total starch (TS), amylose (AM), and resistant starch (RS) contents were determined using K-TSTA-100, K-AMYL, and K-RSTAR standard kits, respectively (Megazyme® International, Bray, Ireland).

2.8. Texture profile

Texture profile analysis (TPA) of the rice samples was performed according to the methodology described by Paiva et al. (2015) using a texture analyzer (TA-XT2, Texture Technologies Corp., UK) with a 5-kg load cell using a two-cycle compression method. The texture analyzer was coupled to a computer that recorded the data via the XT. RA Dimension software (v. 8, Texture Technologies Corp., USA). Each sample was prepared according to the optimal cooking time (t_c). The cooked rice was completely drained using a plastic sieve. Then, a SMS P/25 probe was used to compress 2–3 grains at pre-test and post-test speeds of 1 mm s^{-1} and a test speed of 5.0 mm s^{-1}.

2.9. Microbiological analysis

2.9.1. Isolation of B. cereus

After the determination of optimal cooking time (t_c), the samples (1 kg) were cooked, by three different cooking procedures (item 2.6), using the respective t_c. Twenty-five grams of each sample were stored in sterilized bags at different time (0 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h, 30 days, 60 days and 90 days) and temperature: room temperature (25 °C), cooling temperature (4 °C) and freezing temperature (−20 °C). Cooking was carried out in triplicate. Twenty five (g) of cooked rice were homogenized in 225 mL of peptone water (1 % peptone w/v – Difco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) followed by dilution until 10^{-3}, an aliquot of 0.1 mL of each dilution was spread on to Mannitol-Egg Yolk-Polymyxin Agar (MYP- Becton, Dickinson, Le Pont-de-Claix, France) and the plates were incubated at 30 °C for 24 h (Bennett et al., 2015; Ryser & Schuman, 2015). After incubation, typical colonies were counted and up to 5 colonies were transferred for Tryptic Soy Agar (TSA- Difco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 24 h at 30 °C and kept at 4–7 °C for later identification.

2.9.2. Identification of B.cereus

All *B. cereus* typical colonies were subsequently submitted to biochemical tests for *B. cereus* presumptive identification (Bennett et al., 2015; Fayad et al., 2019). All colonies that presented Gram-positive bacilli morphology, glucose fermentation (+) (HiMedia, Mumbai, Maharashtra, India), nitrate reduction to nitrite (±) (Sigma Aldrich, Darmstadt, Germany), Voges-Proskauer (+) (HiMedia, Mumbai, Maharashtra, India), decomposition of tyrosine (+) (Merck Darmstadt, Germany), crystals production (−) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), motility (±) (Thermo Fisher Scientific, Waltham, Massachusetts, USA), rhizoid growth (−) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and sheep's blood hemolysis (+) (Laborclin, Vargem Grande, Pinhais, PR, Brazil) were considered *B. cereus*.

2.9.3. Molecular identification of enterotoxin genes by PCR

For the extraction of *B. cereus* DNA, the cultures were activated in Brain Heart Infusion broth (BHI) Difco, Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 30 °C for 24 h. After this step the DNA extraction were performed using enzymes, lysozyme, and proteinase K enzymes (Furrer et al., 1991) and the quality was measured used a BioDrop (Biochrom, Cambridge, UK).

The genes encoding the complex of enterotoxin NHE (*nheA*, *nheB*, *nheC*) (Hansen & Hendriksen, 2001) and emetic toxin Ces (*ces*) (Ehling-Schulz et al., 2005) were detected in *B.* cereus using primer sequences (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA) described in Table S1. The PCR were performed according previously described (Ehling-Schulz et al., 2005; Hansen & Hendriksen, 2001). Only for *nheC* detection was the annealing time modified to 50 °C for 45 s. The PCR products were analyzed by electrophoresis in a 1.5% (w/v) agarose gel (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA), with 1.0 × TBE buffer (Sinapse Inc., Hollywood, Los Angeles, USA), stained with Sybr™ Safe Green (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA), and visualized in a UV transilluminator and photo-documented (Gel Logic 200 Imaging System, Kodak, Rochester, New York, USA). The genes were confirmed using positive control of *B. cereus* NCTC 11145 for enterotoxins and *B. cereus* NCTC 11143 for emetic toxins.

2.10. Statistical analysis

The statistical analysis was performed with Tukey's test ($p < 0.05$). One-way ANOVA, Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Radar Chart were employed using the software XLSTAT version 2023.2 (Lumivero, Denver, CO, USA).

3. Results and discussion

3.1. Proximate composition

The CPR sample showed the highest moisture (10.88%), whereas the GBR sample had the lowest value (6.88%), which may be due to difference of the drying conditions. A slight reduction in the contents of ash (11.87%), protein (0.91%), and lipids (27.72%) was observed between the NGBR and GBR samples (Table 1). In fact, germination can decrease the protein content due to protein hydrolysis by the enzyme protease (Cornejo et al., 2015), as well as the lipid content by forming the amylose-lipid complex (Xu et al., 2012). The carbohydrate content (3.97%) and energy value (0.43%) had a slight increase after germination. During germination, the enzymes α - and β-amylases are involved in the transformation of starch into malts and simple sugars (Pino et al., 2022). Regarding the dietary fiber content, the CPR sample showed the lowest value (3.98%), which was expected since fibers are concentrated in the outer part of grains, which is removed during polishing operation. Germination did not increase the content of dietary fibers. However, the GBR sample showed 14.4 % more dietary fibers than the CBR sample.

