

1                   **Use of succinyl chitosan as fat replacer on cake formulations**

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13                  **RUNNING TITLE:** Succinyl chitosan as fat replacer in bakery

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26 **ABSTRACT**

27 Chitosan derivatives have been used in bakeries as dietary fiber source for obtaining healthy  
28 breads, but no other application has been suggested. This research was focused on exploring  
29 the ability of a chitosan derivative (succinyl chitosan, SC) as fat replacer in cakes. SC  
30 suspensions were used to replace different levels of fat (up to absence of fat) and the effect on  
31 batters' consistency and cakes features (color, morphogeometrics properties, texture, moisture  
32 and water activity) were evaluated. SC suspensions (2 g/100 g) were used to replace different  
33 levels of fat in cakes. By adapting batter consistencies, it was possible to obtain fat reduced  
34 batters with the same density, and cakes with high 2D area and lower ratio (width/height)  
35 containing half of the fat present in the reference cake. Crumb structure and texture features  
36 of fat reduced cakes containing SC was similar to that of full fat cakes. During staling, SC  
37 reduced hardening rate of cakes containing half fat, although drying rate was accelerated. The  
38 results indicated that succinyl chitosan has a great potential to be used as partial fat replacer  
39 on cakes, even reducing hardening rate during storage.

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41 **Keywords:** chitosan; fat replacer; cake; batter; quality

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## 44 1. INTRODUCTION

45 Sustainability is one of the main concerns of the food industry, particularly fisheries, owing to  
46 the huge amount of by-products daily generated (Martins, Pinho & Ferreira, 2017). Marine  
47 by-products from the shell of shrimp, krill, crabs and lobsters are rich in the amino-  
48 polysaccharide chitin ( $\beta$  (1–4) linked 2-acetamido-2-deoxy- $\beta$ -d-glucopyranose), the second  
49 most abundant polymer in nature after cellulose (Alishahi & Aider, 2012). Like cellulose,  
50 chitin is highly insoluble due to their extensive hydrogen bonding and high degree of  
51 acetylation, because of that, it is usually deacetylated for increasing its solubility leading to  
52 chitosan ( $\beta$  (1–4) linked 2-acetamido-2-deoxy- $\beta$ -d-glucopyranose and 2- amino-2-deoxy- $\beta$ -d-  
53 glucopyranose) (Pillai, Paul & Sharma, 2009). In addition, different chemical modifications  
54 of their amino and hydroxyl groups have been carried out to improve chitosan solubility, like  
55 quaternization, alkylation, carboxyalkylation and N-acylation (Bashir, Teo, Ramesh, Ramesh  
56 & Khan, 2015). Particularly, succinylation of chitosan enhances water solubility and led to a  
57 biodegradable and non-toxic material (Bashir et al., 2015). Succinyl chitosan has been  
58 reported as water soluble in acidic and alkaline media, high water retention and chelating  
59 abilities, strong antioxidant activity, apoptosis inhibitory activity and so on (Bashir et al.,  
60 2015). Because of that, succinyl chitosan has been widely used in biomedical applications.

61 Application of chitosan derivatives in bakery products has been focused on exploiting their  
62 health promoting properties as dietary fiber with the aim of obtaining functional foods (Kerch,  
63 Zicans & Meri, 2010). Nevertheless, Kerch et al. (2010) incorporated chitosan  
64 oligosaccharide succinate in bread and it was observed that chitosan oligosaccharides (added  
65 up to 3.6 g/110 g) prevented amylose-lipid complexation, accelerated the dehydration of both  
66 gluten and starch and increased the rate of water migration from crumb to bread crust;  
67 similarly to the effect observed with chitosan (Kerch et al. 2008), thus they cannot be used as

68 antistaling agents. However, no previous exploration of their thickening and emulsifying  
69 properties has been reported on bakery products.

70 Cakes are complex bakery products that require sugar, fat and egg yolk to induce freshness,  
71 soft mouthfeel and palatable textures (Karp, Wyrwicz, Kurek & Wierzbicka, 2017; Matos,  
72 Sanz & Rosell, 2014). In cakes formulations, fat is used to obtain cakes with a tender, low  
73 moisture, and yellow crumb. Fat functionality in cake batter is responsible of keeping gaseous  
74 cells into the emulsion (oil-water) and the subsequent volume, softness and flavor of cakes;  
75 besides fats extend the shelf life of the final product (Matsakidou, Blekas & Paraskevopoulou,  
76 2010; Rios, Pessanha, Almeida, Viana & Lannes, 2014; Hesso et al., 2015). Owing to the  
77 awareness to reduce fat content in food products and considering that bakery products are  
78 consumed daily by a wide range of people, some attempts to replace the fat by different types  
79 of ingredients, namely fat substitutes, have been reported (Rodriguez- Sandoval, Prasca-Sierra  
80 & Hernandez, 2017). The role of fat replacers on food matrix, especially in cakes, has been  
81 ascribed to their structuring properties (Psimouli & Oreopoulou, 2013; Rios, Pessanha,  
82 Almeida, Viana & Lannes, 2014). Polymers like hydrocolloids have been used for the purpose  
83 of improving dough-handling properties, the quality of fresh cakes and extend the shelf-life of  
84 stored products. (Rodriguez- Sandoval et al., 2017; Rios et al., 2014)

