1   **MECHANICAL, MICROSTRUCTURE AND PERMEABILITY PROPERTIES**

2   **OF A MODEL BREAD CRUST: EFFECT OF DIFFERENT FOOD ADDITIVES**

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12   **ABSTRACT**

13   The aim of this study was to understand the action of different additives on the crust

14   properties using a layer crust as a model. Moisture content, water vapour barrier

15   properties, water sorption isotherms and mechanical properties were evaluated. Crust

16   model showed multilayer internal structure. Glycerol (10 and 20%) and HPMC-10%

17   increased moisture content, whereas linolenic acid and beeswax, glycerol-1%, HPMC-

18   0.5% and citric acid significantly decreased it. Water vapour permeability (WVP)

19   decreased with lipids and citric acid, due to their hydrophobic nature and crosslinking

20   action, respectively. Hydrophobic additives lowered the WVP of the crust and provided

21   water barrier properties and brittle texture. Crust mechanical properties were greatly

22   correlated with water present as well as with composition of crust layer. Barrier

23   properties of the crust layer were greatly dependent on the hydrophilicity or

24   hydrophobicity of the additives, which determined the internal interactions between

25   starch and proteins and the microstructure and mechanical properties.

26

27   **Key words:** crust layer; additives; water vapour permeability; moisture sorption

28   isotherms; mechanical properties; microstructure.

29

1. INTRODUCTION

Crusty breads are much appreciated due to their crispy texture. Crust is the upper part of the breads formed during baking. Crust is constituted by a network comprising denatured gluten proteins and partially gelatinized starch granules. Different concepts have been applied to define the crust, e.g. dry, hard, dark and dense (Hug-Iten, Escher, & Conde-Petit, 2003). In fresh state, bread crust is dry and crispy and exhibits a brittle noisy fracture, but those properties are transitory and change during staling (Gray & Bemiller, 2003), owing to the steady increase in water content and water activity (Cuq, Abecassis, & Guilbert, 2003). Water acts as a plasticizer and decrease the bread Tg of the material. As a consequence, the mechanical properties of the crust associated to crispness changes and the crust becomes very soft and leathery (Roudaut, Dacremont, & Le Meste, 1998), which cause consumer's rejection. Therefore, bread crust must have low moisture content (3 to 11.5% d.b.) and water activity (0.34 to 0.57) to keep its crispy texture (Cuq et al., 2003). Water uptake kinetic is strongly related to crispiness retention of composite products consisting of a dry crispy part and a more humid and soft part (Meinders & Van Vliet, 2011). Besides, water uptake is usually described by sorption isotherms and several mathematical models have been described for fitting sorption curves. Nevertheless, no approach has been presented considering the crust as a physical barrier and its diffusivity properties.

In addition, the composition of the product, morphology and crust thickness also play an important role in crispy texture perception. Some studies have been focused on strategies for prolonging the bread crust crispiness. With that purpose, enzymes (proteases, transglutaminase, *alpha*-amylase, amyloglucosidase and glucose oxidase) have been sprayed onto dough or bread crust surface (Primo-Martín, Van de Pijpekamp, Van Vliet, De Jongh, Plijter, & Hamer, 2006; Primo-Martin, Beukelaer, Hamer, & Van Vliet, 2008; Altamirano-Fortoul, Hernando & Rosell, 2014). Those enzymes modified

56 the starch-protein network, which had effect on the water holding capacity of the crust

57 and in turn on the crispy texture behaviour and cellular structure of crust. The potential

58 of other additives has not yet been considered.

59 According to previous studies, crust acts as a barrier for water migration. Primo-Martin,

60 Sözer, Hamer, & Van Vliet, (2009) proposed a crust model consisting on a very thin

61 bread to discriminate between the fracture properties of the crust material and the

62 gradient of water in the crust. However, the crust of the bread is not at equilibrium,

63 because it is a complex system in which different reactions as well as changes in water

64 activity/ content occur during breadmaking. Considering that crust is a vitreous surface

65 layer, in this study a model bread crust (crust layer) was developed using pre-gelatinized

66 flour to simulate the bread crust. The aim of the present study was to investigate the

67 effect of different bakery's additives (hydroxypropylmethylcellulose, vital gluten,

68 diacetyl tartaric acid ester of mono-diglycerides, a protease from *Bacillus licheniformis*

69 (Alcalase 2.4 LFG, 2.4 units/g), beeswax, linolenic acid, glycerol and citric acid), on

70 water vapour permeability (WVP), water diffusion, mechanical properties and structure

71 of the model crust layer.

