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Abstract: The suitability of germinated brown rice (GBR) for developing novel multifunctional yogurt-like products was evaluated. Crude brown rice, soaked brown rice and GBR for 48 h and 96 h were fermented (F-CBR, F-SBR, F-GBR48 and F-GBR96, respectively). The viability of the starter culture, acidification pattern, techno-functional properties, content of bioactive compounds [phenolic compounds, gamma-aminobutyric acid (GABA) and gamma-oryzanol], biological activity [antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities] and sensory attributes were evaluated. Fermentation did not modify proximate composition but improved phenolic and GABA contents as well as ACE-inhibitory activity and consistency index of yogurt-like products. Among them, F-GBR96 exhibited the highest phenolic (15.2 mg GAE/100g) and GABA (1.9 mg/100g) concentrations, antioxidant activity (46.9 µg TE/100g) and ACE-inhibition (61.5%) and was well accepted by panellists.

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HIGHLIGHTS

- Yogurt-like products were produced from crude, soaked and germinated brown rice
- Brown rice germination improved content of GABA and phenolics in fermented products
- Germinated brown rice products (F-GBR) exhibited higher ACE-inhibitory activity
- F-GBR showed improved consistency index, density and the best overall acceptability
- F-GBR contained high γ-oryzanol and nutrients levels and large antioxidant activity

Development of a multifunctional yogurt-like product from germinated brown rice.

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ABSTRACT

The suitability of germinated brown rice (GBR) for developing novel multifunctional yogurt-like products was evaluated. Crude brown rice, soaked brown rice and GBR for 48 h and 96 h were fermented (F-CBR, F-SBR, F-GBR48 and F-GBR96, respectively). The viability of the starter culture, acidification pattern, techno-functional properties, content of bioactive compounds [phenolic compounds, γ-aminobutyric acid (GABA) and γ-oryzanol], biological activity [antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities] and sensory attributes were evaluated. Fermentation did not modify proximate composition but improved phenolic and GABA contents as well as ACE-inhibitory activity and consistency index of yogurt-like products. Among them, F-GBR96 exhibited the highest phenolic (15.2 mg GAE/100g) and GABA (1.9 mg/100g) concentrations, antioxidant activity (46.9 µg TE/100g) and ACE-inhibition (61.5%) and was well accepted by panellists.

***Keywords*:** Brown rice, *Oryza sativa*, germination, fermentation, functional foods.

1. Introduction

The increasing consumer demand for healthy and high-quality foods has led both industry and scientific community to develop new functional foods. Whole grains are gaining popularity due to their high nutritional value and bioactive compounds involved in protective effects against chronic diseases (McRae, 2017), making them valuable ingredients for the development of functional foods.

Rice (*Oryza sativa* L.) is one of the most consumed cereals worldwide, but it is mostly consumed as white rice. Brown rice (BR), containing endosperm, embryo and bran, is nutritionally more complete and provides phytochemicals with health-promoting relevance as γ-oryzanol, γ-aminoburyric acid (GABA) and ferulic acid located mainly in the germ and bran layers (Gong et al., 2017). However, BR consumption is limited due to its poor textural and sensory properties. BR germination has been demonstrated to be a cost-effective strategy to improve textural and organoleptic quality, nutrient and phytochemical bioavailability and biological activity of this cereal (Cáceres, Martínez-Villaluenga, Amigo, & Frias, 2014). Germinated BR (GBR) is consumed in salads, boiled, or even it is incorporated as an ingredient in bakery products (Cornejo et al., 2015; Chung, Cho, & Lim, 2014). The development of innovative products is very challenging for food industry to fulfil social demands for natural, gluten-free and vegan products. In this context, ready-to-eat yogurt-like formulations including GBR may expand the range of non-dairy fermented products in the market, which is quite limited in western countries so far. Fermentation with lactic acid bacteria (LAB) may enhance GBR health-promoting properties since LAB are cell factories producing nutrients and bioactive compounds that improve functional features of cereals (Waters et al., 2015). Diverse types of cereals are substrates of lactic-acid fermented beverages, such as oat, maize, rice, barley and sorghum, or a mixture of them (Freire, Ramos, & Schwan, 2017; Salmerón, Thomas, & Pandiella, 2015). Germinated whole grains have been demonstrated to be better substrates for LAB growth compared with non-malted cereals (Nsogning Dongmo et al., 2017). Therefore, the aim of the present study was to explore the suitability of GBR for the production of a healthy and nutritious fermented yogurt-like product, topic that has not been explored so far. Proximate composition, content of bioactive compounds (phenolic compounds, GABA, and γ-oryzanol), biological activities (antioxidant and ACE-inhibitory activities) and sensory quality were evaluated on the GBR products. Non-fermented formulations were also prepared to evaluate the effect of fermentation on nutritional and health-promoting properties of GBR yogurt-like products.

