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1 **TOASTING TIME AND COOKING FORMULATION AFFECT BROWNING**
2 **REACTION PRODUCTS DEVELOPMENT IN CORN FLAKES**
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25 Abstract: During toasting, the last stage of corn flakes production, Maillard reaction
26 takes place, favored by the high temperature and low water content. The cooking
27 formulation ingredients influence color and flavor of the final product and, therefore,
28 consumer acceptance. However, some undesirable components are also formed. The
29 impact of cooking formulation and toasting time on color development and on the
30 formation of chemical markers was investigated. Samples (flakes) were equilibrated at
31 a_w 0.8 and toasted at 230 °C. After extraction of fluorescent pigments with pronase,
32 fluorescence, absorbance at 420 nm and furfurals analysis were performed. Sucrose
33 showed a synergistic interaction with malt and salt. Formulation highly affected the
34 amount of HMF and furfural formed. L* and a* were sensitive variables to measure
35 overall browning reaction. These results allow for further understanding of the influence
36 of formulation used during cooking and would help to mitigate the formation of
37 undesirable compounds.
38

39 1. INTRODUCTION

40 Commercially available corn flakes are usually produced by two different alternative
41 processes: flaking or extrusion. This work focuses on the classical flaking method,
42 which begins with grits mixed with water, sugar, salt and malt extract as formulating

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43 agents. Then the grits are cooked under saturated steam. During this process, the starch
44 is gelatinized and non-enzymatic browning takes place (Farroni & Buera, 2012).
45 Cooked grits are cooled, flaked and then toasted in a stream of air at high temperature
46 (~230°C) for a short time (few minutes) to obtain the final product. During toasting
47 Maillard reaction further develops under conditions at which dehydration reactions
48 occur.

49 At high temperatures sugars decompose into furfural compounds by two possible
50 pathways, both involving a first step of sucrose hydrolysis: caramelization, in which the
51 reducing carbohydrates directly suffer 1–2 enolisation, dehydration and cyclization
52 reactions; and Maillard reaction, in which the Amadori products, (formed by reaction of
53 the amino group of free amino-acids or proteins with reducing carbohydrates) are
54 submitted to isomerization and subsequent dehydration of the sugar moiety (AitAmeur
55 et al., 2006). The intermediate steps of the Maillard reaction include the formation of
56 unsaturated and saturated compounds which are involved in the formation of pigments.
57 Some of these pigments may act as crosslinking agents between protein chains either
58 decreasing food nutritional value or impairing its organoleptic properties (Agudelo-
59 Laverde, Acevedo, Schebor & Buera, 2011). Furthermore, some of the possible
60 resulting compounds such as acrylamide are considered potentially mutagenic (Rufián-
61 Henares, Delgado-Andrade & Morales, 2006).

62 Maillard fluorescent products have been studied in model systems as potential
63 indicators of essential aminoacid damage (Farroni & Buera, 2012).

64 The role of furfurals as indicators of heat treatment and cooking markers was deeply
65 studied (Ramírez-Jimenez, Garcia-Villanova & Guerra-Hernandez, 2000; Ait-Ameur,
66 Trystram & Birlouez-Aragon, 2006). 5-hydroxymethyl-furfural (HMF) is a
67 decomposition product of hexoses, especially when the pH is low (Xu, Templeton &

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68 Reed, 2003), while furfural (F) and 5-methyl-furfural (5MF) are derived mainly from
69 pentoses (Ledl & Sevrin, 1978).

70 There is no previous work regarding HMF formation, advance of Maillard reaction
71 during the toasting of cornflakes, and the effect of different cooking formulations or
72 ingredient interactions. Thus, the aim of this work was to study the progress of the
73 browning reactions in the toasting stage of cornflakes production by tracking HMF,
74 furfural and 5-methylfurfural, color CIELAB, Abs_{420}, and fluorescence, as a function of
75 formulation ingredients and toasting time.