72 **2. MATERIALS AND METHODS**

73 *2.1. Materials*

74 Pre-gelatinized wheat flour, provided by Harinera Villamayor (Huesca, Spain), was

75 used for crust layer formulations. The wheat flour composition was (expressed as dried

76 basis): 10.54% protein content, 10.91% moisture content, 1.03% fats and 0.58% ash

77 content. Additives studied included hydroxypropylmethylcellulose (HPMC K4M) from

78 Dow Chemical (USA), vital gluten provided by Roquette (Keokuk, IL), diacetyl tartaric

79 acid ester of mono-diglycerides (DATEM, Panodan® AB 100 VEG-FS KOSHER) from

80 Danisco (Spain), a protease from *Bacillus licheniformis* (Alcalase 2.4 LFG, 2.4 units/g)

81 provided by Novozymes A/S (Bagsvaerd, Denmark), beeswax from Scharlau

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82   (Barcelona, Spain), linolenic acid provided by Sigma (Barcelona, Spain), glycerol and

83   citric acid from Panreac (Barcelona, Spain).

84   *2.2. Methods*

85   *2.2.1. Crust layer forming solution*

86   Crust layer forming solutions were prepared using pre-gelatinized wheat flour blended

87   with additives at different concentrations (Table 1) and in the presence of calcium

88   propionate (0.1%, w/w) as preservative. All raw materials were mixed mechanically

89   with water during 60 seconds and then were degassed. For beeswax based crust layer,

90   the additive was suspended in 10 ml distilled water and boiled to mix it completely.

91   Crust layers were cast onto plastic trays (25.5cm x 16cm x1.5cm). In each case 134.20 g

92   mixture was poured into each tray to minimize crust layer thickness variations.

93   Preliminary tests were carried out to define the appropriate mixture amount for

94   obtaining model crust of similar thickness to bread crust ($\sim$ 0.5 mm). Mixtures were

95   allowed to dry at 37 ºC for 12 h, after this time, drying continued at 20 ºC for 39 h.

96   Dried crust layers were stored in a desiccator containing saturated magnesium nitrate

97   with 54.4% (RH) at 20 ºC for further analysis. Conditions were selected to avoid

98   microbial growing. Control crust layers were prepared in the same way without the

99   presence of additives. Each crust layer formulation was prepared in duplicate.

100   *2.2.2. Physicochemical analysis*

101   Moisture content was determined following ICC standard method (1994) (ICC 110/1).

102   Thickness of crust layers was determined using a digital micrometer (Mitutoyo,

103   Kanagawa, Japan) with a sensitivity of 2 μm. The mean thickness was calculated from

104   measurements taken at 10 different locations on each crust layer sample.

105   *2.2.3. Water vapor permeability*

106   Water vapor permeability (WVP) of the crust layers was determined according to the

107   method ASTM E96 (ASTM, 1980). A cup having an internal diameter of 3.6 cm was

108   filled with distilled water, sealed with the crust layer and then placed into different

109   desiccators at 20 ºC, and 54.4% RH. Changes in the weight over time were monitored to

110   determine the steady state flux of water vapor through the crust layers. The cups were

111   weighed every day during seven days.

112   *2.2.4. Moisture sorption isotherms*

113   Crust layer pieces of about 3 cm in diameter were transferred into a desiccator

114   containing P_2O_5 to complete drying. Afterwards, crust layer specimens, in duplicate,

115   were placed at 20 ºC in desiccators containing saturated salt solutions with different

116   relative humidity: $LiCl \cdot H_2O$ (11.3%), $KC_2H_3O_2$ (23.1%), $MgCl_2.6H_2O$ (33.1%),

117    $K_2CO_3.2H_2O$ (43.2%), $Mg(NO_3)_2.6H_2O$ (54.4%), $NaCl$ (75.5%), KCl (85.1%),

118    $BaCl_2.2H_2O$ (91.2%) and K_2SO_4 (97.6%). Samples were weighed periodically till

119   constant weight value was reached, where the equilibrium was assumed to be achieved.

120   The experimental values were fitted by the GAB (Guggenheim-Anderson-deBöer)

121   model

122    $EMC = W_m C k a_w / [(1 - k a_w)(1 - k a_w + C k a_w)]$ Eq (1)

123   where EMC is the equilibrium moisture content on a dry basis, W_m represents the water

124   content corresponding to saturation of all primary adsorption sites by one water

125   molecule, and is called monolayer moisture content in BET (Brunauer, Emmett and

126   Teller) theory, C is the Guggenheim constant, k refers to the factor correcting properties

127   of the multilayer molecules corresponding to the bulk liquid, and a_w = water activity.

128   The root mean square (RMS, %) of the fitting is also included for each crust layer.

129    $$\%RMS = \left[\frac{\sqrt{\sum \left[\frac{M^{exp} - M^{cale}}{M^{exp}} \right]^2}}{N} \right] x100 \quad \text{Eq. (2)}$$

130 Where N is the number of experimental points, M_{exp} is the experimental equilibrium

131 moisture content value; M_{calc} is the calculated equilibrium moisture content value.

132 *2.2.5. Mechanical properties: Fracturability test*

133 Crust layers were fractured using a texture analyzer with a 5 kg load (TA XTplus,

134 Stable Micro Systems, Surrey, UK). Experiments were carried out using a HDP/BS

135 blade set at 5 mm/s. The maximum force (N), the area (N/s), and the displacement at

136 fracture (mm) were measured. Twenty replicates of each crust layer were conducted.