2. Materials and Methods

2.1. Chemicals. Unless otherwise stated, all reagents were obtained from Sigma-Aldrich (Barcelona, Spain). MRS agar was obtained from Pronadisa (Madrid, Spain).

2.2. Rice. Commercial BR (*Oryza sativa* subssp. Indica, var. SLF09) was provided by Productora Nacional de Alimentos C.A., INDIA–PRONACA (Ecuador).

2.3. Starter culture. A commercial thermophilic starter culture FD-DVS YC-180 Yo-Flex® containing diverse LAB strains was purchased from Chr. Hansen (Guayaquil, Ecuador). The starter culture was grown in Man Rogosa Sharpe (MRS) broth at 37 ºC for 18 h, and then, bacterial cells were harvested by centrifugation (8,000 × *g*, 10 min), washed twice, and suspended in sterile water, before inoculation in GBR substrates.

2.4. BR germination and flour preparation. BR was hygienised, soaked (SBR) and germinated for 48 h (GBR48) and 96 h (GBR96) as previously described (Caceres et al., 2014). These samples were dried at 50 ± 1°C for 24 h in a temperature-controlled cabin (Memmert SM200) and milled in a cyclone mill (UDY Corporation, USA). Flours from crude BR (CBR) were also obtained and used as reference. Three germination batches were prepared for each experimental condition.

2.5. *Manufacture of fermented products.* Yogurt-like products were manufactured from CBR, SBR, GBR48 and GBR96. Briefly, rice flour samples were supplemented with 7% sucrose (w/v), 5% glucose (w/v) and 0.5% stabilizer gelatin (w/v) and mixed in distilled water at 1:4 ratio (w:v) for 1 h at 20ºC, cooked at 95°C for 30 min, and vacuum-filtered. The resultant slurry fractions were cooled to 42 ºC, placed in sterile Erlenmeyer flasks, and inoculated with the starter culture at 1×10^6 CFU/mL. Lactic-acid fermentations were performed at 42 ºC in agitation until reaching pH 4.4 ± 0.2 and quickly cooled at 4°C. Fermentation experiments were performed in duplicate for each germination condition. Fermented products were coded as follows: F-CBR for that prepared with crude BR, F-SBR for that prepared with soaked BR, and F-GBR48 and F-GBR96 for those obtained from BR germinated for 48 and 96 h, respectively. Non-fermented products were also produced from crude (NF-CBR), soaked (NF-SBR) and germinated BR (NF-GBR48 and NF-GBR96).

2.6. Bacterial growth and pH determination. Bacterial growth was determined in fermented products by plating decimal buffered peptone water dilutions (10^6-10^8) in triplicate onto MRS agar and counting the viable cells after incubation under anaerobic conditions (30 ºC for 48 h). The pH was monitored using a pH-meter Basic 20 (Crison Instruments S. A., Barcelona, Spain).

2.7. Proximate composition. Chemical composition of non-fermented and fermented products was determined following AOAC (2016) methods for moisture, protein, fat and ash. Carbohydrates were calculated by difference. Food energy was calculated using standardized conversion factors (4.0 kcal/g for proteins and carbohydrates, and 9.0 kcal/g for fats) (FAO, 2003).

2.8. Techno functional properties

2.8.1. Consistency and flow behaviour index. The consistency index in Pa.s^n (*K*) and the flow behaviour index (n) were determined using a rotational viscometer Brookfield DV-II+ with spindle for non-Newtonian fluids through the logarithmic linearization of the apparent viscosity (η) curve in Pa.s vs shear rate in s^{-1} (γ) (Steffe,1996).

2.8.2. Total soluble solids and density. Total soluble solids and density were determined by refractometry and gravimetric methods, respectively (AOAC, 2016).

2.9. Determination of total phenolic compounds. Total phenolic content (TPC) was determined using Folin-Ciocalteu's reagent (Cáceres et al., 2014). Results were expressed as mg of gallic acid equivalents (GAE)/100 g product.

2.10. Determination of GABA content. GABA was extracted with methanol and further analysed by RP-HPLC as in Caceres at al. (2014). Results were expressed as mg GABA/100 g product.

2.11. Determination of γ-oryzanol content. The analysis of γ-oryzanol in BR products was performed by RP-HPLC according to Cáceres et al. (2017). Results were expressed in mg γ-oryzanol/100 g product.

2.12. Determination of oxygen radical absorbance capacity (ORAC). Antioxidant activity was determined as ORAC by fluorescence as described recently in Caceres at al., (2014). Results were expressed as mg of Trolox equivalents (TE)/100 g product.

2.13. *Determination of ACE-inhibitory activity.* It was determined in all BR formulations as in Cáceres et al., (2017). Results were presented as % inhibition.

