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3 **Extruded snacks from pigmented rice: nutritional and physical**  
4 **properties**

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19

20 **Abstract**

21 This study arose from the need to meet the request of consumers for healthy, ready-to-eat  
22 foods. The behavior of pigmented rice was investigated during extrusion in terms of  
23 nutritional (i.e., phenolic acids, anthocyanins and antioxidant capacity (AC)) and physical  
24 (i.e., starch pasting properties, texture, porosity and biometric indices) properties. Snacks  
25 were produced from brown, red, and black rice, by means of a co-rotating twin-screw  
26 extruder, and their features were compared with those of snacks from white rice.

27 Although the soluble and cell-wall bound phenolic acid contents of pigmented and brown  
28 rice did not differ, the red and black rice both resulted in a higher AC after extrusion. Black  
29 rice showed the highest anthocyanin and AC contents, even after extrusion. Furthermore,  
30 the high phenolic compound content of black rice affected the starch pasting properties, and  
31 led to a lower viscosity and higher snack porosity than those of brown rice. Nevertheless,  
32 considering both the biometric and textural properties, the best results were obtained from  
33 red rice. Optimizing the extrusion conditions will help reduce the loss of anthocyanins in  
34 snacks made of pigmented rice, increase the nutritional value and improve the physical  
35 properties of the product.

36 **Keywords**

37 phenolic acids; anthocyanins; antioxidant activity; extrusion

38

39 **Abbreviations**

40 ABTS, 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; AC, antioxidant capacity; AC-

41 ABTS, antioxidant capacity obtained by means of an ABTS assay; AC-FRAP, antioxidant

42 capacity obtained by means of a FRAP assay; ANOVA, analysis of variance; CY-3-GLC,

43 cyanidin-3-O-glucoside; cv, cultivar; CWBPA, cell-wall bound phenolic acid; DW, dry weight;

44 FRAP, Ferric Reducing Antioxidant Power; PE-3-GLC, peonidine-3-O-glucoside; REGWF,

45 Ryan-Einot-Gabriel-Welsh F post-hoc test; RP-HPLC-DAD, reverse phase high-

46 performance liquid chromatography-photodiode array detector; SPA, soluble phenolic acid;

47 TAC, total anthocyanin content.

48

## 49 1 Introduction

50 Rice (*Oryza sativa* L.) is a staple food in many countries and is commonly consumed as  
51 white (polished or milled) rice, which is obtained by removing the bran and germ fractions  
52 from the kernels, to guarantee a longer storage and shelf-life and a better palatability after  
53 cooking. Nowadays, the awareness of consumers about the nutritional benefits of whole  
54 grains, which are characterized by a unique blend of bioactive compounds, with several  
55 phytochemicals exerting antioxidant capacity (AC), has led to a significant increase in their  
56 interest (Melini et al., 2019).

57 Rice bran contains large amounts of fiber and other bioactive compounds, including vitamin  
58 B complex, tocopherols, tocotrienols and oryzanols (Hu et al., 2018), whose consumption is  
59 related to several health benefits (e.g., enhancement of the immune system, and reduction  
60 in the risk of developing heart disease and cancer). Phenolic acids are the most common  
61 secondary metabolites in cereals. The large amount of phenolic acids in rice, as well as in  
62 other cereals, exists in an insoluble-bound form (Pang et al., 2018) and they directly  
63 influence their antioxidant capacity.

64 Moreover, some bioactive compounds are responsible for the color of the rice pericarp. For  
65 example, red rice is characterized by the presence of pro-anthocyanidins, which mainly  
66 consist of catechin and epicatechin block units (Gunaratne et al., 2013). Moreover, black  
67 rice contains anthocyanins, such as cyanidin-3-O-glucoside (Cy-3-glc) and peonidine-3-O-  
68 glucoside (Pe-3-glc) (Hiemori et al., 2009).

69 Rice is one of the most frequently used commodities in the gluten-free industry, as it is  
70 suitable for the production of ready-to-eat foods, such as snacks (Dalbhagat et al., 2019).  
71 Increased consumer attention to the nutritional aspects of food products has led to the  
72 demand for healthier and more nutritious snacks than those currently available on the  
73 market. However, healthier snacks that are rich in fiber may present some issues in physical  
74 features (i.e., structure and texture). Indeed, an increased hardness, due to a reduced

75 expansion and density, is among the most critical effects of adding fiber to puffed snacks  
76 (Robin et al., 2012). On the other hand, there is still little information on the effect of fiber-  
77 enrichment on the physical features of co-extruded snacks. The latter are characterized by  
78 a compact structure, with an outer shell that is later filled with either a savory or sweet filling.  
79 Although the characterization of bioactive compounds in pigmented rice has been widely  
80 addressed (Bordiga et al., 2014; Melini et al., 2019; Pang et al., 2018; Shao et al., 2018),  
81 the effect of extrusion has only been investigated occasionally (Hu et al., 2018; Zhang et al.,  
82 2018).

83 Thus, the aim of this study has been to investigate the impact of extrusion on nutritional (i.e.,  
84 the phenolic acid and anthocyanin contents and AC) and physical (including bulk density,  
85 porosity, and texture) features of snacks made of conventional and pigmented rice (i.e., red  
86 and black rice cultivars, *cvs*). The results of the present study could provide new insights  
87 into the raw materials that are more suitable for the production of healthy snacks.

