MECHANICAL, MICROSTRUCTURE AND PERMEABILITY PROPERTIES OF A MODEL BREAD CRUST: EFFECT OF DIFFERENT FOOD ADDITIVES

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ABSTRACT

The aim of this study was to understand the action of different additives on the crust properties using a layer crust as a model. Moisture content, water vapour barrier properties, water sorption isotherms and mechanical properties were evaluated. Crust model showed multilayer internal structure. Glycerol (10 and 20%) and HPMC-10% increased moisture content, whereas linolenic acid and beeswax, glycerol-1%, HPMC-0.5% and citric acid significantly decreased it. Water vapour permeability (WVP) decreased with lipids and citric acid, due to their hydrophobic nature and crosslinking action, respectively. Hydrophobic additives lowered the WVP of the crust and provided water barrier properties and brittle texture. Crust mechanical properties were greatly correlated with water present as well as with composition of crust layer. Barrier properties of the crust layer were greatly dependent on the hydrophilicity or hydrophobicity of the additives, which determined the internal interactions between starch and proteins and the microstructure and mechanical properties.

Key words: crust layer; additives; water vapour permeability; moisture sorption isotherms; mechanical properties; microstructure.

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 the starch-protein network, which had effect on the water holding capacity of the crust and in turn on the crispy texture behaviour and cellular structure of crust. The potential of other additives has not yet been considered.

According to previous studies, crust acts as a barrier for water migration. Primo-Martin, Sözer, Hamer, & Van Vliet, (2009) proposed a crust model consisting on a very thin bread to discriminate between the fracture properties of the crust material and the gradient of water in the crust. However, the crust of the bread is not at equilibrium, because it is a complex system in which different reactions as well as changes in water activity/ content occur during breadmaking. Considering that crust is a vitreous surface layer, in this study a model bread crust (crust layer) was developed using pre-gelatinized flour to simulate the bread crust. The aim of the present study was to investigate the effect of different bakery's additives (hydroxypropylmethylcellulose, vital gluten, diacetyl tartaric acid ester of mono-diglycerides, a protease from *Bacillus licheniformis* (Alcalase 2.4 LFG, 2.4 units/g), beeswax, linolenic acid, glycerol and citric acid), on water vapour permeability (WVP), water diffusion, mechanical properties and structure of the model crust layer.

2. MATERIALS AND METHODS

2.1. Materials

Pre-gelatinized wheat flour, provided by Harinera Villamayor (Huesca, Spain), was used for crust layer formulations. The wheat flour composition was (expressed as dried basis): 10.54% protein content, 10.91% moisture content, 1.03% fats and 0.58% ash content. Additives studied included hydroxypropylmethylcellulose (HPMC K4M) from Dow Chemical (USA), vital gluten provided by Roquette (Keokuk, IL), diacetyl tartaric acid ester of mono-diglycerides (DATEM, Panodan® AB 100 VEG-FS KOSHER) from Danisco (Spain), a protease from *Bacillus licheniformis* (Alcalase 2.4 LFG, 2.4 units/g) provided by Novozymes A/S (Bagsvaerd, Denmark), beeswax from Scharlau

(Barcelona, Spain), linolenic acid provided by Sigma (Barcelona, Spain), glycerol and citric acid from Panreac (Barcelona, Spain).

2.2. Methods

2.2.1. Crust layer forming solution

Crust layer forming solutions were prepared using pre-gelatinized wheat flour blended with additives at different concentrations (Table 1) and in the presence of calcium propionate (0.1%, w/w) as preservative. All raw materials were mixed mechanically with water during 60 seconds and then were degassed. For beeswax based crust layer, the additive was suspended in 10 ml distilled water and boiled to mix it completely.

Crust layers were cast onto plastic trays (25.5cm x 16cm x1.5cm). In each case 134.20 g mixture was poured into each tray to minimize crust layer thickness variations. Preliminary tests were carried out to define the appropriate mixture amount for obtaining model crust of similar thickness to bread crust (~ 0.5 mm). Mixtures were allowed to dry at 37 ºC for 12 h, after this time, drying continued at 20 ºC for 39 h. Dried crust layers were stored in a desiccator containing saturated magnesium nitrate with 54.4% (RH) at 20 ºC for further analysis. Conditions were selected to avoid microbial growing. Control crust layers were prepared in the same way without the presence of additives. Each crust layer formulation was prepared in duplicate.

2.2.2. Physicochemical analysis

Moisture content was determined following ICC standard method (1994) (ICC 110/1). Thickness of crust layers was determined using a digital micrometer (Mitutoyo, Kanagawa, Japan) with a sensitivity of 2 µm. The mean thickness was calculated from measurements taken at 10 different locations on each crust layer sample.