2.14 Sensory evaluation. BR yogurt-like products were submitted to sensory analysis by 8 trained panellists (50% male, 50% female) recruited at Facultad de Ingeniería Mecánica y Ciencias de la Producción (ESPOL, Guayaquil, Ecuador), selected by their regular consumption of yogurt-like products. Panellists evaluated typical attributes for fermented products such as astringency, bitterness, sourness, cereal-type flavour, fermented odour, creaminess, white colour and overall acceptability, using a 9-point hedonic scale, ranging from dislike extremely (1) to like extremely (9). Each panellist received 2 samples of each type of experimental fermented products. F-CBR was considered the reference product, and received a score of 5 for all descriptors. Panellists evaluated the other formulations in comparison with F-CBR.

2.15. Statistical analysis. Experimental data represent the mean value ± standard deviation of two individual experiments of at least three independent sample batches. Analysis of variance (ANOVA) and Duncan's multiple comparison test were used to assess differences among mean values with 95% confidence ($P<0.05$). Statistical analyses were performed with Statgraphics Centurion 16 (Statistical Graphics Corporation, UK).

3. Results

3.1. Microbial growth and pH evolution during fermentation of BR formulations. Figure 1A depicts the pH of fermented products. F-CBR and F-SBR showed similar initial pH values (6.21 and 6.17, respectively), which were higher than those for F-GBR48 and F-GBR96 (5.95 and 5.76, respectively). As expected, a rapid pH drop was observed after 1 h for all substrates, but faster acidification was observed in F-GBR48 and F-GBR96, that reached pH value of 4.4 after 3 h, compared with F-CBR and F-SBR, which reached this pH value after 5h.

The initial microbial density was 7.4-7.7 log CFU/g for all formulations and bacterial growth was substantially larger for F-GBR48 and F-GBR96 than for F-CBR and F-SBR (Figure 1B). At 3h-fermentation, F-GBR48 and F-GBR96 exhibited 9.5 and 9.7 log CFU/g, respectively, similar to F-BR and F-SBR at 5h of fermentation (9.4 and 9.6 log CFU/g, respectively).

3.2. Proximate composition of BR formulations. NF-CBR contained water, protein, fat, carbohydrates and ash of 84.6 g/100 g, 0.89 g/100 g, 0.48 g/100 g, 13.9 g/100 g and 0.22 g/100 g, respectively, providing an energy value of ~64 Kcal/100 g (Table 1). Soaking and germination had a negligible impoact on water, protein, fat and ash contents of BR formulations. Only NF-GBR48 exhibited significantly (p≤0.05) higher carbohydrates content and energy than control (NF-CBR).

Fermentation caused modifications on nutritional composition of BR products. F-CBR and F-GBR48 showed similar concentration of protein and ash than their non-fermented counterparts, while F-SBR and F-GBR96 presented greater content of both nutrients than the respective non-fermented products. No relevant changes were observed regarding carbohydrate content after fermentation, excluding F-CBR that contained higher levels than NF-CBR. Energy of F-CBR was significantly higher while F-GBR48 showed lower energy than their non-fermented counterpart (Table 1).

3.3. Physico-chemical properties of BR formulations. The studied properties were affected by BR substrate (crude, soaked or germinated) and fermentation process (Table 2). Consistency index decreased in NF-SBR and NF-GBR compared to control. Contrarily, NF-CBR and NF-SBR showed lower flow behaviour index and total solids than NF-GBR48 and NF-GBR96. All fermented BR products exhibited higher consistency index and lower pH values than their non-fermented counterparts. Fermentation did not modify flow behaviour index in formulations from SBR and GBR48, while reduced flow behaviour index in F-CBR F-GBR96. Lower total solids and higher density were found in F-GBR96 than the corresponding non-fermented formulation NF-GBR96.

3.4. Content of bioactive compounds in BR formulations. NF-CBR yogurt exhibited a TPC concentration of 9.9 mg GAE/100 g (Table 2). Soaking process significantly ($p \leq 0.05$) reduced TPC while NF-GBR48 contained similar TPC than NF-CBR. NF-GBR96 showed the highest ($p \leq 0.05$) TPC. Fermentation significantly ($p \leq 0.05$) enhanced TPC in all BR formulations compared to their non-fermented counterparts, with the exception of F-GBR96, which showed lower ($p \leq 0.05$) phenolics levels than NF-GBR96.

GABA content was very low in NF-CBR and NF-SBR (0.05 mg/100g) and germination increased GABA significantly ($p \leq 0.05$), showing NF-GBR96 the largest concentration (0.74mg/100g). Fermentation enhanced markedly GABA levels in all BR products.

γ-Oryzanol, however, did not significantly change between non-fermented and fermented BR formulations ($p > 0.05$) (0.13- 0.19 mg/100 g) (Table 2).

NF-CBR, NF-SBR and NF-GBR48 products exhibited similar antioxidant activity (24.2-24.8 µg/100 g) ($p \leq 0.05$) but lower than NF-GBR96 (49.5 µg/100g). Fermentation did not cause relevant changes in this activity and F-GBR96 exhibited the highest ($p \leq 0.05$) ORAC value (46.9 µg/100 g). Regarding the ability of BR products to inhibit ACE activity, non-fermented formulations showed low inhibition (below 30%) but fermentation improved significantly ($p \leq 0.05$) ACE inhibitory activity of BR formulations, showing F-GBR96 the largest activity (61.5%) (Table 2).