## 88 **2 Experimental**

### 89 **2.1 Rice flours**

90 A conventional brown variety (cv Ellebi; Bertone Sementi, Terruggia, Italy; classified as long  
91 B), a pigmented red variety (cv Ermes; Sa.Pi.Se, Vercelli, Italy; classified as long B) and a  
92 pigmented black variety (cv Venere, Sa.Pi.Se, Vercelli, Italy; classified as medium) were  
93 kindly provided by Molino Peila S.p.A. (Valperga, Italy). The rice cvs were cultivated in the  
94 same growing area in North West Italy in the 2018 growing season.

95 De-husked kernels were ground into flour (70% of particle size < 220 µm) in a roller-miller  
96 (Bühler Group, Uzwil Switzerland). Bran was completely removed from the kernel of cv Ellebi  
97 to obtain a white rice that was then ground into flour.

98 Flours were stabilized by means of an infrared heat treatment (120°C for 10 min, RI/1550,  
99 Brovind, Cortemilia, Italy) in order to reduce humidity (<10%) and increase the shelf-life, but  
100 also to act on the bacterial load.

101

### 102 **2.2 Snack production**

103 Co-extruded snacks were produced at an industrial level by Fudex Group S.p.A. (Settimo  
104 Torinese, Italy). Extrusion was performed using a co-rotating twin-screw extruder (model  
105 2FB90; screw speed: 100 rpm; temperature: 120 °C; pressure: 70 bar; feed moisture: 10%).

106 The snacks were stored at room temperature and used without any pre-treatment for the  
107 analysis described in section 2.8. Prior to quantification of the antioxidant capacity and the  
108 antioxidant compound content, all the flour and snack samples were ground to a fine powder  
109 (particle size < 500 µm) in a Cyclotec 1093 sample mill (Foss, Padova, Italy), and stored at  
110 -25°C until the beginning of the analyses.

111

### 112 **2.3 Proximate composition**

113 The moisture content of both the flours and snacks was determined by oven-drying at 105  
114 °C for 24 h. All the results were expressed on a dry weight (dw) basis.

115 The chemical composition was only determined for the rice flours. The total starch content  
116 was measured according to the standard AACC 76-13.01 method (AACC, 2001), the total  
117 protein content according to the AOAC 990.03 (Dumas method conversion factor: 5.7), the  
118 total dietary fiber according to the AOAC 985.29, enzymatic-gravimetric method, while the  
119 fat (AOAC 2003.05, Soxhlet method) and ash (AOAC 923.03, muffle furnace) contents were  
120 determined according to the AOAC (2005) procedures.

121

#### 122 **2.4 Soluble (SPA) and cell wall-bound (CWBPA) phenol acids**

123 The extraction and quantification of the SPAs (free and conjugated) and CWBPAs were  
124 performed according to the procedure reported by Giordano et al. (2019).

125 The phenolic extracts were filtered through a 0.2 µm filter and then analyzed by means of  
126 an Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, USA) high-performance  
127 liquid chromatograph coupled with an Agilent 1200 series diode array detector. Separations  
128 were carried out using a 150 x 4.6 mm, 5 µm particle size Gemini RP-18 column  
129 (Phenomenex, Torrance, CA, U.S.A), as reported by Giordano et al. (2019).

130

#### 131 **2.5 Total anthocyanin content (TAC)**

132 The TAC was determined by means of the pH differential method. Each sample (1 g) was  
133 extracted in 8 mL of ethanol acidified with 1 N HCl (85:15, v/v) for 30 min. After centrifugation  
134 at 20,800 x g for 2 min, the absorbance was read at 540 nm, as reported by Siebenhandl et  
135 al. (2007). TAC was expressed as mg Cy-3-glc equivalents/kg of sample (dw).

136

#### 137 **2.6 Antioxidant capacity (AC)**

138 The total AC was determined by means of FRAP (Ferric Reducing Antioxidant Power) and  
139 ABTS [2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] assays adapted from the  
140 QUENCHER method, as described by Giordano et al. (2019).

141 The results were expressed, through a calibration curve, as mmol Trolox equivalents kg<sup>-1</sup> of  
142 sample (dw).

143

## 144 **2.7 Pasting properties**

145 The pasting properties of both the flours and grinded snacks were evaluated using a Micro  
146 Visco-Amylo-Graph (Brabender GmbH., Duisburg, Germany), according to Bresciani et al.  
147 (2021), using 12 g samples in 100 ml of distilled water and adopting the following  
148 temperature profile: heating from 30 up to 95°C, holding at 95°C for 20 minutes and cooling  
149 from 95 to 30°C with a heat/cooling rate of 3°C min<sup>-1</sup>.

150

## 151 **2.8 Physical properties of the snacks**

152 The section area and inner area of the cross sections of the snacks were measured by  
153 means of image analyses, as reported by Bresciani et al. (2021). Cylindrical shaped snacks  
154 were cut using a cutter, and images of the cross sections were acquired, at 300 dots per  
155 inch, with a digital scanner (Epson Perfection 550 Photo, Seiko Epson Corp., Suwa, Japan).  
156 The images were processed at the gray level (8 bits). Image analyses were performed using  
157 Image ProPlus software (v6; Media Cybernetics, Inc., Rockville, US).

158 Porosity and bulk density were assessed with a Pascal Mercury Porosimeter (P240; Thermo  
159 Fisher Scientific, Waltham, US), as reported by Bresciani et al (2021). Samples were  
160 subjected to an increasing pressure of up to 200 MPa, and pores with a radius of  $3.7 \times 10$   
161  $-3$  to 50  $\mu\text{m}$  were measured. The textural properties of the snacks were determined, as



162 reported by Bresciani et al. (2021), by means of the three-point bend method, using a TA –  
163 XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 10  
164 kg (100 N) load cell.