2.2.3. Water vapor permeability

Water vapor permeability (WVP) of the crust layers was determined according to the method ASTM E96 (ASTM, 1980). A cup having an internal diameter of 3.6 cm was filled with distilled water, sealed with the crust layer and then placed into different desiccators at 20 ºC, and 54.4% RH. Changes in the weight over time were monitored to determine the steady state flux of water vapor through the crust layers. The cups were weighed every day during seven days.

2.2.4. Moisture sorption isotherms

Crust layer pieces of about 3 cm in diameter were transferred into a desiccator containing P_2O_5 to complete drying. Afterwards, crust layer specimens, in duplicate, were placed at 20 ºC in desiccators containing saturated salt solutions with different relative humidity: $LiCl{\cdot}H_2O$ (11.3%), $KC_2H_3O_2$ (23.1%), $MgCl_2.6H_2O$ (33.1%), $K_2CO_3.2H_2O$ (43.2%), Mg $(NO_3)_2.6H_2O$ (54.4%), NaCl (75.5%), KCl (85.1%), $BaCl_2.2H_2O$ (91.2%) and K_2SO_4 (97.6%). Samples were weighed periodically till constant weight value was reached, where the equilibrium was assumed to be achieved. The experimental values were fitted by the GAB (Guggenheim-Anderson-deBöer) model

$$EMC = W_m Cka_w / \left[(1 - ka_w)(1 - ka_w + Cka_w) \right] \quad \text{Eq (1)}$$

where EMC is the equilibrium moisture content on a dry basis, W_m represents the water content corresponding to saturation of all primary adsorption sites by one water molecule, and is called monolayer moisture content in BET (Brunauer, Emmett and Teller) theory, C is the Guggenheim constant, k refers to the factor correcting properties of the multilayer molecules corresponding to the bulk liquid, and a_w = water activity.

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

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

Where N is the number of experimental points, M_{exp} is the experimental equilibrium moisture content value; M_{calc} is the calculated equilibrium moisture content value.

2.2.5. Mechanical properties: Fracturability test

Crust layers were fractured using a texture analyzer with a 5 kg load (TA XTplus, Stable Micro Systems, Surrey, UK). Experiments were carried out using a HDP/BS blade set at 5 mm/s. The maximum force (N), the area (N/s), and the displacement at fracture (mm) were measured. Twenty replicates of each crust layer were conducted.

2.2.6. Microstructure

Structural analysis was performed by scanning electron microscopy (SEM) on samples. Crust layers were freeze-dried previously to the microscopy analysis. Crust layers were fixed with the aid of colloidal silver and then coated with gold (Baltec SCD005) at 10^{-2} Pa and an ionization current of 40 mA. The observation was carried out in a JEOL JSM-5410 (Jeol, Tokyo, Japan) scanning electron microscope at 10 kV.

2.3.Statistical analysis

Data were presented as mean of sample sets. Statistical analysis of the results was performed using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). In order to assess significant differences among samples, a multiple sample comparison was performed.

3. RESULTS AND DISCUSSION

3.1.Moisture content

The model crust or layer crust showed moisture content (7.5 g/100g d.b.). The moisture content of the crust layers were significantly modified due to the presence of additives (P<0.05) (Table 2). The moisture contents ranged from 5.19 to 11.64 g/100g d.b. These values were in agreement with those reported by Cuq et al. (2003) for bread crust. Control sample and crust layer with gluten showed similar moisture content. The polymers (starch and gluten) present in its composition might form a molecular network or matrix with high interaction among them. Likely, the polarity of starch and gluten induced high affinity for water, which was easily integrated in its structure by establishing hydrogen bonds with the polymer molecules. Moisture content increased in the presence of HPMC 10%, glycerol 10% and 20%, which promoted the hydrophilic character of the crust. Hydrophilic plasticizer provides more active sites in layer matrix by exposing its hydroxyl group in which the water molecules could be adsorbed, which agrees with previous observations (Rosell and Foegeding, 2007). Conversely, crust layer containing glycerol 1%, HPMC 0.5%, citric acid, linolenic acid or beeswax presented lower moisture content than the control crust layer. Glycerol and HPMC incorporated in low quantity can have an anti-plasticizing effect in the food matrix, due to interaction of the plasticizer molecules with the starch and gluten, thus decreasing chain mobility (Rosell & Foegeding, 2007; Rosell, Yokoyama & Shoemaker, 2011). Moreover, these additives could compete with water molecules for active sites on the starch-protein matrix, which decreased the moisture content. With regards to linoleic acid and beeswax, these additives due to their hydrophobic nature decreased the water holding capacity of the matrix and thus moisture content. Citric acid could act as a crosslinker in the starch-gluten matrix giving rise to a more compact food matrix limiting its ability to retain water molecules (Olson, Hedenqvist, Johansson & Järnström, 2013).