3.5. Sensory evaluation of BR formulations. F-CBR and F-SBR products scored similar values for astringency, bitterness and sourness, which were lower than those of F-GBR48 and F-GBR96 (Figure 2), having germination time a strong impact on these sensory attributes. Contrarily, cereal-type flavour did not differ significantly ($p > 0.05$) among formulations. Surprisingly, panellists perceived a stronger fermented odour in F-SBR and F-GBR48 formulations. F-CBR exhibited significant higher creaminess scores while F-GBR96 got the least. For white colour, F-SBR received the highest value, followed by F-CBR and F-GBR48, while F-GBR96 presented the lowest. Considering overall acceptability, F-GBR96 received the highest score.

4. Discussion

In the recent years, BR is gaining a great deal of attention due to its lower glycaemic index and better health-promoting properties than polished rice (Sirisoontaralak, Nakornpanom, Koakietdumrongkul, & Panumaswiwath, 2015). Hard texture, poor cooking performance and palatability limit the consumption of BR (Kaur, Asthir, & Mahajan, 2017) and, therefore, innovative approaches overcoming these drawbacks are particularly challenging. Germination emerges as a naturally economically-efficient process to improve palatability, nutritional and techno-functional qualities, as well as health-promoting properties of BR (Cáceres et al., 2014). GBR is an attractive substrate for developing novel foods for consumer's new life choices. Few studies have examined GBR as a nutritious ingredient in the formulation of novel food products such as bread (Cornejo et al., 2015), cookies (Chung, Cho, & Lim, 2014) and noodles (Gong et al., 2017). However, to the best of our knowledge, the suitability of GBR for developing multifunctional yogurt-like products has not been yet explored.

The results presented here revealed that GBR is as attractive substrate for developing functional fermented products, since it promotes the growth of the starter culture. In fact, F-GBR48 and F-GBR96 after 3h of fermentation exhibited similar viable cell counts than F-CBR and F-SBR after 5h. Starch decrease in GBR results in higher fermentable maltose and glucose (Xia et al., 2017) which might enhance the growth of LAB starter culture in GBR formulations. Moreover, germination causes an increase in protein digestibility of BR (Cáceres et al., 2014; Cornejo et al., 2015) and improves the concentration and/or bioaccesibility of micronutrients (Ding et al., 2018) and phenolic compounds in BR (Maksup et al., 2018), which can be used as substrates for the growth of the starter culture, contributing to the higher counts observed in these GBR fermented products. The faster growth found in F-GBR48 and F-GBR96 caused the production of organic acids and, consequently, rapid acidification of the fermented GBR yogurts. This fact explains that shorter acidification period for GBR yogurt-like products.

NF-GBR formulations exhibited higher carbohydrates content and energy value than NF-CBR due to the activation of starch hydrolytic enzymes during germination (Singh et al., 2018). Ash content improved after fermentation in F-SBR and F-GBR96 BR products, which could be related with the enhancement of mineral solubility during fermentation. Certain lactobacilli strains produce phytate-degrading enzymes (Amritha & Venkateswaran, 2017), thus improving the mineral bioavailability in fermented BR products.

Regarding physico-chemical properties, non-fermented formulations exhibited lower consistency than the fermented ones, results compatible with their semi-solid appearance. Non-fermented and fermented formulations containing GBR48 and GBR96 showed lower than those manufactured from SBR and CBR. The thinner consistency of GBR yogurts is consistent with their highest flow behaviour index. The lower consistency index and higher flow behaviour index of yogurts have been associated with lower total solids (Penna, Converti, & De Oliveira, 2006). However, F-GBR formulations exhibited higher total solids and similar protein contents than F-CBR, and their lower consistency could be possibly attributed to lower starch content.

Total phenolic content increased in fermented and non-fermented formulations increased when GBR was used as substrate. The gradual accumulation of soluble phenolic compounds in GBR has been reported (Cáceres et al., 2014; Cáceres et al., 2017; Ti, et al., 2014) and can be attributed to their *novo* synthesis during germination (Cho & Lim, 2018; Maksup et al., 2018) and their release from the cell wall components (Wang et al., 2016). Fermentation enhanced phenolics concentration in BR yogurt-like products, results in accordance with those reported (Liu et al., 2017) in lactic acid fermented rice bran. Among fermented products, those containing GBR showed the highest phenolic content (12.4-15.2 mg GAE/100g), in the range of those reported for liquid formulations containing germinated grains (barley, finger millet and moth bean) (Chavan, Gat, Harmalkar, & Waghmare, 2018), identifying fermentation as a valuable approach for producing TPC-enriched germinated products.

