

Manuscript Number:

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

Article Type: Research paper

Keywords: Brown rice; *Oryza sativa*; germination; fermentation; functional foods

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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

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

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16

17 **ABSTRACT**

18 The suitability of germinated brown rice (GBR) for developing novel multifunctional yogurt-
19 like products was evaluated. Crude brown rice, soaked brown rice and GBR for 48 h and 96 h
20 were fermented (F-CBR, F-SBR, F-GBR48 and F-GBR96, respectively). The viability of the
21 starter culture, acidification pattern, techno-functional properties, content of bioactive
22 compounds [phenolic compounds, γ -aminobutyric acid (GABA) and γ -oryzanol], biological
23 activity [antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities] and
24 sensory attributes were evaluated. Fermentation did not modify proximate composition but
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26 index of yogurt-like products. Among them, F-GBR96 exhibited the highest phenolic (15.2
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36 **1. Introduction**

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

42 Rice (*Oryza sativa* L.) is one of the most consumed cereals worldwide, but it is mostly
43 consumed as white rice. Brown rice (BR), containing endosperm, embryo and bran, is
44 nutritionally more complete and provides phytochemicals with health-promoting relevance as
45 γ -oryzanol, γ -aminobutyric acid (GABA) and ferulic acid located mainly in the germ and bran
46 layers (Gong et al., 2017). However, BR consumption is limited due to its poor textural and
47 sensory properties. BR germination has been demonstrated to be a cost-effective strategy to
48 improve textural and organoleptic quality, nutrient and phytochemical bioavailability and
49 biological activity of this cereal (Cáceres, Martínez-Villaluenga, Amigo, & Frias, 2014).
50 Germinated BR (GBR) is consumed in salads, boiled, or even it is incorporated as an
51 ingredient in bakery products (Cornejo et al., 2015; Chung, Cho, & Lim, 2014). The
52 development of innovative products is very challenging for food industry to fulfil social
53 demands for natural, gluten-free and vegan products. In this context, ready-to-eat yogurt-like
54 formulations including GBR may expand the range of non-dairy fermented products in the
55 market, which is quite limited in western countries so far. Fermentation with lactic acid
56 bacteria (LAB) may enhance GBR health-promoting properties since LAB are cell factories
57 producing nutrients and bioactive compounds that improve functional features of cereals
58 (Waters et al., 2015). Diverse types of cereals are substrates of lactic-acid fermented
59 beverages, such as oat, maize, rice, barley and sorghum, or a mixture of them (Freire, Ramos,
60 & Schwan, 2017; Salmerón, Thomas, & Pandiella, 2015). Germinated whole grains have been

61 demonstrated to be better substrates for LAB growth compared with non-malted cereals
62 (Nsogning Dongmo et al., 2017). Therefore, the aim of the present study was to explore the
63 suitability of GBR for the production of a healthy and nutritious fermented yogurt-like
64 product, topic that has not been explored so far. Proximate composition, content of bioactive
65 compounds (phenolic compounds, GABA, and γ -oryzanol), biological activities (antioxidant
66 and ACE-inhibitory activities) and sensory quality were evaluated on the GBR products. Non-
67 fermented formulations were also prepared to evaluate the effect of fermentation on
68 nutritional and health-promoting properties of GBR yogurt-like products.

69 **2. Materials and Methods**

70 *2.1. Chemicals.* Unless otherwise stated, all reagents were obtained from Sigma-Aldrich
71 (Barcelona, Spain). MRS agar was obtained from Pronadisa (Madrid, Spain).

72 *2.2. Rice.* Commercial BR (*Oryza sativa* subssp. Indica, var. SLF09) was provided by
73 Productora Nacional de Alimentos C.A., INDIA-PRONACA (Ecuador).

74 *2.3. Starter culture.* A commercial thermophilic starter culture FD-DVS YC-180 Yo-Flex[®]
75 containing diverse LAB strains was purchased from Chr. Hansen (Guayaquil, Ecuador). The
76 starter culture was grown in Man Rogosa Sharpe (MRS) broth at 37 °C for 18 h, and then,
77 bacterial cells were harvested by centrifugation (8,000 × g, 10 min), washed twice, and
78 suspended in sterile water, before inoculation in GBR substrates.