165

## 166 **2.9 Statistical analysis**

167 The SPA and CWBPA contents, TAC and AC were measured in triplicate, whereas the  
168 image and texture analysis were carried out on ten and thirty pieces, respectively. The  
169 pasting properties, bulk density and porosity were measured in duplicate.

170 Analysis of variance (ANOVA) was performed with SPSS for Windows statistical package,  
171 Version 24 (SPSS Inc., Chicago, Illinois, USA). Significant differences ( $p < 0.05$ ) between the  
172 samples were determined using the Ryan-Einot-Gabriel-Welsh F (REGWF) test.

173

## 174 **3 Results and discussion**

### 175 **3.1 Proximate composition**

176 The chemical composition of the raw materials is shown in Supplementary Table 1. The  
177 differences between white and brown rice (belonging to the same cv) were consistent with  
178 those reported in the literature. The removal of the bran and germ fractions from the brown  
179 rice in fact led to white rice with lower amounts of lipids, fiber, and ash. The red rice cv  
180 showed the lowest fiber content (3.8 g 100 g<sup>-1</sup>). On the other hand, no differences were  
181 detected for the starch, lipid, or ash contents between the conventional and pigmented rice  
182 cvs. The chemical composition of the pigmented rice agreed with recent findings on the  
183 same cvs (Melini et al., 2019).

184

### 185 **3.2 SPA and CWBPA contents**

186 The effect of the extrusion process on the SPAs and CWBPAs is shown in Figure 1. The  
187 brown, red, and black rice contained more SPAs and CWBPAs than the white rice, since  
188 these compounds are mostly concentrated in the bran fraction (Zhang et al., 2018). Indeed,  
189 the removal of bran from the conventional cv reduced the SPAs and CWBPAs by 71% and  
190 63%, respectively. Ferulic, sinapic and hydroxybenzoic acids are the most abundant SPAs  
191 in white rice, with concentrations of 37%, 28% and 23%, respectively (considering the total  
192 SPA content). Instead, the above-mentioned phenolic acids represent 36%, 39% and 13%,  
193 respectively, of the total SPAs in brown rice. Considering the CWBPAs, ferulic, *p*-cumaric  
194 and sinapic acids are the most abundant compounds in both white and brown rice, with a  
195 higher contribution of *p*-cumaric to brown rice (25%) than to the white one (6%). The  
196 insoluble-bound forms, whereby the acids are esterified to cell walls, are more abundant  
197 than the soluble ones, including soluble-free and soluble-conjugated acids (Shao et al.,  
198 2014b). Ferulic and *p*-cumaric acid are the main phenolic acids in rice, and most of them  
199 are bound to cell wall polysaccharides and lignin (Zaupá et al., 2015).

200 The total phenolic acid content in the brown, red, and black rice was similar, while  
201 differences were observed in their relative composition. The brown and red rice samples  
202 showed a similar profile: sinapic (42%), ferulic (32%) and hydroxybenzoic (13%) acids were  
203 the most abundant SPAs, while ferulic (67%), *p*-cumaric (25%) and sinapic (6%) acids were  
204 the most abundant CWBPAs. Conversely, black rice resulted in a different phenolic acid  
205 composition: sinapic (35%), ferulic (28%) and vanillic (15%) acids were the most abundant  
206 SPAs, while ferulic (68%), protocatechuic (14%) and *p*-cumaric (8%) acids were the most  
207 abundant CWBPAs. In agreement with Shao et al. (2018), larger amounts of protocatechuic  
208 and vanillic acids were detected in black rice rather than in brown rice. The insoluble  
209 protocatechuic acid content (71.5 mg kg<sup>-1</sup>) found in black rice was similar to that reported in  
210 previous works (Zaupa et al., 2015).

211 This is the first study that has directly compared the impact of extrusion on the total phenolic  
212 acid content of conventional (brown and white) and pigmented (red and black) rice cvs. So  
213 far, only black rice has been used to investigate the effects of either different extrusion  
214 temperature (Hu et al., 2018) or milling fractions (Ti et al., 2015) on the phytochemical and  
215 antioxidant properties of rice products.

216 The extrusion process resulted in a significant decrease (-16%) in SPAs, albeit only for black  
217 rice. Furthermore, extrusion also reduced the soluble sinapic acid and ferulic acid in the  
218 conventional white and brown rice cvs. The greatest reduction was detected for sinapic acid,  
219 that is, of 84%, 65% and 43% in white, black and brown rice, respectively. The ferulic acid  
220 content was reduced by 77%, 44% and 30%, in the white, black, and brown rice,  
221 respectively. Hu et al. (2018), studying the role played by the extrusion temperature, showed  
222 a reduction in the soluble sinapic and ferulic acid contents in black rice as the extrusion  
223 temperature increased, although quite similar reduction percentages (-62% and -40% for  
224 soluble sinapic and ferulic acids, respectively) to those of the present study were reached

225 when the extrusion temperature was set at 120 °C. Moreover, Zhang et al. (2018) reported  
226 a significant decrease in soluble ferulic acid of 39%, after extruding brown rice at 120 °C.  
227 The CWBPA content of all the cvs increased following extrusion: +33% for brown rice; +8.5%  
228 for red rice and +20% for black rice, although the increase was only significant for brown  
229 rice. An increase in the insoluble phenolic compounds occurred for brown rice, due to the  
230 polymerization of free phenolic acids, which consequently decreased upon extrusion. This  
231 result could be accentuated by the effect of increased temperature, pressure and shear force  
232 (Zeng et al., 2017). The highest increases detected for the more abundant CWBPAs were  
233 for hydroxybenzoic acid (+153%) and sinapic acid (+141%). A significant increase in  
234 protocatechuic acid (+32%) was only observed for black rice after extrusion. This effect is  
235 supposed to be due to the degradation of Cy-3-Glc, which is converted into protocatechuic  
236 acid (Hiemori et al., 2009). The *p*-cumaric, vanillic and ferulic acid contents remained  
237 approximately stable in all the samples after extrusion, in agreement with Ti et al. (2015).  
238 Zhang et al. (2018) highlighted that, in comparison with other thermal processing methods,  
239 extrusion usually has less effect on the phenolic acid contents of raw materials due to the  
240 short heat exposure time.

