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4	Whey-Bread, an Improved Food Product: Evaluation of					
5	Textural Characteristics					
6	Short Title: TEXTURE CHARACTERISTICS OF WHEY-BREAD					
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Whey-Bread, an Improved Food Product: Evaluation of Textural Characteristics

Short Title: TEXTURE CHARACTERISTICS OF WHEY-BREAD

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24

27 Abstract

28 The diversity in bread all around the world is enormous and enriched breads are a 29 trend to follow in the next years. The aim of this work was to develop new breads 30 incorporating whey residue, and the final products were analysed for their textural 31 properties, as compared with a basic wheat bread. For measurement of texture two types 32 of teste were used (compression and puncture). The results showed that the whey 33 residue could be used to produce bread with good textural properties, particularly for an 34 improved recipe. The improved whey bread showed good textural characteristics, which 35 remained practically unchanged after 24 h, being this true for the properties evaluated 36 through the compression test (hardness, chewiness, resilience, cohesiveness, 37 springiness) and through the puncture test (external firmness, inner firmness, stickiness, 38 adhesiveness). Finally, very strong correlations were found between cohesiveness and 39 resilience and between adhesiveness and stickiness.

40

41 Key words: compression test, puncture test, residue valorisation, textural properties.

42

43 Introduction

44 Bread is undoubtedly recognized as one of the most extensively consumed basic45 foods worldwide. Due to variable chemical composition of flours and utilized baking

46 processes, breads form a food group with highly heterogeneous structures. White wheat 47 bread is a commodity usually baked of starchy endosperm flour. During dough mixing, 48 wheat gluten proteins are transformed into a network in which carbon dioxide generated 49 by yeast fermentation is retained thus producing an expansion during fermentation and 50 baking (Gao, Tay, Koh, & Zhou, 2018; Pentikäinen et al., 2014).

The mechanical properties of the crumb and the crust determine the textural characteristics of bread. Furthermore, the mechanical properties are related to the structural and physical characteristics of the bread matrix. A sequence of texture sensations is perceived by people while chewing bread due to the continuous transformation of its structure, mostly due to the particle size reduction and saliva impregnation processes (Gao et al., 2018; Gao, Wong, Lim, Henry, & Zhou, 2015; Jourdren et al., 2016; Pentikäinen et al., 2014).

58 According to a United Nations report, the world population is expected to surpass 59 9.2 billion by 2050, which brings unquestionably a great challenge of how to feed such 60 an enormous population. Therefore, the efficient use of the available resources is a must 61 that needs to be addressed with urgency. The concept of circular economy contemplates 62 the reutilization and recycling of all types of waste. Therefore, the utilization of organic 63 residues for the production of value added products either for the food, pharmaceutical 64 or cosmetic industries allows the application of the circular economy concept on organic 65 waste management and contributes to the development of a bio-based economy. Many 66 residues and by-products of the food industry can be utilized to produce new foods 67 and/or ingredients with additional nutritional value and improved bioactive properties (de Oliveira, da Silva Lucas, Cadaval, & Mellado, 2017; Keegan, Kretschmer, Elbersen, 68 69 & Panoutsou, 2013; Pleissner et al., 2016).

70 The dairy industry is responsible for the generation of large quantities of liquid 71 effluents, which are also typically characterized by a high organic load (Akhlaghi et al., 72 2017). According to a report about European Commission statistics (European 73 Commission, 2017), the overall amount of dairy products generated in the EU-28 area 74 accounted for more than 100 million tonnes in 2016. Among the main dairy products 75 manufactured that contribute for this waste generation are drinking milk (30.7% of the 76 overall production in 2016), cheese (9.6%), acidified milk (7.9%), cream (2.8%), 77 powder products (2.8%) other products (2.8%), other miscellaneous fresh products 78 (2.6%) and finally butter and other yellow fat products (2.4%) (Akhlaghi et al., 2017; 79 European Commission, 2017).

80 Because there is a growing need to minimize industrial waste by preferably 81 reutilizing the residues and transforming them into products with commercial value, and 82 in particular addressing the needs of some dairy facilities in the central region of 83 Portugal which produce cheese from sheep milk, the traditional Serra da Estrela cheese, 84 it is important to find alternative ways to use the whey residues originating in such dairy 85 facilities. Hence, the objectives of this work were to develop new added-value bakery 86 products incorporating whey residue, in order to take advantage of a resource with 87 nutritional relevance and at the same time minimizing environmental impacts by finding 88 alternative ways to use this residue. Additionally, the textural characteristics of the 89 developed products (breads) were evaluated shortly after baking, and again after 6 and 90 24 hours to observe the evolution of those properties along time.