76 2. MATERIALS AND METHODS

77 *2.1. Sample Preparation*

78 Proximal composition of corn grits in this study were (d.b.): carbohydrates (87 % ± 1),
79 proteins (8.0% ± 0.3), lipids (0.90% ± 0.05), humidity (13.0% ± 0.1), and ash (0.20% ±
80 0.02). Protein value was obtained by Kjeldhal (AOAC 992.23), with a nitrogen to
81 protein conversion factor of 6.25. Lipids value was obtained by Soxhlet extraction
82 (AOAC 945.16). Ashes following AOAC (923.03), humidity by a gravimetric method
83 (AOAC, 925.09), and starch by difference. Corn grits were obtained from a local
84 factory, and stored in freezer until used. The same lot of grits was used for the entire
85 study. Grits were processed simulating the industrial processing conditions.

86 The grits were steam cooked for 2.5 h employing six different formulations with salt,
87 malt extract and sucrose (Table 1). Cooking formulations were selected in order to
88 analyze the effect of the ingredients separately and together. Formulation A was
89 described by Fast (2000) as typical commercial formula and formulation B was
90 provided by a local manufacturing company. In order to analyze the effect of each
91 ingredient, single component formulations were prepared maintaining the proportions

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92 used in A. For each of the formulations, 15 g of raw grits were employed and
93 incorporated to a solution with the ingredients as listed in Table 1. After steam cooking
94 in a pressure cooker pot, the grits were cooled at room temperature for 1 h and flaked
95 using a semi industrial counter rotating hand roller (RD model S-300-M, Argentina). The
96 rollers were 6 cm external diameter and 30 cm long, with a gap of 2 mm between them.

97 In order to perform the toasting experiments under specified conditions for all the
98 samples, the flaked corn grits were freeze dried and equilibrated in an atmosphere of
99 controlled humidity at a_w 0.8 for 3 weeks, which corresponds to a water content close to
100 20.0% (d.b.). This water content is very close to the value found in industrial flaked
101 grits just before the toasting step (Farroni & Buera 2014). The selected a_w to perform
102 the toasting process was chosen taking into account that in amylaceous foods the
103 maximum rate of browning occurs below and close to a_w 0.84 (Acevedo, Schebor &
104 Buera, 2008) and can reflect the changes more sensitively. Once equilibrated, samples
105 were toasted in a convective oven at 230 °C under forced air flow. Three toasting times
106 were studied, 1.5, 2 and 2.5 minutes.

107 *2.2. Experimental design*

108 Experimental design consisted in six formulations, three toasting times, and a single
109 toasting temperature and a_w. A repeated measures design was used, where samples were
110 toasted from zero to the specific time. All extraction procedures for analytical purposes
111 were made in duplicate or triplicate as described in each section.

112 *2.3. Chemicals*

113 5-Hydroxymethylfurfural (HMF), furfural (F) and 5-methyl-furfural (5MF) were
114 analytical standards from Sigma–Aldrich (Saint Louis, USA). Furosine was from
115 Neosystem Laboratories (Strasbourg, France). Pronase E, Heptane sulphonate and

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116 quinine sulphate were from Sigma–Aldrich (Saint Louis, USA). NaCl, HCl,
117 Acetonitrile, Formic Acid and Sucrose were from Biopack (Buenos Aires, Argentina).
118 Corn grits and Malt syrup were provided by a local breakfast cereal manufacturer (Tres
119 Arroyos, S.A., Buenos Aires, Argentina). The commercial malt syrup used consisted in
120 a mixture of sugars and less than 10% protein with no diastatic activity since it was
121 inactivated in the first stage of cooking at 121°C.

122 *2.4. Fluorescence and brown pigments*

123 Browning and fluorescence development as a function of toasting time and cooking
124 formulation were evaluated. Total fluorescent compounds extractable in aqueous media
125 were quantified after sample hydrolysis with pronase E, according to Delgado-Andrade,
126 Rufián-Henares & Morales (2008) employing borate buffered saline, 0.1M at pH 8.2.
127 Pronase E. activity was of 4 Units/mg sample (1U/mg, being 1 U able to hydrolize
128 casein producing an equivalent absorbance to 1.0 μmol (181 μg) of tyrosine/min at pH
129 7.5 and 37 °C measured by the Folin-Ciocalteu method).
130 Fluorescence intensity, with excitation at 340 nm, was measured at 504 nm at which the
131 maximum value of the emission spectrum was observed. A spectrofluorometer Ocean
132 Optics model USB 2000 (Ocean Optics Inc., FL, USA) was used. The samples
133 employed for the determination of fluorescence were first measured at 340 nm and
134 diluted if necessary using phosphate buffer 0.25 M at pH 7, taking as general criteria
135 that the absorbance values should be lower than 0.1 in order to avoid inner filter effect.
136 Quantification of fluorescent compounds was made on the basis of quinine emission
137 equivalents ($\mu g_Q \cdot mg^{-1}$) in dry basis. A calibration curve was constructed with four
138 concentrations of quinine sulphate, in duplicates, with a concentration range between
139 0.05 and 1.00 $\mu g \cdot mL^{-1}$, as described by Matiacevich & Buera (2006). Obtained R^2 was
140 of 0.9980.

