

1 **Influence of the addition of extruded flours on rice bread quality**

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14 **Abstract**

15 The extrusion may improve coeliac bread quality by modifying the functional properties of
16 flour. This study investigates the influence of the substitution of 10% of rice flour by extruded
17 rice flours (three intensities of treatment and two particle sizes) on the characteristics of gluten-
18 free bread (specific volume and texture) at constant consistency. The microstructure and
19 rheology of the doughs obtained and their behaviour during fermentation have also been
20 analysed. The extruded flours increase dough consistency, and the effect is more noticeable with
21 increasing intensities of treatment. The use of extruded flours requires the addition of a larger
22 volume of water to obtain a constant consistency. The addition of extruded flour decreases
23 dough development, producing a lower specific volume and greater bread hardness. This effect
24 is minimized by increasing the particle size. The staling of bread from 24 to 72 hours is less
25 noticeable with a larger particle size.

26 **PRACTICAL APPLICATIONS**

27 This study evidences that the use of extruded flours in rice bread making allows increasing
28 dough hydration and therefore the bread yield while decreasing bread staling. However, the
29 correct selection of extrusion treatment and flour particle size is essential to achieve appropriate
30 results, being preferable the use of coarse flours with more intense extrusion treatment.

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32 Keywords: extrusion, gluten-free bread, particle size, microstructure

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34 **1. Introduction**

35 Gluten-free breads are characterized by their deficient quality and high price compared with
36 traditional breads. This has led to increased interest in developing high quality gluten-free
37 products in recent years (Cureton & Fasano, 2009), with the consequent increase in the volume
38 of research and the number of scientific publications on this subject.

39 Most research studies into the development of gluten-free products have focussed on the
40 substitution of wheat flours by mixtures of gluten-free cereals, starches, proteins and
41 hydrocolloids (Schober, 2009), and on the enzymatic improvement of these formulations
42 (Rosell, 2009). However, less information is available on the use of physical treatments to
43 modify the functional properties of flours used in gluten-free products, and these methods have
44 been less widely employed. Hydrothermal treatments stand out as being among the most
45 effective physical treatments.

46 If starch, the major component of flour, is subjected to a high-temperature treatment with
47 enough moisture it can be gelatinized, increasing the swelling capacity of the granule,
48 decreasing crystallinity and sometimes causing break-up of the granules (Atwell et al., 1988).
49 Hoover & Vasanthan (1994) also demonstrated that starch undergoing thermal treatment at
50 100°C presents a high gelatinization temperature and high resistance to acid hydrolysis due to
51 realignment of the starch chains, although these changes varied depending on the kind of starch
52 used.

53 Pregelatinized rice flour has been widely used as the principal ingredient in many kinds of foods
54 (rice cakes, infant foods, instant rice pudding) due to its thickener properties. It is known that
55 uses of pregelatinized rice flour are determined by its physicochemical and functional properties,
56 which differ depending on the variety of rice and the processing method employed (Hsieh &

57 Luh, 1991; Lu, et al., 1994). These hydrothermal treatments may be performed in various ways,
58 such as drying a paste by atomization or heated drums; but one of the most versatile alternatives
59 is extrusion. Extrusion is a treatment which applies heat and mechanical strain to a flour-water
60 mixture. The main interest of the extrusion of flours and starches is to modify their functional
61 properties, which will vary according to the extrusion conditions applied (Curic, et al., 2009).
62 When flours are extruded, changes take place in starch which modify the rheological behaviour
63 of dough, similar to the changes that occur when dough is subjected to cooling-heating cycles
64 (Hagenimana et al., 2006). However, extrusion causes more intense changes to the starch than
65 traditional cooking methods, as it damages a larger number of starch granules and modifies the
66 cold thickening capacity (Wolf, 2010). This treatment may even rupture amylopectin molecules
67 (Mercier & Feillet, 1975). Colonna et al. (1984) demonstrated that extruded wheat starches
68 contain amylose and amylopectin chains with a lower molecular weight than those obtained by
69 treatment in heated drums. This effect, due to the shear force applied to the starch, is translated
70 into an increase in the solubility and a decrease in the cold thickening capacity (Doublier, et al.,
71 1986).

72 These functional modifications of flour after extrusion could also lead to changes in the baking
73 properties. However, this has not been extensively researched in gluten-free breads.

74 Defloor et al. (1991) found that a mixture of extruded starches and emulsifying agents improved
75 the quality of breads prepared with a mixture of tapioca and soya and a high level of hydration
76 (145%).

77 Sanchez, et al. (2008) observed that the addition of extruded rice flour improved bread volume
78 and crumb structure, but this effect was more noticeable when the waxy varieties of rice were
79 used. However those authors used high percentages of extruded flour (15% and 30%) and

80 modified the quantity of water in the formula according to the penetrometer dough consistency
81 values; those variations led to an increase in dough hydration by more than 30% in the majority
82 of cases. It is also important to note that those authors used over 80% starch in the formula.

83 The effect of the substitution of 10% of the rice flour by extruded non-acidified and acidified
84 rice flour has also been tested (Clerici & El-Dash, 2006) (Clerici, et al., 2009). However, those
85 authors did not use hydrocolloids in their bread-making process and the specific volume of their
86 breads was excessively low in all cases.

87 In summary, some studies do exist on the use of extruded flours in gluten-free breads, but
88 research needs to be extended to include formulae with the addition of hydrocolloids, with
89 hydration percentages below 90%, and with rice flour as the main ingredient.

90 The particle size of the rice flour is also known to have an effect on gluten-free bread-making
91 (Araki, et al., 2009; Nishita & Bean, 1982; Ylimaki, et al., 1988), but there have been no studies
92 to determine the effect of the particle size of extruded rice flour on the functional characteristics
93 of gluten-free breads.

94 In this study of constant consistency gluten-free bread making we have determined the effect of
95 substituting 10% of non-extruded rice flour by extruded rice flour produced using three different
96 extrusion intensities and with two different particle sizes. We analysed the viscous behaviour of
97 flours in a heating-cooling cycle, dough rheology, dough development and gas production
98 during fermentation, the differences in the microstructure of the flours and doughs, and the
99 texture properties and specific volume of the breads.