91

92 Material and methods

94 **Preparation of the breads**

95 Basic wheat bread

96 The water (1700 mL) was warmed at a temperature of about 30 °C, and 40 mL of the 97 warm water was used to dissolve the yeast (40 g of fresh yeast), which was then left to 98 stand for five minutes. After that 2 kg of refined flour type 65 and 40 g of salt were 99 mixed with the water and the yeast. The dough was beaten until the desired elasticity 100 and homogeneity were achieved (about 12-15 min). The dough was left in a stove at 35 101 °C for 20 min to ferment. At the end of the elapsed time, the loaves with about 100 102 grams each were moulded in a round shape, and were taken for more 15 minutes to the 103 stove. Finally, they were baked in the oven, previously heated to 240 °C, for twenty 104 minutes. In the first minutes of the baking process 2 steam baths were performed, to 105 avoid early formation of the crust.

106

107 Bread with whey residue

108 The procedure for the production of this bread was similar to the one described for 109 the basic wheat bread, however some changes were made in the formulation. The same 110 amount of flour was used (2 kg), but the water was replaced by whey residue (1045 mL) 111 and the amount of fresh yeast was changed to 60 g. Also, because the whey residue is 112 obtained from the productive process of making cheese and whey cheese, and therefore 113 already contains some incorporated salt, no salt was added when making the bread. 114 Figure 1 shows the whey residue used, which is considered a waste in the cheese and 115 whey cheese making industry.

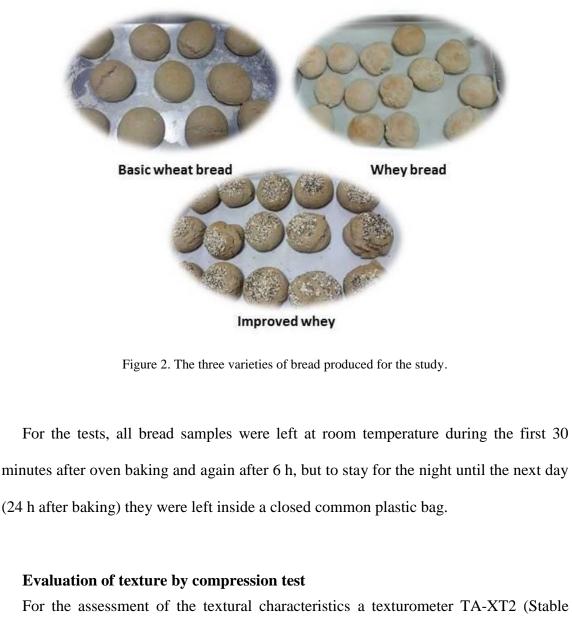


116	
117	Figure 1. Whey residue from the cheese and whey cheese making industry.
118	
119	Improved whey bread
120	The procedure was basically similar to that used for other breads, but more changes
121	were introduced in the formulation so as to produce bread with better nutritional value.
122	Table 1 shows the formulation used to produce the <i>improved whey bread</i> . The pumpkin
123	seeds were slightly dehydrated (approximately 10 min at 240 °C) before incorporation
124	into the dough. The various seeds and the oat flakes were used to produce o cover on
125	the surface of the bread before going to bake in the oven.

Table 1. Amount of ingredients used to produce the improved whey bread.

Ingredient	Amount	Ingredient	Amount	
	(unit)		(unit)	
Refined wheat flour (type	500 (g)	Pumpkin seeds	129 (g)	
65)				
Whole wheat flour	1500 (g)	Chia seeds	8 (g)	
Whey residue	1200 (mL)	Poppy seeds	5 (g)	
Fresh yeast	60 (g)	Sesame seeds	9 (g)	
Oat flakes	11 (g)			

- 129 Figure 2 shows the three types of bread produced.