Crust layers with lipids had the lowest moisture content, which might anticipate crispy crust considering the relationship between moisture content of bread crust with crispy texture (Primo-Martin et al., 2008; Altamirano-Fortoul et al., 2013).

3.2.Water vapor permeability

Thickness in the crust layers showed significant differences among treatment types, it varied from 0.24 mm to 0.58 mm, which agrees with previous studies (Altamirano-Fortoul, Hernando & Rosell, 2013). Water vapor permeability (WVP) of the crust layers showed significant differences, likely attributed to changes in the polymeric matrix due to additives (Table 3). The presence of additives could lead to a structure with or without pores and cracks modifying the permeability. Again, the highest WVP value was presented in the sample with greater glycerol concentration (20%), which acted as a plasticizer. This result agrees with Chillo et al. (2008) findings that indicated an increase in film WVP when increasing plasticizer concentration. At low concentration, glycerol has an anti-plasticizing effect due to the plasticizer-polymer interactions that decrease intermolecular spaces for the diffusion of water molecules through the crust. According to Mali, Karam, Pereira Ramos, and Grossmann (2004), glycerol concentration from 0 to 20% reduced the WVP in cassava starch films produced by casting, as glycerol addition led to a more compacted network without pores or cracks. Hirte et al. (2012) suggested that bread crust with many small cracks had optimal water vapor permeability; however, an excess of cracks could lead to crumb dryness.

Crust layers with HPMC presented the same WVP tendency as glycerol. Crust layer with high concentration of HPMC (10%) had higher water affinity due to the large amount of hydrophilic groups present in HPMC structure, and also it can disrupt starch-protein interactions forming a loose matrix, which favors water vapor permeability. When HPMC was added at low concentration (0.5%), it probably acted as a crosslinker establishing hydrogen bridges between starch and protein polymers, and reduced the number of active sites for water sorption.

Control crust layer and crust layer with gluten exhibited higher WVP, which could be ascribed to their hydrophilic nature. This result agrees with McHugh, Avena-Bustillos and Krochta (1993), who reported that films based on hydrophilic polymers like proteins or polysaccharides are very sensitive to moisture. Moisture sorption exerts a plasticizing effect on the biopolymer matrix increasing polymer free volume and chain mobility, thus facilitating the diffusion of water molecules across the crust.

Incorporation of protease in the crust formulation decreased WVP, which is likely due to the disruption of the crust layer as a consequence of the direct cleavage of the protein-starch structure. Probably, those structural modifications of the polymeric matrix led to a denser structure that hindered water molecules transference through the crust layer. In fact, Primo-Martin et al. (2006) when spraying protease on the surface of the dough, found changes in the crust characteristics that retarded the water content increase.

DATEM, an amphiphilic molecule, decreased the WVP, which could be attributed to its action decreasing interchain spacing between polymer chains promoting a structure with less pores/cracks. This result disagrees with the previous findings of Primo-Martín et al. (2006), who observed an increase of the porosity when adding DATEM. However, the function of DATEM as a crumb softening agent may also reduce water migration from gluten to starch by forming a complex with starch, and be absorbed into the starch surface (Pisesookbunterng & D'Appolonia, 1983).

Citric acid resulted in a decrease in the WVP value, which could be attributed to its crosslinking action, reducing the polymers mobility and increasing their cohesion. According to Moller, Grelier, Pardon and Coma (2004) the addition of a crosslinking agent as citric acid improves the barrier against water vapor.

As expected, crust layer with lipids (linolenic acid and beeswax) showed lower WVP, due to their hydrophobic properties (García, Martino & Zaritzky, 2000). Therefore, non-polar groups yielded a dense structure that slow water migration through the crust layer. Previous studies stated that waxes are the most efficient substances to reduce moisture permeability because of their high hydrophobicity (high content in long chain fatty alcohols and alkanes) (Morillon et al., 2002).

In general, the presence of additives modified the starch-protein matrix structure and moisture sorption properties, which resulted in changes in water vapor permeability of crusts layers. García et al. (2000) reported that WVP depends on many factors such as the ratio between crystalline and amorphous zone, polymeric chain mobility and specific interactions between the functional groups of the polymers in the amorphous zone. According to previous studies, restriction of water uptake by the crust and modification of the proteins in the crust are useful tools to maintain crispy texture in brittle and cellular foods as bread crust (Primo-Martin et al. 2006; Altamirano-Fortoul, et al. 2013).