Table 1
Proximate composition and physical characteristics of rice.

	NGBR	GBR	CBR	CPR
Moisture	8.25 ± 0.67^{bc}	6.88 ± 0.22^{c}	9.10 ± 0.65^{ab}	10.88 ± 0.35^{a}
Ash	1.60 ± 0.09^{a}	1.41 ± 0.07^{ab}	1.16 ± 0.03^{b}	0.24 ± 0.00^{c}
Protein	6.61 ± 0.02^{b}	6.55 ± 0.01^{b}	7.13 ± 0.01^{a}	6.55 ± 0.02^{b}
Lipid	3.39 ± 0.23^{a}	2.45 ± 0.21^{b}	2.14 ± 0.03^{b}	0.31 ± 0.02^{c}
Carbohydrate	73.37 ± 0.16^{c}	76.39 ± 0.85^{b}	74.86 ± 0.52^{c}	78.04 ± 0.26^{a}
Dietary Fiber	6.79 ± 0.02^{a}	6.52 ± 0.01^{a}	5.58 ± 0.02^{b}	3.98 ± 0.01^{c}
Energy value	350.43 ± 0.01^{a}	351.92 ± 0.01^{a}	347.31 ± 0.02^{b}	341.15 ± 0.02^{b}
T_0	58.63 ± 0.31^{a}	59.88 ± 0.83^{a}	43.55 ± 2.38^{b}	61.42 ± 1.07^{a}
T_p	65.66 ± 0.39^{a}	65.68 ± 0.26^{a}	67.32 ± 0.03^{a}	56.75 ± 0.80^{b}
T_c	78.45 ± 1.06^{a}	79.00 ± 0.12^{a}	45.24 ± 0.90^{b}	79.83 ± 0.00^{a}
ΔH (J/g)	8.66 ± 0.71^{a}	8.13 ± 0.34^{a}	0.78 ± 0.35^{b}	9.42 ± 0.64^{a}
***L**	66.13 ± 0.05^{d}	68.10 ± 0.11^{b}	60.97 ± 0.26^{c}	76.20 ± 0.18^{a}
***a**	3.77 ± 0.00^{b}	3.52 ± 0.06^{b}	5.65 ± 0.07^{a}	−0.35 ± 0.50^{c}
***b**	18.35 ± 0.00^{b}	18.40 ± 0.18^{b}	21.11 ± 0.10^{a}	15.30 ± 0.21^{c}
W.I.	62.29 ± 0.00^{b}	63.00 ± 0.19^{b}	55.26 ± 0.14^{c}	71.69 ± 0.11^{a}

Note: Values are mean (n = 3) ± standard deviation. Data with same letters in the same lines did not differ significantly by Tukey test ($p < 0.05$). Where: NGBR= Non-germinated Brown Rice, GBR= Germinated Brown Rice, CBR= Comercial Brown Rice, CPR= Commercial Polished Rice, T_0 = Temperature onset, T_p = Peak temperature, T_c= Conclusion temperature, ΔH = Enthalpy, W.I. = Whiteness index.

3.2. Physical properties

3.2.1. Pasting and gelatinization properties

The pasting curves (RVA) are shown in Fig. 1A. The pasting curves of NGBR and GBR had a similar profile. However, GBR showed a peak viscosity (PV) 41.0 % higher than NGBR and a final viscosity (FV) 6.6 % higher than NGBR. In previous study, Oliveira et al. (2023) reported that under short germination conditions (16 h), GBR sample did not show a reduction in paste viscosity if compared to non-germinated rice (control). It was noteworthy that the impact of germination on rice pasting viscosity depends on (i) time, (ii) cultivar, (iii) temperature, (iv) amylose/amylopectin ratio, and (v) the content of lipids and protein (Li et al., 2020; Oliveira et al., 2022). The initial pasting temperature (PT) ranged from 68.5 (NGBR) to 79.0 °C (CPR). Regarding the commercial samples, CPR showed the highest values of peak viscosity (PV) (2592 cP), minimum viscosity (MV) (960 cP), breakdown (BDV) (1632 cP), final viscosity (FV) (5254 cP), and setback (SBV) (4294 cP). However, the differences found in our study are due to the polishing that removed the pericarp, thus increasing the starch content. Also due to heat treatment (parboiling) that partially gelatinized the starch granules causing changes in the crystallinity profile (Fig. 1B) and PV (Fig. 1A).

There was no statistical difference ($p < 0.05$) between NGBR and GBR for all DSC parameters (T_o, T_p, T_c, and enthalpy) (Table 1), demonstrating that the short processing time was not sufficient to change the thermal properties of the material. The CPR sample showed the highest value of T_o (61.42 °C), T_c (79.83 °C), and enthalpy (9.42 J/g), which was expected since this sample did not have the pericarp, previously removed by polishing, thus increasing the starch content. Our results referring to gelatinization properties corroborate those found for the RVA pasting properties and agree with our previously study (Oliveira et al., 2022). However, these results are contrary to those reported by Pal et al. (2023), that found lower gelatinization temperatures and gelatinization enthalpy in GBR samples after 24 h soaking at 28 ± 1 °C and 48 h germination at 28 ± 1 °C.

