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TESIS DOCTORAL:

ROLE OF STARCH GELATINIZATION AND FRAGMENTATION IN EXTRUDED FLOUR FUNCTIONALITY

Presentada por Laura Román Rivas para optar al grado de Doctor Internacional por la Universidad de Valladolid

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Sé el cambio que quieras ver en el mundo

Mahatma Ghandi

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This Doctoral Thesis is composed of a list of original research papers, according to the Spanish National (R.D. 99/2011, BOE 35/2011) and Regional (BOCYL 243/2012) regulations.

Esta Tesis Doctoral se compone de una lista de trabajos de investigación originales, de acuerdo con las regulaciones nacionales (R.D. 99/2011, BOE 35/2011) y regional (BOCYL 243/2012 españolas.

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Prior to the Thesis defense, this research work has been evaluated by two experts from international research centers and universities, whose research and expertise are directly related to the thesis subject. The changes suggested by the below mentioned evaluators have been included in the final version of this thesis.

Previamente a la defensa de la Tesis, este trabajo ha sido evaluado por dos expertos procedentes de centros de investigación y universidades internacionales, cuya investigación y experiencia están directamente relacionadas con el tema de la tesis. Los cambios sugeridos por los evaluadores mencionados a continuación han sido incluidos en la versión final de esta tesis.

**Dr. Kelvin Goh, Massey Institute of Food Science & Technology, Massey University (New Zealand)*

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RESUMEN

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Los cereales, incluidos el maíz, el arroz y el trigo, constituyen una fuente importante de energía y nutrientes en la dieta humana, ya que son ingredientes ampliamente utilizados (en forma de harina o almidón aislado) en la industria alimentaria. Así, el almidón contribuye con el 50-70% de la energía en la dieta, proporcionando una fuente directa de glucosa. En este sentido, recientemente se está prestando atención a almidones procedente de fuentes más beneficiosas nutricionalmente, como el almidón de plátano, una fuente natural de almidón resistente, que puede tener un efecto fisiológico positivo en la salud humana. Aunque el almidón nativo se utiliza debido a sus capacidades únicas de espesamiento y gelificación, para varias aplicaciones industriales, estas propiedades no cumplen con los requisitos del proceso o del producto. De esta forma, la modificación de la funcionalidad del almidón por tratamientos hidrotérmicos se está volviendo de gran interés, siendo la extrusión una tecnología limpia y rentable para producir la gelatinización y/o fragmentación de almidón.

Esta tesis doctoral se centra en el desarrollo de nuevos productos a base de plantas con un mejor valor físico, organoléptico y/o nutricional mediante la utilización de harinas extruidas como ingrediente funcional. En estos productos se evaluó el efecto de aspectos tales como la fuente de almidón, el tamaño de partícula o la severidad del tratamiento de extrusión de las harinas extruidas.

La primera parte de este estudio evaluó el potencial de las harinas extruidas como sustitutos de grasa en emulsiones alimenticias. En emulsiones tipo mayonesa, el reemplazo de aceite por harina extruida aumentó gradualmente el número y redujo el tamaño de las gotas de aceite y afectó a las propiedades reológicas (mayor carácter elástico y tixotrópico) de la emulsión de aceite en agua. Sin embargo, se concluyó que estas diferencias podrían minimizarse si se eligiera la relación harina-agua adecuada de la pasta extruida (1:3), obteniendo propiedades reológicas similares a las de la mayonesa con el total de grasa, y con una sinéresis tras la congelación-descongelación mejorada. En cuanto a las masas batidas para bizcochos, la sustitución del aceite por harina extruida dio como resultado un batido menos estructurado y viscoelástico, que, a su vez, condujo a una disminución en el volumen del bizcocho reducid en grasa y a una miga

más dura, menos elástica y cohesiva. Sin embargo, estos resultados negativos pueden controlarse parcialmente mediante la acción combinada de harina extruida y un emulsionante.

La mayor capacidad de absorción de agua de las harinas extruidas también puede ser útil para optimizar/incrementar la viscosidad en soluciones frías. Con este objetivo, se evaluó el efecto de la sustitución parcial de la harina de trigo nativa por harinas extruidas sometidas a diferentes severidades de extrusión en la calidad de los batidos para rebozados. El reemplazo de la harina extruida dio como resultado cortezas más crujientes y con un mayor índice de recubrimiento a medida que se incrementaba el porcentaje de harina extruida y la severidad de su tratamiento. También se evaluaron los compuestos volátiles presentes en los rebozados, revelando una menor cantidad de compuestos que otorgan rancidez procedentes de la oxidación de lípidos en las cortezas fritas con harina extruida. Además, las mejores valoraciones en cuanto a la aceptabilidad por parte del consumidor se encontraron en los rebozados hechos con harina extrudida en condiciones intermedias, lo que confirma que la inclusión de harina extruida mejora la calidad de los rebozados fritos. Respecto a las aplicaciones instantáneas, se usaron harinas extruidas completamente pregelatinizadas para la fabricación de salsas. Los resultados obtenidos sugieren que los tamaños de partícula más finos de las harinas extruidas no son preferibles para las salsas preparadas en frío debido a su comportamiento reológico más débil y mayor sinéresis. Por el contrario, las harinas de arroz extruido parecían ser más apropiadas para la fabricación de salsas frías, debido a su menor sensibilidad al cizallamiento y liberación de agua después del proceso de congelación-descongelación.

En la tercera parte de esta investigación, se comparó el comportamiento frente a la retrogradación del almidón procedente de harinas de maíz, trigo y arroz tanto nativas como extruidas a fin de comprender mejor la eficacia de la extrusión a la hora de disminuir la predisposición del almidón en formar interacciones intermoleculares. Las zonas de unión intermoleculares determinan las propiedades mecánicas de la estructura de red de los geles de almidón y, a su vez, la calidad física y sensorial de los alimentos a base de almidón. En todas las pastas de harina, la extrusión fue efectiva al disminuir el endurecimiento de los geles durante el almacenamiento, aunque dos distintos mecanismos parecen ser el motivo para la estructura de gel más débil, basándose en la composición del almidón y la estructura del cereal. Este estudio proporciona una comprensión importante a nivel molecular para la optimización de la funcionalidad de la harina a través de la extrusión con el fin de reducir los fenómenos de retrogradación, como el envejecimiento de los panes o la sinéresis de las salsas.

Finalmente, desde una perspectiva nutricional, se utilizó almidón de plátano extruido como ingrediente funcional en panes sin gluten. El pan blanco (un alimento con alto índice glucémico) sigue siendo la primera opción del consumidor y su miga está formada por almidón altamente susceptible a la digestión enzimática. La velocidad de digestión del almidón completamente gelatinizado presente en el pan se puede ralentizar mediante la manipulación de la estructura molecular del almidón mediante extrusión. Los hallazgos mostraron que el contenido de almidón lentamente digerible (SDS) en la miga gelatinizada que contenía almidón de plátano extruido se quintuplicó, que se atribuyó al aumento de las reasociaciones entre las cadenas A y B1 de la amilopectina de plátano durante el almacenamiento. No se observaron diferencias en cuanto a la valoración global de los panes con plátano extruido y el control, lo que valida la posibilidad de incluir almidón de plátano extruido en los panes. Además, este estudio muestra, por primera vez, que la predisposición de las moléculas de amilopectina para formar SDS estructuralmente motivado se mejora adicionalmente mediante una reducción de su tamaño molecular a través de la extrusión.

ABSTRACT

ABSTRACT

Cereals including maize, rice and wheat, constitute a major source of energy and nutrients in the human diet, since they are widely used ingredients (either in the form of flour or isolated starch) in the food industry. Thus, starch contributes 50-70% of the energy in the human diet, providing a direct source of glucose. In this regard, attention has been recently paid on starch coming from more nutritionally beneficial sources, such as banana starch, a natural source of resistant starch, which may have a physiological effect on human's health. Although native starch is used due to its unique thickening and gelling capacities, for a number of applications, these properties fail to meet process or product requirements. Therefore, the modification of starch functionality by hydrothermal treatments is becoming of great interest, being extrusion a clean, cost-effective technology to produce starch gelatinization and/or fragmentation.

This doctoral thesis focuses on developing novel plant-based products with improved physical, organoleptic and/or nutritional value by utilization of extruded flours as a functional ingredient. The effect of aspects such as the starch source, particle size or severity of the extrusion treatment of the extruded flours were evaluated.

The first part of this study evaluated the potential of extruded flours as fat substitutes in food emulsions. In mayonnaise-like emulsions, oil replacement by extruded flour gradually increased the number and reduced the size of oil droplets and affected the rheological properties (greater elastic and thixotropic character) of the oil-in-water emulsion. However, it was concluded that these differences could be minimized if appropriate flour-water ratio of the extruded paste (1:3) was chosen, obtaining similar rheological properties to the full fat one, and with an improved freeze-thaw syneresis. Regarding cake batters, oil replacement by an extruded flour resulted in a less viscoelastic-structured batter, which, in turn, led to a decrease in the volume of the final cake and a harder, less elastic and cohesive crumb. Nonetheless, these negative results can be partly controlled by the combined action of extruded flour and an emulsifier.

The greater water absorption capacity of extruded flours can also be useful to optimize/enhance viscosity in cold solutions. Thus, the effect of the partial replacement

of native wheat flour by extruded flours subjected to different extrusion severities on the quality of fried nuggets was assessed. Extruded flour replacement resulted in batters with improved pick-up and crispness with increasing percentage and severity of extrusion treatment. Volatile compounds present in coatings were evaluated, revealing a lower amount of rancid volatile compounds from lipids oxidation in fried batters containing extruded flour. In addition, best scores for consumer's acceptability were found in coatings made with intermediate-severity treatment extruded flour, confirming that extruded flour inclusion does improve the quality of deep-fried batters. For instant applications, pregelatinized extruded flours were used in sauces making. Evidence suggests that the finest particle sizes of extruded flours are not preferable in cold-prepared sauces due to their weaker rheological behavior and higher syneresis. Conversely, rice extruded flours seemed to be more appropriate for cold-sauces making, due to their lower sensitivity to shearing and water release after freeze-thaw process.

In the third part of this research, the starch retrogradation behavior of native and extruded maize, wheat and rice flours were compared to better understand the efficacy of extrusion in decreasing the propensity of starch to form inter-molecular interactions. Inter-molecular junction zones determine the mechanical properties of the network structure of starch gels and, in turn, the physical and sensory quality of starch containing foods. In all flour pastes, extrusion was an effective way to decrease gel hardening during storage, although two distinct predominant mechanisms were suggested for the weaker gel structure, based on the starch composition and structure of the cereal source. This study gives important understanding at the molecular level for the optimization of flour functionality through extrusion in order to reduce retrogradation-controlled phenomena such as bread staling or sauce syneresis.

Finally, from a nutritional perspective, banana extruded starch was used as a functional ingredient in gluten-free breads. White bread (a high glycemic food) remains the consumers' first choice and its crumb is formed by starch highly susceptible to enzyme digestion. The digestion rate of the fully gelatinized starch present in bread can be slowed down by the manipulation of the starch molecular structure through extrusion. Findings evidenced a fivefold increase in slowly digestible starch (SDS) content in the

fully gelatinized crumb containing extruded banana starch, which was attributed to increased re-associations involving A and B1 chains of banana amylopectin upon storage. No differences in overall liking between extruded banana and control breads were observed, validating feasibility of inclusion of extruded banana starch in breads. Furthermore, this study shows, for the first time, that the propensity of amylopectin molecules to form structurally driven SDS is further improved by a reduction of their molecular size through extrusion.

LIST OF ORIGINAL PAPERS

List of research papers included in this Doctoral Thesis.

1. Román, L., Martínez, M. M., & Gómez, M. (2015). Assessing of the potential of extruded flour paste as fat replacer in O/W emulsion: a rheological and microstructural study. *Food Research International*, *74*, 72-79.
2. Román, L., Santos, I., Martínez, M. M., & Gómez, M. (2015). Effect of extruded wheat flour as a fat replacer on batter characteristics and cake quality. *Journal of Food Science and Technology*, *52*, 8188-8195.
3. Román, L., Pico, J., Antolín, B., Martinez, M. M., & Gómez, M. (2018). Extruded flour improves batter pick-up, coating crispness and aroma profile. *Food Chemistry*, 260, 106-114.
4. Román, L., Reguilón, M. P., & Gómez, M. (2018). Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source. *Journal of Food Engineering*, *219*, 93-100.
5. Roman, L., Gomez, M., Hamaker, B. R., & Martinez, M. M. (with editor). Shear scission during extrusion diminishes inter-molecular interactions of starch molecules during storage. *Submitted to Journal of Food Engineering.*
6. Roman, L., Gomez, M., Hamaker, B. R., & Martinez, M. M. (with editor). Banana starch and molecular shear fragmentation dramatically increase structurally driven slowly digestible starch in fully gelatinized bread crumb. *Submitted to Food Chemistry.*

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INTRODUCTION

1. INTRODUCTION

Cereals constitute a major source of energy and nutrients in the world, especially among the population of developing countries. Cereals including maize, rice, wheat, barley, rye, oat, and millet make up the majority of the crop production globally. Among these cereals, maize is the main produced one (tons) followed by paddy rice and wheat (FAOSTAT, 2013).