3.3.Moisture sorption isotherms

Moisture sorption isotherms of bread crust incorporating different additives are shown in Figure 1. They exhibited sigmoid shape and three regions could be clearly differentiated. The first region of the curves represents strongly bound water including structural and monolayer water, which is unfreezable and not available as a plasticizer. In this region, the crust layers presented a moderately slight slope at low water activity, similar to the behavior observed for some gluten, starch and cellulose films (Hernández-Muñoz, Kanavouras, Perry, & Gavara, 2003; Al-Hassan & Norziah, 2012). In this stage the physical adhesion of water to active sites of the polymer occurs only in the surface, bound to the polar and hydrophilic groups of polysaccharides, proteins and other component of the film crust (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007).

The second region comprised a linear region of the sorption isotherm, where water molecules bind less firmly than in the first region and they are adsorbed as a multilayer. In this region there is a transition between bound water to free water. The equilibrium moisture content of crust layers increased slightly when increasing water activity up to 0.54, depending on the additive type. Crust layers containing beeswax, linolenic acid and glycerol 1% showed a reduction in the equilibrium moisture content, which confirmed that polarity of lipids affected the capacity to absorb water, and suggest that glycerol incorporated at low concentration (1%) competed with water molecules for hydrophilic sites on crust layer surface avoiding water binding in subsequent layers. Conversely, control and crust containing gluten showed pronounced rise in the equilibrium moisture content. These results could be related to the hydrophilic character of materials presents on polymeric matrix, which led to water mobility within the matrix structure. The third region corresponded to the upper part of the curve, where water molecules are associated to other water molecules forming clusters and loosely binds to food materials; in this region water properties of water molecules are similar to those of free water. For water activities higher than 0.6, all samples presented a rapid increase in moisture content that was attributed to the presence of non-bound or free state water that favored solubilization, which was reflected as swelling of the polymeric matrix. Greater water activities imply a substantial water uptake in the films due to the development of solvent-solvent interactions (Hernández-Muñoz et al., 2003).

Empirical, semi-empirical and theoretical mathematical models of moisture sorption isotherms have been proposed to describe the behavior of food products and other biological materials. The GAB model describes sigmoidal shape isotherms, and it is a refinement of Langmuir and BET theories of physical adsorption. The GAB, model similar to the BET model, describes the monolayer expression in Langmuir's adsorption isotherms and considers the multilayer sorption step. Related to the BET model, this model contains a third constant *k*, but conversely to the BET model it can be used in a wide range of water activities ($0.1 < a_w < 0.9$). GAB model provides an accurate description of moisture sorption of most food materials, thus, experimental data were fitted using this model. Estimated GAB parameters (W_m, C, and k) and root mean square (RMS %) for crust layer are shown in Table 4. Monolayer (W_m) values of control and crust layer with gluten and protease were similar and significantly higher than the ones obtained with other additives. It seemed that they had more active sites due to its hydrophilic group exposition, in which the water molecules can be adsorbed. The monolayer value represents the amount of moisture that is strongly adsorbed to specific sites at sample surface, and at this value the food product is more stable (Andrade, Lemus, & Pérez, 2011). Low values of W_m reflected a reduction in the number of primary active sites and it could be related to chemical, physical and structural modification of the polymeric matrix produced by the additives and the character of these. In this regard, incorporation of gluten, protease did not modifiers values of the monolayer; citric acid reduced the value of the monolayer, which could be related to the formation of physical and/or chemical cross-links with functional groups of starch and proteins, reducing the number of polar and hydrophilic sides for water sorption. It could happen a similar process when added HPMC, DATEM and glycerol. Incorporation of linolenic acid and beeswax reduced notably the monolayer value. The interaction between the polymeric matrix and the linolenic acid or beeswax probably led to an increase in the number of the hydrophobic particles that did not interact with water. Therefore, few active sites were accessible for water adsorption in the polymeric matrix due to the arrangement of the lipid chains.

The parameter *C*, the Guggenheim constant, represents the energy difference between the water molecules attached to primary sorption sites and those absorbed to successive sorption layers (Timmerman, Chirife, & Iglesias, 2001). According to previous studies in biomaterial water sorption isotherms, applying the GAB model, the values for this parameter were comprised between $5.67 \leq C \leq \infty$ (Lewicki, 1997). In the current study, *C* values ranged between 2.98 and 59.17.

The parameter *k* is related to difference in the sorbate's pure liquid state and in the upper layers (Timmerman et al., 2001). Theoretically, the values of *k* should be less than unity (Chirife, Timmermann, Iglesias, & Boquet, 1992). However, in the literature, a huge number of papers presented *k* values higher than unity. Values of parameter *k* obtained for the crust layer with or without additives were between 0.97 and 1.03. Lower value of *k* indicates less structured state of the sorbate in the layers above the monolayer than in the sorbate in the GAB layer. However, proteins and protein-foods present higher values of *k* than starchy foods (Timmermann et al., 2001; Hernández-Muñoz, Kanavouras, Lagaron, & Gavara, 2005).