137 *2.2.6. Microstructure*

138 Structural analysis was performed by scanning electron microscopy (SEM) on samples.

139 Crust layers were freeze-dried previously to the microscopy analysis. Crust layers were

140 fixed with the aid of colloidal silver and then coated with gold (Baltec SCD005) at 10^{-2}

141 Pa and an ionization current of 40 mA. The observation was carried out in a JEOL JSM-

142 5410 (Jeol, Tokyo, Japan) scanning electron microscope at 10 kV.

143 *2.3. Statistical analysis*

144 Data were presented as mean of sample sets. Statistical analysis of the results was

145 performed using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). In

146 order to assess significant differences among samples, a multiple sample comparison

147 was performed.

148 **3. RESULTS AND DISCUSSION**

149 *3.1. Moisture content*

150 The model crust or layer crust showed moisture content (7.5 g/100g d.b.). The moisture

151 content of the crust layers were significantly modified due to the presence of additives

152 ($P<0.05$) (Table 2). The moisture contents ranged from 5.19 to 11.64 g/100g d.b. These

153 values were in agreement with those reported by Cuq et al. (2003) for bread crust.

154 Control sample and crust layer with gluten showed similar moisture content. The

155 polymers (starch and gluten) present in its composition might form a molecular network

156   or matrix with high interaction among them. Likely, the polarity of starch and gluten

157   induced high affinity for water, which was easily integrated in its structure by

158   establishing hydrogen bonds with the polymer molecules. Moisture content increased in

159   the presence of HPMC 10%, glycerol 10% and 20%, which promoted the hydrophilic

160   character of the crust. Hydrophilic plasticizer provides more active sites in layer matrix

161   by exposing its hydroxyl group in which the water molecules could be adsorbed, which

162   agrees with previous observations (Rosell and Foegeding, 2007). Conversely, crust

163   layer containing glycerol 1%, HPMC 0.5%, citric acid, linolenic acid or beeswax

164   presented lower moisture content than the control crust layer. Glycerol and HPMC

165   incorporated in low quantity can have an anti-plasticizing effect in the food matrix, due

166   to interaction of the plasticizer molecules with the starch and gluten, thus decreasing

167   chain mobility (Rosell & Foegeding, 2007; Rosell, Yokoyama & Shoemaker, 2011).

168   Moreover, these additives could compete with water molecules for active sites on the

169   starch-protein matrix, which decreased the moisture content. With regards to linoleic

170   acid and beeswax, these additives due to their hydrophobic nature decreased the water

171   holding capacity of the matrix and thus moisture content. Citric acid could act as a

172   crosslinker in the starch-gluten matrix giving rise to a more compact food matrix

173   limiting its ability to retain water molecules (Olson, Hedenqvist, Johansson &

174   Järnström, 2013).

175 Crust layers with lipids had the lowest moisture content, which might anticipate crispy

176 crust considering the relationship between moisture content of bread crust with crispy

177 texture (Primo-Martin et al., 2008; Altamirano-Fortoul et al., 2013).

178 *3.2.Water vapor permeability*

179 Thickness in the crust layers showed significant differences among treatment types, it

180 varied from 0.24 mm to 0.58 mm, which agrees with previous studies (Altamirano-

181 Fortoul, Hernando & Rosell, 2013). Water vapor permeability (WVP) of the crust layers

182   showed significant differences, likely attributed to changes in the polymeric matrix due

183   to additives (Table 3). The presence of additives could lead to a structure with or

184   without pores and cracks modifying the permeability. Again, the highest WVP value

185   was presented in the sample with greater glycerol concentration (20%), which acted as a

186   plasticizer. This result agrees with Chillo et al. (2008) findings that indicated an

187   increase in film WVP when increasing plasticizer concentration. At low concentration,

188   glycerol has an anti-plasticizing effect due to the plasticizer-polymer interactions that

189   decrease intermolecular spaces for the diffusion of water molecules through the crust.

190   According to Mali, Karam, Pereira Ramos, and Grossmann (2004), glycerol

191   concentration from 0 to 20% reduced the WVP in cassava starch films produced by

192   casting, as glycerol addition led to a more compacted network without pores or cracks.

193   Hirte et al. (2012) suggested that bread crust with many small cracks had optimal water

194   vapor permeability; however, an excess of cracks could lead to crumb dryness.

195   Crust layers with HPMC presented the same WVP tendency as glycerol. Crust layer

196   with high concentration of HPMC (10%) had higher water affinity due to the large

197   amount of hydrophilic groups present in HPMC structure, and also it can disrupt starch-

198   protein interactions forming a loose matrix, which favors water vapor permeability.

199   When HPMC was added at low concentration (0.5%), it probably acted as a crosslinker

200   establishing hydrogen bridges between starch and protein polymers, and reduced the

201   number of active sites for water sorption.