85 Therefore, the aim of this research was to explore the ability of a chitosan derivative (succinyl  
86 chitosan) as fat replacer in cakes. Previously, the concentration of chitosan derivative to be  
87 incorporated as fat replacer was determined based on its emulsion ability (activity and  
88 stability). The selected concentration of succinyl chitosan was employed to replace different  
89 levels of fat and the effect of succinyl chitosan was evaluated at batter and cakes level.

## 90 **2. MATERIALS AND METHODS**

### 91 ***2.1. Materials***

92 Commercial wheat flour was supplied by Harinera La Meta S.A. (Lleida, Spain). Whole dried  
93 egg was acquired from EPSA Aditivos Alimentaria Emilio Peña S.A. (Valencia, Spain).  
94 Sunflower oil, sugar, skimmed dried milk and baking powder (sodium bicarbonate, tartaric  
95 and malic acids) were purchased from the local market.

## 96 **2.2. Methods**

### 97 **2.2.1. Succinyl chitosan preparation and assessment**

98 Succinyl chitosan (SC) was obtained from *N*-succinylation reaction of chitosan using succinic  
99 anhydride following the method of Mello, Bernusso, Pitombo & Polakiewicz (2006).  
100 Chitosan and succinyl chitosan were characterized by Alpha infrared spectrophotometer  
101 (Bruker, Germany), with ATR-FTIR (Attenuance Total Reflectance – Fourier Transformed  
102 Infrared) module in the range of 4000/ cm – 600/ cm. The degree of deacetylation of chitosan  
103 was calculated according to Kasaai (2010).

### 104 **2.2.2. Emulsifying properties**

105 The emulsifying properties of the wheat flour and the blends of succinyl chitosan and  
106 wheat flour were measured by the method of Gujral & Rosell (2004) with some  
107 modifications. Blends containing succinyl chitosan at 0.5 g/100 g, 1.0 g/100 g, 2.0 g/100 g  
108 and 3.0 g/100 g (flour basis) were tested. To prepare the emulsions, 2 mL of refined sunflower  
109 oil and 6 mL of samples suspensions (0.005: 1 w/v) in 0.1 mol/L phosphate buffer (pH 7.0)  
110 were mixed together and homogenized in a T18 Ultra Turrax (Wilmington, NC, USA) at  
111 2,300 rad/s for 1 min at 20 °C. Aliquots (30 µL) of the emulsion were taken at regular  
112 intervals (10 min up to 60 min) and diluted with 3 mL of sodium dodecylsulphate water  
113 solution (0.001:1, v/v). The absorbance of the diluted emulsion was then determined at 500

114 nm in a spectrophotometer. The emulsifying activity was expressed as measured at 0 min.

115 Value was the average of three replicates. The emulsion stability was expressed as:

116 
$$ES (\%) = (Abs_{60 \text{ min}}/Abs_{0 \text{ min}}) \times 100 \quad [\text{Equation 1}]$$

### 117 **2.2.3. Cake batter preparation and characterization**

118 Cakes were prepared as described Matos et al. (2014) with slight variations. The solids for  
119 batter formulation included 300 g wheat flour; 214.4 g sugar; 32.1 g whole dried egg; 10.8 g  
120 skimmed dried milk and baking powder (sodium bicarbonate 4.2 g; 2.2 g tartaric and malic  
121 acids). In reference samples, 171.3 g sunflower oil was added and 350 g of water; they were  
122 referred as full fat sample, where no SC was added. A solution of succinyl chitosan was used  
123 as fat replacer (2 g/100 g SC, flour basis), progressively reducing the amount of fat in the  
124 reference formulation. The amount of oil in fat-reduced recipes was 128.5 g, 85.65 g, 42.8 g,  
125 and 0.0 g to give 25%, 50%, 75% and 100% fat reduction samples, respectively. To guarantee  
126 the same batter consistency in all formulations, the amount of added water was adjusted based  
127 on the batter viscosity using RVA-measurements. Whenever present, 6 g succinyl chitosan  
128 were previously diluted in part of the water (171.3 g) by heating at 60 °C for 30 min. Total  
129 water added to each recipe were 348 g, 365 g, 390 g, and 421 g of water to obtain 25%, 50%,  
130 75% and 100% fat-reduced batters, respectively.

