UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Modulation of the physico-chemical and functional properties of bread by applying surface treatments

Modulación de las propiedades fisico-químicas o funcionales del pan mediante la aplicación de tratamientos en superficie

Tesis doctoral

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Valencia, octubre de 2013

A mi familia con mucho amor y cariño les dedico todo mi esfuerzo y trabajo puesto para la realización de esta tesis

AGRADECIMIENTOS/ ACKNOWLEDGEMENTS

Esta investigación ha sido posible gracias a la financiación procedente del Consejo Superior de Investigaciones Científicas (CSIC), el Ministerio de Economía y Competitividad (AGL2011-23802), la Generalitat Valenciana (Proyecto Grupo de Excelencia Prometeo 2012/064), Fondos Europeos para el Desarrollo (FEDER) y a la beca predoctoral JAEPredoc (CSIC).

Agradezco también al Instituto de Agroquímica y Tecnología de alimentos por los recursos proporcionados para la realización de esta investigación.

A la empresa Forns Valencians S.A, por suministrar los panes precocidos utilizados en gran parte de esta investigación.

Quisiera agradecer a la Dra. Cristina Molina Rosell, directora de esta tesis, por todo el tiempo dedicado a la realización de esta investigación, por el apoyo en todo momento, por la paciencia y por todas las enseñanzas.

De la misma forma quiero expresar mi gratitud a la Dra. Concepción Collar y Dra. Mónica Haros, así como a las técnicos Consuelo, Fina, Encarna y Elvira. Por sus enseñanzas y porque siempre estuvieron dispuestas a ayudarme.

A la Dra. Isabel Hernando, tutora académica de la UPV; por su apoyo, dedicación y confianza.

I would like to thank Dr. Alain Le Bail from the École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation University, for having me in your lab.

Special thanks to Dr. Wioletta Blasczak and Prof. Józef Fornal from the Department of Chemical and Physical Properties of Food, Institute of Animal Reproduction and Food Research in Poland. Thanks for having me in your lab.

To Prof. Maria Soral-Smietana, Dr.Gosia, Dr.Ula and Natalia of Functional Properties of Food at the Institute of Animal Reproduction and Food Research in Poland. Thanks for helping in my first stay in Olsztyn.

A Dios nuestro señor, por brindarme la fuerza necesaria para salir adelante, ya que después de muchos tropiezos y de estar a punto de renunciar a todo, él nunca me abandonó y por haber puesto en mi camino a aquellas personas que han sido mi soporte y compañía durante todo este tiempo.

Muy especialmente quiero agradecer a mis padres, a pesar de no estar presentes físicamente, sin su apoyo, colaboración e inspiración habría sido imposible haber llegado hasta aquí. Estoy infinitamente agradecida con ustedes, los amo.

A mi esposo por su incondicional apoyo mantenido a través de estos años. Por compartir conmigo cada momento vivido durante esta tesis.

A mi pequeña hija Gemma Guadalupe. Su nacimiento ha coincidido con el final te la Tesis. Ella con su luz ha iluminado mi vida y hace mi camino mas claro.

A mi hermano y sobrina, porque a pesar de la distancia, el ánimo, apoyo y alegría que me brindan me dan la fortaleza necesaria para seguir adelante.

A Laura Laguna y Eleonora Harries, compañeras de los cursos de doctorado y grandes amigas, gracias por su apoyo tanto en el plano profesional como personal. Las echaré de menos, pero siempre las llevaré en mi corazón.

A la Dra. María Eugenia Bárcenas, profesora de mi alma máter (Universidad de las Americas-Puebla) por encaminarme en el mundo de la investigación.

A mí querida compañera y amiga Raquel Garzón, gracias por tu apoyo durante este tiempo, especialmente en la recta final de este largo camino.

A todos y cada uno de mis compañeros que han pasado por el grupo de cereales: Eva, Alessandro, Pablo, Juan Mario, Marcia, Myriam y María Estela, y a los que aún siguen en este grupo: Dulce, Martha, Ade, Carolina, Ángela, Izaskun y Esther. Por todos los buenos momentos que pasamos en el laboratorio, y por su gran apoyo.

Aquellos amigos que han compartido conmigo los altos y bajos en el plano personal durante este tiempo; Rosa, Quique, Nancy e Inma, les agradezco el haberme brindado todo el apoyo, ánimo, cariño y amistad.

En general quisiera agradecer a todas y cada una de las personas que han vivido conmigo la realización de esta tesis doctoral.

"Las ideas son como las estrellas, no llegarás a tocarlas con las manos, pero como el marinero en el desierto de las aguas, las eliges como guía y si las sigues alcanzarás tu destino".

Carl Schurz

RESUMEN

El pan es un alimento de gran consumo desde la antigüedad, obtenido de un proceso de panificación dinámico. Una de las innovaciones de mayor éxito en panificación ha sido el pan parcialmente cocido obtenido por la tecnología de panificación interrumpida que proporciona pan crujiente en cualquier momento del día. La crujibilidad es el atributo más demandado por los consumidores en el pan crujiente fresco. Desafortunadamente, la crujibilidad es percibida por corto tiempo después del horneado y la pérdida de ésta es uno de los atributos que causa rechazo en los consumidores. El objetivo de esta investigación fue la modulación de las propiedades físico-químicas y funcionales del pan mediante la aplicación de tratamientos de superficie con el fin de comprender las características de la corteza y el desarrollo de productos de pan con un valor agregado.

Para abordar dicho objetivo se han realizado estudios para determinar el impacto de la adición de vapor, durante el horneado, sobre las propiedades mecánicas de la corteza y para evaluar la influencia de estas propiedades en los parámetros de calidad utilizando diferentes panes comerciales. Además se han realizado estudios para modular las propiedades de la corteza dirigidos a entender su microestructura y modificarla mediante tratamientos enzimáticos con amiloglucosidasa o con un recubrimiento

funcional, con la finalidad de modificar la permeabilidad de la corteza u obtener panes probióticos.

Los resultados mostraron que la cantidad de vapor utilizada durante la cocción (100, 200 y 400 ml) modificaron las propiedades físico-químicas y mecánicas así como la estructura de la corteza del pan. Los parámetros de calidad permitieron la diferenciación de variedades de pan parcialmente cocido, específicamente las propiedades mecánicas de la corteza junto con el volumen específico, dureza y estructura de la miga.

Sin embargo, la textura de la corteza de pan fue significativamente dependiente de las condiciones de ensayo (velocidad y sección de la sonda). Los resultados mostraron que la velocidad más baja (0,5 mm /s) dio información acerca de la estructura celular de la corteza relacionada con la textura crujiente. Por otra parte, la estructura celular fue modificada por la amiloglucosidasa pulverizada sobre la superficie del pan antes de la cocción, lo que condujo a una disminución en el contenido de agua y actividad del agua de la corteza, lo cual se requiere para extender crujibilidad. La aplicación de recubrimientos comestibles con *L. acidophilus* microencapsulado sobre la superficie pan (disperso o en multicapa) garantizó la supervivencia del microorganismo después del tiempo de cocción y almacenamiento, a pesar de que estos disminuyeron la fuerza de fractura y actividad de

agua de la corteza. El análisis de microestructura demostró la presencia de microcápsulas dispersas en la corteza de pan. Por lo tanto, *L. acidophilus* incluido en microcápsulas pueden ser incorporado en la superficie de pan a través de recubrimientos comestibles, abriendo la posibilidad de obtener panes funcionales.

RESUM

El pa és un aliment de gran consum des de l'antiguitat, obtingut d'un procés de panificació dinàmic. Una de les innovacions de major èxit en panificació ha estat el pa parcialment cuit obtingut per la tecnologia de panificació interrompuda que proporciona pa cruixent en qualsevol moment del dia. El caràcter cruixent és l'atribut més demandat pels consumidors en el pa cruixent fresc. Desafortunadament, el caràcter cruixent és percebuda per curt temps després del fornejat i la pèrdua d'aquesta és un dels atributs que causa rebuig en els consumidors. L'objectiu d'aquesta investigació va ser la modulació de les propietats físic-químiques i funcionals del pa mitjançant l'aplicació de tractaments de superfície amb la finalitat de comprendre les característiques de l'escorça i el desenvolupament de productes de pa amb un valor agregat. Per a abordar dit objectiu s'han realitzat estudis per a determinar l'impacte de l'addició de vapor, durant el fornejat, sobre les propietats mecàniques de l'escorça i per a avaluar la influència d'aquestes propietats en els paràmetres de qualitat utilitzant diferents pans comercials. A més s'han realitzat estudis per a modular les propietats de l'escorça dirigits a entendre la seva microestructura i modificar-la mitjançant tractaments enzimàtics amb amiloglucosidasa o amb un recobriment funcional, amb la finalitat de modificar la permeabilitat de l'escorça o obtenir pans probiòtics. Els resultats van mostrar

que la quantitat de vapor utilitzada durant la cocció (100, 200 i 400 ml) van modificar les propietats físic-químiques i mecàniques així com l'estructura de l'escorça del pa. Els paràmetres de qualitat van permetre la diferenciació de varietats de pa parcialment cuit, específicament les propietats mecàniques de l'escorça juntament amb el volum específic, duresa i estructura de la molla. No obstant això, la teixidura de l'escorça de pa va ser significativament depenent de les condicions d'assaig (velocitat i secció de la sonda). Els resultats van mostrar que la velocitat més baixa (0,5 mm /s) va donar informació sobre l'estructura cel·lular de l'escorça relacionada amb la teixidura cruixent. Per altra banda, l'estructura cel·lular va ser modificada per la amiloglucosidasa polvoritzada sobre la superfície del pa abans de la cocció, el que va conduir a una disminució en el contingut d'aigua i activitat de l'aigua de l'escorça, la qual cosa es requereix per a estendre el caràcter cruixent. L'aplicació de recobriments comestibles amb *L. acidophilus* microencapsulat sobre la superfície pa (dispers o en multicapa) va garantir la supervivència del microorganisme després del temps de cocció i emmagatzematge, a pesar que aquests van disminuir la força de fractura i activitat d'aigua de l'escorça. L'anàlisi de microestructura va demostrar la presència de microcapsulas disperses en l'escorça de pa. Per tant, *L. acidophilus* inclòs en microcàpsules pot ser incorporat en la superfície de pa a través de recobriments comestibles, obrint la possibilitat d'obtenir pans funcionals.

ABSTRACT

Bread is an ancient widely consumed food resulting from a dynamic breadmaking process. One of the most successful innovations in breadmaking has been the partially baked bread obtained by the bake off technology which provides crispy bread at any time of the day. Crispness is the most consumers' demanded attribute in fresh crispy bread. Unfortunately, crispness is rapidly lost after baking and then bread is rejected by consumers. The objective of this research was the modulation of the physico-chemical and functional properties of bread by applying surface treatments in order to understand crust features and to develop value-added bread products. Studies have been conducted to identify the impact of steaming during baking on the crust mechanical properties and to assess the weight of crust mechanical properties on the overall quality parameters using different commercial breads. Further on studies were focused on modulate crust properties by understanding its microstructure and modify it by enzymatic treatments with amyloglucosidase or functional coating to modify the crust permeability or to obtain probiotic breads, respectively.

Results showed that the amount of steaming used during baking (100, 200 and 400 ml) modified the physico-chemical and mechanical properties as well as structure of bread crust. Quality parameters allowed the differentiation of bread type, specifically crust mechanical properties together with specific volume, crumb hardness and structure. Nevertheless, texture of the bread crust was significantly dependent on the tests conditions (speed and punch cross-section). Results showed that low speed (0.5 mm/s) gave information about the cellular structure of the crust related to crispness texture. Moreover, that cellular structure was modified by amyloglucosidase sprayed on the bread surface prior to the bake off technology, leading to a decrease in the moisture content and water activity of the crust, which is required for extending the crispness shelf life. The application of edible coating with microencapsulated *L. acidophilus* onto the bread surface (dispersed or multilayer) guaranteed microorganisms survival after baking and storage time, although they reduced failure force and water activity of the crust. Microstructure analysis showed the presence of scattered microcapsules onto the bread crust. Therefore, *L. acidophilus* included in microcapsules can be incorporated to bread surface through edible coatings, leading a prospect future for obtaining a functional bread.

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Introduction

INTRODUCTION

Bread: Importance and consumption

Bread is considered to be one of the oldest processed foods. The first report about bread appeared around 10,000 years BC or over 12,000 years in the past, where this product was developed by deliberate experimentation with water and grain flour. Egyptians were the pioneers in extended the art of bread from the Nile to all parts of Europe, and thereafter bread production was seen as valuable tool since it was used as a means of exercising political influence (Mondal and Datta, 2008). According to Spanish Real Decreto 1137/84 (1984), bread is the product resulting from the cooking of dough obtained by mixing flour and water, with or without addition of edible salt and fermented by microorganism species from bread fermentation.

Nowadays, bread is an important staple food in both developed and developing countries; since it has been an essential element of human diets because it constitutes important source of complex carbohydrates, proteins, minerals and vitamins (Rosell, 2007). Bread is consumed in large quantities in the world in different types and forms depending on cultural habits and consumers demands. For example, there is a wide diversity of leavened breads, such as crusty bread (baguette, rustic and ciabatta), pan bread, partially baked bread, and fiber-enriched bread.

Nevertheless, during a long time the increased consumption of bread in the world has been almost parallel to the growth of the world population. But from the twentieth century, there has been a significant decrease consumption, which does not comply with the recommendations of the World Health Organization (WHO) that recommends the daily intake of 250 g of bread per day per person (Serra-Majem and Quintana, 2010).

The European bread industry produces 25 million tons of bread per year (Baking+Biscuit International, 2011). Germany is the largest producer of bread within the European market, followed by the UK, France, Italy, and Spain. The average consumption of bread in Europe at present is 65 kg per capita per year (GIRA, 2008).

In Spain, there are also many different and popular forms of bread. However, as a result of the strong impact of European eating habits, the classic baguette can be found all over the country. Although bread is a mainstay of the Mediterranean diet, during the period 2007-2011 its consumption has fallen to 7.2 kg/person (CEOPAN, 2011). In spite of this, the Spanish Ministry of Agriculture, Food and Environment reported that during the last year, bread consumption in households increased by 1.2% (MAGRAMA, 2013).

In Europe, bread produced by the “bake off technology” (BOT) is growing very rapidly (10% a year). Likewise, Spain is one of the countries that increasingly consume the bread obtained by this technology (Research and Innovation, 2012). Therefore, until 2014, the bread market has experienced a polarized growth: a premium segment including added value products, healthy varieties or other special breads (Baking+Biscuit International, 2011).

Breadmaking process: formation of bread crumb and crust

Despite the many different types of breads worldwide, the basis of the bread making is common. Bread is the resulting product of a dynamic process in which mechanical as well as biochemical changes are involved leading a soft and light product. During breadmaking, several chemical, biochemical and physical transformation occur, which affect the final product. Breadmaking stages include mixing the ingredients, dough resting, dividing and shaping, proofing and baking, with some variations depending on the type of bread.

Mixing

This stage comprises the homogenization of the ingredients (water, flour and others), and some sub-processes that have a direct consequence on the quality of the end product.

1. Uniform mixing, dissolution and hydration of ingredients, particularly flour proteins (Cauvain,

2012). Hydration is very important because wheat gluten protein pass through their glass transition phase, inducing an increase in protein molecular chain mobility. In the same way, grain hydration involves the swelling of starch granules and glass transition for amorphous molecular organizations (Cuq et al., 2003).

2. The development of a gluten structure in the dough is the result of the distribution of materials, hydration of flour protein and input of mechanical energy (Cauvain, 2012). This input of mechanical energy is responsible for distributing particles and components, inducing interaction between proteins and, finally, leads to the formation of a continuous macromolecular viscoelastic structure (Cuq et al., 2003; Sigh and MacRitchie, 2001). Most of the studies on doughs have been on the mixing, because of the rheological changes that occur in the gluten viscoelastic network during this sub-process (Dobraszczyk and Morgenstern, 2003; Rosell et al., 2013).
3. The incorporation of air bubbles within the dough to provide the gas bubble nuclei for the carbon dioxide that will be generated by yeast fermentation and oxygen for oxidation and yeast activity. During the nucleation, there are numerous changes in the solid phase such as development and fixing of the gas cells,

which will permit to retain the gas cell structure in the final product (Hayman et al., 1998). However, this dependens on the processing conditions employed; for example, a higher pressure in the mixer headspace during mixing leads to more bubbles (Cauvain and Young, 2007; Campbell and Shah, 1999). On the other hand, some authors suggest that good quality bread should have a high porosity with a regular gas cell structure in the crumb (Hayman et al., 1998; van Vliet, 1999).

Dough resting

After mixing, the dough pieces are usually rested for 10-30 minutes. Dough resting helps to water penetration into dough particles evenly, resulting in smoother structure. Besides, during this step, the gluten structure relaxes (Cauvain, 2012).

Dividing and shaping

During this stage, the bulk of the dough is divided into small pieces according to the final weight of the bread. Dividing is a critical process because when cutting portions of dough, is necessary to preserve the precise weight, gluten structure and gas retention. Also, shaping plays an important role in this stage. During this step is essential to provide minimum pressure and stress on the dough.

Proofing

Proofing is carried out under temperature and humidity control to avoid that the mass undergoes a dehydration, which would eventually affect product quality. During proofing, dough is relaxing while it ferments and expands. In this stage, the yeast consumes sugars and generates carbon dioxide, causing the dough doubles in volume. The expansion of the air bubbles previously incorporated during mixing provides the characteristic aerated structure of bread, which is relevant to its appeal (Cauvain, 2012). Coalescence of gas cell, due to rupture of inter-cell dough, is the main instability mechanism towards the end of proofing. Therefore, extensive coalescence as well as an increasing proof time gives rise to an irregular and coarse crumb structure (Zghal et al., 2002; Hayman et al., 1998).

Baking

Baking is the last stage but the most important in bread making. Baking conditions (rate and amount of heat application, the humidity level in a baking chamber and the baking time) are a significant influence on the final product quality. During baking, dough undergoes physical, chemical and biochemical transformations, leading the formation of crumb and crust. Several conversion activities take place during baking such as evaporation of water, formation of porous structure, starch gelatinization, protein denaturation, melting of fat crystals, volume expansion and browning reaction.

As mentioned above, the temperature determines the quality and shelf life of the bread products. During baking, the dough surface is exposed to high temperatures (> 200 °C) and quick dehydration of the dough surface will take place (Cuq et al., 2003). The temperature rise is sufficient to induce protein denaturation and starch gelatinization, which affect the water diffusion by releasing and absorbing water, contributing to the transformation from dough to crumb (Mondal and Datta, 2008).

On the other hand, it is important to consider that heat transport induces water transport and water distribution in two directions, towards the center of the product and towards the surface. In the surface, water evaporates from the warmer region, absorbing latent heat of vaporization and the surface layers start to dry. Due to diffusive flow of liquid water from the core is less rapid than evaporation flow at the surface, a drying zone is developed, which gradually intensifies its thickness and forms the crust (Vanin et al., 2009; Wagner et al., 2007; Therdthai et al., 2002; Zanoni et al., 1994). The loss of water on crust is quick, because of that the starch is not completely gelatinized, which suggest that bread crust is a network of gluten protein with starch granules embedded

(Vanin et al., 2009; Primo-Martín et al., 2007; van Nieuwenhuijzen et al., 2008).

Furthermore, surface temperature conditions, give place to different kinds of reactions such as dextrinization, caramelization, and non-enzymatic browning that lead to irreversible changes. These reactions result in the formation of a brown colour on the crust and aromatic substances that give the typical aroma of fresh baked bread (Poinot et al., 2008; Ahrné et al., 2007; Wählby and Skjöldebrand, 2002; Zanoni et al., 1995).

Besides, some products like crispy bread require the addition of steam before baking to increase the volume and make the crust crispy. Steaming injection reduce the total water at the onset of baking, causing modification in drying rate of the crust and therefore the elasticity (Le Bail et al., 2011). Some authors have studied the incorporation of steam injection; they found that steaming affects the thickness of the upper crust and the cellular structure as well as fracture behavior (Besbes et al., 2013a; Le Bail et al., 2011; Schirmer et al., 2011).

Breadmaking processes: bake off technology

Traditional baking processes are sometimes too limited and inflexible to fully satisfy manufacturer’s requirements and consumer's demands. One of the most significant technological developments in the bakery sector in the last few decades is the bake off technology applied to frozen or low temperature stored products allowing a continuous growth for easily prepared bakery items. Low temperature technology has been initially applied to bakery products to solve the economical losses associated with the bread staling problem that produces a decrease of consumer acceptance (Hebeda et al., 1990).

The partially baked bread (part-baked, part baked bread or pre baked bread) is produced in large plant bakeries following the conventional process, with the exception of baking. This technology is also called bake off technology (BOT) and it has proved itself as a good method for preventing the staling process and obtaining a product at any time of the day whose quality is close to that of fresh bread. Since bake off technology does not require highly trained people, it saves costs and ensures a product of uniform quality at any time. The partial baking or interrupted baking method consists in baking the bread dough untill the structure is fixed, giving a product with structured crumb but without a crispy crust. In the retail bakery, the partially baked bread only requires a very short baking time to generate the crust and to release an attractive flavor. The market of partially baked bread is rapidly growing because the product is already sized, shaped and partially baked. Studies conducted on the partially baked bread have been focused on assessing the effect of proofing (Le Bail et al., 2005), partial baking (Fik and Surowka, 2002; Bárcenas et al., 2003), chilling and freezing conditions (Bárcenas and Rosell, 2006a, b) on bread quality. Optimal time for the initial partial baking lies within the range from 74 to 86% of the time needed for the full-baking in conventional breadmaking (Fik and Surowka, 2002). Par-baked frozen bread is suited to produce crusty bread. Nevertheless, no attempt has been made to develop innovative and added value products applying the bake off technology.

Differences between bread crumb and crust

There have been various attempts to define bread crumb and crust. These definitions are based on physical structure although both parts come from the same dough, but their final properties differ according to a distinct local heat-moisture treatment.

Bread crumb is defined as anisotropic, open-cell solid, with a wide range of cell sizes, exhibiting a hierarchical organization of structure in both void and solid phase (Liu and Scanlon, 2003). Whereas Loiste et al. (2004) understand the crumb as structure with viscous behavior. Crumb quality is dependent on several rheological and physical properties evaluated using different parameters. Furthermore, as mentioned previously, its properties are affected by breadmaking process giving place to grand diversity of breads.

In the case of bread crust, different concepts have been used for its definition. It has been described as dryer, harder, darker and denser (Hug-Iten et al., 2003; Vanin et al., 2009). Nevertheless, other authors preferred to define it as a part of bread near the surface (Wählby and Skjöldebrand, 2002; Jefferson et al., 2006; Purlis and Salvadori, 2009). Della Valle (2012) and Primo-Martín et al. (2009) described crust as compact and external layer. In spite of the existence of a great variety of definitions, crust has been considered an important factor for the bread quality assessment.

As mentioned above, breadmaking process is responsible of the crumb specific properties that distinguish it from the crust. For example, crust presents an insufficient starch gelatinization, and it is in a glassy state that differs from the rubbery state of the crumb. Also, water content of bread crumb and crust are significantly different. During cooling, the crust shows very low water content (between 3 and 7%), which contrasted with the high water content of the crumb (between 35 and 40%), responsible of its smooth texture (Cuq et al., 2003).

Bread crust

Importance of bread crust to consumers

Consumers are attracted by bread that is freshly baked, because its quality is easily recognized in terms of appealing aroma, crispy crust and moist, soft crumb. However, the perception of fresh bread may be influenced by social, demographic and product experiences.

With the purpose of better understand the attitude of the European innovations in bread, Lambert et al. (2009) carried out a survey of consumers considering five countries (Belgium, Croatia, Spain, France and Poland). They reported two main groups, which were defined as (1) frequent (daily) buyers with a focus on quality and pleasure and (2) less frequent buyers (once a week) with a more pronounced interest in nutrition, shelf life and energy (process). The first group was named the “crust group” and the second one the “crumb group.” Therefore as we can see, crust and crumb can even divide consumer attitudes.

Consumer habits have undergone great changes, motivated by the new social lifestyles, which have promoted the increase in the consumption of crispy bread. Moreover, is considered that about one third of the dry mass of bread is located in the crust, which means that the nutritional value, quality and taste are widely associated with the crust (Le Bail et al., 2005).

Moreover, for the consumer's appreciation, crispness character of the crust in crispy bread is an important sensory characteristic for the purchasing attitude of this type of product. In fact, the tactile, visual and auditory sensations help people to verify whether this characteristic is as expected.

Crispness is one of the key textural attributes of interest, and appears to be the most versatile and universally liked texture characteristic. It is really a complex attribute resulting from multiple sensations that involves numerous physical parameters, combining molecular, structural and manufacturing process as well as storage conditions (Primo-Martin et al., 2006; Roudaut et al., 2002). Nevertheless, all baked products have a very small shelf life and their quality is highly dependent on the period of time between baking and consumption (Bárcenas et al., 2003).

Effect of storage in bread crust

The bread quality is quickly lost due to staling that begins when the bread pieces are taken out from the oven and persist along the storage. Staling is a very complex multistage dynamic process affected by many factors and involving multiple mechanisms operating at different space and time scale (Cauvain, 2012; Gray and Bemiller, 2003; He and Hoseney, 1990). The changes occurring during staling include loss of flavor, an increase of crumb hardness and loss of crispness of the crust.

The loss of crispy texture could be associated with slight increase in water content, which induces glass transition in amorphous regions in polymers that were initially in the glassy state (Luyten et al., 2004; Cuq et al., 2003).

Unfortunately, the crispness is rapidly lost after baking and then bread is rejected by consumers. In fresh state, bread crust is dry and crispy and exhibits a brittle noisy fracture. In the stored bread, the water activity (Aw) of the crust increase due to migration of water from the wet crumb to the dry crust, producing a soft and tough crust (Primo-Martín et al., 2006; Luyten et al., 2004; Roudaut et al., 1998). Furthermore, the water content of the environment, expressed as relative humidity, also plays an important role. Specifically, water migration to the crust induces a transition from the glassy to the rubbery state of the main crust macromolecules.

A few studies have been focused on strategies for extending the bread crust shelf life. Primo-Martín et al. (2006) sprayed protease, transglutaminase and alpha-amylase on the dough surface to selectively modify the bread crust, they found that modification of the protein network can be used to regulate the water holding capacity of the crust, and consequently the retention of crispness. Another study, reported the effect of different enzymes and additives that changed crust behaviour during storage (Primo-Martín et al., 2008).

van Nieuwenhuijzen et al. (2008), with the purpose of study the relationship between the glass transition of bread and the sensorial loss of the crispness, carried out oscillatory sorption experiments to change the characteristic diffusion time and to get insights in the contribution of polymer matrix relaxation to the water sorption. Others authors claim that the increase of the shelf life of bread crust relies on its permeability. Because of that, they support the hypothesis that the crust has low water vapour permeability and acts as a barrier to water vapour migration. Therefore, an increase in the permeability of the crust would facilitate water migration through the crust. In consequence, the effective diffusion coefficient would increase because the decrease in the resistance of the crust to water migration. In conducting this study, authors increased the permeability of the bread crust by creating small channels through the crust. Finally, they concluded that an increase in the permeability of the crust meant an increase in the retention of crispness (Hirte et al., 2010).

Despite the mentioned attempts to understand the crust properties and modified the permeability, the impact of crust microstructure on its permeability and mechanical properties is not completely understood.

Application of analysis to assess the properties of bread crust

Quality of a bread is related to its sensorial (shape, size, colour) and mechanical (texture) characteristics. These features are strongly affected by the product structural organization. Hence, some techniques have been applied to study properties of bread crust such as sensory perception by judged training, microstructure and texture.

Sensory analysis

The qualitative aspects of a product comprise aroma, appearance, flavour, texture, aftertaste and sound properties of a product. Sensory judges score those aspects in order to facilitate description of the perceived product attributes. A panel with some degree of training or orientation is required for that assessment. The training phase begins with the development of a common language, which comprehensively and accurately describes the product attributes (Murray et al., 2001).

Concerning to the sensory properties of baked products, in terms of appearance, odour, taste and texture, without any doubt they strongly contribute to consumer freshness perceptions (Heenan et al., 2009; Gambaro et al., 2002). Many surveys have been focused on determining consumer perceptions and preferences for bread products (Lambert et al., 2009; Heenan et al., 2008). However, sensory terms used are often subjective descriptors rather than objective measurements. In order to validate those measurements with more objective measurements, some authors have tried to correlate sensory parameters with texture analysis (Fizman et al., 2005; Gambaro et al., 2002). With respect to crispy foods, crispness is strongly appreciated by consumers and some instrumentals techniques have been tested for measuring the consumer perception (Castro-Prada et al., 2009; Primo-Martín et al., 2009; Roudaut et al., 1998).