79 *2.4. BR germination and flour preparation.* BR was hygienised, soaked (SBR) and
80 germinated for 48 h (GBR48) and 96 h (GBR96) as previously described (Caceres et al.,
81 2014). These samples were dried at $50 \pm 1^\circ\text{C}$ for 24 h in a temperature-controlled cabin
82 (Memmert SM200) and milled in a cyclone mill (UDY Corporation, USA). Flours from crude
83 BR (CBR) were also obtained and used as reference. Three germination batches were
84 prepared for each experimental condition.

85 *2.5. Manufacture of fermented products.* Yogurt-like products were manufactured from CBR,
86 SBR, GBR48 and GBR96. Briefly, rice flour samples were supplemented with 7% sucrose
87 (w/v), 5% glucose (w/v) and 0.5% stabilizer gelatin (w/v) and mixed in distilled water at 1:4
88 ratio (w:v) for 1 h at 20°C, cooked at 95°C for 30 min, and vacuum-filtered. The resultant
89 slurry fractions were cooled to 42 °C, placed in sterile Erlenmeyer flasks, and inoculated with
90 the starter culture at 1×10^6 CFU/mL. Lactic-acid fermentations were performed at 42 °C in
91 agitation until reaching pH 4.4 ± 0.2 and quickly cooled at 4°C. Fermentation experiments
92 were performed in duplicate for each germination condition. Fermented products were coded
93 as follows: F-CBR for that prepared with crude BR, F-SBR for that prepared with soaked BR,
94 and F-GBR48 and F-GBR96 for those obtained from BR germinated for 48 and 96 h,
95 respectively. Non-fermented products were also produced from crude (NF-CBR), soaked
96 (NF-SBR) and germinated BR (NF-GBR48 and NF-GBR96).

97 *2.6. Bacterial growth and pH determination.* Bacterial growth was determined in fermented
98 products by plating decimal buffered peptone water dilutions (10^6 - 10^8) in triplicate onto MRS
99 agar and counting the viable cells after incubation under anaerobic conditions (30 °C for 48
100 h). The pH was monitored using a pH-meter Basic 20 (Crison Instruments S. A., Barcelona,
101 Spain).

102 *2.7. Proximate composition.* Chemical composition of non-fermented and fermented products
103 was determined following AOAC (2016) methods for moisture, protein, fat and ash.
104 Carbohydrates were calculated by difference. Food energy was calculated using standardized
105 conversion factors (4.0 kcal/g for proteins and carbohydrates, and 9.0 kcal/g for fats) (FAO,
106 2003).

107 *2.8. Techno functional properties*

108 *2.8.1. Consistency and flow behaviour index.* The consistency index in Pa.sⁿ (*K*) and the flow
109 behaviour index (*n*) were determined using a rotational viscometer Brookfield DV-II+ with

110 spindle for non-Newtonian fluids through the logarithmic linearization of the apparent
111 viscosity (η) curve in Pa.s vs shear rate in s^{-1} ($\dot{\gamma}$) (Steffe,1996).

112 *2.8.2. Total soluble solids and density.* Total soluble solids and density were determined by
113 refractometry and gravimetric methods, respectively (AOAC, 2016).

114 *2.9. Determination of total phenolic compounds.* Total phenolic content (TPC) was
115 determined using Folin-Ciocalteu's reagent (Cáceres et al., 2014). Results were expressed as
116 mg of gallic acid equivalents (GAE)/100 g product.

117 *2.10. Determination of GABA content.* GABA was extracted with methanol and further
118 analysed by RP-HPLC as in Cáceres et al. (2014). Results were expressed as mg GABA/100
119 g product.

120 *2.11. Determination of γ -oryzanol content.* The analysis of γ -oryzanol in BR products was
121 performed by RP-HPLC according to Cáceres et al. (2017). Results were expressed in mg γ -
122 oryzanol/100 g product.