140 Microsystems) was used and two types of test were performed: compression test and 141 puncture test.

142 The texture profile analysis was carried out by a compression test involving two 143 compression cycles between parallel plates performed on the samples using a flat compression probe 75 mm in diameter (P/75), with 5 seconds of interval between cycles. The parameters used for the test were: 30 kg force load cell, pre-test, test and post-test speeds equal to 1.0 mm/s, distance 4 mm and trigger force 0.1 N. The textural properties: hardness, resilience, springiness, cohesiveness and chewiness were calculated after equations (1) to (5) (see Figure 3) (Correia et al., 2017):

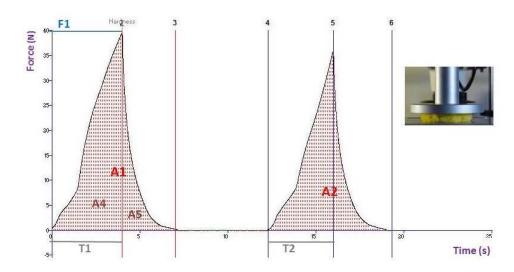
149 Hardness (N) =
$$F_1$$
 (1)

150 Resilience (%) =
$$(A_5/A_4) \times 100$$
 (2)

- 151 Springiness (%) = $(T_2/T_1) \times 100$
- 152 Cohesiveness (%) = $(A_2/A_1) \times 100$ (4)

Chewiness (N) =
$$F_1 x (T_2/T_1) x (A_2/A_1)$$
 (5)

(3)



154

153

155 Figure 3. Example of a texture profile analysis for bread, obtained by compression with a P/75 probe.

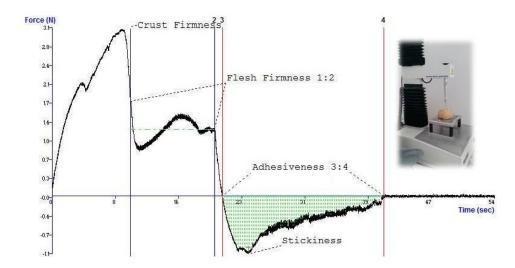
156

For these textural evaluations 8 units of each type of bread were used, and for each bread one measurement was made on the top and another on the bottom faces of the bread. Furthermore, the samples were evaluated 30 minutes after baking, and again 6 hours and 24 hours later, since this is a bread without preservatives, destined to be consumed within 24 hours of baking, preferably. The results were processed using Exponent software TEE from Stable Micro Systems.

164 **Evaluation of texture by puncture test**

To undertake the puncture test a drilling rig with 2 mm diameter was used. The pre-test speed was 2 mm/s and the test and post-test speeds were 1 mm/s. The perforation distance was 10 mm and the trigger force 0.05 N. The texture parameters evaluated with this test were: crust firmness, flesh firmness, adhesiveness and stickiness, as indicated in Figure 4, and defined in equations (6) to (9):

- 170 Crust firmness (N) = maximum force (6)
- 171 Inner firmness (N) = average force between 1 and 2 (7)
- 172 Adhesiveness (N.s) = negative area (8)
- 173 Stickiness (N) = minimum force
- 174



(9)



176

Figure 4. Example of a texture profile for bread, obtained by puncture with a P/2 probe.

177

For these textural evaluations 8 units of each type of bread were used, and for each bread six measurements were made on the top and another six on the bottom faces of the bread. The results were also processed with TEE software.

182 Statistical analysis

183 To validate the results obtained for the mean values calculated, a comparison of 184 means was performed by an analysis of variance (ANOVA), with the Post-Hoc Tukey 185 HSD (Honestly Significant Difference) test for identification of differences between 186 samples. Also the Pearson correlation coefficients were used to evaluate the possible 187 associations between properties. For absolute value of r = 0 there is no correlation, for r 188 $\in]0.0, 0.2[$ the correlation is very weak, for $r \in [0.2, 0.4]$ the correlation is weak, for r 189 $\in [0.4, 0.6]$ the correlation is moderate, for r $\in [0.6, 0.8]$ the correlation is strong, for r 190 $\in [0.8, 1.0]$ the correlation is very strong, for r = 1 the correlation is perfect (Maroco, 191 2012; Pestana & Gageiro, 2014).

For all statistical analyses was used the software SPSS version 24 (IBM, Inc.) and the level of significance considered was 5% (p < 0.05).