Maize (*Zea mays* L.) is a worldwide-cultivated crop with multiple end-uses in the food industry. Grit and meal maize are used in the production of snacks and breakfast cereals, whereas maize flour or starch are mostly used in blends with wheat flours in bakery products or as a thickening agent in many dishes such as soups, sauces, infant products and gravies (Roman, Martinez, Rosell, & Gomez, 2015).

Rice (*Oryza Sativa L.*) is mostly orientated to be consumed as intact kernels, supporting more than half of the global population as a dietary energy source, particularly in Asia and some African countries (Nakamura, 2017). Nonetheless, a recent significant raise in the rice flour production has been observed due to its utilization in novel foods such as baby foods (hypoallergenic nature) and gluten-free based goods (de la Hera, Gómez & Rosell, 2013).

Regarding wheat, the majority of the wheat grown in the world for food belongs to two botanical species, *Triticum durum* and *Triticum aestivum*. The resultant products from the milling process of the former are mainly used as regular raw material for pasta production, while the latter is orientated to both non-bread making (cakes, cookies) and bread making purposes (Pareyt & Delcour, 2008). Wheat is the most versatile cereal because of the ability of its storage proteins to interact and develop the gluten network, needed for the making of many bakery products and pasta.

Wheat, rice and maize grains (also referred as kernels or caryopsis) present, in general, similar features, and same constituting parts are found in approximately same relationships. The caryopsis develops within floral envelopes and consists of a fruit coat (pericarp) and a seed composed of several parts (i.e. seed coat and nucellar epidermis enclosing the endosperm and germ). In maize and wheat, unlike rice, the grain and hull separate easily during threshing, leaving the kernel as an uncovered caryopsis (naked grains), and facilitating further milling (Delcour & Hoseney, 2010). Milling process is a several-stage process combining a sequence of breaking, sieving, and size-reducing processes, through which flour is extracted. In the milling process, bran and germ, as well as other seed layers are separated from the endosperm cells, which are broken into a very fine product, constituting the flour. Although bran and germ can also form part of the final flour, in general, refined flours rather than whole flours are used, which have lost a great part of nutrients such as fibers, vitamins and minerals (Rosell, 2012).

Regarding the chemical composition of cereal grains, and, in turn, its processed resultant products, it is worth mentioning the remarkably high content of starch, being this polysaccharide the major component and accounting for around 60-75% of the weight of the grain. Starch contributes 50-70% of the energy in the human diet, providing a direct source of glucose, which is an essential substrate in brain and red blood cells for generating metabolic energy (Copeland, Blazek, Salman, & Tang, 2009). Starch importance in the human diet lies in the fact that it is a common ingredient (either in flour or isolated form) widely used in the food industry. Starch is a macro-constituent of many food products, including bread, pastry, breakfast cereals, cooked rice, pasta, sauces, etc. Starch possesses unique thermal, structural and functional properties being responsible for several physical changes in food products such as the gelling of puddings, the thickening of sauces, the setting of the crumb in bakery products and their staling, the formation of a crispy continuous coating over a food substrate, etc. Thus, being able to predict starch functionality from knowledge of its structure, and explain how starch interacts with other components in a food system remains a challenge in the food industry.

Other than flours coming from cereals, a great deal of attention has been paid on flours coming from other plant sources rich in starch, such as green fruits. Among these novel alternative flours, banana (genus *Musa*) is a prominent starch source material, because it can be easily obtained from cull bananas discarded by large banana plantations. The fruit obtained from banana trees is one of the most consumed tropical fruits in the world. With a global production of banana in 2014 of 10.4×10^7 tons (FAOSTAT, 2017), about one-fifth of all bananas harvested become culls. In this sense, bananas too small for shipping, along with those presenting damaged or spoiled areas are removed and normally disposed of improperly (Segundo, Roman, Gomez, & Martinez, 2017). Thus, culled bananas can be used for the production of low-cost banana flour and starch, helping solve this problem. For instance, the production costs, essentially of cartage and starch extraction, are estimated to give a market price that approaches or equals that of maize starch (Schwartz & Whistler, 2009). The flour obtained from green banana pulp is rich in starch (61-76.5%) and fiber (6-15.5%) while it contains low amounts of protein (less than 4%) and lipids (less than 1%) [da Mota, Lajolo, Cordenunsi, & Ciacco, 2000]. The high starch content in green banana pulp is comparable to that in the endosperm of maize grain and the pulp of white potato (Zhang, Whistler, BeMiller, & Hamaker, 2005). Furthermore, the trigger for the industrial use of banana flour could be also based on its nutritional properties. Banana flour contains an important fraction of dietary fiber [mainly pectin (soluble fraction) and cellulose, lignin and hemicellulose (insoluble fraction)] (Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997) and it is also a natural source of resistant starch (RS) in its raw state (Englyst & Cummings, 1986; Faisant et al., 1995). So far, several studies have been done on the application of green banana flour for the production of bakery goods (Agama-Acevedo, Islas-Hernández, Pacheco-Vargas, Osorio-Díaz, & Bello-Pérez, 2012; Ho, Aziz, & Azahari, 2013; Juarez-García, Agama-Acevedo, Sáyago-Ayerdi, Rodriguez-Ambriz, & Bello-Perez, 2006; Segundo et al., 2017).

In this introduction, the molecular structure of starch components, the architecture of starch granules and dependent properties will be addressed to later understand the starch structure-function relationships and their modifications when further subjected to hydrothermal treatments. Subsequently, an overview on the effect of physical treatments, and in particular extrusion, on starch structure and functionality will be approached. To conclude, the application of extruded starchy materials as ingredients in food applications will be covered.

1.1. STARCH STRUCTURE AND COMPOSITION

The functionality of starch is dependent on many factors such as the proportions of its amylose and amylopectin components, molecular size and structures, starch granule properties, and to some degree, other non-starch components (lipids and proteins) associated with the granules.

1.1.1. Starch granule size and appearance

Starch is present as intracellular water-insoluble granules differing in sizes and shapes depending on the botanical origin (Table 1). Starch granules are mainly found in seeds, roots and tubers, but are also found in stems, leaves and fruits. Starch granules occur in all shapes and sizes (spheres, ellipsoids, polygons, platelets, irregular tubules); and their dimensions range from 0.1 to at least 200 µm (Perez, Baldwin, & Gallant, 2009). For most starches, the external surface of starch granules is the first barrier to processes such as granule hydration, enzyme attack and chemical reaction with modifying agents. Consequently, the nature of the granule surface, and particularly the presence of surface proteins and lipids, may have significant effects on the properties of the starch (Hoover, 2001; Morrison, 1995).

Endosperm starch is synthesized in a specialized plastid called amyloplast (Perez et al., 2009). Each amyloplast can contain one simple starch granule, as it happens in wheat, maize or banana. In contrast, in rice, many granules are found in each amyloplast, and it is said to have compound starch granules (Delcour & Hoseney, 2010).

Table 1. Properties of certain starches

Starch source	Gelatinization temperature range (ºC)	Granule Shape	Granule size (µm)
Wheat	51-60	Lenticular Round	20-35 2-10
Maize	62-72	Round Polyhedral	15
Rice[a]	68-78	Polygonal	3-8
Green banana	74-81	Elongated ovals Spheroid	20-50[b] 15-40

Adapted from Lineback (1984) and Zhang et al. (2005). [a]Data for isolated individual granules instead of for compound granules. [b]Elongated granules are ~ 7–25 mm in width and 20–50 mm in length

In wheat starch, two distinct types of granules exist that differ in size and shape, i.e. large, lenticular (A-type) and small, spherical (B-type) granules (Table 1, Figure 1). These wheat starch granules also differ in composition and properties. Generally, A-type granules have higher amylose content and lower gelatinization temperature, whereas B-type granules have higher lipid content and water absorption capacity (Kim & Huber 2010; Maningat, Seib, Bassi, Woo, & Lasater, 2009).

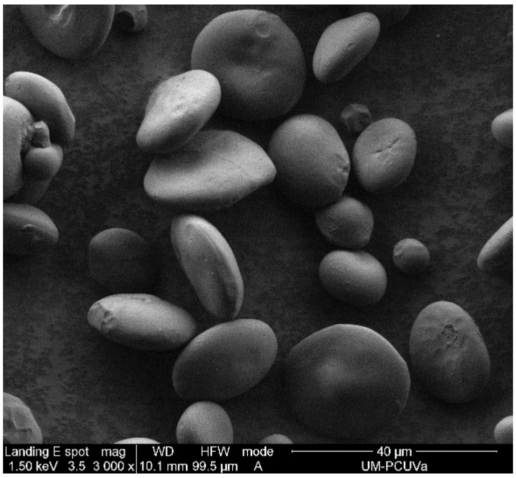

Figure 1. Environmental scanning microscope showing A and B-type wheat starch granules

The starch granules of maize are similar to each other in shape, presenting an unimodal size distribution. They average about 20 µm in diameter while their shape varies from polygonal to almost spherical (Figure 2). Starch granules in the more outside cells of the kernel (vitreous endosperm) tend to be polygonal, whereas those in the inner cells (opaque endosperm) tend to be spherical. As far as it is known, the properties of the differently shaped granules are the same (Delcour & Hoseney, 2010).

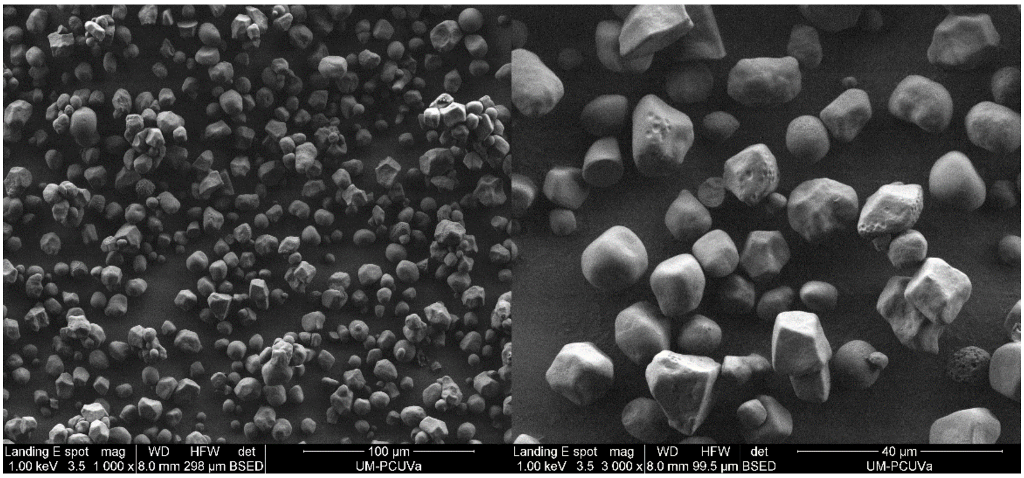

Figure 2. Environmental scanning microscope showing round and polyhedral maize starch granules

Rice starch granules are the smallest known to exist in cereal grains, with the size reported in the range of 2 to 7 µm (Vandeputte & Delcour, 2004). These granules have smooth surface but angular and polygonal shapes (Figure 3).