GABA exhibits several well-recognized physiological functions as antihypertensive, immunomodulatory, hypocholesterolemic, anticarcinogenic and antidiabetic activities (Diana, Quílez, & Rafecas, 2014). Long-term consumption of GABA-enriched foods such as soaked or germinated BR prevented hypertension and hypercholesterolemia in animal models (Kawakami et al., 2018). Our results showed higher GABA levels in fermented and non-fermented formulations when they contained GBR. Previous studies showed also a time-dependent GABA accumulation during BR germination (Cáceres et al., 2014; Cáceres et al., 2017). GABA is primarily synthesised by glutamate decarboxylase (GAD) which is activated during rice germination (Khwanchai, Chinprahast, Pichyangkura, & Chaiwanichsiri, 2014). GABA can be also formed from polyamines through diamine oxidase (DAO) and its activity increased during grain germination (Yang, Chen, & Gu, 2011), outcomes consistent with the results obtained in the present study. Moreover, fermentation promotes the accumulation of GABA in all F-BR products possibly due to the ability of *Lactobacillus* strains to produce GABA throughout expression of GAD (Yunes et al., 2016). The increase of glutamic acid during germination and further GAD activation during fermentation might increase GABA concentration in BR yogurt-like products. The regular intake of F-GBR formulations obtained here could provide enough GABA amount to improve consumer's health, but further *in vivo* studies are required.

γ-oryzanol is a mixture of phytosteryl ferulates located in rice bran. This compound shows a wide spectrum of health promoting effects such as antioxidant, anticarcinogenic, antihyperlipidemic, anti-inflammatory and neuroprotective properties (Francisqueti et al., 2017). Non-fermented and fermented GBR products provide γ-oryzanol and fermentation did not modify notably its levels, suggesting that LAB strains used for fermentation were unable to synthetize γ-oryzanol, likely due to the lack of an efficient system for its transport (Esteban-Torres est al., 2013).

Among NF-formulations, NF-GBR96 exhibited the highest antioxidant activity, results in accordance with the time-dependent enhancement of antioxidant activity during BR germination (Cho & Lim, 2018). In fact, the current study revealed that antioxidants such as phenolic compounds and GABA increased remarkably during germination in a time-dependent manner (Table 3). These compounds undoubtedly contribute to the antioxidant activity of GBR formulations, but the contribution of other soluble compounds as antioxidant vitamins that increase during germination (Sun et al., 2018) cannot be ruled out.

Since hypertension is the main cause of mortality worldwide (Ciau-Solís, Acevedo-Fernández, & Betancur-Ancona, 2018), *in vitro* antihypertensive activity of GBR yogurt-like formulations was studied. NF-GBR formulations exhibited stronger ACE inhibitory activity than NF-CBR and NF-SBR. Phenolic acids and flavonols, which can be released and/or synthesized during germination (Wang et al., 2016) exhibit ACE inhibitory effects *in vitro* (Al Shukor et al., 2013). ACE inhibitory peptides can be also generated from germinated grains (Mamilla & Mishra, 2017), contributing to the antihypertensive activity of GBR products observed. Fermentation caused improvements (1.5-2.4-fold) on ACE inhibition in all BR formulations, and F-GBR96 showed the highest activity. These finding is consistent with the observed rise of soluble phenolics caused by the action of esterases during LAB fermentation (Esteban-Torres et al., 2015) and match with the findings of other authors in rice beverages fermented by different LAB (Ghosh et al., 2015; Rashid et al., 2015).

Fermented GBR formulations were further subjected to sensorial analysis to evaluate consumer acceptability. F-GBR yogurts exhibited higher average scores for astringency, bitterness and sourness than non-germinated ones. Bitterness is related with lipid oxidation that can occur during germination (Kince et al., 2017), while perceived sourness is associated with their lower pH values. However, these attributes were appreciated as desirable by panellists, which perceived them as typical yogurts features. No differences were found among BR-formulations for cereal-type flavour, recognized by consumers as a pleasant flavour of whole-grains derived products (Miocinovic et al., 2018). F-GBR96 received the lowest score regarding white colour since it was slightly yellowish. However, this formulation scored the highest fermented odour, a desirable attribute for a yogurt-like product. F-GBR formulations displayed lower creaminess than non-germinated ones, possible due to low carbohydrate content since the addition of starch improves the creaminess and mouthfeel of yogurts (Brückner-Gühmann, Benthin, & Drusch, 2018). In general, the overall impression of GBR yogurt-like products was better that that for formulations containing crude or soaked BR.

5. Conclusions

The suitability of GBR as substrate for the development of multifunctional yogurt-like products was demonstrated. Fermentation modified slightly the proximal composition of BR formulations and improved the content of bioactive compounds (phenolic compounds and GABA), ACE-inhibitory activity, consistency index and density. F-GBR96 yogurt-like product showed the highest biological and techno-functional properties, as well as overall acceptability. F-GBR formulations can be considered as natural healthy food choice for vegans and consumers interested in reducing animal protein intake.

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Figure captions.