131 In a RM8 mixer (Robot Coupe U.S.A., USA), all of the dry ingredients, except the baking  
132 powder, were pre-mixed for 1 min for complete homogenization. Thereafter, the sunflower oil  
133 and / or SC (depending on the formulation) were added, starting the mixing. During the  
134 mixing process, the water was gradually incorporated until a homogeneous batter was  
135 obtained; finally, the baking powder was added at the end of the process. The total mixing

136 time was 5 min until getting a spongy batter. Part of the batter (50 mL) was used for further  
137 determining density and pasting properties. Two batches were carried for each recipe.

138 The density of the batter was measured as the ratio of the weight of a graduated cylinder filled  
139 with batter to that one filled with water. Three replicates were determined.

140 The pasting and viscosity properties of the batter were measured using a Rapid Visco™  
141 (RVA 4500, Perten Instruments, New South Wales, Australia) as previously described (Xing,  
142 Niu, Su & Yang, 2016) to record the changes of a complex system like batters when  
143 transforming from a mixing suspension to a gel-like structure. Eight grams of batter were  
144 diluted in 12 g of distilled water inside of an aluminum canister and maintained at 50 °C for 1  
145 min, then heated from 50 to 95 °C at 12 K/min and held at 95 °C for 1 min. After that, the  
146 batter was cooled at 12 K/min and held at 50 °C for 2 min. The mixing speed was 63 rad/s  
147 and the viscosity was monitored (mPa.s). The experiment was conducted twice per sample.  
148 Pasting parameters such as onset temperature, peak viscosity, trough, breakdown (peak  
149 viscosity-trough), final viscosity, setback (final viscosity-trough) were recorded using  
150 Thermocline software for Windows (Perten Instruments, Hägersten, Sweden).

#### 151 **2.2.4. Cakes preparation and evaluation**

152 Batter prepared as described in section 2.2.3 was poured into the disposable bags and 30 g  
153 were dispensed in each cake paper cup (45 mm x 27 mm) and baked in a conventional oven  
154 model Labe (Salva Industrial S.A., Gipuzkoa, Spain) for 12 min at 180 °C. Cakes were cooled  
155 down at room temperature during 90 min. Fresh cakes were characterized for texture, color,  
156 image, water activity and moisture content. Part of the cakes were packed in 15 cm x 30 cm  
157 polyethylene plastic bags and stored in a temperature controlled chamber at 25 °C (Gabarro,

158 Barcelona, Spain) for staling study during seven days. Two batches were made for each  
159 formulation on different days.

160 Texture was determined in fresh and stored cakes (at days 0, 2, 4 and 7 after baking). The  
161 texture profile analysis of the cake crumb was made with a TA.XT.plus Texture Analyzer  
162 (Stable Microsystems, Godalming, UK) provided with Texture expert software. Cakes were  
163 horizontally cut giving a section of 1.5 cm thickness. A double compression test (texture  
164 profile analysis) was performed with a 75 mm diameter flat-ended cylindrical probe (P/75)  
165 and compression to 50% of the initial height at a speed of 1 mm/s with 5 s waiting time  
166 between the two cycles. Parameters recorded included: hardness, springiness, cohesiveness,  
167 chewiness and resilience. Results were the mean values of at least four replicates for each  
168 sample. Moisture content was determined in fresh and stored cakes (at days 0, 2, 4 and 7 after  
169 baking) following the two-step oven method of the AACCI method 44-15A. The water  
170 activity was measured in an Aqua Lab model 3T (Decagon Devices, Inc., USA). Hardening  
171 rate (g/day) and drying rate (g/100 g per day), during the 7-days storage, were calculated  
172 using the hardness increase or moisture content decrease, respectively.

173 Color was determined on cake crumb using a Konica Minolta CM-3500 spectrophotometer.  
174 Illumination (D65) with a 10° viewing angle and a 30 mm port size was used. The CIE-  
175  $L^*a^*b^*$  values of cake crumb were recorded:  $L^*$  (lightness/darkness),  $a^*$  (redness/greenness),  
176 and  $b^*$  (yellowness/blueness). The measurements were made in three different regions on the  
177 surface of the crumb previously cut from the central part.

178 Fresh cakes were cut in longitudinal and transversal mode. High resolution images (600 dpi)  
179 of the cakes were captured by HP Scanjet G3110 (Hewlett-Packard, USA). A single 30 x 30  
180 mm square field was cropped and analysed by Fiji Image J software (Schindelin et al. 2012)  
181 for each image. Color channels were separated to improve differences between cells and



182 crumb, the pre-defined “otsu” algorithm were applied to establish the threshold and then  
183 particles were analyzed. The studied parameters were mean cell area ( $\text{mm}^2$ ), cell density or  
184 number of cells for  $\text{cm}^2$  ( $\text{cell}/\text{cm}^2$ ), surface porosity (calculated as total cell area/cross section  
185 area, expressed in percentage) and cell circularity (from 0 (square) to 1 (perfect circle)). To  
186 study the whole morphogeometric characteristics of cakes, width and height, 2D area ( $\text{mm}^2$ ),  
187 2D perimeter (mm) and the ratio (width/height) of each slice were calculated using the same  
188 software.