194

195 **Results and discussion**

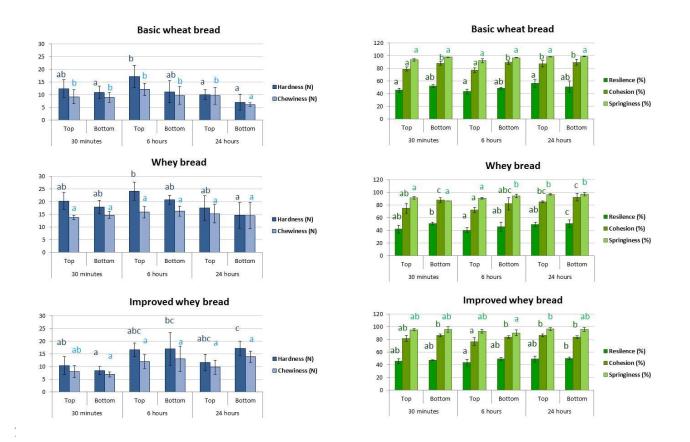
196

Textural properties – compression test

198 The textural properties evaluated by the compression test were hardness, resilience, 199 springiness, cohesiveness and chewiness. Hardness represents the force necessary to 200 compress a food between the teeth or between the palate and the tong. Chewiness 201 measures the energy required to disintegrate a food to a state suitable to swallow. 202 Springiness is associated with the ability to recover shape after compression, being 203 equal to the rate at which the product returns to the initial point after removal of the 204 deforming force. Resilience is the energy used when applying a force to a material 205 without occurring rupture, with or without any residual strain, and corresponds to an 206 instant springiness. Cohesiveness represents the internal forces inside the food that stop

the sample from disintegrating (Cruz, Guiné, & Gonçalves, 2015; R. P. F. Guiné,
Henriques, & Barroca, 2014; Raquel P. F. Guiné, Almeida, Correia, & Gonçalves,
209 2015).

Figure 5 shows the mean values of the textural properties evaluated by the compression test and the corresponding standard deviation. The results were subject to a statistical analysis to verify if significant differences were found in the mean values for each property.



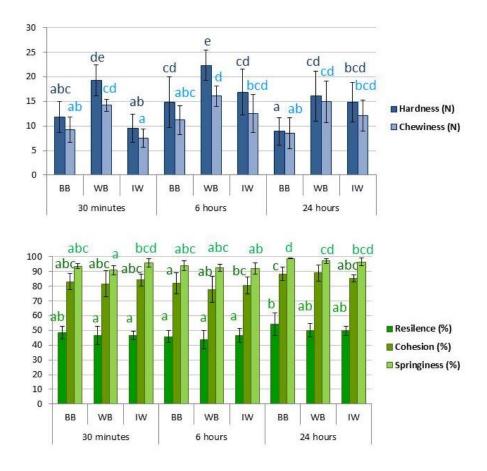
215Figure 5. Textural properties evaluated by the compression test, separated according to the sides of the216bread samples (Bars with the same letter are not significantly different: ANOVA with Tukey post-hoc test,217p > 0.005).

The results in Figure 5 show that the *whey bread* presented in general highest hardness and chewiness as compared with the *basic wheat bread* and the *improved whey bread*. The *improved whey bread*, which is intended to be marketed, presented uniform

hardness and chewiness considering both sides of the loaf (the top and the bottom), varying from 8-10 and 6-8 N, respectively, 30 minutes after oven baking. Furthermore, as time elapsed the texture became harder after 6 hours and remained similar after 24 hours. This is important, since it is expected that the bread is consumed within 24 hours after baking, for optimum textural characteristics. As for resilience, cohesiveness and springiness, the 3 types of bread were not much different, and no apparent differences were seen also between the top and bottom sides of the loaves.

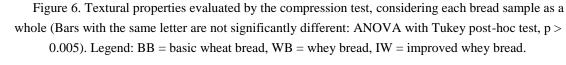
229 Figure 6 shows the results obtained for the textural properties of the compression 230 tests, but considering the bread samples as whole, i.e., not differentiating the top from 231 the bottom sides. The results indicate that the *improved whey* bread was softer after 30 232 min of baking, but regained some firmness after some hours and maintained it for 24 233 hours, this being evident on the values of hardness and chewiness, which were 15 and 234 12 N, respectively. According to Ozturk and Mert (2018) solubilized proteins, which are 235 present in the whey residue, can produce a more homogenous structure, providing softer 236 products. On the other hand, as time passes the texture of bread is expected to change, 237 namely by increasing hardness (Barbosa-Ríos et al., 2018).

