

1 **Broccoli by-product as an attractive ingredient of gluten-free mini sponge cakes: evaluation of**
2 **batter viscosity and characteristics of the final product**

3

4 Urszula Krupa-Kozak^{1*}, Natalia Drabińska^{1,2}, Cristina M. Rosell², Angelo Lobina^{1,3},
5 Costantino Fadda³, Andrzej Anders⁴, Tomasz Jeliński⁵, Anita Ostaszyk⁶

6

7 ¹ *Department of Chemistry and Biodynamics of Food, Institute of Animal Reproduction and*
8 *Food Research of Polish Academy of Sciences, Olsztyn, Poland; u.krupa-*
9 *kozak@pan.olsztyn.pl; n.drabinska@pan.olsztyn.pl*

10 ² *Food Science Department, Institute of Agrochemistry and Food Technology (IATA-CSIC),*
11 *Paterna, Valencia, Spain; crosell@iata.csic.es*

12 ³ *Department of Agriculture, University of Sassari, Sassari, Italy; cfadda@uniss.it;*
13 *30034169@studenti.uniss.it*

14 ⁴ *Department of Engineering and Natural Raw Materials, University of Warmia and Mazury,*
15 *Olsztyn, Poland; anders@uwm.edu.pl*

16 ⁵ *Department of Chemical and Physical Properties of Food, Institute of Animal Reproduction*
17 *and Food Research of Polish Academy of Sciences, Olsztyn, Poland; t.jelinski@pan.olsztyn.pl*

18 ⁶ *Sensory Laboratory, Institute of Animal Reproduction and Food Research of Polish*
19 *Academy of Sciences, Olsztyn, Poland; a.ostaszyk@pan.olsztyn.pl*

20

21

22 * Corresponding author: Urszula Krupa-Kozak, tel: +48 89 523 46 18; fax: +48 89 524 01 24;
23 e-mail:u.krupa-kozak@pan.olsztyn.pl

24

25 **ABSTRACT**

26 The nutritional improvement of gluten free foods is still a challenge, because celiac patients
27 are looking for foods similar to gluten containing foods but providing the supplements they
28 need for ameliorating their deficiencies. This study aimed to assess the influence of broccoli
29 leaf powder (BLP) of previously confirmed nutraceutical properties on batter viscosity,
30 technological characteristics and sensory quality of gluten-free mini sponge cake (GFS). BLP
31 (2.5%, 5, 7.5 %; w/w) was incorporated into GFS formulation by replacing an equivalent
32 amount of starches. BLP decreased significantly ($p < 0.05$) the viscosity of experimental
33 batters and increased the instrumental firmness of GFS. Nevertheless, elasticity, crustiness,
34 mastication and adhesiveness of broccoli GFS were similar to the control GFS. GFS with
35 2.5% BLP was characterized by attractive green colour, small size and proper distribution of
36 pores, and was distinguished for its desirable sensory quality, although a slight cabbage aroma
37 and taste were perceived. As a conclusion, BLP addition should not exceed moderate
38 amounts to preserve good quality and palatability of GFS.

39

40 **Keywords**

41 Broccoli leaves; gluten-free; confectionary products; batter consistency; technological
42 properties; sensory quality

43

44

45 **1. Introduction**

46 Recent years have witnessed an exponential growth of the gluten-free (GF) market which has
47 become one of the most profitable segments of the food industry. This dynamic growth is the
48 result of the increasing detection of a spectrum of gluten-related disorders (Sapone et al.,
49 2012), in particular coeliac disease (CD). For those patients, GF products are the cornerstone
50 of therapy because the elimination of gluten from the daily diet is still the only available and
51 effective therapeutic method. A growing number of patients/consumers expect continuous
52 improvement in the nutritional value and palatability of GF products. The enhancement of the
53 quality of GF foods should be of fundamental interest to producers who are faced with a
54 growing demand for products of the type. Therefore, studies aiming to improve the quality
55 and nutritional value of GF products are of great importance.

56 In comparison with conventional wheat dough, GF batter is less cohesive and flexible, more
57 viscous and difficult to handle. Moreover, GF baked products are characterised by lower
58 technological quality, in particular by firm and crumbling texture, unattractive colour, faster
59 staling, and poorer consumer acceptance (Drabińska, Rosell, & Krupa-Kozak, 2018;
60 Drabińska, Zieliński, & Krupa-Kozak, 2016; Gallagher, Gormley, & Arendt, 2004). GF
61 products are also characterised by insufficient nutrients content (Saturni, Ferretti, & Bacchetti,
62 2010; T. Thompson, 1999; Tricia Thompson, Dennis, Higgins, Lee, & Sharrett, 2005; Matos
63 and Rosell, 2011). However, numerous studies have demonstrated that the technological and
64 sensory properties and the nutritional value of GF products can be improved by applying
65 natural GF cereals (millet, sorghum), pseudocereals (buckwheat, amaranth, quinoa) (Alvarez-
66 Jubete, Auty, Arendt, & Gallagher, 2009; Capriles & Arêas, 2014; Krupa-Kozak,
67 Wronkowska, & Soral-Śmietana, 2011), and proteins of animal and vegetable origin (Krupa-
68 Kozak, Baczek, & Rosell, 2013; Nunes, Ryan, & Arendt, 2009).

69 Sponge cake, muffins and biscuits are widely consumed around the world as breakfast or
70 evening snacks. These confectionary products are particularly popular among children and
71 adolescents on account of their taste and spongy texture. The quality of cakes is determined
72 by balanced formulae, batter aeration, stability of liquid batters in the early stage of baking
73 and the thermal-setting stage (Cauvain, 2003). In the production of GF cakes, wheat flour has
74 to be replaced with GF flours. Rice flour is the principal ingredient of GF muffins and cakes
75 (Marco & Rosell, 2008b; Turabi, Sumnu, & Sahin, 2008a). Other approaches of GF cake
76 production involve the application of corn and potato starches (Ronda & Roos, 2011).
77 However, the use of alternative ingredients in the production of GF cake remains rare
78 (Majzoobi, Poor, Jamalain, & Farahnaky, 2016; Talens, Álvarez-Sabatel, Rios, & Rodríguez,
79 2017). By-products from fruit and vegetable processing could be incorporated into GF
80 products as a low-cost source of nutrients and functional ingredients (O'Shea, Arendt, &
81 Gallagher, 2014). Hemp seed oil press-cake and decaffeinated green tea leaves have been
82 used to improve the antioxidant properties and nutritional value of GF crackers (Radočaj,
83 Dimić, & Tsao, 2014). Majzoobi et al. (2016) demonstrated that carrot pomace powder (up to
84 30%) had a positive effect on the quality of batter and the acceptability of GF sponge cake. In
85 a recent study by Talens et al. (2017), a microwave-dried orange by-product was incorporated
86 into the GF formulation to produce muffins characterised by an attractive colour, flavour and
87 texture.