202   Control crust layer and crust layer with gluten exhibited higher WVP, which could be

203   ascribed to their hydrophilic nature. This result agrees with McHugh, Avena-Bustillos

204   and Krochta (1993), who reported that films based on hydrophilic polymers like

205   proteins or polysaccharides are very sensitive to moisture. Moisture sorption exerts a

206   plasticizing effect on the biopolymer matrix increasing polymer free volume and chain

207   mobility, thus facilitating the diffusion of water molecules across the crust.

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208 Incorporation of protease in the crust formulation decreased WVP, which is likely due

209 to the disruption of the crust layer as a consequence of the direct cleavage of the

210 protein-starch structure. Probably, those structural modifications of the polymeric

211 matrix led to a denser structure that hindered water molecules transference through the

212 crust layer. In fact, Primo-Martin et al. (2006) when spraying protease on the surface of

213 the dough, found changes in the crust characteristics that retarded the water content

214 increase.

215 DATEM, an amphiphilic molecule, decreased the WVP, which could be attributed to its

216 action decreasing interchain spacing between polymer chains promoting a structure with

217 less pores/cracks. This result disagrees with the previous findings of Primo-Martín et al.

218 (2006), who observed an increase of the porosity when adding DATEM. However, the

219 function of DATEM as a crumb softening agent may also reduce water migration from

220 gluten to starch by forming a complex with starch, and be absorbed into the starch

221 surface (Pisesookbunterng & D'Appolonia, 1983).

222 Citric acid resulted in a decrease in the WVP value, which could be attributed to its

223 crosslinking action, reducing the polymers mobility and increasing their cohesion.

224 According to Moller, Grelier, Pardon and Coma (2004) the addition of a crosslinking

225 agent as citric acid improves the barrier against water vapor.

226 As expected, crust layer with lipids (linolenic acid and beeswax) showed lower WVP,

227 due to their hydrophobic properties (García, Martino & Zaritzky, 2000). Therefore, non-

228 polar groups yielded a dense structure that slow water migration through the crust layer.

229 Previous studies stated that waxes are the most efficient substances to reduce moisture

230 permeability because of their high hydrophobicity (high content in long chain fatty

231 alcohols and alkanes) (Morillon et al., 2002).

232 In general, the presence of additives modified the starch-protein matrix structure and

233 moisture sorption properties, which resulted in changes in water vapor permeability of

234   crusts layers. García et al. (2000) reported that WVP depends on many factors such as

235   the ratio between crystalline and amorphous zone, polymeric chain mobility and

236   specific interactions between the functional groups of the polymers in the amorphous

237   zone. According to previous studies, restriction of water uptake by the crust and

238   modification of the proteins in the crust are useful tools to maintain crispy texture in

239   brittle and cellular foods as bread crust (Primo-Martin et al. 2006; Altamirano-Fortoul,

240   et al. 2013).

241   *3.3.Moisture sorption isotherms*

242   Moisture sorption isotherms of bread crust incorporating different additives are shown

243   in Figure 1. They exhibited sigmoid shape and three regions could be clearly

244   differentiated. The first region of the curves represents strongly bound water including

245   structural and monolayer water, which is unfreezable and not available as a plasticizer.

246   In this region, the crust layers presented a moderately slight slope at low water activity,

247   similar to the behavior observed for some gluten, starch and cellulose films (Hernández-

248   Muñoz, Kanavouras, Perry, & Gavara, 2003; Al-Hassan & Norziah, 2012). In this stage

249   the physical adhesion of water to active sites of the polymer occurs only in the surface,

250   bound to the polar and hydrophilic groups of polysaccharides, proteins and other

251   component of the film crust (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007).

252   The second region comprised a linear region of the sorption isotherm, where water

253   molecules bind less firmly than in the first region and they are adsorbed as a multilayer.

254   In this region there is a transition between bound water to free water. The equilibrium

255   moisture content of crust layers increased slightly when increasing water activity up to

256   0.54, depending on the additive type. Crust layers containing beeswax, linolenic acid

257   and glycerol 1% showed a reduction in the equilibrium moisture content, which

258   confirmed that polarity of lipids affected the capacity to absorb water, and suggest that

259   glycerol incorporated at low concentration (1%) competed with water molecules for

260 hydrophilic sites on crust layer surface avoiding water binding in subsequent layers.

261 Conversely, control and crust containing gluten showed pronounced rise in the

262 equilibrium moisture content. These results could be related to the hydrophilic character

263 of materials presents on polymeric matrix, which led to water mobility within the matrix

264 structure. The third region corresponded to the upper part of the curve, where water

265 molecules are associated to other water molecules forming clusters and loosely binds to

266 food materials; in this region water properties of water molecules are similar to those of

267 free water. For water activities higher than 0.6, all samples presented a rapid increase in

268 moisture content that was attributed to the presence of non-bound or free state water that

269 favored solubilization, which was reflected as swelling of the polymeric matrix. Greater

270 water activities imply a substantial water uptake in the films due to the development of

271 solvent-solvent interactions (Hernández-Muñoz et al., 2003).