Structure analysis

Food structure indicates the organization of their constituents at multiple spatial scales and their interactions. Many food properties are related to structure, which helps to understand texture perception, chemistry stability and transport phenomena. Both the macro and the micro structure of the crispy food are potentially important factors that affect the mechanical behavior and jaggedness of mechanical parameters related to crispness. In particular, microstructure and interactions of components, such as protein, starch, and fat, determine the texture of a food. Bread crust have a porous structure composed by cell walls; which will determine the adhesion between cell, its stiffness as well as mechanical properties influencing the fracture properties of the whole product (Luyten et al., 2004).

Microscopy techniques vary in method of image production, resolution and type of signal detected, giving a particular type of structural information. Special techniques such as light microscopy, confocal laser scanning microscopy, scanning electron microscopy and X-ray microtomography are used to assess microstructure.

The light microscopy employs visible light and magnifying lenses to detect small objects. However, advanced instrumentation has been developed like confocal laser-scanning microscopy; which is a valuable tool for obtaining high resolution images and 3-D reconstructions (Claxton et al., 2005). Scanning electron microscopy (SEM) is another common and important method for understanding the microstructure of bakery products. Sample preparation is fairly easy and introduces fewer artefacts than light microscopy. When the electron beam strikes an ultra-fine sample section (100nm), some of the incident electrons are transmitted to form an image with the impression of three dimensions (Aguilera and Stanley, 1999). SEM has been used to study changes that occur during baking in bread crust (Besbes et al., 2013b; Turabi et al., 2010; Ozkoc et al., 2009). The use of other techniques as X-ray microtomography proves to be a very useful technique for the 3D visualization and quantitative analysis of bubbles in food products, providing information about the cellular matrix. In order to study the role of the morphology of the bread on the migration of water during storage, Primo-Martín et al. (2010) used X-ray microtomography. These authors reported that the size, shape, connectivity and distribution of air bubbles and their cell walls are seen as critical factors influencing the moisture transport within the cellular structure and therefore having an impact on the crispness.

Principles and types of instrumental texture analysis as applied to bread crust.

Texture is a major factor in determining consumer acceptability of food. As mentioned above, the textural characteristics of bread are commonly described in terms of their sensory properties; but these are subjective descriptions, hence is required the use of objective measurements. The sensory character experienced through the fingers and with the eyes can be readily confirmed in the mouth. Therefore, many of the objective tests employed to evaluate bread are based on tests of compression or deformation. Some attempts have been made to correlate instrumental parameters as loudness, crispness and firmness with peak force, slope, force-deformation, fracture and Young’s Modulus (Carr et al., 2006; Mazumder et al., 2007; Valles-Pamies et al., 2000; Sauvageot and Blond, 1991;Vickers and Christensen, 1980).

A general characteristic of crispy food is that they fracture at low work of mastication with different successive fracture events which are accompanied by sound emission (Luyten et al., 2004). The texture of bread crust has been assessed using puncture or penetration test, owing to its similarity to bite, using either a needle or a small diameter cylinder (Rosell and Altamirano-Fortoul, 2011; Primo-Martín et al., 2009). During this test, several sudden force reductions are observed; which mainly correspond to different fracture events. The number of force reduction depends on the pores size and cell walls in food (Valles-Pamies et al., 2000; van Hecke et al., 1998). The response obtained in this test is usually recorded in the form of force-deformation plots, which represent curves with a series of sharp force peaks, each corresponding to a fracture event (Roudaut et al., 2002; Vincent, 1998).

Additionally, on a crispy food if the force increases, so does the deformation. The rising part of this curve is a function of the stiffness both by effect of the material as well as the structural (thickness, shape, density or degree of aeration). The force peak depends on the material composition and structure. The area under the graph is related to the total work done. After the first break, If the material is brittle (i.e.

has a low work to fracture) fracture will travel quickly, resulting in sudden unloading of the force. The fracture is inhibited when material is deformed further (Vincent, 1998).

Van Hecke et al. (1998) and Valles-Pamies et al. (2000) studied the texture in cellular foods by puncturing them, and force-displacement plots were obtained after initial fracture. Those authors calculated the average of the so-called puncturing force (integral of force–time), the number of spatial ruptures, the average specific force of structural ruptures and the crispness work.

Other test to assess the texture includes both instrumental parameters and sound. Some authors report that sound emission during fracture of a crisp food is of importance for the crispy sensation, because when a crispy material is broken each fracture corresponds to an acoustic event. In those cases, the sound emission during fracturing has been quantified (Hirte et al., 2010; Primo-Martín et al., 2008; Luyten and van Vliet, 2006; Roudaut et al., 1998). The combination of acoustic analysis and mechanical testing results in a more controlled and objective way of analyzing sound emission and allows the extraction of a number of parameters as force, sound pressure, peaks numbers and sound level.

However, independently of the technique used to determine the texture of the bread crust, it is necessary to take into account that texture evaluation is a complex subject due to food’s texture is a result of its underlying structure and the mechanical properties depend of this.

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Objectives

OBJECTIVES

Main Objective

Modulation of the physico-chemical and functional properties of bread by applying surface treatments in order to understand crust features and to develop added value bread products.

Specific objectives

- To select bread quality parameters that permit rapid characterization of crust and crumb and also discriminate among different bread specialties obtained by applying bake-off technologies.
- To evaluate the influence of the amount of steaming on the physico-chemical, mechanical and microstructural properties of bread crust, especially water vapour transmission rate and water vapour permeability.
- To study crust features by setting up reliable mechanical tests that correlate with microstructure morphology.
- To modulate the mechanical properties of bread crust by enzymatic treatments understanding the microstructure modifications.
- To develop probiotic breads by evaluating the viability of different types of functional coatings applied onto the surface of partially baked breads before the full baking step.

Chapter 1

PHYSICO-CHEMICAL CHANGES IN BREADS FROM BAKE OFF TECHNOLOGIES DURING STORAGE

Rossana Altamirano-Fortoul, Cristina M. Rosell

ABSTRACT

Quality of several bread specialties from frozen partially baked breads was assessed to define main quality features. Loss of crust freshness shortly after baking was also determined. Quality parameters that characterize bread crust and crumb were determined by instrumental methods in nine different (regarding to formulation and bake off duration) bread types obtained from frozen partially baked breads. Principal component analysis (PCA) allowed discriminating among bread specialties. Quality parameters that enable the differentiation of wheat bread types were crust mechanical properties together with specific volume, crumb hardness and structure. Crust flaking barely represented a problem in the studied types of bread. Crust mechanical properties were rapidly lost during the first 4h after baking and the rate of the process was greatly dependent on the bread type. The force to promote crust fracture underwent increase up to 6h after baking and those changes occurred in the A_w range of 0.50-0.74 or moisture content 9-15 g/100 g.

Key words: bread; instrumental analysis; crust properties; frozen part-baked.

INTRODUCTION

Bread has an important contribution in a well balanced diet, due to its content on starch and complex carbohydrates. Partially baked bread is an alternative bakery product that is gaining market share every year. It offers several advantages at retails, such as materials and equipment reductions, less production space is required, greater variety of bread, and also at the consumers, which have fresh bread available at any time of the day (Rosell, 2009). Breadmaking technology with partial baking, also referred as bake off technology (BOT), consists in two baking stages (Bárcenas and Rosell, 2006a, b; Fik and Surowka, 2002). First baking gives a product (partially baked bread) with aerated crumb but without crunchy and colored crust. After that bread can be kept refrigerated or frozen. Second or full baking favors water evaporation from the surface layers, and in consequence the crust development, and the Maillard reaction responsible for the coloration and the release of flavors takes place during this stage (Farahnaky and Majzoobi, 2008; Le Bail et al., 2005). Similarly to conventional breadmaking, main quality attributes of bread obtained from BOT process are texture and taste. Breads obtained from frozen partially baked breads are frequently connected to negative aspects, mainly associated to mechanical changes in the crust that led to flaking and very rapid crispiness loss (Le Bail et al., 2005). However, studies reported on those effects have been focused on French baguette, which avoid making a general statement about this type of technology.

Industrial practice suggests that the freezing step is responsible of crust flaking, one of the major quality problems of frozen part-baked breads (Hamdami, et al., 2007). Crust flaking resulting from the detachment of some part of the crust constitutes an important drawback, which has been related to excessive drying of the bread surface at the end of the post-baking chilling and freezing process (Lucas et al., 2005). Crust flaking has been related to concentration of water as ice below the crust during freezing and the mechanical damages due to the intense thermo-mechanical shock during chilling- freezing and final baking (Hamdami et al., 2007).

Additionally, other problem associated to all bakery products is their relative very short shelf life, since certain physical and chemical changes affecting to crust and crumb occur during their storage, which are known as staling (He and Hoseney, 1990). Breads from frozen partially baked bread are particularly sensitive to changes related to crust crispiness. Crust characteristics are decisive for the purchasing attitude of consumers of this type of product, which is based on the subjective fingers sensory perception (Lambert et al., 2009). A crispy texture is originated when starch and gluten matrix are in glassy state and it has been associated with low moisture content and water activity (Stokes and Donald, 2000).

Crispiness is a complex attribute resulting from multiple sensations and influenced by numerous physical parameters, combining molecular, structural and manufacturing process as well as storage conditions (Roudaut et al., 2002; for additional information see review of Luyten et al., 2004). Crispiness is perceived only for a short time after baking and it is the main attribute that causes consumer rejection (Duizer, 2001). Lately, different studies have been focused on extending the crust mechanical properties by either modifying the bread formulation or the cooling conditions (Primo-Martín et al., 2008a,b). However, scarce information has been reported on the variation of the crust mechanical properties of breads obtained from partially baked technologies.

The aim of this study was to select quality parameters that permit a rapid characterization and differentiation of different bread specialties obtained by applying bake-off technologies. For that purpose, diverse instrumental quality parameters related to crust and crumb were assessed. Short term storage behavior was also followed to evaluate crust changes during staling in different bread specialties obtained from BOT processes.

MATERIALS AND METHODS

Nine partially baked breads were selected to represent a range of different bread specialties currently available in the Spanish market. Part-baked frozen breads were directly provided by the producers (Forns Valencians S.A., Valencia, Spain) and stored at -18^{o} C until use. A description of the bread ingredients is given in Table 1.

Table 1. Bread product sample and listed ingredients (information supplied by producer). Special ingredients for each type of bread appeared highlighted.

Product	Ingredients
Pulguita	Wheat flour, water, **lard**, yeast, salt, bread improver
Small ciabatta	Wheat flour, water, rye flour, salt, bread improver, **freeze dried sourdough**, yeast and gluten
Ximos	Wheat flour, water, **lard**, yeast, salt, bread improver
Small brioches	Wheat flour, water, **sugar**, **lard**, **milk powder**, yeast, salt, bread improver
Rustic	Wheat flour, water, rye flour, yeast, salt, bread improver
Brioche	Wheat flour, water, **sugar**, **lard**, **milk powder**, yeast, salt, bread improver
White loaf	Wheat flour, water, rye flour, yeast, salt, bread improver
Baguette	Wheat flour, water, rye flour, yeast, salt, bread improver
Ciabatta	Wheat flour, water, rye flour, salt, bread improver, **freeze dried sourdough**, yeast and **gluten**

Full baking process and storage

Loaves removed from the freezer were thawed at ambient temperature (18-20º C) for 50 min, and baked in an electric oven (Eurofours, France). Baking conditions (provided by the company) varied with the specialty and were as follows: 180º C for 11 min in the case of pulguita, small ciabatta, small brioche, rustic and brioche, 180º C for 14 min for ximos, and 180º C during 18 min for white loaf, baguette and ciabatta.

After baking, breads were cooled down and stored in a cabinet with controlled both temperature (18º C) and relative humidity (57%) till further characterization. Duplicates of each sample were baked in separate days.

Fresh loaves (0.5 h after baking) and stored loaves (2, 4, 6 and 24 h) were tested for specific volume, water activity, moisture content, crust and crumb color, width/height ratio of the slices, textural characteristics, crust mass ratio, crust flaking, crust section and crumb cell analysis.

Physico-chemical analysis

Chemical composition was determined following ICC standard methods (1994) for moisture (ICC 110/1), protein (ICC 105/2), fat (ICC 136) and ash (ICC 104/1). Carbohydrates were determined by difference.

Bread volume was determined by the rapeseed displacement method. The rest of the physicochemical parameters were independently determined in the crust (according to crust section analysis described below) and crumb, which were separated using a razor blade. Colour parameters of the crumb and crust were measured at three different locations by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate (L^* = 96.9, a^* = -0.04, b^* = 1.84). The colour was recorded using CIE-L^* a^* b^* uniform colour space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis. Moisture content of the crust and crumb was determined following the ICC Method (110/1, 1994). Water activity (Aw) of crust and crumb samples was measured using an Aqua Lab Series 3 (Decagon devices, Pullman, USA) at 22^{o} C. Crust mass ratio was calculated as the ratio of crust weight and crumb weight.

Puncture tests

The peak force and the peak deformation point of the crust were calculated by punching the sample at three different points of bread surface: in the middle of the crust area and at 2 cm distance on both sides. The average value was determined for each bread variety. Experiments were assessed using a texture analyzer (TA XT plus, Stable Micro Systems, Surrey, UK). A cylindrical probe of 4 mm diameter was used at 40 mm/s cross-speed. This speed was initially chosen by Primo-Martín et al. (2008a) to simulate biting with the front teeth (Vincent, 1998). The failure force was calculated as the peak force observed according to studies by Jackman and Stanley (1992). The failure deformation, defined as the deformation at the peak point, was also calculated. Three loaves were used for each bread variety.

Crumb hardness test

Crumb hardness was carried out in a texture analyzer (TA XT plus, Stable Micro Systems, Surrey, UK). A 1 cm-thick slice was compressed with a 25 mm diameter cylindrical stainless steel probe, up to 50% penetration of its original height at a crosshead speed of 1 mm/s speed.

Crust flaking evaluation

Crust flaking test was carried out in specific crushing system developed by Le Bail et al. (2005). Bread was crushed on its flanks and on its base by 30% of its diameter and height in crushing system. Pieces of the crust were collected and weighed, after that a digital picture of crust pieces was taken. Total number of flakes, total area in mm^2, average size in mm^2 and area fraction in percent were determined Using an Image J software.

Crust section and crumb cell analysis

Crust section and crumb cell analysis were performed by scanning longitudinal and cross section of bread sample, 10 mm thick, on flatbed scanner (HP Scanjet 4400c). The crust section was calculated from the scanned samples at the upper and bottom side using an image analysis program (UTHSCSA Image Tool software). For crumb cell, the images were analyzed by Image J software according to Gonzales-Barron and Butler (2006), number of cell, average cell per mm^2, average diameter per mm^2 and circularity were calculated.

Statistical analysis

For each quality parameter, a one way analysis of variance (ANOVA) was applied using Statgraphisc Plus V 7.1 (Statistical Graphics Corporation, UK), to assess significant differences (P<0.05) among samples that might allow discrimination among them. Principal component analysis (PCA) was also performed to determine the number of principal components that significantly (P<0.05) discriminated breads.

RESULTS AND DISCUSSION

Characterization of fresh breads

Nine bread specialties with rather similar recipes (Table 1) were used to evaluate crust behavior during staling. Physico-chemical characteristics were initially determined, in order to find the main discriminating factors among the specialties that could help to understand possible differences during storage. Table 2 shows the macronutrients composition of the nine bread specialties that agrees with common bread proximate composition. Fat content was higher in the bread varieties (pulguita, ximos, small brioches and brioches) that contained lard, and specialties with higher protein content were brioches and small brioches that had milk powder added.

Table 2. Chemical proximate composition (expressed as g/100 g as is) of nine breads obtained from frozen partially baked breads.

Product	Carbohydrates	Fats	Proteins	Minerals
Pulguitas	53.2	5.5	9.6	0.70
Small ciabatta	54.7	1.5	6.8	0.50
Ximos	53.2	5.5	9.6	0.70
Small brioches	46.7	5.8	9.6	0.60
Rustic	51.5	2.9	6.9	0.50
Brioches	46.7	5.8	9.6	0.60
White loaf	53.0	1.8	9.8	0.50
Baguette	53.0	1.8	9.9	0.70
Ciabatta	54.7	1.5	6.8	0.50
Mean	51.9	3.6	8.7	0.59
SD	3.1	2.0	1.4	0.09

Several technological parameters have been assessed to identify the most discriminating parameter that allows the characterization of the breads. Preliminary analysis of data collected using ANOVA showed that all physicochemical characteristics significantly ($P<0.05$) discriminated between the bread types tested (Table 3 and 4). The highest specific volume was observed in the brioche type followed by rustic bread (Table 3). Crust properties have been considered essential features for bread quality assessment (Luyten et al., 2004), because of that, special emphasis was put in assessing crust physicochemical properties. Significant differences ($P<0.05$) were observed in both the crust and crumb moisture content, which ranged from 4.8 to 11.5 g/100 g and from 31.3 to 45.3 g/100 g, respectively. Similarly, water activity in the crust and in the crumb showed significant differences among bread types; it varied from 0.34 to 0.58 in the crust and from 0.95 to 0.98 in the crumb. Those values agree with previous findings for breads (Baik and Chinachoti, 2000). Moisture content and A_w greatly contribute to crust mechanical properties (Katz and Labuza, 1981).

Regarding crust flaking, significant ($P<0.05$) differences were observed among the bread types, but in all cases very low flaking (less than 2%) was observed. Crust flaking has been mainly investigated on partially baked French baguette stored under frozen conditions (Le Bail et al., 2005).

Table 3. Technological quality parameters of nine bread specialties obtained from frozen partially baked breads frozen partially baked breads.

Product	Specific volume	Moisture content (g/100g)		Aw		Crust color			CM ratio	Flakes count	Flakes area	Cells number	Cells area
	mL/g	Crust	Crumb	Crust	Crumb	*L**	*a**	*b**	g crust /100g		mm^2		mm^2
Pulguita	3.3 ab	5.3 b	39.7 d	0.57 f	0.97 cd	63.4 e	3.9 a	29.9 b	0.61 a	1230 a	504 a	386 c	0.65 b
Small ciabatta	3.0 a	11.5 h	39.4 c	0.54 e	0.97 cd	61.6 e	9.0 b	33.7 cd	0.43 a	5852 h	120981 i	177 b	2.23 e
Ximos	3.0 a	9.5 f	31.3 h	0.43 c	0.97 cd	67.4 f	8.8 b	38.7 e	0.47 ab	1819 d	2501 h	75 a	0.45 a
Small brioches	3.5 b	7.6 d	38.7 b	0.46 c	0.95 a	47.8 a	16.1 e	31.3 bc	0.48 ab	1588 b	1480 e	436 e	1.08 d
Rustic	5.6 d	6.9 c	45.3 g	0.58 f	0.98 e	56.5 d	11.2 bc	28.6 b	0.45 a	1729 c	934 d	416 d	2.28 ef
Brioche	6.0 d	8.5 e	38.7 b	0.45 c	0.96 b	53.2 bc	15.0 de	36.1 de	0.55 bc	4063 f	1497 f	607 f	0.64 b
White loaf	3.4 ab	5.3 b	43.5 e	0.34 a	0.98 de	56.5 cd	12.3 cd	36.2 de	0.47 a	3504 e	830 c	607 f	2.33 f
Baguette	4.9 c	4.8 a	44.5 f	0.38 b	0.97 c	52.6 b	14.8 de	34.7 d	0.45 a	4431 g	2068 g	375 c	0.72 c
Ciabatta	3.4 ab	10.8 g	37.3 a	0.52 d	0.97 ce	45.6 a	13.6 cde	20.9 a	0.64 d	1816 d	783 b	74 a	7.64 g
P- value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

CM ratio: crust mass ratio: g crust/100 g bread. Means sharing the same letter within a column were not significantly different ($P < 0.05$) (n=3).

Only the small ciabatta yielded high amount of flakes, which also showed the highest size. Crumb structure analysis of breads confirmed that bread specialties had significantly ($P<0.05$) different structure, having very big void spaces or gas cells the ciabatta, white loaf, rustic bread and small ciabatta. Those types of breads also showed higher crumb hardness (Table 4), suggesting coarser crumb structure with thicker cell walls. The presence of rye flour in their formulation could be responsible of the different crumb structure; although baguette would be an exception, because it contains rye flour and did not exhibited the same crumb behavior.

Crust mechanical properties were assessed by a puncture test, where sufficient strain energy was applied to penetrate in the crispy crust (Table 4). The failure force was related to the hardness necessary to break a brittle material in the mouth during the first bite. A force-deflection curve was obtained, showing a force increase up to a major rupture and a drop to zero when pieces fall away (Vincent, 1998). Higher values of failure force were observed for ciabatta and small ciabatta, followed by baguette and white loaf, which required higher force to promote crust fracture. Presumably, the presence of additional gluten and freeze dried sourdough in the recipe of ciabatta and small ciabatta could be responsible of their harder crust. Considering that crispy texture is generated when materials in a glassy state interrupted with empty spaces (cells or air pockets leading to heterogeneous structure) are subjected to enough energy (Vincent, 1998), higher force in the fresh bread suggests more rigid and firm crust with less cracks in it.

Table 4. Crust and crumb textural properties of nine bread specialties obtained from frozen partially baked breads

Product	Hardness	Failure force	Area	Failure deformation	Moisture content at 4 h storage	Aw at 4 h storage	Failure force at 4 h storage
	N	N	N/m	mm	g/100g		N
Pulguita	1.66 bc	12.06 b	3.17 abc	5.4 c	13.5 c	0.67 c	14.41 b
Small ciabatta	2.05 e	21.62 d	4.88 de	4.93 bc	14.3 d	0.68 c	25.28 c
Ximos	1.91 de	7.85 a	2.16 a	5.13 c	13.1 c	0.68 c	11.42 a
Small brioches	1.59 b	8.33 a	2.38 ab	2.93 a	9.9 b	0.53 b	11.2 a
Rustic	2.39 f	11.52 b	3.53 bc	7.07 d	8.3 a	0.66 c	14.53 b
Brioche	1.82 cd	8.70 a	2.00 a	3.73 ab	9.1 ab	0.54 b	12.22 a
White loaf	1.96 de	17.75 c	5.46 de	6.93 d	8.7 a	0.45 a	32.19 d
Baguette	1.39 a	18.06 c	4.36 cd	6.27 cd	9.4 b	0.45 a	26.9 c
Ciabatta	3.82 g	22.54 d	5.79 e	6.70 d	15.1 e	0.74 d	37.57 e
P - value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Means sharing the same letter within a column were not significantly different ($P < 0.05$) (n=3).

On the opposite side, ximos together with brioches, small brioches and pulguita exhibited the lowest failure force with low values of failure deformation, due to thinner crust section (values not showed), likely due to the presence of added fat in their formulation that acts as plasticizer. Failure deformation is referred to the applied deformation that has been related to the teeth displacement (Vincent, 1998). High values of failure deformation have been related to less stiff crust texture when the effect of different hydrocolloids on bread physical properties were studied (Mandala, 2005). However, in the present study where different bread types were evaluated, breads with thick crust gave high values of failure deformation suggesting stiffer crust structure.

Significant quality parameters analyzed by PCA indicated that six principal components significantly ($P<0.05$) discriminated between bread specialties, which accounted for 95% of the variability in the original data (data not showed). This analysis described 32% and 22% of variation on principal components 1 (PC1) and 2 (PC2), respectively (Figs. 1 and 2). Component 1 was defined by b^* color parameter of the crust, flakes count, specific volume and number of cells along the positive axis, which were present in brioche, small brioche, baguette, and ximos. Along the negative axes, PC1 was described by cell diameter, hardness, area and failure force that were present in ciabatta.

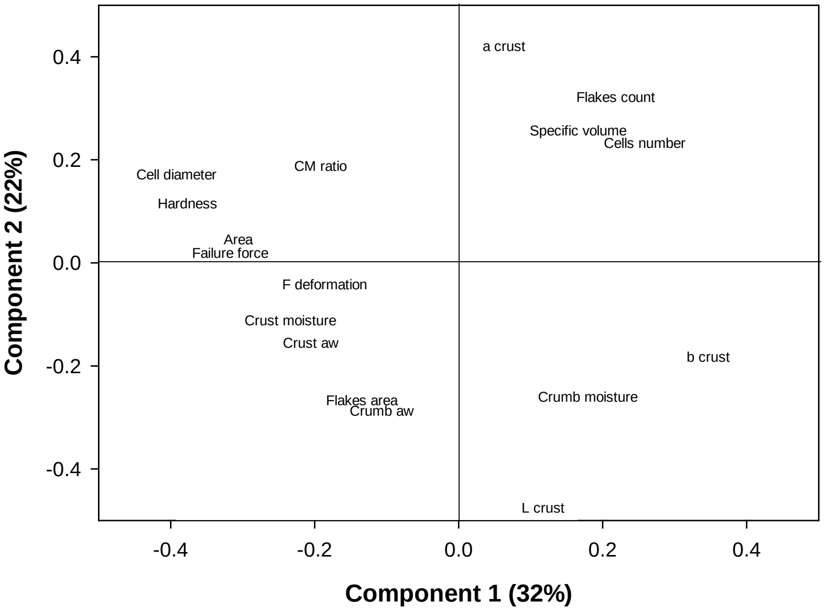

Fig. 1 Correlation loadings plot from principal component analysis showing the technological quality parameters of nine bread specialties.

Parameters like crust moisture, flakes area and A_w of crumb were present in small ciabatta bread. Conversely, the component 2 was mainly defined by the crust colour parameters *a** and *L**, along the positive and negative axes of PC1, respectively. Fresh samples were located in different positions (Fig. 2), brioche, small brioche, baguette and white loaf breads were positively located along PC1 and PC2. On the other hand, the breads located along the negative axis of PC1 were ciabatta and small ciabatta. Ximos bread and small ciabatta were located along the negative axis of PC2. Therefore, PCA allowed discriminating among bread specialties and it showed that brioches, small brioches and baguette were similar in terms of specific volume, flaking behavior and crumb structure, although they had different ingredients in their formulation. In addition, bread types with similar ingredient composition, like baguette, white loaf and rustic, could be discriminated with the quality parameters from instrumental analysis.

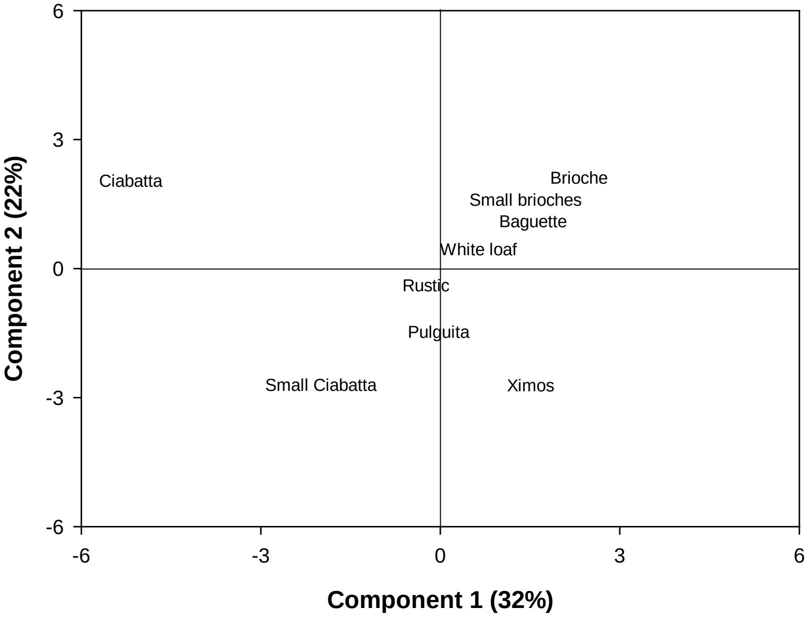

Fig. 2 Scores plot from principal component analysis of the nine bread types evaluated for technological quality.

Descriptive sensory attributes have been reported for discriminating among different bread types (Heenan et al., 2008). In that study, porous appearance and odour attributes were the most important descriptors. The same authors indicated that freshness perception varied widely with consumers, making necessary to segregate in consumer clusters to obtain a reliable approach for predicting freshness. Main quality parameters obtained from instrumental analysis that influence the consumers' acceptability have been recently defined (Curic et al., 2008). The instrumental methods that describe bread appearance, structure and texture were selected to identify and quantify the main discrepancies of wheat bread produced by different breadmaking processes (Curic et al., 2008). In the present study, wider range of instrumental parameters for characterizing crust and crumb has been considered and they made possible to differentiate the bread types obtained from frozen partially baked breads.

Characterization of stored breads

Moisture content increases as bread ages, due to water sorption from the atmosphere and by mass transport from neighboring components of the crumb. The overall result was an increase of crumb hardness and moreover, the crust initially dry and crispy became soft and rubbery (Katz and Labuza, 1981). Water distribution between crust and crumb also contributes largely to the organoleptic perception of freshness (Baik and Chinachoti, 2000). Therefore, the influence of storage time on the water activity, moisture content and mechanical crust properties were determined in different bread specialties.