### 189 **2.2.5. Statistical analysis**

190 In order to assess significant difference among samples, a one-way ANOVA analysis of  
191 samples was performed using the program Statgraphics Centurion XVI Version 16.2.04.  
192 Fisher's least significant differences (LSD) test was used to describe means with 95%  
193 confidence.

## 194 **3. RESULTS AND DISCUSSIONS**

### 195 **3.1. Succinyl chitosan characterization on ATR-FTIR and emulsifying properties**

196 The deacetylation degree of the chitosan used was 77.0%, based on the peak area values of  
197 the absorption bands at  $1655/\text{cm}$  and  $3450/\text{cm}$  that are associated with carbonyl and hydroxyl  
198 groups, respectively (Kasaai 2010). The calculated ATR-FTIR results for chitosan and  
199 succinyl chitosan are presented in Table 1. The main difference of the spectra was  
200 displacement of the absorption band observed at  $1568/\text{cm}$  attributed to amide II and the  
201 carbonyl stretching ( $\text{C}=\text{O}$ ) at  $1635/\text{cm}$ . Also, the presence of the symmetric stretching ( $\nu_s$ ) of  
202 carboxyl group  $\nu_s(\text{COO}^-)$  is a direct evidence of substitution, as well as absent in the chitosan  
203 structure, and confirms the presence of the succinated group.

204 The effect of different concentrations of succinyl chitosan on the emulsifying activity (EA)  
205 and emulsifying stability (ES) are summarized on Table 2. As expected, EA of the blends  
206 increased with the SC concentration, reaching a maximum when containing 2 g SC /100 g  
207 flour. The emulsifying stability also increased in the presence of SC, but only in the range 1-2  
208 g/100 g concentration. It has been reported that functionality of other biopolymers changes  
209 when increasing concentration due to phase separation processes as has been observed with  
210 rice starch and ionic hydrocolloids (Rosell, Yokoyama & Shoemaker, 2011). Presumably,  
211 only up to 2 g/100 g SC is needed to decrease the superficial tension and to maintain the  
212 emulsion stable for a long period. Therefore, the emulsifying properties suggested the use of 2  
213 g SC /100 g flour basis in solution (in aqueous medium) the most adequate to stabilize  
214 biphasic systems.

### 215 ***3.2. Effect of succinyl chitosan on pasting properties and density of the cake batter***

216 The addition of 2 g SC /100 g flour was used to obtain reduced fat batters. With that purpose,  
217 batters with different levels of oil were prepared in order to determine the amount of fat that  
218 the SC was able to replace. At the same time, hydration was individually adapted for each  
219 batter to keep a steady consistency when decreasing fat levels, because it is already known  
220 that excessive batter consistency might limit its expansion (Gularte, de la Hera, Gómez &  
221 Rosell, 2012).

222 Viscosities of the batters were compared during a heating-cooling cycle and pasting  
223 parameters were determined (Table 3). It is well stated that fats affect the properties of starch  
224 during gelatinization and retrogradation mainly due to the complex formation of lipids with  
225 starch, namely amylose, which produces an increase in the breakdown (Gujral & Rosell  
226 2004). Accordingly, the batters containing different percentages of fat reduction (25, 50, 75,  
227 and 100%) showed significant differences in the resulting breakdown of the batters, compared

228 to the batter with full fat content (0% reduction), but no significant differences ( $P > 0.05$ )  
229 were observed on onset temperature, peak viscosity, trough, final viscosity and setback  
230 parameters. Regarding breakdown, a significant decrease was observed up to 50% fat  
231 reduction, but beyond that level a progressive increase was detected (Table 3). Fats increase  
232 the breakdown related to the stability of the starch during cooking, thus it was expected that a  
233 decrease in the fat content induces a decline in this parameter. Nevertheless, it seems that at  
234 higher fat reduction ( $> 50\%$ ), strong interaction between the SC structure and the starch  
235 molecules was produced stabilizing the integrity of the starch granule during heating, as has  
236 been described for ionic hydrocolloids like xanthan gum (Diao et al. 2017; Rosell et al., 2011)  
237 Anyway, interactions of starch with hydrocolloids during gelatinization lead to different  
238 network structures, whose pasting and rheological properties depend on the hydrocolloid and  
239 its concentration (Diao et al. 2017; Shi & BeMiller 2002). Chitosan-containing emulsion was  
240 found to be dependent on molecular weight, concentration and deacetylated degree  
241 contributing to the viscosity enhancement (Klinkesorn, 2013; Kerch et al., 2008). Therefore,  
242 the SC with 77% degree of deacetylation showed a behavior like ionic hydrocolloids.