272 Empirical, semi-empirical and theoretical mathematical models of moisture sorption

273 isotherms have been proposed to describe the behavior of food products and other

274 biological materials. The GAB model describes sigmoidal shape isotherms, and it is a

275 refinement of Langmuir and BET theories of physical adsorption. The GAB, model

276 similar to the BET model, describes the monolayer expression in Langmuir's adsorption

277 isotherms and considers the multilayer sorption step. Related to the BET model, this

278 model contains a third constant k, but conversely to the BET model it can be used in a

279 wide range of water activities (0.1 < a_w < 0.9). GAB model provides an accurate

280 description of moisture sorption of most food materials, thus, experimental data were

281 fitted using this model. Estimated GAB parameters (W_m, C, and k) and root mean square

282 (RMS %) for crust layer are shown in Table 4. Monolayer (W_m) values of control and

283 crust layer with gluten and protease were similar and significantly higher than the ones

284 obtained with other additives. It seemed that they had more active sites due to its

285 hydrophilic group exposition, in which the water molecules can be adsorbed. The

286 monolayer value represents the amount of moisture that is strongly adsorbed to specific

287 sites at sample surface, and at this value the food product is more stable (Andrade,

288 Lemus, & Pérez, 2011). Low values of W_m reflected a reduction in the number of

289 primary active sites and it could be related to chemical, physical and structural

290 modification of the polymeric matrix produced by the additives and the character of

291 these. In this regard, incorporation of gluten, protease did not modifiers values of the

292 monolayer; citric acid reduced the value of the monolayer, which could be related to the

293 formation of physical and/or chemical cross-links with functional groups of starch and

294 proteins, reducing the number of polar and hydrophilic sides for water sorption. It could

295 happen a similar process when added HPMC, DATEM and glycerol. Incorporation of

296 linolenic acid and beeswax reduced notably the monolayer value. The interaction

297 between the polymeric matrix and the linolenic acid or beeswax probably led to an

298 increase in the number of the hydrophobic particles that did not interact with water.

299 Therefore, few active sites were accessible for water adsorption in the polymeric matrix

300 due to the arrangement of the lipid chains.

301 The parameter C, the Guggenheim constant, represents the energy difference between

302 the water molecules attached to primary sorption sites and those absorbed to successive

303 sorption layers (Timmerman, Chirife, & Iglesias, 2001). According to previous studies

304 in biomaterial water sorption isotherms, applying the GAB model, the values for this

305 parameter were comprised between $5.67 \leq C \leq \infty$ (Lewicki, 1997). In the current study,

306 C values ranged between 2.98 and 59.17.

307

308 The parameter k is related to difference in the sorbate's pure liquid state and in the

309 upper layers (Timmerman et al., 2001). Theoretically, the values of k should be less than

310 unity (Chirife, Timmermann, Iglesias, & Boquet, 1992). However, in the literature, a

311 huge number of papers presented k values higher than unity. Values of parameter k

312   obtained for the crust layer with or without additives were between 0.97 and 1.03.

313   Lower value of k indicates less structured state of the sorbate in the layers above the

314   monolayer than in the sorbate in the GAB layer. However, proteins and protein-foods

315   present higher values of k than starchy foods (Timmermann et al., 2001; Hernández-

316   Muñoz, Kanavouras, Lagaron, & Gavara, 2005).

317   Results showed that additives can modify the water affinity of the polymeric matrix and

318   therefore the water sorption.

319

320   *3.4. Mechanical properties*

321   Any food's texture is mainly connected to its mechanical properties, which in principle

322   reflect the internal food microstructure. It is well known that moisture content and water

323   distribution have a strong effect on mechanical properties of brittle and cellular foods

324   such as bread crust. The crispy texture is related to properties of the product such as

325   hardness, brittleness and fracturability. Therefore, a crispy product must be stiff or

326   brittle with a fast fracture (Van Vliet & Luyten, 1995). Results of mechanical properties

327   of the crust layers are shown in Table 5. The flat structure of the crust model facilitated

328   the assessment of the mechanical properties.

329   In this study the hardness term was used to describe a product which displays

330   substantial resistance to breaking. As expected, the control sample presented high value

331   of hardness due to its components, since the hydroxyl group of the polar

332   macromolecules (proteins and starch) bound water via hydrogen bonds resulting a

333   plasticized polymeric matrix.

334   Crust layer with protease presented higher hardness (maximum force) than the rest of

335   the additives. The protease breaks the network and water interacted with the protein and

336   released chains creating a more cohesive structure, very flexible and more difficult to

337   fracture.

14

338   Presumably, other additives weakened the polymeric matrix, requiring less force for its

339   fracture. Nevertheless, this effect depended on the additive, ie. glycerol (10 and 20%) or

340   HPMC 10% and DATEM could have increased the mobility of polymer chains, due to

341   water absorbed into the polymer, which made the crust layers somewhat flexible.