100

101 **2. Materials and methods**

102 **2.1 Materials**

103 The rice flour used in this study was provided by Harinera Los Pisones (Zamora, Spain). Rice
104 flour was extruded using an industrial Buhler Basf single-screw extruder (Buhler S.A., Uzwil,
105 Switzerland). Three kinds of flour were used. Flour 1 was extruded with the addition of 2%
106 moisture with a maximum temperature at the end of the extruder of 110°C. Flour 2 was extruded
107 with the addition of 15% moisture and a maximum temperature of 110°C. For flour 3, 10%
108 moisture was added and the maximum temperature in the extruder was 140°C. The resulting
109 products were ground by compression rollers and sieved to obtain flours with two different
110 particle sizes, fine and coarse. Fine flours (f) were obtained by sieving through a 132 micron
111 screen, and coarse flours (c) were retained between a 132 micron sieve and a 200 micron sieve.
112 Depending on the thermal treatment and the particle size, extruded rice flours were referred to
113 by a number (1, 2, 3) and a letter (f, c). Non-extruded rice flour was use as the control. Due to
114 the fact that the chemical composition of coarse and fine flour could be changed by shieving the
115 flour into fractions, chemical composition is attached (table 1).

116 Saf-Instant yeast (Lesaffre, Lille, France), dry refined salt (Esco European Salt Company,
117 Niedersachsen, Germany), local tap water, white sugar (Acor, Valladolid, Spain), refined
118 sunflower oil (Coosur, Vilches, Spain) and hydroxypropylmethylcellulose (HPMC) Methocel
119 K4M Food grade (Dow Wolf Celulosics, Bitterfeld, Germany) were also used in the bread-
120 making.

121

122 **2.2 Methods**

123 **2.2.1. Flour characterisation**

124 The viscous behaviour of flours during the heating-cooling cycle was measured with the Rapid
125 Visco Analyzer (RVA) (Newport Scientific, Warriewood, Australia), following AACC method
126 61.02.01 (AACC, 2012). The microstructure of the flours was analysed with an environmental
127 scanning electron microscope (ESEM) (FEI, Quanta 200FEG, Oregon, USA) with integrated x-
128 ray microanalysis using an EDAX Genesis XM2i, which enables wet samples to be analysed at
129 ambient pressure without superficial metallization.

130 **2.2.2. Dough rheology, gas production and dough microstructure.**

131 The rheological behaviour of dough at constant moisture was studied using a Thermo Scientific
132 Haake RheoStress1 controlled strain rheometer (Thermo Fisher Scientific, Schwerte, Germany)
133 and a Phoenix II P1-C25P water bath which controlled the analysis temperature (set at 25°C).
134 The rheometer was equipped with parallel-plate geometry (60 mm diameter titanium serrated
135 plate-PP60 Ti) with 3 mm gap. After adjustment of the 3 mm gap, the excess batter was
136 removed and vaseline oil (Panreac, Panreac Química SA, Castellar del Vallés, Spain) was
137 applied to cover the exposed sample surfaces. Dough was rested for 300 seconds before
138 measuring. Samples were analysed in duplicate and without yeast. First, a strain sweep test was
139 performed at 25°C with a strain range of 0.1 - 100 Pa and a constant frequency of 1Hz to
140 identify the linear viscoelastic region. On the basis of the results obtained, a strain value
141 included into the linear viscoelastic region was used in a frequency sweep test at 25°C with a
142 frequency range of 100-0.1Hz. Values of the complex modulus (G^* [Pa]), elastic modulus
143 (G' [Pa]), viscous modulus (G'' [Pa]) and tangent δ (G''/G') were obtained for different
144 frequency values (ω [Hz]) (Dobraszczyk & Morgenstern, 2003).

145 A rheofermentometer (Chopin, Tripette and Renaud, France) was used to analyse dough height,
146 gas production and gas liberation related to the fermentation time following the method
147 described by Czuchajowska & Pomeranz, (1993). However, the authors adapted the method for
148 gluten-free dough. Only 200g of dough were placed into the rheofermentometer container, the 2
149 kg weight indicated by the method was removed, 3% yeast was added to the formula, and
150 fermentation in the rheofermentometer was performed at 30°C.

151 Dough microstructure was studied with an ESEM microscope (FEI, Quanta 200FEG, Oregon,
152 USA). The doughs did not contain yeast to avoid alterations in image visualization.

153

154 **2.2.3. Bread making**

155 The following ingredients (g/100g flour) were used in the bread making: Saf-Instant yeast
156 (3g/100g), salt (1.8g/100g), refined sunflower oil (6g/100g), HPMC (2g/100g) and white sugar
157 (5g/100g). The quantity of water in the doughs made with extruded flour was regulated in each
158 sample to obtain a G* value equal to the G* value of the control dough.

159 The water temperature in all the tests was between 20°C and 22°C. Rice flour was substituted by
160 extruded rice flour at a rate of 10g/100g. Control breads containing no extruded rice flour were
161 also prepared. After mixing all the ingredients for 8 minutes in a Kitchen Aid 5KPM50 mixer
162 (Kitchen Aid, Michigan, USA), 250 g of the bread dough were placed in model 151090
163 aluminium pans measuring 108 mm by 232 mm (ALU-Schale, Wiklarn, Germany).
164 Fermentation was performed at 30°C and 75% RH for 90 minutes in an FC-K proofer (Salva,
165 Lezo, Spain). After fermentation, doughs were baked in an electric modular oven (Salva, Lezo,
166 Spain) for 40 minutes at 190°C. The loaves were removed from the moulds after a 60-minute
167 cooling period and were weighted. The measurements on the breads were performed 24 hours

168 after baking, except for the texture parameters which were measured at 1, 24 and 72 hours after
169 baking. The loaves were introduced into polyethylene plastic bags and stored at 20°C until
170 analysis. All the elaborations were performed twice.

171

172 **2.2.4. Bread characteristics**

173 Bread volume was determined using a laser sensor with the BVM-L 370 volume analyser
174 (Perten Instruments, Hägersten, Sweden). The volume measurements were performed in
175 duplicate on two loaves from each elaboration. The specific volume was calculated as the ratio
176 of bread volume to its mass.