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141 The development of extractable brown pigments was followed by measuring
142 absorbance units at 420 nm in a UV-Vis spectrophotometer Jasco model V630 (Jasco
143 Corporation, Japan). This was also performed after the hydrolysis with pronase.
144 Absorbance values were corrected by dilution and normalized to 125 mg of dry sample
145 weight (Abs_{420}).
146 Duplicate extraction and duplicate assays of each extract were run. The average values
147 were informed.
148
149 *2.5. Furfurals content*
150 HMF, furfural and 5-MF were quantified in hydrolyzed samples by RP-HPLC in a
151 modification of the procedure proposed by Ait-Ameur et al. (2006). An Alliance HPLC
152 system equipped with a diode array detector Waters 2995 was used (Waters, Milford,
153 USA). Samples were separated in a Waters X-Bridge C_{18} column 2.1 mm x 100 mm and
154 3.5 µm particle diameter operating at 25 °C. The mobile phase consisted of 5 %
155 acetonitrile at 0.1 $mL \cdot min^{-1}$. 10 µL of sample were injected and the absorbance was
156 monitored at 284 nm. Retention times were 7.4 min for HMF, 8.8 min for Furfural, and
157 15.3 min for 5MF. Calibration curves were constructed for each analyte using the
158 external standard method by measuring 5 points in duplicates, in the range of 0.1 to 2.5
159 ppm diluted in mobile phase (R^2= 0.9999). The quantification limit was calculated using
160 the signal to noise relation and was below 0.05 ppm for all compounds and the relative
161 standard deviation was 3 %. Average recovery was 85 % which is in the acceptable
162 range for most of the values found in this work according to AOAC guidelines (AOAC,
163 2002). The three standards used were from Sigma Aldrich. Duplicate extraction of the
164 same sample was performed, duplicate assays of each extract were run, and the average
165 values were informed expressed in milligrams per kilogram of dry sample (ppm db).

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167 *2.6 Furosine determination by HPLC*

168 The method used by Delgado-Andrade, Rufián-Henares & Morales, F. J. (2007) was
169 followed. 30 mg of ground sample were hydrolysed with 4 ml of 7.95M HCl at 110 °C
170 for 23 h in a Pyrex test tubes with PTFE-faced septa. Hydrolysis tubes were sealed
171 under nitrogen. The hydrolysates were cooled at room temperature and subsequently
172 centrifuged at 14,000g for 10 min. A 0.5 ml portion of the supernant was applied to a
173 Sep-pak C18 cartridge (Millipore) pre-wetted with 5 ml of methanol and 10 ml of
174 deionized water, and was then eluted with 3 ml of 3M HCl. Solvent was evaporated in
175 roto-vap at 35 °C and the sample was then resuspended in 1 ml of ACN 5%. A similar
176 Alliance HPLC system with an Extrasyl-ODS2 analytical column (25 · 0.40 cm, 5 µm
177 particle size, Tecknokroma, Barcelona, Spain) was used at 32°C, isocratically
178 eluted at a 1.0 ml/min flow rate.
179 Mobile phase consisted of 5 mM sodium heptane sulphonate containing 20% of
180 acetonitrile and 0.2% of formic acid. The injection volume was of 20 µL and detection
181 set at 280 nm. Furosine was quantified by the external standard method. Calibration
182 curve was built in the range between 2.6 –39 mg/l, in duplicates. (R^2 = 0.9988).
183 *2.7. Water content*

184 In order to express the results in a dry matter basis, water content of the studied samples
185 was determined gravimetrically. Samples were grinded in a mill and aliquots of about
186 500 mg were dried at 130 °C in 2 cm diameter aluminum crucibles for 1 h (AOAC, M.
187 14.003, 1980). Triplicates were performed and the average of results was employed to
188 calculate dry matter.