The analysis of the breads during storage revealed that moisture content of the crust increased in all types of bread along storage, and the most rapid enhancement was observed during the first 4 h after baking (Fig. 3, Table 4). Fresh samples had an average moisture content of 4.84-11.50 g/100 g and after 24-h storage their moisture content ranged from 10.07 to 17.66 g/100g.

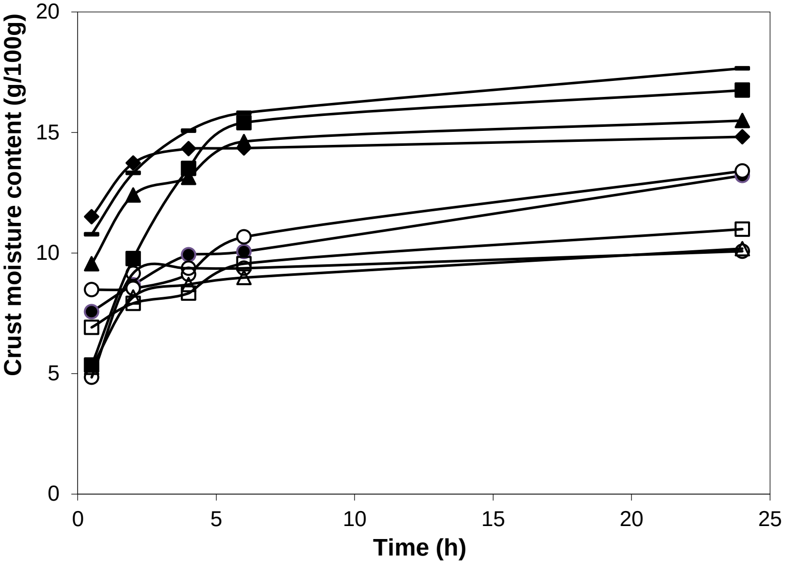

Fig. 3 Changes in moisture content of the crust from nine bread types during storage. Symbols: ■: pulguita; ♦: small ciabatta: ▲ : ximo; ●:small brioche; □: rustic; ○: brioche; Δ: white loaf; ◊: baguette; ▬: ciabatta.

The slope of the curves that was an indication of the speed of water uptake showed great variation depending on the bread type. Taking into account that all breads have been stored in the same conditions, and thus they will have similar water sorption from the atmosphere, divergence observed in the curve slopes must be due to variation in the moisture gradient between crust and crumb. However, no relationship was found between the slope of the curves and the gradient of water content between the inner crumb and the outer crust in the different fresh bread types (Table 3). It has been reported that although factors like water content/activity play a significant role, other features like molecular structure, porosity, and crust boundary (Luyten et al., 2004), greatly influenced the water migration through the crumb to the crust, and remains unclear the contribution of each factor.

Water activity in the crust also increased during storage (Fig.4). Again, the most pronounced changes were observed during the first 4 h after baking (Table 4). The major increase was observed in pulguita, small ciabatta and ciabatta breads. Values for water activity after 24-h storage varied between 0.59 (brioche) and 0.78 (pulguita).

A crispy texture has been associated with low values of moisture content and water activity, when starch and gluten matrix are in a glassy state and thus cell walls more susceptible to fracture (Stokes and Donald, 2000).

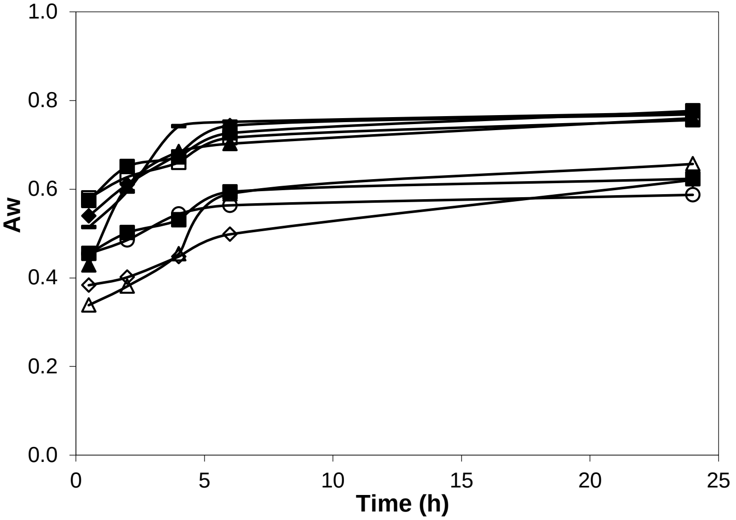

Fig. 4 Water activity in bread as function of storage time for different specialty breads. Symbols: ■: pulguita; ♦: small ciabatta:▲: ximo;●:small brioche; □: rustic; ○: brioche; Δ: white loaf; ◊: baguette; ▬: ciabatta.

The crust failure force during storage (Fig. 5, Table 4) was much higher in the aged breads, due to moisture redistribution (water migration from crumb to crust) that leads to a toughen crust (He and Hoseney, 1990). The highest increase in the crust rupture force was observed for white loaf and ciabatta. In general, the failure force showed a steady increase in all bread specialties up to 6 h storage, reaching an asymptotic plateau, that suggests the completely loss of crispy texture. Crispness loss during storage seems to be greatly dependent on the water migration from crumb to crust and from the atmosphere.

Primo-Martín et al. (2007) stated that amylopectin retrogradation, which is the main process responsible for the staling of bread crumb, cannot be responsible for crispness deterioration of the crust as amylopectin retrogradation upon storage of breads. Those authors confirmed by X-ray and differential scanning calorimetry (DSC) that starch retrogradation of the crust could only be measured in the crust after 2 days storage.

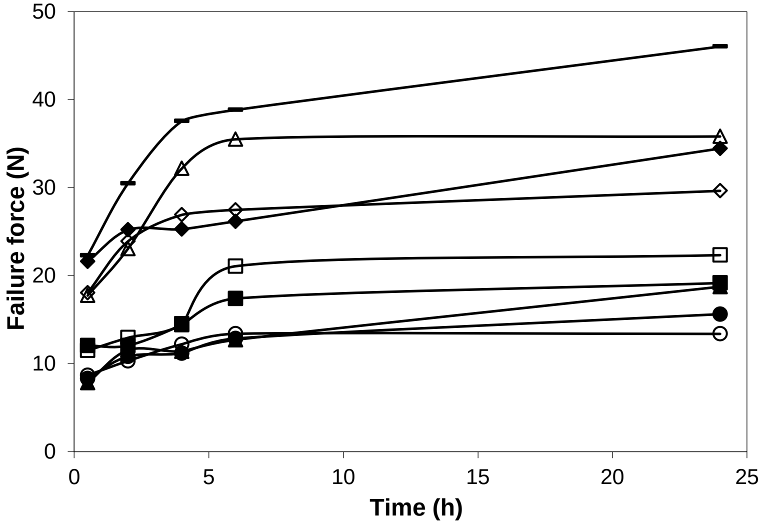

Fig. 5 Evolution of failure force of the crust for different specialty breads during storage. Symbols: ■: pulguita; ♦: small ciabatta: ▲: ximo; ●:small brioche; □: rustic; ○: brioche; Δ: white loaf; ◊: baguette; ▬: ciabatta.

Changes in the curve trend occurred between 0.50 and 0.74 A_w or 9-15% moisture content, depending on the bread type. These results agree with previous findings of Primo-Martín et al. (2009), who reported that a significant increase of the force required breaking the crust was observed at A_w of 0.65 or higher when using a crust model with similar structure to bread crust.

The instrumental methods applied for bread quality assessment allowed discriminating among bread specialties from frozen partially baked breads. Crust mechanical properties together with specific volume, crumb hardness and structure were the quality parameters that permitted bread differentiation. In general, crust flaking did not represent a problem in the types of bread tested.

Regarding the evolution of the crust physico-chemical properties, they changed shortly after baking. Specifically, water content/activity showed a steady increase during the first 4 h after baking. In addition, the force to promote crust fracture varied up to 6 h and those changes occurred in the A_w range of 0.50-0.74 or moisture content 9-15%.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of Spanish Ministerio de Ciencia e Innovación (Project AGL 2008-00092 ALI) and the Consejo Superior de Investigaciones Científicas (CSIC). R. Altamirano-Fortoul would like to thank her grant to

CSIC. The authors also thank Forns Valencians S.A. (Spain) for supplying commercial frozen partially baked breads.

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Chapter 2

EFFECT OF THE AMOUNT OF STEAM DURING BAKING ON BREAD CRUST FEATURES AND WATER DIFFUSION

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Journal of Food Engineering 108 (2012) 128–134

ABSTRACT

Surface mechanical properties in fresh bread and during storage are greatly affected by the water plasticizing effect. However, the incidence of steaming during baking on the crust mechanical properties remains still unclear. The impact of the amount of steam (100, 200 and 400 ml) during baking on the crust features and water diffusivity was investigated. The amount of steam significantly (P<0.05) affected the crust colour, glossiness and mechanical properties. An increase in the amount of steam led to reduced colour, failure force and failure firmness, whereas increased glossiness. Water vapour transfer rate and water vapour permeability of the bread crust significantly decreased when increasing the amount of steam applied during baking. Crust microstructure studies carried out by scanning electron microscopy and X-ray microtomography confirmed that the amount of steam greatly affected the surface starch gelatinization and also the protein-starch network.

Key words: bread; crust; baking; microstructure; tomography.

INTRODUCTION

Wheat bread is one of the most commonly consumed food products in the world. Fresh bread usually presents an appealing brownish, crispy and crunchy crust besides pleasant aroma. In bakery products, crust quality attributes as crispy texture and colour are the most important attributes for consumer's perception. Jefferson et al. (2005) defined the crust as the part of bread near its surface, where the density is significantly higher than elsewhere, which is formed during the final baking.

Baking is an important stage in bread making; because it implies many physical and chemical changes, e.g. evaporation of water, formation of crumb structure, volume expansion, protein denaturation and starch gelatinization. In this final stage of the bread making process, the temperature in the oven chamber and amount of steaming are essentially to give bread with main characteristics as open gas cell structure, expansion volume, and a crust envelop. Steaming is applied at the beginning of the baking process to improve crust texture and colour (Ahrné et al., 2007). Steam is responsible of preserving the extensibility of the surface of the dough during the oven-spring phase and giving a nice gloss of the crust (Chang, 2006), improving crust colour, and promoting heat penetration into the loaf interior (Ahrné et al., 2007). Dried oven conditions will yield rapid evaporation of water from the dough exposed surface, causing premature formation of a dry inelastic shell on the bread surface. As a consequence, the oven spring is restricted, which increases the risk of tearing of the surface of the crust in the finished bread (Hui et al., 2006). Conversely, it is expected that excessive moisture in the oven atmosphere will favour the expansion during baking (oven rise) (Le Bail et al., 2011) but no definitive scientific information is available.

Instrumental measurements of bread crust texture provide force-displacement plots that are related to the surface structure. Mechanical surface properties have been mainly associated to water migration from the crumb to the crust (Luyten et al., 2004). Water transport through crumb to the crust is complex and it may occur that water evaporates at one end of a pore and condenses at the other end. Water vapour diffuses through the interconnected pores towards the surface, under the influence of the gradient of water vapour concentration. In fact, liquid water gradient is formed and ensures the diffusive transfer of water from the core to the surface (Luyten et al., 2004). Diffusion and solubilisation of molecules through the material matrix are clearly dependent on the permeability of the product. Water vapour permeability (WVP) is defined as the rate of water vapour transmission per area unit of flat material or thickness unit under specified temperature and humidity conditions. The most common method used for the determination of WVP on films or edible packaging is the “cup method” based on the gravimetric technique (Debeaufort, et al., 1998). However, the reliability of this method in brittle materials is questioned.

The objective of this research was to evaluate the influence of the amount of steaming (100, 200 and 400 ml used in a baking chamber, which will correspond to 0.33 l/m^3, 0.66 l/m^3 and 1.33 l/m^3, respectively on the physico-chemical) and mechanical properties of bread crust. Special emphasis was put on assessing water vapour transmission rate and water vapour permeability in crust features during the post baking storage. The effect of steaming on the crust microstructure was investigated by scanning electron microscopy and X-ray microtomography.

EXPERIMENTAL

Materials

Commercial wheat flour was supplied by Paulic Minotiers SA (France). The flour had the following properties: 14.51% moisture content, 11.30% protein, 0.51 % d.m. ash content, Zeleny index 33 ml, alveograph parameters W=243x10^{-4} J and P/L=0.95.

Bread making procedure

Dough was prepared on a flour weight basis, for 100 g flour, 63 g water, 2g compressed baker’s yeast (l’Hirondelle, France) and 2g table salt (Cérebos, France). Ingredients were mixed in a SP10 spiral mixer (VMI, Montaigu, France) for 4 min at 100 rpm and 4 min at 200 rpm, and rested for 20 min while covered with a plastic film to avoid drying. Dough was divided (75 g) and moulded in a divider-moulder (Bongard, Holtzheim, France), then dough pieces were rolled mechanically in MB230 moulder (L'Artisanne, France). Nine dough pieces were placed on aluminium trays (size 40 cm x 60 cm) and proofed at 25° C and 95% relative humidity up to three dough volume increase (versus the initial volume of the dough) in a fermentation cabinet (ARG68 HENGEL, France). Next, dough pieces were baked in an electrical deck oven (MIWE CO 1.1208-Germany) with a surface of 1.02 m^2 (0.85 x 1.2 m). All baking was performed at fixed oven temperature of 230° C for 18 min. The steam injection was programmed to 100, 200 and 400 ml, which will correspond to 0.33 l/m^3, 0.66 l/m^3 and 1.33 l/m^3, respectively. From hereafter, milliliters will be used to refer to the different amount of steam applied at the beginning of the baking process.

The selection of the amount of steaming applied was based on results of a previous study where the impact of steaming on the kinetics of heating of crispy rolls was assessed (Le Bail et al., 2011).

After baking, breads were allowed to cool down for 1h at room temperature. Four sets of each sample were performed in separate days.

Fresh loaves were tested for colour and crust mechanical properties one hour after baking. Bread crust was used for further water vapour transmission rate and water permeability determinations.

Chemical and physical analyses

Colour parameters and glossiness of bread crust were measured using a CM-3500d spectrophotometer (Minolta, Co. Japan) after standardization with a white calibration plate (L^* = 93.4, a^* = -1.8, b^* = 4.4). The colour was recorded using CIE-L^* a^* b^* uniform colour space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b* indicates hue on a blue (-) to yellow (+) axis.

Glossiness is an indication of the degree to which the coated surface simulates a perfect mirror in its capacity to reflect incident light (Yang and Zhang, 2004). The value L^* was determined between specular component included (SCI) and specular component excluded (SCE) measurements using CM-3500d spectrophotometer (Minolta, Co. Japan). Crust glossiness was calculated as the subtraction of *L** (SCE) and *L** (SCI) values.

Water vapour permeability (WVP) determination

Water vapour permeability of the crust was determined according to the method ASTM E96 (ASTM, 1980). First, the crust was separated from the bread one hour after cooling using a razor blade. The crust was sealed to a cup partially filled with distilled (100% RH; 2.38×10^3 Pa of vapour pressure at 20ºC) leaving an air gap beneath the crust as shown in Fig. 1. Sealing was carried out with gaffer tape of grade that met packaging certification standards (ASTM D5486). The test cup was then placed into a chamber (VC 7018 Vötsch, Industrietechnik, Germany) with controlled temperature and relative humidity at 61.2% RH and 20°C, respectively. Changes in the weight over time were monitored to determine the steady state flux of water vapour through the crust. The cups were weighed every day during five days. The thickness of the crust (Table 1) was measured using a micrometer, and the area of crust exposed in the test cup was 3.14 cm^2. Three replicates were determined for each measurement.

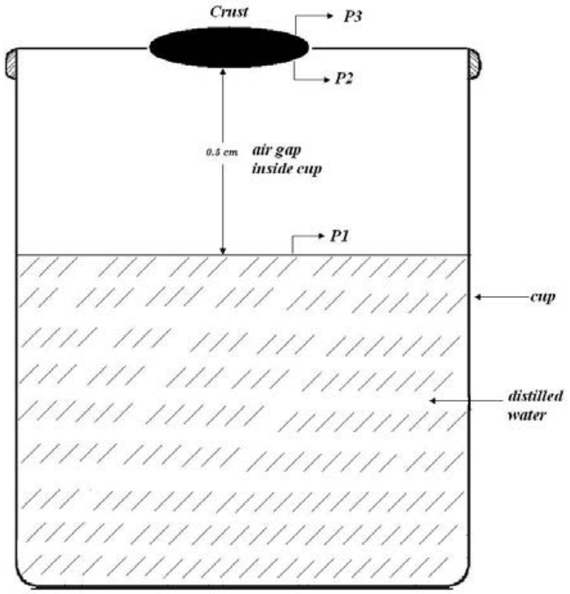

Fig. 1 Schematic diagram of water vapour permeability measurement cup indicating locations of water vapour pressure values and air gap.

Water vapour transmission rate (WVTR) calculations

Water vapour transmission rate (WVTR) was determined using Eq. (1) (ASTM E96 method, 1980). The slope of the weight versus time plot was divided by the effective crust area (3.14 cm^2) to obtain the WVTR.

$$WVTR = \frac{Slope}{Crust} \qquad \text{Eq (1)}$$

Water vapour permeability (WVP) correction method

Water vapour permeability was calculated by determining mass transfer through the stagnant air layer in the test cup (Bird et al., 1960; Krochta, 1992). Eq. (2) enables calculation of the corrected water vapour partial pressure at the crust inner surface (P2) (McHugh et al., 1993).

$$WVTR = \frac{P * D * Ln\left[\left(P - P2\right)/\left(P - P1\right)\right]}{R * T * \Delta z} \qquad \text{Eq (2)}$$

Where:

P= Total pressure

D= Diffusivity of water through air at 20° C ($2.53\times10^{-5)}$

R= Gas law constant (8.32 Pa m^3 mol^{-1} K^{-1})

T= Absolute temperature 293.25 K

Δz =Mean stagnant air gap height ($z_{original}$+z_{final})/2 (m)

P1= Water vapour partial pressure at solution surface (Pa)

P2= Corrected water vapour partial pressure at crust inner surface in cup (Pa).

Water vapour permeance could then be calculated using Eq. (3). The corrected partial pressure at the inner surface of the crust; P2 was employed in Eq. (3) to calculate true water vapour permeance.

$$Permeance = \frac{WVTR}{P2 - P3} \qquad \left(\frac{g}{m^2 sPa}\right)$$ (gmm/m^2s Pa) Eq (3)

Where:

P3= Water vapour partial pressure at crust outer surface in the cabinet. Eq. (4) could then be used to calculate true WVP of the crust; by multiplying the cored permeance and average crust thickness. The thickness of the crust was measured using a flat parallel surface micrometer with 1p.m resolution. This procedure, using Eqs. (2)-(4), was defined as the WVP Correction Method.

$$Permeability = Permeance * Thickness$$ Eq (4)

Puncture tests

Fresh breads after one hour cooling were puncture tested at a deformation speed of 100 mm/min using a 4mm diameter cylindrical probe. This speed was chosen because low puncturing rate allows penetrate successively cell walls of the brittle cellular materials (Vickers and Bourne, 1976). Experiments were performed using a texture analyzer (Lloyd Instrument LR5K, Southampton, UK). The peak force and the peak deformation point of the crust were calculated by punching the samples at five different points on the bread crust: looking down on the top surface, punching was at the midpoint and 2 cm from that point at 12, 3, 6, and 9 o’clock. Three different samples were used, which gave 15 measurements and the average value was calculated. Preliminary studies were run to confirm that peak force values responded to crust failure by following the crust rupture over 6-h period after baking. Data confirmed that puncture test carried out in this type of bread within two hours after baking give information about stiff crust and not toughness. The failure force was calculated as the peak force observed according to studies by Jackman and Stanley (1992). The failure deformation, defined as the deformation at the peak point and the failure firmness, defined as the average slope of load displacement curve from zero to the point of rupture or failure (Shafiee et al., 2008), were also determined.

Scanning electron microscopy (SEM) and x-ray Microtomography

The structure of the crust was observed with environmental SEM at 15 kV (ZEISS). The environmental microscopy permits to observe the sample without any treatment usually done for most SEM protocols (drying + gold plating). Observation of the sample was placed from a “fresh” piece of crust. The sample is placed on a temperature controlled sample holder. The temperature of the sample was adjusted as well as the partial vapour pressure of water above the sample to prevent dehydration.

In addtion, X-ray micromicrotomography was used, since it seems to be a very powerful tool in the study of materials microstructure, it is non destructive and provides understanding of the physical structure of the product from an engineering perspective (Frisullo et al., 2009). The characterization of the cellular crust structure by X-ray microtomography was performed using the Skyscan 1174 (Skyscan, Belgium). The crust sample was placed on a plate; 10 mm thickness was evaluated starting from the outer crust surface. The source and the detector were fixed, while the sample was rotated during measurement. Power settings of 50kV and a CCD camera of 12 bits 1280 x 1024 pixels were used. A set of flat cross-section images was obtained for each sample after tomographical reconstruction (NRecon software, Skyscan) of projection images acquired under different rotations (0.7 degree steps) over 180 degrees. The size of the gas cells or porosity was analyzed from X-ray microtomography images obtained at a magnification factor of 20x (1 pixel = 13.5 µm), the volume analysed was 10 x 6 x 1.5 mm^3. Porosity was determined from the pixel ratio (solid domain versus void domain).

Statistical analysis

All determinations were carried out in triplicate. The results presented are averages of all available replicates. For each parameter, a one way analysis of variance (ANOVA) was applied using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK), to assess significant differences ($P<0.05$).

RESULTS AND DISCUSSION

Effect of the amount of steaming on the physico-chemical properties of crust

The impact of different amount of steaming applied during baking on the physico-chemical properties of crust was evaluated (Table 1). The surface colour of bread is an important quality for consumer’s acceptance. The steaming conditions induced significant differences ($P < 0.05$) in the crust colour parameters. The lightness ($L*$) increased with the steaming amount, observing significantly higher values with the highest amount of steaming (400 ml), also these samples had the highest yellowish colour ($b*$).The reddish colour was observed with the lowest amount of steaming (100 ml). Therefore, crust browning during baking is significantly affected by the steaming amount during baking. In fact, Xue and Walker (2003) reported that cakes baked in highly humid air showed higher crust lightness.

Glossiness was also affected significantly ($P<0.05$) by the amount of steaming used during baking. There was an increase of the glossiness on the crust when increases the steam (Table 1). The breads baked with the lowest steam amount (100 ml) gave an opaque crust, whereas the breads baked with 400 ml steam displayed a glossy crust. This effect could be attributed to the level of gelatinized starch granules on the crust, because the glossy appearance is due to the water vapour from steaming, which allows the formation of a starch paste that will gelatinize, form dextrins and finally caramelize to give both colour and gloss (Cauvain and Young, 2007).

Table 1. Crust physico-chemical and mechanical properties obtained from bread baked with different amount of steaming (100 ml, 200 ml and 400 ml corresponded to 0.33 l/m^3, 0.66 l/m^3 and 1.33 l/m^3, respectively).

	Amount of steaming (ml)		
	100	200	400
Color parameter values:			
*L**	60.05±0.98 a	61.11±0.47 a	64.71±0.34 b
*a**	13.04±0.65 b	8.24±0.51 a	7.83±0.34 a
*b**	32.72±0.67 a	33.62±0.69 b	34.84±0.62 c
Glossiness	0.32±0.01 a	0.51±0.01 b	0.60±0.01 c
WVTR (g/m^2s)	1.11E-03±8.91E-06 c	7.76E-04±3.43E-05 b	6.24E-04±1.27E-05 a
WVP (gmm/m2sPa	7.34E-07±5.91E-09 c	5.49E-07±2.43E-08 b	4.65E-07±9.43E-09 a
WVP correction (gmm/m2sPa)	1.88E-06±1.51E-08 c	1.41E-06±6.21E-08 b	1.20E-06±2.42E-08 a
Thickness crust (mm)	0.90±0.01 a	1.0±.01 b	1.0±0.01 b
Failure Force (N)	4.20±0.23 a	4.70±0.04 b	5.73±0.20 c
Failure Deformation (mm)	17.70±1.04 b	16.57±1.26 b	15.43±3.23 a
Failure Firmness (N/mm)	1.45±0.19 a	1.90±0.34 b	2.30±0.03 c

Means sharing the same letter within a row were not significantly different ($P<0.05$)

WVTR: water vapour transmission rate

WVP: water vapour permeability

The loss of water through each sample stored in the chamber was plotted as function of time (Fig. 2), thus exhibiting significantly different plots according to the steam amount used for baking. In the breads baked with the lowest steam level (100 ml), the crust had a greater rate of weight loss (slope) during storage. When higher amounts of steam were used the differences were reduced. The slope of the curve in the linear region was used to calculate the water vapour transfer rate (WVTR) and water vapour permeability (Table 1). Differences in WVTR were found as a result of the amount of steaming used during baking. In consequence greater WVTR was obtained with lower amount of steam, which could be expected, taking into account that lower amount of steam limits the starch gelatinization and gelification yielding a more porous network comprised of protein, intact granules and partially gelatinized starch granules. When a dried solid polymer material like starch is brought into contact with water, the water diffuses into polymer and diffusion will increase dramatically with increasing water activity (Luyten et al., 2004). In the breads baked with the highest steam level (400 ml), the crust showed the lowest weight loss after storage (Fig. 2), and in consequence, the lowest WVTR, thus the resulting crust network appears to decrease the rate of water diffusion.

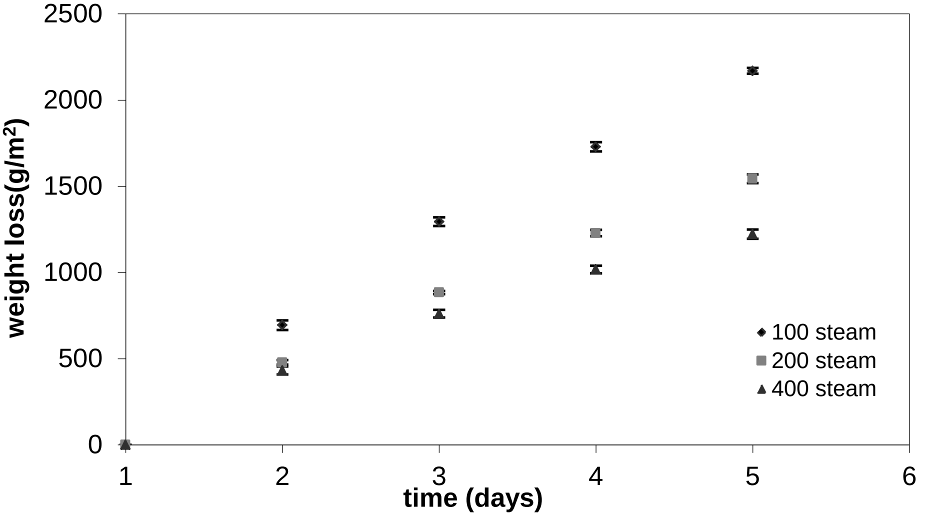

Fig. 2 The effect of amount of steam on the water vapour transfer rate (WVTR) as weight loss during the experimental time.

The water vapour permeability (WVP) results reported in Table1 showed significant (*P*<0.05) changes with the amount of steam. There was a steady decrease in WVP of the crust when increasing the amount of steam used during baking. Likely, the amount of steam modifies the heat penetration into the loaf from the surface to the inner parts, which will affect the crust thickness. In fact, low steaming produced thinner crust that favoured the water diffusion, whereas 200 and 400 ml of steam led to thicker crust that retains more moisture inside the loaf. Besides during baking there exists a concentration gradient of water vapour between the product surface and air. Therefore, water vapour is transferred through mass convection (Zhou and Therdthai, 2008). Changes in WVP values have been associated to structural changes in the polymeric matrix due to modification in the density of the network (Carvalho and Grosso, 2004).

WVP correction method was also applied to obtain corrected WVP using experimental WVTR data (Eq. (2)-(4)). For the WVP values without correction, it is assumed that the resistance to water vapour transport of the air gap between the crust and the surface of the liquid inside the cup is negligible. Conversely, correction method considers the water vapour partial pressure gradient in the air gap, where the driving force will be P2-P3. The corrected WVP values of the crust are displayed in Table 1. The corrected values of WVP were much lower than those without any correction. Thus in the case of breads it is important to consider the resistance to water vapour transport of the air gap between the crust and the liquid when calculating the water vapour permeability. This result agrees with McHugh et al. (1993), who reported that films based on hydrophilic polymers like proteins or polysaccharides are very sensitive to the moisture content diffusion and the air gap could drive to erroneous values. An increase of the amount of steam used in the baking decreased the corrected WVP.