3.2.2. Crystalline structures

As expected, CBR showed a reduction in peak intensity since this commercial sample is parboiled rice (Fig. 1B). The peaks at 15°, 17°, and around 23° demonstrate a typical A-type polymorphic form, and the peak around 23° represents the amylopectin molecules, the complex formed between starch and the endosperm cell wall (Pal et al., 2023). Germination did not reduce crystallinity in the samples (Fig. 1B), which was attributed to the non-activation of amylolytic enzymes. He et al. (2022) observed a reduction in relative crystallinity (RC) (32.18–28.59%) after 12 h of germination and greater reduction after 48 h of the process (32.18–12.08 %). The reduction in relative crystallinity during germination may be due to the increase in α-amylase activity, resulting in the disruption of double-helical structures.

3.2.3. Color measurement

The lightness (*L) ranged from 60.79 (CBR) to 76.20 (CPR), and the W.I. ranged from 55.26 (CBR) to 71.69 (CPR), as expected since the components of pericarp tends to attribute browning aspect. In addition, no statistical difference ($p < 0.05$) was observed between the NGBR and GBR samples in all parameters (Table 1). The short soaking and short germination time (<24 h) were not sufficient to cause significant changes in grain color, except *L that showed a high value (68.1). The changes in the color parameters could be attributed to the enzymatic hydrolysis that took place during germination (Chinma et al., 2015), e.g., the Maillard reaction between reducing sugars and proteins (Chung et al., 2012) and the diffusion of pigmented compounds present in the bran layer into the grain core during the aqueous phase (soaking) (Xia & Li, 2018).

3.3. Bioactive compounds

The results of ferulic, sinapic, *p*-coumaric, and gamma-aminobutyric acid (GABA) are presented in Fig. 2. CBR showed the highest value of ferulic, sinapic, and *p*-coumaric acid, whereas CPR showed the lowest values. High levels of phenolic acids are present in rice bran and husk (90%) compared to other grain parts (Ding et al., 2018). This layer is removed during polishing, reducing the content of phenolic acids in the free and bound fractions (Liu et al., 2015).

Results showed that there was no statistical difference ($p < 0.05$) between NGBR and GBR regarding ferulic acid content. However, germination increased (54%) sinapic acid and significantly reduced (87%) the content of *p*-coumaric acid. Furthermore, although soluble phenolic acids can be leached out during soaking, these compounds could increase in the final stages of germination in longer process (96 h). Germination induces the activity of two enzymes associated with the synthesis of phenolic compounds in the GBR: (i) phenylalanine ammonia-lyase (PAL), which reaches its peak 48 h after germination, and (ii) cell wall peroxidase (CW-PRX), activated in the early stage (Cho & Lim, 2018).

Regarding the GABA contents, the CPR sample showed the lowest value (4.28 mg GABA/100g), as expected, with the short germination time increasing this parameter by 91% (12.85–160 mg GABA/100g). In the case of CBR, parboiling is an important strategy to favor the diffusion of ferulic, sinapic, and *p*-coumaric acid (14.5 μg/g, 1.8 μg/g, and 23.5 μg/g) from the pericarp to the endosperm. Parboiling increases the phenolic acid content due to the instability of the cell-wall structure caused by heat treatment, consequently releasing these acids and its