88 Brassica vegetables, including broccoli, are a good source of proteins, dietary fibre and
89 bioactive compounds, in particular polyphenols which contribute to the prevention of diseases
90 associated with oxidative stress, such as cardiovascular and neurodegenerative diseases and
91 cancer (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005; Zhang et al., 2011).
92 Cruciferous vegetables also contain glucosinolates with chemopreventive properties (Higdon,
93 Delage, Williams, & Dashwood, 2007). Several studies have demonstrated that broccoli by-

94 products, in particular leaves, have similar chemical composition to broccoli florets (Campas-
95 Baypoli et al., 2009; Domínguez-Perles, Martínez-Ballesta, Carvajal, García-Viguera, &
96 Moreno, 2010), which suggests that these materials could be valuable functional food
97 additives. In our previous study (Drabińska, Ciska, Szmatowicz, & Krupa-Kozak, 2017),
98 broccoli leaf powder (BLP) obtained in laboratory conditions was determined as an ingredient
99 of a high antioxidant capacity and characterised by a high content of biologically active
100 glucosinolates. Moreover, the use of BLP in the production of GF mini sponge cakes (GFS)
101 significantly increased their antioxidant capacity, while the glucosinolate content of GFS was
102 higher than expected, which suggests the presence of a synergistic interaction between these
103 bioactive compounds and the food matrix. In view of our previous findings, the objective of
104 the present study was to assess the impact of BLP on batter viscosity characteristics,
105 technological parameters and sensory attributes of experimental GFS.

106

107 **2. Materials and Methods**

108 *2.1. Preparation of broccoli leaf powder*

109 Preparation of a broccoli leaf powder (BLP) was described previously (Drabińska et al.,
110 2017). Briefly, mature leaves of broccoli (*Brassica oleracea* L. var. italica cv. Sebastian)
111 without damage were washed and blanched in hot water for 1 min to inactivate enzymes
112 hydrolysing biologically active compounds. Afterwards, blanched leaf blades without petioles
113 and main midribs were freeze-dried and ground to obtain powder of particle size ≤ 0.60 mm.
114 The powder was stored in a tightly closed container for further application in experimental
115 gluten-free mini sponge cake (GFS) formulation.

116

117 *2.2. Preparation of experimental gluten-free mini sponge cakes*

118 Potato (30.6%) and corn (7.8%) starches, egg (43%), sugar (14%), sunflower oil (3.7%), salt
119 (0.2%) and gluten-free baking powder (0.7%) were the main ingredients of a control GF mini
120 sponge cake (GFS) formulation (Drabińska et al., 2017). BLP was incorporated into GFS in
121 the following proportions: B1 – 2.5%, B2 – 5%, B3 – 7.5% (w/w) by replacing an equivalent
122 amount of potato and corn starches in the control formulation. To prepare the GFS, egg white
123 and salt were mixed for 2 min at a high speed to form foam in a stainless steel bowl using a
124 planetary mixer KitchenAid Professional K45SS (KitchenAid Europa, Inc, Brussels,
125 Belgium). Then, egg yolk and sugar were added and vigorous mixing was continued for
126 another 3 min. Finally, starches, baking powder and oil were added, and all the ingredients
127 were mixed at a low speed for 3 min to obtain smooth homogenous batter. Batter portions of
128 30 g was dosed into a paper mould (50 mm diameter x 35 mm high). Twelve moulds were
129 arranged in three rows each of four mini sponge cakes on a baking tray and baked at 180 °C
130 for 25 min in electric oven (SVEBA DAHLEN, AB model DC-21, Sweden). Baked GFSs
131 were left to cool at room temperature for 1 h, then were packed in a clip-on polyethylene bags
132 and analysed at the day of preparation. Products of two independent batches were analysed.

133

134 *2.3. Pasting characteristic*

135 The pasting behaviour of GFS batter was evaluated using a Rapid Visco-Analyser (RVA-
136 4800; Perten Instruments, Madrid, Spain). A batter sample (8 g) was dispersed in 12 mL of
137 distilled water. Suspensions were stirred thoroughly at 600 rpm for 1 min at 30 °C. Then, the
138 temperature raised to 95 °C at a rate of 12 °C/min. The sample was maintained at 95 °C for 30
139 s, cooled to 50 °C at a rate of 12 °C/min and finally maintained at 50 °C for 2 min. The peak
140 temperature (P_{temp} , °C), peak viscosity (PV, cP), hot paste viscosity (HPV; cP), cold paste
141 (final) viscosity (CPV; cP), breakdown (PV-HPV; cP) and setback (CPV-HPV; cP) were
142 recorded. The experiments were conducted in duplicate.

143

144 *2.4. Physical parameters and instrumental colour analysis*

145 In order to determine the physical parameters of GFSs, weight (using a digital balance),
146 volume and surface area (using 3D NextEngine Scanner; NextEngine, Inc., Santa Monica,
147 USA) were determined. Weight loss was calculated as the percentage of the ratio between
148 weight of batter in the mould and the weight of the cake after baking, whereas a specific
149 volume was calculated as the ratio of volume and weight. The instrumental measurement of
150 colour of crumb and crust of experimental GFSs was evaluated using a HunterLab ColorFlex
151 (Hunter Associates Laboratory, Inc, Virginia, USA), and the results were expressed in
152 accordance with the CIELAB system. The parameters determined were L^* ($L^* = 0$ (black) and
153 $L^* = 100$ (white)), a^* ($-a^*$ = greenness and $+a^*$ = redness), and b^* ($-b^*$ = blueness and $+b^*$ =
154 yellowness). Values were the mean of twelve replicates.

155

156 *2.5. Instrumental texture analysis*

157 Textural properties of experimental GFSs were assessed on the day of preparation. Firmness
158 and springiness were determined using TA.HD Plus Texture Analyser (Stable Micro Systems
159 Ltd., UK) equipped with 5 kg load cell. Values obtained were the mean of six replicates.
160 The GFS samples were removed from clip-on polyethylene bag just prior to testing to retain
161 moisture. To standardise the procedure, the GFS were cut horizontally at the height of the
162 mould to form a flat surface, the upper part was discarded and the 2 cm-high lower part were
163 analysed. Sample was placed centrally under AACC 36 mm cylinder probe with radius
164 (P/36R). The GFS sample was compressed at a constant rate of 1.0 mm/s at a distance of 5
165 mm. Probe holds at this distance for 30 seconds and then withdraws from the sample and
166 returns to its starting position. Each kind of GFSs was tested in six replications. In this
167 experiment, firmness was defined as the force in grams, required to compress the sample at