241

### 242 **3.3 Total anthocyanin content (TAC)**

243 The black rice showed a significantly higher TAC than the red and brown rice, in agreement  
244 with Bordiga et al. (2014). Previous works regarding the qualitative profile of anthocyanins  
245 in black rice consistently reported the prevalence of Cy-3-glc, which represents more than  
246 80% of the total anthocyanin content in some varieties, and the presence of Pe-3-glc as the  
247 second most relevant anthocyanin (Melini et al., 2019; Shao et al., 2014a).

248 The TAC in red rice did not differ significantly from the value found in both the brown and  
249 white rice. There are conflicting data in the literature on the presence of anthocyanins in red  
250 rice. The TAC of red rice (17.8 mg kg<sup>-1</sup>) in this study was similar to the one registered by

251 Ziegler et al. (2018) (13.8 mg kg<sup>-1</sup>). Melini et al. (2019) found traces of malvidin in one of the  
252 three varieties of red rice they tested, and Pereira-Caro et al. (2013) observed Cy-3-glc in a  
253 red rice cultivar from France. In contrast, no anthocyanins were found in red rice varieties  
254 from Korea (Kim et al., 2010) or Sri Lanka (Gunaratne et al., 2013).  
255 The extrusion of black rice significantly reduced the TAC by 90%, in agreement with Ti et al.  
256 (2015), who reported a TAC reduction of 88%. Despite the overall loss in TAC, the snacks  
257 from black rice maintained the highest value (Figure 2). Hu et al. (2018) instead reported  
258 that increasing the extrusion temperature from 90 °C to 110 °C did not affect the TAC  
259 content. They also observed a significant decrease in Cy-3-glc and Pe-3-glc contents when  
260 black rice was extruded at 90 °C. It has been observed that the cooking temperature is the  
261 factor that influences the stability of anthocyanins the most (Hiemori et al., 2009). The high  
262 extrusion temperature (120°C), together with high shear forces applied in the present study,  
263 may have reduced the TAC more consistently than other cooking processes reported in the  
264 literature.

265

### 266 **3.4 Antioxidant capacity (AC)**

267 The AC of the brown rice was significantly higher (+273% and +169% for FRAP and ABTS)  
268 than that of the white rice. A further significant increase in AC was detected in the red and  
269 black rice (+169% and + 154% for FRAP and ABTS, respectively), in accordance with  
270 previous studies (Pang et al., 2018; Shao et al. 2018). However, no differences were found  
271 between the red and black rice cvs (Figure 3). Whereas, in other studies, black rice exhibited  
272 a higher AC than red or brown rice (Zaupa et al., 2015).

273 The extrusion of the white rice did not affect the AC to any great extent. On the other hand,  
274 the AC<sub>ABTS</sub> of the brown rice increased (+16%) after extrusion, likely due to the production  
275 of dark color pigments (particularly melanoidins), which are known to have antioxidant  
276 activity (Patil and Kaur, 2018). Conversely, as assessed in both of the AC assays, the

277 extrusion process caused a decrease in AC of the red rice of -20% (ABTS) and -33%  
278 (FRAP). The AC of the black rice instead showed no significant differences after extrusion,  
279 although Ti et al. (2015) showed a decrease (-15%).

280 Phenolic compounds are among the substances that are mainly responsible for the  
281 antioxidant activity of rice (Shao et al., 2014b). The significant reduction in TAC and SPAs  
282 in the black rice following extrusion was possibly compensated by the increase in CWBPAs  
283 and the formation of other antioxidant compounds as a result of the Maillard reaction.  
284 Moreover, other classes of non-phenolic compounds (such as carotenoids and  $\gamma$ -oryzanols)  
285 present in rice can contribute to its antioxidant capacity. According to Melini et al. (2019),  
286 black rice has a greater nutraceutical potential than red rice. Focusing on the  
287 phytochemicals found in extruded snacks, our study has clearly highlighted the higher AC  
288 exerted in the final products of pigmented rice cvs than in conventional ones. The black rice  
289 in particular, despite the reduction observed after production, maintained a good  
290 anthocyanin content in the snacks (Figure 2). Moreover, this raw material seemed to prevent  
291 a drop in AC after the extrusion process, as observed for other cvs (Figure 3).