342   Conversely, the presence of lipids, gluten, citric acid, glycerol 1% and HPMC 0.5%

343   resulted in crust layers with lower hardness values. Incorporating lipids in the crust

344   layer could interfere with interaction of polymers chains leading to a discontinuity

345   within the protein-starch matrix. Furthermore, the lipids as beeswax exhibited low

346   cohesiveness and structural integrity, which makes them very brittle. Gluten probably

347   increased intermolecular forces along the polymer chain and this led to a decrease of the

348   flexibility within the polymeric matrix structure. Inclusion of citric acid into the

349   polymeric matrix led to a decrease of hardness. This fact might be attributed to a

350   reduction in the molecules mobility and a decrease in the absorbed water promoted by

351   the crosslinking effect of the citric acid, resulting in a rigid crust layer (Olson et al.,

352   2013). When HPMC 0.5% was incorporated, probably intermolecular associations

353   among the polymer chains were inhibited by the HPMC and the crust layer had a stiff

354   structure, which required low force to fracture.

355   Fracturability is an important characteristic of brittle products. In general, the

356   fracturability of crust layers decreased with addition of the additives (Table 5), with the

357   exception of protease and glycerol 20%. Therefore, additives might modify the

358   polymeric matrix affecting fracturability. According to Primo-Martín et al. (2006) the

359   distribution of protein and partially gelatinized starch in the bread crust as well as the

360   water content alters the way the crust fractures. With respect to area value, control crust

361   and that with protease had much higher area, indicating their increased toughness, thus

362   these needed high work to fracture the crust layers.

363   The presence of additives in the model crust modified the water uptake and also the

364   mechanical properties related to crispy texture, and the most prominent effect was

365   observed with protease and lipids.

366   *3.5. Microstructure*

367   Microstructure of the cross section of crust models was analyzed to explain the effect of

368   additives on the mechanical properties and water vapor permeability behavior. SEM

369   micrographs confirmed microstructure differences promoting by additives (Figure 2).

370   Control crust layer showed a continuous veil-like film that revealed the underlying

371   structures, lenticular shape and circular starch granules of various sizes, likely

372   surrounded by protein matrix (Figure 2a), like it has been described for bread structure

373   (Rojas et al. 2001). The effect of additives in the crust layers was evident. The crust

374   layer with gluten revealed large starch granules and some small slightly deformed starch

375   granules embedded completely in the protein network (Figure 2b). Likely, this structure

376   might result from the covalent bonds as well as non-covalent interactions between

377   gluten proteins and starch. Thus, this allows a significant change in molecular motion of

378   proteins; and thus crust layer presented less resistance to break in spite of its capacity

379   for water diffusion.

380   Crust layer with protease was characterized by compact structure, with higher

381   deformation of starch granules and a more distorted gluten network (Figure 2c), which

382   agrees with the protease action splitting the protein strands of the gluten molecule that

383   leads first to a softening and then to a complete collapse of the structure. The crust layer

384   with HPMC 0.5% led to a smooth, compact and cracked structure (Figure 2d).

385   Conversely, crust layer with HPMC 10% presented irregular starch granules within a

386   disrupted and discontinuous protein network (Figure 2e). Therefore, HPMC could be

387   integrated in the molecular structure of the layer or formed a biphasic system leading

388   stiff structure depending on the addition level. These results agree with those observed

16

389 in hardness and fracturability parameters, and WVP. The structures containing glycerol

390 were significantly different and the extent of the changes was dependent on the glycerol

391 concentration. Crust layer with glycerol 1% revealed a structure masked by a

392 continuous gel and relatively smooth with obvious cracks as well as holes formation

393 (Figure 2f), suggesting a brittle fracture. Altamirano-Fortoul et al. (2013) suggested that

394 a cracking structure gives brittle bread crust behavior. The opposite effect was observed

395 in crust layer with glycerol 10%, where a compact and heterogeneous microstructure

396 was observed (Figure 2g). While the addition of glycerol 20% led to crust layer with

397 greater force to fracture as result of an apparent swelling of starch granules with

398 distorted structure and embedded in a protein network (Figure 2h). The addition of

399 plasticizers as glycerol produced a more flexible film with soft structure due to

400 hydrophillicity of plasticizers molecules, which favors the sorption of water.

401 Crust layer with DATEM exhibited a structure where starch granules lost their identity

402 and were covered with alternate continuous veil-like film and some cracks (Figure 2i).

403 Crust layer with citric acid was similar to sample containing DATEM, with alternate

404 continuous zones, besides a polymeric matrix with areas of protein aggregates (Figure

405 2j). This pattern might be attributed to the crosslinking action that led to less flexible

406 and brittle layer supporting hardness and fracturability results. The citric acid promotes

407 fragmentation of starch granules and also causes disruption of the bridges of inter and

408 intramolecular hydrogen, leading to a matrix with homogeneous appearance (Olson et

409 al., 2013).