168 maximum distance (5 mm). To determine springiness, the force after 30 seconds was recorded
169 ($F_{30 \text{ sec}}$), divided by the force at maximum distance (F_{max}) and multiplied by 100%:

$$170 \quad \text{Springiness} = (F_{30}/F_{\text{max}}) \times 100\%$$

171

172 *2.6. Sensory analysis*

173 *2.6.1. Sensory methods and evaluation conditions*

174 Sensory characteristics of the experimental GFS were determined using a quantitative
175 descriptive analysis (QDA) (Lawless & Heymann, 2010). Prior to the analysis, vocabularies
176 of the sensory attributes were developed by the panel in a round-table session, using a
177 standardised procedure (ISO/DIS, 1998). Fifteen attributes were evaluated: porosity (pore
178 collocation and dimension), aroma (sponge cake, sweet, cabbage), taste (sponge cake, sweet,
179 cabbage, aftertaste), texture by finger (elasticity, crustiness) and texture by mouth feel
180 (mastication, adhesiveness) and overall quality (overall appearance and palatability). The
181 panellists evaluated the intensity perceived for each sensory attribute on unstructured
182 graphical scales. The scales were 10 cm long and verbally anchored at each end, and the
183 results were converted to numerical values (from 0 to 10 units) by a computer. The samples of
184 experimental GFS were coded with a three-digit number and presented to the panellists all
185 together in random order in transparent plastic boxes. Mineral water was offered and
186 suggested to drink between each sample evaluation. The assessments were carried out at a
187 sensory laboratory room, which fulfils the requirements of the ISO standards (ISO, 1998).
188 The results were collected using a computerised system ANALSENS (IAR&FRPAS, Olsztyn,
189 Poland). GFSs were tested in two replications.

190

191 *2.6.2. Sensory panel*

192 Sensory assessments was carried out by the expert panel consisting of 6 members (5 females;
193 1 male) previously selected and trained according to ISO guidelines (ISO, 1993). Prior to their
194 participation in the experiments, the panellists were trained on sensory descriptors for gluten-
195 free sponge cake baked from commercial mix (Celiko S.A., Poznań, Poland) purchased in a
196 local supermarket.

197

198 *2.7. Statistical analysis*

199 The results were processed by one-way analysis of variance (ANOVA). The significance of
200 differences between the samples was determined by Fisher's Least Significant Difference
201 (LSD) Post Hoc Test with $p < 0.05$ set as a significance point. To verify significant
202 differences between the products in each sensory attribute the statistical analyses were
203 performed using software FIZZ, Biosystemes version 2.47B, and XLSTAT Version 19.01.
204 The Pearson correlation coefficient between rheological and technological parameters of the
205 samples as well as the proteins content (Drabińska et al., 2017) were calculated. The same
206 parameters were analysed using principal component analysis (PCA). All the analyses were
207 performed in Statistica v. 12 software (StatSoft, USA).

208

209 **3. Results and Discussion**

210 Measurements of the apparent viscosity of batter with a RVA can provide valuable insights
211 into the quality of the final product. In the cake-making process, starch is the key structure-
212 forming component, and the addition of sugar and liquids modify the gelatinisation properties
213 of starch and affects the subsequently formed crumb structure (Cauvain, 2003). The pasting
214 characteristics of the experimental GFS batters are shown in Figure 1. In general, shapes of
215 RVA plots obtained during mixing, pasting and gelling of experimental GFS batters
216 composed of potato and corn starches did not differ meaningfully. During the initial mixing, a

217 consistency of all experimental GFS batters was similarly low, but after a rapid increase in
218 batter viscosity was observed during heating that led to the gelatinisation of starches and,
219 ultimately, the rupture of starch granules when the maximum consistency was achieved. The
220 experimental batters achieved the peak viscosity at similar temperature regardless of the
221 presence of BLP in GFS formulation (Table 1), but the formulations containing BLP differed
222 in viscosity relative to the control batter. The peak viscosity of all samples containing BLP
223 was significantly ($p < 0.05$) lower than in the control batter, in particular in sample B3
224 containing the highest amount of BLP (Table 1). The immediate explanation for that
225 reduction could be the starch dilution that is produced when replacing starch by BLP. To
226 confirm that a batter without BLP and containing the amount of starches present in B3 were
227 tested. Indeed, the replacement of starches, brought about a reduction in the maximum peak
228 viscosity, but some additional reduction was observed, likely due to the BLP composition.
229 Regarding the proteins content in BLP (Drabińska et al., 2017), the observed differences
230 could be attributed in some extent to the thermal properties of proteins added with BLP as a
231 correlation between the peak viscosity and proteins content was noted ($R = - 0.65$) (Figure 2).
232 Results obtained in the present study correspond with a previous study by Wang et al. (2002),
233 where a negative correlation between proteins content and peak viscosity was noted in rice
234 flour. Moreover, Marco and Rosell (2008a) analysed the effect of different protein isolates
235 (pea, soybean, egg albumen and whey proteins) on the rheological properties of rice flour
236 dough and reported that pea, soybean and whey proteins promoted a decrease in peak
237 viscosity. Breakdown viscosity is influenced by starch resistance to high temperature and
238 shear stress (Marco & Rosell, 2008a). In samples with higher BLP content, a significant (p
239 < 0.05) decrease in breakdown viscosity was determined relative to the control batter (Table
240 1), which agree with starch dilution. In the last stage of RVA analysis when temperature
241 decreased, the viscosity of GFS batters increased gradually, but significant differences in final

242 viscosity were not detected. However, final viscosity resulting from starch dilution was much
243 lower, thus BLP conferred additional viscosity during cooling. However, setback viscosity,
244 the amplitude between final and hot paste viscosity, changed in response to BLP application.
245 The highest setback was determined in sample B3 containing the highest amount of BLP
246 (Table 1). The increase in viscosity during setback is typically associated with the
247 crystallisation of amylose chains; however, in this case, it could also be affected by the
248 reorganisation of denatured proteins from BLP (correlation between protein content and
249 setback, $R = 0.89$). An analysis of changes in batter viscosity during heating-cooling cycles
250 could provide important information about the baking properties and technological
251 characteristics of GFS.