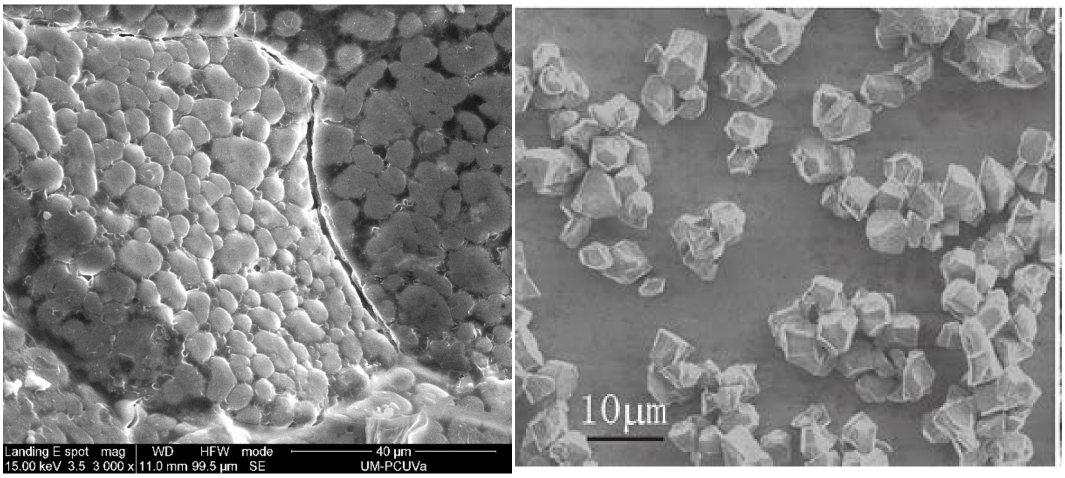

Figure 3. Environmental scanning microscope showing compound starch granules in a rice flour (left) and in isolated form (right)

In general, starch granules from various banana types, while being irregular in shape, appear microscopically as elongated ovals with ridges (Zhang et al., 2005) (Figure 4). In these elongated granules major axes range from 6 to 80 µm, mostly between 20 and 60 µm. Among these irregular shaped granules, spheroid forms are also visible, whose diameters vary from 15 to 40 µm (Table 1). Microscopic observations also revealed irregularly shaped starch granules with smooth surfaces. A smooth and dense surface of native banana starch granules could partially account for their higher resistance to be digested (Zhang & Hamaker, 2012).

Figure 4. Environmental scanning microscope showing various shaped banana starch granules

1.1.2. Composition and architecture of granular starch

Starch is a polymer of glucose molecules linked to one another through the C_1 oxygen and C_4 hydroxyl group, known as the α-glycosidic linkage. At the end of the polymeric chain, an aldehyde group is present, which forms the reducing end and confers reducing power to the molecule. Depending of their architecture, two types of glucose polymers are present in starch, amylose (AM) and amylopectin (AP).

AM is an essentially linear molecule, with much longer chains than AP molecule. It consists of α-(1,4)-linked D-glucopyranosyl units with a degree of polymerization (DP) in the range of 500–6000 glucose residues. Thus, its molecular weight varies between about $8x10^4$ and about $1x10^6$ (Delcour & Hoseney, 2010). There is a fraction of AM slightly branched (α-1,6 branch points) which has between 5 and 20 chains, depending on the source of starch (Takeda, Hizukuri, Takeda, & Suzuki, 1987). However, the branches of amylose are so long and so few that, in many ways, the molecule acts as an unbranched entity (Delcour & Hoseney, 2010).

Cereal starches, typically possess amylose contents in the 20–25% range, although there are starch types with amylose content close to 0 (waxy varieties) and amylotypes with amylose contents in the range of 50-70%. On the other hand, amylose contents in banana starch were mostly in the 10–20% range, although amylose contents up to 40% have been found (Zhang et al., 2005).

In contrast, AP, which is typically the major component in most of starches, is a very large, highly branched polysaccharide with a DP ranging from $3x10^5$ to $3x10^6$ glucose units (Goesaert et al., 2005). The extensively branched structure is composed of short chains of α-(1,4)-linked D-glucosyl units that are interconnected through α-(1,6)-linkages (Bertoft, Piyachomkwan, Chatakanonda, & Sriroth, 2008; Takeda, Shibahara, & Hanashiro, 2003). Amylopectin is branched to a much greater extent than is amylose, with 4–5% of the glycosidic bonds being α-1,6 bonds. This level of branching means that the average unit chain in amylopectin is only 20–25 glucose units long (Delcour & Hoseney, 2010). The molecular weight of amylopectin has been reported to be as high as 10^8, with an average chain length (CL) of only 18–27 glucose residues. The chains are of two major types, namely short chains with CL 6-36 and long chains with CL > 36. Unbranched chains are referred as A-chains, whereas chains that carry other chains are B-chains. Furthermore, there is a single C chain per molecule containing the sole reducing end (Peat, Whelan, & Thomas, 1956), which is otherwise similar to the B-chains. All A-chains are external chains while B-chains, consist of an external chain segment and an internal part.

Amylopectin structure is remarkably complex and several models have tried to explain its chain organization. At present, the actual organization is not clarified, but two major hypothesis exist; the cluster model and the building block backbone model. The cluster model (Figure 5a) suggests that the short (A and B1) chains form double helices, which are organized in discrete clusters, while the longer B chains extend into several clusters (interconnecting the clusters) (French, 1972; Hizukuri, 1986).

Conversely, the building block backbone model (Bertoft, 2013) suggests that the clusters are built up from still smaller structural units called building blocks (units consisting of several chains with varying glucose residues) (see Figure 5b). The building blocks were suggested to be outspread along a backbone consisting mostly, but not entirely, of the long unit chains in AP.

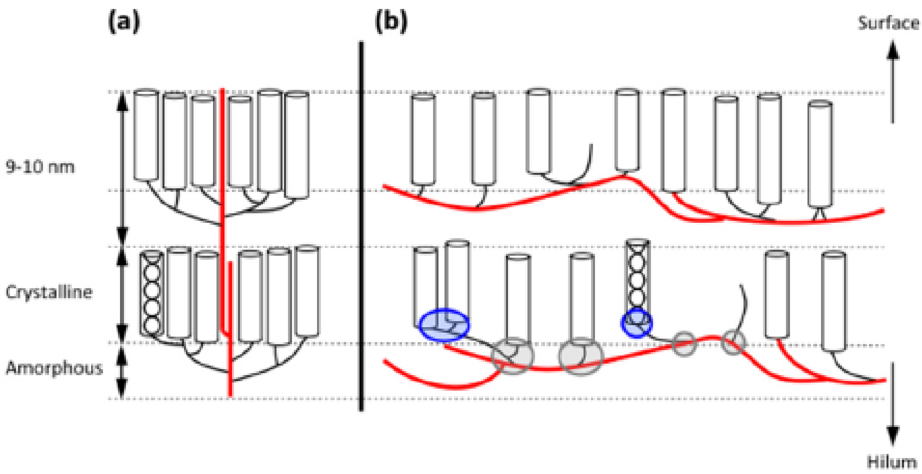

Figure 5. Amylopectin the semicrystalline growth ring depicted based on the cluster model (a) and the building block backbone model (b). Long B-chains (red lines) and short chains (black lines) are shown. In b, the backbone carries internal building blocks (grey circles) and can also carry short B chains (BSmajor) that form branches to the backbone and connect to external building blocks (blue circles). Cylinders symbolize double-helices formed by external chains. Single chains introduce defects in the crystalline lattice (Bertoft, 2017).

The hierarchical structure of starch can be described at least into six levels of organization (from nanometer to micrometer scale) as seen in Figure 6: individual linear branches of starch molecules linked by α-1,4 glycosidic bonds (level 1); macromolecular branched structure (level 2) where the linear glucan branches are linked by α-1,6 glycosidic bonds to form amylopectin and amylose molecules; semicrystalline lamellae (level 3); growth rings or shells (level 4) formed by several alternating crystalline and amorphous lamellae, granular structure (level 5) consisting of several growth rings, and grain structure (level 6) where starch granules interact with other grain components (Buleón, Colonna, Planchot, & Ball, 1998; Tran et al., 2011).

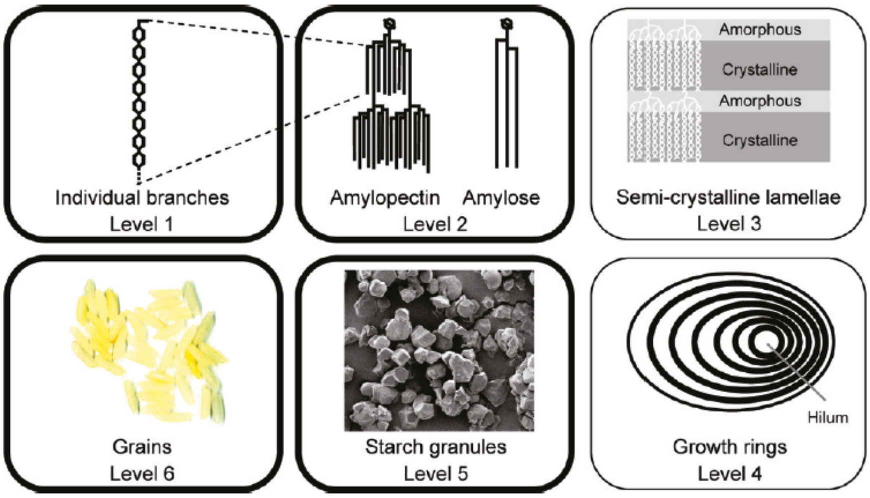

Figure 6. Six levels of starch organization (Tran et al., 2011)

Albeit the structure of the amorphous rings is still not well known, it is broadly assumed that a large part of AM is in the amorphous state and, hence, found in these rings (Glaring, Koch, & Blennow, 2006; Morrison, Tester, & Guidley, 1994). However, AP is probably a part of these rings, since the rings also exist in amylose-free granules. On the other hand, the short, external chains of AP form double helices, that can result in A- or B-type allomorphs (Imberty & Perez, 1989), as will be discussed below, build up the crystalline lamellae (see Figure 7).

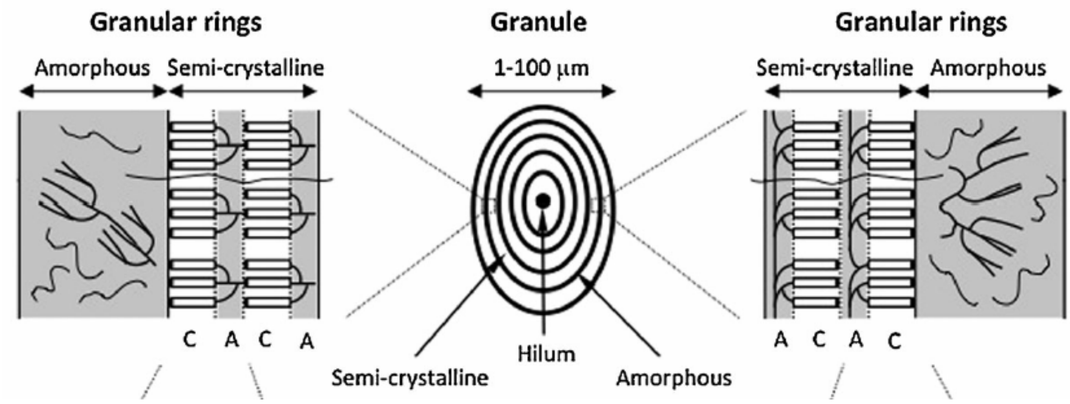

Figure 7. Starch granule consisting of growth rings with a hilum, normally considered as amorphous and centered to their middle. The principal arrangement of the semi-crystalline rings according to the cluster model (left) and building block backbone (right) of AP (Vamadevan & Bertoft, 2015)

1.1.3. Starch birefringence and crystallinity

Native starch granules show 'birefringence' when viewed under polarized light indicating a high degree of order in the starch granule. The concentric pattern of semi-crystalline layers is responsible for the birefringence (Copeland et al., 2009). The refraction of polarized light by the crystalline regions in starch results in a 'Maltese cross' characteristic of an orientation of the macromolecules perpendicular to the surface of the granule (Buleón et al., 1998). Thus, the birefringent cross supports the idea of radially arranged growth rings of amylopectin within the starch granule structure (French 1984; Perez et al., 2009; Tester, Karkalas, & Qi, 2004).

Native starch exists in a form of semi-crystalline granules with a crystallinity varying from 15 % to 45 % (Perez et al., 2009), thus, most native starch granules exhibit a Maltese cross under polarized light. The degree and type of crystallinity of raw starch are mainly related to the structural characteristics of amylopectin, although retrograded (re-associated) amylose also leads to a unique type of crystalline structure (Delcour & Hoseney, 2010). Three types (A, B, and C) of crystalline structures are distinguished for starch granules. Most cereal starches show A patterns; tuber, amylose-rich (amylotypes), and retrograded starch yield B patterns; and certain root and seed starches, such as pea and bean starches, show C patterns (Copeland et al., 2009; Zhang et al., 2005). The C pattern is an intermediate form, a mixture of the A and B polymorphs. Native banana starch has been reported to have either B or C polymorph (Zhang et al., 2005). Another crystal polymorph observed in starch is V-type (Figure 8) which is a pattern shown by amylose-lipid complexes (Biliaderis, 2009). The double helical structures within the A- and B-type crystalline forms are essentially the same (Gidley,1987; Imberty, Buleón, Tran, & Perez, 1991), but the packing of the helices in the A-type crystalline structure is more compact than in B-type crystallites, with the later having a more open structure with a hydrated core (Copeland et al., 2009).