177 Crumb texture was measured with a TA-XT2 texture analyzer (Stable Microsystems, Surrey,
178 UK) fitted with the “*Texture Expert*” software. A 25-mm diameter cylindrical aluminium probe
179 was used in a “*Texture Profile Analysis*” (TPA) double compression test to penetrate to 50% of
180 the sample depth at test speed of 2 mm/s and with a 30 second delay between first and second
181 compressions. Hardness (N), cohesiveness and springiness were calculated from the TPA graph
182 (Gómez et al., 2007). Texture analyses were performed on 30 mm thick slices at 1, 24 and 72
183 hours after baking. Analyses were performed on two slices from two loaves (2x2) from each
184 type of elaboration, taking the average of the 4 measurements made.

185 **2.2.5. Statistical analysis**

186 All data were presented as mean values and analysed using an analysis of unidirectional
187 parametric variance (ANOVA) using Fisher’s least significant difference (LSD) ($p < 0.05$). The
188 analyses were performed using the Statgraphics Centurion XVI statistical package (StatPoint
189 Technologies Inc, Warrenton, USA). Additionally, in order to plot the hardness values over
190 time, an analysis of variance was also performed taking into account time as a factor for

191 repeated measures using Fisher's least significant difference ($p < 0.05$). The Statistica 6 software
192 (Statsoft Inc, Tulsa, USA) was used for this analysis.

193

194 **3. Results and Discussion**

195 **3.1. Electron microscopy of flours**

196 Figure 1 shows the microstructure of the flours used in this study. It may be observed that the
197 particles of non-extruded rice flour (a) was formed by compound starch granules connected by a
198 compact protein structure. In contrast, in the extruded rice flour with the most intense treatment
199 (d), the starch granules lose their integrity and a paste is formed in which the different
200 components cannot be distinguished in the resultant particles. In the extruded flours with low-
201 intensity hydrothermal treatment, intermediate structures are observed. Whilst swollen starch
202 granules, which represent a phase prior to gelatinization, are observed in flour 1 (b), fusion of
203 the different components is observed in flour 2 (c).

204 Our observations coincide with those reported by Yeh et al. (1999) and by Chao-Chi Chuang &
205 Yeh (2003), who studied the morphological changes of rice starch during heating extrusion. Yeh
206 et al. (1999) showed that non-extruded rice flour had a powder-like appearance and that starch
207 granules swelled as they advanced through the cold zone of the extruder but without losing the
208 powder-like appearance. When the rice flour reached the heating zone of the extruder, the starch
209 granules started to melt and formed a continuous matrix. However, those authors obtained their
210 samples from intermediate zones of the extruder and without milling. Our samples were
211 obtained at the end of the extrusion treatment after drying, milling and sieving. Chao-Chi
212 Chuang & Yeh (2003) showed that starch gelatinization increased with increases in the
213 temperature and duration of treatment.

214

215 **3.2. Viscous behaviour of flours during a heating-cooling cycle.**

216 The RVA parameters for non-extruded rice flour and for 10% substituted extruded flours are
217 shown in Table 2. It can be seen that the more intense the extrusion treatment, the lower the
218 values of pasting viscosity (PV), breakdown (BR), trough (TR), setback (ST) and final viscosity
219 (FV). No significant differences were observed in relation to particle size. Dough viscosity
220 decreased throughout the heating-cooling cycle as the treatment intensity increased. However,
221 no significant differences were observed between the milder treatments (1 and 2). It is known
222 that changes in the physicochemical properties of starch during extrusion develop as a result of
223 morphological changes of the starch granules and the degree of gelatinization (Camire et al.,
224 1990; Yeh & Li, 1996). It is also known that dough viscosity depends mainly on the degree of
225 gelatinization of the starch granules and the degree of rupture of the molecular chains (El-Dash
226 et al., 1983). Previous studies related high PV values to a high quantity of non-gelatinized
227 starch, whereas low PV values indicated a proportion of gelatinized starch which is attributable
228 to the variation in the degree of depolymerization and the molecular tangle resulting from the
229 processing conditions (Barres et al., 1990; McPherson et al., 2000). The decrease in PV, BR and
230 TR observed when the intensity of treatment increased, previously observed by Hagenimana et
231 al. (2006), could indicate degradation and gelatinization of the starch. High values of these
232 parameters in the non-extruded rice flour would be related to the presence of non-gelatinized
233 starch. These modifications in the starches and in the flour particles have been studied on the
234 photomicrographs, and the flours undergoing the most intense treatment (flours 3) contained the
235 highest quantity of gelatinized starch.

236 The fall in FV and ST values in the extruded flours has already been observed by Doublier et al.
237 (1986) and by Mercier & Feillet (1975). These values indicate the degree of retrogradation that
238 occurs after heating. When the hot gels are cooled, the increase of viscosity depends on the
239 tendency of starch to reassociate. The extruded rice flours that had undergone the milder
240 treatments showed higher FV values than the same flours extruded with more intense treatments.
241 Although it seems that starch gelatinization is the main factor responsible for changes in the
242 RVA curve, other authors have also observed modifications in the amylose/amylopectin ratio
243 during the extrusion treatments of corn flour (Chinnaswamy & Hannah, 1990) and wheat flour
244 (Colonna et al., 1984). Those authors state that fragmentation of amylose and amylopectin
245 chains takes place during extrusion, and that this is more intense in the amylopectin chains; this
246 will therefore modify the behaviour of the flours during the heating-cooling cycle.

247

248 **3.3. Dough rheology and gas production**

249 **3.3.1. Dynamic rheology of dough.**

250 Figures 2 and 3 show the rheological properties of gluten-free doughs at constant moisture.
251 There was a clear tendency to an increase in G' , G'' and G^* , and a decrease in $\tan \delta$ when
252 extruded rice flours were added to the formula. The most marked effect was observed after the
253 addition of flours 2 and 3; flour 2 was associated with the highest G' and G^* values and the
254 lowest $\tan \delta$ values. Particle size, on the other hand, produced no clear differences. Thus, when
255 increasing the intensity of the extrusion process, doughs became more consistent and elastic, and
256 this effect was most noticeable with flour 2. G' values were higher than G'' values in all cases
257 and over the whole frequency range, which indicated behaviour to be more elastic than viscous.