252 The physical and technological parameters of GFS are shown in Table 2. Visual differences
253 between the control sample and broccoli GFS can also be observed in Figure 3. The analysis
254 of physical properties revealed that the control GFS was characterised by the greatest weight
255 loss and significantly ($p < 0.05$) higher specific volume and surface area than the GFS
256 containing BLP (Table 2). Cakes with higher specific volume generally lose more water
257 during baking because they have a greater surface area that comes into contact with air (Zhou
258 & Therdthai, 2008). The occlusion of air cells in batter during mixing produces an aerated
259 emulsion or foam which is converted to a semi-solid, porous and soft structure during baking.
260 The final cake volume depends on air incorporation and retention, which is influenced by
261 batter viscosity and the distribution of air cells inside the batter matrix (Edoura-Gaena, Allais,
262 Trystram, & Gros, 2007; Turabi, Sumnu, & Sahin, 2008b). In the current study, the results of
263 the statistical analysis revealed that specific volume and surface area were clearly dependent
264 on batter viscosity, and a high positive correlation was noted with most RVA parameters
265 (Figure 2). The application of BLP in the experimental formulations significantly ($p < 0.05$)
266 decreased the specific volume and surface area of baked GFS, but their specific gravity was

267 nearly twice higher than in the control GFS. In conventional wheat cakes, the quality of flour,
268 in particular its starch and protein content (Wilderjans, Luyts, Goesaert, Brijs, & Delcour,
269 2010) and the size of flour particles (Yamazaki & Donelson, 1972), directly influences the
270 quality attributes of the final product. BLP was characterised by relatively large particles (\leq
271 0.60 mm) and a relatively high content of proteins and minerals (Drabińska et al., 2017). It
272 might be that BLP compounds decrease the amount of water available for starch gelatinisation
273 and interact with starch to weaken the structure and lower the quality of GFS.

274 The results of the instrumental texture analysis are shown in Table 2. The experimental GFS
275 did not differ in springiness, however considerable variations in firmness were noted between
276 the softest control GFS (572.5 g) and the significantly ($p < 0.05$) firmer GFS containing BLP
277 (Table 2). Furthermore, a gradual increase in firmness was observed in broccoli GFS, ranging
278 from 947.2 in B1 to 1505.7g in B3, which indicates that firmness depended on the quantity of
279 BLP in the formulation. The firmness of the experimental GFS was affected by batter
280 viscosity and revealed a strong negative correlation with peak ($R = - 0.97$) and breakdown (R
281 $= - 0.97$) viscosity (Figure 2). Moreover, firmness increased with a rise in the specific gravity
282 of cakes ($R = 0.81$) which was influenced by the amount of BLP in the formulation. In a study
283 by Majzoobi et al (2016) investigating GF sponge cake with carrot pomace powder, high-
284 density cakes were also characterised by harder texture. Therefore, the properties of the
285 applied flours and ingredients, as well as physical properties of batter (viscosity) and cake
286 (moisture content, density, volume) should be evaluated in the cake-making process because
287 they considerably influence the textural properties and the quality of the final product (de la
288 Hera, Martinez, Oliete, & Gómez, 2013; Talens et al., 2017).

289 The results of the instrumental colour analysis of the experimental GFS are shown in Table 2.
290 The values of parameters L , a^* and b^* for the crust and crumb of the control sample were
291 significantly ($p < 0.05$) different from those noted in the GFS with BLP. The crust and crumb

292 of the control GFS were characterised by the highest lightness (69.61 and 81.20, respectively)
293 and redness (11.99 and 4.83, respectively), but yellowness (positive b^* value) was
294 significantly ($p < 0.05$) lower in comparison with the GFS containing BLP. As expected, due
295 to its vivid green colour, BLP applied in formulation significantly influenced the colour of the
296 final GFS (Table 2). In gluten-free cake containing carrot pomace powder colour changed
297 from pale to slightly orange (due to a high beta-carotene content in carrot powder) however,
298 these changes had a positive effect on the overall acceptability of the evaluated products
299 (Majzoobi et al., 2016). In the present study, a significant ($p < 0.05$) decrease in crust and
300 crumb lightness was related to BLP content (Table 2). Moreover, the crust and crumb of the
301 experimental GFS containing BLP were also characterised by the highest values of greenness
302 (parameter a^*) that increased gradually with a rise in the content of vividly green BLP.
303 However, the yellowness of the crust and crumb decreased with an increase in the amount of
304 BLP in the formulation. Pigmented ingredients (orange by-products) significantly influence
305 the colour of the final product (Talens et al., 2017). The visual differences between the
306 typically creamy control GFS and broccoli GFS (B1, B2, B3) of light-green colour can be
307 seen at Figure 3.

308 The differences in firmness of the experimental GFS noted in the instrumental texture
309 analysis (Table 2) were not perceived in sensory analysis conducted by expert's panel (Table
310 3). The results of texture analysis by QDA revealed that the elasticity, crustiness, mastication
311 and adhesiveness of all broccoli GFS were comparable to the control GFS (Table 3).

312 Moreover, the porosity of GFS with BLP, in particular the distribution and size of air bubbles,
313 was similar to the porosity of the control GFS (Table 3). High-quality sponge cakes are
314 characterised by a large number of small air bubbles rather than a small number of large
315 bubbles (Sahi, 1994). In all experimental broccoli GFS, air bubbles were small (≤ 2) and
316 homogeneously distributed that is a feature of a good quality of sponge cake. QDA revealed

317 significant differences ($p < 0.05$) in colour, aroma and taste of the experimental GFS (Table
318 3). The control GFS was creamy and had a typical for sponge cake aroma and taste. Sample
319 B1 containing the lowest amount of BLP was characterised by a light-green colour, while
320 sponge cake aroma and sweet taste was masked by a slight cabbage aroma and aftertaste. As
321 expected, in samples B2 and B3 with a higher content of BLP, cabbage aroma and taste were
322 perceived as more intensive. The scores of overall quality, representing the general sensory
323 acceptability, indicated however that the appearance and palatability of sample B1 was
324 comparably high to that of the control GFS.

325

326 **4. Conclusions**

327 BLP obtained in laboratory conditions was applied as a component of GFS formulation. The
328 results obtained indicated that BLP influenced the batter viscosity characteristics and selected
329 technological and sensory parameters of final GFS. Sample containing the lowest amount of
330 BLP (B1) was characterized by attractive green colour, proper size and distribution of pores,
331 and was distinguished for its desirable sensory quality, although a slight cabbage aroma and
332 taste were perceived. On the other hand, the results of the instrumental texture analysis and
333 QDA suggested that BLP addition should not exceed moderate amounts to preserve good
334 quality and palatability of GFS. As a conclusion, the adequate amount of experimental BLP
335 could be an attractive novel ingredient suitable in GFS.

336

337 **Acknowledgements**

338 The study was financed from the statutory funds of the Department of Chemistry and
339 Biodynamics of Food in the Institute of Animal Reproduction and Food Research PAS and
340 from the KNOW Consortium: “Healthy Animal – Safe Food” (Ministry of Sciences and
341 Higher Education; Dec: 05-1/KNOW2/2015), and Generalitat Valenciana (Prometeo

342 2017/189. Authors would kindly thank to Fruit and Vegetables Warehouse GEMIX (Olsztyn,
343 Poland) for donating broccoli leaves used in this work.