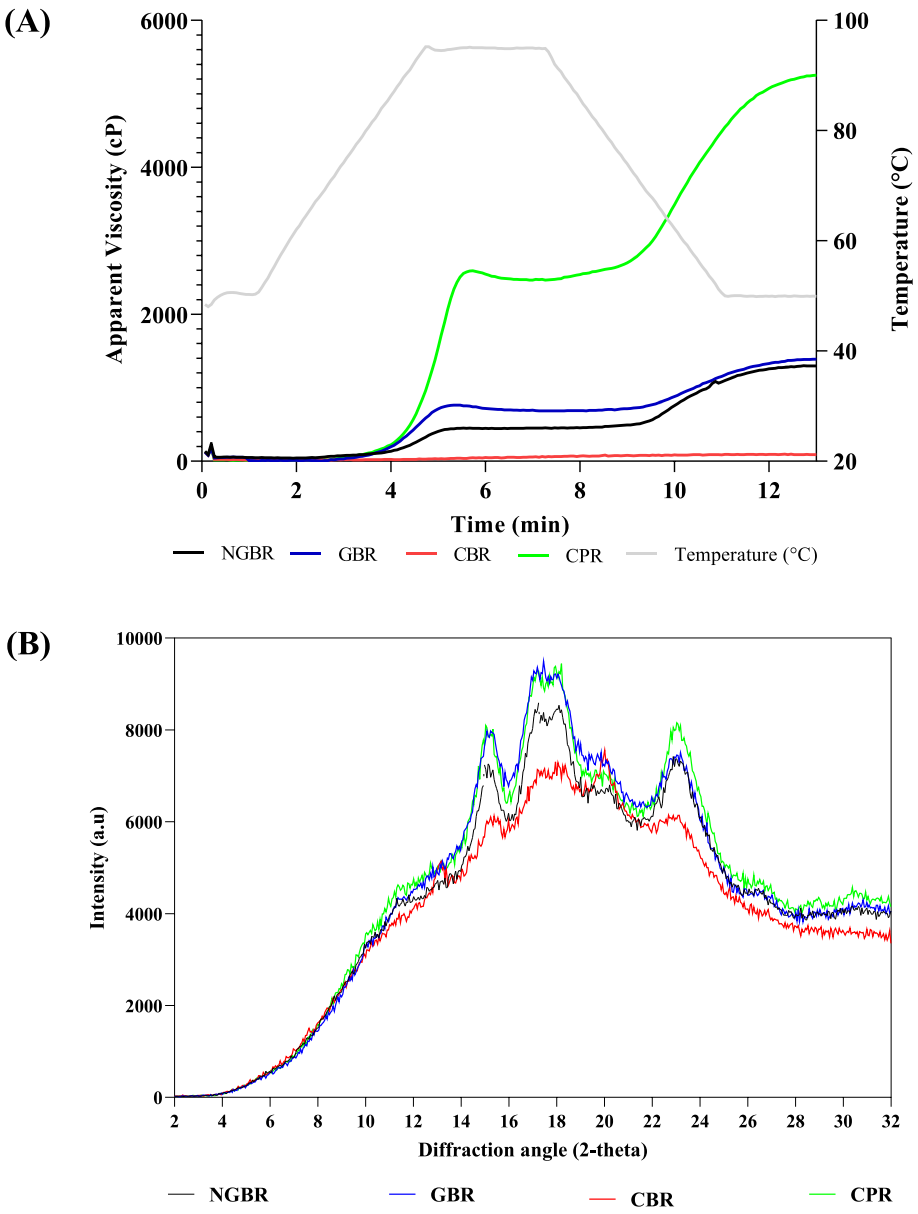

Fig. 1. (A) Rapid visco-analyzer (RVA) curves and (B) X-ray diffraction spectra. Where: NGBR= Non-germinated Brown Rice; GBR= Germinated Brown Rice; CBR= Commercial Brown Rice; CPR= Commercial Polished Rice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

derivatives from the wall cells into the starchy endosperm (Thammapat et al., 2016).

3.4. Cooking stage and carbohydrate evaluation

In general, the cooking method determines the cooking time necessary to reach a complete cooking point of rice and results showed statistical difference between them ($p < 0.05$). Regarding the commercial samples, CBR showed the highest value for conventional pan cooking (37 min) as expected, in contrast to electric rice cookers (20 min). CPR showed the lowest cooking time and the highest water uptake ratio regardless of the method used. Regarding GBR, short germination was effective in reducing the cooking time (30 %) using conventional pan (Table 2).

In relation to the water uptake ratio, CPR (168.42–206.85%) showed the highest value, probably due to the high starch contents (79.27–83.79 %) (Table S2), contrary to GBR cooked by the microwave method (101.85 %). Short germination induced an increase of 40 % in the water uptake ratio using the electric rice cooker, which can be related to an increment of amylose content (14.74–15.70%) (Table S2). Changes observed in the cooking quality of GBR are probably due to the hydrolysis of high molecular weight polymers by hydrolytic enzymes activated during germination (Sirisoontaralak et al., 2015). Du et al. (2019) observed a reduction (26–21 min) in cooking time and water absorption in germinated-parboiled rice compared to polished rice (330–197 %), attributing this phenomenon to partial starch gelatinization.

The literature has not reported changes in TS, AM, and RS under different storage conditions. In this study, the TS ranged from 57.72% (GBR microwave) to 85.22 % (NGBR microwave) immediately after cooking (t = 0). At cooling temperature (4 °C, t = 24 h), the TS ranged from 71.86% (NGBR) to 91.38 % (CPR) for both using electric rice cooker. Under freezing temperature, (−20 °C, t = 30 days), the TS ranged from 63.45% (GBR) to 84.95 % (CPR) by conventional pan. Regarding AM, germination decreases its contents regardless of the cooking method used. Respecting the commercial samples, a distinct reduction was observed in microwave-cooked rice after t = 24 h,