258 Chao-Chi-Chuang & Yeh (2002), studying the extrusion process, and Kim et al. (2009), using
259 the steam cooking method, observed that the moisture content in hydrothermal treatments was
260 the key to the variation in G' , G'' and $\tan \delta$ values. In particular, Chao-Chi-Chuang & Yeh
261 (2002) detected higher G' and G'' values in the treatments with lower moisture; our
262 observations did not coincide with their findings, though it should be recognized that those
263 authors subjected the flours to higher moisture contents (45%-55%) than were employed in our
264 study, with lower extrusion temperatures (20°C-100°C) and they used waxy varieties of rice.
265 However, those authors did observe that an increase in the mechanical energy applied during
266 extrusion produced an increase in G' and G'' values and a fall in $\tan \delta$ values. It is already
267 known that an increase in the consumption of mechanical energy usually leads to extruded
268 samples with a higher degree of cooking (González et al., 2000). In our case, the flours with
269 lower viscosity values in the RVA curves, and thus a higher degree of cooking, were those that
270 obtained the highest G' , G'' and G^* values.

271 These changes in rheology may be related to changes in the starch during the extrusion
272 treatment, as Shim & Mulvaney (1999) found that the balance between the intact starch granules
273 and gelatinized or damaged ones affected G' values. It has already been shown that extruded
274 flours contain a higher quantity of pregelatinized starch than non-extruded flours and, according
275 to Slade & Levine (1994), this greater degree of gelatinization increases the water absorption
276 capacity of doughs. These rheological changes may also be related to the internal structure of the
277 dough, which may be seen in Figure 4. In the two doughs studied (control dough and dough with
278 flour 3c), the structure is composed of large flour particles covered by small simple starch
279 granules, compacted by a matrix formed of water, hydrocolloids and dissolved substances.
280 However, in the case of dough with flour 3c, there was a smaller quantity of simple starch

281 granules, which is consistent with what was observed in the microstructure of the extruded
282 flours (the starch granules had lost their integrity). The starch granules observed correspond to
283 starch granules from the non-extruded flour. It could therefore be thought that extruded flour is
284 mixed with the network formed by water and hydrocolloids, and this will modify the
285 viscoelastic properties of dough.

286 Additionally, an increase in the quantity of damaged starch granules was observed in the flour
287 3c sample compared with the control, as can be seen in Figures 1 and 4 (starch granules with
288 small holes and breaks) and in table 1.

289 The amount of water needed in bread-making at constant consistency ($G^*=15500\pm1550$) is
290 shown in Table 3. The addition of extruded flours increased the quantity of water required to
291 obtain doughs with constant consistency, and thus they can increase the bread yield. This
292 increase was greater in doughs prepared with flours 2 and 3 (the more intense extrusion
293 treatments) than doughs prepared with flour 1. This finding may be explained by a higher degree
294 of damage caused to the starch granules and to the greater degree of gelatinization in the
295 extrusion process with more intense processing conditions (temperature and moisture) (Mercier
296 and Feillet, 1975). However no clear difference was observed between the doughs made with
297 flours 2 and 3 or with flours with different particle sizes.

298 **3.3.2. Dough height and gas production.**

299 The curves of dough height during fermentation at constant consistency are shown in Figure 5.
300 Regarding gas production, no significant differences were seen in any case (data not shown).
301 Greater height was observed in doughs made with non-extruded flours than in doughs with
302 extruded flours, but the differences only became evident after a certain duration of fermentation
303 (around minute 90). The poor dough height over the whole fermentation process when using

304 flour 1f, was noticeable. The differences between the other extruded flours were minimal but
305 doughs made with flour 3 were somewhat more stable if over-fermentation occurred. Greatest
306 height was obtained in doughs made with flours 1c and 2c, although over-fermentation had a
307 negative effect in both cases. Doughs with flour 3 showed a higher stability than the other
308 doughs, and higher G' and G^* values and lower $\tan \delta$ values; they are thus more consistent and
309 elastic doughs. This could be related to starch gelatinization and damage during the extrusion
310 treatment (Shim & Mulvaney, 1999), as these changes increase the water absorption capacity of
311 dough, as indicated previously (Slade & Levine, 1994).

312 Changes in the dough height during fermentation can be also related to the internal structure of
313 the dough (Figure 4). The dough prepared with flour 3c, which was the dough with the highest
314 stability in the case of excess fermentation, had the lowest quantity of simple starch granules.
315 This is consistent with the results observed in the microstructure of extruded flours, which
316 showed a loss of integrity of the starch granules. It can therefore be assumed that extruded flours
317 form a mixture with the network produced by the water and hydrocolloid, modifying dough
318 height during fermentation.

319

320 **3.4. Bread properties**

321 **3.4.1. Specific volume and weight lost**

322 Table 3 shows the specific volume of breads made at constant consistency. A decrease in the
323 specific volume was observed when extruded flours were added to the formula, except after the
324 addition of flour 1c, which produced a specific volume equal to that of the control. Only the
325 breads prepared with flours 1c and 2c stood out, showing higher specific volumes than the other
326 extruded flours.

327 No differences in weight loss were observed in the doughs during baking (data not shown).
328 In previous studies it has been found that both acidified (Clerici et al., 2009) and non-acidified
329 (Clerici and El-Dash, 2006) extruded rice flours could improve the specific volume of gluten-
330 free breads, depending on the extrusion conditions. However, those authors did not use
331 hydrocolloids in the formula, and the specific volume values obtained were much lower than in
332 our case. This result could indicate some kind of interaction between the hydrocolloids and the
333 extruded flours or their components. Sanchez et al. (2008) made bread with extruded waxy rice
334 flours and also observed an increase in the specific volume of breads when using the extruded
335 flours with the highest intensity extrusion treatments. It is important to note the difference in the
336 moisture values between the bread obtained by those authors as they prepared bread at constant
337 consistency using a penetrometer. In contrast to our study, those authors used a high percentage
338 of starch in the formula and considered that the increase in volume was related to the increase in
339 the quantity of soluble solids, as those components increased the consistency of the aqueous
340 phase, improving the viscoelastic characteristics of doughs.

341 Defloor et al. (1991) also found that the addition of extruded starch improved the volume of
342 breads prepared with a mixture of tapioca and soya flour. It is important to note that these
343 authors used high levels of hydration (145%), and their doughs were therefore less consistent
344 than ours. In their case, extruded starches increased the consistency of very soft doughs (in
345 contrast to our study, in which we used more consistent doughs with lower hydration) and made
346 it possible to increase bread volume.