243 Batter density was assessed (Table 3) and no changes were observed due to fat reduction in  
244 the presence of SC. Similar density values indicated comparable amount of air incorporated  
245 into the batter, which was ascribed to the consistency adjustment previously carried out.  
246 Conversely, Psimouli & Oreopoulou (2013) reported that the addition of carbohydrate-based  
247 compounds (maltodextrin, oligofructose, pectin, inulin and microparticulated whey protein  
248 concentrate) to replace 35, 65 or 100% of fat on cake batter significantly increased batter  
249 density and decreased viscosity, likely due to the different fat replacer structure and recipes.  
250 Nevertheless, in contrast with the present study, those authors did not adjust the water content  
251 of the recipes to guarantee the same batter viscosity.

252 **3.3. Effect of fat reduction on morphogeometrics characteristics and colour parameters of**  
253 **fresh cakes**

254 The fat reduction by adding succinyl chitosan confers marked differences in  
255 morphogeometrics characteristics of the cakes (Table 4) particularly significant in 2D area  
256 and ratio width/height. Variations in 2D area, perimeter and ratio were observed on the cross-  
257 section of cakes in Figure 1. In general, the reduction of fat content gave high volume cakes  
258 (high 2D area); although in the case of 75% reduction was not significant ( $P > 0.05$ ). This  
259 result was opposite to those obtained when adding chitosan to bread, where either a volume  
260 decrease (Lafarga, Gallagher, Walsh, Valverde & Hayes, 2013) or no significant effect was  
261 observed (Kerch et al., 2010), depending on the chitosan derivative. Hence, results suggested  
262 a proper functionality of chitosan derivative as fat replacer in cakes but no as improver in  
263 bread. The improving effect of SC was also observed in the reduction of the ratio width/height  
264 (Table 4, Fig. 1), but only up to 50% fat reduction. Despite the similar viscosity encountered  
265 on the batters during heating (Table 3), some significant changes were observed on the shape  
266 and volume of the cakes, suggesting that SC affected the gas incorporation into the batter, and  
267 also the ability of the structure to expand during baking.

268 Regarding crumb color, the addition of SC significantly modified the luminosity ( $L^*$ ) and  
269 slightly the redness tonality ( $a^*$ ). In general, SC-containing cakes exhibited significantly  
270 lighter crumb and no trend was observed with the level of fat reduction.

271 Internal structure of the cakes was evaluated by digital analysis (Table 4) and results  
272 confirmed that no significant effect was observed on either, cell density, mean cell area or  
273 surface porosity when fat content was reduced in SC containing cakes. As expected, constant  
274 batter consistency ensures the same air incorporation obtaining cakes with similar cell density  
275 and cells size. Only significant effect was observed on the circularity of the internal cells, the

276 reduction of fat content up to 50% yielded rounded gas cells. That result agrees with  
277 observation on batter pasting properties, supporting that SC contributes to the integrity of the  
278 starch granules and their stability during cooking when sufficient reduction of fat was  
279 produced, which could favor expansion in more uniform cells. The properties of SC  
280 contribute to the performance of the porosity characteristics (Klinkesorn, 2013). But it seems  
281 that in excess of water (with higher fat reduction) limited interactions between starch and SC  
282 were produced due to starch dilution or water molecules competing with SC for interacting  
283 with starch.

284 In parallel to the fat reduction, an increase in the amount of water was carried out to keep  
285 batters' consistency. Consequently, the cake moisture content showed a progressive increase  
286 with the fat reduction (Table 4). The presence of SC in the cakes did not modify the amount  
287 of free water available in the crumbs, as has been described when 2 g/100 g of chitosan was  
288 added in breads (Kerch et al., 2008). In fact, the 25% fat reduction, which hardly require a  
289 change in the amount of water (348 ml vs 350 ml in control), led to similar moisture content  
290 to that obtained in the full fat cake (0%). Nevertheless, significant changes were observed in  
291 the water activity ( $a_w$ ) (Table 4). A decline in the  $a_w$  was observed in the cakes that were made  
292 with up to 50% fat reduction, likely due to the water binding capacity of SC (Lafarga, 2013).  
293 However, the maximum fat reduction (100%) resulted in higher  $a_w$  than the full fat cake.