Effect of the amount of steam on crust mechanical properties

Crust mechanical properties were measured by a puncture test (Jackman and Stanley, 1992), being dependent on the crust hardness and also on its microstructure. These parameters have been selected for characterizing crust of bread. Lately, high values of failure deformation have been related to stiffer crust structure due to the presence of thick crusts (Altamirano-Fortoul and Rosell, 2011). The effect of steaming on mechanical properties of the crust is shown in Table 1. The failure force was significantly ($P<0.05$) affected. The increase of the amount of steaming used during baking caused an increase in the failure force, lower value has been observed for lower steaming (100 ml). Therefore, lower values in failure force indicate soft crust, whereas high force suggests more brittle and rigid crust. In addition, the failure deformation decreased significantly ($P<0.05$) with the increase of the amount steaming. This may be explained by the distribution and size of starch granules, due to varied moisture content across the crust, which affect the mechanical properties of the cell walls of cereal-based food (Attenburrow et al., 1989).

The failure firmness augmented significantly (P<0.05) with the increase of the amount of steam (Table 1), probably due to the changes induced in the starch on the crust surface by the steam. The firmness is caused mainly by the formation of cross-links between partially solubilized starch and gluten proteins (Martin et al., 1991).

Effect of the amount of steaming on crust microstructure

Microstructure was studied in order to explain the effect of different amounts of steam used during baking on the mechanical properties of the crust, because the morphology of the crust in crispy foods determines the fracture behaviour (Luyten et al., 2004). Scanning electron microscopy (SEM) was applied to the crust surface. The micrographs of the morphology of the top and side crust sections are presented in Figs. 3 and 4. Bread baked with 100 ml of steam (Figs. 3a and b) showed a continuous sheet of clearly visible starch granules with homogeneous distribution, whereas, in the side or inner section (Fig. 4a) it was observed starch granules with lenticular shape, besides starch granules that preserved their integrity.

Bread baked with 200 ml of steam revealed a less compact structure (Fig.3c), also the starch granules were less spherical due to its swelling occurred during gelatinization (Fig. 3d), thus the amount of steaming used during baking resulted in a different starch structure. In the side section crust showed some swollen starch granules and these were surrounded by protein matrix (Fig. 4b). When 400 ml of steam were used, some deformed starch structure could be envisaged integrated in a gelled structure (Fig. 3e and f); the crust had smoother and uneven structure compared to the lower steam levels tested. The side section of this crust showed less intact starch granules on its surface, which were embedded in a very smooth film resulted from the gelatinization of the starch (Fig.4c).

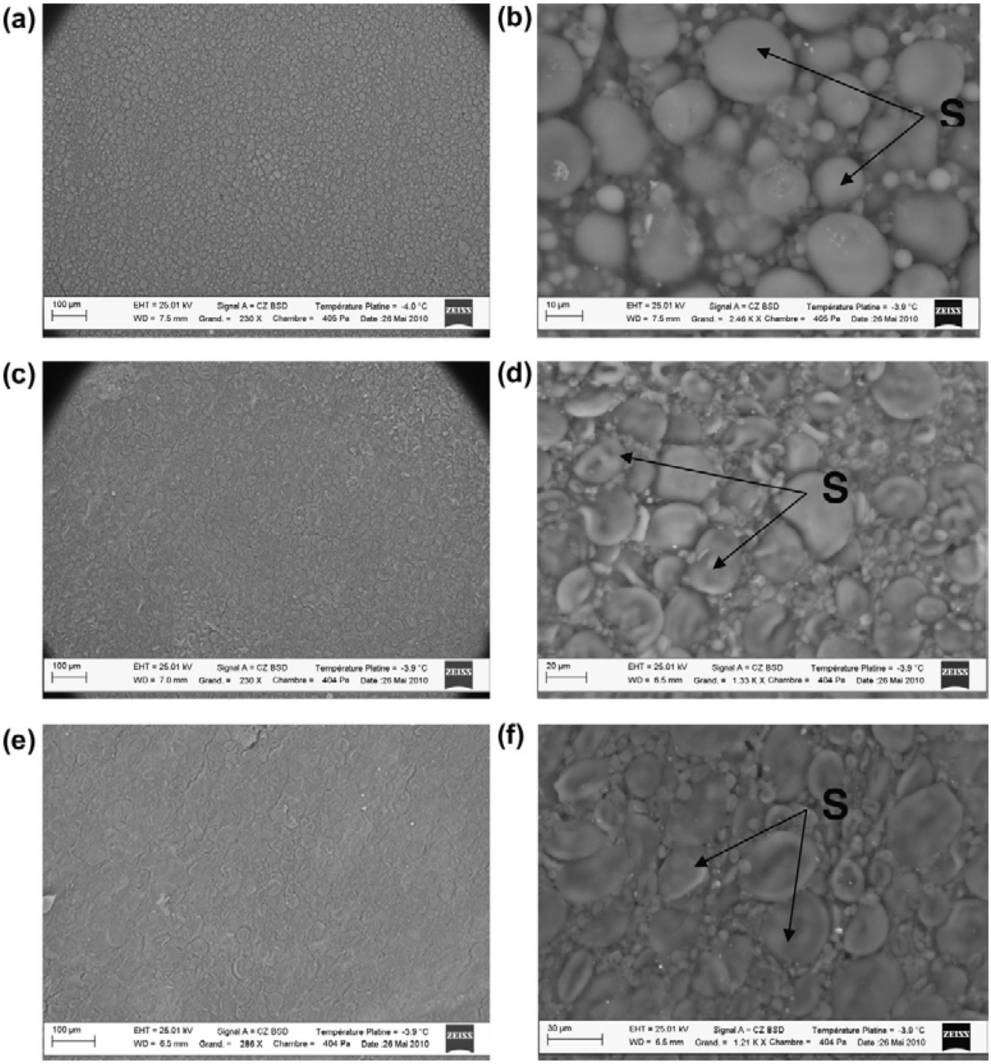

Fig. 3 Scanning electron micrographs of top crust section at different magnification. Top crust section in the left side (a, c, e) using a magnification of 250x and top crust section crust section in the right side (b, d, f) using adjusted magnification to show the starch granules. Pictures corresponded to: 100 ml of steam (a, b), 200 ml of steam (c, d), 400 ml of steam (e, f). Arrows show starch granules (S).

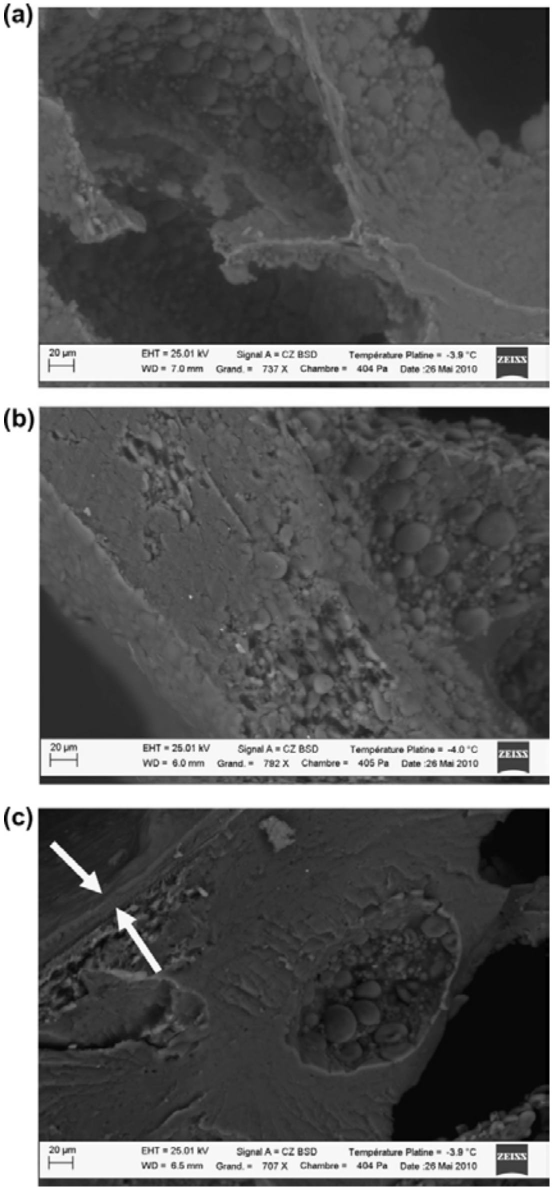

Fig.4 Scanning electron micrographs of side crust section using a magnification of 1000. Pictures corresponded to 100 ml (a), 200 ml (b), 300 ml (c). The arrows indicate the location of the crust outer surface, which appears to be thicker for the high steaming.

3D-images of the crust of breads baked with different amounts of steam obtained by X-ray microtomography are presented on Fig. 5. Different cell structures were observed depending on the amount of steam applied. Breads baked with 100 ml of steam showed more homogeneous and spherical crust cellular structure, the volume of the matrix being rendered in grey levels (Fig.5a). Very thin crust was observed on the surface. Breads baked with 200 ml of steam presented more heterogeneous cellular structure and cell size distribution, the previously spherical bubbles became elongated and a population of bigger bubbles coexisted with smaller ones mainly located towards the surface (Fig.5b). A denser zone was observed close to the surface indicating thicker crust. When much steam was applied during baking (samples with 400 ml), bubbles were more elongated, with a deformed appearance, it seems that their resistance to expansion was exceeded and the protein-starch matrix network was disturbed and broken in several places (Fig.5c). Thickness of the dense zone greatly increased.

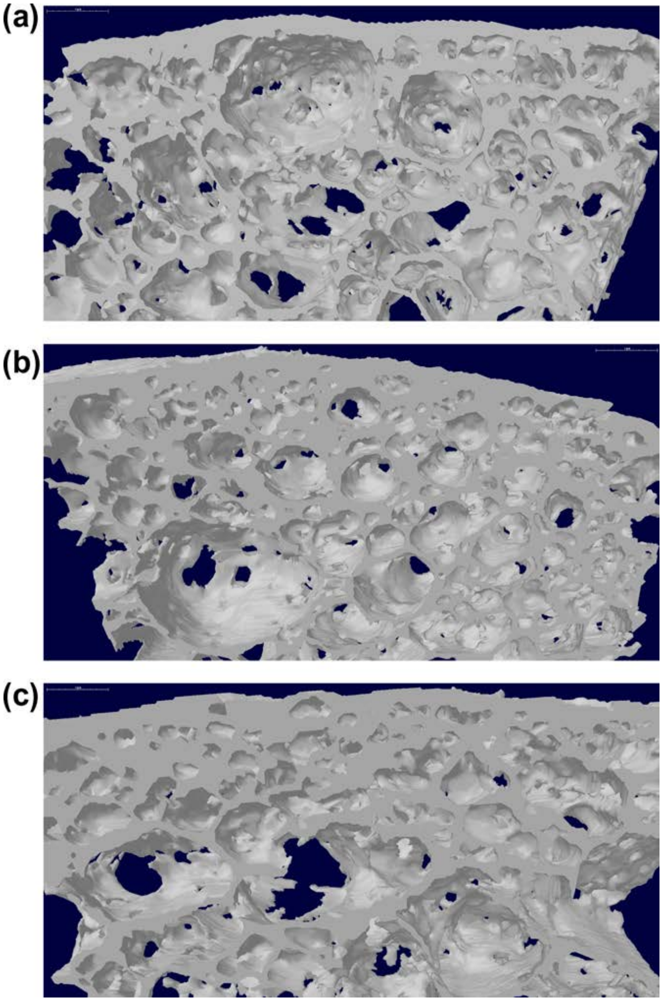

Fig. 5 Effect of amount of steaming on the crust microstructure by X-ray microtomography. 3D surface rendering of 100 vertical cross sections of images obtained at a magnification of 20x (1 pixel = 13.5 µm), volume size = 10 x 6 x 1.5 mm3: a) 100 ml of steam, b) 200 ml of steam and c) 400 ml of steam.

The evaluation of the local porosity has been calculated from X-rays microtomography images (Fig. 6). It appeared that baking with 100 ml of steam resulted in higher porosity than in the case of 200 ml and 400 ml. The higher porosity observed for 100 ml steamed samples is in agreement with the moisture permeability results observed for crust in comparison to values obtained for higher amount of steam. Indeed, higher porosity combined with thinner outer crust is supposed to favour moisture diffusion in comparison to a denser domain (lower porosity) such as those observed for 200 and 400 ml. Therefore, microstructure analysis confirmed that the amount of steaming used during baking greatly modifies the crust structure, affecting the bubbles shape and size, the proteins-starch network and surface density.

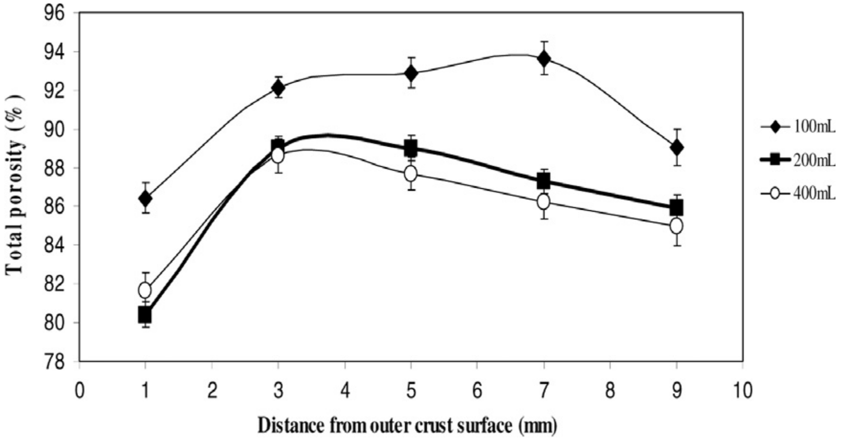

Fig. 6 Crust porosity expressed as percentage (calculated from X-ray microtomography images) in function of the distance from the outer crust surface.

CONCLUSION

Crust properties greatly determine the perception of the fresh bread, especially crispness. Those properties are significantly affected by the amount of steam applied during the baking process, particularly colour and glossiness, thickness, microstructure and texture properties. Low amounts of steam gave thinner crusts with high vapour transmission rate and permeability. Thus, lower permeability attributed to the crust can be directly related to porosity, which acted as a barrier affecting this property. On the other hand, it is necessary to apply the right amount of steam to obtain brittle and firm crusts in the fresh bread. Based on present results, it seems that a medium to low amount of steam results in a thinner crust, which in turn is more permeable to moisture during storage. Therefore, it is expected that such crust will remain brittle for longer time during storage after baking.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of Spanish Ministerio de Ciencia e Innovación (Project AGL 2008-00092 ALI) and the Consejo Superior de Investigaciones Científicas (CSIC). R. Altamirano-Fortoul would like to thank her grant to CSIC. Acknowledgements to Annick PERRONNET and Guillaume ROELENS from the Institut de Recherche en Génie Civil et Mécanique (GeM) UMR CNRS 6183 for SEM pictures.

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Chapter 3

TEXTURE OF BREAD CRUST: PUNCTURING SETTINGS EFFECT AND ITS RELATIONSHIP TO MICROSTRUCTURE

R. Altamirano-Fortoul, I. Hernando, C.M. Rosell

ABSTRACT

The effect of the puncturing settings (crosshead speed and punch cross-section) on the crust mechanical parameters was investigated using breads with two different crust thickness. Results showed that, greater punch cross-section was associated to compression behavior, which reduced the sensibility to detect changes in the crust structure. Moreover, low crosshead speed (0.5 mm/s) puncture test provided information about the cellular structure of the crust. The relationship between the puncturing parameters and the water activity and moisture content together with the crust microstructure analysis revealed that for obtaining reliable information about the structural ruptures related to crispiness texture, it is necessary to use low crosshead speeds (0.5 mm/s) and low punch cross-section (3 mm^2). Crust microstructure observations indicate that the crust layers and the size and shape of the air cells are responsible of the puncturing behavior.

***Key words*:** bread; crust texture; microstructure; moisture content; puncturing; water activity

INTRODUCTION

Texture is an important component for the consumer's perception of bread quality. Any food's texture is largely connected to its mechanical properties, which in principle are a direct consequence of the internal food microstructure. Texture is considered a multidimensional attribute that comprised a number of different textural properties (Bourne, 2002). In a more specific definition, texture is primarily the response of tactile senses to physical stimuli that result from contact between some part of the body and the food. In case of bread the textural attributes that better defined its quality and even freshness are crust crispness and crumb firmness. Different methods have been proposed to measure food mechanical features, which involve techniques like compression, penetration, acoustical procedure and sensory analysis (Van Vliet and Primo-Martín, 2011). Texture evaluation in crispy food is a complex subject, because of that the combination of techniques involving sensory analysis, acoustical procedure and instrumental analysis has been performed. Many authors use these techniques, for instance to evaluate the relationship between texture and sensory attributes on potato chips (Jaworska and Hoffmann, 2008). On the other hand, Duizier (2001) and Varela et al. (2008) used the combination of acoustic analysis with mechanical testing for analyzing crispness. Nevertheless, instrumental measurements are generally preferred to evaluate objectively the attributes related to the mechanical properties (Valles-Pamies et al., 2000). In fact, loudness, crispness and firmness are correlated with peak force, slope, force-deformation and Young's Modulus (Vickers and Christensen, 1980; Sauvageot and Blond, 1991; Valles-Pamies et al., 2000; Carr et al., 2006; Mazumder et al., 2007).

Regardless the instrumental technique used to characterize food texture, the puncture test, is one of the most widely applied for texture evaluation and its application in food texture measurement has been comprehensively studied (Georget et al., 1995; Van Hecke et al., 1998; Cheng et al., 2007; Arimi et al., 2010; Altamirano-Fortoul and Rosell 2011). Considering that the textural properties of a food depend on its structural elements, the puncture test has been used for defining the texture in cellular foods (Van Hecke et al., 1998; Valles Pamies et al., 2000). From the recorded force-displacement plots, obtained after fracture, it can be calculated the average of the so-called puncturing force (integral of force–time), the number of spatial ruptures, the average specific force of structural ruptures and the crispness work (Van Hecke et al.,1998; Valles Pamies et al., 2000). The puncture test measures the force required to push a punch or probe into a food. The test is characterized by (1) the force measuring instrument; (2) penetration of the probe into the food causing irreversible damage or failure results; and (3) the depth of penetration (Bourne,2002). Regarding crust mechanical properties, different instruments and settings have been used without applying any objective criteria for selecting those. Van Nieuwenhuijzen et al. (2008) used a texture analyzer (TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) to evaluate a model crust, where the pieces were punctured with a cylinder of 2 mm diameter at a speed of 0.1 mm/s. While, Primo-Martín et al. (2009) used a 32 mm diameter probe and a compression speed of 40 mm/s. The crust of crispy breads obtained from part-baked has been investigated by puncturing with a cylindrical probe (4 mm diameter) at crosshead speed of 40 mm/s (Altamirano-Fortoul and Rosell, 2011) or 50 mm/min (Mandala, 2005). Despite the wide use of puncturing for assessing crust features, there is no information about the incidence of punching settings on the bread crust mechanical parameters and neither the impact of the crust microstructure on those measurements.

The texture of food is a result of its core structure and the mechanical properties of this, being that some crispy foods are cellular solids, and they contain holes filled with gas or liquid (bread or vegetables and fruit). Nevertheless, it must be in mind that the mechanical behavior of the crispy food crusts is affected by their morphology and structure. Consequently, the use of instrumental methods and microstructure analysis would certainly be an alternative for providing more information about the texture of crispy food crust.

The objective of this study was to determine the effect of the puncturing settings (crosshead speed and punch cross section) on the crust mechanical parameters. Moreover, the study aimed to select the appropriate puncturing settings for identifying crust features based on water activity and moisture content of the crust and also its relationship to microstructure analysis. Scanning electron microscopy (SEM) of the crust section was used to confirm the reliability of the mechanical parameters. With that purpose, breads with two different crust thicknesses were used.

MATERIALS AND METHODS

Materials

Two different specialties of part-baked frozen breads provided by Forns Valencians S.A. (Valencia, Spain) were used. Those specialties were selected for giving breads with different crust section, thus hereafter they will be referred as thin and thick crusts. Chemical proximate composition of bread with thin crust was 30.1 g/100g moisture content, 60 g/100g carbohydrates, 2.74 g/100g fats and 6.41 g/100g proteins. The composition of the one with thick crust was 34.3 g/100g moisture content, 59 g/100g carbohydrates, 0.72 g/100g fats and 5.41 g/100g proteins.

Full baking process and storage

Part-baked breads were removed from the freezer (-18º C) and thawed at room temperature until the center of the loaf reached 5º C. Then, they were baked in an electric oven (Eurofours, France). Baking conditions varied with specialty and were as follows: 180° C for 11 min in the case of bread with thick crust, 180° C during 18 min for the one with thin crust. Both specialties required a preheated oven at 220° C.

For each specialty, two sets of samples were used, which were baked in separate days. Fresh loaves (0.5 h after baking) were tested for textural characteristics (mechanical properties), water activity, moisture content, crust section and structure.

Physico-chemical analysis

Moisture content and water activity were determined in the crust and crumb of breads. Crust and crumb were separated using a razor blade based on white versus brown color. Moisture content was determined following ICC standard method (1994) (ICC 110/1). Water activities were measured using a water activity unit (Aqua Lab Series 3, Decagon devices, Pullman, USA) at 25° C.

Crust section analysis was performed by scanning cross sections of bread sample, 10 mm thick, in a flat bed scanner equipped with the software HP PrecisoScan Pro version 3.1

(HP scanjet 4400C, Hewlett-Packard, Madrid, Spain). The default settings for brightness (midtones 2.2) and contrast (highlights 240, midtones 2.2, shadows 5) of the scanner software were used for acquiring the images. The crust section was calculated from the scanned samples at the upper and bottom side using an image analysis program (UTHSCSA Image Tool software, TX).

All determinations were carried out in triplicate for each set of samples.

Puncture tests

Loaves were puncture tested using a texture analyzer with a 5 kg load (TA XTplus, Stable Micro Systems, Surrey, UK). The analysis consisted in recording the force required to penetrate the bread crust by punching the sample at three different points of bread surface: in the middle of the crust area and at 2 cm distance on both sides. Experiments were carried out using cylindrical probes with diameters 2 mm (punching area=3 mm^2), and 6 mm (punching area= 28 mm^2) at varied crosshead speed that included 0.5, 1, 10, 20, 30 and 40 mm/s.

The data were analyzed using the method proposed by Van Hecke et al. (1998). This method is based on the peak analysis of the force-deformation curves. From the force-deformation curve, the following puncturing parameters were determined:

Average puncturing force $Fm(N) = \frac{A}{d}$ Eq. (1)

Spatial frequency of structural ruptures $N_{wr}(m^{-1}) = \frac{No}{d}$ Eq. (2)

Average specific force of structural ruptures $f_{wr}(N) = \sum \frac{\Delta F}{No}$ Eq. (3)

Crispness work $W_c(N.m) = \frac{Fm}{N_{wr}}$ Eq. (4)

Where: No is the total number of peaks, d is the distance of penetration (mm), ΔF is the individual force drops for each peak (N) and A is the area under the force-deformation curve.

Four breads from each set were used for carrying on the puncture test, obtaining 24 individual measurements for each experimental point.

SEM of bread crust

Scanning electron microscopy was used to examine the crust of bread. Slices of bread were freeze-dried previously to the microscopy analysis. Sample cubes (1cm^3) were fixed with the aid of colloidal silver and then coated with gold (Baltec SCD005) at 10^{-2} Pa and an ionisation current of 40 mA. The observation was carried out in a JEOL JSM-5410 scanning electronic microscope at 10 kV.

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, Cambridge, UK). In order to assess significant differences among samples, a multiple sample comparison was performed. The analysis of variance was carried out to decompose the variance of the data into two components: a between-group component and a within-group component and a within-group component. When the *P*-value of the *F*-test was lesser than 0.05, there was statistically significant difference between the means of the two groups at the 95.0% confidence level. Multiple range test was used to determine which means were significantly different from which others and Fisher's least significant difference procedure was used to discriminate among the means. A correlation matrix was established to analyze the relationship among variables. The correlation matrix shows Pearson’s product moment correlations between each pair of variables (Rodgers and Nicewander, 1988).

RESULTS AND DISCUSSION

Physicochemical characteristics of fresh bread

The crust section was initially determined in the specialties selected to confirm that they obey the criteria of significant different crust width. The upper crust of the sample identified as thick crust had a section of 5.10 mm, which was significant (P <0.05) different than that in the bread with thin crust (2.94 mm). Sluimer (2005) reported that in the case of a soft bun or pan bread, the thickness of the crust is restricted to 2-5 mm on the top crust and the side and bottom crust are thinner.

Numerous studies have been carried out stating the importance of the moisture content and water activity on the crust mechanical properties (Van Hecke et al., 1998; Kawas and Moreira, 2001; Lewiki, 2004; Varela et al., 2008). Water is a constituent of food which affects food stability, quality and textural properties; because of that, special emphasis was put in assessing moisture content in the loaves. A significant difference was determined at a confidence level of 95% between the water activity and moisture content of the two breads. In the bread with thin crust, the water activity of the crust was 0.522 and 0.971 for the crumb. On the opposite side, the bread with thick crust exhibited the highest (0.540) water activity in the crust with also high values in crumb (0.976). Castro-Prada et al. (2009) reported that increment in water activity of crispy products induces the transition from brittle to ductile fracture behavior. Water leads to plasticization and thus alters the mechanical properties of the product. Previous studies in bread reported that a_w of 0.68-0.69 would be the point where the mechanical transition (brittle to tough) of crust occurs (Altamirano-Fortoul and Rosell, 2010). In the present study, the water activity values for both crust thickness were lower than that range, which ensures that experimental assessment of crust mechanical properties were carried out when they behave as crispy material.

Similarly, moisture content in the crust and in the crumb showed significant differences; being 11.46 g/100 g and 40.89 g/100 g for the bread with thick crust and 9.67 g/100 g and 39.16 g/100 g for the bread with thin crust, respectively. Those values were within the range of previous findings for breads (Baik and Chinachoti, 2000; Altamirano-Fortoul and Rosell, 2011).

Effect of punching settings on the puncturing parameters of thin and thick bread crust

Bread crust texture was measured by a puncture test and the method proposed by Van Hecke et al. (1998) was used for assessing puncturing parameters from the force-time or deformation curves. A multiple comparison analysis was performed to determine the possible effect of the punch cross-section (probe) and crosshead speed and to have greater understanding of the factors that influence the puncturing parameters. The puncturing conditions (punch cross-section and speed) affected significantly (*P* <0.05) all mechanical parameters used to characterize the bread crust in two types of samples (breads with thin and thick crusts).

The first parameter defined from the force-time or deformation curve was the average puncturing force (*F*m), which represents the mechanical resistance that has a product when a force is applied. Fig. 1 shows the influence of punch cross-section and test speed on *F*m parameter when the texture of crust was determined in two types of breads which differed on the crust thickness.

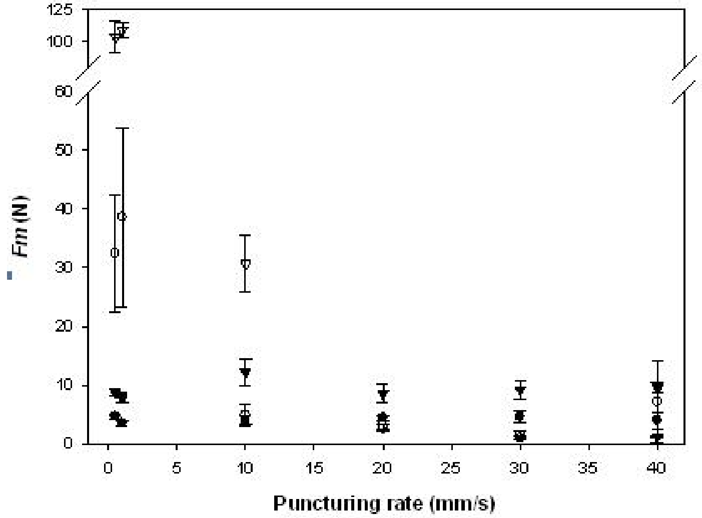

Fig. 1 Effect of texture settings (crosshead speed and punch cross-section) on the average puncturing force (*F*m) of thick (open symbols) and thin (closed symbols) crust bread. Legends: 3mm^2 punch cross-section (●), 28 mm^2 punch cross-section (▼).