344

345 **Author Contributions**

346 UKK and CMR conceived and designed the study; ND, AL, AA, and CF performed the
347 experiments; TJ performed the texture analysis; AO performed the sensory analysis; UKK,
348 CMR and ND analysed the data; UKK wrote the manuscript. All authors read and approved
349 the final version of manuscript.

350

351 **References:**

- 352 Alvarez-Jubete, L., Auty, M., Arendt, E. K., & Gallagher, E. (2009). Baking properties and
353 microstructure of pseudocereal flours in gluten-free bread formulations. *European Food*
354 *Research and Technology*, 230(3), 437–445. <https://doi.org/10.1007/s00217-009-1184-z>
- 355 Campas-Baypoli, O. N., Snchez-Machado, D. I., Bueno-Solano, C., Núñez-Gastélum, J. A.,
356 Reyes-Moreno, C., & López-Cervantes, J. (2009). Biochemical composition and
357 physicochemical properties of broccoli flours. *International Journal of Food Sciences*
358 *and Nutrition*, 60(SUPPL.4), 163–173. <https://doi.org/10.1080/09637480802702015>
- 359 Capriles, V. D., & Arêas, J. A. G. (2014). Novel approaches in gluten-free breadmaking:
360 Interface between food science, nutrition, and health. *Comprehensive Reviews in Food*
361 *Science and Food Safety*, 13(5), 871–890. <https://doi.org/10.1111/1541-4337.12091>
- 362 Cauvain, S. P. (2003). Cakes: Nature of Cakes. In *Encyclopedia of Food Sciences and*
363 *Nutrition* (pp. 751–756). London: Elsevier Science.
- 364 de la Hera, E., Martinez, M., Oliete, B., & Gómez, M. (2013). Influence of Flour Particle Size
365 on Quality of Gluten-Free Rice Cakes. *Food and Bioprocess Technology*, 6(9), 2280–
366 2288. <https://doi.org/10.1007/s11947-012-0922-6>

367 Domínguez-Perles, R., Martínez-Ballesta, M. C., Carvajal, M., García-Viguera, C., &
368 Moreno, D. a. (2010). Broccoli-derived by-products--a promising source of bioactive
369 ingredients. *Journal of Food Science*, 75(4), C383-92. [https://doi.org/10.1111/j.1750-](https://doi.org/10.1111/j.1750-3841.2010.01606.x)
370 [3841.2010.01606.x](https://doi.org/10.1111/j.1750-3841.2010.01606.x)

371 Drabińska, N., Ciska, E., Szmatowicz, B., & Krupa-Kozak, U. (2017). Broccoli by-products
372 improve the nutraceutical potential of gluten-free mini sponge cakes. *Food Chemistry*.
373 <https://doi.org/10.1016/J.FOODCHEM.2017.08.119>

374 Drabińska, N., Rosell, C. M., & Krupa-Kozak, U. (2018). Inulin-Type Fructans Application in
375 Gluten-Free Products: Functionality and Health Benefits. In J.-M. Mérillon & K. G.
376 Ramawat (Eds.), *Bioactive Molecules in Food* (pp. 1–40). Cham: Springer International
377 Publishing. https://doi.org/10.1007/978-3-319-54528-8_2-1

378 Drabińska, N., Zieliński, H., & Krupa-Kozak, U. (2016). Technological benefits of inulin-
379 type fructans application in gluten-free products – A review. *Trends in Food Science and*
380 *Technology*. <https://doi.org/10.1016/j.tifs.2016.08.015>

381 Edoura-Gaena, R. B., Allais, I., Trystram, G., & Gros, J. B. (2007). Influence of aeration
382 conditions on physical and sensory properties of aerated cake batter and biscuits. *Journal*
383 *of Food Engineering*, 79(3), 1020–1032. <https://doi.org/10.1016/j.jfoodeng.2006.04.001>

384 Gallagher, E., Gormley, T. R., & Arendt, E. K. (2004). Recent advances in the formulation of
385 gluten-free cereal-based products. *Trends in Food Science and Technology*.
386 <https://doi.org/10.1016/j.tifs.2003.09.012>

387 Higdon, J. V., Delage, B., Williams, D. E., & Dashwood, R. H. (2007). Cruciferous
388 vegetables and human cancer risk: epidemiologic evidence and mechanistic basis.
389 *Pharmacological Research*. <https://doi.org/10.1016/j.phrs.2007.01.009>

390 ISO. (1993). 8586–1: Sensory analysis—general guidance for the selection, training and
391 monitoring of assessors—Part 1: selected assessors.

392 ISO. (1998). 8589: Sensory analysis—general guidance for the design of test rooms.
393 ISO/DIS. (1998). 13299: Sensory analysis—methodology—general guidance for establishing
394 a sensory profile.

395 Krupa-Kozak, U., Baczek, N., & Rosell, C. M. (2013). Application of dairy proteins as
396 technological and nutritional improvers of calcium-supplemented gluten-free bread.
397 *Nutrients*, 5(11), 4503–4520. <https://doi.org/10.3390/nu5114503>

398 Krupa-Kozak, U., Wronkowska, M., & Soral-Śmietana, M. (2011). Effect of buckwheat flour
399 on microelements and proteins contents in gluten-free bread. *Czech Journal of Food*
400 *Sciences*, 29(2), 103–108.

401 Lawless, H. T., & Heymann, H. (2010). Sensory Evaluation of Food - Principles and
402 Practices. In *Sensory Evaluation of Food - Principles and Practices* (pp. 473–478).
403 <https://doi.org/10.1007/978-1-4419-6488-5>

404 Majzoobi, M., Poor, Z. V., Jamalian, J., & Farahnaky, A. (2016). Improvement of the quality
405 of gluten-free sponge cake using different levels and particle sizes of carrot pomace
406 powder. *International Journal of Food Science and Technology*, 51(6), 1369–1377.
407 <https://doi.org/10.1111/ijfs.13104>

408 Marco, C., & Rosell, C. M. (2008a). Effect of different protein isolates and transglutaminase
409 on rice flour properties. *Journal of Food Engineering*, 84(1), 132–139.
410 <https://doi.org/10.1016/j.jfoodeng.2007.05.003>

411 Marco, C., & Rosell, C. M. (2008b). Functional and rheological properties of protein enriched
412 gluten free composite flours. *Journal of Food Engineering*, 88(1), 94–103.
413 <https://doi.org/10.1016/j.jfoodeng.2008.01.018>

414 Nunes, M. H. B., Ryan, L. A. M., & Arendt, E. K. (2009). Effect of low lactose dairy powder
415 addition on the properties of gluten-free batters and bread quality. *European Food*
416 *Research and Technology*, 229(1), 31–41. <https://doi.org/10.1007/s00217-009-1023-2>