Results showed that additives can modify the water affinity of the polymeric matrix and therefore the water sorption.

3.4. Mechanical properties

Any food's texture is mainly connected to its mechanical properties, which in principle reflect the internal food microstructure. It is well known that moisture content and water distribution have a strong effect on mechanical properties of brittle and cellular foods such as bread crust. The crispy texture is related to properties of the product such as hardness, brittleness and fracturability. Therefore, a crispy product must be stiff or brittle with a fast fracture (Van Vliet & Luyten, 1995). Results of mechanical properties of the crust layers are shown in Table 5. The flat structure of the crust model facilitated the assessment of the mechanical properties.

In this study the hardness term was used to describe a product which displays substantial resistance to breaking. As expected, the control sample presented high value of hardness due to its components, since the hydroxyl group of the polar macromolecules (proteins and starch) bound water via hydrogen bonds resulting a plasticized polymeric matrix.

Crust layer with protease presented higher hardness (maximum force) than the rest of the additives. The protease breaks the network and water interacted with the protein and released chains creating a more cohesive structure, very flexible and more difficult to fracture.

Presumably, other additives weakened the polymeric matrix, requiring less force for its fracture. Nevertheless, this effect depended on the additive, ie. glycerol (10 and 20%) or HPMC 10% and DATEM could have increased the mobility of polymer chains, due to water absorbed into the polymer, which made the crust layers somewhat flexible. Conversely, the presence of lipids, gluten, citric acid, glycerol 1% and HPMC 0.5% resulted in crust layers with lower hardness values. Incorporating lipids in the crust layer could interfere with interaction of polymers chains leading to a discontinuity within the protein-starch matrix. Furthermore, the lipids as beeswax exhibited low cohesiveness and structural integrity, which makes them very brittle. Gluten probably increased intermolecular forces along the polymer chain and this led to a decrease of the flexibility within the polymeric matrix structure. Inclusion of citric acid into the polymeric matrix led to a decrease of hardness. This fact might be attributed to a reduction in the molecules mobility and a decrease in the absorbed water promoted by the crosslinking effect of the citric acid, resulting in a rigid crust layer (Olson et al., 2013). When HPMC 0.5% was incorporated, probably intermolecular associations among the polymer chains were inhibited by the HPMC and the crust layer had a stiff structure, which required low force to fracture.

Fracturability is an important characteristic of brittle products. In general, the fracturability of crust layers decreased with addition of the additives (Table 5), with the exception of protease and glycerol 20%. Therefore, additives might modify the polymeric matrix affecting fracturability. According to Primo-Martín et al. (2006) the distribution of protein and partially gelatinized starch in the bread crust as well as the water content alters the way the crust fractures. With respect to area value, control crust and that with protease had much higher area, indicating their increased toughness, thus these needed high work to fracture the crust layers.

The presence of additives in the model crust modified the water uptake and also the mechanical properties related to crispy texture, and the most prominent effect was observed with protease and lipids.

3.5. Microstructure

Microstructure of the cross section of crust models was analyzed to explain the effect of additives on the mechanical properties and water vapor permeability behavior. SEM micrographs confirmed microstructure differences promoting by additives (Figure 2). Control crust layer showed a continuous veil-like film that revealed the underlying structures, lenticular shape and circular starch granules of various sizes, likely surrounded by protein matrix (Figure 2a), like it has been described for bread structure (Rojas et al. 2001). The effect of additives in the crust layers was evident. The crust layer with gluten revealed large starch granules and some small slightly deformed starch granules embedded completely in the protein network (Figure 2b). Likely, this structure might result from the covalent bonds as well as non-covalent interactions between gluten proteins and starch. Thus, this allows a significant change in molecular motion of proteins; and thus crust layer presented less resistance to break in spite of its capacity for water diffusion.