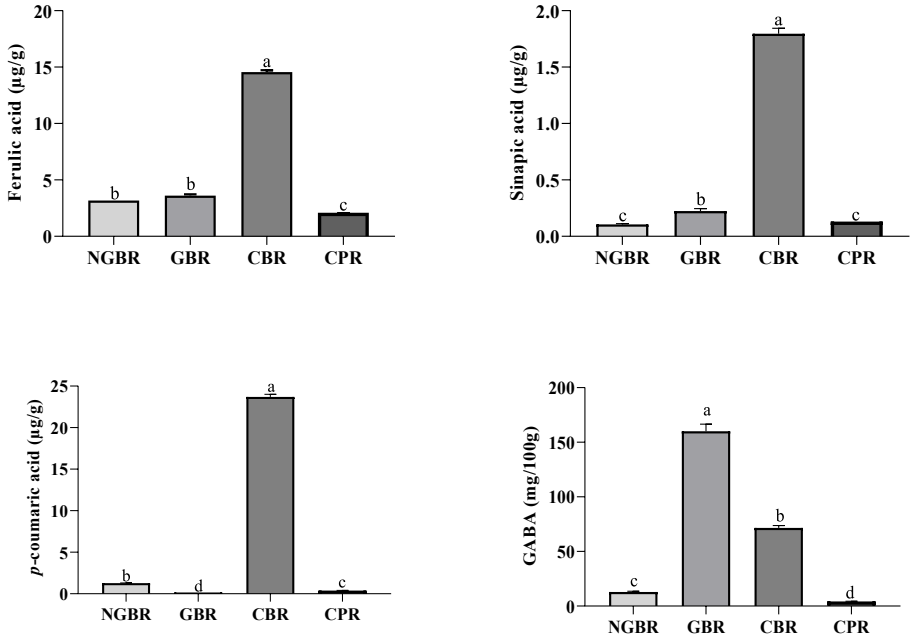

Fig. 2. Bioactive compounds in different types of rice. The values are the mean (n = 3 repetitions) and error bars represent standard deviation. Different letters represented a significant difference ($p < 0.05$). Where: NGBR= Non-germinated Brown Rice; GBR= Germinated Brown Rice; CBR= Commercial Brown Rice; CPR= Commercial Polished Rice.

Table 2
Cooking time and water uptake ratio of rice.

Sample	Cooking time (min)			Water uptake ratio (%)		
	Conventional pan	Microwave	Electric rice cookers	Conventional Pan	Microwave	Electric rice cookers
NGBR	31 ± 0.15^{bB}	23 ± 2.15^{bD}	23 ± 0.12^{aD}	153.55 ± 1.39^{cD}	109.29 ± 6.73^{bcG}	76.82 ± 9.32^{cH}
GBR	22 ± 1.14^{cD}	23 ± 0.01^{bD}	24 ± 0.00^{aC}	143.12 ± 3.81^{dDE}	101.85 ± 10.06^{cG}	129.80 ± 0.35^{bF}
CBR	37 ± 0.00^{aA}	25 ± 0.32^{aC}	20 ± 0.04^{bE}	186.13 ± 1.42^{bB}	137.19 ± 2.86^{bE}	114.07 ± 0.33^{bFG}
CPR	18 ± 0.00^{cdE}	12 ± 0.00^{cG}	16 ± 0.01^{cF}	206.85 ± 0.93^{aA}	168.42 ± 8.62^{aC}	202.05 ± 7.55^{aA}

Note: Values are mean (n = 3) ± standard deviation. Different lowercase and uppercase letters mean a significant difference ($p < 0.05$) between the samples and the cooking methods, respectively. Where: NGBR= Non-germinated Brown Rice, GBR= Germinated Brown Rice, CBR= Commercial Brown Rice, CPR= Commercial Polished Rice.

especially in the case of CBR (22.60–12.22 %). However, CBR stored under freezing increased the AM value (48%).

CPR showed AM reductions regardless of the cooking method and storage condition. In this study, the RS ranged from 1.7 to 2.9 % (*BRS Catiana*) and from 0.4 to 2.5 % (commercial samples), with these values agree with Kim et al. (2019) and Reed et al. (2013), that reported values ranging from 0.7 to 2.7 %. Interestingly, the RS content was only detected in rice samples cooked by conventional pan and microwave (t = 0 and t = 24 h). In the case of electric rice cooker, the most common preparation method in Asia, the RS content was only observed after 30 days under freezing, in which a significant increase (100 %) was observed, characterizing a time-dependent phenomenon. Samples cooked by electric rice cookers obtained similar results in the contents of total starch, amylose and resistant starch but the same behavior did not occur on the other methods (Fig. 3A and B).

When starch is gelatinized during cooking, its original crystalline structure is converted into an amorphous structure and subsequently retrograded. Refrigeration can facilitate starch retrogradation, resulting in low digestibility of cooked and processed starchy foods. The amorphous structure becomes rearranged and ordered during a prolonged storage period (Singh et al., 2010). Many factors affect the RS content, e.g., degree of polishing and cooking method, with the *Indica* genotype showing the greatest value due to its largest amylose content (Reed et al., 2013). Germination and parboiling increased the RS content due to starch retrogradation and cooling during storage (Du et al., 2019).

3.5. Texture profile

At t = 0 h, the combination of germination and cooking by electric rice cooker led to the highest values of hardness (19.7 N) and chewiness (24.6 N) ($p < 0.05$). The same behavior was also observed in CBR (commercial sample) (Fig. 4A and Table S3). In addition, cooling (t = 24 h) also induced an increase in hardness (17.5–32.3 N) and chewiness (7.4–17.4 N) in the CBR sample regardless off the culinary method (Fig. 4B and Table S3), which was expected due to starch retrogradation.