In general, higher values were observed with larger punch cross-section (28 mm^2) in both samples. Therefore, regardless the thickness of the crust, *F*m parameter was dependent on the punch cross section. With major probe section higher number of cells is compressed leading to wider force-deformation curve, where the maximum force is an indication of the peak resistance offered by crust during compression. An increase in the section to be compressed enhances the force and consequently the other parameters related to the force. In fact, Van Hecke et al. (1998), when studied the mechanical parameters in extruded products, found that at higher punch cross-section, the compression acquires major importance. In addition, the average puncturing force was crosshead speed dependent, obtaining higher values for *F*m at lower speed, and that effect was more noticeable with thick crust. The speed of travel of the punch is an important factor when testing viscoelastic foods because they are strain-rate sensitive (Bourne 2002). As expected, thick crust required great force (*F*m) for puncturing than thin crust, but that was only correct for puncturing test carried out at crosshead speed up to 10mm/s, and beyond that speed the trend was reversed.

In thin bread crust, the average puncturing force was more dependent of the probe than the speed, obtaining higher *F*m values at higher punch cross-section, but values were barely constant when modifying the crosshead speed. In the case of thick bread crust, independently of the punching section, the maximum *F*m values were obtained at lower speeds, and a progressive decreased of this parameter was observed when increasing the speed. The low speed produced jagged force-displacement plots, resulting slow speed of deformation, where the molecules presented conformational changes in direction of applied force, resulting in higher mechanical resistance. These results agree with the explication of Tsukakoshi et al. (2008) that observed differences in force-deformation curves based on the plunger or probe speed.

The N_{wr} parameter, or spatial frequency of structural ruptures, is related to the number of peaks caused during the fracture of the sample. Therefore, a highly jagged curve profile with many peaks because of numerous fracture events is often produced by products that are perceived as crispy. In fact, if the sample is undergoing a large amount of fracture events, this is related to a more crispy texture (Vincent, 1998). Considering has been mentioned earlier, if during the fracture events there is major number of structural ruptures (high value of N_{wr}), it indicates that the sample is more crispy. In Figure 2, it is shown the effect of the punch cross-section and speed on N_{wr} parameter. The effect of the puncturing conditions on the N_{wr} was dependent on the crust section. In the thick bread crust, the N_{wr} versus speed plot showed a bell shape trend, with maxima values within 20 mm/s to 30 mm/s speeds, and significantly higher values were obtained when using lower punch cross-section (3 mm^2). Those results demonstrate that a greater punch cross-section caused the decrease of the jaggedness of the force-deformation curve as well as the peaks present, and consequently there were fewer structural ruptures. Considering that a thickness of more than 5 mm is a good level for crusty bread (Sluimer, 2005); this thick bread crust might have more cellular structure, therefore there were more structural ruptures in short time.

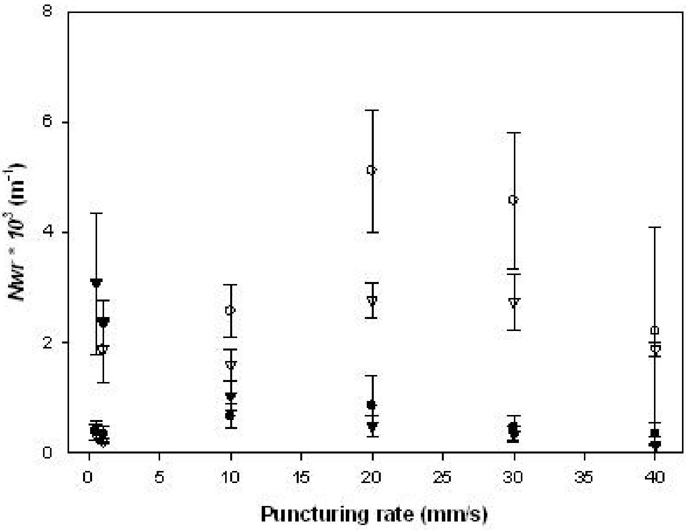

Fig. 2 Effect of texture settings (crosshead speed and punch cross-section) on the frequency of structural ruptures (N_{wr}) of thick (open symbols) and thin (closed symbols) crust bread. Legends: 3mm^2 punch cross-section (●), 28 mm^2 punch cross-section (▼).

In thin bread crust, the highest value of this parameter was obtained when using greater punch cross-section (28 mm^2), but the tendency reversed with increasing speed (puncturing speed ≥20 mm/s). With low punch cross-section (3 mm^2) the N_{wr} parameter showed the same bell shape plot that was observed with the thick bread crust.

Overall results suggest that with smaller punch cross-section, major number of peaks corresponding to structural ruptures can be detected within less deformation and thus causes greater values of N_{wr} parameter.

Concerning the f_{wr} parameter that relates the specific force with the structural ruptures, higher values were obtained with great punch cross-section (28 mm^2) (Fig. 3). Therefore, greater punch section caused a major structural rupture because it encompasses fractures in the walls and small microscopic structures. Since, it is difficult to attribute one observed fracture to a single wall beneath the center of the plunger, as was observed by Tsukakoshi et al. (2008) when studied mechanical properties of snacks by using a wide plunger.

In the thin bread crust, f_{wr} parameter increased significantly when using the punch cross-section of 28 mm^2 at speed higher than 1 mm/s speed. Nevertheless, with smaller punch cross-section (3 mm^2) this parameter exhibited a slightly different behaviour; it showed maximum value at 0.5 mm/s crosshead speed and after a steep decrease, it showed a steady increase from 20 mm/s. In the thick bread crust, a more erratic behavior was observed, but it was possible to detect that the values obtained with the low punch cross-section were less dependent on the crosshead speed than those obtained with the punch cross-section of 28 mm^2. Therefore, results on average specific force with the structural ruptures show that independently of crust thickness, at higher speeds and punch section the mechanical resistance in the cell walls increases.

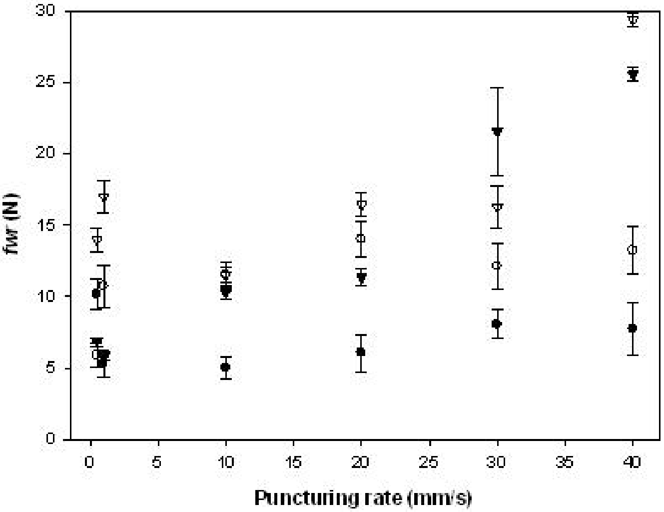

Fig. 3 Effect of texture settings (crosshead speed and punch cross-section) on the average specific force of structural ruptures (f_{wr}) of thick (open symbols) and thin (closed symbols) crust bread. Legends: 3mm^2 punch cross-section (●), 28 mm^2 punch cross-section (▼).

The parameter crispness work, W_c, was significantly dependent on the punch cross-section and the crosshead speed used for carried out the assessment (Fig. 4). Crispness work decreased with the crosshead speed of puncturing assay, excepting when thin bread crust was punctured with large punch cross-section (28 mm^2). With the large punch cross-section, W_c parameter showed a steady increased with the crosshead speed when assessing thin bread crust mechanical properties. Dan and Kohyama (2007) found that the increase in fracture work associated with the test speed is due to higher fracture force. However, present results indicate that large punch cross-section can be responsible of that behaviour, where pure compression force could mask the real fracture force. Crispness work results suggest that only by using low puncturing speed is it possible to assess this parameter, independently of the punch cross-section used, reducing the interference of the compression.

The high value of crispness work indicates that the structure is difficult to fracture. Considering that thick bread crust has a more crispy texture, with stiff structure that collapse with a brittle fracture, the fracture of the crust would need more work.

Therefore, puncturing settings play a fundamental role when determining the mechanical parameters of bread crust. Crosshead speed of the puncture test and the punch cross-section affected the mechanical parameters and the effect was largely noticeable with thick bread crusts.

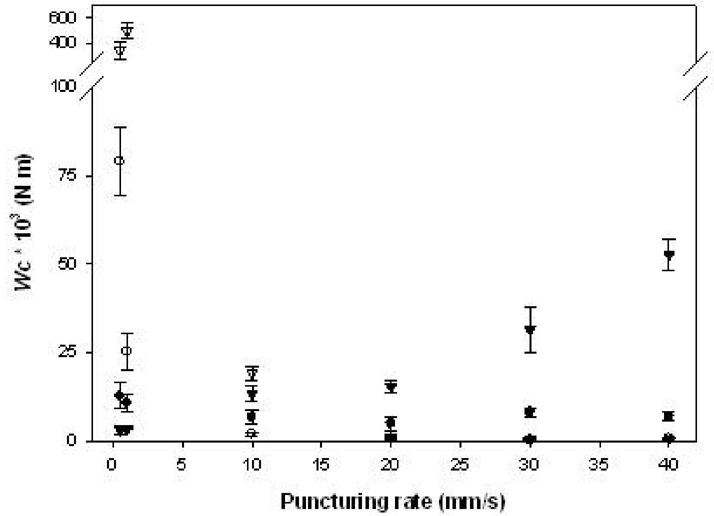

Fig. 4 Effect of texture settings (crosshead speed and punch cross-section) on the crispness work (W_c) of thick (open symbols) and thin (closed symbols) crust bread. Legends: 3mm^2 punch cross-section (●), 28 mm^2 punch cross-section (▼).

Relationship between crust puncturing parameters and water activity and moisture content

It has been previously stated the role of water in the mechanical properties of foodstuff. However, because the values of the mechanical parameters are greatly dependent on the setting conditions, a possible relationship between the puncturing parameters and the water activity and moisture content must be established.

A multivariate analysis was applied to determine the possible correlation among the water and the puncturing parameters that defined the mechanical properties of the crust (Table 1 and 2) using the two punch cross-section tested and the lowest (0.5 mm/s) and highest (40 mm/s) crosshead speeds used.

Table 1. Correlation Matrix.

Parameters	Parameters $Nwr\ (mm^{-1})$	fwr (N)	Wc (Nmm)	a_w	Moisture (%)
Fm (N)	-0.3935 *	0.7144 ***	0.9703 ***	0.5044 **	0.7309 ***
$Nwr\ (mm^{-1})$		-0.4157 *	-0.4244 *	-0.456 **	-0.5272 ***
fwr (N)			0.7559 ***	0.1636	0.2274
Wc				0.4852 **	0.695 ***
a_w					0.6083 ***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Correlation coefficients and *P*-value within the physical and puncturing paramaters of the thin and thick crust bread determined at crosshead speed of 0.5 mm/s and with 3mm^2 punch cross-section.

Using the experimental data obtained at the low speed and punch cross-section, regardless of the crust thickness (Table 1), a significant positive relationship was obtained between the *F*m parameter and water activity and moisture content. A significant negative relationship was observed with the spatial frequency of structural ruptures (N_{wr}) and the water activity and moisture content. In addition, a significant positive relationship was obtained between the crispness work, W_c parameter, and moisture content and water activity. No significant relationships were observed between f_{wr} and neither water activity or moisture content.

Some divergences were obtained when the experimental data obtained at high speed and punch cross-section were used for obtaining the correlation matrix (Table 2). Again, the *F*m showed a significant positive relationship with the water activity and moisture content, and no relationship was observed with f_{wr}. Conversely to results obtained at low speed and low punch cross-section, a significant positive relationship was observed with the spatial frequency of structural ruptures (N_{wr}) and the water activity and moisture content and the correlation coefficients indicated a moderately strong relationship between the variables.

Table 2. Correlation Matrix.

Parameters	Parameters				
	Nwr (mm^{-1})	fwr (N)	Wc (Nmm)	a_w	Moisture (%)
Fm (N)	-0.6131 ***	0.3102 *	0.9686 ***	0.6572 **	0.9837 ***
Nwr (mm^{-1})		0.4424 *	-0.6232 ***	0.5139 **	0.8138 ***
fwr (N)			0.3488 *	0.1725	0.2553
Wc				-0.4111 *	-0.6541 ***
a_w					0.6083 ***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Correlation coefficients and P-value within the physical and puncturing paramaters of the thin and thick crust bread determined at crosshead speed of 40 mm/s and with 28mm^2 punch cross-section.

Whereas W_c parameter showed a significant negative relationships with water activity and moisture content.

According to the results presented in Table 1 and 2, it should be remarked that water influences the mechanical properties of bread crusts as well as it affected their response to force required for crust fracture. The positive relationship Fm with the water activity and moisture content independently of the test conditions might be consequence of the strong interaction of water molecules with the rest of the macromolecules that constitute the bread crust. Conversely, the frequency of structural ruptures (N_{wr}) and the crispness work were more sensitive to experimental settings. Taking into account that water molecules act as plasticizer and soften the starch–protein matrix, besides that an increase in water activity or in the moisture content decreases the frequency of structural ruptures (N_{wr}) (Van Hecke et al., 1998), it seems that mechanical properties of bread crust might be only accurately determined when low crosshead speed and low punch cross-section are used (Table1), where N_{wr} and W_c show negative relationship with the water activity and water moisture content.

Crust structure

In order to support the puncturing parameters results, the microstructure of the cross-section in each bread was analyzed by SEM. Micrographs confirmed microstructure differences between thin and thick crusts of bread (Fig. 5). Two different structural zones were detected in the so-called bread crust, a dry crust and a sub-crust. The sub-crust is of great importance because it gives rise to chemical transition between the crust and the crumb and also allows to show where ends the crust and where begins the crumb. Figure 5a,b show the micrographs of crust zones in both samples. The swollen granules and partially solubilized starch as well as proteins act as essential structural elements of bread. Therefore, bread crust is formed by a network of gluten protein with starch granules (Cuq et al., 2003). Thin bread crust presented a small section of zone 1 than the thick bread crust. In addition, thin bread crust showed a smooth zone 2 (Fig. 5a), whereas thick bread crust exhibited an irregular zone 2 with cracking structure (Fig. 5b). Therefore, the differences observed when assessing the mechanical parameters responded to microstructural differences. The puncture plot of the thick bread crust displayed irregular peaks in the force curves, which is characteristic of a crispy product. This crust presented multiple fracture events, which could be the result of cracks and irregular morphology.

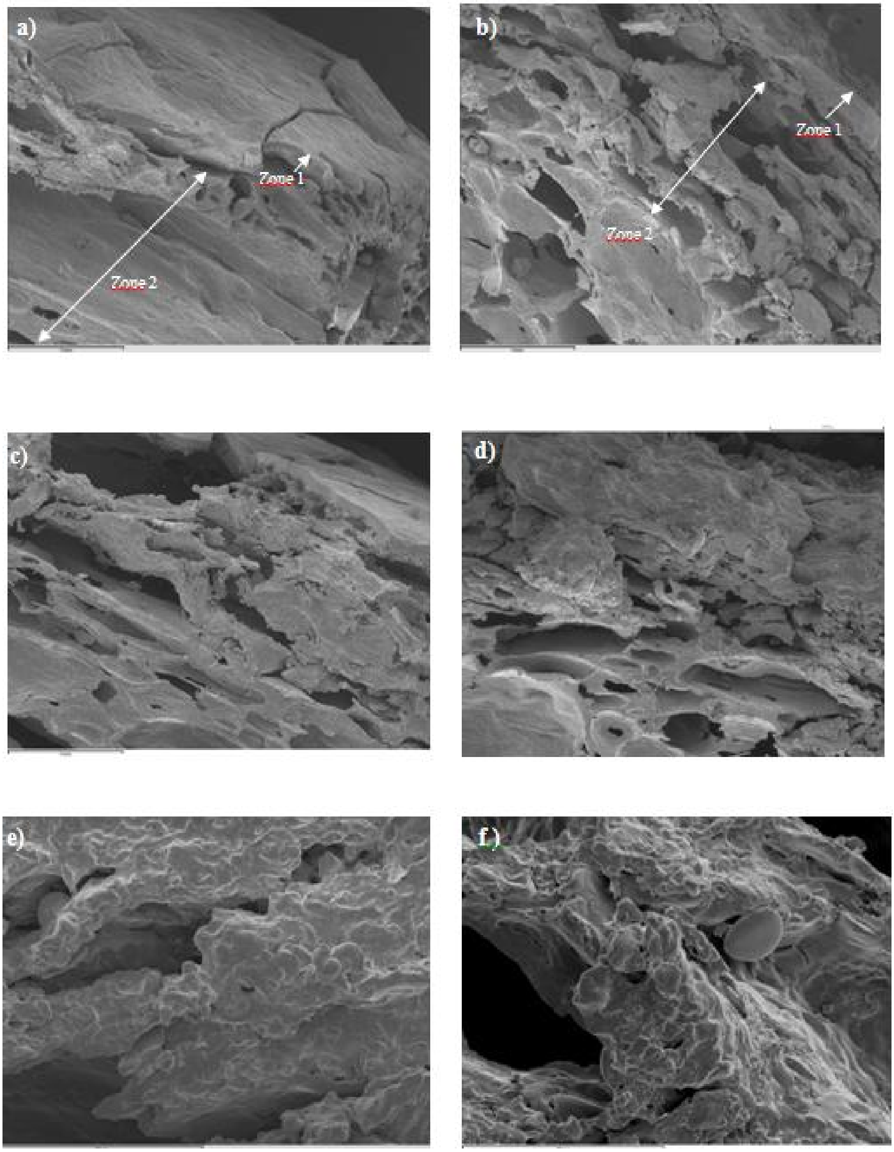

Fig. 5 Scanning Electron Micrographs of crust cross-section**.** Images correspond to cross-section of breads with thin (a,c,e) and thick (b,d,f) crust. Micrographs of zone of cross-section of bread crust (a,b), inner zone 1 (c,d) at high (750x) magnification (e,f) are show.

In zone 1, it was possible to detect the layers and air cells with varying dimensions, where the differences in the cellular structure were readily apparent. The thin bread crust showed few layers (Fig. 5c), whereas the thick bread crust had more layers and contain more irregular and larger cells (Fig. 5d). That observation is in accordance with the puncturing results, because greater values were obtained for thicker bread crust, because the presence of air cells more fully stretched required greater force to fracture them.

At higher magnification, in the inner zone of the crust, the thin crust showed a homogeneous structure, where starch granules were embedded in a lipoprotein network and covered by a coating (Fig. 5e). However, thick crust showed some loose starch granules on the matrix surface, giving place to a more heterogeneous starch structure with the presence of cells and discontinuous phase (Fig. 5f). This effect might be responsible of the mechanical behavior of thick crust, which was much less resistant to the compression and collapsed as a result of the compression force on the surface; therefore the fracture occurred rapidly. The morphology of the crust in crispy foods determines the behavior of the mechanical properties (Luyten et al., 2004). Sam et al. (2001) found that chips with thicker cell walls and larger internal voids were judged crispier, which is consistent with the thick crust results.

Possibly, the differences in the structure between the samples were ameliorated by the water molecules present in the breads due to a plasticizing effect. Martinez-Navarrete et al. (2004) found that water leads to plasticization and softening of the starch-protein matrix and thus alters the strength of the product. On the other hand, the starch granule may therefore contribute to the product architecture in different ways.

CONCLUSIONS

When studying the mechanical properties of bread crust by puncturing, the test settings, namely crosshead speed and punch cross-section, exert great influence in the data recorded, and that effect is magnified when increases crust thickness. The relationship between the puncturing parameters and the water activity and moisture content together with the crust microstructure analysis allow selecting the most appropriate texture settings for obtaining reliable mechanical parameters. Strong positive relationship is observed between the average puncturing force (*F*m) and the water activity and moisture content. However, for obtaining reliable information about the cellular structure of the crust, that is the amount of fracture events or structural ruptures related to crispiness texture, it is necessary to use low crosshead speeds (0.5 mm/s) and low punch cross-section (3 mm^2), which will reduce the incidence of the compression. Crust microstructure observations indicate that the crust layers and the size and shape of the air cells are responsible of the puncturing behavior.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of Spanish Ministerio de Ciencia e Innovación (Project AGL2008-00092/ALI and AGL2011-23802) and the Consejo Superior de Investigaciones Científicas (CSIC). Altamirano-Fortoul would like to thank her PhD grant (JAE pre) to CSIC. The authors also thank Forns Valencians S.A. (Spain) for supplying commercial frozen partially baked breads.

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Chapter 4

INFLUENCE OF AMYLOGLUCOSIDASE IN BREAD CRUST PROPERTIES

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ABSTRACT

Enzymes are used in baking as a useful tool for improving the processing behavior or properties of baked products. A number of enzymes have been proposed for improving specific volume, imparting softness or extend the shelf life of breads, but scarce studies have been focused on bread crust. The aim of this study was to determine the use of amyloglucosidase for modulating the properties of the bread crust and increase its crispness. Increasing levels of enzyme were applied onto the surface of two different partially bake breads (thin and thick crust bread). Amyloglucosidase treatment affected significantly ($P<0.05$) the colour of the crust and decreased the moisture content and water activity of the crusts. Mechanical properties were modified by amyloglucosidase, namely increasing levels of enzyme promoted a decrease in the force (Fm) required for crust rupture and an increase in the number of fracture events (N_{wr}) related to crispy products. Crust microstructure analysis confirmed that enzymatic treatment caused changes in the bread crust structure, leading to a disruption of the structure, by removing the starchy layer that covered the granules and increasing the number of voids, which agree with the texture fragility.

Key words: bread crust; amyloglucosidase; colour properties; water activity; puncturing; microstructure.

INTRODUCTION

Bread is considered worldwide a staple food; being one of the most important sources for human nutrition that provides starch and complex carbohydrates, proteins, minerals and vitamins (Rosell, 2007; 2011). Current consumption trends show that consumers demand fresh bread all day along and freshness is pointed as an essential attribute (Heenan et al., 2008). Fresh bread usually presents an appealing brownish, crispy crust, besides a pleasant aroma and a soft and elastic crumb texture. Nevertheless, those attributes, particularly the crust crispiness, are very rapidly lost. Crust refers to the part of bread near the surface, which is formed during the final baking. Crust has very low water content (Wählby and Skjöldebrand, 2002), because of that it is relatively dry, crisp and brittle in the fresh state (Hug-Iten et al., 2003). Water migration from the crumb and the atmosphere surroundings to the crust induces a transition from the glassy to the rubbery state of the main crust macromolecules (Gondek et al., 2006; Jakubczyk et al., 2008; Van Nieuwenhuijzen et al., 2008; Castro-Prada et al., 2009; Arimi et al., 2010). As consequence, the mechanical properties of the crust associated to crispness changes and crust becomes very soft and leathery (Roudaut et al., 1998), which causes consumer’s rejection.

Texture has been widely used for assessing bread freshness either by determining crumb hardness or crust crispiness, both of those directly related to bread acceptability. Texture of the bread crust is an important parameter used to define the quality of crispy breads and their freshness, in which multiple sensations involving numerous physical parameters, combining molecular, structural and manufacturing process are implicated (Roudaut et al., 2002, Luyten et al., 2004). Crispy bread crust is originated when starch and gluten matrix are in glassy state and it has been associated with low moisture content and water activity (Stokes and Donald, 2000). Different methods have been proposed for assessing the mechanical properties of the bread crust, although punching is a common feature in all of them. Recently, Altamirano-Fortoul et al. (2013) defined the optimal punching settings for assessing the crust mechanical properties providing information about the internal cell structure. Their results were also supported by water activity and moisture content determinations, and scanning electron microscopy of the crust section, which confirmed the reliability of the mechanical parameters.

Although very much attention has been paid to bread crumb and alternatives to soften it, scarce information has been reported about the crust behavior and how to modulate its mechanical properties. Primo-Martín et al. (2006) reported the effect of different enzymes (endoprotease, transglutaminase, alpha-amylase), sprayed onto the dough surface, as possible strategy for extending crust crispiness. Those authors observed that protease activity led to crust with lower water content, stating that protein network has a main role in the crispness perception. In order to control crust moisture diffusion and water uptake to preserve crispy texture, some attempts using hydrocolloids and enzymes have been reported (Altamirano-Fortoul and Rosell, 2010; Rosell et al., 2011). Nevertheless, the possible role of the starch on the mechanical texture of crust is far from understood.

Amyloglucosidase is also used in bakery applications, because its hydrolytic activity on starch yields faster fermentations (Sharma and Singh, 2010), improves bread crust colour (Van Oort, 2010), and enhances flavor in crackers (Heiniö et al., 2012). Also, this enzyme is suggested to delay bread staling due to decreasing retrogradation of amylopectin (Würsch and Gumy, 1994). In fact, anti-staling effects of amyloglucosidase in baking are claimed in some patents (Vidal and Guerrety, 1979; Van Eijk, 1991; Van Benschop et al., 2012).

The aim of the present research was to determine whether amyloglucosidase could be used to modulate the mechanical properties of bread crust. For that purpose, the effect of different concentrations of amyloglucosidase on the physicochemical, mechanical properties and the crust structure were tested. Enzyme solutions were applied onto the surface of two specialties of partially baked bread and the crust features of the full baked breads were assessed. Scanning electron microscopy (SEM) of the crust section was used to confirm the reliability of the mechanical parameters.

MATERIALS AND METHODS

Two different specialties of part-baked frozen breads provided by Forns Valencians S.A. (Valencia, Spain) were used. Those specialties were selected for giving breads with different crust section, thus hereafter they will be mentioned as thin and thick crusts. Chemical proximate composition of bread with thin crust was 30.1 g/100g moisture content, 60 g/100g carbohydrates (calculated by difference), 2.74 g/100g fats and 6.41 g/100g proteins. The composition of the bread with thick crust was 34.3 g/100g moisture content, 59 g/100g carbohydrates (calculated by difference), 0.72 g/100g fats and 5.41 g/100g proteins.

A food grade commercial amyloglucosidase from *Aspergillus niger (*Amyloglucosidase 1100BG, 1100AGU/g) was provided by Novozyme A/S (Bagsvaerd, Denmark).

Enzymatic treatments

Amyloglucosidase was used to selectively modify the crust starchy components. Enzymatic solutions were prepared by suspending the commercial enzyme in distilled water at the levels described in Table 1.

Table1. Enzyme concentrations applied onto the bread surface (2 ml were applied per loaf).

Treatment	Code	Description	Dosage
Control	C	Distilled water	0mg/10 ml
AMG	A1	Amyloglucosidase	100mg/10ml
	A2	Amyloglucosidase	250mg/10ml
	A3	Amyloglucosidase	500mg/10ml
	A4	Amyloglucosidase	1000mg/10ml

Full baking process and storage

Part-baked breads were taken from the freezer (-18ºC) and were placed at room temperature. Loaves were spread evenly with enzymatic solutions over the top surface before baking. The amount of enzyme solution (2 ml) used per piece of bread was sufficient to cover the whole top surface (118.3± 1 cm^2). Dosages were calculated based on previous studies (Altamirano-Fortoul and Rosell, 2010). Control bread was similarly treated but without enzyme. Loaves were thawed at room temperature till the center of the loaf reached 5ºC. After thawing, loaves were baked off in a forced convection oven (Eurofours, Gommegnies, France). Baking conditions varied with specialty and were as follows: 180° C for 11 min in the case of bread with thick crust, 180° C during 16 min for the one with thin crust. Both specialties required a preheated oven at 220°C. For each specialty, three sets of samples were performed for each treatment, which were baked in separate days.

Fresh loaves (0.5 h after baking) were tested for textural characteristics (mechanical properties), water activity, moisture content, crust section, crust colour and structure.

Physico-chemical analysis

Moisture content and water activity were determined in the crust and crumb of breads. Crust and crumb were separated using a razor blade based on white versus brown colour.

Moisture content was determined following ICC standard method (1994) (ICC 110/1). Water activities were measured using a water activity unit (Aqua Lab Series 3, Decagon Devices, Pullman, USA) at 25°C. Crust section analysis was performed by scanning cross section of bread sample, 10 mm thick, in a flat bed scanner equipped with the software HP PrecisoScan Pro version 3.1 (HP scanjet 4400C, Hewlett-Packard, USA). The default settings for brightness (midtones 2.2) and contrast (highlights 240, midtones 2.2, shadows 5) of the scanner software were used for acquiring the images. The crust section was calculated from the scanned samples at the upper and bottom side using an image analysis program (UTHSCSA Image Tool software, TX, USA).

Colour parameters of bread crust were measured at three different locations by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate (L^* = 96.9, a^* = -0.04, b^* = 1.84). The colour was recorded using CIE-L^* a^* b^* uniform colour space (CIE-Lab) and D65 illuminant, where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis. The results were reported in the form of total colour difference using Eq (1).

$$\Delta E = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)}^{2} \qquad \text{Eq. (1)}$$

Where: ΔL^*, $\Delta a^*$and Δb^* are the differences between the L^*, a^* and b^* values of the sample and white plate calibration.