417 O'Shea, N., Arendt, E., & Gallagher, E. (2014). Enhancing an Extruded Puffed Snack by
418 Optimising Die Head Temperature, Screw Speed and Apple Pomace Inclusion. *Food and*
419 *Bioprocess Technology*, 7(6), 1767–1782. <https://doi.org/10.1007/s11947-013-1181-x>

420 Radočaj, O., Dimić, E., & Tsao, R. (2014). Effects of Hemp (*Cannabis sativa* L.) Seed Oil
421 Press-Cake and Decaffeinated Green Tea Leaves (*Camellia sinensis*) on Functional
422 Characteristics of Gluten-Free Crackers. *Journal of Food Science*, 79(3).
423 <https://doi.org/10.1111/1750-3841.12370>

424 Ronda, F., & Roos, Y. H. (2011). Staling of fresh and frozen gluten-free bread. *Journal of*
425 *Cereal Science*, 53(3), 340–346. <https://doi.org/10.1016/j.jcs.2011.02.004>

426 Sahi, S. S. (1994). Interfacial properties of the aqueous phases of wheat flour doughs. *Journal*
427 *of Cereal Science*, 20(2), 119–127. <https://doi.org/10.1006/jcrs.1994.1052>

428 Sapone, A., Bai, J. C., Ciacci, C., Dolinsek, J., Green, P. H. R., Hadjivassiliou, M., ... Fasano,
429 A. (2012). Spectrum of gluten-related disorders: Consensus on new nomenclature and
430 classification. *BMC Medicine*, 10. <https://doi.org/10.1186/1741-7015-10-13>

431 Saturni, L., Ferretti, G., & Bacchetti, T. (2010). The gluten-free diet: Safety and nutritional
432 quality. *Nutrients*. <https://doi.org/10.3390/nu2010016>

433 Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary
434 polyphenols and the prevention of diseases. *Critical Reviews in Food Science and*
435 *Nutrition*. <https://doi.org/10.1080/1040869059096>

436 Talens, C., Álvarez-Sabatel, S., Rios, Y., & Rodríguez, R. (2017). Effect of a new
437 microwave-dried orange fibre ingredient vs. a commercial citrus fibre on texture and
438 sensory properties of gluten-free muffins. *Innovative Food Science and Emerging*
439 *Technologies*, 44, 83–88. <https://doi.org/10.1016/j.ifset.2017.07.011>

440 Thompson, T. (1999). Thiamin, riboflavin, and niacin contents of the gluten-free diet: Is there
441 cause for concern? *Journal of the American Dietetic Association*, 99(7), 858–862.

442 [https://doi.org/10.1016/S0002-8223\(99\)00205-9](https://doi.org/10.1016/S0002-8223(99)00205-9)

443 Thompson, T., Dennis, M., Higgins, L. A., Lee, A. R., & Sharrett, M. K. (2005). Gluten-free
444 diet survey: Are Americans with coeliac disease consuming recommended amounts of
445 fibre, iron, calcium and grain foods? *Journal of Human Nutrition and Dietetics*, *18*(3),
446 163–169. <https://doi.org/10.1111/j.1365-277X.2005.00607.x>

447 Turabi, E., Sumnu, G., & Sahin, S. (2008a). Optimization of baking of rice cakes in infrared-
448 microwave combination oven by response surface methodology. *Food and Bioprocess
449 Technology*, *1*(1), 64–73. <https://doi.org/10.1007/s11947-007-0003-4>

450 Turabi, E., Sumnu, G., & Sahin, S. (2008b). Rheological properties and quality of rice cakes
451 formulated with different gums and an emulsifier blend. *Food Hydrocolloids*, *22*(2),
452 305–312. <https://doi.org/10.1016/j.foodhyd.2006.11.016>

453 Wang, Z. H., Wang, Y., Cui, H. R., Xia, Y. W., Altosaar, I., & Shu, Q. Y. (2002). Factor
454 analysis of physicochemical properties of 63 rice varieties. *Journal of the Science of
455 Food and Agriculture*, *82*(7), 745–752. <https://doi.org/10.1002/jsfa.1094>

456 Wilderjans, E., Luyts, A., Goesaert, H., Brijs, K., & Delcour, J. A. (2010). A model approach
457 to starch and protein functionality in a pound cake system. *Food Chemistry*, *120*(1), 44–
458 51. <https://doi.org/10.1016/j.foodchem.2009.09.067>

459 Wilderjans, E., Pareyt, B., Goesaert, H., Brijs, K., & Delcour, J. A. (2008). The role of gluten
460 in a pound cake system: A model approach based on gluten-starch blends. *Food
461 Chemistry*, *110*(4), 909–915. <https://doi.org/10.1016/j.foodchem.2008.02.079>

462 Yamazaki, W. T., & Donelson, D. H. (1972). The relationship between flour particle size and
463 cake-volume potential among eastern soft wheats. *Cereal Chemistry*, *49*, 649–653.

464 Zhang, X., Shu, X., Xiang, Y., Yang, G., Li, H., Gao, J., ... Zheng, W. (2011). Cruciferous
465 vegetable consumption is associated with a reduced risk of total and cardiovascular
466 disease mortality. *American Journal of Clinical Nutrition*, *94*(1), 240–246.

467 <https://doi.org/10.3945/ajcn.110.009340.1>

468 Zhou, W., & Therdthai, N. (2008). Heat and mass transfers during baking of sweet goods. In

469 *Food Engineering Aspects of Baking Sweet Goods* (pp. 173–190).

470 <https://doi.org/doi:10.1201/9781420052770.ch9> 10.1201/9781420052770.ch9

471

472 **Table 1** Effects of BLP on the RVA parameters of GFS batters

473

	Control	B1	B2	B3
Onset temperature (°C)	64.3±0.1	64.5±0.5	64.3±0.1	65.3±0.4
Peak temperature (°C)	76.7±0.7	77.9±0.1	79.2±2.4	77.1±0.9
PV (cP)	3947.5±16.3 ^a	3736.3±85.4 ^b	3652.0±72.3 ^{bc}	3535.0±89.6 ^c
HPV (cP)	1866.5±14.85	1796.0±36.4	1809.7±45.24	1725.3±61.8
Breakdown (cP)	2081.0±1.41 ^a	1940.3±54.3 ^{ab}	1842.3±27.6 ^b	1809.7±76.5 ^b
Final PV (cP)	2811.0±49.5	2722.0±47.7	2785.7±28.0	2769.0±49.1
Setback (cP)	944.5±64.4 ^b	926.0±30.6 ^b	976.0±19.0 ^b	1043.7±29.2 ^a

474 ^a Values followed by different letters in the same row are significantly different (P < 0.05).

475

476 **Table 2.** The effect of BLP on the physical and textural properties and the colour of GFS.