Considering the other textural properties, the differences between types of bread or times of evaluation were not so representative. Ozturk and Mert (2018) observed that springiness values in samples of gluten-free corn breads decreased over time, indicating elasticity loss, but that was not the case with the present breads analysed, who were able to maintain the textural properties for a period of 24 hours, which is the expected period for consumption at optimum conditions of bread formulated without preservatives.





246 247 248



250 **Textural properties – puncture test**

251 The puncture test gives information about the external and internal firmness, i.e., the 252 resistance of the crust and of the inner crumbs, as well as the stickiness and 253 adhesiveness. Adhesiveness corresponds to the negative area after the probe was 254 removed from the sample and corresponds to the force required to remove the material 255 that adheres to a specific surface (e.g., lips, palate, teeth). Stickiness is also related to 256 adhesiveness and corresponds to the minimum force (negative value) registered by the 257 probe right before starting to retract from the sample.

258 The results presented in Figure 7 correspond to the evaluation of the bread loaves 259 separately as top and bottom faces, and they indicate that the crust firmness was always

higher when compared to the inner firmness, which is in accordance with the fact that 260 261 during baking a crust is formed on the bread producing a harder surface. However, it 262 was observed that after 24 h the firmness tended to diminish for all types of bread. It 263 was reported that bread crust loses its crispness within a few hours after baking due to 264 water uptake from the soft and moist crumb, and hence, the crispy texture of the crust is 265 directly related to the water uptake kinetics (Meinders & van Vliet, 2011). The 266 *improved whey bread* allowed a better preservation of both the external and inner 267 firmness, as compared with the other bread samples evaluated. The adhesiveness was 268 considerable for all three types of bread, but stickiness was low in all cases. The 269 *improved whey bread* showed a more uniform trend for adhesiveness along time when 270 compared to the basic wheat bread or the whey bread.

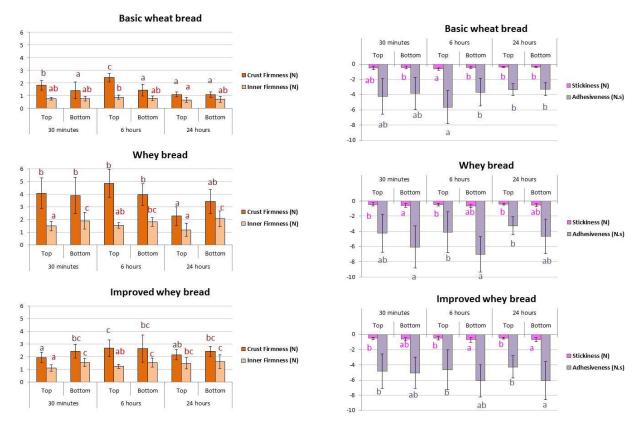


Figure 7. Textural properties evaluated by the puncture test, separated according to the sides of the 273 bread samples (Bars with the same letter are not significantly different: ANOVA with Tukey post-hoc test, 274 p > 0.005).

276 Figure 8 presents the values obtained for the textural properties through the puncture 277 test, but considering each bread sample as a whole. The sample whey bread was the 278 hardest, for all moments of evaluation, being significantly different from the others in 279 terms of external and internal firmness. Regarding the improved whey bread, the 280 firmness was just slightly increased from the 30 min to the 6 h and after that was kept 281 approximately constant, which indicates its suitability for preservation of the desired 282 textural properties. This sample (IW) also showed a constant adhesiveness and 283 stickiness over the time of evaluation, thus confirming the ability to maintain the textural characteristics for the desired period of 24 h. 284

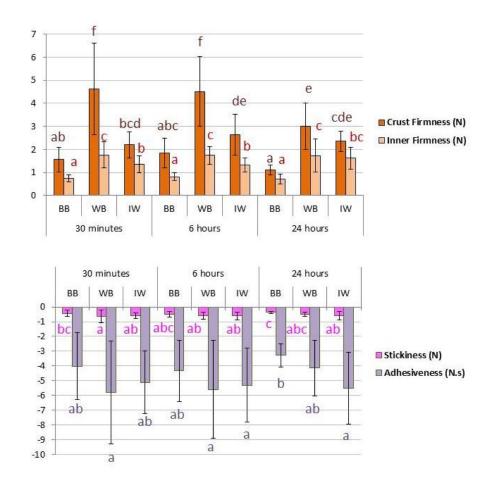


Figure 8. Textural properties evaluated by the puncture test, considering each bread sample as a whole
(Bars with the same letter are not significantly different: ANOVA with Tukey post-hoc test, p > 0.005).
Legend: BB = basic wheat bread, WB = whey bread, IW = improved whey bread.