#### 294 ***3.4. Effect of fat reduction on textural parameters of SC-containing cakes***

295 When the level of fat was reduced in the cakes containing succinyl chitosan, significant  
296 changes were observed in the texture related parameters, with exception of springiness (Table  
297 5). The complete removal of fat (100% fat reduction) resulted in the softest cake and no  
298 changes were detected with the other reduction levels, compared to the full fat cake. Previous  
299 studies carried out with breads reported that the addition of chitosan increased the firmness of

300 bread crumb and in the case of chitosan oligosaccharides the effect depended on the  
301 oligosaccharide molecular weight (Kerch et al., 2010). Considering cohesiveness and  
302 resilience, the progressive fat reduction induced a steady increase of these parameters, which  
303 are indicative of food structure with higher ability to recover after compression ( Matos et al.,  
304 2014). Usually, those have been associated to crumbs with higher number of gas cells, but  
305 taking into account that crumb porosity was similar in all the cakes, the result obtained might  
306 be attributed to the plasticizer role of the water molecules in the fresh cakes. In fact, during  
307 storage of the cakes, when a significant dehydration was observed (Table 5), the texture  
308 behaved significantly different. Hardening rate of SC-containing cakes decreased, but only up  
309 to 50% fat reduction cake. Above this percentage (75% and 100% fat reduction), an opposite  
310 tendency was observed. Therefore, SC was able to act as anti-staling agent in cakes and  
311 replacing up to 50% the fat content of the cakes. The use of chitosan and its derivatives have  
312 been solely studied in bread formulations, which are quite distinct. In breads, chitosan is  
313 adsorbed onto the starch surface preventing the amylose-lipid complexation and accelerating  
314 the aging of bread (Kerch et al. 2008; Rakcejeva, Rusa, Dukalska & Kerch, 2011) and  
315 increasing crumb hardening (Lafarga et al. 2013). Based on that, present results might be  
316 attributed to its synergy with the lipids portion of the formulation, since SC could act as an  
317 amphiphilic surfactant (hydrophilic and hydrophobic groups) (Bashir et al., 2015) capable of  
318 binding fats, besides proteins and polysaccharides presents in the cakes recipe.

319 In addition, results on the drying rate (Table 5) during the storage time, showed higher values  
320 in cakes with reduced fat, with the exception of non-fat cake (100% reduction). The drying  
321 rate decreased when increasing the level of fat reduction, thus the presence of SC speeded up  
322 the release of water molecules. It seems that interaction of SC with fat structure accelerated  
323 the drying during storage, which was partially compensated with the water increase for batter  
324 consistency adjustment. But in the absence of fat, SC reduce the drying, likely due to chitosan

325 retards the redistribution of water between the bread components (gluten and starch) although  
326 at the same time accelerates the dehydration of gluten and starch and the migration of water  
327 from the crumb to the crust (Kerch et al. 2008; Rakcejeva et al., 2011).

#### 328 **4. CONCLUSIONS**

329 Succinyl chitosan, a water soluble biopolymer, with 77% degree of the deacetylation  
330 increased the emulsifying capacity of wheat flour and that effect was maximum when 2 g SC  
331 /100 g flour was blended with flour. In consequence, the ability of SC as fat replacer was  
332 checked in a bakery product with high fat content like cake. Batters of comparable  
333 consistency were obtained with different levels of fat content (0-100%) in the presence of 2 g  
334 SC /100 g flour. When producing fat reduced cakes, SC (2 g/100 g) was able to replace up to  
335 50% of the cakes fat content, without altering batter pasting properties, only breakdown.  
336 Crumb structure of fat reduced cakes containing SC was similar to that of full fat cakes, even  
337 SC improved cell circularity. Regarding texture properties, effect of SC was readily evident  
338 during cake storage, reducing hardening rate, although drying rate was accelerated. Succinyl  
339 chitosan could be considered a potential fat substitute for cake formulation, although more  
340 investigations regarding consumer acceptance should be carried out.

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- 422

**Table 1.** ATR-FTIR results of chitosan and succinyl chitosan.

Group characteristic	Chitosan (wave number 1/cm)	Succinyl chitosan (wave number 1/cm)
v(OH),(NH)	3369 s	3381 s
v(CH)	2910 m	2918 m
Amide II	1568 w	1556 s
v(C=O)	1635 vw	1646 m
v <sub>s</sub> (COO <sup>-</sup> )	-	1401 s
Saccharide backbone	1152 vs	1068 vs

s = strong; vs = very strong; m = medium; w = weak; vw = very weak.

**Table 2.** Emulsifying and foaming properties of wheat flour-succinyl chitosan blends.

Samples	EA (AU)	ES (%)
wheat flour	0.12 ± 0.05	37.8 ± 1.4
SC 0.5%	0.51 ± 0.13	20.3 ± 5.0
SC 1.0%	0.56 ± 0.05	46.4 ± 5.6
SC 2.0%	0.80 ± 0.01	62,6 ± 6.7
SC 3.0%	0.60 ± 0.02	39.5 ± 2.8

EA = emulsion activity; ES = emulsion stability;

SC = succinyl chitosan. AU = absorbance units (nm).