However, germination led to a reduction of 10 % in hardness of GBR prepared by electric rice cooker, which is desirable in terms of sensory quality. Likewise, germination also reduced chewiness by 14 % compared to the CBR sample cooked by electric rice cooker. On the other hand, it was not observed statistical differences ($p < 0.05$) neither in refrigerated during 24 h nor in frozen samples for 30 days (Fig. 4B and C and Table S3). These findings did not corroborate with the highest AM content (24.2 %) observed in the samples prepared in electric rice cookers after cooling (Table S3). Germination can change the texture parameters of rice, but this effect depends mainly on the type of cultivar/genotype. In fact, non-glutinous rice (13.7–22.7 % of amylose content) has a greater hardness reduction compared to glutinous rice (2.7–4.1 % of amylose content) (Chao et al., 2022).

Unexpectedly, freezing (t = 30 days) decreased hardness (12.7–19.2 N) and chewiness (5.3–17.1 N) compared to commercial and non-germinated materials (Fig. 4 C) regardless of the preparation method.

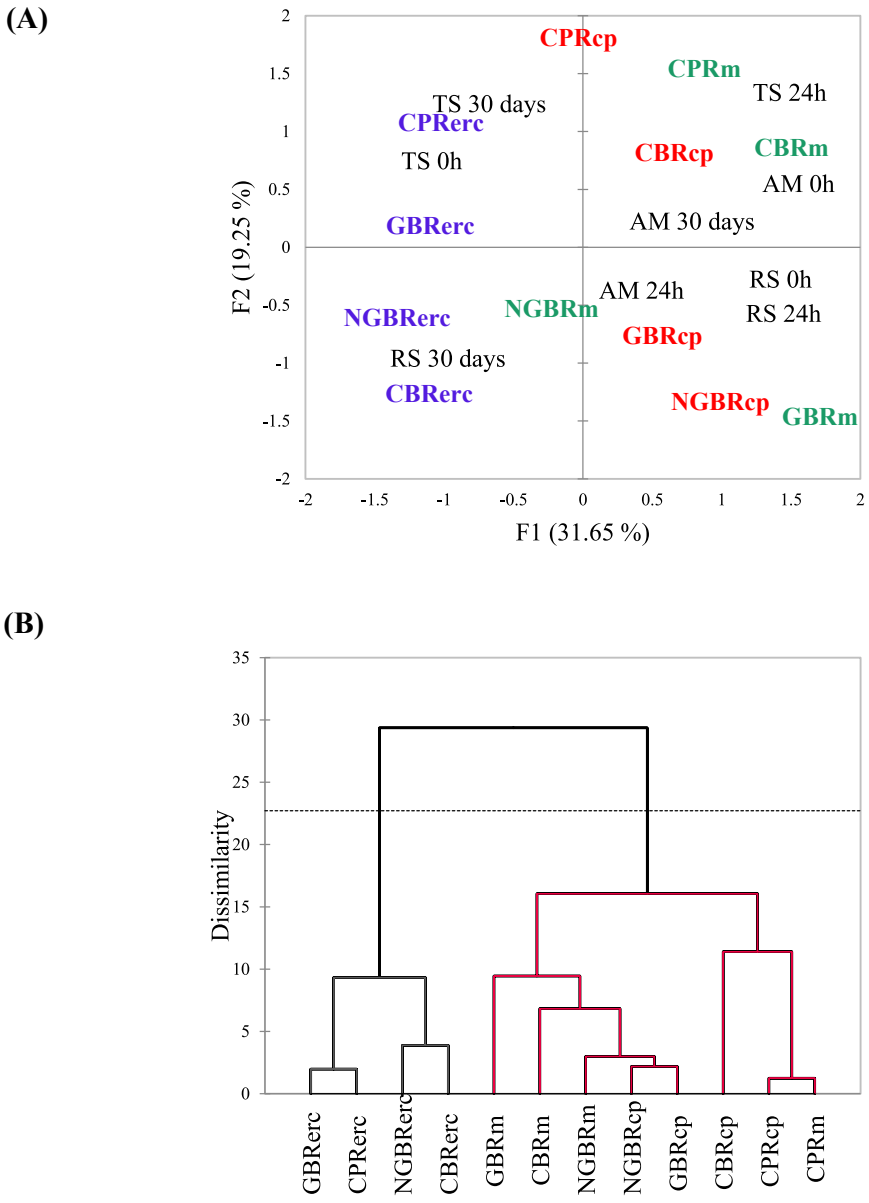

Fig. 3. (A) Principal Componet Analysis (PCA) and (B) Hierarchical Cluster Analysis (HCA) of carbohydrate characteristics. Where: cp = conventional pan; m = microwave, erc = electric rice cookers; TS = Total Starch; AM = Amylose, RS= Resistant Starch; NGBR= Non-germinated brown rice; GBR= Germinated brown rice; CBR= Commercial brown rice and CPR= Commercial polished rice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