292

### 293 **3.5 Pasting properties**

294 The pasting profiles of the raw materials are reported in Figure 4. The brown rice showed a  
295 higher peak viscosity ( $538.5 \pm 20.5$  BU) than the related white rice ( $429.5 \pm 26.1$  BU), although  
296 the removal of bran from kernels generally leads to an increase in peak viscosity (Mariotti et  
297 al., 2009). The observed differences in peak viscosity could be due to bran, which absorbs  
298 water during heating, thus increasing the paste viscosity. On the other hand, the viscosity  
299 after cooling (final viscosity) was higher in white rice ( $733 \pm 35.3$  vs  $668 \pm 14.1$  BU). This  
300 could be due to the higher starch content in white rice than in brown rice (Supplementary  
301 Table 1). The brown and red rice showed a higher peak viscosity ( $538.5 \pm 20.5$  BU and  
302  $416 \pm 2.8$  BU, respectively) than the black rice ( $306 \pm 31.1$  BU). During cooling, the lowest

303 final viscosity and setback (i.e., the difference between the final viscosity at 30°C and the  
304 viscosity reached at the end of the holding period) were observed in the black rice ( $418 \pm 0.1$   
305 BU and  $285 \pm 7.7$  BU, respectively), followed by the red ( $587.5 \pm 3.5$  BU and  $385.5 \pm 4.9$  BU,  
306 respectively) and brown ( $733 \pm 35.3$  BU and  $489.5 \pm 43.13$  BU, respectively) rice.

307 Similar results have been reported by Lang et al. (2020), who attributed the differences in  
308 pasting properties in pigmented rice to differences in the phenolic compounds (Zhu, 2015).  
309 Indeed, the black rice used in this study was characterized by the highest SPAs (Fig. 1a)  
310 and TAC (Fig. 2). Phenolic compounds may compete with starch for water, thus affecting  
311 starch water absorption and gelatinization (Zhu et al., 2008), thereby resulting in a low  
312 viscosity. In addition, the hydroxyl groups of polyphenols may interact directly with starch,  
313 thus altering its properties (Zhu et al., 2008). During cooling, due to their ability to interact  
314 with both water and starch hydroxyl groups, phenolic compounds may limit starch  
315 interactions and thus its retrogradation (Zhu, 2015). Moreover, certain phenolic compounds  
316 may have a great ability to complex with amylose and/or proteins (Lang et al., 2020). Overall,  
317 the effect of these compounds on the pasting properties mainly appears to be dependent on  
318 the type of starch, the structure of the specific phenolic compound, and their capability to  
319 absorb water, as recently reviewed by Zhu (2015).

320 The pasting profile of the snacks (not shown here) indicated a total loss of viscosity after  
321 extrusion, irrespective of the type of rice, thus suggesting the loss of the crystalline order in  
322 starch granules. Similar results were obtained when extrusion was applied to various  
323 matrices, including corn (Bresciani et al., 2021).

324

### 325 **3.6 Snack characteristics**

326 So far, the interest in using pigmented rice for the production of snacks has almost  
327 exclusively concerned, although not in a systematic way, the optimization of the extrusion  
328 conditions and their effects on the phenolic profiles and/or antioxidant activity of the

329 extrudates. Little attention has been paid to the physical characteristics of such snacks. In  
330 addition, the wide range of products – which are different in shape, size and structure -  
331 obtained from extrusion makes the comparison of the results with previous findings difficult.  
332 The present study has focused on a co-extruded snack, characterized by an outer shell that  
333 can be filled with either a savory or sweet filling. Since the filling needs to be contained within  
334 the snack, a compact structure is needed.

335 The features of the snack (i.e., section and inner area, porosity, bulk density and hardness)  
336 are shown in Table 1. The section area could be considered an index of the expansion  
337 degree, considering that the extruder die was not changed during the extrusion trials.  
338 Compared to the snacks from white rice, the section area and bulk density of those obtained  
339 when using brown rice decreased significantly. This result may be attributed to differences  
340 in the chemical composition of the rice samples (Supplementary Table 1). Indeed, the fiber  
341 content – thanks to its high water affinity - decreased the expansion capability (Dalbhagat  
342 et al., 2019). Lipids could act as an inhibitor by reducing the degree of gelatinization, thus  
343 reducing the expansion of snacks (Lin et al. 1997). Moreover, the high protein content –  
344 from the formation of intermolecular disulphide linkages as a result of the heat treatment –  
345 might reduce starch swelling and thus the expansion of extrudates (Dalbhagat et al., 2019).  
346 It was not possible to determine the porosity of the snacks made from the white rice because  
347 the macropores were larger than the measurement range. In addition, large pores were  
348 dissected by the cutter, thus creating open cavities that the instrument considered as non-  
349 pores.

350 When comparing the snacks from the brown, red and black rice, the highest section area  
351 and the lowest inner area were found in the brown rice samples, a result that is consistent  
352 with the lowest hardness value (Table 1). On the other hand, the biometric characteristics  
353 of the snacks made of red rice (i.e., low section area and high inner area) suggested a  
354 compact wall area (i.e., the part that separates the cavity from the outer wall) accounting for



355 high resistance to breaking. The negative relationship between expansion capability (or  
356 section area) and hardness has already been shown (Bresciani et al., 2021; Robin et al.,  
357 2012). Similar hardness values to the control (white rice snack), together with a high inner  
358 area, make the red rice snacks ideal products for use on the co-extruded filled snack market.  
359 As far as porosity is concerned, the bulk density of all the samples increased as the value  
360 decreased. The black rice samples showed the highest porosity and therefore the lowest  
361 bulk density. Porosity makes a snack structure less resistant to breaking; indeed, this  
362 sample showed a low degree of hardness. Although the red and brown rice snacks showed  
363 similar porosity values, the bulk density was different. The red rice snacks recorded a lower  
364 bulk density than the brown rice snacks and resulted in a more compact structure, which  
365 was more resistant to breaking. This can also be noted from the section area values; the red  
366 rice snacks showed a lower degree of expansion and therefore a more compact structure  
367 than the brown rice snacks. Apart from the differences in chemical composition and starch  
368 properties, as well as the differences in kernel features might account for the differences in  
369 the snack characteristics. Indeed, it has been shown that rice obtained from varieties with a  
370 low length-breadth ratio (such as black rice, 2.1) undergo a greater expansion than products  
371 obtained from varieties with a higher length-breadth ratio (such as red rice, 3.7) (Gujral et  
372 al., 2002).