Values are given as mean ( $n = 3$ ) ± standard deviation.

**Table 3**

Effects of succinyl chitosan on pasting properties and density of cake batters obtained with different levels of fat reduction.

Fat reduction (%)	Onset (°C)	Peak viscosity (mPa.s)	Trough (mPa.s)	Breakdown (mPa.s)	Final viscosity (mPa.s)	Setback (mPa.s)	Batter density (g/mL)
0	65.72 ± 0.40	1363 ± 165	580 ± 40	858 ± 110 <sup>c</sup>	1103 ± 64	548 ± 4	1.10 ± 0.10
25	65.76 ± 0.66	1301 ± 41	529 ± 11	758 ± 22 <sup>ab</sup>	1124 ± 21	595 ± 19	1.00 ± 0.03
50	65.72 ± 0.36	1282 ± 26	532 ± 18	749 ± 34 <sup>a</sup>	1138 ± 28	608 ± 23	0.98 ± 0.03
75	65.92 ± 0.81	1365 ± 91	549 ± 63	816 ± 38 <sup>bc</sup>	1141 ± 101	592 ± 46	1.06 ± 0.10
100	65.53 ± 0.38	1358 ± 64	526 ± 30	832 ± 46 <sup>c</sup>	1135 ± 62	609 ± 36	0.93 ± 0.07
<i>P</i> -value	0.8852	0.3262	0.4736	<b>0.0204</b>	0.893	0.1432	0.2902

Values are given as mean ± standard deviation. ( $n = 4$ ). Same letter within a column are not significantly different ( $P > 0.05$ ) for each specific parameter.

**Table 4**

Effect of fat reduction on shape and crumb color of succinyl chitosan containing cakes.

Fat reduction (%)	Crumb morphogeometric characteristics			Crumb colour		
	2D area (cm <sup>2</sup> )	2D Perimeter (cm)	Ratio (width/height)	<i>L</i> *	<i>a</i> *	<i>b</i> *
0	13.83 ± 1.54 <sup>a</sup>	30.07 ± 5.78	1.31 ± 0.07 <sup>b</sup>	61.40 ± 3.53 <sup>a</sup>	-2.65 ± 0.23 <sup>ab</sup>	19.95 ± 3.26
25	17.56 ± 0.54 <sup>bc</sup>	30.05 ± 2.18	1.09 ± 0.03 <sup>a</sup>	68.65 ± 2.52 <sup>c</sup>	-2.51 ± 0.25 <sup>b</sup>	20.73 ± 0.71
50	19.05 ± 1.19 <sup>c</sup>	29.35 ± 2.93	1.02 ± 0.04 <sup>a</sup>	65.18 ± 2.98 <sup>b</sup>	-2.42 ± 0.29 <sup>bc</sup>	19.98 ± 1.34
75	14.51 ± 1.62 <sup>ab</sup>	30.40 ± 10.41	1.32 ± 0.14 <sup>b</sup>	64.05 ± 2.08 <sup>b</sup>	-2.28 ± 0.16 <sup>c</sup>	21.25 ± 1.37
100	18.35 ± 4.85 <sup>c</sup>	24.18 ± 2.46	1.29 ± 0.10 <sup>b</sup>	64.81 ± 2.32 <sup>b</sup>	-2.72 ± 0.30 <sup>a</sup>	21.26 ± 0.93
<i>P</i> -value	<b>0.0272</b>	0.4587	<b>0.0004</b>	<b>0.0000</b>	<b>0.0009</b>	0.3680
	Crumb structure				Physicochemical parameters	
Fat reduction (%)	Cell density (cell/cm <sup>2</sup> )	Mean cell area (mm <sup>2</sup> )	Surface porosity (%)	Circularity	Moisture content (g/100 g)	Water activity
0	9.58 ± 1.40	1.78 ± 0.20	16.78 ± 1.21	0.63 ± 0.04 <sup>a</sup>	32.28 ± 0.96 <sup>a</sup>	0.931 ± 0.012 <sup>bc</sup>
25	7.33 ± 1.58	2.25 ± 0.70	15.65 ± 1.76	0.70 ± 0.02 <sup>b</sup>	32.69 ± 0.52 <sup>a</sup>	0.925 ± 0.004 <sup>b</sup>
50	9.47 ± 1.36	1.87 ± 0.50	17.18 ± 2.28	0.71 ± 0.02 <sup>b</sup>	35.13 ± 0.80 <sup>b</sup>	0.911 ± 0.011 <sup>a</sup>
75	8.75 ± 0.62	2.13 ± 0.17	18.61 ± 1.82	0.66 ± 0.04 <sup>ab</sup>	37.07 ± 1.85 <sup>b</sup>	0.938 ± 0.009 <sup>cd</sup>
100	8.06 ± 0.86	2.01 ± 0.07	16.14 ± 1.15	0.62 ± 0.04 <sup>a</sup>	40.81 ± 3.72 <sup>c</sup>	0.943 ± 0.005 <sup>d</sup>
<i>P</i> -value	0.2164	0.5767	0.2644	<b>0.0115</b>	<b>0.0000</b>	<b>0.0000</b>

Values are given as mean ± standard deviation. (*n* = 4). Same letter within a column are not significantly different (*P* > 0.05) for each specific parameter.