123 *2.12. Determination of oxygen radical absorbance capacity (ORAC).* Antioxidant activity was
124 determined as ORAC by fluorescence as described recently in Cáceres et al., (2014). Results
125 were expressed as mg of Trolox equivalents (TE)/100 g product.

126 *2.13. Determination of ACE-inhibitory activity.* It was determined in all BR formulations as in
127 Cáceres et al., (2017). Results were presented as % inhibition.

128 *2.14 Sensory evaluation.* BR yogurt-like products were submitted to sensory analysis by 8
129 trained panellists (50% male, 50% female) recruited at Facultad de Ingeniería Mecánica y
130 Ciencias de la Producción (ESPOL, Guayaquil, Ecuador), selected by their regular
131 consumption of yogurt-like products. Panellists evaluated typical attributes for fermented
132 products such as astringency, bitterness, sourness, cereal-type flavour, fermented odour,
133 creaminess, white colour and overall acceptability, using a 9-point hedonic scale, ranging
134 from dislike extremely (1) to like extremely (9). Each panellist received 2 samples of each

135 type of experimental fermented products. F-CBR was considered the reference product, and
136 received a score of 5 for all descriptors. Panellists evaluated the other formulations in
137 comparison with F-CBR.

138 *2.15. Statistical analysis.* Experimental data represent the mean value \pm standard deviation of
139 two individual experiments of at least three independent sample batches. Analysis of variance
140 (ANOVA) and Duncan's multiple comparison test were used to assess differences among
141 mean values with 95% confidence ($P < 0.05$). Statistical analyses were performed with
142 Statgraphics Centurion 16 (Statistical Graphics Corporation, UK).

143 **3. Results**

144 *3.1. Microbial growth and pH evolution during fermentation of BR formulations.* Figure 1A
145 depicts the pH of fermented products. F-CBR and F-SBR showed similar initial pH values
146 (6.21 and 6.17, respectively), which were higher than those for F-GBR48 and F-GBR96 (5.95
147 and 5.76, respectively). As expected, a rapid pH drop was observed after 1 h for all substrates,
148 but faster acidification was observed in F-GBR48 and F-GBR96, that reached pH value of 4.4
149 after 3 h, compared with F-CBR and F-SBR, which reached this pH value after 5h.

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

155 *3.2. Proximate composition of BR formulations.* NF-CBR contained water, protein, fat,
156 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
157 g/100 g, respectively, providing an energy value of ~64 Kcal/100 g (Table 1). Soaking and
158 germination had a negligible impact on water, protein, fat and ash contents of BR

159 formulations. Only NF-GBR48 exhibited significantly ($p \leq 0.05$) higher carbohydrates content
160 and energy than control (NF-CBR).

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

168 *3.3. Physico-chemical properties of BR formulations.* The studied properties were affected by
169 BR substrate (crude, soaked or germinated) and fermentation process (Table 2). Consistency
170 index decreased in NF-SBR and NF-GBR compared to control. Contrarily, NF-CBR and NF-
171 SBR showed lower flow behaviour index and total solids than NF-GBR48 and NF-GBR96.
172 All fermented BR products exhibited higher consistency index and lower pH values than their
173 non-fermented counterparts. Fermentation did not modify flow behaviour index in
174 formulations from SBR and GBR48, while reduced flow behaviour index in F-CBR F-
175 GBR96. Lower total solids and higher density were found in F-GBR96 than the
176 corresponding non-fermented formulation NF-GBR96.

177 *3.4. Content of bioactive compounds in BR formulations.* NF-CBR yogurt exhibited a TPC
178 concentration of 9.9 mg GAE/100 g (Table 2). Soaking process significantly ($p \leq 0.05$) reduced
179 TPC while NF-GBR48 contained similar TPC than NF-CBR. NF-GBR96 showed the highest
180 ($p \leq 0.05$) TPC. Fermentation significantly ($p \leq 0.05$) enhanced TPC in all BR formulations
181 compared to their non-fermented counterparts, with the exception of F-GBR96, which
182 showed lower ($p \leq 0.05$) phenolics levels than NF-GBR96.