189 *2.8. Color measurement*

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190 Color changes during toasting were determined by image analysis under standardized
191 conditions. A computer vision system was employed, consisting of a standard gray box
192 (luminance 50 Munsell scale). The interior of the box had a D65 illumination system
193 located in the upper part designed to simulate daylight (Lawless & Heymann, 1998).
194 Images were taken with a digital camera (EOS 40D, Canon, Inc., USA) located at an
195 angle of 45° with sample plane and at a distance of 60 cm. Image acquisition was
196 performed using the remote capture Eos Utility (Canon Inc., USA). The camera settings
197 were: shutter speed 1/8 seconds (no zoom, no flash), macro focus mode, opening f = 6.3
198 and ISO 100. The images were obtained with a resolution of 3888 x 2592 pixels and
199 saved in JPEG format. The photographs obtained were analyzed using the software
200 Adobe PhotoShopCS4 and color parameters were expressed in CIELAB space (L^*, a^*
201 and b^*) (CIE, 1976) according to the procedure described by Yam & Papadakis (2004).
202 Photo shop software allowed to precisely select the sample area for color quantification
203 avoiding background and shadowed zones. Samples were placed in plastic capsules of
204 1.5 cm height, at which the system behaves as opaque. Three photos were taken of each
205 sample, rotating 90° in between, and results were averaged.

206 *2.9. Statistical Analysis*

207 Mathematical modeling of the kinetic data was done with GraphPad software.
208 Split-plot ANOVA was performed with INFOSTAT software (InfoStat version 2012.
209 FCA, Universidad Nacional de Córdoba, Argentina). Post hoc Tukey test was used to
210 evaluate for significant differences with $p<0.05$.

211

212 3. RESULTS AND DISCUSSION

213 *3.1. Furfurals development*

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214 Table 2 shows the values of HMF concentration obtained for all samples at different
215 toasting times. HMF content of the cooked samples was around 10 ppm for all
216 formulations before toasting. The toasting process produced a significant increment on
217 HMF content for A and B formulation at 1.5 min and 2 min. At 2.5 min toasting time
218 only A formulation showed a significant increment in HMF. Interaction between the
219 formulation and the toasting time was found statistically significant. In extruded-corn
220 breakfast cereals HMF values around 45 ppm in dry basis (db) were reported by Rufián-
221 Henares et al. (2006). These values are comparable to those found in the present work
222 for formulations prepared with only salt, malt or sucrose and up to 1.5 minutes of
223 toasting.
224 When sucrose was added to the formulation it could be seen a tendency of increasing
225 HMF values in comparison with control (C), salt (S) and malt-containing (M) systems
226 that cannot be confirmed statistically. It is interesting to notice that a difference of 30
227 seconds in the first toasting interval produced a large increase in HMF levels, indicating
228 that this stage is critical and must be carefully controlled.
229 The ingredients used didn't show a significant effect on HMF formation when added
230 individually, however after 2 min toasting, systems A and B containing the three
231 ingredients developed the highest HMF levels. These data indicate that sucrose, malt
232 and salt had a synergistic effect.
233 HMF concentrations increased up to around 2500 ppm in formulations A and B, after
234 2.5 min toasting. These values are similar to the ones found by Ait-Ameur et al. (2006),
235 during model-cookies baking at temperatures over 250 °C, at a_w values close to zero. As
236 drying occurred during cooking, Ait-Ameur et al. (2006) detected HMF formation when
237 a_w values decreased below 0.4. It is known that a_w is a fundamental parameter in HMF
238 production (Kroh, 1994). In this a_w range the formation of one mole of HMF from