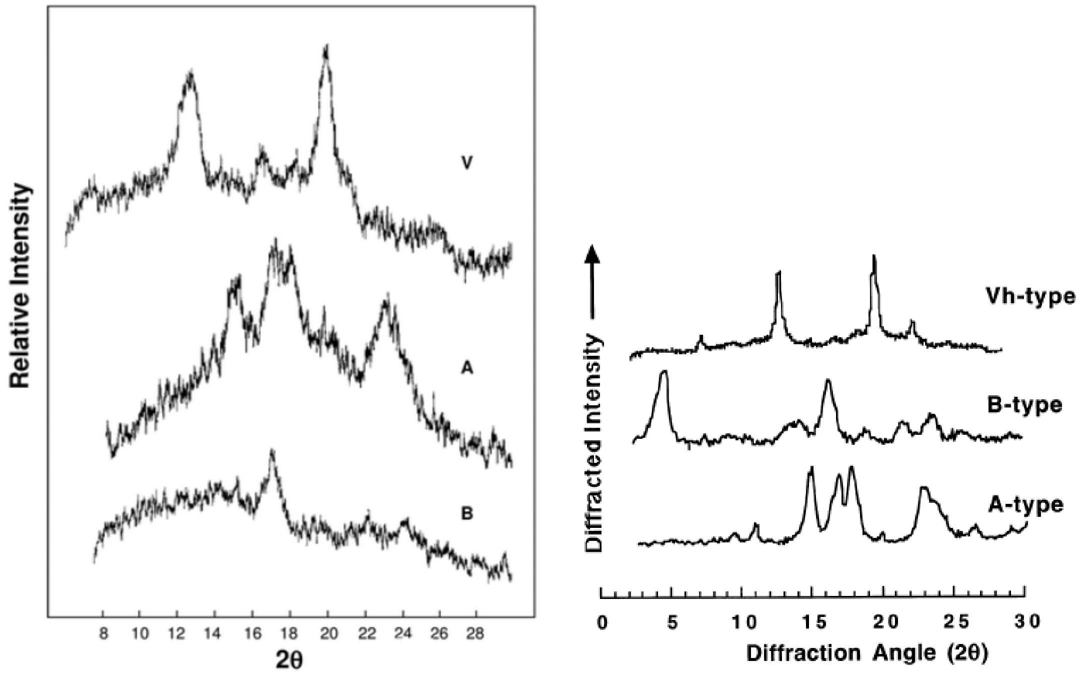

Figure 8. X-Ray diffracion diagrams of A-, B- and V-type starch at 2θ diffraction angle (Delcour & Hoseney, 2010/ Buleón et al., 1998)

X-ray diffractometry is a technique commonly accepted to study the crystalline structure of starch granules. In Table 2, approximate peak locations for different types of crystallinity are given, as reported by Htoon et al. (2009) and Lopez-Rubio, Clarke, Scherer, Topping and Gilbert (2009).

Table 2. X-ray Diffraction peak locations for different types of starch crystallinity

Crystallinity	Strong Peaks	Weak Peaks
A-type	15.1°, 17.1°, 18.1°, 22.5°, 23.6° 2θ	11.4°, 20.6°, 26.9°, 30.3° 2θ
B-type	5.5°, 17°, 22.3°, 23.7° 2θ	15°, 21° 2θ
C-type	17.2°, 18.1°,23.1° 2θ	15.1°,5.5° 2θ
V-type	7°, 13°, 20°	

1.2. STARCH FUNCTIONALITY

In the preparation of most cereal-based food systems, the starch-containing fractions thereof are, at some point, heated in the presence of water and cooled before consumption. The unique character of many of these foods results from the changes that starch undergoes when it is heated and subsequently cooled. Some examples are the viscosity and mouthfeel of gravies and sauces and the texture of crumbs in breads and cakes. It is therefore important to understand what happens to starch under heating-cooling conditions. In many food applications, the functionality of starch is largely related to its water absorption, gelatinization and pasting characteristics.

1.2.1. Swelling power and solubility

The water absorption capacity, solubility pattern and swelling behavior of starch granules are crucial in many industrial applications. Starch granules are generally insoluble in cold water, but when heated in excess water, they absorb water and swell.

Amylose to amylopectin ratio, granule shape and size, amylopectin fine structure and the presence of other non-carbohydrate constituents such as proteins or lipids have been shown to influence the swelling pattern of starch granules (Vamadevan & Bertoft, 2015). In addition, the extent of damaged starch (partial disruption of granular integrity) that occurs primarily as a result of milling also affects starch granule hydration, swelling, and solubility (Roman, Gomez, Li, Hamaker, & Martinez, 2017). Swelling is primarily a property of amylopectin with the packing arrangement of the double helices playing a dominant role in granular swelling, while amylose is said to reduce swelling (Tester & Morrison, 1990). Other flour components, such as proteins and lipids are also said to reduce the rate of swelling. Furthermore, flours with coarser particle sizes tend to present a more delayed and restricted granular swelling (Roman et al., 2017).

1.2.2. Starch gelatinization

In the presence of water acting as plasticizer, starch has the unique property to gelatinize when a specific temperature is reached. This phenomenon includes the disruption of the molecular order, resulting in loss of birefringence, and is accompanied by the melting of amylopectin (AP) crystallites, water absorption, granule swelling and progressive starch solubilization (Delcour & Hoseney, 2010). During gelatinization process, the amorphous regions of the starch granule are the first to swell. Continued heating of the starch granule in sufficient water will eventually break intermolecular hydrogen bonds, melt the crystalline regions, solubilize and leach amylose, and ultimately result in the complete disruption of the starch granule (BeMiller & Whistler, 1996; Morris, 1990). In this way, starch–water mixture undergoes the reversible glass transition in the amorphous background, which is a temperature-induced transition of an amorphous “glassy” state to a progressively more rubbery state (Schirmer, Jekle, & Becker, 2015), prior to the irreversible destruction of molecular order within the starch granule or gelatinization.

Several methods can be used to determine gelatinization temperatures of starch and the resulting properties of the gelatinized starch. Differential scanning calorimetry (DSC) is widely used to measure the endothermic thermal transition that occurs during starch gelatinization (Figure 9) and provide both gelatinization temperatures [onset (To), melting (Tm), and conclusion (Tc)], and enthalpy of gelatinization [rupture of the H-bonds between glucan strands (loss of double helical structures)]. Generally, a high gelatinization temperature and a narrow endothermic peak suggest a higher molecular order or more stable crystals (Vamadevan, Bertoft, & Seetharaman, 2013).

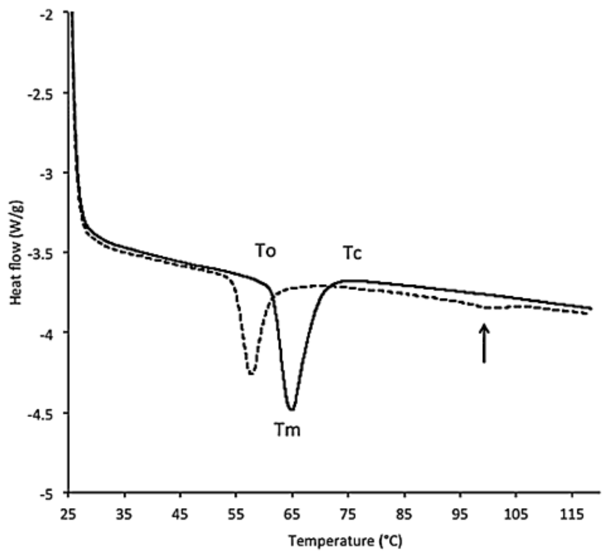

Figure 9. DSC thermograms of barley (broken line) and potato (continuous line) starches. Onset (To), melting (Tm) and conclusion (Tc) temperatures of gelatinization. The peak area is a measure of the energy (enthalpy, ΔH) required for the transition from an ordered to a disordered state (crystalline to melted state). The arrow indicates AM-lipid transition endotherm (Vamadevan & Bertoft, 2015).

The gelatinization temperature range and extent of gelatinization is influenced by factors such as the starch botanical source, granule size and shape, amylose/amylopectin content, amylopectin fine structure, degree of crystallinity, starch granule-associated lipids and proteins, starch damage or water content (Becker, Hill, & Mitchell, 2001; Lin, Aboubacar, Zehr, & Hamaker, 2002; Whistler & BeMiller, 1997). In the case of flours, intracellular (protein matrix) and extracellular components (cell walls) of endosperm play an important role since these physical structures encapsulating starch granules can restrict starch gelatinization during cooking to a major extent, limiting the heat, water or space required for granular swelling (Roman et al., 2017).

DSC is a useful technique to follow starch gelatinization as well as retrogradation, and other endothermic transitions of starch polymers, such as dissociation and melting enthalpies of amylose-lipid complexes (type I and type II) that affect pasting behavior (Figure 9). DSC can measure the enthalpies (heat flows) associated with melting of both crystalline amylopectin and amylose. Type I amylose-lipid complexes typically give rise to an endothermic transition at about 95-105°C, while type II gives rise to an endothermic transition typically at about 110-120 °C (Biliaderis, 2009; Schirmer, Jekle, & Becker, 2015). The first type corresponds to less ordered complexes while the second, higher-melting endotherm can be ascribed to the melting of semicrystalline amylose-lipid complexes (Delcour & Hoseney, 2010; Schirmer, Jekle, & Becker, 2015).

Hot stage microscope with polarized light have been also used to monitor the phase transition or structural changes during gelatinization, detecting the loss of birefringence in starch granules as well as swelling and disappearance of starch granules.

1.2.3. Pasting properties

Viscometric methods, as exemplified in the Rapid ViscoAnalyser (RVA) and Micro Visco Amylograph, are often used to observe changes in viscosity due to the gelatinization of starch granules through a programmed heating-cooling cycle. Assessing the viscosity profile of starch in presence of water in this cycle can help predict the suitability of those flours for the elaboration of different products. The technique implies heating a starch-water suspension in a canister under constant shear applied with a paddle and measuring the changes in viscosity as torque on the paddle. A pasting profile is a curve monitoring the changes in viscosity as a function of temperature and time, and different pasting properties are reported. Typical pasting profiles of a native and pregelatinized starch are depicted in Figure 10.

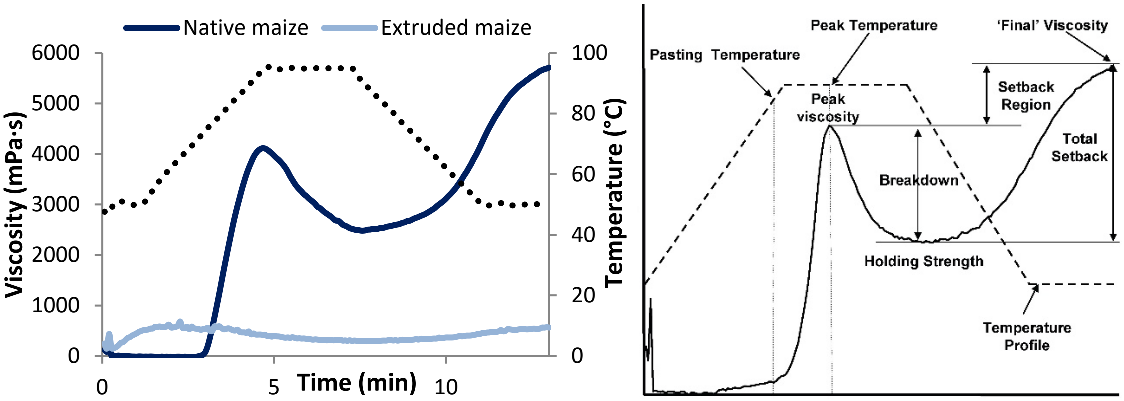

Figure 10. Pasting profile of native and extruded maize flours (left). In extruded flour, the pasting curve of a completely gelatinized starch is visible. Pasting profile highlighting the main pasting parameters (right).

In this pasting profile, the pasting temperature is the temperature at which starch begins to thicken. The peak viscosity is the highest viscosity level reached during the heating cycle (maximum swelling). After this peak, the viscosity of the starch slurry decreases due to the continued shearing that occurs during the 95°C holding period, during which highly swollen granules disintegrate. As long as the granules remain intact as they take up water and release amylose, there is an increase in the relative viscosity of the system. However, from a given moment onward, the relative viscosity of the starch system decreases markedly. The decrease in viscosity is caused by the molecules of soluble starch orienting themselves in the direction that the system is being stirred, as well as by shear-induced destruction of the (not necessarily fully) swollen (and hence fragile) granules (Delcour & Hoseney, 2010). Breakdown, calculated by subtracting the trough viscosity (minimum viscosity after holding at 95°C) from the peak viscosity, indicates the stability of the paste during cooking. When the hot paste is cooled down to 50°C, glucan chains start to reassociate, which causes a further increase in the viscosity. Setback, a measure of the retrogradation and syneresis of the starch upon the cooling of the cooked starch paste, is calculated by subtracting the trough viscosity from the final viscosity (Vamadevan & Bertoft, 2015).