Crust darkness was determined on as 100-L^* (100-L^* =0, white and 100-L^* = 100, black) (Sahlström and Brathen, 1997).

Three measurements were performed in each bread and three breads from each treatment were used for this determination. Crust samples were freeze dried and kept for further microstructure studies. Preliminary tests were carried out to confirm that freeze drying was not affecting the crust microstructure.

Puncture tests

Loaves were puncture tested using a texture analyzer with a 5 kg load (TA XTplus, Stable Micro Systems, Surrey, UK). The analysis consisted in recording the force required to penetrate the bread crust by punching the sample at three different locations: in the middle of the crust area and at 2 cm distance on both sides. Experiments were carried out using two distinct cylindrical probes: 2 mm diameter (punching area=3 mm^2) at 0.5 mm/s, and 6 mm diameter (punching area= 28 mm^2) at crosshead speed 40 mm/s, following the settings suggested by Altamirano-Fortoul et al. (2013).

The data were analyzed using the method proposed by Van Hecke et al. (1998). This method is based on the peak analysis of the force-deformation curves. From the force-deformation curve recorded, the following puncturing parameters were determined:

Average puncturing force: $Fm(N) = \frac{A}{d}$ Eq. (2)

Spatial frequency of structural ruptures: $N_{wr}\left(m^{-1}\right) = \frac{No}{d}$ Eq. (3)

Average specific force of structural ruptures: $f_{wr}(N) = \sum \frac{\Delta F}{No}$ Eq. (4)

Crispness work: $W_c(N.m) = \frac{Fm}{N_{wr}}$ Eq. (5)

Where: No is the total number of peaks, d is the distance of penetration (mm), ΔF is the individual force drops for each peak (N) and A is the area under the force-deformation curve.

Four breads from each set were used for carrying on the puncture test, obtaining 24 individual measurements for each experimental point.

SEM of bread crust

Scanning electron microscopy was used to examine the crust of bread. Slices of bread were freeze-dried previously to the microscopy analysis. Sample cubes (1 cm^3) were fixed with the aid of colloidal silver and then coated with gold (Baltec SCD005) at 10-2 Pa and an ionisation current of 40 mA. The observation was carried out in a JEOL JSM-

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

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. The analysis of variance was carried out to decompose the variance of the data into two components: a between-group component and a within-group component. When the *P*-value of the *F*-test was less than 0.05, there was statistically significant difference between the means of the 2 groups at the 95.0% confidence level.

Multiple range test was used to determine which means were significantly different from each other and Fisher's least significant difference (LSD) procedure was used to discriminate among the means. A multifactor analysis of variance was performed to determine which factors have a statistically significant effect on mechanical parameters. Pearson product moment correlations between each pair of variables were also carried out.

RESULTS AND DISCUSSION

Effect of enzymatic treatments on the physico-chemical properties of bread crust

Amyloglucosidase was sprayed onto the surface of frozen partially baked breads and the effect of increasing levels of enzyme on the physical and chemical properties of bread crusts was studied. Two different bread specialties with diverse crust thickness were selected for determining the ability of the enzyme to penetrate through the crust. The upper crust of the sample identified as thick crust had a section of 5.10 mm, which was significantly (P <0.05) different than that in the bread with thin crust (2.94 mm).

The values obtained for bread crust colour, water activity and moisture content are shown in Table 2. The enzyme concentration had a significant effect on bread crust colour parameters and crust darkness. Comparing breads without enzymatic treatment, the thick crust showed higher L^* (lightness) and lower a^* (redness), with no significant differences on b^*. Those differences could be derived from their different composition and/or processing conditions. In general, regardless the type of crust, the enzymatic treatment decreased lightness, and larger effect was observed on the thick crust that underwent a drop in L^* with the presence of the lowest level of amyloglucosidase. Regarding a^*, the value slightly increased in the thick bread crust due to the addition of amyloglucosidase, but no trend was observed in the case of thin bread crust. In both samples, the b^* value decreased due to the enzyme activity.

Concerning to the total colour difference (ΔE) (Table 2), in general, the enzymatic treatment induced a progressive increase of the values of this parameter when increasing the enzyme concentration, with the exception of the thin crust sample treated with 250mg/10ml amyloglucosidase.

Table 2. Effect of amyloglucosidase on the physicochemical properties of thin and thick bread crust.

Bread crust	Enzyme concentration (mg/10ml)	Aw crust	Moisture content (%)	*L**	*a**	*b**	Δ*E*	Darkness crust
Thin	0	0.516 ±0.02 c	9.67 ±0.10 g	54.26 ±0.53 e	14.87 ±0.40 e	37.42 ±0.56 c	0 ±0 a	45.74 ±0.53 b
	100	0.498 ±0.01 bc	6.22 ±0.12 d	55.12 ±1.77 e	14.09 ±0.90 de	36.49 ±1.03 c	2.65 ±0.58 b	44.88 ±0.77 b
	250	0.481 ±0.01 b	5.43 ±0.32 c	48.44 ±0.40 c	12.72 ±0.01 ab	20.53 ±0.68 a	18.10 ±1.15 e	51.56 ±0.04 d
	500	0.552 ±0.03 d	6.73 ±0.08 d	49.22 ±0.70 cd	13.96 ±0.23 d	27.29 ±0.55 b	11.44 ±0.68 c	50.78 ±0.70 cd
	1000	0.505 ±0.01 c	8.02 ±0.10 f	44.86 ±0.59 a	13.70 ±0.69 cd	27.04 ±0.48 b	14.06 ±0.86 d	55.14 ±0.59 f
Thick	0	0.540 ±0.00 d	11.46 ±0.22 h	60.82 ±0.24 f	11.85 ±0.45 a	35.21 ±0.46 c	0 ±0 a	39.18 ±0.25 a
	100	0.540 ±0.04 d	6.10 ±0.05 d	49.98 ±0.21 d	12.42 ±0.21 ab	20.82 ±0.75 a	18.06 ±1.01 e	50.02 ±0.21 c
	250	0.507 ±0.01 c	5.27 ±0.15 b	48.51 ±0.55 c	12.99 ±0.83 bc	20.97 ±0.87 a	18.86 ±0.83 ef	51.49 ±0.56 d
	500	0.459 ±0.01 a	5.30 ±0.03 bc	46.74 ±0.08 b	12.92 ±0.53 bc	21.80 ±0.36 a	19.50 ±0.84 f	53.26 ±0.26 e
	1000	0.452 ±0.01 a	4.93 ±0.02 a	45.85 ±0.12 ab	12.40 ±0.74 ab	23.39 ±0.79 a	19.18 ±0.78 f	54.15 ±0.13 ef

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

Enzymatically treated samples were significantly darker than breads without enzymatic treatment, and the darkness augmented with the level of enzyme added. As it was expected, the enzyme level of amyloglucosidase increased the release of glucose from the hydrolysis of amylose and amylopectin, providing additional glucose that accelerates the Maillard reaction. In fact, Sharma and Singh (2010) reported the use of amyloglucosidase to enhance bread crust colour. Furthermore, colour of bread is an important quality associated with aroma, texture and appearance features which are decisive for consumers.

No differences were detected in the water activity and moisture content of the crumb in the different samples due to enzymatic treatments (results not showed). The enzymatic treatment at levels higher than 100mg/10ml promoted a significant ($P<0.05$) decrease in the crust water activity of the thick crust bread, and the reduction increased with the level of enzyme. Considering that water activity refers to unbound or free water in a system available to support biological and chemical reactions (Potter and Hotchkiss, 1998), it seems that the enzyme consumes molecules of water in the reaction of hydrolysis of 1,4 and 1,6-α linkages of the starch, which reduces the amount of free water in the bread crust. In the case of the thin crust bread, water activity showed a decrease when amyloglucosidase was added up to 250mg/10ml, but the trend was reversed when higher enzyme concentrations were added. A plausible explanation could be that the enzyme penetrates the thin bread crust at high concentrations reaching the bread crumb, which had significantly higher moisture content (40,9% in thick bread and 42.4% in thin bread) than the crust, facilitating the water molecules diffusion from the crumb to the crust and leading an increase of the water activity.

Similar trend was observed when assessing the moisture content of the bread crust. The enzymatic treatment produced a significant ($P<0.05$) decrease in the moisture content; probably due to the participation of the water molecules in the hydrolysis reaction, which led to drier crusts. Again, in thin crust bread the addition of up to 250mg/10ml amyloglucosidase resulted in the lowest moisture content, which increased at higher enzyme levels. In the thick crust bread, the effect was dependent on the enzyme level, moisture content showed lower values with higher concentration. Therefore, greater enzyme levels were required for diffusing through the crust in breads with thicker crust. Water is the predominant constituent in most foods and it is a direct reactant in hydrolytic processes. Moreover, the change of cross link and entanglements between amylose and amylopectin caused by enzymatic treatment might increase the porosity, which favors the water release during the full baking yielding drier bread crust. According to Esveld et al. (2012), the moisture diffusion in cereal cellular products involves diffusive transport in the gas phase and in the solid phase, and both depend on the morphological details of the structure. Xiong et al. (1991) indicate that the mobility of water in solid foods is strongly dependent on the porosity of the structure. Porosity is intuitively related to macroscopic vapor transport rate while sorption rate in the solids seems more related to the local microscopic thickness of the solid (Esveld et al., 2012), which could explain the differences observed in the two specialties due to bread crust thickness.

A reduction in water activity and moisture content of the bread crust was previously observed by Altamirano-Fortoul and Rosell (2010) and Primo-Martín et al. (2006) when different enzymes were sprayed or added to study their effect in bread crust characteristics.

Mechanical properties

Altamirano-Fortoul et al. (2013) reported that the use of smaller punch cross section and low speed allow obtaining reliable information about the cellular structure of the bread crust. On the contrary, compression becomes more important with the use of greater punch cross section and high speed. According to the above, two sets of conditions (punch cross section of 3 mm^2 and 28 mm^2) were applied for determining the mechanical properties of the crust to obtain information about the cellular structure and the compression behavior. Table 3 shows the mean values obtained for the mechanical parameters for each level of the factors (crust type, enzyme concentration, punch cross section) and the statistical significance of each of the factors. It also shows the standard error of each mean, which is a measure of its sampling variability. Regardless the punch cross section used in the test, the enzymatic treatment produced significant changes on all mechanical parameters used to define texture of the crust in the two bread specialties studied (Table 3). The *F*m parameter was not significantly affected by the punch cross section. Thick bread crust required greater force (*F*m) for breaking crust than thin crust.

Table 3. Effect of enzyme level and punch cross section on puncturing parameters in two different bread specialties.

Factor	Fm (N)		N_{wr} (m^{-1})		f_{wr} (N)	
	Mean	SE	Mean	SE	Mean	SE
GRAND MEAN	7.53		1.57		12.04	
Bread crust	***		***		***	
Thick	9.82	± 0.71	2.36	± 0.16	13.59	± 0.42
Thin	5.24	± 0.71	0.79	± 0.16	10.50	± 0.42
Enzyme concentration (mg/10ml	***		***		***	
0	11.94	± 1.13	0.71	± 0.26	20.31	± 0.67
100	6.78	± 1.13	1.94	± 0.26	11.46	± 0.67
250	6.30	± 1.13	2.17	± 0.26	9.96	± 0.67
500	6.51	± 1.13	1.20	± 0.26	9.81	± 0.67
1000	6.10	± 1.13	1.85	± 0.26	8.68	± 0.67
Cross setion (mm^2)			***		***	
3	6.57	± 0.71	2.76	± 0.16	6.22	± 0.42
28	8.48	± 0.71	0.39	± 0.16	17.87	± 0.42

Means values + standard error (SE). The standard error of each mean is a measure of its sampling variability.

* Significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

The increase of enzyme level slightly reduced the puncturing force (*F*m), and that effect was more evident in thin bread crust, independently of the punch cross section and speed (Fig. 1). Thus, the amyloglucosidase was acting on the thin and thick bread crust inducing changes at cellular structure level, leading fragile structure. In fact, Luyten et al. (2004) describe that the force depends on the composition and the structure of the food.

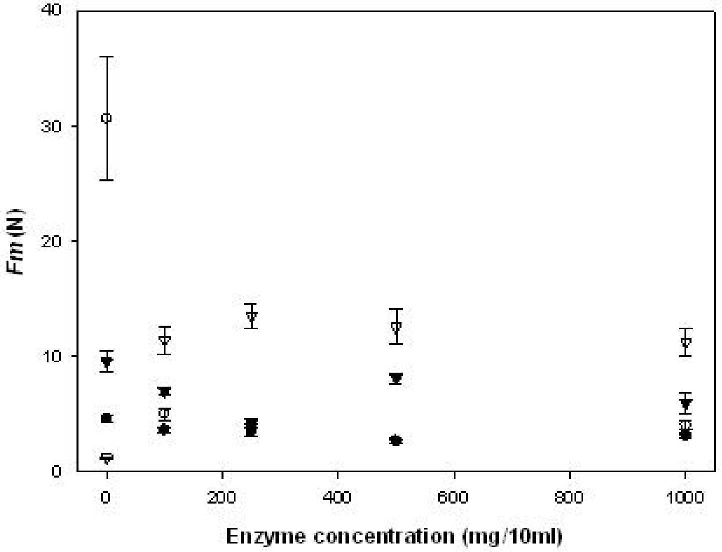

Fig. 1 Effect of enzyme treatment on the puncturing force (*F*m) of thin (closed symbols, ●, ▼) and thick (open symbols, o,▽) bread crust. Legends: 3 mm^2 punch cross section at 0.5 mm/s (●), 28 mm^2 punch cross section at 40 mm/s (▼).

The reduction observed in the puncturing force due to enzyme action could be related to the decrease in the moisture content and water activity. As mentioned above, water leads to plasticization and softening of the starch-protein matrix and thus alters the mechanical properties, and an increase in water content increases the response to force (Jakubczyk et al., 2008). In the case of bread crust, Primo-Martín et al. (2009) described that at a_w of 0.75 bread crust was fully plasticized, and the transition from glassy state to rubbery state occurs at a_w of 0.68-0.69 leading an increase in the rupturing force (Altamirano-Fortoul and Rosell, 2010).

Greater punch cross section (28 mm^2) and higher speed produced significantly (P<0.001) less structural ruptures in all the samples (Table 3). Therefore, more information about cellular structure was obtained at lower punch cross section and slower speed. Spatial frequency of structural ruptures (N_{wr}) in the thick bread crust showed significantly (P<0.001) higher values than thin bread crust (Table 3). In the case of thick bread crust, at both punch cross sections and speeds, the number of structural ruptures increased with the enzyme level up to 250mg/10ml amyloglucosidase, but lower number of structural ruptures was observed at higher enzyme levels (Fig. 2). Considering that high number of fracture events is produced by crispy products, the enzymatic treatment resulted in samples crispier than the control crust. Similar positive effect was observed by Altamirano-Fortoul et al. (2013). Newly, these results might be related to the decrease in water activity and an increase of the porosity due to the action of the treatment. The decrease in water activity resulted in an increase of the jaggedness of the force-displacement curve. Some authors reported that the increase of moisture content or water activity of crispy food promote the loss in jaggedness of force-displacement curve and consequently the frequency distribution of number of fracture (Van Hecke, 1998; Jakubczyk et al., 2008; Tsukakoshi et al., 2008; Castro-Prada et al., 2009; Arimi et al, 2010)

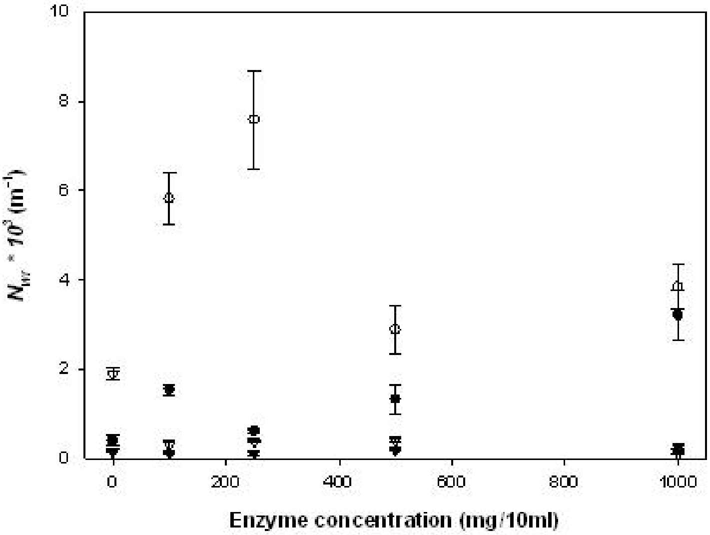

Fig. 2 Effect of enzyme treatment on the frequency of structural ruptures (N_{wr}) of thin (closed symbols, ●, ▼) and thick (open symbols, o, ▽) bread crust. Legends: 3 mm^2 punch cross section at 0.5 mm/s (●), 28 mm^2 punch cross section at 40 mm/s (▼).

With respect to f_{wr} parameter, a significant (P<0.001) decrease was obtained with enzyme treatment in both samples in comparison to their respective controls (Table 3). Thick bread crust presented significantly higher values in this parameter than the thin bread crust. Again, the effect of punch cross section showed an increase in f_{wr} parameter when using 28 mm^2 compared with punch cross section of 3 mm^2. Considering f_{wr} parameter relates the specific force with the structural ruptures, if treated bread crusts required lower force to promote the fracture as well as showed greater number of ruptures, it would be expected that this parameter will be lower than that in the control bread crust. Amyloglucosidase sequentially detaches the glucose units allowing the polysaccharide breakdown, which might have modified the cell wall associated to starch within the crust matrix. Consequently, the addition of enzyme reduced the mechanical resistance in the cell walls, leading to lower values of this parameter.

Recently, Altamirano-Fortoul et al. (2013) suggested that only by using low puncturing speed is possible to assess the crispness work, because of that it is only shown the values obtained by puncturing with small punch cross section and low speed (Fig. 3). Crispness work parameter (W_c) showed a decrease with increasing the enzyme level in both bread specialties. Results obtained showed that with those puncturing settings was possible to detect the effect of enzymatic treatment on the mechanical behaviour of the crust. The observed effect could be related to the amyloglucosidase hydrolysis of long-chain polysaccharides causing an increase in the number of the pores, and in consequence less crispness work was needed. In fact, some authors reported that pores play a main role in the crispness and texture of foods (Goedeken and Tong, 1993; Tsukakoshi et al., 2008).

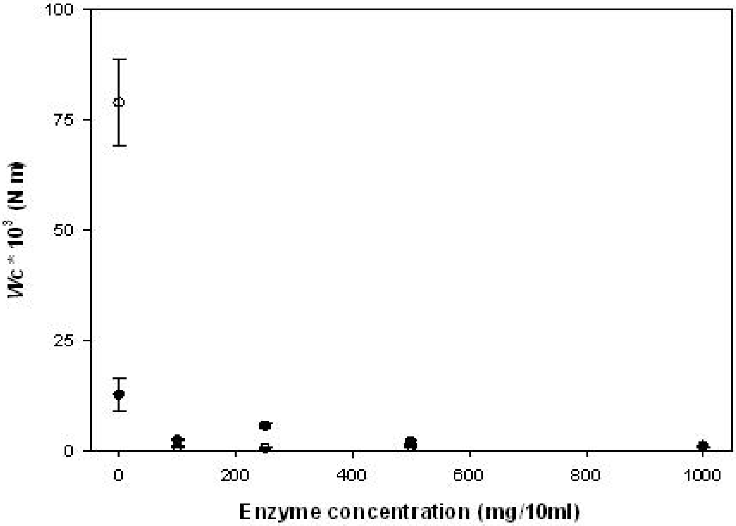

Fig. 3 Effect of enzyme treatment on the crispness work (W_c) of thin (closed symbols,●) and thick (open symbols, o) crust breads.

A multivariate analysis was applied to determine the possible correlation among the physicochemical properties and the parameters that defined the mechanical properties of the crust obtained with the 3 mm^2 punch cross section. Fm parameter showed a significant positive relationship with crispiness work (W_c) (r=0.9225), moisture content (r = 0.7041), and also significant but very weak correlation with water activity (r = 0.3145). A significant positive relationship was observed between the spatial frequency of structural ruptures (N_{wr}) and the total colour difference (r=0.6352). In addition, a significant positive relationship was obtained between the crispness work (W_c) and moisture content (r=0.7939).

Crust Structure

To achieve a better understanding of the enzyme action on the mechanical behaviour, the microstructure of the crust cross-section was analyzed by SEM. The bread crust with lowest and highest enzyme concentrations were selected for microstructure studies with the purpose of observing the effect of the dosage of added enzyme.

Micrographs of control bread crusts and also those treated with amyloglucosidase 100mg/10ml (A1) and amyloglucosidase 1000mg/10ml (A4) are showed (Fig 4 and 5). Bread crusts with and without treatment revealed two different structural zones: a dry crust and sub-crust. Similar structures were observed by Altamirano-Fortoul et al. (2012), who suggested that the sub-crust is of great importance due to it lends rise to chemical transition between the crust and the crumb, as well as this indicates where the crust begins and ends. Figure 4a shows the thin bread crust without added enzyme (control), where an uniform structure was presented, and at higher magnification (Fig. 5a) it was observed a smooth layer due to gelatinised starch, which covers quite well the ungelatinized starch granules around the air cell. However, in untreated thick bread crust (control) a cracking structure with a thicker zone 1 and bigger cells were displayed (Fig. 4b), besides starch granules could be clearly envisaged under a smooth film showing little distorted structures (Fig. 5b). Therefore, the distinct mechanical properties observed in both specialties could be ascribed to their cellular structure. Moreover, it is important to consider that the bread crust properties are dependent on the breadmaking process and of many factors including for instance lower water content, extent of heat and mass transfer at the bottom and top surfaces (Vanin and Trystram, 2009).

Enzyme treatment modified the bread crust structure in both samples. Thin bread crust treated with A1 revealed a more disordered structure with small irregular voids and great cracks (Fig. 4c). At higher magnification it seems that the starchy gel, that initially covered the structure, was thinner, revealing underneath structures (Fig. 5c). In thin bread crust treated with A4, the structure was significantly different with apparent compact structure but with sub-holes within the cells (Fig. 4e), which agrees with previous observations of Rojas et al. (2000) when bread were formulated with alfa amylase. Besides, the network was not continuous and sharper surface was detected (Fig. 5e), likely due to the hydrolysis of starchy compounds. Treatment A4 led to bread crust with greater spatial frequency of small structural ruptures as result of a non-homogeneous structure and the numerous sub-holes, which agrees with mechanical results (N_{wr} parameter).

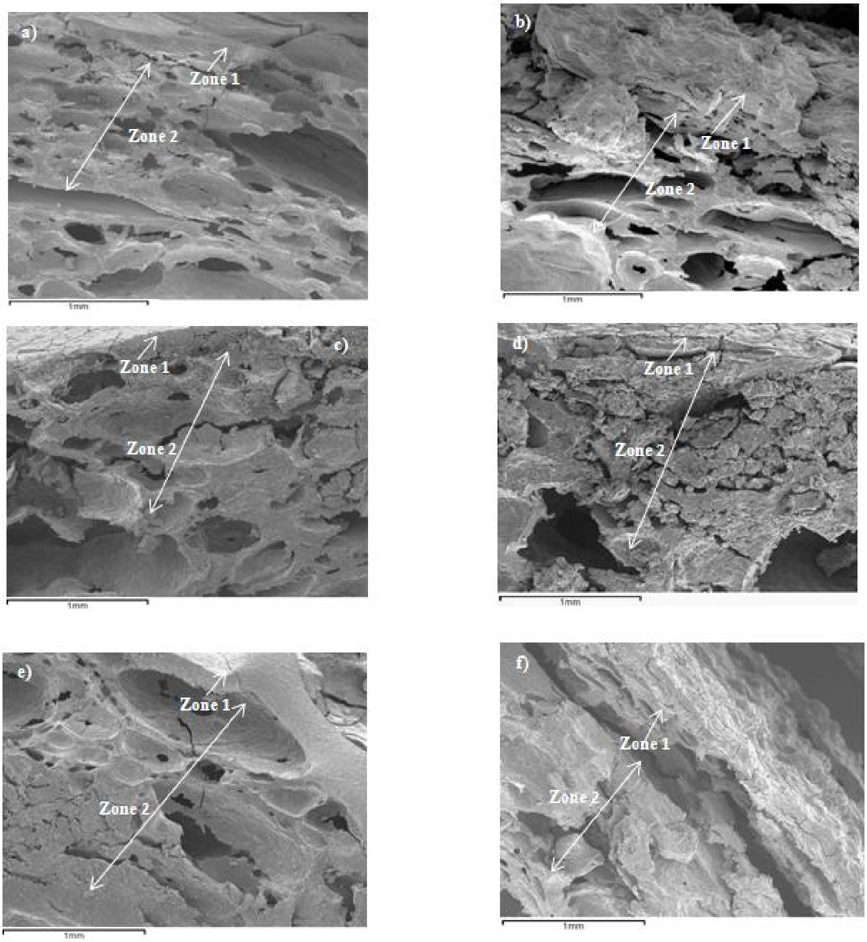

Fig. 4 Scanning electron micrographs of crust cross section. Magnification of 50x. Images correspond to cross section of breads with thin (a, c, e) and thick (b, d, f) crusts. Micrographs of control crust (a, b), crust treated with amyloglucosidase 100mg/10ml (c,d) and amyloglucosidase 1000mg/10ml (e, f).

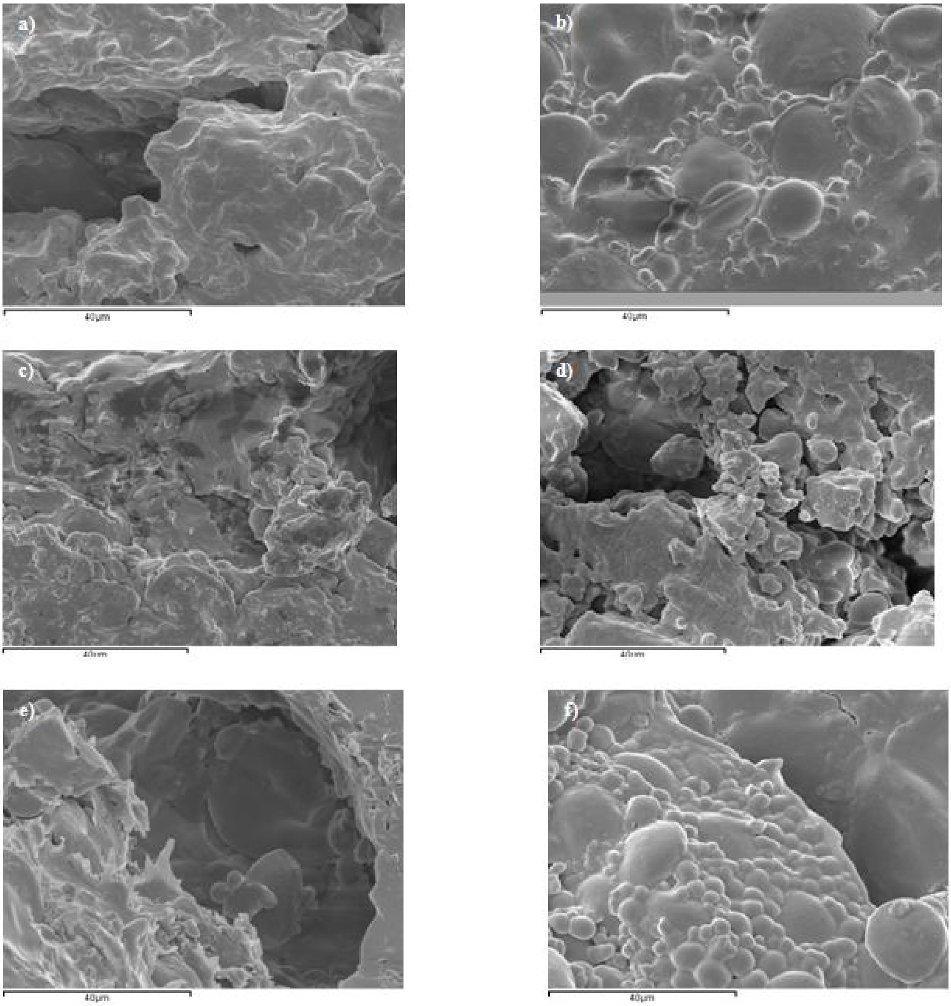

Fig. 5 Scanning electron micrographs of crust cross section at high (1500x) magnification. Images correspond to cross section of breads with thin (a, c, e) and thick (b, d, f) crusts. Micrographs of control crust (a, b), crust treated with amyloglucosidase 100mg/10ml (c, d) and amyloglucosidase 1000mg/10ml (e, f).

These changes were mainly related to greater starch hydrolysis, which altered the starch structure, resulting in a more porous structure. Therefore if the microstructure is more porous, it gives brittle behavior (Goedeken and Tong, 1993).