373

#### 374 **4 Conclusions**

375 Pigmented rice could be a suitable raw material for the production of healthy and gluten-free  
376 co-extruded snacks. The comparison of white and brown rice of the same cv has indicated  
377 that the latter has a higher bioactive compound content and higher antioxidant activities.  
378 Although the final SPA and CWBPA contents in the pigmented and brown rice snacks did  
379 not differ, the red and black cvs both resulted in a higher AC. The black rice (cv Venere) was  
380 the rice richest in anthocyanins and also the one with the highest AC, even after extrusion.  
381 Furthermore, the higher phenolic compound content in pigmented rice may affect the pasting  
382 properties, and contribute to lowering the viscosity in raw-materials and increasing the  
383 porosity of the snacks, compared to brown rice. Considering both the biometric and textural  
384 properties, the best results were obtained when using red rice (cv Ermes).

385 Such information may be used successfully by industries interested in producing rice  
386 products which are rich in health-promoting phytochemicals. Optimizing the extrusion  
387 conditions for the production of snacks from pigmented rice will allow the loss of phenolic  
388 compounds to be reduced, the nutritional value to be increased and the physical properties  
389 of the snacks to be improved. However, the acceptability of such snacks by consumers still  
390 needs to be further investigated to better understand how the process can be modeled to  
391 satisfy the consumers' needs.

392

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399

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1 **Figure captions**

2

3 **Figure 1.**

4 Soluble phenolic acid (SPAs; panel A) and cell wall-bound phenolic acid (CWBPA; panel  
5 B) contents of the raw material and snacks obtained from different rice cultivars.

6 Data are expressed on a dry weight basis. Bars with different letters are significantly different ( $p < 0.001$ ).

7

8 **Figure 2.**

9 Total anthocyanin content (TAC) in the raw material and snacks obtained from different rice  
10 cultivars.

11 Data are expressed on a dry weight basis. Bars with different letters are significantly different ( $p < 0.001$ ).

12

13 **Figure 3.**

14 Antioxidant capacity (AC), ABTS (panel A) and FRAP (panel B) assays in the raw material  
15 and snacks obtained from different rice cultivars.

16 Data are expressed on a dry weight basis. Bars with different letters are significantly different ( $p < 0.001$ ).

17

18 **Figure 4.**

19 Pasting profiles of the white, brown, red and black cultivars.

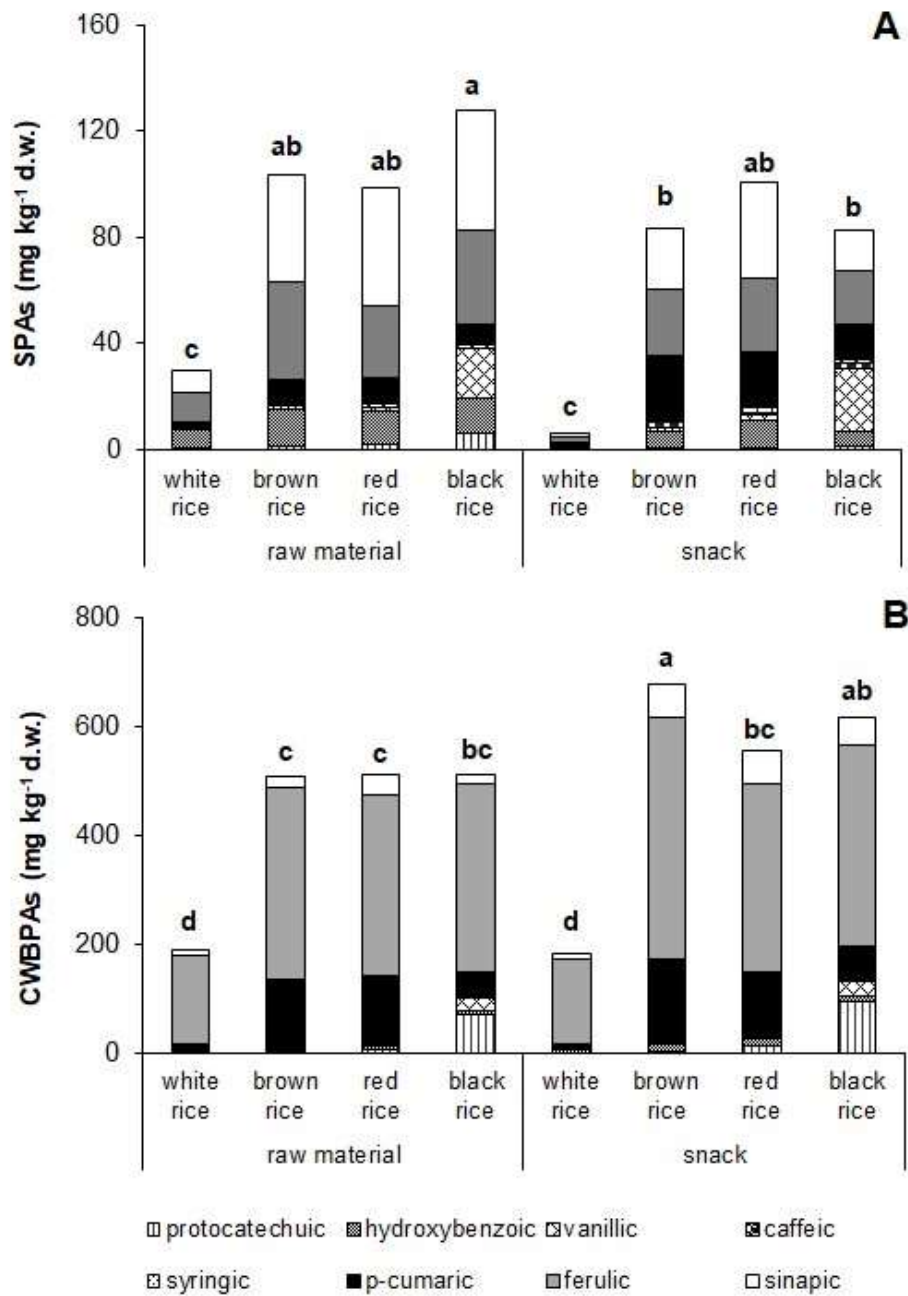
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21 Figures