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

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

189 NF-CBR, NF-SBR and NF-GBR48 products exhibited similar antioxidant activity
190 (24.2-24.8 $\mu\text{g}/100 \text{ g}$) ($p \leq 0.05$) but lower than NF-GBR96 (49.5 $\mu\text{g}/100\text{g}$). Fermentation did
191 not cause relevant changes in this activity and F-GBR96 exhibited the highest ($p \leq 0.05$)
192 ORAC value (46.9 $\mu\text{g}/100 \text{ g}$). Regarding the ability of BR products to inhibit ACE activity,
193 non-fermented formulations showed low inhibition (below 30%) but fermentation improved
194 significantly ($p \leq 0.05$) ACE inhibitory activity of BR formulations, showing F-GBR96 the
195 largest activity (61.5%) (Table 2).

196 *3.5. Sensory evaluation of BR formulations.* F-CBR and F-SBR products scored similar values
197 for astringency, bitterness and sourness, which were lower than those of F-GBR48 and F-
198 GBR96 (Figure 2), having germination time a strong impact on these sensory attributes.
199 Contrarily, cereal-type flavour did not differ significantly ($p > 0.05$) among formulations.
200 Surprisingly, panellists perceived a stronger fermented odour in F-SBR and F-GBR48
201 formulations. F-CBR exhibited significant higher creaminess scores while F-GBR96 got the
202 least. For white colour, F-SBR received the highest value, followed by F-CBR and F-GBR48,
203 while F-GBR96 presented the lowest. Considering overall acceptability, F-GBR96 received
204 the highest score.

205 **4. Discussion**

206 In the recent years, BR is gaining a great deal of attention due to its lower glycaemic
207 index and better health-promoting properties than polished rice (Sirisoontaralak,

208 Nakornpanom, Koakietdumrongkul, & Panumaswiwath, 2015). Hard texture, poor cooking
209 performance and palatability limit the consumption of BR (Kaur, Asthir, & Mahajan, 2017)
210 and, therefore, innovative approaches overcoming these drawbacks are particularly
211 challenging. Germination emerges as a naturally economically-efficient process to improve
212 palatability, nutritional and techno-functional qualities, as well as health-promoting properties
213 of BR (Cáceres et al., 2014). GBR is an attractive substrate for developing novel foods for
214 consumer's new life choices. Few studies have examined GBR as a nutritious ingredient in
215 the formulation of novel food products such as bread (Cornejo et al., 2015), cookies (Chung,
216 Cho, & Lim, 2014) and noodles (Gong et al., 2017). However, to the best of our knowledge,
217 the suitability of GBR for developing multifunctional yogurt-like products has not been yet
218 explored.

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

232 NF-GBR formulations exhibited higher carbohydrates content and energy value than
233 NF-CBR due to the activation of starch hydrolytic enzymes during germination (Singh et al.,
234 2018). Ash content improved after fermentation in F-SBR and F-GBR96 BR products, which
235 could be related with the enhancement of mineral solubility during fermentation. Certain
236 lactobacilli strains produce phytate-degrading enzymes (Amritha & Venkateswaran, 2017),
237 thus improving the mineral bioavailability in fermented BR products.

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

247 Total phenolic content increased in fermented and non-fermented formulations
248 increased when GBR was used as substrate. The gradual accumulation of soluble phenolic
249 compounds in GBR has been reported (Cáceres et al., 2014; Cáceres et al., 2017; Ti, et al.,
250 2014) and can be attributed to their *novo* synthesis during germination (Cho & Lim, 2018;
251 Maksup et al., 2018) and their release from the cell wall components (Wang et al., 2016).
252 Fermentation enhanced phenolics concentration in BR yogurt-like products, results in
253 accordance with those reported (Liu et al., 2017) in lactic acid fermented rice bran. Among
254 fermented products, those containing GBR showed the highest phenolic content (12.4-15.2
255 mg GAE/100g), in the range of those reported for liquid formulations containing germinated
256 grains (barley, finger millet and moth bean) (Chavan, Gat, Harmalkar, & Waghmare, 2018),

257 identifying fermentation as a valuable approach for producing TPC-enriched germinated
258 products.