Figure 1. Acidification profile during fermentation (A) and viable cell counts (B) of F-CBR, F-SBR, F-GBR48 and F-GBR96 products. —◆— CBR;···△··· SBR; —■— GBR48;. --⊗-- GBR96

Figure 2. Sensory analysis of fermented formulations elaborated from crude (F-CBR), soaked (F-SBR) and germinated for 48 and 96 h (F-GBR48 and F-GBR96) brown rice.

Tables and Figures

Figure 1.

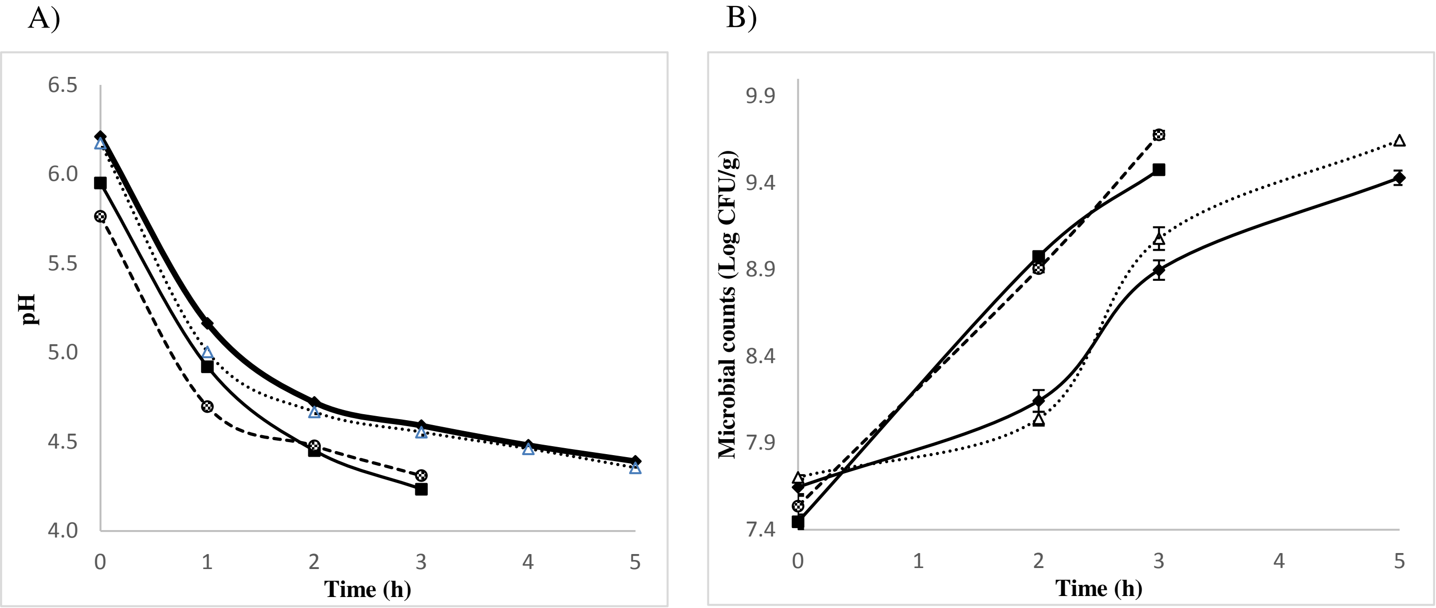
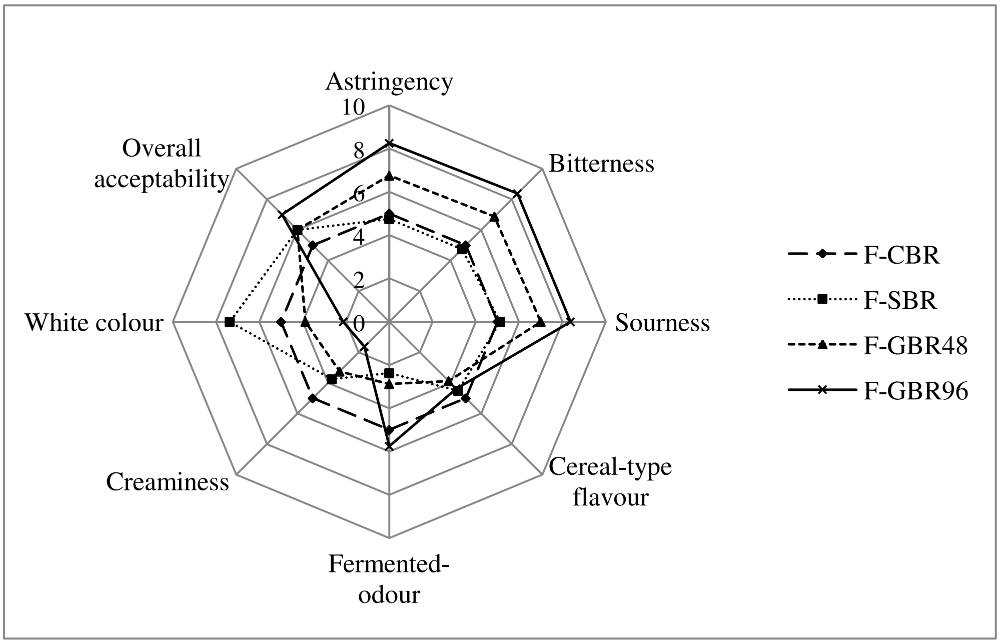


Figure 2.