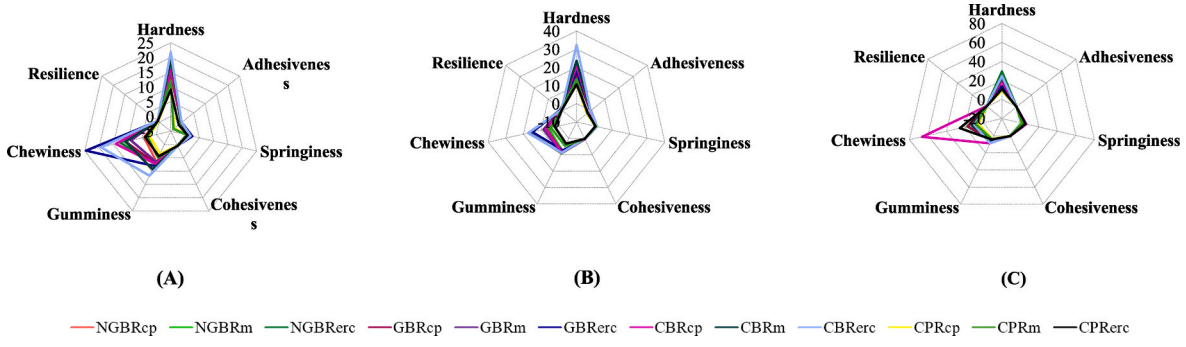

Fig. 4. TPA profile of rice at t = 0 h (A); t = 24 h (B) and t = 30 days (C). Where: cp = conventional pan; erc = electric rice cookers; m = microwave; NGBR= Non-germinated Brown Rice; GBR= Germinated Brown Rice; CBR= Commercial Brown Rice; CPR= Commercial Polished Rice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

According to GBR, freezing was more effective in reducing hardness (64 %) than cooling (t = 24 h), with the electric rice cooker resulting in the highest values for commercial samples, i.e., CBR (66.4 N) and CPR (25.5 N) ($p < 0.05$). Our results are in agreement with Yu et al., 2010 that reported a decrease of hardness and an increase of adhesiveness in rice processed with higher freezing rates (1.45 °C/min), varied temperatures and times (4 °C for 14 days and 18 °C for up to 7 months).

3.6. Microbiological analysis

3.6.1. Growth and characterization of B. cereus in cooked rice

The growth of *B. cereus* was detected solely in GBR samples stored at room temperature (25 °C). During the storage time (48 h) at 25 °C, the number of *B. cereus* increased significantly ranging between 1.30 and 5.38 CFU/g (Fig. 5A; Table S4). There wasstatistical difference ($p < 0.05$) among the cooking methods. The GBRcp sample had higher growth of *B. cereus* (5.38 CFU/g) especially after 12 h of storage ($p < 0.05$), which is the most used culinary method at home. Although, there was no difference between the GBRerc and GBRm samples ($p < 0.05$). *B. cereus* was not detected (<1 log CFU/g) in non-germinated rice and commercial samples (Table S4).

Concerning microwave technique, this method could inactivate *B. cereus* by (i) cleaning of bacterial nuclear chromatin; (ii) increasing the permeability and disrupting the integrity of membrane and (iii) disordering the expression of proteins (Cao et al., 2018). In addition to these factors, the binomial time x temperature was decisive on the *B. cereus* growing. Indeed, the absence of *B. cereus* viable cells in cooked and fried rice were observed at 4 °C and 10 °C of storage temperature (Cronin & Wilkinson, 2009; Huang & Hwang, 2022).

3.6.2. Virulence genes in B. cereus strains

The 45 confirmed isolates as *B. cereus* by the biochemical tests were submitted to the PCR technique for the detection of virulence of *nheA*, *nheB*, *nheC*, encoders of NHE, responsible for diarrheal syndrome and *ces* responsible for emetic disease. The *ces* gene was not found in any strain. Among these isolates, 80% exhibited the presence of *nheB* (34/45), 75% showed the presence of *nheA* (34/45) and 24% had the presence of the gene *nheC* (11/45) (Table S5). 6 strains showed the presence of all genes, 20 strains showed the presence of *nheA* and *nheB* and 5 strains showed the presence of *nheB* and *nheC* genes (Fig. 5 B).

Navaneethan and Effarizah (2021) evaluated the prevalence of toxigenic profiles of *B. cereus* isolated from 100 ready-to eat cooked rice samples purchased from food outlets comprising of cafeterias, family or casual dining restaurants, and food courts located in 5 different regions of Penang Island (Malaysia). They observed a high percentage (82.4 %) of the isolates that demonstrated at least one toxin gene with high detection rates of most diarrheal genes (58.8–76.5 %) than emetic toxin gene (14.7 %). In this study, the emetic toxin gene (*ces*) was detected in a total of only five (14.7 %) and the frequency of *nheA*, *nheB* and *nheC* were 38.24 %, 67.65 % and 64.71 % respectively. The detection rates of