290 **Correlations**

291 Table 1 presents the Pearson correlations between the textural variables studied, i.e., 292 those obtained with the compression test and with the puncture test. Generically, the 293 properties from the compression test do not correlate with those from the puncture test, 294 which would be predictable given the highly different nature of each test: one 295 corresponding to compression on the surface and the other comprising penetration 296 inside the sample. On the other hand, for each of the tests separately, there are important 297 correlations, as highlighted for example by the correlation between cohesiveness and 298 resilience (r = 0.939) for the compression test and between adhesiveness and stickiness 299 (r = 0.858) for the puncture test, which are considered very strong $(0.8 \le r < 1)$. There 300 are also some strong correlations ($0.6 \le r < 0.8$) like in the case of the chewiness and 301 hardness (r = 0.637) and springiness and cohesiveness (r = 0.639), in the compression 302 test, or even between internal firmness and crust firmness (r = 0.767) for the puncture 303 test. 304

		Compression test					Puncture test			
		НА	RE	СО	SP	СН	CF	IF	ST	AD
Compression	HA	1								
	RE	0.052	1							
	СО	-0.013	0.939**	1						
	SP	-0.099	0.577**	0.639**	1					
	СН	0.637**	0.444**	0.414**	0.594**	1				
Puncture	CF	0.504**	0.133	-0.092	-0.126	0.273**	1			
	IF	0.424**	0.055	0.070	-0.092	0.287**	0.767**	1		
	ST	-0.130	0.020	-0.013	0.156	0.011	-0.378**	-0.381**	1	
	AD	-0.104	0.061	0.034	0.161	0.062	-0.321**	-0.267**	0.858**	1

308 Notes:

309 HA = Hardness, RE = Resilience, CO = Cohesiveness, SP = Springiness, CH = Chewiness, CF =

310 Crust firmness, IF = Inner Firmness, ST = Stickiness, AD = Adhesiveness.

311 **Correlation is significant at the 0.01 level.

312

313 **Conclusions**

314 This work allowed concluding that whey residue can be used to produce bread with 315 good textural properties, and an improved formulation with whey residue and some 316 other functional ingredients was developed. The improved whey bread tended to 317 become harder after 6 h of baking but did not change any more after 24 h. Resilience, 318 cohesiveness and springiness were not variable with time over an evaluation period of 319 24 h. Furthermore, the textural properties of the puncture test (external and internal 320 firmness, stickiness and adhesiveness) were approximately constant over time. Finally, 321 some very strong correlations were encountered between the textural properties

evaluated, namely between cohesiveness and resilience and between adhesiveness andstickiness.

324

325 **Implications and future work**

326 The success in utilizing the whey residue originating from the numerous dairy 327 facilities the used sheep milk to produce cheese in the central region of Portugal has a 328 first impact by greatly minimizing the amount of liquid effluents that are sent every day 329 to the sewage treatment plants, thus minimizing the environmental impact and reducing 330 operating costs for treatment of residues. Furthermore, for the owners of the dairy 331 facilities this provides additional revenue, because they sell the whey residue instead of 332 discarding it. Regarding the bakery industries, they are able to produce bread with whey 333 residue, especially by following the improved formulation hereby developed, and this 334 bread proved to have good textural properties, and therefore may have good 335 acceptability by the consumers.

Because this work focused on developing bread products and evaluating at first the textural properties, the work undertaken so far should be complemented with further work to evaluate the chemical and nutritional properties, having in mind that it was developed an improved recipe with potentially functional ingredients. Also, the developed products could be submitted to a sensory analysis for a better knowledge of the acceptability by the potential future consumers. Finally, other bakery products could be developed incorporating whey residue, like biscuits or cookies.

343

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