259 GABA exhibits several well-recognized physiological functions as antihypertensive,
260 immunomodulatory, hypocholesterolemic, anticarcinogenic and antidiabetic activities (Diana,
261 Quílez, & Rafecas, 2014). Long-term consumption of GABA-enriched foods such as soaked
262 or germinated BR prevented hypertension and hypercholesterolemia in animal models
263 (Kawakami et al., 2018). Our results showed higher GABA levels in fermented and non-
264 fermented formulations when they contained GBR. Previous studies showed also a time-
265 dependent GABA accumulation during BR germination (Cáceres et al., 2014; Cáceres et al.,
266 2017). GABA is primarily synthesised by glutamate decarboxylase (GAD) which is activated
267 during rice germination (Khwanchai, Chinprahast, Pichyangkura, & Chaiwanichsiri, 2014).
268 GABA can be also formed from polyamines through diamine oxidase (DAO) and its activity
269 increased during grain germination (Yang, Chen, & Gu, 2011), outcomes consistent with the
270 results obtained in the present study. Moreover, fermentation promotes the accumulation of
271 GABA in all F-BR products possibly due to the ability of *Lactobacillus* strains to produce
272 GABA throughout expression of GAD (Yunes et al., 2016). The increase of glutamic acid
273 during germination and further GAD activation during fermentation might increase GABA
274 concentration in BR yogurt-like products. The regular intake of F-GBR formulations obtained
275 here could provide enough GABA amount to improve consumer's health, but further *in vivo*
276 studies are required.

277 γ -oryzanol is a mixture of phytosteryl ferulates located in rice bran. This compound
278 shows a wide spectrum of health promoting effects such as antioxidant, anticarcinogenic,
279 antihyperlipidemic, anti-inflammatory and neuroprotective properties (Francisqueti et al.,
280 2017). Non-fermented and fermented GBR products provide γ -oryzanol and fermentation did
281 not modify notably its levels, suggesting that LAB strains used for fermentation were unable

282 to synthesize γ -oryzanol, likely due to the lack of an efficient system for its transport
283 (Esteban-Torres et al., 2013).

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

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

303 Fermented GBR formulations were further subjected to sensorial analysis to evaluate
304 consumer acceptability. F-GBR yogurts exhibited higher average scores for astringency,
305 bitterness and sourness than non-germinated ones. Bitterness is related with lipid oxidation
306 that can occur during germination (Kince et al., 2017), while perceived sourness is associated

307 with their lower pH values. However, these attributes were appreciated as desirable by
308 panellists, which perceived them as typical yogurts features. No differences were found
309 among BR-formulations for cereal-type flavour, recognized by consumers as a pleasant
310 flavour of whole-grains derived products (Miocinovic et al., 2018). F-GBR96 received the
311 lowest score regarding white colour since it was slightly yellowish. However, this formulation
312 scored the highest fermented odour, a desirable attribute for a yogurt-like product. F-GBR
313 formulations displayed lower creaminess than non-germinated ones, possible due to low
314 carbohydrate content since the addition of starch improves the creaminess and mouthfeel of
315 yogurts (Brückner-Gühmann, Benthin, & Drusch, 2018). In general, the overall impression of
316 GBR yogurt-like products was better than that for formulations containing crude or soaked
317 BR.

318

319 **5. Conclusions**

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

327

328 **Acknowledgments**

329 This research received funding from Ministry Economy, Industry and Competitiveness, Spain
330 (MINEICO, Spain) and FEDER, European Commission program (grant numbers AGL2015-
331 67598-R and AGL2017-83718-R). Authors also acknowledge the National Autonomous

332 Institute of Agricultural Research from Ecuador (INIAP) for providing the BR grains. E.
333 Peñas thanks the Spanish Ministry of Economy, Industry and Competitiveness and European
334 Social Fund for “Ramón y Cajal” contract (grant number RYC-2013-14354).