1.2.4. Retrogradation

Starch retrogradation is a physical process in which starch molecules (both amylose and amylopectin) reassociate through H-bonding to form ordered structures of double helices and crystallites during the time period of cooling and storage after starch gelatinization (Hoover, 1995). The retrogradation process comprises three successive steps: nucleation, propagation and maturation.

Amylose recrystallization occurs rapidly because of its linear nature and is called short-term retrogradation, whereas highly branched amylopectin molecules need a longer time to associate, a process commonly called long-term retrogradation. Amylopectin retrogradation rate is related to its fine structure, in such a way that starches with longer AP chains (potato, pea, banana) retrograde more quickly than those with short AP chains (cereals) [Kalichevsky, Orford, & Ring, 1990; Fredriksson, Silverio, Andersson, Eliasson, & Åman, 1998; Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000]. AP retrogradation is thermally reversible at temperatures of cooking (Morris, 1990). Retrograded AP melts at lower transition temperatures (~40-60 °C) than does its native counterpart while retrograded AM melts at temperatures between 130-160 °C. Starch-lipid complexes have a lower tendency toward retrogradation. Thus, amylose crystallization determines to a great extent the initial hardness of a starch gel, while amylopectin retrogradation determines the long-term development of gel structure and crystallinity in starch systems (Delcour & Hoseney, 2010).

Although starch polymer retrogradation has been reported to be one reason for baked products staling during storage (Gray & BeMiller, 2003), it is also known to result in a reduction of the rate and extension of starch digestion, depending on the main constituent involved (Zhang, Sofyan, & Hamaker, 2008). Thus, amylose double helices are enzymatically resistant and yield resistant starch (RS III) [Haralampu, 2000; Patel et al., 2017], while retrograded amylopectin has been attributed to the formation of slowly digestible starch (SDS) [Borah, Deka, & Duary, 2017; Cui & Oates, 1997; Farhat et al., 2001; Zhang et al., 2008].

1.2.5. Freeze-thaw stability

The freeze-thaw stability of starch plays an important role in the sensory attributes of starch-based frozen foods, and, then, in their overall quality. Freeze-thaw cycles are known to enhance starch retrogradation, leading to a release of bound water from the glucan polymer network (Yuan & Thompson, 1998). In this way, when a frozen food is thawed, water is readily separated from the matrix. The separation of the gel and water phases is known as syneresis, which can be evaluated through DSC or by measuring the amount of liquid that has separated from the gel (Yuan & Thompson, 1998). As previously mentioned, starches with high AM content have a greater propensity for retrogradation, whereas AM-free starches have less tendency toward this behavior, and, then, to produce syneresis (Vamadevan & Bertoft, 2015). Chemical substitution of starch has been shown to improve its freeze-thaw stability, by preventing reassociation of the glucan chains (Wu & Seib, 1990). Likewise, hydrocolloids are used to improve the freeze-thaw stability of starches, reducing the syneresis due to their great water-holding capacity (BeMiller, 2011).

1.3. PHYSICAL MODIFICATION OF STARCH. EXTRUSION

Native starch is used because of its unique thickening and gelling capacities, which make it suitable for numerous food applications. However, for a number of applications, properties of native starches fail to meet process or product requirements. Native starches present many disadvantages, among which a high tendency of their pastes to retrograde and synerese, and the poor freeze-thaw stability and cohesiveness of their pastes stand out, thus limiting their application in food products (Wurtzburg, 1986). In this regard, many starchy materials (i.e., flours or pure starches) available commercially have been modified physically (mainly via pregelatinization), chemically (phosphorylated, hydroxypropylated or cross-linked, etc) or enzymatically to overcome some of these drawbacks and hence fulfill the industry needs (Chiu & Solarek, 2009).

Other than modifications by means of chemical or/and enzymatic modification of starch, physical methods for starchy materials modification are gaining importance, also in the view of clean label products (Jacobs & Delcour, 1998). Among physical modifications, particle size classification, fine grinding/air classification, drying, annealing (ANN) and heat moisture treatment (HMT) and extrusion can be highlighted as depicted in Figure 11.

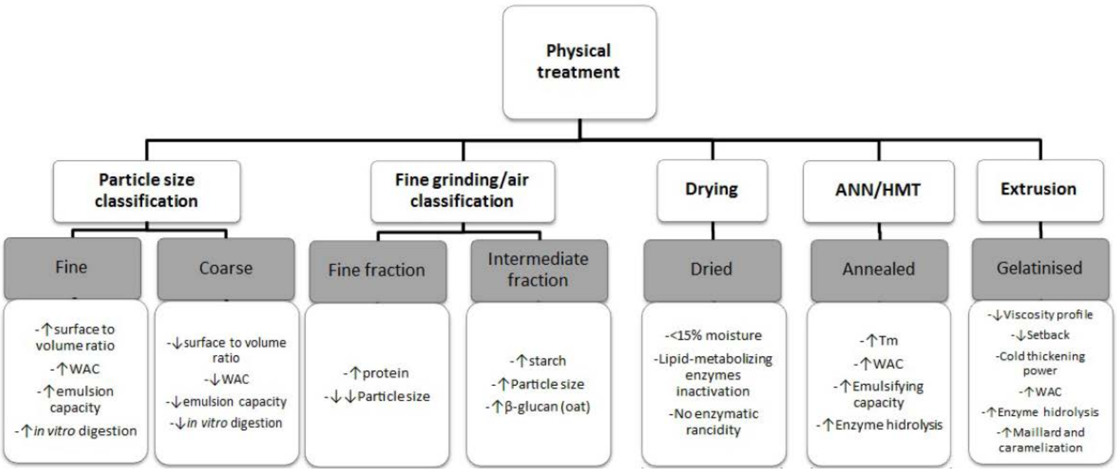

Figure 11. Schematic representation of the main physicochemical properties that could be influenced after physical treatments of flours. Grey boxes represent the different flours obtained by the physical treatments. WAC, water absorption capacity; Tm, gelatinization temperature (Gómez & Martinez, 2016).

Heat treatments appear to be one of the simplest physical methods of treating flour and its components, namely starch, in a controlled way. HMT (low moisture levels and high temperatures, above gelatinization) and ANN (excess of moisture and temperature between glass transition and gelatinization) are physical modifications that alter the physicochemical properties of starch without destroying its granular structure (da Rosa Zavareze & Dias, 2011). These treatments confer heat-moisture-treated and annealed starches an improved thermal stability and decreased retrogradation trend (Adebowale Afolabi, & Olu-Owolabi, 2005). Nonetheless, when starches or flours are subjected to hydrothermal treatments carried out above gelatinization temperature in the presence of enough moisture, the molecular order of the starch granule undergoes irreversible breakage, making starch more prone to swell and thicken in contact with water (Martinez, Calviño, Rosell, & Gómez, 2014a; Martinez, Macias, Belorio, & Gómez, 2015a; Martinez, Rosell, & Gómez, 2014b; Mason, 2009). Starch gelatinization can be industrially carried out in several ways, such as drying a starch paste by means of atomization or heated drums; although one of the most versatile alternatives is extrusion (Chiu & Solarek, 2009; Doublier, Colonna, & Mercier, 1986; Hagenimana, Ding, & Fang, 2006). Since extrusion modification of flours and their application was the main endpoint in this thesis, it will be further described in this introduction. More information regarding other physical and, more specifically, hydrothermal treatments is reviewed elsewhere (da Rosa Zavareze & Dias, 2011; Gómez & Martinez, 2016, Jacobs & Delcour, 1998) and will not be further discussed in this thesis, since these means were not used for starch modification.

1.3.1. Extrusion cooking

Extrusion is widely used throughout the food and polymer industries because of the ability to design continuous processes with short processing times, and the ability to produce a wide range of unique products (Chinnaswamy & Hanna, 1988). More especifically, extrusion is a common commercial processing technique for starch-based foods such as pasta, snacks and breakfast cereals, as well as to obtain flours or starches with new desired functionality.

Physical modification through extrusion process is a cost-effective technology to improve the properties of native starches without using any chemicals (Hagenimana, Ding, & Fang, 2006) and in a “label-friendly” way (Jacobs & Delcour, 1998), more acceptable to consumers. Flours modified by extrusion could also be used in food products as thickening and gelling agents (Mason, 2009), allowing their utilization as fat replacers, viscosity enhancers or texturizing agents among others. An extra advantage of the use of flours is that the process of obtaining flour (milling and shieving) is more economical with a lower environmental impact than starch extraction process (Eckhoff & Watson, 2009). However, it is important to bear in mind that the functionality of extruded flours can slightly differ to that of starches, due to the presence and interactions of other non starch components such as proteins or higher amounts of lipids.

Among the different types of extruders configurations, single screw and intermeshing co-rotating twin screw extruders are the two main types of extruders found in the manufacturing of extruded starches and flours. Screw extruders are composed of four main parts, generally supported by a frame. The kinematics (motor and gearbox, in particular), which delivers the mechanical power required by the extrusion process; The screw-barrel assembly, in which the material is converted (Figure 12); The die assembly, through which the converted material is shaped, formed, or textured, depending on the targeted products; The central operating cabinet or "brain" of the equipment, which monitors equipment operation (Bouvier & Campanella, 2014).

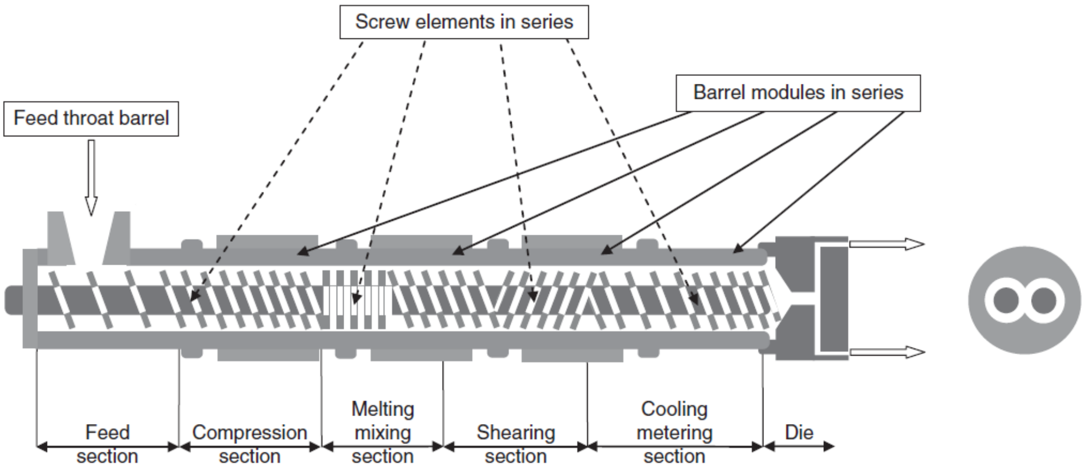

Figure 12. Schematic view of a modular screw barrel assembly with operating sections (Bouvier & Campanella, 2014).

Extrusion cooking is considered a high-termperature-short-time (HTST) process combining several operations of feed transport, mixing, working and forming. During extrusion cooking, starchy materials are processed at relatively high temperatures and high pressures and shear forces at relatively low moisture contents [ranging roughly from 14% to 32% (wet weight basis)] (Camire, Camire, & Krumhar, 1990). The combination of a high temperature with a large amount of mechanical energy input during a short residence time can be used to promote structural changes of starch such as gelatinization, melting, degradation (or dextrinization) and fragmentation (Lai & Kokini, 1991). Apart from these structural changes in starch, other physicochemical reactions occuring during extrusion-cooking include protein denaturation and aggregation (especially important when extruding flours), enzyme (in)activation, color reactions (Maillard) and complexations between ingredients (such as amylose–lipid complexation) (Camire et al., 1990; Wen, Rodis, & Wasserman, 1990). The extent of these structural changes increases with increasing severity of processing conditions, determining extruded starch or flour functionality. Thus, product quality can vary considerably, depending on the extrusion processing characteristics, such as extruder type, screw configuration, feed moisture, and temperature profile in the barrel sections, screw speed and feed rate (Thymi, Krokida, Pappa, & Maroulis, 2005).