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239 fructose or glucose promotes the release of three moles of water, and thus water, being a
240 reaction product, acts as an inhibitor (Ait-Ameur et al., 2006). The effect of salt on
241 increasing HMF concentration in baked food was already described (Van Der Fels, et al.
242 2014, Mesías, Holgado, Márquez-Ruiz & Morales, 2015). The mechanism underlying
243 this effect is objet of discussion. Addition of salts can retain water in low moisture
244 systems, thus reducing the inhibition by products in dehydration reactions (Acevedo,
245 Briones, Buera & Aguilera, 2008). Although the initial a_w of the samples studied in
246 present work is quite high (0.8), flakes are thin and offer a large surface exposed at high
247 temperatures. Thus the a_w decreased fast down to final values between 0.4 and 0.5
248 during toasting. It can be seen in table 2 that the main water loss occurred in the first 1.5
249 minutes. At similar a_w values high HMF formation was reported on model cookies (Ait-
250 Ameur 2006). Two mechanisms have been proposed for the formation of HMF from
251 sucrose, one of them via Maillard reaction pathway starting from glucose or fructose
252 and aminoacids through several steps (Van Der Fels et al. 2014) and the other with
253 sucrose degrading into glucose and a reactive fructofuranosyl cation, that can be
254 effectively converted directly into HMF (Locas & Yaylayan, 2008). Regarding furfural,
255 only samples with added sugar had values above the quantification limit at 1.5 min
256 toasting time, and the C, S and M formulations showed values below 0.05 ppm. For 2.0
257 and 2.5 min toasting times no statistical differences were found among C, S, SUC and
258 M containing samples (range 0.65 to 27 ppm). As with HMF, A and B formulations
259 presented furfural values significantly higher than the rest of the systems, showing a
260 range between 99 and 131 ppm thus confirming the synergistic effect of ingredients in
261 furfurals formation.

262 Petisca et al. (2014) studied the occurrence of HMF and furfural in baked bread and
263 cookies. It is interesting to note that a similar order of magnitude difference between

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264 both compounds was usually found in samples with low concentrations of HMF, and
265 sometimes even furfural concentration exceeded HMF. This behaviour was also
266 observed in the model samples of present work. Nevertheless, as soon as HMF
267 concentration rose above 20 ppm, the concentration difference between both
268 compounds increased drastically. This could be related to the different reaction kinetics,
269 but also to the difference in molecular volatility between them. It has been stated that
270 one of the related strategies to remove furfurals from food systems is by heating in open
271 vessels (Anese & Suman, 2013) taking advantage of their characteristic volatility.
272 5MF was formed at very low concentrations (between 1.5 and 3 ppm) in all systems.
273 The maximum 5-MF concentration values were 2 orders of magnitude lower than those
274 corresponding to the maximum furfural values, and 3 orders lower than the maximum
275 HMF values. No apparent relation could be observed between the 5MF values, neither
276 with the toasting times nor the cooking formulations, and no statistical differences were
277 observed between samples.
278 HMF was the most representative compound of the studied furfurals, showing the
279 highest concentrations. 5MF was detected in all samples, and the furfural variation was
280 statistically significant and quantified in the samples A and B at 2 and 2.5 min toasting
281 times, but difference between samples was not significant at lower toasting times for the
282 formulations having a single ingredient and water.
283 *3.2 Furosine production*
284 Furosine as a specific early Maillard reaction marker did not show significant changes
285 during toasting also the interaction between toasting time and cooking formulation was
286 not significant. Control sample showed a concentration of 8.5 μg/100g. The addition of
287 other ingredients did not promote major differences in furosine content at this stage.

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288 Complex formulations A and B surprisingly showed the lowest quantities of furosine
289 (6.1 and 2.3 μg/100g respectively) and an amount of HMF over 150 ppm.
290 Our results suggest that during toasting furosine as an early Maillard reaction marker is
291 not suffering major changes. A more detailed study of the Maillard reaction could study
292 other advance reaction products, even though it has been previously reported that during
293 the industrial processing of flakes, HMF levels increased drastically in the toasting
294 process while other markers as carboximethyllisine (CML) did not show significant
295 differences at this stage, so that the amount of CML was not changed after the toasting
296 of corn flakes (Farroni & Buera 2012).
297
298 *3.3. Color changes*
299 The chromatic variables L^* (luminosity) and a^* (redness) presented significant
300 differences ($p<0.01$) among the different formulations and toasting times. Interaction
301 between formulation and toasting time was also significant. Changes in the chromatic
302 coordinate b^* *(*yellowness*)* were less pronounced. In Figure 1a the darkening of samples
303 was represented by plotting the difference in luminosity before and after toasting
304 process ($L_o^*-L^*$) vs. toasting time Formulations S and M showed significant darkening
305 after 2.5 m toasting while SUC, A and B showed significant darkening after 1.5 min.
306 Samples containing all ingredients (A and B) showed the most important darkening at
307 2.5 min. Figure 1b shows that a^* values increased significantly with the toasting time
308 and also were highly dependent on the formulations following a very similar tendency
309 as found for ($L_o^*-L^*$). For the first 1.5 min, b^* values were in a range of 38.2- 42.8 and
310 did not show differences between formulations. After 2.0 and 2.5 min toasting, the b*
311 values were between 37-45 and still did not show significant differences between
312 samples, except for two values at 2.5 min that were significantly lower, A (23.7) and B