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475

476 **Figure captions.**

477 **Figure 1.** Acidification profile during fermentation (A) and viable cell counts (B) of F-CBR,

478 F-SBR, F-GBR48 and F-GBR96 products. —◆— CBR; ...△... SBR; —■— GBR48; .-●- GBR96

479

480 **Figure 2.** Sensory analysis of fermented formulations elaborated from crude (F-CBR), soaked

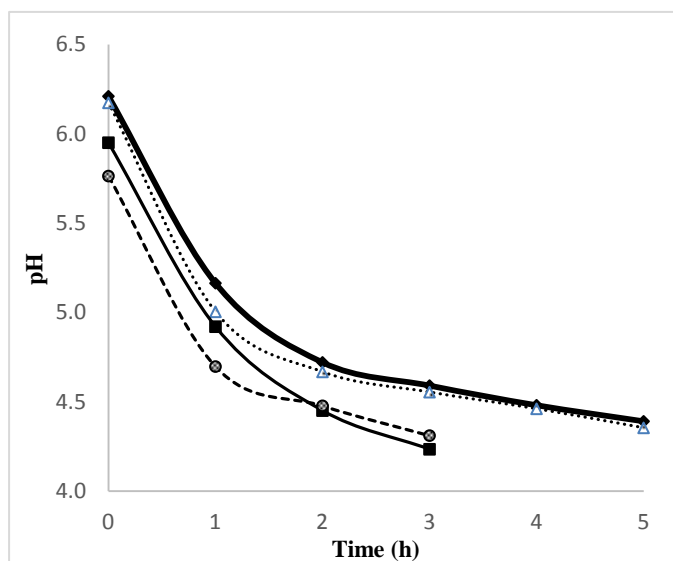
481 (F-SBR) and germinated for 48 and 96 h (F-GBR48 and F-GBR96) brown rice.

482

483

Figure 1.

A)



B)

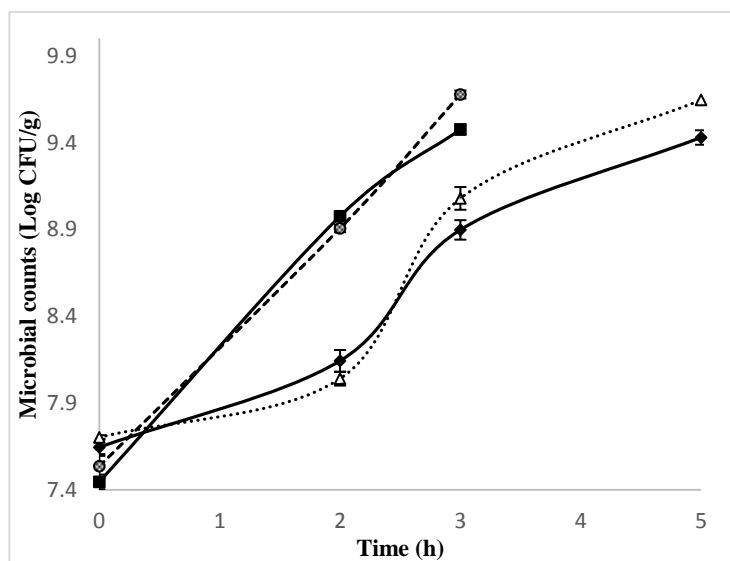


Figure 2.