The degree of starch conversion and phyisicochemical characteristics of starchy products can be evaluated by means of various analysis techniques such as optical microscopy (loss of birefringence), DSC (loss of gelatinization endotherm), Water Solubility Index or WSI (solubilized polysaccharides), Water Absorption Index or WAI (gel phase of polysaccharides), alkaline viscosity (starch depolymerization), and Rapid ViscoAnalyzer or RVA (pasting properties) [Bouvier & Campanella, 2015; Hagenimana et al., 2006; Martínez et al., 2014a,b]. Size exclusion chromatography (SEC) is also an effective method for monitoring starch fragmentation (decrease in molecular weight or size). The molecular weight of the starch polymers significantly affects the physical properties of starch, such as solubility and viscosity. Therefore, determining the degree of degradation of starch during extrusion is essential for establishing processing-structure-property relationships (Ye et al., 2018). From SEC studies it has been concluded that amylopectin, not amylose, is the component most affected by extrusion processing (Zhang et al., 2015). The intense shear within the extruder can cause the cleavage of α-(1-4) and α-(1-6) bonds as well as starch ordered structures such as crystallites and double helices. Amylopectin (highly branched large molecule) is degraded to a larger extent than the essentially linear and lower molecular weight amylose, and the degradation could occur in the inner part/close to the center of the molecule (Liu, Halley, & Gilbert, 2010). In this sense, Shrestha et al. (2010) reported that extruded high-amylose maize starch retains its crystallinity, while low-amylose maize starch becomes mostly amorphous after extrusion under the same operation conditions. The mechanical/shear force induced by extrusion processing is believed to randomly cleave glycosidic bonds in branches of amylopectin (Zhang et al., 2015), although the glycosidic bonds near or at the branching points, sometimes termed building blocks (Dhital, Shrestha, & Gidley, 2010), are apparently more susceptible to shear degradation (Liu et al., 2016).

1.3.2. Physicochemical properties of extruded starches and flours

When starch is gelatinized by extrusion, its crystalline structure is disrupted due to the breaking of inter- and intra- molecular hydrogen bonds. This results in more exposed hydroxyl groups for forming hydrogen bonds with water and the presence of starch in an amorphous state (water molecules can diffuse easier) (Ye et al., 2018). Thus, WAI of extruded starch is normally higher than that of native starch, albeit it can be lower if extensive dextrinization or melting takes place (Altan, McCarthy, & Maskan, 2009; Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006; Kadan, Bryant, Pepperman, 2003; Sarawong, Schoenlechner, Sekiguchi, Berghofer, & Ng, 2014). Regarding WSI, it has been observed that, in all cases, the WSI of extruded starch is much higher than that of native starch (Altan et al., 2009; Ding et al., 2006; Kadan et al., 2003; Sarawong et al., 2014). Nonetheless, WAI and WSI are dependent on the severity of the extrusion processing. For instance, if the material is extruded at a low feed moisture and a high screw speed (increase in shearing and starch degradation) the extruded product will have a low WAI and high WSI.

Martínez et al. (2014a, 2014b) reported changes in the emulsifying capacity and stability of rice and wheat extruded flours as a function of the severity extrusion conditions (namely screw speed, feed rate, moisture content and barrel temperature). If severe enough conditions were applied, an increase in the emulsifying ability was observed, which was attributed to both the protein unfolding and aggregation and starch gelatinization increasing the number of hydroxyl groups available to form hydrogen bonds with the proteins. These authors also observed an increase in the foaming capacity and stability of extruded rice flours.

Extrusion leads to changes in the structure of starch and significantly transforms its pasting profile (see Figure 10 in Pasting properties section). Pasting properties are also strongly influenced by the intensity of the extrusion conditions. In general, the viscosity during heating and cooling together with the breakdown and setback decreases with the extrusion intensity (Hagenimana et al., 2006; Martínez et al., 2014a, 2014b). Nevertheless, if extrusion conditions are severe enough to produce a sufficient amount of gelatinized starch, a cold-swelling peak is observed, while gelatinization peak is reduced or even disappears. Different RVA responses can be obtained depending upon the range of process specific mechanical energy (SME) as shown in Figure 13. The intensity of thermomechanical processing is typically estimated by the SME input.

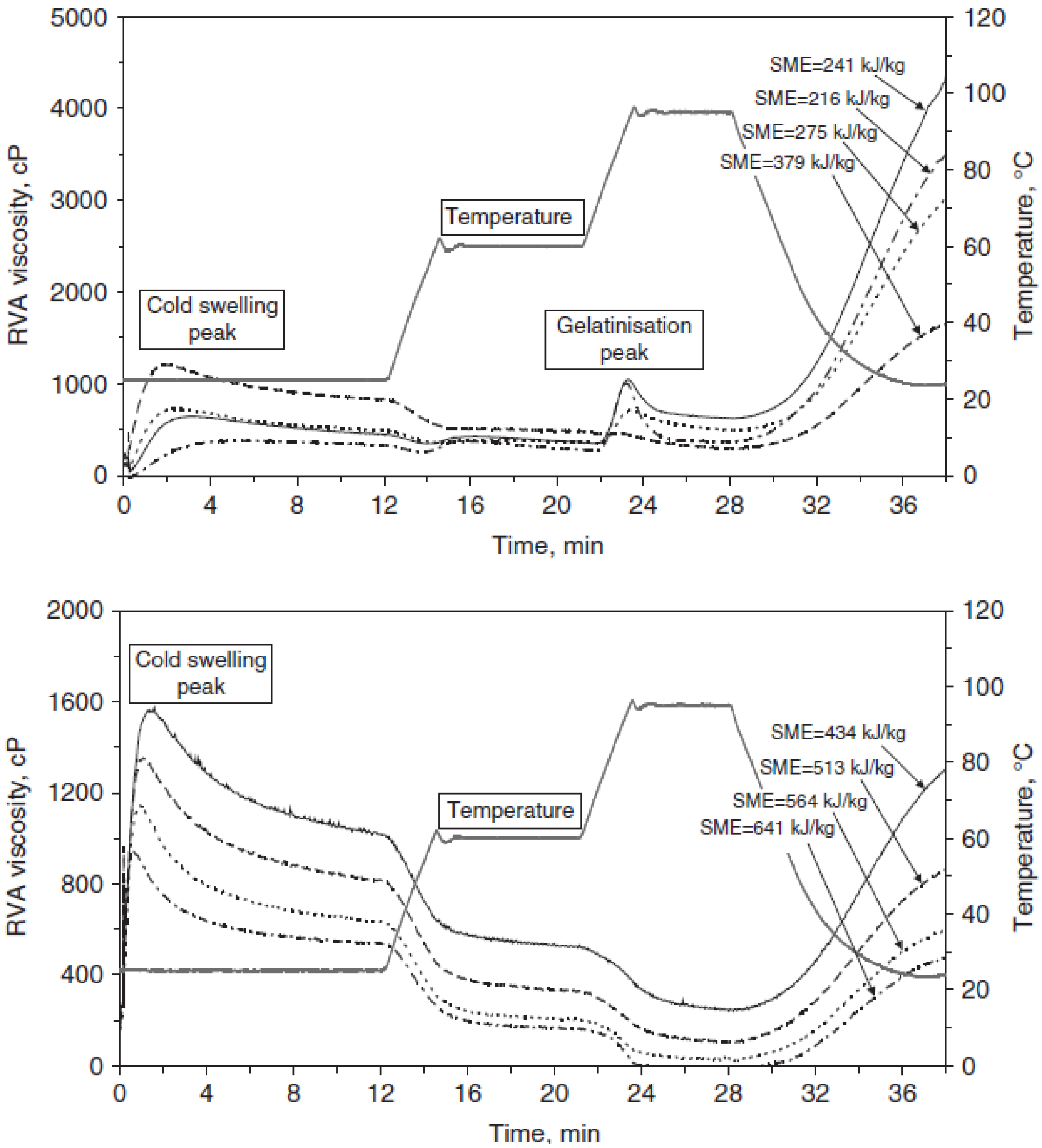

Figure 13. RVA responses of extrusion-processed maize-based material under intermediate range of process SME (200 kJ/kg < SME <400 kJ/kg) and high-SME extrusion-processed corn-based material (Bouvier & Campanella, 2014).

1.3.3. Application of extruded starches and flours

The suitability of extruded flours for a particular application depends on their water absorption, water solubility and oil absorption indexes, and cold-paste viscosity. Extrusion is known to generally result in higher water solubility and water absorption of the extruded starchy material. In this sense, a higher water solubility is useful in products cooked at lower temperatures, such as soups, sauces, puddings, and desserts (Ye et al., 2018). Meanwhile, a high water absorption and cold-viscosity allows the addition of more water to the dough/batter system, improving its handling characteristics (increasing dough consistency) and reducing the typical dryness of products during storage (Ye at al., 2018), something that can be desirable for bakery products or instant food applications.

In bread making applications, Defloor, De Geest, Schellekens, Martens and Delcour (1991) reported that a mixture of extruded starches and emulsifiers improved the quality of breads prepared with a mixture of tapioca and soy and a high level of hydration. Meanwhile, Sanchez, Gonzalez, Osella, Torres and De La Torre (2008) found that the addition of extruded rice flour improved bread volume and crumb structure, especially when waxy rice varieties were used. However, it is worth noting that these authors had to increase the amount of water in the formula when using extruded flours in order to compensate their higher consistency. The effect of the substitution of rice flour by extruded non-acidified and acidified rice flour has also been tested (Clerici & El-Dash, 2006; Clerici, Arioldi, & El-Dash, 2009). More recently, Martinez, Oliete, Roman and Gomez (2014c) observed that greater severity of the extrusion conditions gave rise to an increased dough viscoelastic moduli and consistency in gluten-free breads containing extruded rice flour. Therefore, larger amounts of water were necessary to achieve a constant consistency and increase the bakery yield, which was obtained by Martinez, Oliete and Gomez (2013b) when adding extruded wheat flour to wheat based breads. Martinez and coworkers (Martínez, Marcos, & Gomez, 2013a) also observed that the substitution of native rice flour by extruded rice flour, in gluten-free breads, increased bread volume while decreased initial hardness and delayed bread staling. In this regard, extruded rice flour has also been used to improve the texture attributes of gluten-free rice based cakes (Jeong, Kang, & Shin, 2013).

Several cereal-based products need to be cooked to develop their viscous properties, based on the gelatinization of starch granules. To ease the preparation and remove or reduce this heating stage, pregelatinized starches and flours obtained by extrusion cooking can be utilized. Thus, pregelatinized flours and starches can be used for production of ready-to-eat (instant) products with no need for heating, such as porridges, soups, creams (Fiorda, Soares Jr, da Silva, de Moura, & Grossmann, 2015; Onyango, Noetzold, Bley, & Henle, 2004; Pelembe, Erasmus, & Taylor, 2002) or corn tortillas (Arambula-Villa, Gonzalez-Hernandez, & Ordorica-Falomir, 2001). Other industrial applications of extruded starch include the production of starches with low retrogradation (Zhang et al., 2014) and good freeze-thaw stability (Ye et al., 2016) features that are convenient for extending product shelf life. Due to their reduced retrogradation and breakdown, and good freeze-thaw stability, extruded starches can also be used as thermally stable ingredients in canned and frozen foods (Zavareze & Dias, 2011). On the other hand, extruded flours with low WAI and high WSI usually have low viscosity and stickiness, being suitable for children's food and beverages (Sarawong et al., 2014).

The disruption of the starch granules (gelatinization) during extrusion also enhances their chemical reactivity towards hydrolytic enzymes (Zhang et al., 2015), and, in turn, the digestion rate of the extruded starch (Hagenimana et al., 2006). The higher susceptibility to enzymatic action of extruded matrices has already been taken into account to produce flours rich in cyclodextrins (Roman, Dura, Martinez, Rosell & Gómez, 2016). Cyclodextrins are used for different applications such as food additives, encapsulation of molecules (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gandara, 2009; Astray, Mejuto, Morales, Rial-Otero, & Simal-Gandara, 2010) and as a source of dietary fiber (Artiss, Brogan, Brucal, Moghaddam, & Jen, 2006). Furthermore, the enzymatic amylolysis with both α–amylase and glucoamylase was also carried out to get flours with different functionality and high oligosaccharide content (Martinez, Pico, & Gomez, 2015b). This starch hydrolysis sugars act as fermentation substrates in bread-making application and they are one of the principal compounds participating in Maillard and caramelization reactions ocurring during thermal processes such as baking (Purlis, 2010; Salim-ur-Rehman, Paterson, & Piggott, 2006). Nevertheless, since the high susceptibility to the enzymatic hydrolysis can also increase the action of the pancreatic α-amylase, efforts have been made to reduce the digestibility of these flours. Thus, a synergistic maltogenic α-amylase and branching treatment to produce enzyme-resistant molecular and supramolecular structures in extruded maize matrices was carried out in views of attenuating the starch digestion properties of extruded flours (Martinez, Pico & Gómez, 2016). However, this enzymatic treatment was found to be ineffective in slowing down the starch digestion properties may be due to changes produced at larger hierarchical levels in the starch structure of enzymatically modified extruded flours (Roman, Martinez, Rosell & Gómez, 2016).