Astringency
10
8
6
4
2
0
Bitterness
Sourness
Cereal-type flavour
Fermented-odour
Creaminess
White colour
Overall acceptability
F-CBR
F-SBR
F-GBR48
F-GBR96

Table 1. Proximate composition (g/100 g) and energy (Kcal/100 g) of non-fermented (NF) and fermented (F) formulations obtained from crude brown rice (CBR), soaked brown rice (SBR) and germinated brown rice for 48h (GBR48) and 96h (GBR96).

	Water	Protein	Fat	Carbohydrates	Ash	Energy
Non-fermented products						
NF-CBR	$84.57\pm0.40^{b}_{2}$	$0.89\pm0.07^{a}_{1}$	$0.48\pm0.00^{a}_{1}$	$13.85\pm0.36^{a}_{1}$	$0.22\pm0.03^{a}_{1}$	$64.25\pm1.89^{ab}_{1}$
NF-SBR	$84.40\pm0.53^{b}_{1}$	$0.80\pm0.02^{a}_{1}$	$0.37\pm0.06^{a}_{1}$	$14.26\pm0.46^{ab}_{1}$	$0.18\pm0.02^{a}_{1}$	$64.11\pm1.84^{a}_{1}$
NF-GBR48	$83.33\pm0.58^{a}_{1}$	$0.80\pm0.06^{a}_{1}$	$0.46\pm0.08^{a}_{1}$	$15.24\pm0.73^{b}_{1}$	$0.16\pm0.03^{a}_{1}$	$69.28\pm2.28^{c}_{2}$
NF-GBR96	$83.67\pm0.58^{ab}_{1}$	$0.81\pm0.04^{a}_{1}$	$0.49\pm0.06^{a}_{1}$	$14.91\pm0.54^{b}_{1}$	$0.12\pm0.01^{a}_{1}$	$68.00\pm1.99^{bc}_{1}$
Fermented products						
F-CBR	$82.97\pm0.46^{a}_{1}$	$0.97\pm0.02^{b}_{1}$	$0.48\pm0.06^{b}_{1}$	$15.37\pm0.51^{b}_{2}$	$0.21\pm0.02^{bc}_{1}$	$70.29\pm2.14^{b}_{2}$
F-SBR	$84.43\pm0.51^{b}_{1}$	$0.88\pm0.02^{a}_{2}$	$0.39\pm0.05^{a}_{1}$	$14.08\pm0.51^{a}_{1}$	$0.22\pm0.01^{c}_{2}$	$63.87\pm2.01^{a}_{1}$
F-GBR48	$84.27\pm0.75^{b}_{1}$	0.86 ± 0.06^{a1}	$0.49\pm0.03^{b}_{1}$	$14.20\pm0.77^{ab}_{1}$	$0.18\pm0.02^{ab}_{1}$	$65.13\pm2.24^{a}_{1}$
F-GBR96	$83.83\pm0.76^{ab}_{1}$	$0.92\pm0.03^{ab}_{2}$	$0.54\pm0.03^{b}_{1}$	$14.54\pm0.72^{ab}_{1}$	$0.17\pm0.02^{a}_{2}$	$67.03\pm2.26^{ab}_{1}$

Data are the mean ± standard deviation of independent experiments analyzed in duplicate. Different superscript letters within a column indicate significant differences among samples for the same time of product (fermented or not fermented) ($P < 0.05$. Duncan's test). Different subscript numbers within a column indicate significant differences among fermented and non-fermented products elaborated with the same type of brown rice.

Table 2. Physico-chemical characterization of non-fermented (NF) and fermented (F) formulations obtained from crude brown rice (CBR), soaked brown rice (SBR) and germinated brown rice for 48h (GBR48) and 96h (GBR96).