Crust layer with protease was characterized by compact structure, with higher deformation of starch granules and a more distorted gluten network (Figure 2c), which agrees with the protease action splitting the protein strands of the gluten molecule that leads first to a softening and then to a complete collapse of the structure. The crust layer with HPMC 0.5% led to a smooth, compact and cracked structure (Figure 2d). Conversely, crust layer with HPMC 10% presented irregular starch granules within a disrupted and discontinuous protein network (Figure 2e). Therefore, HPMC could be integrated in the molecular structure of the layer or formed a biphasic system leading stiff structure depending on the addition level. These results agree with those observed in hardness and fracturability parameters, and WVP. The structures containing glycerol were significantly different and the extent of the changes was dependent on the glycerol concentration. Crust layer with glycerol 1% revealed a structure masked by a continuous gel and relatively smooth with obvious cracks as well as holes formation (Figure 2f), suggesting a brittle fracture. Altamirano-Fortoul et al. (2013) suggested that a cracking structure gives brittle bread crust behavior. The opposite effect was observed in crust layer with glycerol 10%, where a compact and heterogeneous microstructure was observed (Figure 2g). While the addition of glycerol 20% led to crust layer with greater force to fracture as result of an apparent swelling of starch granules with distorted structure and embedded in a protein network (Figure 2h). The addition of plasticizers as glycerol produced a more flexible film with soft structure due to hydrophillicity of plasticizers molecules, which favors the sorption of water.

Crust layer with DATEM exhibited a structure where starch granules lost their identity and were covered with alternate continuous veil-like film and some cracks (Figure 2i). Crust layer with citric acid was similar to sample containing DATEM, with alternate continuous zones, besides a polymeric matrix with areas of protein aggregates (Figure 2j). This pattern might be attributed to the crosslinking action that led to less flexible and brittle layer supporting hardness and fracturability results. The citric acid promotes fragmentation of starch granules and also causes disruption of the bridges of inter and intramolecular hydrogen, leading to a matrix with homogeneous appearance (Olson et al., 2013).

In the case of samples with lipids, crust layers presented a smooth and nonporous structure, and no phase separation was observed (Figure 2k and 2l). In fact, an almost continuous structure with aligned constituents could be appreciated, readily evident in the crust layer with linolenic acid. The crust layer containing beeswax showed no individual crystals.

In general, the composition of the crust layer could influence strongly the molecular level of the microstructure and, therefore, its mechanical and in some extent moisture barrier behavior.

CONCLUSIONS

The crust model (crust layer) was a good approach to understand bread mechanical properties and microstructure. Crust layers were significantly affected by the additives. Sorption isotherms indicated that additives modified the water uptake. In general an increase in monolayer value (W_m) was observed when gluten and protease were added. However, lipids (linolenic acid and beeswax) promoted few active sites, decreasing the monolayer values in comparison with control sample and the rest of the samples. Therefore, crust layer with lipids provides a barrier. In relation to mechanical properties, control sample and crust layers with greater glycerol concentration showed resistance to fracture; these mainly due to the amount of water present into the polymeric matrix. Opposite effect was observed with the crust layers with lipids, which indicated brittle or stiff products. Thus, crispy texture was correlated closely with water present as well as with composition of crust layer. Therefore, considering all results, crust layer with HPMC 0.5% as well as with citric acid would be the best alternative additives to be used for changing the crispiness behavior of bread crust. SEM analysis also confirmed the effect of the additives.

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FIGURE CAPTIONS:

Figure 1. Effect of different additives of equilibrium moisture content of crust layer at 20°C fitted with the GAB model. Control crust layer:▲, control crust layer-GAB ,crust layer with gluten **x**, crust layer with gluten-GAB , crust layer with protease **O**, crust layer with protease-GAB , crust layer with HPMC 0.5% ◊, crust layer with HPMC 0.5%-GAB , crust layer with DATEM □, crust layer with DATEM-GAB , crust layer with glycerol 1% ●, crust layer with glycerol 1%-GAB , crust layer with citric acid +, crust layer with citric acid-GAB , crust layer with linolenic acid ♦, crust layer with linolenic acid-GAB , crust layer with beeswax ■, crust layer with beeswax-GAB .

Figure 2. Scanning electron micrographs of crust layer cross section at high (3500x) magnification. Images correspond to the following crust layer with additives: control crust layer (a), crust layer with gluten (b), crust layer with protease (c), crust layer with HPMC 0.5% (d), crust layer with HMPC 10% (e), crust layer with glycerol 1% (f), crust layer with glycerol 10% (g), crust layer with glycerol 20% (h),crust layer with DATEM (i), crust layer with citric acid (j), crust layer with linolenic acid (k), crust layer with beeswax (l),

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

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

Means and standard deviations sharing the same letter within a column were not significantly different ($P < 0.05$).

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

Sample	WVP **(g•mm/m²•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

Means and standard deviations sharing the same letter within a column were not significantly different ($P < 0.05$).

Table 4. Estimated parameters from the GAB model.

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

Means and standard deviations sharing the same letter within a column were not significantly different ($P < 0.05$).

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

Means and standard deviations sharing the same letter within a column were not significantly different ($P < 0.05$).

Figure 1.