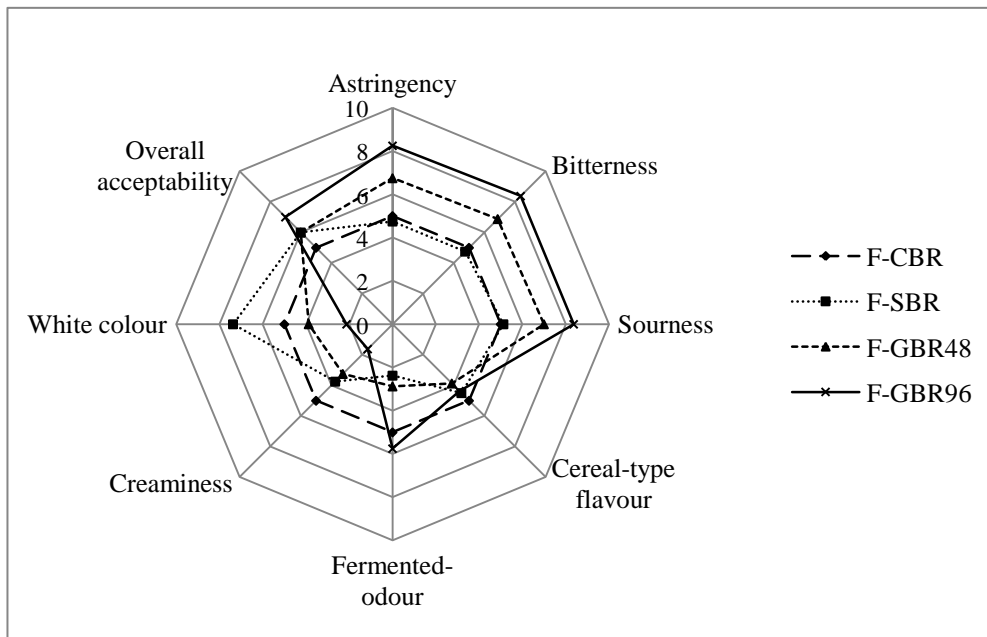


Table 1. Proximate composition (g/100 g) and energy (Kcal/100 g) of non-fermented (NF) and fermented (F) formulations obtained from crude brown rice (CBR), soaked brown rice (SBR) and germinated brown rice for 48h (GBR48) and 96h (GBR96).