347 Gallagher et al. (2003), on the other hand, observed that an increase in dough hydration
348 increased bread volume. In our study we also found that the level of hydration could alter the
349 effect of including certain components that increase dough consistency, such as extruded flours,

350 and particularly extruded coarser flours, which have a high amount of damaged starch that could
351 contribute greatly to increase dough hydration, as can be seen in table 1 and in figure 1 and 4. In
352 excessively soft doughs, a certain increase in consistency could therefore be useful, though this
353 can have a negative effect in more consistent doughs with lower hydration. Nevertheless, this
354 factor alone cannot explain the changes observed, as breads with a constant consistency were
355 also found to have a different specific volume.

356

357 **3.4.2. Texture analysis**

358 The texture parameters of bread at constant consistency are presented in Table 3.

359 A tendency to increased hardness was observed as the extrusion intensity of the flours increased.

360 Higher hardness values were also found when using fine flours compared to coarse ones,
361 although significant differences were only detected with loaves elaborated using flours 2.

362 No significant differences were found in the springiness or cohesiveness of loaves made with
363 extruded flours compared to control loaves. As in our study, Clerici & El-Dash (2006) reported
364 higher hardness values in breads made with extruded flours. Maleki et al. (1980) and Morad &
365 Wakeil (1976) found that starch retrogradation was strongly influenced by the moisture content
366 of the product. The reduction of starch retrogradation through increased moisture content would
367 therefore produce softer breads. It is also known that bread hardness correlates with bread
368 volume (Gómez et al., 2011), and thus the explanation of the differences in hardness could be
369 related to differences in the specific volume.

370 The changes in hardness over time are shown in Figure 6. Loaves made with the control flour
371 and with flours 1f and 2f showed the fastest rates of hardening. Loaves made with flours 1c and
372 2c showed the lowest hardness values and their hardening curves had a low gradient. Loaves

373 made with flours 3 had the highest hardness values up to 24 hours, but the values subsequently
374 remained almost constant or even fell, indicating a decrease in the rate of staling.
375 Changes in crumb properties associated with staling include an increase in starch crystallinity
376 and crumb hardness and a decrease in aroma, soluble starch and crumb hydration capacity
377 (D'Appolonia & Morad, 1981). Rogers et al. (1988) stated that the main cause of bread staling is
378 starch retrogradation, which increases with increased moisture content of breads. As gluten-free
379 breads have a high moisture content, starch retrogradation may progress faster during storage
380 than in gluten breads. Extruded flours have a higher water-retention capacity than non-extruded
381 flours and they could thus delay water migration from the crumb to the crust, decreasing the rate
382 of staling. Furthermore, the extrusion process may even break the amylopectin chains (Mercier
383 and Feillet, 1975) and Colonna et al. (1984) demonstrated that extruded wheat starches contain
384 amylose and amylopectin chains with lower molecular weights than drum-dryer starches; this
385 could be another factor that decreases retrogradation and the rate of staling of bread.

386

387 **4. Conclusion**

388 The use of extruded flours in the elaboration of gluten-free bread offers an interesting alternative
389 approach to improve gluten-free breads. We found that the addition of extruded flours subjected
390 to high intensity extrusion treatments produced doughs with a higher elastic modulus and
391 consistency, and that it was necessary to add larger volumes of water to achieve constant
392 consistency. The bakery yield is therefore increased. However, the development of doughs
393 prepared with extruded flours was lower, but these doughs showed higher stability to over-
394 fermentation. In general, the addition of extruded flours reduced the specific volume of breads
395 and increased hardness, but these effects were minimized by using the coarse flour fractions,

396 which also reduced the rate of staling. Future studies should look in detail at the effect of these
397 extruded flours on the acceptability of breads and their influence in breads with higher levels of
398 hydration.

399

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403

404 **References**

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503 response surface methodology to the development of rice flour yeast breads: objective
504 measurements. *J Food Sci*, 53, 1800-1805.

505

506 Table 1: Chemical composition of different types of flour of non-extruded flour with 10%
 507 substituted extruded flour.

Flours	Damage Starch (%)	Protein (%)	Moisture (%)
Flour 3f	29,81±2.50	7,85±1.24	9,56±0.40
Flour 3c	36,57±0.28	8,42±1.50	10,23±0.65
Flour 2f	19,78±2.60	8,25±1.30	13,96±2.10
Flour 2c	30,29±4.22	8,11±1.11	14,04±1.74
Flour 1f	11,09±2.91	8,74±0.98	10,72±3.65
Flour 1c	10,88±1.64	8,04±1.01	11,2±2.64
Control	5,75±0.97	7,81±0.99	13,45±3.05

508

509 Table 2: Viscous behaviour in a heating-cooling cycle of non-extruded flour with 10%
 510 substituted extruded flour.

Flours	PV (cp)	BR (cp)	TR (cp)	ST (cp)	FV (cp)
Flour 3f	3793a±94	1296a±2	2496a±93	2510b±6	5006a±98
Flour 3c	3913a±46	1224a±182	2688ab±135	2306a±106	4994a±29
Flour 2f	4596b±64	1759bc±19	2837b±84	2773cd±17	5610b±67
Flour 2c	4571b±16	1690bc±160	2881b±176	2656bc±121	5537b±56
Flour 1f	4444b±77	1663b±35	2781ab±42	2777cd±26	5558b±16
Flour 1c	4682b±244	1706bc±11	2975bc±234	2706c±6	5681b±228
Control	5175c±142	1947c±192	3228c±49	2925d±62	6153c±12