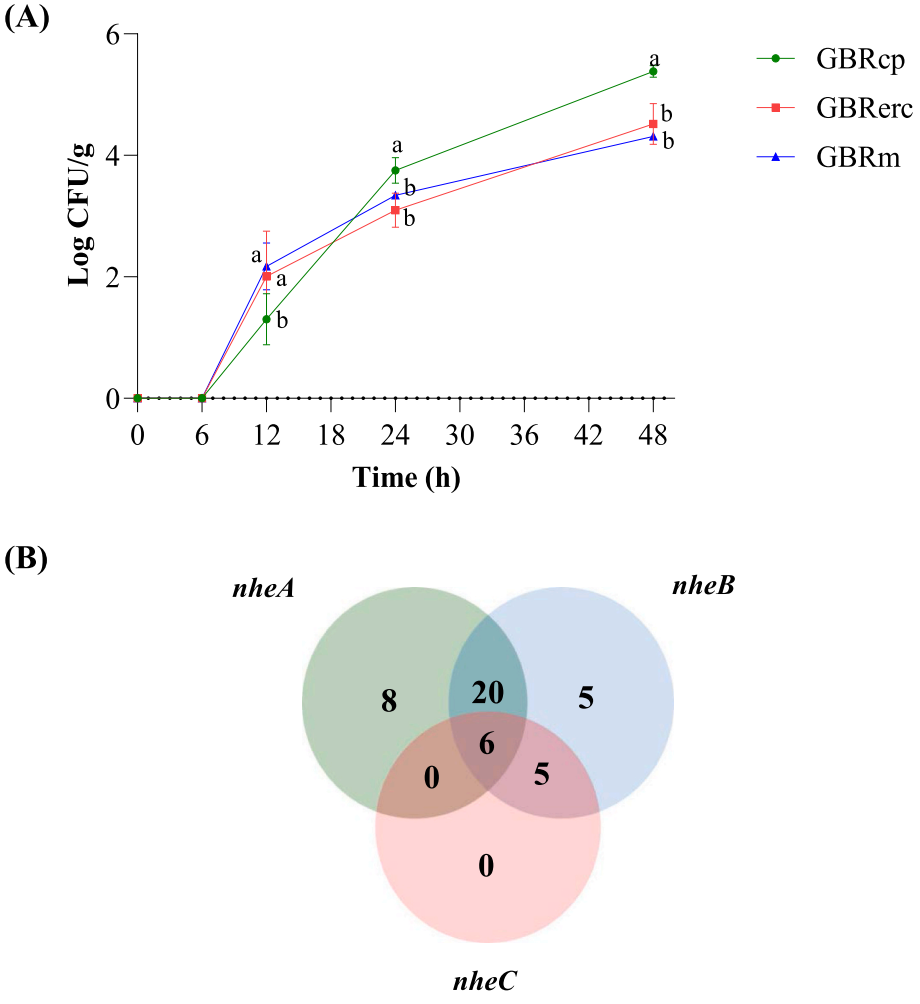

Fig. 5. (A) Growth of *B. cereus* after different methods of cooking and storage at 25 °C. The values are the mean (n = 3 repetitions) and error bars represent standard deviation. Different letters represented a significant difference ($p < 0.05$); (B) Veen diagram of the distribution of NHE complex in 45 sequenced *B. cereus* group strains. Where: cp = conventional pan; erc = electric rice cookers; m = microwave; GBR= Germinated Brown Rice. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

nheBC (29.4 %) were much higher than that of *nheAB* (2.9 %), *nheAC* (5.9 %) and any isolate containing only a single *Nhe*-associated virulence gene (5.9 %). Further, Chen et al. (2022) studied the prevalence and antimicrobial-resistant characterization of *B. cereus* isolated from 1071 ready-to-eat rice products (756 boiled rice dishes, 91 boiled rice noodles, 105 fried rice noodles, 33 sticky rice rolls, 64 boiled sticky rice dishes, and 22 fried rice cakes) in Eastern China and revealed the *hblACD* and *nheABC* occurrence rate of 36.3 % and 47.3 % and 84.6 %, respectively, that showed at least one enterotoxin or emetic toxin gene.

4. Conclusion

Short germination increased sinapic acid and GABA whereas reduced the cooking time in the case of the use of conventional pan, which is the most traditional cooking method used. Resistant starch increased after 30 days freezing only when cooked in electric rice cooker, characterizing a time-dependent phenomenon. Germinated rice prepared by electric rice cooker presented the highest values of hardness and chewiness at time 0 h, although freezing followed by cooling temperature reduced hardness and chewiness compared to non-germinated material, which is an appealing rice consume feature. By keeping the material under refrigeration and freezing temperatures after all cooking methods did not increase the growth of *B. cereus* and may be a way to prevent it in germinated brown rice. Considering the predominant market of polished rice, freezing was effective to improve convenience, increase of resistant starch and risk reduction of *B. cereus* incidence in germinated rice. These results shed light on understanding the effects of germination and storage conditions on different types of rice.

CRediT authorship contribution statement

Maria Eugenia Araujo Silva Oliveira: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Monique Barreto Santos:** Writing – review & editing, Formal analysis, Data curation. **Gabriel Gozzi:** Formal analysis. **Carine da Fonseca Cechin:** Formal analysis. **Leda Maria Fortes Gottschalk:** Writing – review & editing, Methodology, Formal analysis. **Priscila Zaczuk Bassinello:** Writing – review & editing, Project administration. **José Manoel Colombari Filho:** Project administration. **Dirce Yorika Kabuki:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Carlos Wanderlei Piler de Carvalho:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Cristina Yoshie Takeiti:** Writing – review & editing, Supervision, Project administration.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2025.111164.

Data availability

Data will be made available on request.

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