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313 (30.1). An interaction between time and formulations was also observed, but no
314 apparent relationship between time-formulation and samples could be extracted.

315 The CIELAB color coordinates (L_o*-L*, and a*) increased linearly with the toasting
316 time, indicating a zero order reaction kinetics. In general, it was possible to visually
317 observe that, for the formulations containing all ingredients, toasting times higher than
318 1.5 min promoted browning, generating a very dark color that could be unacceptable by
319 a consumer. This could be related also to the high amount of HMF detected in these
320 samples. A significant (negative) correlation between L* and HMF was previously
321 reported by Kowalski, Lukasiewicz, Juszczak & Kutyla-Kupidura (2013).

322

323 *3.4. Fluorescence and Browning*

324 The obtained results of brown pigments ln (Abs_{420}) and fluorescence ($\mu g_Q \cdot mg^{-1}$) for the
325 pronase-treated samples at the different toasting times are shown in Figure 2a and b,
326 respectively. The initial Absorbance of samples before toasting was below 0.1 and was
327 not significantly different between sample systems. When Absorbance data was
328 analyzed statistically, interaction between time and formulation was significant. While
329 at 1.5 min toasting the obtained ln (Abs_{420}) values were not statistically different among
330 samples, at 2 and 2.5 min toasting the ln (Abs_{420}) values for formulations A and B were
331 significantly higher compared to the rest of samples except for malt formulation at 2.5
332 min.

333 Before toasting, fluorescence was below limit of quantification for all six flaked
334 samples. After, it showed an initial increment with toasting time at 1.5 min. It increased
335 ~~linearly~~ with toasting time for Control and Salt formulations. Malt, Sucrose, A and B
336 formulations showed a rapid initial increment and then tended to reach a plateau. This

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337 behavior is expected because of their role as reaction intermediates (Morales, F. J.,
338 Romero, C., & Jiménez-Pérez, S. (1996) and has been already described by Patton &
339 Chism (1952) where they stated that fluorescence formation showed an induction period
340 followed by a period of increasing concentration until a maximum was reached.
341 We have not observed any lag period in our systems, in concordance with (Morales, F.
342 J., Romero, C., & Jiménez-Pérez, S. (1996). The observation may be due to the high
343 temperature conditions used in our experiment as described also by this authors.
344 The interaction between time and formulation was not significant. The effect of time
345 showed significant differences ($p<0.05$) between the average value obtained at 1.5 min
346 and the higher value obtained at 2.5 min. Also, individual formulations showed
347 significant differences between them. Control and Salt systems showed the lowest
348 average values, statistically different from the average values found for the other
349 formulations with a $p<0.05$.
350 Overall, when comparing formulations A and B no significant differences in
351 the intermediates concentration was detected, in spite B contained about 10 times
352 less salt and 300 times less malt than A. Only a slight difference was observed
353 between both systems in the color coordinate a*, but it was attributable to the intrinsic
354 color of malt since it was also detected at the initial time. It can be concluded that the
355 synergistic effect of malt and salt combined with sucrose is evident even at low
356 salt or malt concentrations as its variation was similar in B and in A systems. At
357 the same time, sucrose individually showed a significant lower development of
358 browning than A and B.

359 4. CONCLUSIONS

360 The combined ingredients (sucrose+salt+malt) had a synergistic effect. The individual
361 addition of small amounts of salt, malt or sucrose did not accelerate the browning

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362 process, being this synergistic effect more important than the difference in the amounts
363 added. Therefore, the results obtained allow to better understand the influence of
364 formulation used during cooking and the effect of the toasting time in browning
365 intermediates production. This may help to select conditions to mitigate the loss of
366 nutrients and the generation of undesirable components.