511 Values with different letters in the same parameter are significantly different (p<0.05).

512 Data shown are the mean of two repetitions for each type of simple

513 PV = Pasting temperature; BR = Breakdown; TR = Trough; ST = Setback; FV = Final viscosity

514

515 Table 3: Specific volume, texture properties and percentage hydration of breads made at
 516 constant consistency.

Flour	Specific volume (m ³ /kg)	Hardness (N)	Springiness	Cohesiveness	% Hydration
Flour 3f	2.807a±0.121	22.018e±1,155	0.689a±0,030	0.244a±0,001	76.67d±0,01
Flour 3c	2.813a±0.192	21.370e±0,588	0.738a±0,045	0.314a±0,102	74.55c±0,31
Flour 2f	2.987a±0.171	13.675d±0,583	0.706a±0,015	0.271a±0,005	74.86c±0,08
Flour 2c	3.637b±0.259	6.430c±1,649	0.635a±0,070	0.307a±0,061	78.92c±0,23
Flour 1f	3.041a±0.311	4.153b±0,181	0.615a±0,007	0.286a±0,003	72.98b±0,01
Flour 1c	4.597c±0.121	2.431b±0,378	0.590a±0,125	0.297a±0,013	73.36b±0,33
Control	4.802c±0.044	1.723a±0,693	0.656a±0,067	0.348a±0,018	70.00a±1,17

517 Values with different letters in the same parameter are significantly different (p<0.05).

518 Data shown are the means of two repetitions for each kind of simple

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521 Figure captions:

522 Figure 1: Photomicrographs from the environmental scanning electron microscope (ESEM)
523 ($\times 2000$) study of the control flour and flours extruded with different intensities of treatment
524 (analysis made in triplicate with subsequent selection of the most representative
525 photomicrographs). a) control flour (non-extruded), b) extruded flour 1f, c) extruded flour 2f, d)
526 extruded flour 3f. 1) compound starch granule, 2) swollen starch granule, 3) gelatinized starch
527 granules.

528 Figure 2: Mechanical spectrum of doughs. G' and G'' values according to oscillation frequency
529 (ω) in fine flours (a) and coarse flours (b). G' values are represented by filled symbols and G''
530 values are represented by unfilled symbols. Control (diamond), flour 1 (square), flour 2
531 (triangle), flour 3 (circle).

532 Figure 3: Mechanical spectrum of dough. $\tan \delta$ (a) and G^* (b) according to oscillation frequency
533 (ω). Extruded fine flours are represented by unfilled symbols and extruded coarse flours by
534 filled symbols. Control (cross), flour 1f (unfilled square), flour 2f (unfilled triangle), 3f (unfilled
535 circle), 1c (filled square), 2c (filled triangle), 3c (filled circle)

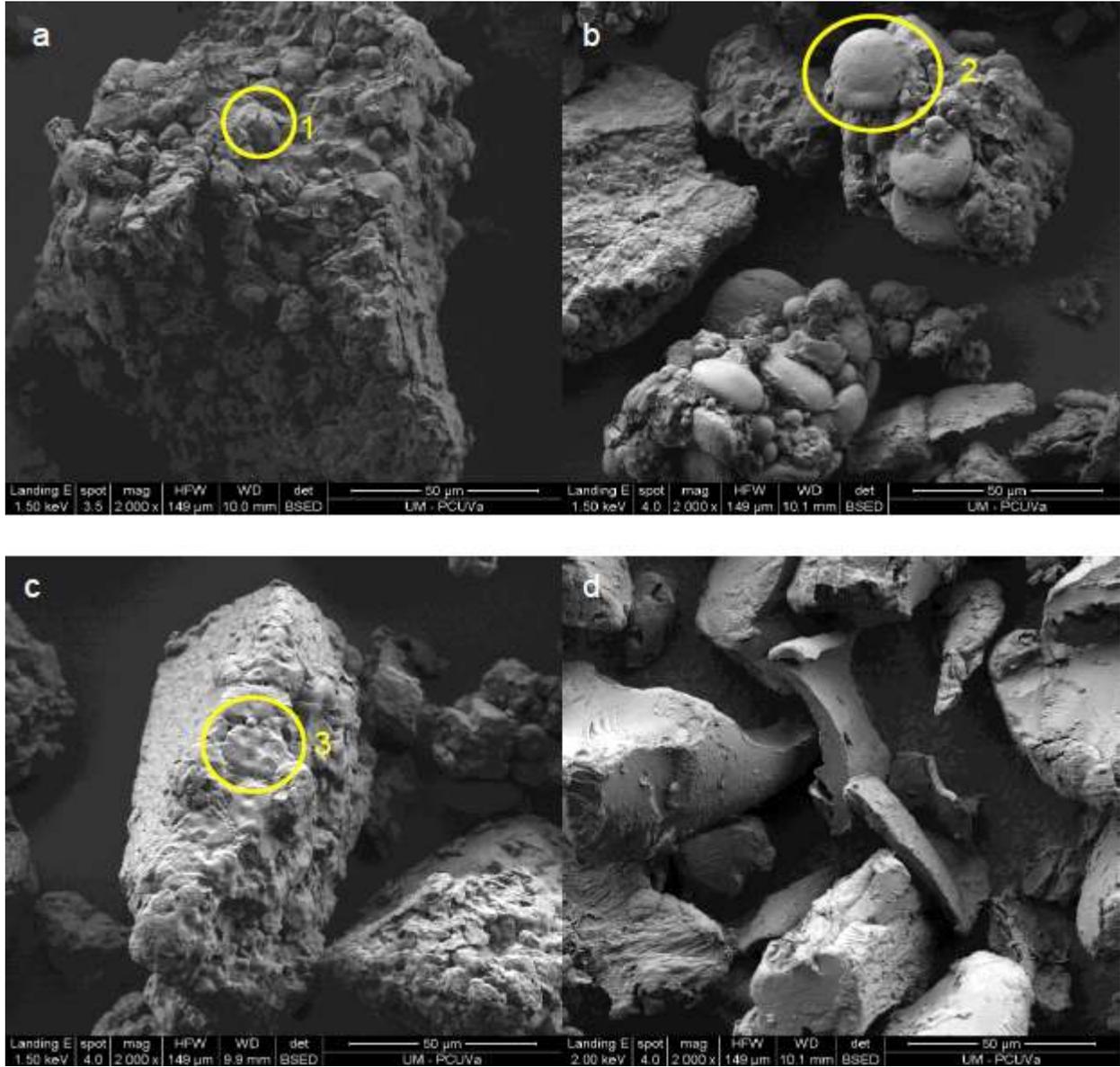
536 Figure 4: Photomicrographs of the scanning electronic microscope (SEM) ($\times 1000$) of doughs
537 prepared with non-extruded flour with 10% substitution by extruded flour (analysis performed in
538 triplicate with subsequent selection of the most representative photomicrographs). a) control, b)
539 dough made with flour 3c,. Arrows indicate damaged starch granules.