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OBJECTIVES

3. OBJECTIVES

The main objective of this work is to develop novel plant-based products with improved physical, organoleptic and/or nutritional value by means of the utilization of extruded flours as a functional ingredient.

The aim of this objective is to obtain scientific knowledge of flour constituents and of the impact of extrusion treatment and conditions on their functionality, as well as obtaining novel products with more desired properties that can increase the end-uses of extruded flours.

In order to achieve the main objective, several specific objectives were also established:

1. To study the potential of extruded flours as fat substitutes in different food emulsions.
2. To study the effect of the addition of extruded flours subjected to different extrusion conditions on the quality of batter coatings.
3. To study the influence of particle size and starch source of extruded flours on the quality of cold-prepared sauces.
4. To understand the retrogradation mechanisms of extruded flours from different sources that determine the mechanical properties of the network structure in starch gels.
5. To study the effect of shear fragmentation produced during extrusion to increase structurally driven slowly digestible starch in bread crumb.

STRUCTURE

4. STRUCTURE

The present doctoral thesis is structured in chapters containing scientific publications, resulting from the research carried out during the thesis, which respond to the objectives proposed. The content of this research is presented in four chapters:

Chapter 1: Exploiting the potential of extruded flours as fat substitute in food applications

- Román, L., Martínez, M. M., & Gómez, M. (2015). Assessing of the potential of extruded flour paste as fat replacer in O/W emulsion: a rheological and microstructural study. *Food Research International*, *74*, 72-79.
- Román, L., Santos, I., Martínez, M. M., & Gómez, M. (2015). Effect of extruded wheat flour as a fat replacer on batter characteristics and cake quality. *Journal of Food Science and Technology*, *52*, 8188-8195.

Chapter 2: Exploiting the potential of extruded flours as viscosity enhancer in food applications

- Román, L., Pico, J., Antolín, B., Martínez, M. M., & Gómez, M. (2018). Extruded flour improves batter pick-up, coating crispness and aroma profile. *Food Chemistry*, 260, 106-114.
- Román, L., Reguilón, M. P., & Gómez, M. (2018). Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source. *Journal of Food Engineering*, *219*, 93-100.

Chapter 3: Exploiting the potential of extruded flours to reduce gel hardening in starch-based foods

- Román, L., Gómez, M., Hamaker, B. R., & Martínez, M. M. (with editor). Shear scission during extrusion diminishes inter-molecular interactions of starch molecules during storage. *Submitted to Journal of Food Engineering.*

Chapter 4: Exploiting the potential of molecular shear fragmentation during extrusion to increase slowly digestible starch in gelatinized baked goods

- Román, L., Gómez, M., Hamaker, B. R., & Martínez, M. M. (with editor). Banana starch and molecular shear fragmentation dramatically increase structurally driven slowly digestible starch in fully gelatinized bread crumb. *Submitted to Food Chemistry.*

The articles of the thesis have been structured based on the extruded flour functionality.

Chapter 1 focuses on the replacement of oil by pregelatinized maize and wheat extruded flours in high-caloric products, such as mayonnaises and cakes. Regarding mayonnaises, changes in the microstructure, rheological properties and stability of the oil in water emulsion were assessed with the partial substitution of fat by an extruded flour paste. In the case of cakes, the effect of partial and total substitution of oil was assessed in both cake batter (microstructure and rheological behaviour) and final cake (specific volume, weight loss and textural parameters) quality. Furthermore, a sensory test to determine consumer's acceptability of reduced-fat cakes was carried out.

In **chapter 2**, the thickening properties of extruded flours were exploited to determine the effect of their incorporation during processing of batters for coating and cold-prepared sauces. Wheat flour subjected to different extrusion conditions was used in batters for coating with the aim of improving the coating of the food matrix due to an enhanced batter viscosity and stickiness. The effect of partial replacement of native wheat flour by extruded wheat counterpart on the characteristics of the resulting fried coatings was investigated. Thus, analyses for batter-pick-up, coating microstructure, fat and moisture content, color, crispiness and acceptability consumer's test were carried out. Furthermore, in this study, for the first time, volatile compounds in batter systems before and after frying were evaluated. This section also deals with the study of rheological and textural properties, microstructure and stability of sauces prepared with no need of heating ("instant" sauces) using extruded flours as main ingredient. In this regard, the effect of extruded flours with distinct physicochemical characteristics based on their particle size and starch source were analyzed.

In **chapter 3**, it was hypothesized that molecular shear fragmentation of starch during extrusion could decrease starch inter-molecular associations responsible for affecting negatively the mechanical properties of extruded flour gels and, therefore, starch-based foods. The starch retrogradation behavior of native and extruded maize, wheat and rice flours was studied to better understand the efficacy of extrusion (same SME conditions) to modify their propensity to form inter-molecular interactions. Therefore, native and extruded flours were analyzed for starch fragmentation, gelatinization, retrogradation and gelation properties over 7 days storage.

Finally, **chapter 4** was approached from a nutritional perspective, based on the novel molecular structure-digestion relationships recently discovered by Martinez, Okoniewska, Mukherjee, Vellucci, and Hamaker (submitted). These authors found that, certain starches containing long A and B1 amylopectin chains, such as banana starch, are prone to form structurally-driven slowly digestible starch in fully gelatinized material, such as a bread crumb. In the present thesis, we hypothesized that a molecular size reduction would improve mobility and alignment properties of banana amylopectin molecules resulting in crumbs with significantly higher slowly digestible starch. Thus, the role of native and extruded banana starch in slowing down the starch digestibility of gluten-free bread crumb and crust was investigated. To do so, *in vitro* starch digestion fractions, the degree of starch gelatinization and retrogradation in crumb and crust, and the physical and sensory properties of breads were analyzed.

Besides the aforementioned publications that are included in this doctoral thesis, work with extruded flours and enzymes also resulted in two publications (Roman, Dura, Martinez, Rosell & Gómez, 2016; Roman, Martinez, Rosell & Gómez, 2017). However, these are not included in this thesis since they are not directly connected with existing food applications. A brief description of these works has been included at the end of the introduction section.

Martinez, M. M., Li, C., Okoniewska, M. Mukherjee, N., Vellucci, D., & Hamaker, B. R. (submitted). Structure-digestion relationships of fully gelatinized starch: a novel approach to slow down the starch digestibility in hydrothermally-treated foods. Carbohydrate Polymers (submitted).

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CHAPTER 1

Exploiting the potential of extruded flours as fat substitute in food applications

* Román, L., Martínez, M. M., & Gómez, M. (2015). Assessing of the potential of extruded flour paste as fat replacer in O/W emulsion: a rheological and microstructural study. *Food Research International*, *74*, 72-79.

* Román, L., Santos, I., Martínez, M. M., & Gómez, M. (2015). Effect of extruded wheat flour as a fat replacer on batter characteristics and cake quality. *Journal of Food Science and Technology*, *52*, 8188-8195.

This chapter includes two scientific articles published in SCI journals addressing the use of extruded flours as fat replacer in two different food emulsions. The role of an extruded flour paste as oil substitute was assessed in mayonnaises and cake batters. The two related publications, addressed in this chapter in the same order as below mentioned, are the following:

1. Román, L., Martínez, M. M., & Gómez, M. (2015). Assessing of the potential of extruded flour paste as fat replacer in O/W emulsion: a rheological and microstructural study. *Food Research International,* 74, 72-79.
2. Román, L., Santos, I., Martínez, M. M., & Gómez, M. (2015). Effect of extruded wheat flour as a fat replacer on batter characteristics and cake quality. *Journal of Food Science and Technology*, 52, 8188-8195.

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Assessing of the potential of extruded flour paste as fat replacer in O/W emulsion: A rheological and microstructural study

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ABSTRACT

Extruded flour represents an economical and environmental friendly alternative as fat replacer. In this research, the potential use of an extruded flour–water paste as fat replacer in an oil-in-water emulsion was studied. The effect of flour–water ratio and level of oil replacement (30, 50 and 70%) on the microstructure, rheological properties and stability of mayonnaise-like emulsion was evaluated. Fat replacement by extruded flour gradually increased the number and reduced the size of oil droplets. All the emulsion samples showed a pseudoplastic behaviour. At low shear rates a Newtonian region characterised by Carreau model appeared ($R^2 > 0.99$). In general, the limiting viscosity of the Newtonian region and the consistency index increased with the decreased water content of the paste and increased the level of oil substitution. A decrease in oil concentration led to a greater thixotropic behaviour. Oscillatory test revealed that predominance of the continuous or dispersed phase influenced viscoelastic behaviour. Reduction in oil content resulted in an increased freeze–thaw stability. Results suggested that if the flour–water ratio of the paste is controlled, extruded flour is appropriate for preparing reduced-fat oil-in-water emulsion with similar rheological properties to the full fat and greater freeze–thaw stability.

1. Introduction

Mayonnaise is probably one of the oldest and most widely used sauces in the world. It is an oil-in-water emulsion in semi-solid form with oil droplets closely and strongly packed (Depree & Savage, 2001). The oil content of traditional mayonnaises is more than 65% (w/w); hence, it is generally regarded as a high-fat and high-calorie food (Ma & Boye, 2013). Currently, there is an increasing tendency toward reducing the fat content in foods in order to satisfy the demands of consumers, who are more concerned about health problems. Actually, overconsumption of fat leads to obesity (Bray, Paeratakul, & Popkin, 2004) and is associated with other human health problems such as cardiovascular diseases (Astrup et al., 2011; Schwingshackl & Hoffmann, 2013) and even with several types of cancers (Rothstein, 2006).

Nonetheless, it is difficult to imitate traditional product quality when preparing reduced-fat foods. Removal of fat can cause significant changes in the sensory and bulk physicochemical properties of mayonnaises which may be undesirable for consumers (Ma & Boye, 2013), since factors which affect the oil-in-water emulsion stability of mayonnaise include the amount of oil and the relative volume of the oil phase to the aqueous phase (Harrison & Cunningham, 1985). Therefore, to maintain these properties of low-fat mayonnaises, it is necessary to use different types of ingredients or additives as fat replacers. These ingredients are mainly added to stabilise the emulsion and to increase the viscosity of light mayonnaise (Nikzade, Mazaheri-Tehrani, & Saadatmand-Tarzjan, 2012). A wide variety of gums have been employed, like xanthan, konjac, guar and pectin (Li, Wang, Jin, Zhou, & Li, 2014; Liu, Xu, & Guo, 2007; Ma & Barbosa-Canovas, 1995; Su, Lien, Lee, & Ho, 2010). In addition, some soluble fibres (Su et al., 2010; Worrasinchai, Suphantharika, Pinjai, & Jamnong, 2006) are also used as fat replacers in mayonnaises. These products usually have a thickening effect, increasing the viscosity of the continuous phase and therefore, slowing down the droplet movement, which contribute to the long-term stability of the product (McClements & Demetriades, 1998).

Modified starches are often included in the formulation of reduced-fat products because of their low cost and their unique creamy texture (Cho, Lim, Park, Hwang, & Lim, 1999), as well as to stabilise dressings and to provide the desired flow character (Mason, 2009). Therefore, chemically (Teklehaimanot, Duodu, & Emmambux, 2013), enzymatically (Mun et al., 2009) and physically (Lee, Lee, Lee, & Ko, 2013) modified starches are also proposed as fat replacers in low-fat mayonnaises.

In hydrothermal treatments, such as extrusion, if starch is subjected to high moisture and temperatures, it can be pre-gelatinised (pre-cooked), with a rupture of the structure of granules, an increase of the swelling power of the granule, and a loss of crystallinity (Atwell, Hood, Lineback, Varriano-Marston, & Zobel, 1988). This starch swells and thickens on contact with water (Chiu & Solarek, 2009), resulting in an increase of cold water absorption, as well as solubility (Doublier,

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Colonna, & Mercier, 1986) and a smooth texture (Mason, 2009). The intensity of the extrusion treatment, concerning to temperature, initial moisture content and screw speed modify flour behaviour by affecting its hydration, thermal and pasting properties (Martínez, Calviño, Rosell, & Gómez, 2014). Consequently, these functional properties are the origin of many applications in the food industry. Physical modification through extrusion process is an alternative way to improve the properties of native starches without using any chemicals (Hagenimana, Ding, & Fang, 2006) and with a "label-friendly" (Jacobs & Delcour, 1998), which is more acceptable to consumers. Flours modified by extrusion could also be used in food products as thickening and gelling agents (Mason, 2009), allowing their utilisation as fat replacer in oil-in-water emulsions. Moreover, the process of obtaining flour is more economical with a lower environmental impact when compared to the starch extraction process (Eckhoff & Watson, 2009). Although other pre-gelatinised starches have been used in the production of low-fat mayonnaises, no information concerning the effect of extruded pre-gelatinised flours as fat replacer in mayonnaise was available.