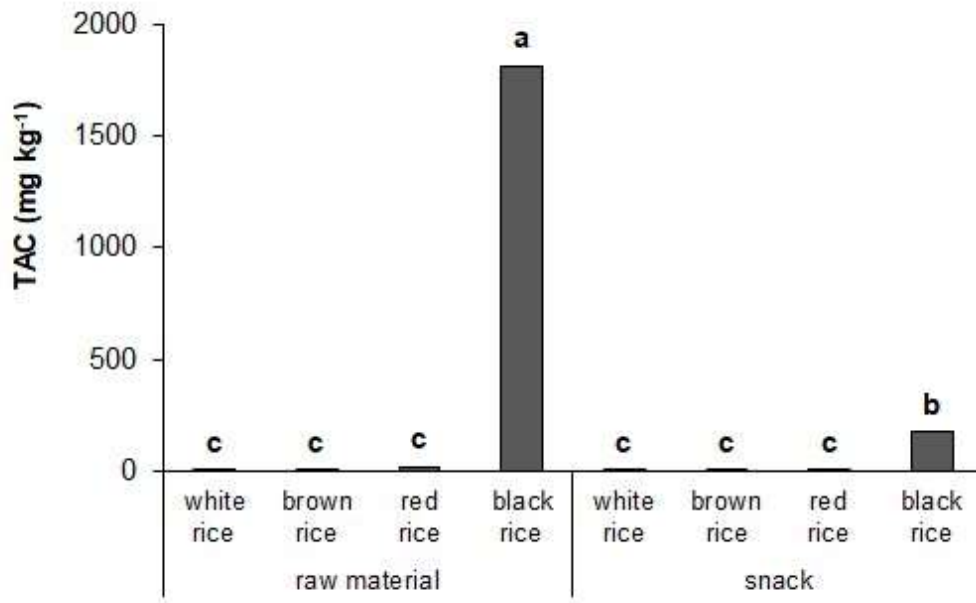
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23 Figure 1.



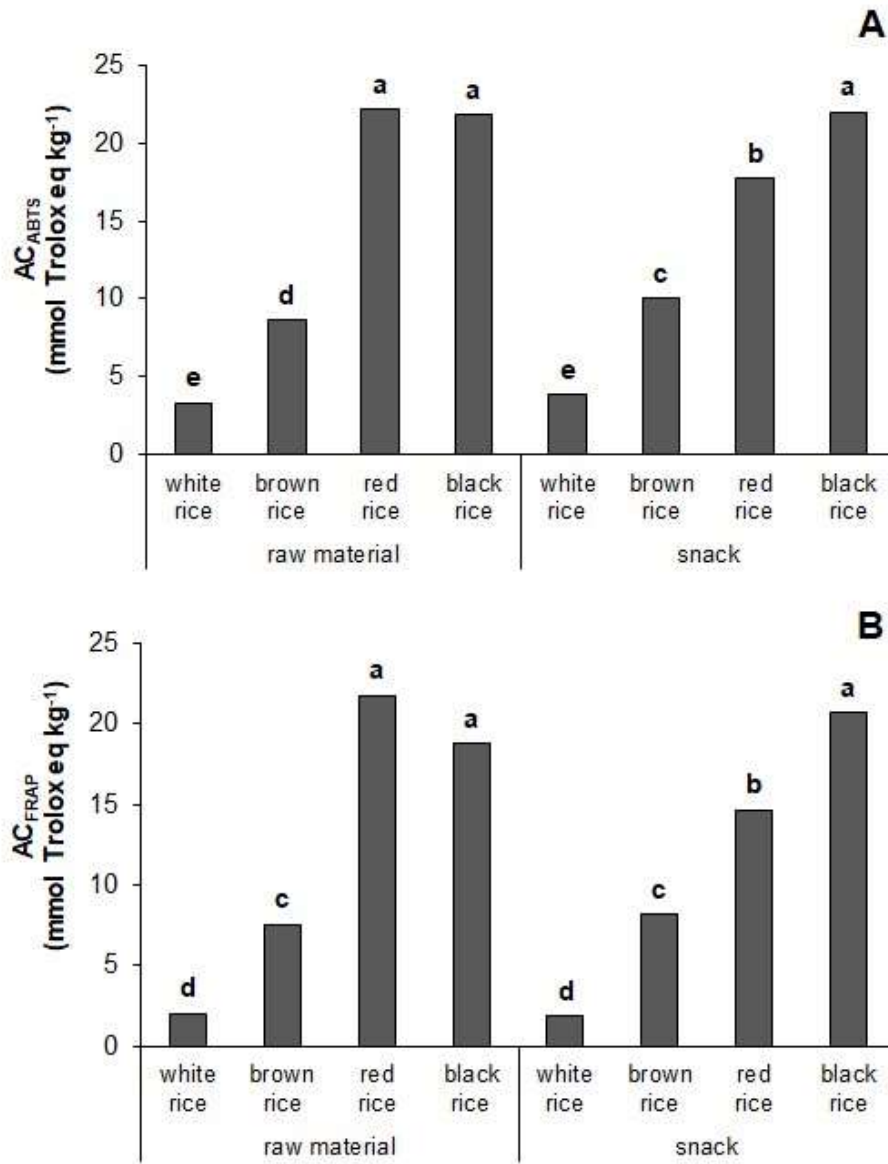
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25 **Figure 2.**