In the case of thick bread crust treated with A1 an amorphous, disrupted and cracked structure was observed (Fig. 4d). Higher magnification allowed detecting some deformed starch structure due to the partly disappearance of the covering layer, and even some remnant intact starch granules (Fig. 5d). In samples treated with A4 a layered and fragmented structure was observed (Fig. 4f). Likely, this different structure might result from the intense hydrolysis through the crust that reach the crumb, allowing some water molecules to diffuse and in consequence change the structure. This different structure was confirmed at higher magnification (Fig. 5f). Moreover, these results agree with those observed in N_{wr} where different trend was observed at higher enzyme activity. Most probably, enzyme level affected the protein-starch interactions as well as the interaction between starch chains and water molecules, and in consequence the granule’s gelatinization. According to Guerrieri et al. (1997) certain proteins (purified gluten, gliadin and high molecular weight glutenin subunits) modified amyloglucosidase activity in model systems. The proteins had an effect on the starch hydrolysis, which is related to protein-starch interaction, especially when producing starch gelatinization. In addition, considering that the enzyme treatment reduced the amount of water available in the bread crust, starch gelatinization would be rather limited. Altamirano-Fortoul et al. (2012) found that lower amount of water present in the bread crust limited the gelatinization, which yield a more porous network with intact granules and partially gelatinized starch granules. Consequently, those effects can be related with the formation of successive structure layers (sandwich-like structures) in the sample treated with A4. The sample composed of long cell walls disrupted more easily when performing the fracture, resulting in lower values in the puncturing force parameter as were detected when texture was determined with small puncturing at low punching speed. Stokes and Donald (2000) indicated that when starch and gluten matrix are in a glassy state cell walls become more prone to fracture.

In general, the effect of the enzyme on microstructure of bread crust was dependent on enzyme dosage and the type of bread crust (thin or thick). Amyloglucosidase action resulted in a more disrupted structure with partly removal of the gelled film that covered the starch granules. Previous studies showed that enzyme treatment modified the morphology and characteristics of bread crust (Primo-Martín et al., 2006; Altamirano-Fortoul and Rosell, 2010). Therefore, it is of special interest to know the microstructure of the bread crust because it is responsible for the puncturing behavior.

CONCLUSIONS

The present study shows that enzymatic treatment of the bread crust decreased the moisture content and water activity, due to an increase in the crust porosity besides the removal of water participating in the hydrolysis reaction. Enzyme addition affected the colour crust; in general an increase in the total colour difference was observed when enhancing the enzyme concentration. Regarding mechanical properties, overall results indicate that the enzymatic treatment resulted in crust with reduced resistance to puncture and high number of fracture events, indicating crispy products. In addition, crispness work parameter was lower as consequence of the fragility of the crust. The correlation matrix revealed the positive relationship of the moisture content with Fm and W_c when studying the effect of amyloglucosidase on the crust.

Furthermore, the results of the SEM analysis also confirmed the effect of the enzymatic treatment. Amyloglucosidase hydrolyzed the starchy gel of the crust exposing the starch granules and resulting in a more irregular and uneven structure. This study suggest that the enzyme produced an important modification on the starch-protein matrix structure, related to the steady removal of the gelatinized starchy layer that cemented the matrix, which validate the results on the physicochemical and puncturing parameters. The enzyme level required for modulating crust structure was dependent on the crust thickness.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of Spanish Ministry of Economy and Sustainability (Project AGL2011-23802), the European Regional Development Fund (FEDER), Generalitat Valenciana (Project Prometeo 2012/064) and the Consejo Superior de Investigaciones Científicas (CSIC). R. Altamirano-Fortoul would like to thank her grant to CSIC. The authors also thank Forns Valencians S.A. (Spain) for supplying commercial frozen partially baked breads.

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Chapter 5

VIABILITY OF SOME PROBIOTIC COATINGS IN BREAD AND ITS EFFECT ON THE CRUST MECHANICAL PROPERTIES

R. Altamirano-Fortoul, R. Moreno-Terrazas, A. Quezada-Gallo and C. M. Rosell

ABSTRACT

The objective of this study was to obtain functional bread combining the microencapsulation of *Lactobacillus acidophilus* and starch based coatings. Different probiotic coatings (dispersed or multilayer) were applied onto the surface of partially baked breads. In all treatments, microencapsulated Lactobacillus acidophilus survived after baking and storage time, although reduction was higher in the sandwich treatment (starch solution/sprayed microcapsules/starch solution). Despite coatings significantly affected the physicochemical properties of the crust, increasing water activity and reducing the failure force, the sensory evaluation revealed a good acceptability of the functional breads. Scanning electron microscopy revealed the presence of scattered microcapsules onto the bread crust, being highly covered in the sandwich coating. Therefore, *Lactobacillus acidophilus* included in microcapsules can be incorporated to bread surface through edible coatings, leading functional bread with similar characteristics to common bread, but with additional healthy benefits.

Key words: starch; probiotic coatings; bread; microstructure; crust

INTRODUCTION

Bread is staple food in many countries, since it constitutes an important source of complex carbohydrates, proteins, minerals and vitamins (Rosell, 2007). In recent years, consumers' interest in the role of nutrition for health and wellbeing has increased. Therefore, today, the priority of the industry is to innovate, meet and satisfy consumer requirements. Concerning baking industry, that trend has prompted the development of baked goods keeping in mind the healthy concept. All the whole meal products or the fiber enriched baked goods would fall in this category (Redgwell and Fischer, 2005). However, functional breads containing viable microorganisms have not been developed yet due to the high temperature reached during baking.

Guarner and Schaafsma (1998) defined probiotic as a live microorganism, which upon ingestion in certain numbers, exerts health benefits beyond inherent basic nutrition. Various species of genera *Lactobacillus and Bifidobacterium* have been used as probiotics (Lavermicocca et al., 2005; Lian, et al., 2002). Sometimes survival of many probiotic bacteria during processing and storage is insufficient and limits its usefulness in food applications. Therefore, alternative for providing viable microorganisms are the microencapsulation technique. The encapsulation protects probiotic from environmental and physiological degradation (Capela et al., 2006; Lian et al., 2002).

Edible coatings are materials which can be consumed and provides a barrier to moisture, oxygen and solute movement for the food. Edible coatings are particular forms of films directly used onto the surface of materials, which become an element of the ending product (Cuq et al., 1995). This type of coatings is prepared with biological materials such as proteins, lipids and polysaccharides (Tharanathan, 2003). However, starch is one of the preferred types of coatings because it is abundant, cheap and biodegradable. Moreover, the incorporation of functional ingredients on the edible coatings would be an alternative for protecting the microorganisms. In bakery products, microencapsulation has been extensively used for protecting iron salts and increasing its bioavailability (Cocato et al., 2007). Partially baked bread is an alternative product that shows an expansion trend, owing to provide fresh bread available all time of day (Rosell, 2009). This bread only requires a short baking for obtaining full baked bread. Therefore, it constitutes a potential food for obtaining functional bread combining the microencapsulation and coating technologies.

The objective of this study was to determine the viability of different types of functional coatings applied onto the surface of partially baked breads before the full baking step. The survival of microorganisms (*Lactobacillus acidophilus*) was assessed after baking and after a short storage (24 hours). Fresh breads were sensory evaluated and the physical and chemical properties of bread crust were determined. Special attention has been paid to the coatings and bread crust microstructures.

MATERIALS AND METHODS

The strain of *Lactobacillus acidophilus* used for microencapsulation was obtained from Danisco Ingredients México, S.A. (México). Whey protein isolates (WPI) from Davisco Foods International Inc. (EUA), carboxymethylcellulose (CMC, Aqualon® Cellulose gum, 7LF PH) from Hércules Incorporated (EUA), low methoxyl citric pectin (P) (Grindsted, Pectin RS 400) from Danisco Méxicana, S. A. (México), inulin (I) from Quantum Natura S. A. (México), fresh agave sap without fermentation (aguamiel) from San Juan de las Manzanas (México) were used for encapsulation. Commercially available corn starch (Maizena) from Unilever Food solutions (México) was used for coating. Some corn starch characteristics are as follows: maximum viscosity during heating 1847cP, viscosity at 50º C 1531cP, gelatinization peak temperature 66º C, gelatinization enthalpy 10 J/g.

A partially baked bread specialty available in the Spanish market was used. Part-baked frozen breads were provided by

Forns Valencians S.A. (Valencia, Spain) and stored at -18° C until use. The qualitative composition of the breads includes breadmaking wheat flour, water, yeast, salt and bread improver. Its chemical proximate composition was: 30.1% moisture content, 60% carbohydrates, 6.41% proteins and 2.74 % fats.

Microbial encapsulation

Microcapsules formation was based on previous results (Rodríguez-Huezo et al., 2007; Villa-García et al., 2010; Pedroza-Islas et al., 2010). Briefly, encapsulating agents were prepared by dispersing whey protein isolates, CMC, pectin, inulin and fresh agave sap in a proportion of 45.67 : 11.72 : 18.72 : 22.83 : 1.06., in order to obtain a suspension concentration of 6.57% (w/w).

Lactobacillus acidophilus was used as probiotic. The pure culture of L. acidophilus were developed in MRS medium (de Man, Rogosa & Sharp, DIFCO) with low oxygen tension. After growing, they were transferred to 0.1% peptone water.

One litre of watery dispersions of encapsulating agents was combined with the inoculum, previously adjusted to 5 McFarland standard of turbidity (bioMérieux). The mixture was homogenized during for 10 min at room temperature and then spray dried by a Niro Atomizer dryer, provided with a rotatory atomizer set on an input/output temperature of 130° C/65° C, with a 2 bar pressure and a feeding of 15 ml/min.

Edible coating preparation and characterization

Edible coating preparation

Three different treatments were prepared (S1, S2, S3), which differed in the number of coating layers applied onto the bread surface (Table 1). Starch suspension (5%, w/v) was used as coating material. Preliminary tests were carried out to optimize the level of microcapsules in each treatment in order to have similar microbes' survival after baking. Treatment S1 consisted in the starch solution (5%, w/v) containing microcapsules (1%, w/v). Treatment S2 was as described in S1 plus a coating with starch solution (5%, w/v). In treatments S1 and S2, microcapsules (1%, w/v) were added to the starch suspension and kept under magnetic stirring for ensuring uniform dispersion. Treatment S3 consisted in a coating of starch solution (5%, w/v), followed by dispersing microcapsules (2% w/w, which corresponded to 0.2 g/bread), and a final coating with starch solution (5%, w/v), like a sandwich. Treatment S3 required double microbes' concentration than treatment S1 and S2 to obtain similar survival after subjected to baking.

Table1. Probiotic coatings concentrations applied onto the bread surface

Sample	Probiotic Coatings	Dosage
S1	1) Starch-microcapsules solution	5% starch containing 1% microcapsules
S2	1) Starch-microcapsules solution	5% starch containing 1% microcapsules
	2) Starch solution without microcapsules	5% starch
S3	1) Starch solution without microcapsules	5% starch
	2) Microcapsules sprayed	0.2 g microcapsules/bread
	3) Starch solution without microcapsules	5% starch

Coating Properties

Mechanical properties, film thickness and morphology of the coating were determined. Test filmstrips (6 X 2.0 cm) were cut from preconditioned samples (23° C; 75% RH) and mounted between the grips of the probe A/TGT of the TA.XT2i texturometer (Stable Micro Systems, UK). The tests were conducted according to the ASTM D882-00 (2001) method (Veiga-Santos et al., 2005). Ten specimens were tested for each formulation.

Average film thickness of the preconditioned samples (7% RH, 25° C) was obtained using a flat parallel surface micrometer with 1 picometer resolution. Five measurements were taken at three different randomly selected positions.

The coatings were placed on glass plates and the scanning photography was carried out on flatbed scanner (HP Scanjet 4400c). One representative sample (of three) from each of the coatings was chosen for digital documentation.

Cross sections of the starch film and microcapsules powder samples were sprinkled onto double-backed cellophane tape attached to a stub. They were vacuum coated by evaporation with silver and examined by means of a JEOL JSM-5310LV scanning electron microscope (SEM) (JEOL Korea Ltd., Korea) at an accelerating voltage of 12 kV.

Full baking process and storage

Part-baked breads were removed from the freezer and thawed at room temperature till the center of the loaf reached 5º C. Breads were baked off in a forced convection oven (Eurofours, Gommegnies, France) under the following conditions: preheating of the oven at 220° C, and convection during 16 min at 180° C. Then, 10 ml of probiotic coating solution were evenly sprayed over the top surface (118.3± 1cm^2) of the breads before baking. When various coatings were applied (S2 and S3) they were sprayed successively onto the surface of the partially baked bread (Table 1).

After bake off, breads were allowed to cool down and stored in a cabinet at 25° C and relative humidity (RH) of 61%. Three sets of loaves were prepared for each treatment and they were baked in separate days.

Microbiological analysis

The amount of viable *Lactobacillus acidophilus* in the bread surface was determined after full baking 0.5 h (fresh bread), and after 24 h storage. A bread crust portion (1 g) was aseptically diluted in 9 ml of sterile peptone water solution (Scharlau Chemie, Barcelona, Spain) and mixed for 1 min in Lab Blender 400 Stomacher (Seward Medical, London, UK). Serial dilutions were made in sterile peptone water and plated following the surface technique onto De Man, Rogosa and Sharpe (MRS, Scharlau Chemie, Barcelona, Spain) agar supplemented with 10% sterile skim milk. The culture medium contained a second layer of MRS agar used for generating anaerobic conditions. The agar plates were incubated at 32° C for 5 days. After the respective incubation times, results were recorded as colony-forming units (CFU)/g of product.

Chemical and physical analyses

Analysis of the bread samples was performed at 0.5h (fresh bread), 2, 4, 6, 8 and 24 h after baking. Bread volume was determined by the rapeseed displacement method. Crust colour parameters were measured at three different locations of the surface by using a Minolta colorimeter (Chroma Meter CR-400/410, Konica Minolta, Japan) after standardization with a white calibration plate (L^* = 96.9, a^* = -0.04, b^* = 1.84). The colour was recorded using CIE-L^* a^* b^* uniform colour space (CIE-Lab), where L^* indicates lightness, a^* indicates hue on a green (-) to red (+) axis, and b^* indicates hue on a blue (-) to yellow (+) axis.

Crust moisture content and water activities were followed during short bread storage. Crust was separated using a razor blade. Moisture content was determined according to the ICC Method (110/1, 1994). Water activities were measured using a water activity unit (Aqua Lab Series 3, Decagon devices, Pullman, USA) at 25° C.

All determinations were carried out in triplicate. The results presented are averages of all available replicates.

Puncture tests

Breads were puncture tested at a deformation speed of 40 mm/s using a 4mm diameter cylindrical probe. Experiments were performed using a texture analyzer (TA XTplus, Stable Micro Systems, Surrey, UK). The peak force and the peak deformation point of the crust were calculated by punching the samples at eight different points of bread surface: left and right sides, 2 cm distance from the middle point. The average value was calculated for each sample. The failure force was calculated as the peak force observed according to studies by Jackman and Stanley (1992). The failure deformation, defined as the deformation at the peak point, was also calculated. The failure firmness, defined as the slope of load displacement curve from zero to the point of rupture or failure, was calculated according to studies by Shafiee et al. (2008). Three bread samples were used for each measurement.

SEM of bread crust

The structure of the treated crusts were analysed by scanning electron microscopy. Freeze-dried samples of the crust were mounted on metal stubs and the samples were coated with a gold and palladium layer (100–200 Å) by Ion Sputter (Bio-Rad SC-500). All samples were examined using an accelerating voltage of 10 kV with a scanning electron microscope (S-4100, Hitachi, Ibaraki, Japan) equipped with a field emission gun, a back-secondary electron detector and an EMIP 3.0 image data acquisition system (Rontec, Normanton, UK) from the SCSIE Department of the University of Valencia.

Sensory evaluation

Sensory evaluation was carried out by a trained panel of eight judges and scored on a scale of 1 (dislike extremely) to 5 (like extremely). Sensory tests were carried out under normal lighting conditions and at room temperature. The experience of the judges in this type of analysis for bread products varied from 3 to 20 years. Preliminary training was performed to evaluate crust appearance, odour, crust colour, crispness and crumb hardness. For each one of these attributes, the average response was reported.

Statistical analysis

All data were presented as mean values of at least three replicates. Statistical analysis of the results was performed using Statgraphics Plus V 7.1 (Statistical Graphics Corporation, UK). Data were analyzed by nonparametric one-way analysis of variance (ANOVA). When ANOVA indicated significant F values, multiple sample comparison was also performed by Tukey HSD test in order to detect significant differences.

RESULTS AND DISCUSSION

Probiotic coatings

Lactobacillus acidophilus is a microorganism that requires low oxygen tension, and essential nutrients as carbohydrates, proteins, vitamins of the B-Complex, nucleic acids and minerals. Thus, these nutrients must be available in the medium for growth and establishment of a predominant microflora of lactobacilli (Gomes and Malcata, 1999). Microcapsules composition was selected based on reported results of activation energy (E_a) that is a good parameter for the selection of spray drying encapsulated materials. Higher values of this parameter deliver better microcapsules (Rodríguez-Huezo et al., 2007). Previous findings showed that the mix of protein and agave sap largely increases the activation energy (35.7 kJ/mol) and that the agave sap, as well as the inulin, provides major E_a 37.92 kJ/mol when they are combined (De Jonge et al., 2007; Martínez and Morales, 2007; Villa-García et al., 2010). Moreover, the addition of hydrocolloids like pectin and CMC to the previous mix further increases the E_a (40.3kJ/mol). It seems that the combination of the inulin and agave sap with the selected hydrocolloids could have some interaction with the lipids of the cell membrane of the microorganisms and help to protect the probiotics during drying in the encapsulation process.

Figure 1 presents the digital images of probiotic coatings. Probiotic coating S1 showed a moderately regular surface and cohesive layer (Fig. 1a), while a slightly fragmented and opaque layer was observed in S2 (Fig. 1b) resulted from the double layer. When microcapsules were sprayed, as in coating S3, a non-cohesive and an uneven as well as fragmented surface was obtained (Fig. 1c). Mechanical characterization of probiotic films showed that S3 had the highest tensile strength

(1.86 ± 0.74 kg), followed by S1 (0.69 ± 0.52 kg) and S2 (0.41 ± 0.19 kg), despite no significant differences were detected among the thickness values (77.67±15.37µm, 56.89±10.43µm, 66.78±6.53µm for S1, S2 and S3, respectively) (Fig. 2). It should remark that films were prepared without swelling the starch granules, thus no significant change in the film thickness was initially expected. Likely, the incorporation of microcapsules within the film, which occurred in S1 and S2, interrupts the starch based film structure leading to a decrease in the mechanical resistance.

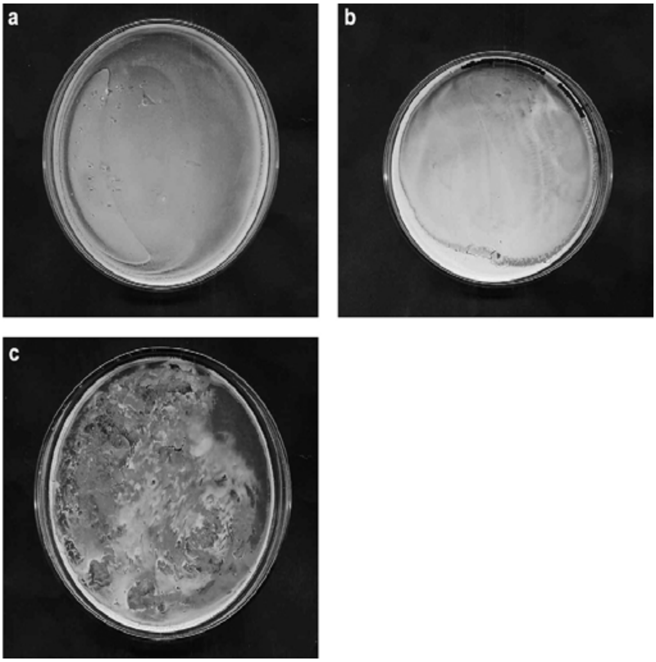

Fig. 1 Digital images of probiotic coatings. The composition of a) S1, b) S2 and c) S3 is detailed in materials and methods section.

The microstructure analysis (Fig. 2) showed two different populations of microcapsules (Fig. 2a), one with diameters ranging from 2-5µm and the other with diameter around 20µm. The small population had smooth and corrugated surface, forming like-tube cages surrounding cell agglomerations, and the large microcapsules were more spherical with smooth surface enclosing microcapsules as described before. Apparently L. acidophilus protection is complete because free bacteria were not observed. Previous findings suggested that the rough surface gives protection to probiotics (De Jonge et al., 2007; Martínez and Morales, 2007; Villa-García et al., 2010). Probiotic films showed continuous and smooth surface but microcapsules surrounded by a layer of starch were envisaged (Fig. 2b). Comparing the cross sections of the three films, it was observed that S1 (Fig. 2c) presents a continue network of starch very similar to the one of the film without microcapsules. S2 had denser cross section exhibiting accumulations of microcapsules at the surface (Fig. 2d). In addition, film S3 (Fig. 2e) showed an interior network of starch, and two layers of higher density at both sides of the film.

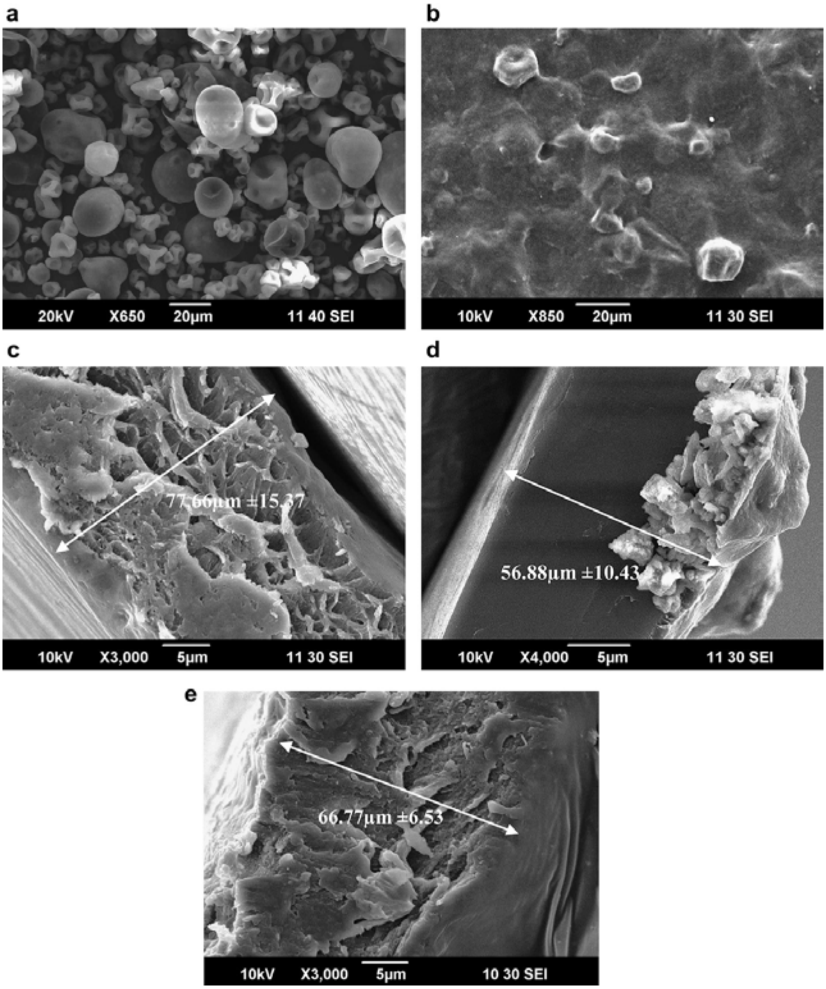

Fig. 2 Scanning electron micrograph of starch-based coating film. Legends: (a): Microcapsules, (b): Surface of a starch coating film containing microcapsules, (c): Cut side of S1 coating film, (d): Cut side of S2 film, (e): Cut side of S3 film.

Viability of Lactobacillus acidophilus in bread crust

The microbial count was determined after baking and after 24h storage to detect the microbial viability after baking and also its stability during bread storage (Table 2). Viable microorganisms remained after the baking process in all the coatings. Therefore, those coatings could be used for obtaining viable microorganisms containing breads. However, the temperature reached during the full baking process of the partially baked bread affected in different extent depending on the coatings studied. Breads with S2 treatment kept 63.2% of the counts after baking. Considering that breads with treatment S3 had more microcapsules concentration, the treatment S3 was the most sensitive to baking temperature. Quezada-Gallo et al. (2004) showed that when comparing the functional properties of dextrans, starch and four highly purified biopolymers (xanthan gum, sodium alginate, carboxymethylcellulose and tragacanthin) dextrans and starch showed better properties as gas barrier.

Table 2. Survival of Lactobacillus acidophilus after 24 h of short-term storage.

Samples	Concentration of *Lactobacillus acidophilus*[a] in each solution	Fresh bread (CFU/bread)	24 h storage bread (CFU/bread)
Control	0.00E+00	0.00E+00	0.00E+00
S1	4.83E+07	2.40E+07	1.70E+06
S2	4.83E+07	3.05E+07	1.15E+06
S3	9.66E+07	2.75E+07	1.22E+06

[a]The initial concentration of Lactobacillus acidophilus in the microcapsule was 4.83E+08 UFC/g.

In addition, when starch-based coating was applied onto white bread and doughnuts, results showed that starch coatings controlled additives liberation to the product as a function of its water activity (Quezada-Gallo et al., 2004). It seems that coatings somewhat protects the microorganisms viability even after the baking process. In addition, the stability of the microorganism during short storage of the breads was investigated. The short-term storage caused a reduction in the total colony counts of microencapsulated *L. acidophilus* in all treatments. The reduction in the microbial counts during the storage period was similar in all the treated breads, independently of the coating treatment (Table 2). Therefore, it seems that the immediate surroundings of the microbes are responsible of this result, and only the microcapsules composition, which provides the essential micronutrients, determines the viability of the microbes during storage. Despite the reduction, microbes' survival indicates that probiotic coatings can be applied to the bread crust for obtaining functional breads.

Effect of probiotic coatings on the physicochemical properties and sensory evaluation of bread

Loaves used in this study have half-cylindrical geometry of size roughly 15±0.03 x 2.5±0.1 x 3.0±0.1 cm, weight 70±2 g and crust thickness was roughly 2.96±0.45 mm. Breads were sensory evaluated and no difference in taste was detected due to the presence of coatings. All the breads were accepted and no significant differences were observed regarding the attributes scored (Table 3). Perhaps the most affected attribute was the crust colour, namely in S3 due to the presence of some brown spots that corresponded to the microcapsules.

Table 3. Characteristics of fresh bread treated with probiotic coatings.

Sample	Control	S1	S2	S3
Specific volume ml/g	2.9 b	2.9 b	2.8 a	2.9 b
*L**	61.7 c	61.3 c	57.9 a	56.4 b
*a**	11.9 b	12.1 b	10.4 a	10.3 a
*b**	37.0 b	37.6 b	25.8 a	24.5 a
Crust A_w	0.4 a	0.5 a	0.4 a	0.6 a
Crust Moisture content (%)	7.9 b	6.8 a	6.6 a	9.9 c
Failure force (N)	13.1 d	11.4 c	9.6 a	10.6 b
Failure deformation mm	4.3 b	3.4 a	5.1 c	4.9 c
Failure firmness (N/mm)	2.6 c	3.4 d	1.9 a	2.2 b
Odour	3.7 a	3.7 a	3.1 a	3.9 a
Crust colour	4.0 a	3.3 a	3.6 a	2.7 a
Crispness	4.3 a	3.9 a	3.1 a	3.9 a
Crumb hardness	4.1 a	3.4 a	4.0 a	3.9 a

Means sharing the same letter within a row were not significantly different ($P<0.05$).

Some physical and chemical properties of bread were studied. The values obtained for crust colour, specific volume, water activity and moisture content are showed in Table 3. No differences were detected in the moisture content of the crumb, neither in the water activity or texture properties of the crumb due to the different coating treatments (results not showed). Unexpectedly, the treatment S2 produced a significant ($P<0.05$) decrease in the specific volume of bread; whereas, the control and breads with treatments S1 and S3 showed the same specific volume of the bread. Coatings were applied onto the partially baked breads surface, thus it seems that treatment S2 impairs any expansion that could take place during full baking.

The colour of the bread crust was also affected by treatments with probiotic coatings. The loaves with treatment S3 had the lowest lightness ($L*$), which might be attributed to the spraying of microcapsules between two coatings of starch solution, and the uneven mixing with the coatings produced an increase of opaque colour. Craig et al. (1989), when studied the visual characteristic of aqueous starch paste, observed that during gelatinization the starch granules swell and more light passes through the granules instead of being reflected. Therefore, the ability of the granules to reflect light diminishes, whereas, the transmitted light passing through swollen granules is refracted and the degree of refraction decreases with increasing swelling of the granules.