540 Figure 5: Dough height during fermentation.

541 Figure 6: Changes in hardness over time. Extruded fine flours are represented by unfilled
542 symbols and discontinuous lines. Extruded coarse flours are represented by filled symbols and

543 continuous lines. Control (cross), flour 1f (unfilled square), flour 2f (unfilled triangle), 3f
544 (unfilled circle), 1c (filled square), 2c (filled triangle), 3c (filled circle)
545

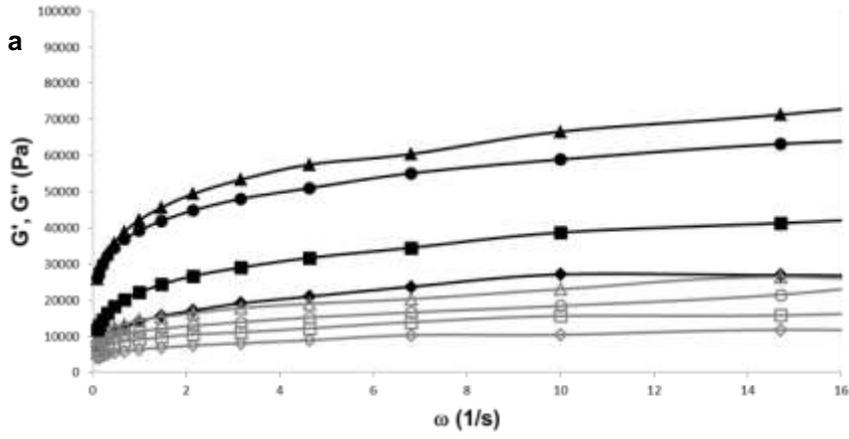
546 Figure 1:



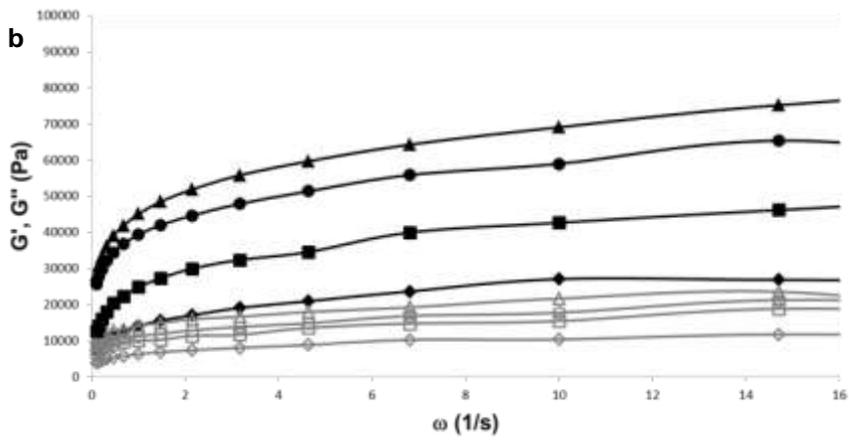
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549 Figure 2:



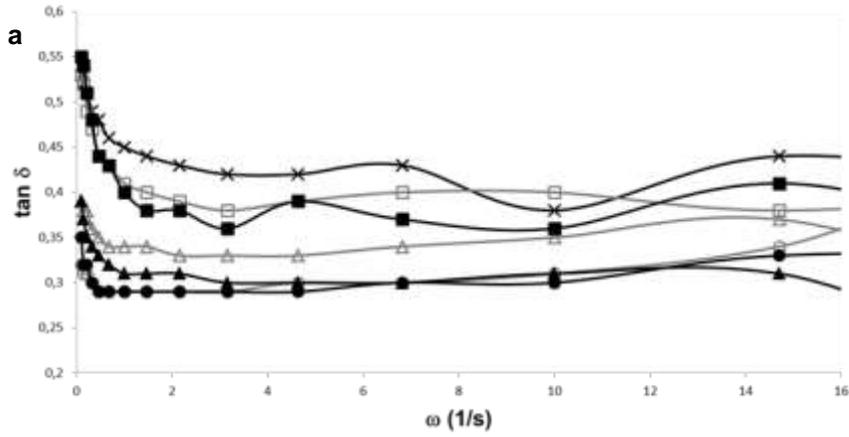
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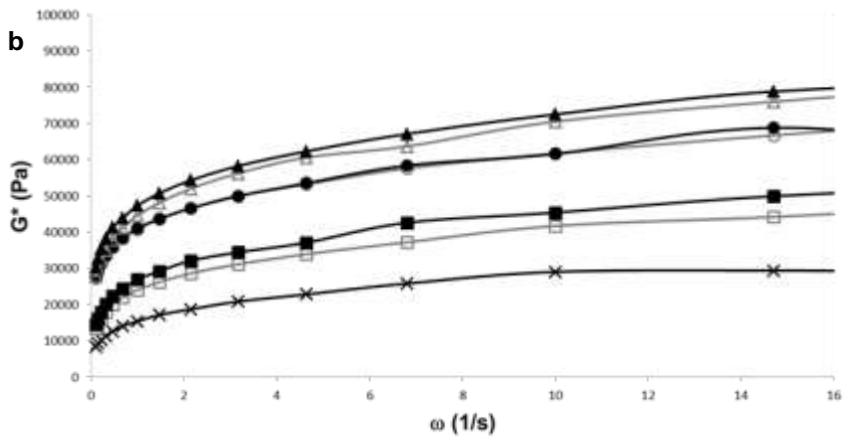
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553 Figure 3:



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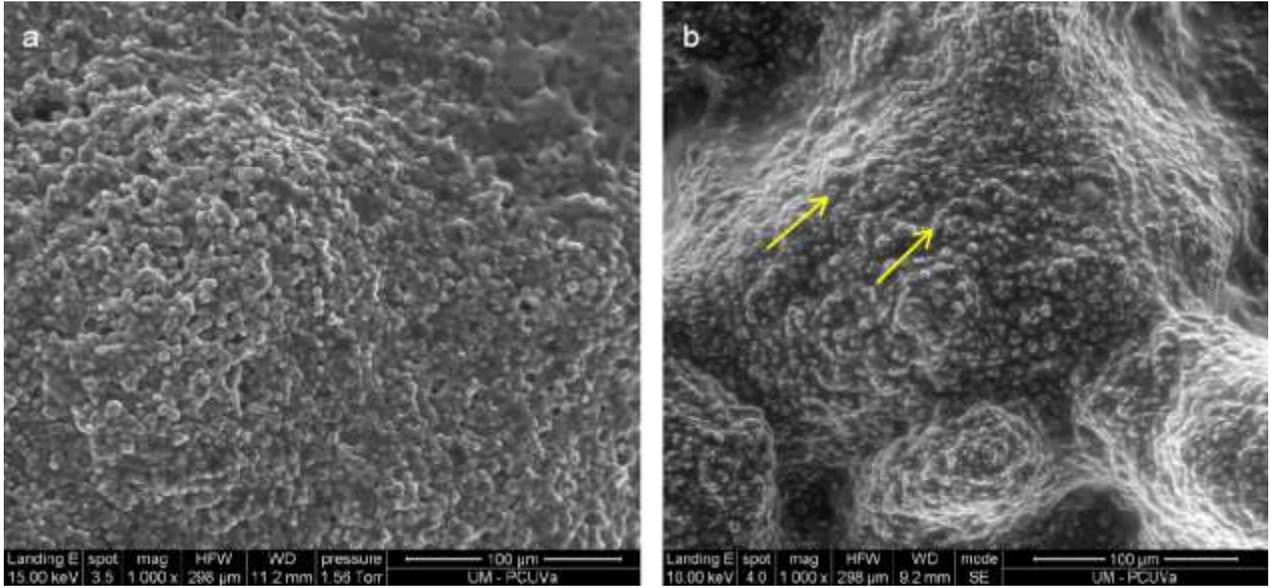
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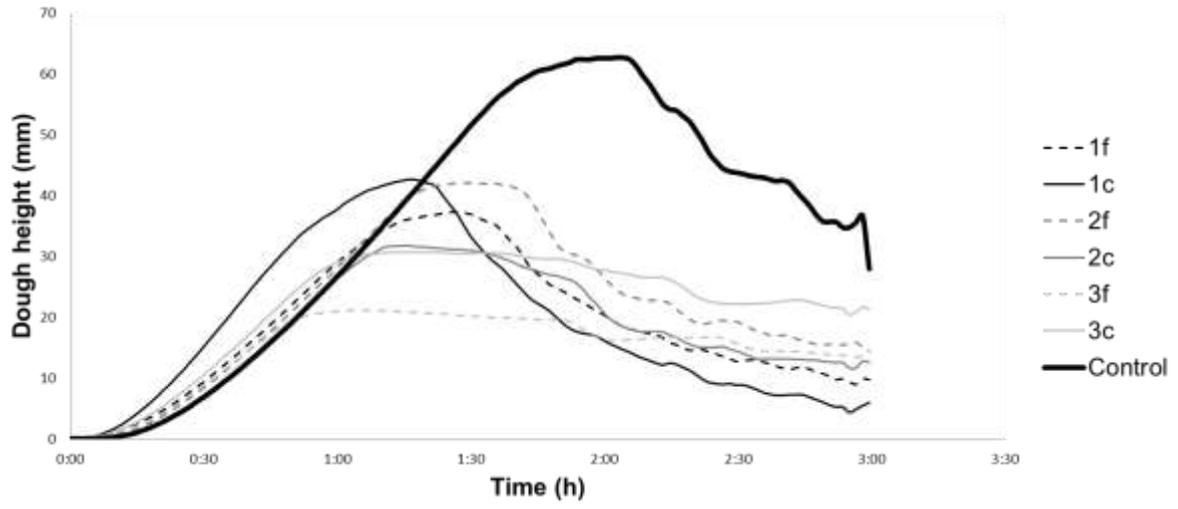
563 Figure 4:



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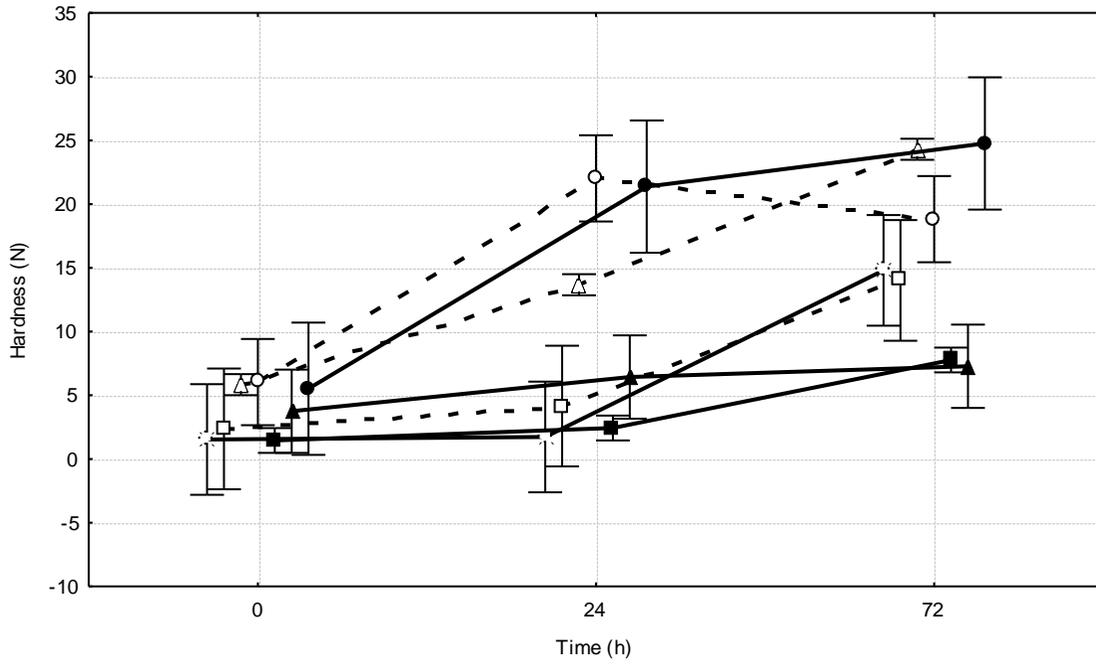
566 Figure 5:



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569 Figure 6:



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