367 Although not specific, during toasting of flaked corn grits the CIELAB coordinates
368 luminosity expressed as (L_o*-L*) and redness (a*) showed to promptly evaluate the
369 degree of browning reaction showing a zero order rate constant.

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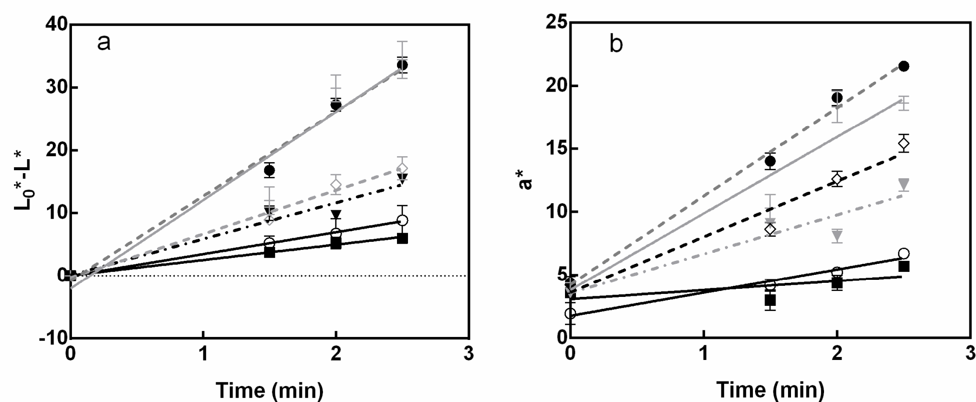

439

440 Fig. 1(a) and (b) correspond to (Lo*-L*) and a* as a function of time, respectively.

441 Curves for the different formulations at times: 1.5 min; 2 min and 2.5 min. Symbols for

442 each formulation: (—■—) Control, (—○—) Salt, (–•–▼–•–) Malt, (– –◊– –) Sucrose, (—

443 ●—) A, (– –┼– –) B. Lines represent the predicted curves (best fit model) for all

444 different formulations. Error bars correspond to the standard deviation.

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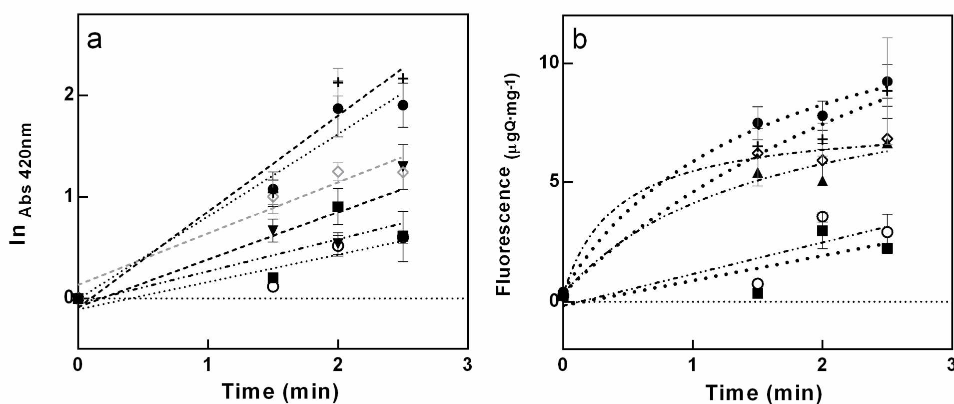

447

448 Fig. 2(a) and (b) correspond to ln (Abs420) and Fluorescence (μgQ•mg-1) for the

449 different toasting times (1.5 min; 2 min and 2.5 min). Symbols are the mean values for

450 each formulation: (—■—) Control, (—○—) Salt, (–•–▼–•–) Malt, (– –◊– –) Sucrose, (—

451 ●—) A, (– –┼– –) B. Error bars correspond to the standard deviation. Connecting lines

452 present the best mathematical fit.

Table 1: Systems composition: mass of each ingredient mixed with 15 g of grits for steam cooking formulations.