477

		Control	B1	B2	B3
Moisture (%)		33.73±0.23	33.63±0.42	33.60±0.03	33.23±0.75
Weight loss (%)		14.9±1.2	13.9±0.8	13.8±1.0	13.4±1.3
Specific volume (cm ³ /g)		3.99±0.06 ^a	2.56±0.17 ^b	2.44±0.07 ^b	2.50±0.11 ^b
Surface area (cm ²)		113±1 ^a	84±2 ^c	83±1 ^{bc}	88±2 ^b
Specific gravity (g/cm ³)		0.25±0.01 ^b	0.39±0.03 ^a	0.41±0.01 ^a	0.40±0.02 ^a
Firmness (g)		572.5±71.0 ^c	947.2±127.7 ^b	1109.7±109.4 ^b	1505.7±87.3 ^a
Springiness (%)		54.1±1.4	56.1±1.6	54.7±2.2	55.4±1.6
Crust colour	<i>L</i> [*]	69.61±1.30 ^a	52.53±3.99 ^b	44.38±2.91 ^c	40.67±0.26 ^d
	<i>a</i> [*]	11.99±0.26 ^a	-3.38±1.33 ^b	-7.73±2.39 ^c	-9.83±2.44 ^d
	<i>b</i> [*]	34.73±0.43 ^d	40.18±0.63 ^a	38.91±1.65 ^b	37.65±0.89 ^c
Crumb colour	<i>L</i> [*]	81.20±0.65 ^a	69.61±1.47 ^b	47.42±1.50 ^c	42.98±0.49 ^d
	<i>a</i> [*]	4.83±0.35 ^a	-11.99±4.40 ^b	-12.72±0.34 ^c	-13.02±0.09 ^c
	<i>b</i> [*]	25.31±0.42 ^c	41.04±1.20 ^a	39.61±0.35 ^a	34.73±0.71 ^b

478 ^a Values followed by different letters in the same row are significantly different (P < 0.05).

479 **Table 3.** QDA sensory analysis of experimental GFS with BLP

		Control	B1	B2	B3
Colour	creamy colour	3.2±0.8 ^a	0.0 ^b	0.0 ^b	0.0 ^b
	green colour	0.0 ^d	3.0±1.0 ^c	5.7±0.9 ^b	7.7±1.3 ^a
Aroma	sponge cake	8.2±1.3 ^a	4.8±1.6 ^b	3.2±1.8 ^c	2.2±1.5 ^c
	sweet	6.3±1.6 ^a	4.5±1.2 ^b	3.3±1.5 ^{bc}	3.0±1.5 ^c
	cabbage	0.0 ^d	2.3±0.9 ^c	3.6±1.6 ^b	5.1±1.9 ^a
Taste	sponge cake	8.2±1.2 ^a	5.3±1.7 ^b	4.5±1.5 ^{bc}	3.7±1.6 ^c
	sweet	5.2±1.2 ^a	4.1±1.0 ^b	4.0±0.9 ^b	3.9±0.9 ^b
	cabbage	0.0 ^d	1.6±0.5 ^c	2.7±0.8 ^b	3.8±1.4 ^a
Porosity	aftertaste	1.7±0.8 ^c	2.3±0.7 ^{bc}	2.7±0.8 ^{ab}	3.1±1.1 ^a
	collocation	6.6±2.0	6.9±2.5	7.0±2.5	5.2±2.9
	dimension	2.0±0.9	1.6±0.7	1.7±0.5	1.9±0.6
Texture (by finger)	elasticity	6.4±1.7	4.9±2.9	4.6±2.9	4.3±2.9
	crustiness	1.7±1.1	2.1±1.4	2.3±1.6	2.7±1.7
Texture (mouth fell)	mastication	3.6±2.5	3.6±2.1	3.6±2.2	3.6±2.0
	adhesiveness	3.5±2.5	3.6±2.3	3.5±2.4	3.4±2.4
Overall quality		8.3±1.0 ^a	6.8±1.6 ^{ab}	6.1±1.7 ^b	5.43±1.7 ^b

480 ^a Values followed by different letters in the same row are significantly different (P < 0.05).