410 In the case of samples with lipids, crust layers presented a smooth and nonporous

411 structure, and no phase separation was observed (Figure 2k and 2l). In fact, an almost

412 continuous structure with aligned constituents could be appreciated, readily evident in

413 the crust layer with linolenic acid. The crust layer containing beeswax showed no

414 individual crystals.

415 In general, the composition of the crust layer could influence strongly the molecular

416 level of the microstructure and, therefore, its mechanical and in some extent moisture

417 barrier behavior.

418 **CONCLUSIONS**

419 The crust model (crust layer) was a good approach to understand bread mechanical

420 properties and microstructure. Crust layers were significantly affected by the additives.

421 Sorption isotherms indicated that additives modified the water uptake. In general an

422 increase in monolayer value (W_m) was observed when gluten and protease were added.

423 However, lipids (linolenic acid and beeswax) promoted few active sites, decreasing the

424 monolayer values in comparison with control sample and the rest of the samples.

425 Therefore, crust layer with lipids provides a barrier. In relation to mechanical properties,

426 control sample and crust layers with greater glycerol concentration showed resistance to

427 fracture; these mainly due to the amount of water present into the polymeric matrix.

428 Opposite effect was observed with the crust layers with lipids, which indicated brittle or

429 stiff products. Thus, crispy texture was correlated closely with water present as well as

430 with composition of crust layer. Therefore, considering all results, crust layer with

431 HPMC 0.5% as well as with citric acid would be the best alternative additives to be

432 used for changing the crispiness behavior of bread crust. SEM analysis also confirmed

433 the effect of the additives.

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FIGURE CAPTIONS:

544   **Figure 1.** Effect of different additives of equilibrium moisture content of crust layer at

545   20°C fitted with the GAB model. Control crust layer:▲, control crust layer-GAB

546   ———,crust layer with gluten **x**, crust layer with gluten-GAB ·········· , crust layer with

547   protease **O**, crust layer with protease-GAB ━━━━ , crust layer with HPMC 0.5% ◊,

548   crust layer with HPMC 0.5%-GAB ━·━· , crust layer with DATEM ☐, crust layer

549   with DATEM-GAB ━ ━ , crust layer with glycerol 1% ●, crust layer with glycerol

550   1%-GAB ━·━ , crust layer with citric acid ⁺, crust layer with citric acid-GAB

551   ━··━··, crust layer with linolenic acid ♦, crust layer with linolenic acid-GAB ------ ,

552   crust layer with beeswax ■, crust layer with beeswax-GAB ━━━━ .

551   **Figure 2.** Scanning electron micrographs of crust layer cross section at high (3500x)

552   magnification. Images correspond to the following crust layer with additives: control

553   crust layer (a), crust layer with gluten (b), crust layer with protease (c), crust layer with

554   HPMC 0.5% (d), crust layer with HMPC 10% (e), crust layer with glycerol 1% (f),

555   crust layer with glycerol 10% (g), crust layer with glycerol 20% (h),crust layer with

556   DATEM (i), crust layer with citric acid (j), crust layer with linolenic acid (k), crust layer

557   with beeswax (l),

552

552 **Table1**. Additives concentrations applied in crust layer formulation

Sample	Dosage % (w/w) flour basis
Control	------
Gluten	1
Protease	0.8
Hydroxypropylmethylcellulose (HPMC)	0.5 10
Diacetyl tartaric acid ester of mono-diglycerides (DATEM)	0.3
	1
Glycerol	10
	20
Citric acid	1
Linolenic acid	0.3
Beeswax	0.3

553

554   **Table 2**. Effect of additives on the moisture content of crust layers.

Sample	Moisture content (g/100g d.b)		
Control	7.51	± 0.08	d
Gluten (1%)	7.63	± 0.05	d
Protease (0.8%)	7.22	± 0.04	cd
HPMC (0.5%)	5.19	± 0.13	a
HMPC (10%)	9.70	± 0.01	e
DATEM (0.3%)	7.26	± 0.12	cd
Glycerol (1%)	5.51	± 0.12	ab
Glycerol (10%)	9.81	± 0.22	e
Glycerol (20%)	11.64	± 0.04	f
Citric acid (1%)	6.88	± 0.23	c
Linolenic acid (0.3%)	5.57	± 0.84	ab
Beeswax (0.3%)	5.68	± 0.20	b

556

557   Means and standard deviations sharing the same letter within a column were not significantly

558   different ($P < 0.05$).

559

25

560 **Table 3.** Effect of additives on the water vapour permeability (WVP) of crust layers.