System name →	Control (C)	Salt (S)	Malt (M)	Sucrose (SUC)	Commercial formulation A	Commercial formulation B
Water (g)	9	9	9	9	9	6
NaCl (g)		0.33			0.33	0.01
Malt (g)			0.33		0.33	0.001
Sucrose (g)				1.23	1.23	1.23

Table 2: Furfurals and Furosine values for the 6 different cooking formulations toasted at three toasting times.

Toasting time	System	Humidity (%) Wet basis	HMF ppm dry matter	Furfural ppm dry matter	5-MF ppm dry matter	Furosine µg/100g dry matter
1.5 min	control	$9.6^{g} \pm 0.4$	$14^{a} \pm 3$	$<0.02^{a}$	2.0 ± 0.3	$9^{ab} \pm 5$
	NaCl	$8.4^{defg} \pm 0.4$	$8.7^{a} \pm 0.3$	$<0.02^{a}$	1.8 ± 0.1	$8^{ab} \pm 2$
	malt	$7.7^{cde} \pm 0.2$	$20^{a} \pm 2$	$<0.02^{a}$	2.6 ± 0.2	$6.9^{ab} \pm 0.4$
	sucrose	$6.6^{abc} \pm 0.5$	$53.9^{a} \pm 0.9$	$7.1^{a} \pm 0.1$	2.47 ± 0.01	$11^{b} \pm 2$
	A	$8.0^{cdef} \pm 0.1$	$396^{a} \pm 13$	$27^{a} \pm 1$	1.8 ± 0.3	$6.^{ab} \pm 4$
	B	$7.3^{bcde} \pm 0.6$	$154^{a} \pm 16$	$10^{a} \pm 8$	1.98 ± 0.09	$2.3^{a} \pm 0.5$
2 min.	control	$8.6^{efg} \pm 0.4$	$11^{a} \pm 4$	$0.7^{a} \pm 0.6$	3.2 ± 0.2	$5^{ab} \pm 1$
	NaCl	$9.4^{fg} \pm 0.6$	$22^{a} \pm 3$	$1.7^{a} \pm 0.3$	2.8 ± 0.5	$3.6^{ab} \pm 0.9$
	malt	$8.3^{defg} \pm 0.2$	$26^{a} \pm 7$	$2.83^{a} \pm 1.17$	2.5 ± 0.1	$1.6^{a} \pm 0.4$
	sucrose	$6.9^{abcd} \pm 0.6$	$137^{a} \pm 22$	$16^{a} \pm 5$	2.1 ± 0.4	$3.9^{ab} \pm 0.2$
	A	$6.5^{abc} \pm 0.2$	$1797^{b} \pm 71$	$99^{b} \pm 7$	1.5 ± 0.4	$3.2^{a} \pm 0.3$
	B	$7.0^{bcd} \pm 0.4$	$2336^{c} \pm 72$	$131^{b} \pm 21$	2.4 ± 0.2	$2.1^{a} \pm 0.4$
2.5 min.	control	$8.4^{defg} \pm 0.3$	$15^{a} \pm 1$	$1.2^{a} \pm 0.3$	2.55 ± 0.04	$2.6^{a} \pm 0.3$
	NaCl	$7.8^{cde} \pm 0.1$	$42^{a} \pm 23$	$4^{a} \pm 2$	2.4 ± 0.4	$3.2^{a} \pm 0.6$
	malt	$7.5^{cde} \pm 0.3$	$72^{a} \pm 5$	$10^{a} \pm 7$	2.0 ± 0.3	$2.35^{a} \pm 0.07$
	sucrose	$5.9^{ab} \pm 0.6$	$114^{a} \pm 9$	$14^{a} \pm 1$	2.3 ± 0.2	$2.8^{a} \pm 0.3$
	A	$5.5^{a} \pm 0.6$	$2639^{c} \pm 186$	$125^{b} \pm 58$	1.7 ± 0.6	$6.1^{ab} \pm 0.6$
	B	$7.4^{bcde} \pm 0.2$	$2398^{c} \pm 460$	$131^{b} \pm 5$	1.9 ± 0.7	$4.4^{ab} \pm 2.1$

Different letters refer to statistically significant differences within the same variable, for $\alpha = 0.05$. 5-MF values did not show significant differences between them.

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