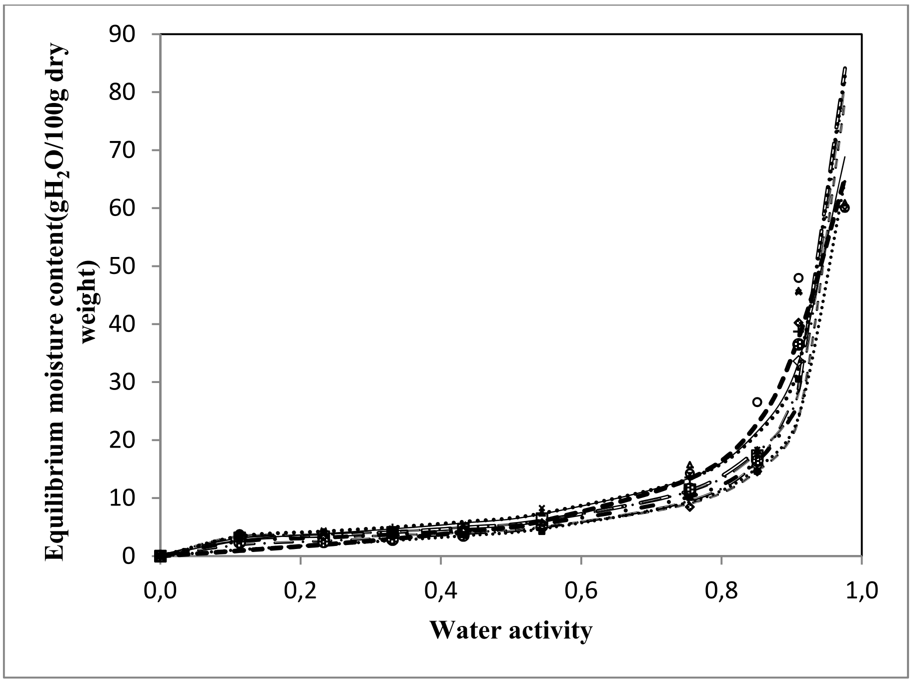
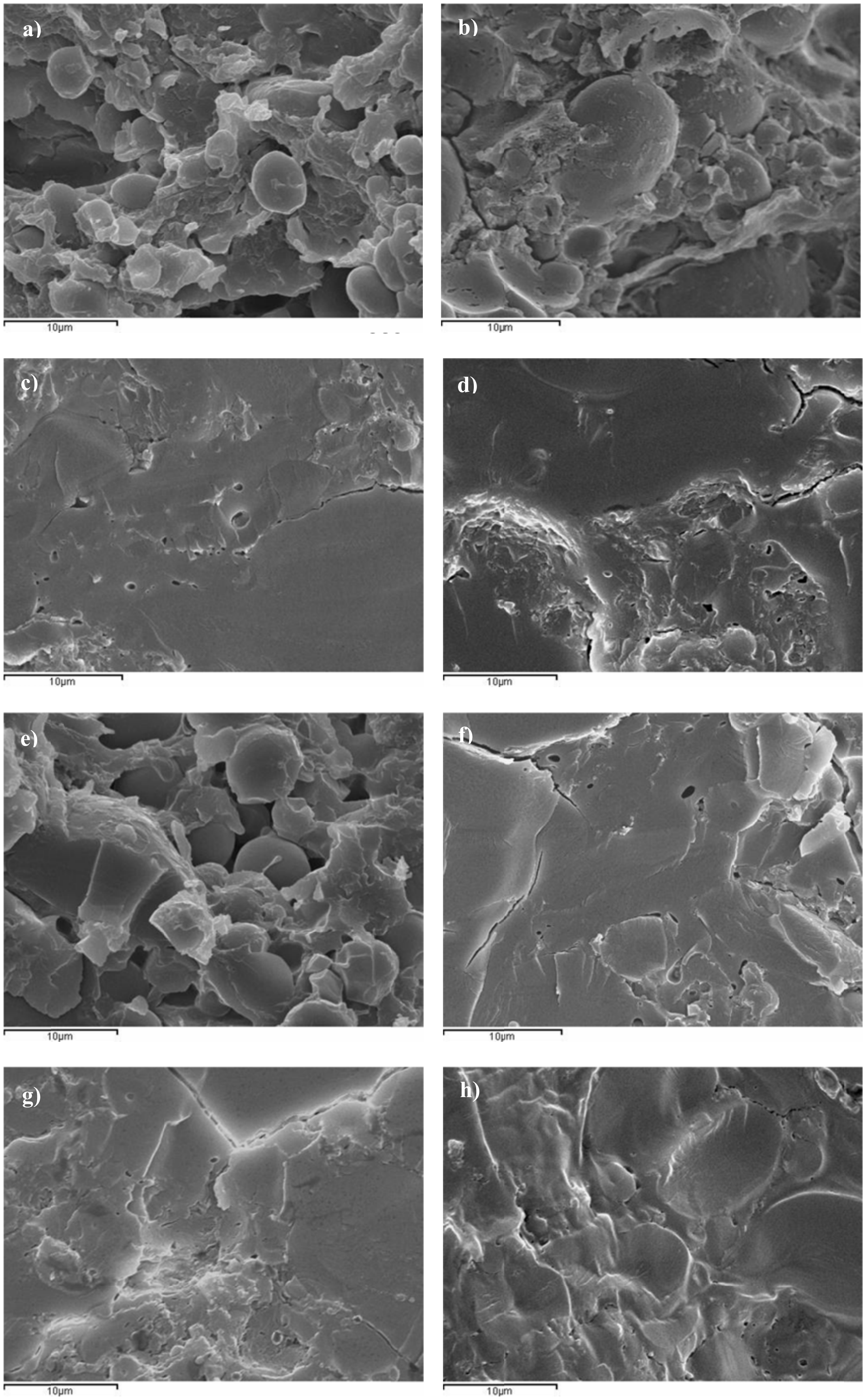
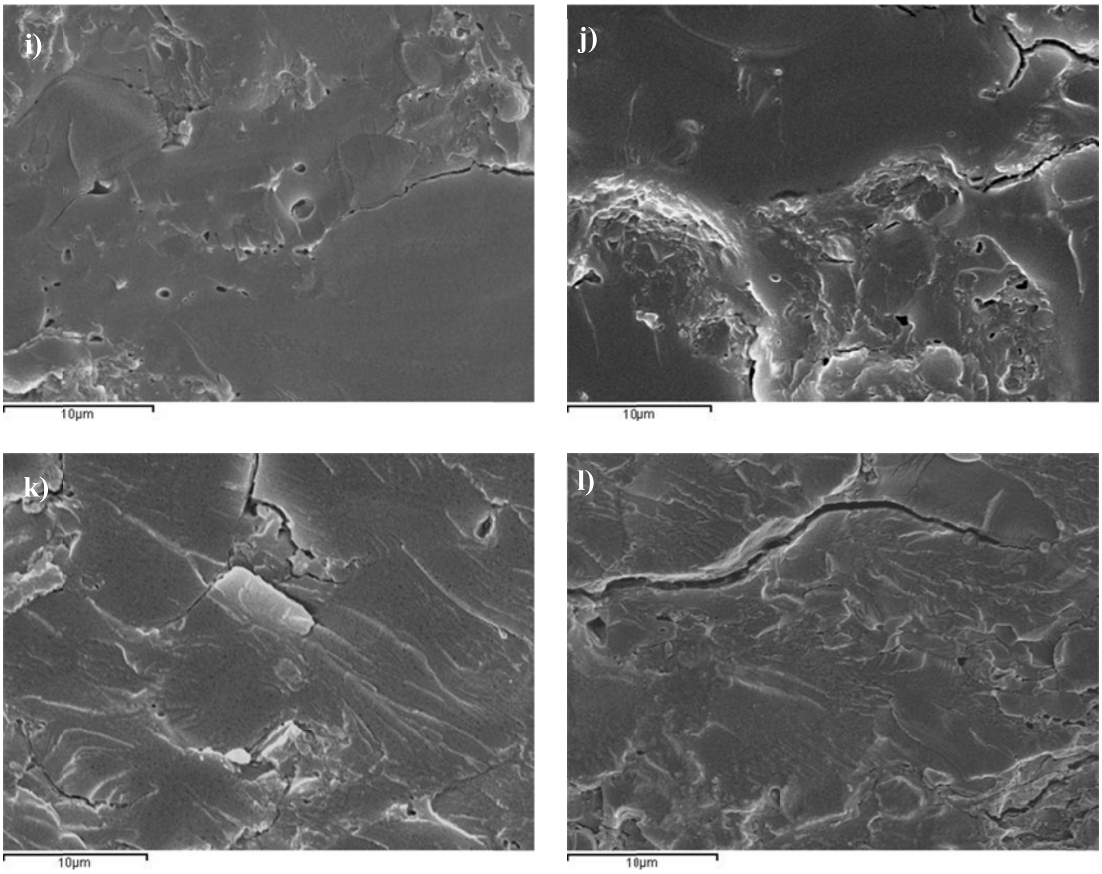

Equilibrium moisture content(gH2O/100g dry weight)
90
80
70
60
50
40
30
20
10
0
0,0
0,2
0,4
0,6
0,8
1,0
Water activity

Figure 2.




i)
10µm
j)
10µm
k)
10µm
l)
10µm