	Consistency index ($Pa.s^{n}$)	Flow index	Total solids (*Brix)	pH	Density (Kg/m^{3})
Non-fermented products					
NF-CBR	$0.54\pm0.05^{c}_{1}$	$0.53\pm0.02^{a}_{2}$	$14.97\pm0.15^{a}_{1}$	$6.20\pm0.20^{b}_{2}$	$1,054.7\pm4.51^{b}_{1}$
NF-SBR	$0.42\pm0.05^{b}_{1}$	$0.53\pm0.03^{a}_{1}$	$15.00\pm0.10^{a}_{1}$	$6.17\pm0.21^{b}_{2}$	$1,053.3\pm4.16^{ab}_{1}$
NF-GBR48	$0.20\pm0.03^{a}_{1}$	$0.62\pm0.03^{b}_{1}$	$15.27\pm0.06^{b}_{1}$	$5.97\pm0.06^{ab}_{2}$	$1,047.3\pm3.06^{a}_{1}$
NF-GBR96	$0.13\pm0.02^{a}_{1}$	$0.82\pm0.05^{c}_{2}$	$15.37\pm0.06^{b}_{2}$	$5.77\pm0.06^{a}_{2}$	$1,047.0\pm1.00^{a}_{1}$
Fermented products					
F-CBR	$2.07\pm0.25^{c}_{2}$	$0.42\pm0.02^{a}_{1}$	$14.93\pm0.06^{a}_{1}$	$4.33\pm0.06^{a}_{1}$	$1,053.0\pm3.46^{a}_{1}$
F-SBR	$1.59\pm0.04^{b}_{2}$	$0.52\pm0.04^{b}_{1}$	$14.93\pm0.06^{a}_{1}$	$4.30\pm0.10^{a}_{1}$	$1,054.3\pm2.08^{a}_{1}$
F-GBR48	$1.45\pm0.10^{ab}_{2}$	$0.57\pm0.01^{c}_{1}$	$15.13\pm0.15^{b}_{1}$	$4.23\pm0.06^{a}_{1}$	$1,052.0\pm5.29^{a}_{1}$
F-GBR96	$1.27\pm0.04^{a}_{2}$	$0.72\pm0.03^{d}_{1}$	$15.23\pm0.06^{b}_{1}$	$4.27\pm0.06^{a}_{1}$	$1,053.3\pm2.08^{a}_{2}$

Data are the mean ± standard deviation of independent experiments analyzed in duplicate. Different superscript letters within a column indicate significant differences among samples for the same type of product (fermented or not fermented) ($p\leq0.05$., Duncan's test). Different subscript numbers within a column indicate significant differences among fermented and non-fermented products elaborated with the same type of brown rice.

Table 3. Changes in phenolic compounds, GABA and γ-oryzanol contents, antioxidant activity (ORAC) and ACE-inhibitory activity of non-fermented (NF) and fermented (F) formulations obtained from crude brown rice (CBR), soaked brown rice (SBR) and germinated brown rice for 48 h (GBR48) and 96 h (GBR96).

	TPC (mg GAE/100g)	GABA (mg/100g)	γ-oryzanol (mg/100g)	ORAC (μg TE/100g)	ACE-inhibitory activity (%)
<u>Non-fermented products</u>					
NF-CBR	$9.89\pm0.26^{b}_{1}$	$0.05\pm0.01^{a}_{1}$	$0.16\pm0.01^{c}_{1}$	$28.22\pm3.94^{a}_{1}$	$10.73\pm1.65^{a}_{1}$
NF-SBR	$8.57\pm0.68^{a}_{1}$	$0.05\pm0.01^{a}_{1}$	$0.13\pm0.01^{a}_{1}$	$24.23\pm1.57^{a}_{2}$	$12.73\pm1.40^{a}_{1}$
NF-GBR48	$9.81\pm0.52^{b}_{1}$	$0.47\pm0.03^{b}_{1}$	$0.14\pm0.01^{b}_{1}$	$24.78\pm3.09^{a}_{1}$	$25.17\pm2.32^{b}_{1}$
NF-GBR96	$16.19\pm0.37^{c}_{1}$	$0.74\pm0.11^{c}_{1}$	$0.16\pm0.01^{c}_{1}$	$49.48\pm0.48^{b}_{1}$	$26.05\pm1.91^{b}_{1}$
<u>Fermented products</u>					
F-BR	$13.49\pm0.76^{c}_{2}$	$0.28\pm0.02^{a}_{1}$	$0.19\pm0.01^{d}_{2}$	$32.46\pm2.17^{b}_{2}$	$16.46\pm1.99^{a}_{2}$
F-SBR	$9.89\pm0.48^{a}_{2}$	$0.36\pm0.08^{a}_{2}$	$0.12\pm0.01^{a}_{1}$	$21.18\pm2.37^{a}_{1}$	$24.62\pm1.79^{b}_{2}$
F-GBR48	$12.41\pm0.55^{b}_{2}$	$0.88\pm0.10^{b}_{2}$	$0.15\pm0.01^{b}_{1}$	$23.51\pm2.04^{a}_{1}$	$44.04\pm3.07^{c}_{2}$
F-GBR96	$15.20\pm0.65^{d}_{1}$	$1.86\pm0.19^{c}_{2}$	$0.16\pm0.01^{c}_{1}$	$46.93\pm3.54^{c}_{1}$	$61.47\pm1.61^{d}_{2}$

Data are the mean ± standard deviation of independent experiments analyzed in duplicate. Different superscript letters within a column indicate significant differences among samples for the same type of product (fermented or not fermented) ($p\leq0.05$., Duncan's test). Different subscript numbers within a column indicate significant differences among fermented and non-fermented products elaborated with the same type of brown rice.