	Water	Protein	Fat	Carbohydrates	Ash	Energy
<u>Non-fermented products</u>						
NF-CBR	84.57±0.40 ^b ₂	0.89±0.07 ^a ₁	0.48±0.00 ^a ₁	13.85±0.36 ^a ₁	0.22±0.03 ^a ₁	64.25±1.89 ^{ab} ₁
NF-SBR	84.40±0.53 ^b ₁	0.80±0.02 ^a ₁	0.37±0.06 ^a ₁	14.26±0.46 ^{ab} ₁	0.18±0.02 ^a ₁	64.11±1.84 ^a ₁
NF-GBR48	83.33±0.58 ^a ₁	0.80±0.06 ^a ₁	0.46±0.08 ^a ₁	15.24±0.73 ^b ₁	0.16±0.03 ^a ₁	69.28±2.28 ^c ₂
NF-GBR96	83.67±0.58 ^{ab} ₁	0.81±0.04 ^a ₁	0.49±0.06 ^a ₁	14.91±0.54 ^b ₁	0.12±0.01 ^a ₁	68.00±1.99 ^{bc} ₁
<u>Fermented products</u>						
F-CBR	82.97±0.46 ^a ₁	0.97±0.02 ^b ₁	0.48±0.06 ^b ₁	15.37±0.51 ^b ₂	0.21±0.02 ^{bc} ₁	70.29±2.14 ^b ₂
F-SBR	84.43±0.51 ^b ₁	0.88±0.02 ^a ₂	0.39±0.05 ^a ₁	14.08±0.51 ^a ₁	0.22±0.01 ^c ₂	63.87±2.01 ^a ₁
F-GBR48	84.27±0.75 ^b ₁	0.86±0.06 ^{a1}	0.49±0.03 ^b ₁	14.20±0.77 ^{ab} ₁	0.18±0.02 ^{ab} ₁	65.13±2.24 ^a ₁
F-GBR96	83.83±0.76 ^{ab} ₁	0.92±0.03 ^{ab} ₂	0.54±0.03 ^b ₁	14.54±0.72 ^{ab} ₁	0.17±0.02 ^a ₂	67.03±2.26 ^{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)
<u>Non-fermented products</u>					
NF-CBR	0.54±0.05 ^c ₁	0.53±0.02 ^a ₂	14.97±0.15 ^a ₁	6.20±0.20 ^b ₂	1,054.7±4.51 ^b ₁
NF-SBR	0.42±0.05 ^b ₁	0.53±0.03 ^a ₁	15.00±0.10 ^a ₁	6.17±0.21 ^b ₂	1,053.3±4.16 ^{ab} ₁
NF-GBR48	0.20±0.03 ^a ₁	0.62±0.03 ^b ₁	15.27±0.06 ^b ₁	5.97±0.06 ^{ab} ₂	1,047.3±3.06 ^a ₁
NF-GBR96	0.13±0.02 ^a ₁	0.82±0.05 ^c ₂	15.37±0.06 ^b ₂	5.77±0.06 ^a ₂	1,047.0±1.00 ^a ₁
<u>Fermented products</u>					
F-CBR	2.07±0.25 ^c ₂	0.42±0.02 ^a ₁	14.93±0.06 ^a ₁	4.33±0.06 ^a ₁	1,053.0±3.46 ^a ₁
F-SBR	1.59±0.04 ^b ₂	0.52±0.04 ^b ₁	14.93±0.06 ^a ₁	4.30±0.10 ^a ₁	1,054.3±2.08 ^a ₁
F-GBR48	1.45±0.10 ^{ab} ₂	0.57±0.01 ^c ₁	15.13±0.15 ^b ₁	4.23±0.06 ^a ₁	1,052.0±5.29 ^a ₁
F-GBR96	1.27±0.04 ^a ₂	0.72±0.03 ^d ₁	15.23±0.06 ^b ₁	4.27±0.06 ^a ₁	1,053.3±2.08 ^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) ($p \leq 0.05$, 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 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 (μ g TE/100g)	ACE-inhibitory activity (%)
<u>Non-fermented products</u>					
NF-CBR	9.89 \pm 0.26 ^b ₁	0.05 \pm 0.01 ^a ₁	0.16 \pm 0.01 ^c ₁	28.22 \pm 3.94 ^a ₁	10.73 \pm 1.65 ^a ₁
NF-SBR	8.57 \pm 0.68 ^a ₁	0.05 \pm 0.01 ^a ₁	0.13 \pm 0.01 ^a ₁	24.23 \pm 1.57 ^a ₂	12.73 \pm 1.40 ^a ₁
NF-GBR48	9.81 \pm 0.52 ^b ₁	0.47 \pm 0.03 ^b ₁	0.14 \pm 0.01 ^b ₁	24.78 \pm 3.09 ^a ₁	25.17 \pm 2.32 ^b ₁
NF-GBR96	16.19 \pm 0.37 ^c ₁	0.74 \pm 0.11 ^c ₁	0.16 \pm 0.01 ^c ₁	49.48 \pm 0.48 ^b ₁	26.05 \pm 1.91 ^b ₁
<u>Fermented products</u>					
F-BR	13.49 \pm 0.76 ^c ₂	0.28 \pm 0.02 ^a ₁	0.19 \pm 0.01 ^d ₂	32.46 \pm 2.17 ^b ₂	16.46 \pm 1.99 ^a ₂
F-SBR	9.89 \pm 0.48 ^a ₂	0.36 \pm 0.08 ^a ₂	0.12 \pm 0.01 ^a ₁	21.18 \pm 2.37 ^a ₁	24.62 \pm 1.79 ^b ₂
F-GBR48	12.41 \pm 0.55 ^b ₂	0.88 \pm 0.10 ^b ₂	0.15 \pm 0.01 ^b ₁	23.51 \pm 2.04 ^a ₁	44.04 \pm 3.07 ^c ₂
F-GBR96	15.20 \pm 0.65 ^d ₁	1.86 \pm 0.19 ^c ₂	0.16 \pm 0.01 ^c ₁	46.93 \pm 3.54 ^c ₁	61.47 \pm 1.61 ^d ₂

Data are the mean \pm 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) ($p \leq 0.05$, 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.