481

482 **Figure captions:**

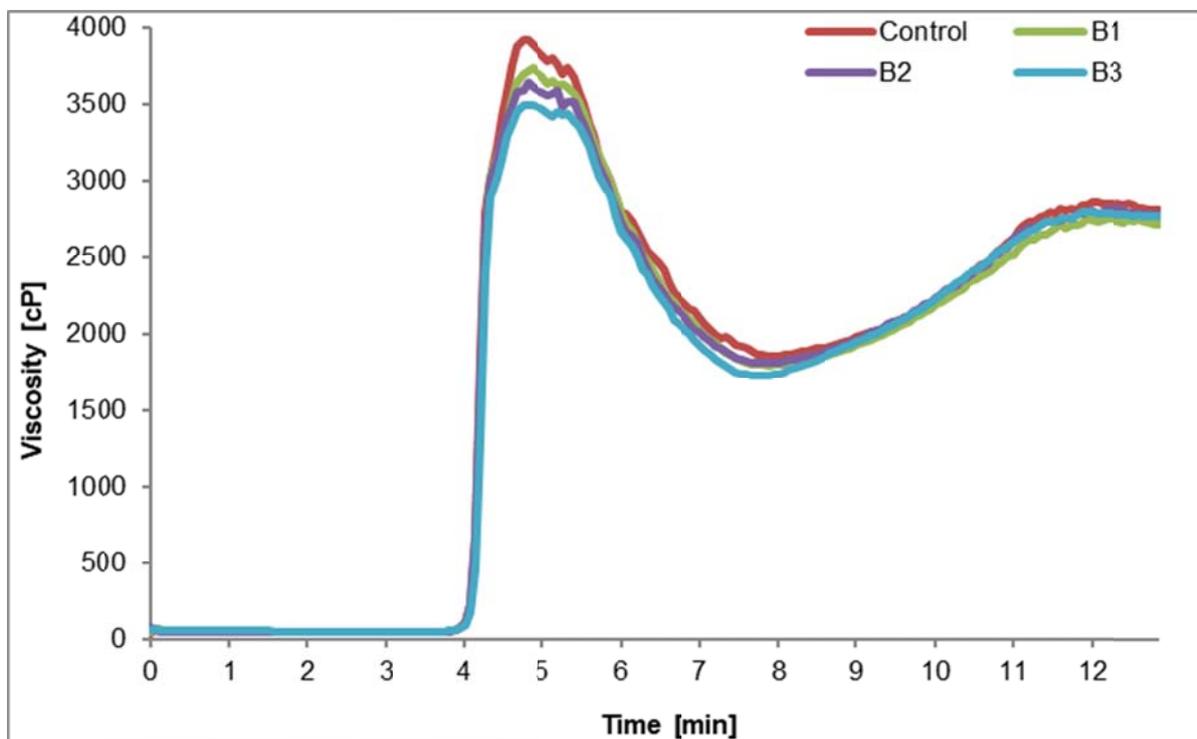
483

484 Figure 1. Effect of BLP on the plots of viscometric profile recorded with the rapid
485 viscoanalyzer

486 Figure 2. Principal components plot of physical and technological features of GFSs.

487 Figure 3. Example pictures of appearance, cross-section and 3D scans of experimental GFS
488 with BLP.

489 Figure 1. Pasting properties of starches affected by broccoli leaves.

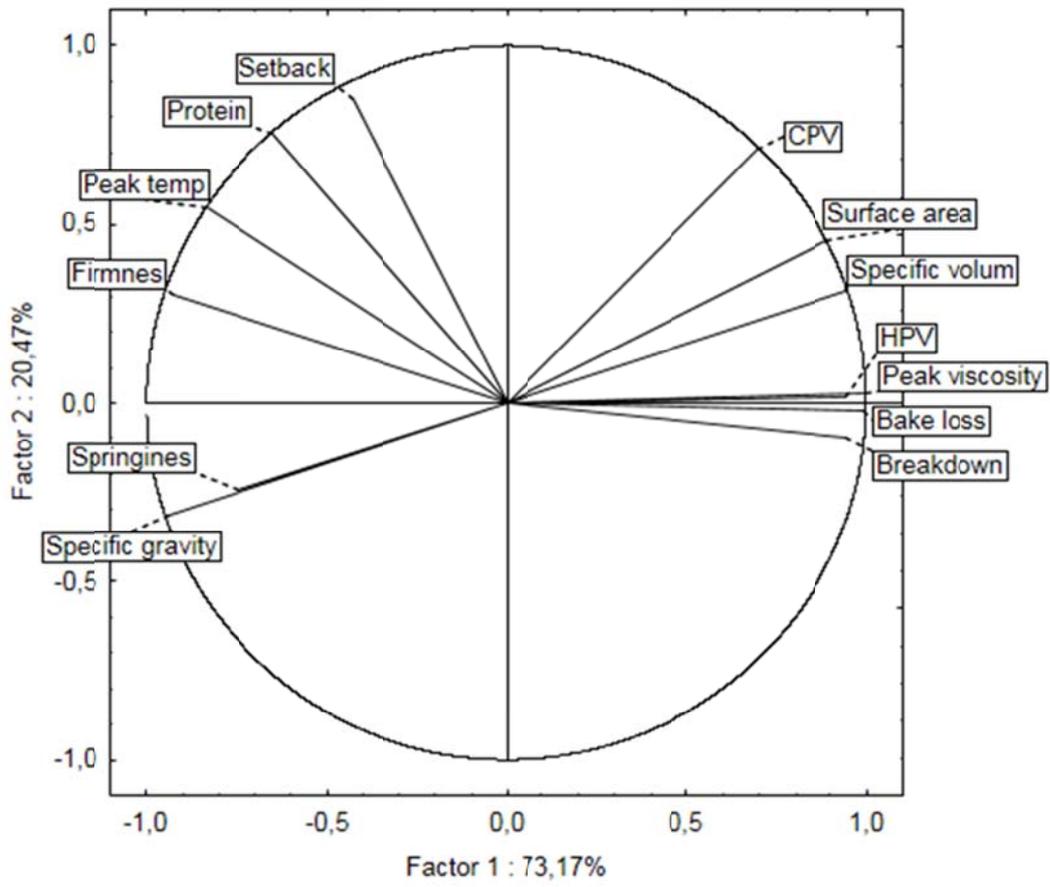


490

491

492

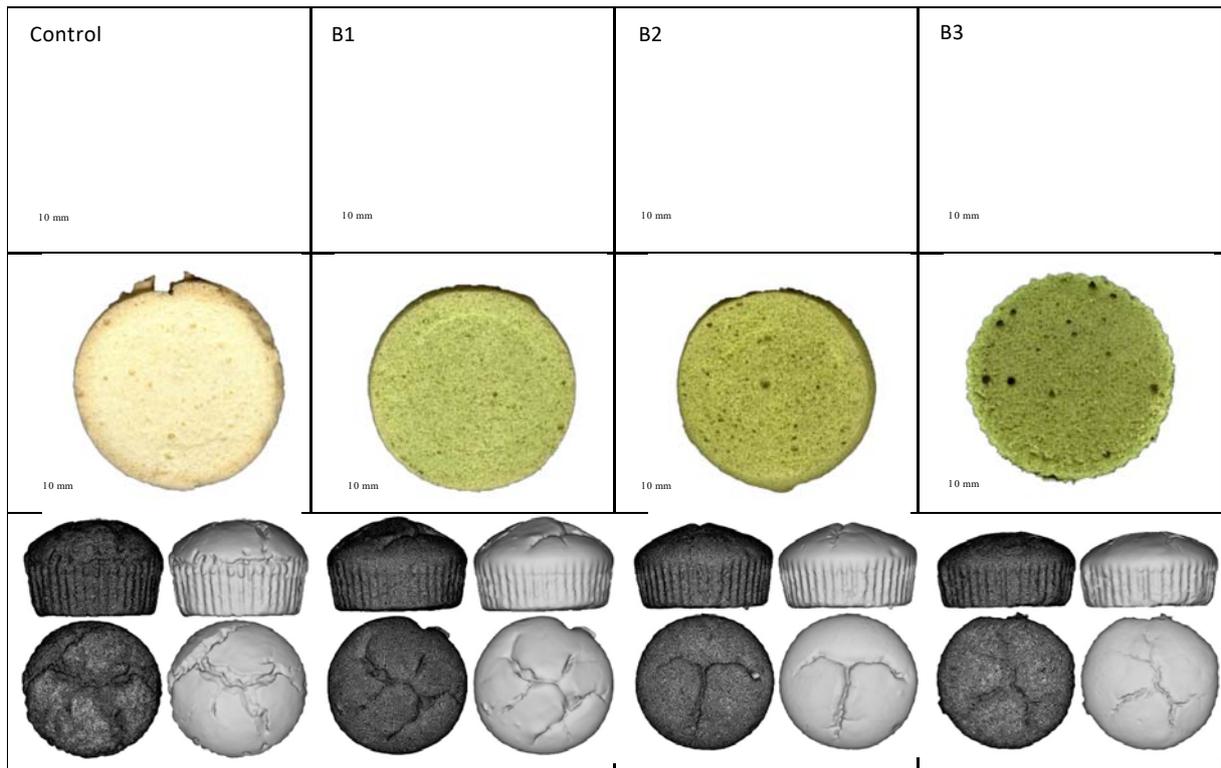
493 Figure 2.



494

495

496 Figure 3.



497