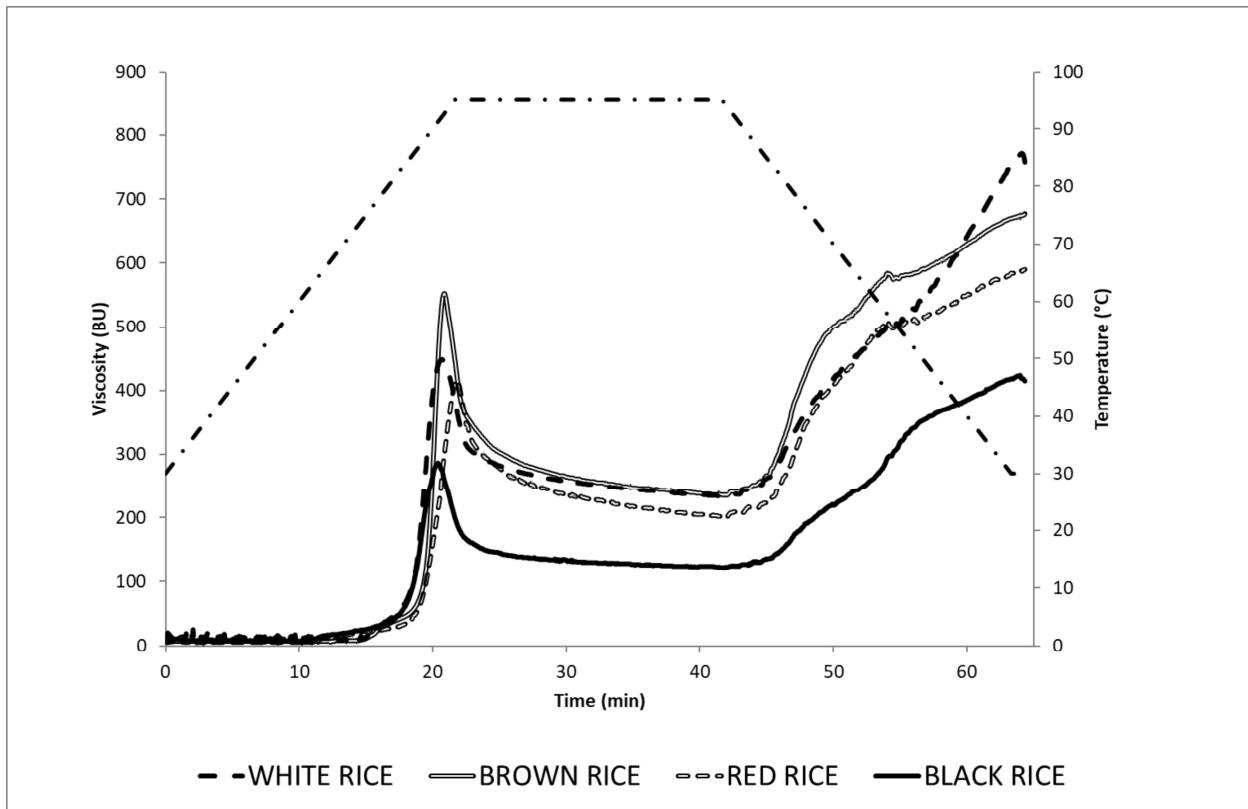


26

27 **Figure 3.**



29 **Figure 4.**



30

1 **Tables**

2

3 **Table 1.** Snack features of different rice cvs.

Parameters	White rice	Brown rice	Red rice	Black rice
Section area (mm <sup>2</sup> )	135.6 <sup>a</sup>	126 <sup>b</sup>	100 <sup>c</sup>	118 <sup>bc</sup>
Inner area (mm <sup>2</sup> )	15.3 <sup>bc</sup>	9.5 <sup>c</sup>	24.5 <sup>a</sup>	21.6 <sup>ab</sup>
Bulk density (gcm <sup>-3</sup> )	1.1 <sup>a</sup>	0.84 <sup>b</sup>	0.75 <sup>c</sup>	0.72 <sup>c</sup>
Porosity (%)	ND	45 <sup>b</sup>	47 <sup>b</sup>	56 <sup>a</sup>
Hardness (N)	46.3 <sup>a</sup>	35.1 <sup>b</sup>	43.9 <sup>a</sup>	36.9 <sup>b</sup>

4

5 Different letters in the same row are significantly different, according to the REGW-Q test ( $p < 0.001$ ).6 The reported values are based on thirty replications for harness, ten replications for section area and inner area and two  
7 replications for bulk density and porosity. ND, not determinable