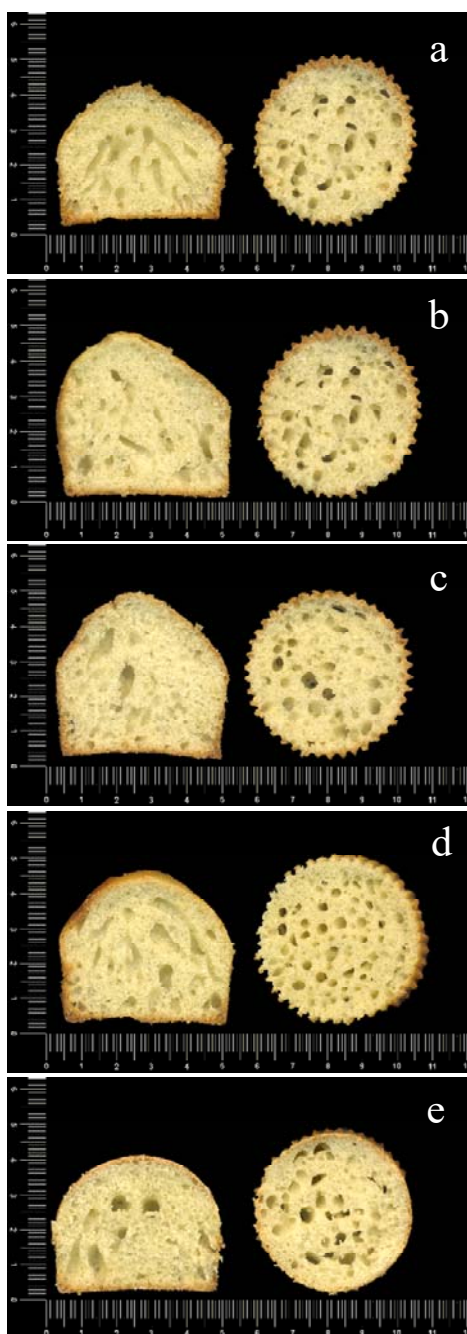
**Table 5**

Effect of fat reduction on crumb texture and the rate of hardening and drying of succinyl chitosan containing cakes.

Fat reduction (%)	Hardness (N)	Springiness (-)	Cohesiveness (-)	Chewiness (g)	Resilience (-)	Hardening rate (N/day)	Drying rate (g/100 g per day)
0	130 ± 10 <sup>b</sup>	0.981 ± 0.012	0.724 ± 0.013 <sup>a</sup>	922 ± 71 <sup>a</sup>	0.307 ± 0.009 <sup>a</sup>	27.8	3.85
25	132 ± 7 <sup>b</sup>	0.993 ± 0.015	0.758 ± 0.010 <sup>b</sup>	1029 ± 72 <sup>a</sup>	0.308 ± 0.009 <sup>a</sup>	26.1	5.64
50	130 ± 10 <sup>b</sup>	1.009 ± 0.051	0.759 ± 0.026 <sup>b</sup>	985 ± 37 <sup>a</sup>	0.303 ± 0.015 <sup>a</sup>	14.0	5.00
75	129 ± 11 <sup>b</sup>	1.007 ± 0.037	0.798 ± 0.017 <sup>c</sup>	1186 ± 90 <sup>b</sup>	0.332 ± 0.020 <sup>b</sup>	35.4	4.00
100	111 ± 4 <sup>a</sup>	0.997 ± 0.012	0.862 ± 0.031 <sup>d</sup>	960 ± 23 <sup>a</sup>	0.432 ± 0.022 <sup>c</sup>	49.2	3.46
<i>P</i> -value	<b>0.0003</b>	0.0697	<b>0.0000</b>	<b>0.0095</b>	<b>0.0000</b>	-	-

Values are given as mean ± standard deviation. (n = 4). Same letter within a column are not significantly different ( $P > 0.05$ ) for each specific parameter.

Figure 1.



**Fig. 1** Captured images (600 dpi) of the longitudinal and cross section of the succinyl chitosan containing cakes obtained with different levels of fat reduction: a: 0%, b: 25%, c: 50%, d: 75%, e: 100%. Two batches were carried out for each sample.