Sample	WVP (g•mm/m^2•s•Pa)		
Control	8.26E-07	± 3.94E-08	h
Gluten (1%)	8.00E-07	± 5.37E-08	gh
Protease (0.8%)	6.34E-07	± 4.39E-08	d
HPMC (0.5%)	5.00E-07	± 2.76E-08	bc
HMPC (10%)	7.26E-07	± 4.83E-08	f
DATEM (0.3%)	6.77E-07	± 3.34E-08	e
Glycerol (1%)	4.82E-07	± 1.22E-08	b
Glycerol (10%)	7.74E-07	± 2.57E-08	g
Glycerol (20%)	9.61E-07	± 2.49E-08	i
Citric acid (1%)	5.26E-07	± 2.50E-08	c
Linolenic acid (0.3%)	3.70E-07	± 2.59E-08	a
Beeswax (0.3%)	4.91E-07	± 1.89E-08	bc

561

562 Means and standard deviations sharing the same letter within a column were not significantly

563 different ($P < 0.05$).

564

565   **Table 4.** Estimated parameters from the GAB model.

566

Sample	W_m (g H_2O/100 g dry weight)	C	k	RMS (%)
Control	3.53	17.53	0.98	0.203
Gluten (1%)	3.50	49.63	0.98	0.111
Protease (0.8%)	3.61	2.98	1.00	0.855
HPMC 0.5%	2.61	22.22	0.97	0.347
DATEM (0.3%)	2.86	35.26	1.00	0.061
Glycerol 1%	2.63	6.14	1.00	0.285
Citric acid (1%)	3.07	27.62	0.98	0.217
Linolenic acid (0.3%)	2.36	8.61	0.99	0.592
Beeswax (0.3%)	2.00	59.17	1.03	0.244

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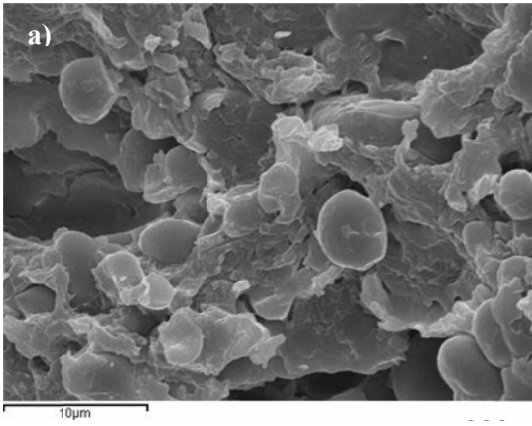568   Means and standard deviations sharing the same letter within a column were not significantly

569   different ($P < 0.05$).

570

27

571   **Table 5.** Mechanical properties of the crust layer.

Sample	Hardness (N)	Fracturability (mm)	Area (N.s)
Control	2.45 ± 0.76 ef	1.32 ± 0.36 ef	3.43 ± 0.51 e
Gluten (1%)	1.51 ± 0.13 b	1.03 ± 0.34 ab	0.88 ± 0.32 a
Protease (0.8%)	2.54 ± 0.04 f	1.48 ± 0.48 e	3.52 ± 0.03 e
HPMC (0.5%)	1.83 ± 0.35 a	1.09 ± 0.22 b	0.84 ± 0.02 a
HPMC (10%)	2.27 ± 0.07 c	1.23 ± 0.34 c	2.07 ± 0.15 c
DATEM (0.3%)	2.26 ± 0.21 d	1.19 ± 0.42 bc	3.02 ± 0.48 d
Glycerol (1%)	1.03 ± 0.46 a	1.04 ± 0.12 ab	0.84 ± 0.05 a
Glycerol (10%)	2.22 ± 0.25 d	1.22 ± 0.13 c	1.33 ± 0.61 b
Glycerol (20%)	2.51 ± 0.37 d	1.34 ± 0.06 f	3.05 ± 0.6 d
Citric acid (1%)	1.95 ± 0.6 c	1.10 ± 0.33 b	0.83 ± 0.03 a
Linolenic acid (0.3%)	1.09 ± 0.53 a	0.98 ± 0.00 a	0.83 ± 0.13 a
Beeswax (0.3%)	1.18 ± 0.11 a	1.08 ± 0.08 ab	0.84 ± 0.03 a

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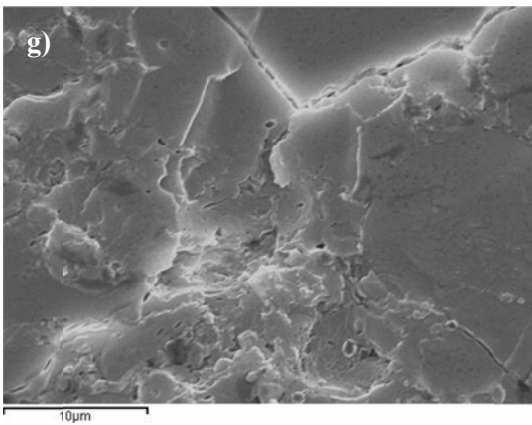573   Means and standard deviations sharing the same letter within a column were not significantly

574   different ($P < 0.05$).

575 **Figure 1.**

576

Figure 2.

583

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585