The treatment S1 did not promote any effect on the $a*$ parameter of the crust with respect to control bread; but the double addition of starch solution in treatment S2 and S3 produced a decrease in this parameter. Again, the presence of double starch solution yielded the lowest $b*$ values of the crust.

The water activity and the moisture content of the crust ranged from 0.43 to 0.56, and 6.6% to 9.9%, respectively. Coatings significantly ($P<0.05$) increased the crust water activity. Sample S3 showed the highest water activity, likely due to the hydrophilic structure of the starch based coatings. The moisture content of the crust significantly ($P<0.05$) increased with treatments S1 and S3. In opposition, treatment S2 showed lower moisture content than the control. It seems that in coating S2 the incorporation of microcapsules on starch solution and the application of a second starch solution over crust bread become more rigid and difficult to disperse, yielding microcapsules accumulation (Fig. 2d). Likely a more cracking film was obtained which favored the water diffusion (Müller et al., 2009).

The probiotic coatings applied on the bread surface produced significant changes on the mechanical properties of the crust (Table 3). The failure force or force necessary to induce the crust fracture significantly ($P<0.05$) decreased with the coatings applied. The treatment S2 resulted in the lowest force for fracture, despite its lower water content. Considering that at $A_w<0.6$ high failure force indicated brittle crust. Goedeken (1993) found that if microstructure is more porous, it gives brittle behavior and eases the water diffusion through the crust, strongly affecting the permeability of porous materials. A crispy texture has been associated with low values of moisture content and water activity, when starch and gluten matrix are in a glassy state and thus cell walls become more susceptible to fracture (Stokes and Donald, 2000). Therefore, starch based coatings decreased the mechanical properties associated to crispness, but sensory analysis indicated that those changes did not induce significant differences in the perceived attributes.

The failure deformation increased significantly ($P<0.05$) in the treated breads, with the exception of breads treatment with S1, which presented lowest deformation value than control bread. High values of failure deformation have been obtained in breads with thick crust suggesting stiffer crust structure (Altamirano-Fortoul and Rosell, 2011). Although no significant differences were found in the coatings thickness, the higher failure deformation observed in the crust with double starch coating suggested the presence of thicker crust in those samples, which would be expected after the swelling occurred during full baking.

Effect of storage conditions

No crust separation was observed during the bread storage, neither in the control or the treated bread. The moisture content of the crust increased with the storage time (Fig. 3a), observing the most rapid increase during the first 4 h after baking, which agrees with previous results of Altamirano-Fortoul and Rosell (2011). No significant effect was observed in the presence of the different coatings, the only distinguishable effect was observed in S2 that had lower initial slope, but higher moisture content when reaching the plateau. If the initial slope of the plots is taken as a measure of the speed of water uptake by the crust, the coating S2 reduced that speed, which has been related to higher porosity of the bread crust (Primo-Martín et al., 2008). This observation agrees with the coating microstructure of S2 that had accumulation or agglomerates of microcapsules, which could facilitate water diffusion from the crumb and the atmosphere surroundings. However, after prolonged storage high water uptake was obtained likely due to the agglomerates hydration.

Crust water activity was also affected by probiotic coatings during storage time (Fig. 3b). This parameter also presented a rapid initial increase during the first 4 h after baking. Control crust showed the highest increase of water activity. It has been described that at $A_w = 0.6$ water content starts to increase in an exponential fashion producing film structural changes which allow a facilitated water transport phenomenon (Bertuzzi et al., 2007). Coatings decreased the slope of the curves, being the highest reduction observed with treatment S2. Considering together the results obtained of treatment S2 for moisture content and water activity, it seems that at short storage period microcapsule agglomerates reduced the ability of the crust to retain water.

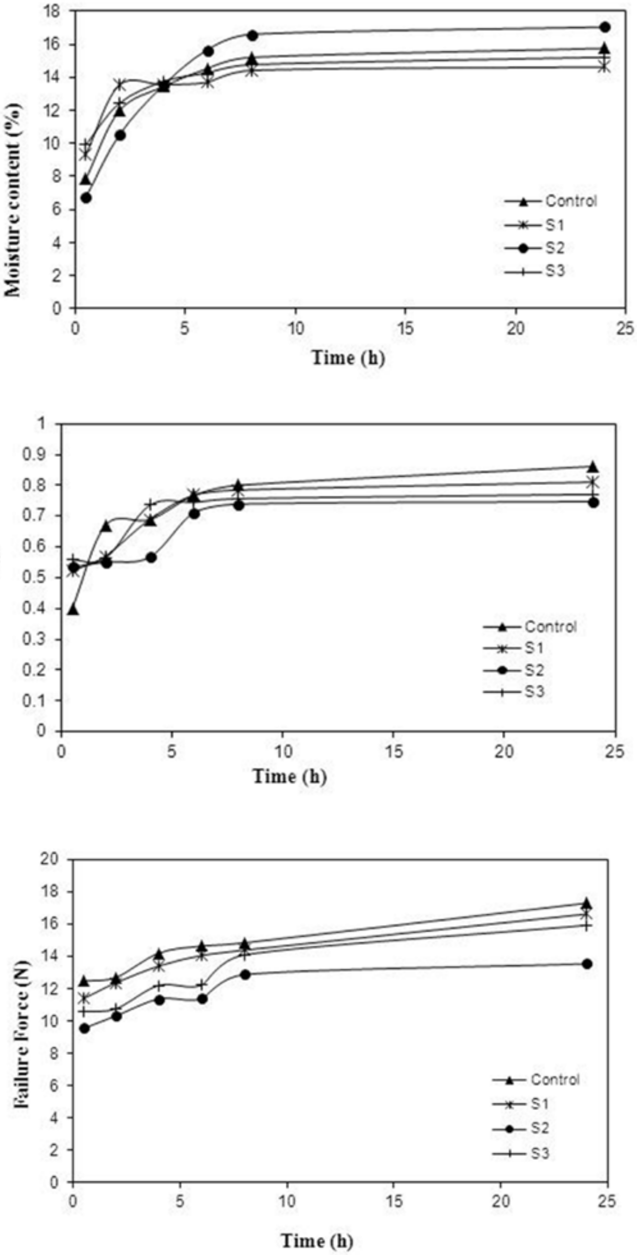

Fig. 3 Physicochemical properties of the probiotic bread crusts during a short storage at 25ºC. (a): moisture content vs. time, (b): water activity vs. time, (c): failure force vs. time. S1, S2, S3 are referred to the different probiotic coatings applied to the bread surface.

However, after 4 h storage agglomerates hydration might be responsible of higher crust moisture content with tightly bound water molecules, as suggest the lower water activity observed.

With respect to the mechanical properties of bread with probiotic coatings, failure force increased throughout the time of storage (Fig. 3c). The crust on control bread presented an increase in failure force as a result of moisture migration from the crumb to the crust and from the surrounding atmosphere to the crust. Consequently, the initially crispy crust becomes soft and leathery within very short period of storage. Samples with coatings showed initially lower crust failure force than the control, and the same trend was observed along the storage period. Loaves with treatment S2 presented the lowest value of failure force after 6 and 24 h of storage, thus coating S2 showed small increase of the failure force during storage. Coatings modified the crust structure, due to the new layers addition, which decreased the failure force in the fresh bread, but they did not significantly modify the trend during bread storage.

Crust structure

Scanning electron microscopy observations of surface and cross-section of probiotic crusts are showed in Fig. 4. Control crust showed a continuous veil-like film that revealed a dominant presence of the partially gelatinized starch granules

(Fig. 4a), whereas the cross section showed a compact structure resulting from starch gelatinization and protein denaturation (Fig. 4b).

Probiotic coatings induced significant differences in the crust microstructure. The crust with coating S1 showed a smooth and homogenous background due to gelatinized starch, together with some agglomerates of roughly polyhedral microcapsules (Fig. 4c). The micrograph of crust coated with S2 also revealed a homogenous background of starch, but the microcapsules appeared more concentrated, leading to denser zones, as was observed in the coating microstructure (Fig. 4e). The sample treated with S3 showed higher density of microcapsules over the surface (Fig. 4g) comparing with the other samples, which might be attributed to the high concentration of microcapsules used in this treatment. Less difference was observed in the cross section micrographs (Fig. 4 b, d, f, h). A compact cross section with some small void spaces were observed in all the micrographs, although less void spaces were observed in the samples with double starch layers (Fig. 4 f, h). In the crust cross sections the microcapsules were observed onto the surface, and in sample with S3 the microcapsules were more embedded in the gelatinized matrix. Thus it seems that the microcapsules were better covered when they were located between two starch layers, which agree with the survival results described in section 3.2.

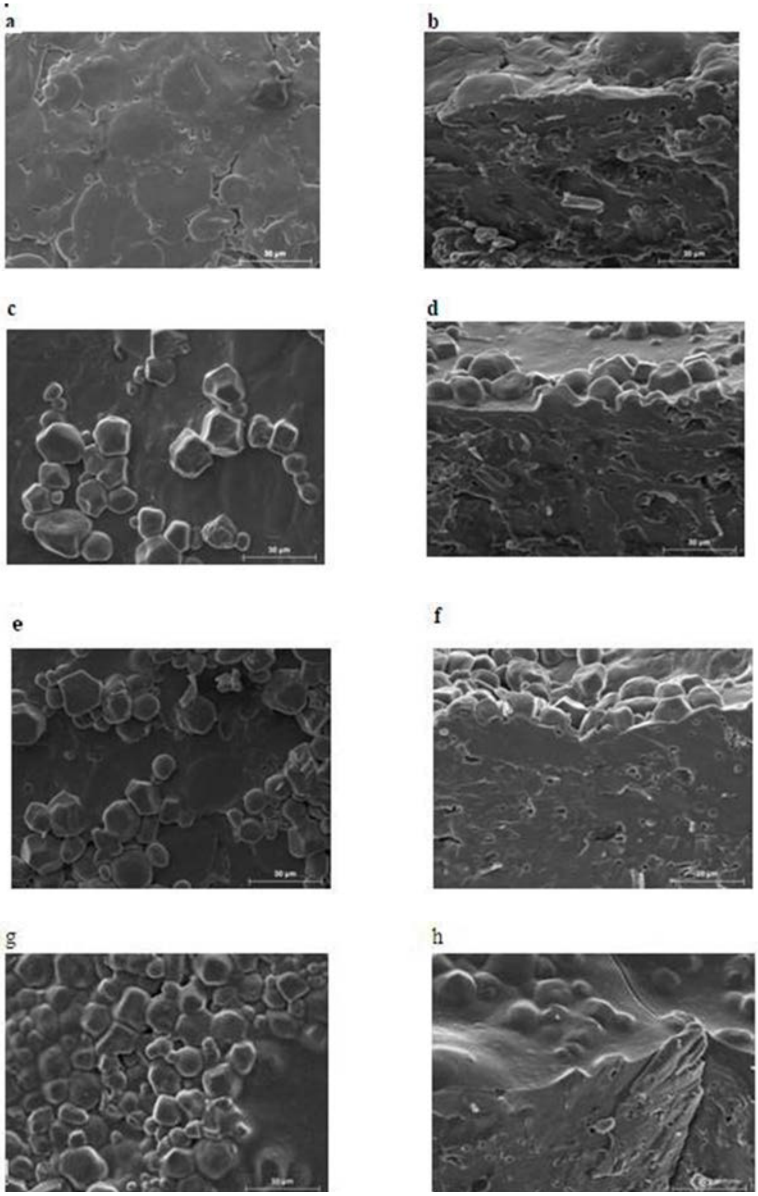

Fig. 4 Scanning electron micrographs of bread crust surface (a, c, e, g) and cross section (b, d, f, h). Images correspond to the following probiotic coatings treatments: (a, b): Crust without probiotic coating, (c, d): crust with S1 treatment, (e, f): crust with S2 treatment, (g, h): crust with S3 treatment. Scale bars of 30µm.

CONCLUSIONS

Overall results show that *L. acidophilus* included in microcapsules can be incorporated to bread surface through edible coatings, leading to bread with similar characteristics to common bread, but with additional healthy benefits. Edible coatings have been used as a vehicle for microorganisms and the physical properties of the resulting bread confirmed the potential use of this procedure for obtaining healthier baked goods. The survival of microencapsulated *Lactobacillus acidophilus* demonstrated the ability of starch solution to protect the microcapsules during baking and storage time, likely due to the adhesion of the microcapsules to the starch macromolecules. This study also shows that the functionality of edible coatings depends on their composition (suspension constituents) and the coating procedure (monolayer, successive layer or multi coating) onto the product. Considering the microorganism survival, the physicochemical properties of the bread crust and the economy of the process, the treatments S1 and S2 would be the best alternative for carrying the microcapsules. Currently, studies are undertaken to confirm the probiotic effect of these breads by carrying out *in vitro* and *in vivo* studies.

ACKNOWLEDGEMENTS

Authors acknowledge the financial support of Spanish Ministry of Economy and Sustainability (Project AGL 2011-23802) and the Consejo Superior de Investigaciones Científicas (CSIC). Authors thank Mr. Alfonso Martínez from CICATA, IPM (México) for his contribution with the electron microscopy analysis. R. Altamirano-Fortoul would like to thank her PhD grant (JAE pre) to CSIC. The authors also thank Forns Valencians S.A. (Spain) for supplying commercial frozen partially baked breads.

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General discussion

GENERAL DISCUSSION

The bread crust due to its crispy character is an important characteristic for the consumer's appreciation (Luyten et al., 2004). Nevertheless, bread has very short shelf life with rapid loss of freshness, which has a negative influence in quality of product affecting both flavor and desirable crispy character (Primo-Martín et al., 2006). Therefore, the study of properties of crust and its ability to retain its freshness is of great interest from the consumers' point of view and also for reducing the economic losses associated to bread waste. Crust is formed during baking, because of that the initial understanding of crust formation is necessary prior to propose any treatment for inducing its modification. In this regard, the present investigation describes a number of studies for understanding bread crust features regarding its formation, characterization and modification. Specifically, studies have been conducted to assess the effect of steam condition on bread crust, to define the main quality features that allow differentiation among different bread specialties, to determine the best conditions to study the crust's mechanical properties for providing information about the internal cell structure, and finally use of bread crust surface for obtaining added value baked products.

Bread quality is highly dependent on the breadmaking process. In crispy bread, baking is a crucial stage that has a vital influence on crust formation as well as on its properties. Besides temperature and baking time, steaming is greatly responsible of crust features. The precise impact of the amount of steam injected in the oven at the beginning of baking is not always very well indicated. Steam injection results in an increase of the humidity in the oven. The moisture condenses on the cold fermented dough pieces (Zhang et al., 2007; Hui et al., 2006); therefore, a strong interaction is foreseen between the amount of steaming and the oven rise (loaf expansion during the first minutes of baking). However, the amount of steam present in the oven determines the drying rate of the crust and its elasticity (Le Bail et al., 2011).

As mentioned earlier, this research investigated the effect of the amount of steaming (100, 200 and 400 ml) during baking on bread crust feature. Results supported that the amount of steam affected significantly ($P<0.05$) the properties of the crust colour parameters and glossiness. The lightness ($L*$), yellowish colour ($b*$) and glossiness increased with the steaming amount, contrary to what observed with the reddish colour ($a*$). The application of steam improves favorably the crust colour causing the formation of a brown colour and aromatic substances that give the typical smell of fresh baked bread (Ahrné et al., 2007). Moreover, water vapour permeability (WVP) of the bread crust significantly ($P<0.05$) decreased when increasing the amount of the steam applied during baking. Crust permeability changes, induced by the amount of steam applied, indicated that crust permeability can be modulated by the amount of steam because it modifies the heat penetration into the loaf from the surface to the inner parts, which will affect the bread crust thickness. In fact, in the literature some studies reported a relationship between the thickness and WPV. Particularly, McHugh et al. (1993) observed that when thickness increases, the polymeric structure increased the resistance to mass transfer across it. Other authors attributed thickness effect to starch swelling as a result of attractive forces between polymer and water (Park et al., 1993).

Additionally, the increase of the amount of steam caused an increase in bread crust mechanical properties (failure force and firmness), related to a more brittle and rigid bread crust. Furthermore, the values obtained in this study were supported with bread crust microstructure analysis. Using Scanning Electron Microscopy (SEM), results showed that non gelatinized starch was visible for the low steaming crust whereas evidence of starch gelatinization was visible for high steaming; which could be attributed to the presence of condensed steam at the surface of the bread. Microstructure analyzed by X-Ray microtomography showed 3D-images of the bread crust, determining the porosity profile in the bread crust; where higher porosity was observed for 100 ml steamed samples than in the case of 200 and 400 ml.

Partially baked bread obtained from the bake off processing, allows obtaining bread without the brownish crust formation, which is finally formed during the final baking. This breadmaking system provides a very uniform product for studying the crust features immediately after baking. Because of that, frozen partially baked products were selected as raw material for assessing crust features. But previously a characterization of different bread specialties was carried out to identify the quality parameters that were able to discriminate among breads. Nine partially baked breads (with different ingredients in their formulation) were selected on the basis that these represent a range of bread specialties currently disposable in the Spanish market. Diverse quality parameters that characterize bread crust and crumb were determined by instrumental methods to define the main quality features. Results obtained were analyzed by principal component analysis (PCA). Quality parameters that enable the differentiation of bread types were crust mechanical properties together with specific volume, crumb hardness and structure. Also, in this study breads were characterized during the storage. The crust moisture content and water activity increased in all types of bread during storage; however, the most rapid increase was showed during the first 4 h after baking. These increases were primarily due to water migration between crumb, crust, and environment. Increasing water content can lead to plasticizing effect because water acquires properties of solvent and promotes mobility of polymer chains. This plasticization of polymer chains facilitates changes in features like molecular structure, porosity and texture of brittle or crispy products (Lewiki, 2004; Luyten et al., 2004; Hug-Iten et al., 2003; Harris and Peleg, 1996; He and Hoseney, 1990; Katz and Labuza, 1981).

In addition, crust mechanical properties were rapidly lost during the first 4 h after baking and the rate of the process change was greatly dependent on the bread type. The force to promote crust fracture increased up to 6 h after baking and those changes occurred in the water activity range of 0.50 to 0.74 or moisture content 9 to 15g/100 g. Anew, this is caused by migration of moisture from the crumb to the crust. The crust is hygroscopic and readily absorbs moisture which diffuses outwards and becomes soft and leathery. Bread crust in the fresh state is dry and crispy, exhibiting brittle facture. The loss of crispy texture observed during storage could be due to hydration of the components, which induce glass transition in amorphous regions in polymers that were initially in the glass state, causing a soft and tough crust (Primo-Martín et al., 2006; Cuq et al., 2003).

According to the previous paragraph, the texture in crispy bread is an important component for the perception of its quality. Nevertheless, numerous physical parameters combined with the microstructure of food determine its texture. Therefore, different instrumental measurements are generally used to evaluate the mechanical properties related to texture. In the present investigation, the effect of setting conditions, such as speed (0.5, 1, 10, 20, 30 and 20 mm/s) and punch cross-section (3 and 28 mm^2), on punching parameters and their relationship with microstructure of bread crust were determined. Two specialties of bread with different crust thickness were selected for study mechanical properties of the bread crust, because they would allow validating the reliability of the texture assessment. After baking the water content and water activity of the bread crust is low allowing the main components to undergo glass transition (Cuq et al., 2003). It is clear that water plays a critical role in mechanical properties of bread crust but it happens till water activity values around 0.70, further increase in water activity leads to little changes in these properties (Castro-Prada et al., 2009; van Nieuwenhuijzen et al., 2008).

With respect to punching settings on the puncturing parameters, average puncture force (Fm) was affected by punch cross-section and test speed in both types of bread crust thickness. Thick bread crust had more layers and contain more irregular and larger cell. Slumier (2005) reported that for crusty bread, the thickness of the crust is important and a thickness of more than 5 mm is a good level for crust in this type of bread. Thick bread crust showed more structure rupture in short time due to it has higher cellular structure that thin bread crust. A highly jagged curve profile with many peaks due to numerous fracture events is often produced by products that are perceived as crispy (Valles-Pamies et al., 2000; van Hecke et al., 1998; Vincent, 1998). Results demonstrated that a greater punch cross-section caused the decrease of the jaggedness of the force-deformation curve. In this way, with smaller punch cross-section major number of peaks can be detected and thus greater values of N_{wr} parameter. The specific force of structural rupture parameter (f_{wr}) was independent of crust thickness, at higher speeds and punch cross-section the mechanical resistance in the cell walls increase. Luyten and van Vliet (2006) study the fracture on dry crispy foods using 0.2-40 mm/s speeds, and they found that the estimation of the rupture rate could be faster than 100m/s, which means that the rupture propagates 1 mm in 10 µs. The crispness work parameter (W_{c}) was significantly dependent on the punch cross-section and speed. However, results suggest that independently of the punch cross-section, only by using low speed is it possible to assess this parameter due to the interface of the compression is reduced.

In general, all results demonstrated that to find information about the cellular structure of the crust, it is necessary to use both low speed (0.5 mm/s) and low punch cross-section. Scanning Electron Microscopy (SEM) allows differing between thin and thick bread crust. According to Bourne (2002), the texture of foods is derived from their structure.

A proposed strategy for modifying the microstructure of the crust was the application of different concentrations (0, 100, 250, 500, 100 mg/10ml) of amyloglucosidase. In this research, two different bread specialties with diverse thickness (thin and thick) were selected for determining the capacity of the enzyme to penetrate through the crust. The enzyme concentration applied onto the bread surface significantly affected the physicochemical properties of crust. In general, total color difference (ΔE) increased progressively due to the enzymatic treatment; causing a darkness bread crust than the breads without treatment. This increase might be consequence of the acceleration of Maillard reaction due to the additional glucose released by amyloglucosidase, which agree with results of Sharma and Singh (2010), who reported the use of amyloglucosidase to improve bread crust color. Enzyme treatments at levels higher than 100 mg/10 ml decreased significantly the water activity in the thick crust bread; contrary occurred in the thin crust. Possibly, enzyme treatments changed the cross-link of protein-starch network forming the crust, which altered the crust microstructure making it with more porous. Therefore, water migrates very quickly. In fact, in a porous domain, molecules are allowed to transfer more quickly because of several mechanisms of moisture transfer; the smaller the pore sizes in the matrix the slower the moisture migration (Labuza and Hayman, 1998).

Enzyme treatments reduced the puncturing force, spatial force of structural ruptures and crispness work compared to the control bread. With respect to spatial frequency of structural ruptures treatments with amyloglucosidase promoted a significant increase in this parameter. Microstructure analysis by SEM confirmed that the level of enzyme treatment modifies the crust structure, and the effect was dependent on the thickness of the crust. In thin bread crust treated with greater enzyme concentration, the structure was more compact with sub-holes within the cells. However, in thick bread crust a layer and fragmented structure was showed. The enzyme level induced changes in the protein-starch interactions and between starch chains and water molecules.

The development of functional foods seems to be the trend of the food industry that is looking for innovation in a traditional sector. The term functional food is used for referring to healthful foods or food ingredients that have a potential health benefit beyond their nutrient content when consumed regularly in typical quantities as part of a varied diet (American Dietetic Association, 1999; Hasler, 2000). In

1998, European Commission’s Concerted Action on Functional Food Science in Europe (FUFOSE), which actively involved a large number of the most prominent European experts in nutrition and related sciences, reached a consensus about the concept of functional foods defining that they produces beneficial effects beyond the usual nutritional effects in a sense relevant to the welfare state and health or disease risk reduction (Roberfroid, 2000). Basically, functional foods include positive physiological effects of prebiotics, probiotics and synbiotics. The expert committee of FAO/WHO recommends defining probiotic as a live microorganism which when ingested in sufficient amounts by humans confer a health benefit on the host (FAO/WHO, 2001; Brown and Valere, 2004; Sanz and Dalmau, 2008). Some authors recommend a daily dosage of 106-109 viable organisms reaching the intestinal tract in humans (Sanz and Dalmau, 2008).

Despite the importance of cereals and baked goods in human nutrition, so far no probiotic baked goods has been developed mainly due to the high temperature reached during baking. The combination of partially baked bread technology with the microorganism encapsulation and biopolymers coating could be used for obtaining probiotic breads.

Partially baked bread could be an alternative that allow obtaining probiotic bread due to only requires a very short baking time. Edible coatings (containing *Lactobacillus acidophilus* microencapsulated) might allow obtaining a probiotic product and improving the crust mechanical properties. Results demonstrated differences between the coatings S1 (starch-microcapsule solution), S2 (starch-microcapsules solution with starch solution without microcapsules) and S3 (starch solution without microcapsules with microcapsules sprayed and starch solution without microcapsules) in their mechanical properties, thickness and morphology. The choice of ingredients and preparation method has a significant impact on the material properties of starch-based films or coatings (Koch et al., 2010). Respect to viability of *L. acidophilus* in bread crust after full baking process, the temperature affected in different extent depending on the coating. Therefore, the bread crust with treatment S2 kept higher counts after baking. That could be due to double starch solution protected the microcapsules. While treatment S3 was the most sensitive to baking temperature due to microcapsules were less protected. In fact, microscopy analysis demonstrated that microcapsules were embedded in the gelatinized matrix and thus it was not covered. In the case of short storage, the reduction in total colony counts of *L. acidophilus* was similar in all treatments.

Coatings significantly ($P<0.05$) increase the crust water activity, bread crust with treatment S3 presented the highest value in this parameter; probably to the hydrophilic structure of the starch based coating. Significant changes on the mechanical properties were produced by all treatments. The force necessary to induce the crust fracture decreased with the treatments applied, which seems to be related to the hydrophilic nature of the coatings that increased the moisture content (Hirte et al., 2010; van Nieuwenhuijzen et al., 2008; Mazumder et al., 2007); thus, an increase in moisture content increases maximum force.

During short storage time, all the samples were affected. Moisture content and water activity of the crust treated increased with the storage, but the increase was less with respect to control crust. This might be consequence to treatments allow better water diffusion between the crumb and external atmosphere. Namely, this was corroborated by the microstructure, where a compact structure with a continuous veil-like film was showed. As mentioned above many factors affect the texture of cellular food products during storage. For example, the loss of crispness perception can be caused by an increase of moisture due to water sorption during storage of crisp bread (Jakubczyk et al., 2008; Primo-Martín, 2008, 2006).

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Conclusions

CONCLUSION

A continuación se presentan las conclusiones más relevantes de esta Tesis Doctoral:

- The instrumental methods applied for bread quality assessment allowed discriminating among bread specialties from frozen partially baked breads. Crust mechanical properties together with specific volume, crumb hardness and structure were the quality parameters that permitted bread differentiation. Regarding the evolution of the crust physicochemical properties, they changed shortly after baking. Specifically, water content/activity showed a steady increase during the first 4 h after baking. In addition, the force to promote crust fracture varied up to 6 h and those changes occurred in the A_w range of 0.50-0.74 or moisture content 9-15%.
- Crust properties are significantly affected by the amount of steam applied during the baking process, particularly colour and glossiness, thickness, microstructure and texture properties. Low amounts of steam give thinner crusts with high vapour transmission rate and permeability. Based on present results, it seems that a medium to low amount of steam results in a thinner crust, which in turn is more permeable to moisture during storage. Therefore, it is

expected that such crust will remain brittle for longer time during storage after baking.

- When studying the mechanical properties of bread crust by puncturing, the test settings, namely crosshead speed and punch cross-section, exert great influence in the data recorded, and that effect is magnified when increases crust thickness. The relationship between the puncturing parameters and the water activity and moisture content together with the crust microstructure analysis allow selecting the most appropriate texture settings for obtaining reliable mechanical parameters. Crust microstructure observations indicate that the crust layers and the size and shape of the air cells are responsible of the puncturing behavior.
- Enzymatic treatment of the bread crust can decrease the moisture content and water activity, due to an increase in the crust porosity besides the removal of water participating in the hydrolysis reaction. Regarding mechanical properties, overall results indicate that the enzymatic treatment resulted in crust with reduced resistance to puncture and high number of fracture events, indicating crispy products. Amyloglucosidase produces an important modification on the starch-protein matrix structure, related to the steady removal of the gelatinized starchy layer. The

enzyme level required for modulating crust structure was dependent on the crust thickness.

- *L. acidophilus* included in microcapsules can be incorporated to bread surface through edible coatings, leading to bread with similar characteristics to common bread, but with additional healthy benefits. The survival of microencapsulated *Lactobacillus acidophilus* demonstrated the ability of starch solution to protect the microcapsules during baking and storage time. Considering the microorganism survival, the physicochemical properties of the bread crust and the economy of the process, the treatments S1 and S2 would be the best alternative for carrying the microcapsules.