The aim of this work was to study the effect of extruded maize flour incorporation as fat replacer in reduced-fat mayonnaise-like emulsion. Mixtures of extruded flour and water at 1:3, 1:3.5 and 1:4 ratios were used to replace fat (30%, 50% and 70% of the fat content). The microscopy, rheological properties and stability of mayonnaises were studied.

2. Materials and methods

2.1. Materials

The extruded maize flour (Zoc 90) used in this study was provided by Molendum Ingredients (Zamora, Spain). The extrusion treatment was performed with an industrial Bühler Basf single screw extruder (Bühler S.A., Uzwil, Switzerland). The length to diameter (L/D) ratio for the extruder was 20:1. The extrusion was carried out with a further water addition of 12% at a maximum barrel temperature of 160 °C, a feed rate of 500 kg/h and a screw speed of 453 rpm. The extruded product was dried by convection air until 10.09% moisture and then ground with a compression roller to a particle size below 200 μm. The extruded flour resulted after milling had a mean particle size of 99.37 μm.

Other ingredients in the mayonnaise formulation, such as sunflower oil, vinegar, salt and pasteurised whole egg, were all purchased from a local supermarket.

2.2. Methods

2.2.1. Mayonnaise preparation

Mayonnaises were prepared for 200 g of sample. Full-fat mayonnaise formulation (w/w), used as control, contained sunflower oil (65%), whole pasteurised egg (31%) vinegar (3.5%) and salt (0.5%). All the ingredients of the full-fat mayonnaise were added, the oil last of all, to the plastic beaker of an InfinyForce Active flow (700W) mixer (Moulinex, Écully, France). The mayonnaise blender accessory of the mixer was used, which allows the sauce to be homogenised. This slurry was stirred at turbo speed for 20 s. Reduced-fat mayonnaises were prepared by replacing the oil with 30%, 50% and 70% of a flour paste (a mixture of extruded flour and water) in three different flour-water ratios (1:3; 1:3.5 and 1:4, respectively). In these samples, extruded flour and water were previously mixed until complete dispersion of flour and then the flour paste was added to the plastic beaker with the rest of the ingredients. The mayonnaises were transferred to a plastic sealed jar and stored at 20 °C until further analysis. All mayonnaises were prepared twice. Reduced-fat mayonnaises were labelled with the corresponding percentage of oil replacement and a number according to the flour–water ratio used.

2.2.2. Optical microscope observation

Mayonnaise samples were examined with a DM750 microscope (Leica Microsystems, Wetzlar, Germany) with 1000 times magnification, fitted with an EC3 video camera and images were captured with LAS-EZ software (Leica Microsystems, Wetzlar, Germany). First, the mayonnaise sample was placed on a glass microscope slide and covered with a cover slip. The slides were compressed under a 1 kg weight to create a sample layer of uniform thickness. Then, immersion oil was spread out to obtain photomicrographs. Micrographs were taken at least twice in two random points of each sample. Optical observation was carried out after 24 h of mayonnaise preparation. The images were analysed in a 2048 × 1536 pixel format with ImageJ software (National Institute of Health, Bethesda, Md., USA) for the number of droplets, mean size of oil droplets, and total area of droplets. Picture studied area was 18.72 × 89.04μm^2.

2.2.3. Rheological analysis

Rheological properties of mayonnaises were determined with a Thermo Scientific Haake RheoStress1 rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P unit to control the temperature, which was fixed at 20 °C for all analyses. Measurements were conducted at a gap distance of 1 mm. All tests were monitored with RheoWin 4 Job Manager software and rheological parameters were calculated with Rheowin 4 Data Manager software (Thermo Fisher Scientific, Schwerte, Germany). Mayonnaise surface exposed to air between the plates was covered with vaseline oil to prevent moisture loss. In order to determine the minimum resting time to allow structure recovery and temperature equilibration in further analysis, a time sweep (1 mm gap) at 20 °C within the linear viscoelastic region (at a stress value within the linear viscoelastic region from the stress sweep) for 1800 s was carried out. Then resting time of 500 s was selected for all rheological tests. Rheological measurements were performed twice after 24 h of storage.

2.2.3.1. Steady flow behaviour. The Newtonian region was determined with a titanium parallel plate system (diameter: 35 mm). Rheological measurement was carried out in control stress mode by increasing the shear stress from 0.5 to 100 Pa until steady state was reached at each point. The Carreau model is commonly used to describe oil-in-water emulsion behaviour (Batista, Raymundo, Sousa, & Empis, 2006; Moros, Franco, & Gallegos, 2002) and therefore the flow curve data were fitted (R^2 equal to or higher than 0.99) to the following equation:

$$\eta = \frac{\eta_o}{\left[1+\left(\frac{\Upsilon}{\Upsilon_c}\right)^2\right]^s}.$$

In Carreau equation η (Pa·s) is the apparent viscosity, η_o (Pa·s) is the limiting viscosity for the first Newtonian region, Υ (s^{-1}) is the shear rate, Υ_c (s^{-1}) is the critical shear rate for the onset of the shear thinning behaviour and s is a parameter related to the slope of this region (Laca, Sáenz, Paredes, & Díaz, 2010; Liu et al., 2007).

The flow behaviour measurements of mayonnaises were carried out in control rate mode with a titanium parallel plate system with a 35 mm diameter. The shear rate was increased logarithmically from 1 to 150 s^{-1} for 100 s (up curve), then the shear rate was maintained for 150 s and was finally reduced to 1 s^{-1} in a further period of 100 s (down curve). The data of the up curve were adjusted to Ostwald de Waele equation (Nikzade et al., 2012; Worrasinchai et al., 2006) since it was the best fit to the experimental data (R^2 equal to or higher than 0.99):

$$\sigma = K(\Upsilon)^n$$

where σ is the shear stress (Pa), Υ is the shear rate (s^{-1}), K is the consistency index (Pa·s^n) and n is the flow behaviour index. Moreover, the "relative thixotropic area" was calculated as the area between the up and down curves divided by the area under the up curve, which allows a correct comparison of the rate of internal structural breakdown in systems with different viscosities (Dolz, González, Delegido, Hernández, & Pellicer, 2000).

2.2.3.2. Viscoelastic behaviour. Viscoelastic properties of mayonnaises were measured with a serrated titanium parallel plate system with a diameter of 60 mm. The linear viscoelastic region was determined by performing a stress sweep (0.01 to 10 Pa) at a frequency of 1 Hz. Subsequently, a dynamic frequency sweep was executed using a shear stress value within the linear viscoelastic region, over a frequency range between 10 and 0.1 Hz. The elastic modulus (G′), loss modulus (G″) and loss tangent (tan $\delta = G''/G'$) were obtained. Furthermore, Bohlin's parameters (A and z) were assessed starting from the complex modulus (G^* in Pa) and frequency (ω in s^{-1}), following the next equation:

$$G* = A\omega^{1/z}.$$

According to Bohli's theory of flow as a cooperative phenomenon (Bohlin, 1980), emulsions are modelled as a network of rheological units, which interact by establishing a system structure. The coordination number (z) is a measure of the number of rheological units correlated with one another in the three-dimensional structure, and the proportional coefficient (A) is a parameter (G^* at 1 Hz in Pa $s^{1/z}$) related to the strength of the interaction between those units (Manoi & Rizvi, 2009; Peressini, Sensidoni, & de Cindio, 1998).

2.2.4. Emulsion stability and freeze–thaw stability

Emulsion and freeze–thaw stability measurements were performed in duplicate. Emulsion stability of mayonnaise was determined following the method of Mun et al. (2009) with slight modifications. Samples of 15.00 g ± 0.01 g (F_0) of mayonnaise after 24 h of storage at 20 °C were placed in falcon centrifuge tubes (50 ml) and left in a chamber at 80 °C for 30 min to promote destabilisation. Heated samples were centrifuged for 10 min at 4000 ×g, and the separated layer was then removed and weighed (F_1). The emulsion stability was characterised as follows:

$$\text{Emulsion stability} = (F_1/F_0) \times 100$$

Freeze–thaw stability, after storage of the mayonnaise at −40 °C for 24 h, was measured and characterised following the emulsion stability test method.

2.2.5. Statistical analysis

The obtained data were subjected to a one-way analysis of variance (ANOVA) using the LSD Fisher test ($p < 0.05$). All analyses were performed with Statgraphics Centurion XVI software (StatPoint Technologies Inc., Warrenton, USA).

3. Results and discussion

3.1. Microstructure

The microstructure of the mayonnaise samples is shown in Fig. 1. Full-fat mayonnaise showed a defined and closely packed structure. Nevertheless, as the oil was replaced and, thus, the proportion of the aqueous phase increased, smaller oil droplets were observed. The amount and distribution of the emulsifying agent, the type and level of ingredients, the oil concentration, the size of the droplets, and the viscosity of the aqueous phase are important factors which determine mayonnaise microstructure (Ma & Boye, 2013; Mun et al., 2009). Table 1 shows the statistical analysis reported that the general trend indicated a more number of oil droplets with smaller mean area as the oil was gradually replaced by the extruded flour. These observations are consistent with the findings of Mun et al. (2009) and Teklehaimanot et al. (2013) for mayonnaises in which the oil was replaced by enzymatically and chemically modified starches, respectively. Regarding the amount of water of the paste, the mean area of the droplets did not show significant differences for any of the levels of oil substitution. Nevertheless, less number of oil droplets was observed for 1:4 ratio (higher water content) in 30% and 50% reduced-fat mayonnaises, showing a tendency to increase the oil droplet diameter with the amount of water in the formulation. The final size of the droplets produced during homogenisation depends on the rapid stabilisation of these droplets against bridging flocculation and re-coalescence once they are formed (McClements, 2005). Flour compounds, such as carbohydrates (starch or fibre) may also enhance emulsion stability by acting as bulky barriers between the oil droplets, preventing or slowing down the rate of oil droplet coalescence (Aluko, Mofolasayo, & Watts, 2009). Thus, in extruded flours the great amount of pregelatinised starch, with a major thickening effect (Mason, 2009), would cause a higher viscosity of the continuous phase. This higher viscosity would promote less movement and therefore would prevent these particles from sticking together (Manoj, Fillery-Travis, Watson, Hibberd, & Robins, 1998; McClements & Demetriades, 1998). In addition, proteins facilitate stable oil droplet formation by lowering interfacial tension and through development of interfacial membranes (Aluko et al., 2009; Chen, Chen, Ren, & Zhao, 2011). Extrusion treatment promoted molecular reorganisation of proteins, increasing its superficial hydrophobicity, affecting its interfacial properties and improving its emulsifying behaviour (Martínez et al., 2014; Silva, Arêas, Silva, & Arêas, 2010).

Taking into account the total area of oil droplets, a gradually decrease in the area occupied by the droplets was observed as oil substitution increased, with no significant differences according to the flour–water ratio of the paste. This trend is in agreement with Fig. 1, which showed larger areas of the aqueous phase as oil substitution increased, especially in samples of 70% substitution of oil as a result of the drastic reduction in oil concentration. It has already been observed in other studies in which the oil was replaced by hydrocolloids (Li et al., 2014; Ma & Barbosa-Canovas, 1995; Worrasinchai et al., 2006).

3.2. Newtonian region

The flow behaviour of mayonnaises was characterised by the Carreau model (Table 2). During the transport and storage of these products, low shear rate situations are produced (Izidoro, Scheer, Sierakowski, & Haminiuk, 2008). At low shear rates, a Newtonian region characterised by a constant limiting viscosity (η_o) is manifested. Conversely, the critical shear rate (Υ_c) sets the limit between Newtonian and pseudoplastic behaviour.

Although in general η_o tended to increase as the water concentration in the paste decreased, significant differences were only found for the ratios of the 50% and 70% level of substitution. This trend could be motivated by the viscosity reduction that took place in the continuous phase of the emulsion as the water content increased. Regarding the level of oil substitution, differences were only found in the 1:3 ratio, with the higher value for the higher level of substitution, which could be related to the smaller particle size of the oil droplets (Moros et al., 2002; Pal, 1996). In general, Υ_c decreased with reducing the concentration of water in the paste and increasing the oil substitution level, though the decrease was more evident as the concentration of water in the paste (1:4 ratio) was increased, with differences between all the levels of substitution. A lower value of Υ_c means a lower resistance of the emulsion microstructure to the shear-induced breakdown process (Moros et al., 2002) and this is related to an increased shear sensitivity in more structured emulsions (Raymundo, Franco, Empis, & Sousa, 2002).

Concerning the value of the s index, which describes the pseudoplastic character (Batista et al., 2006), no clear trend was reported. Nevertheless, all emulsions exhibited a similar behaviour, with values consistent

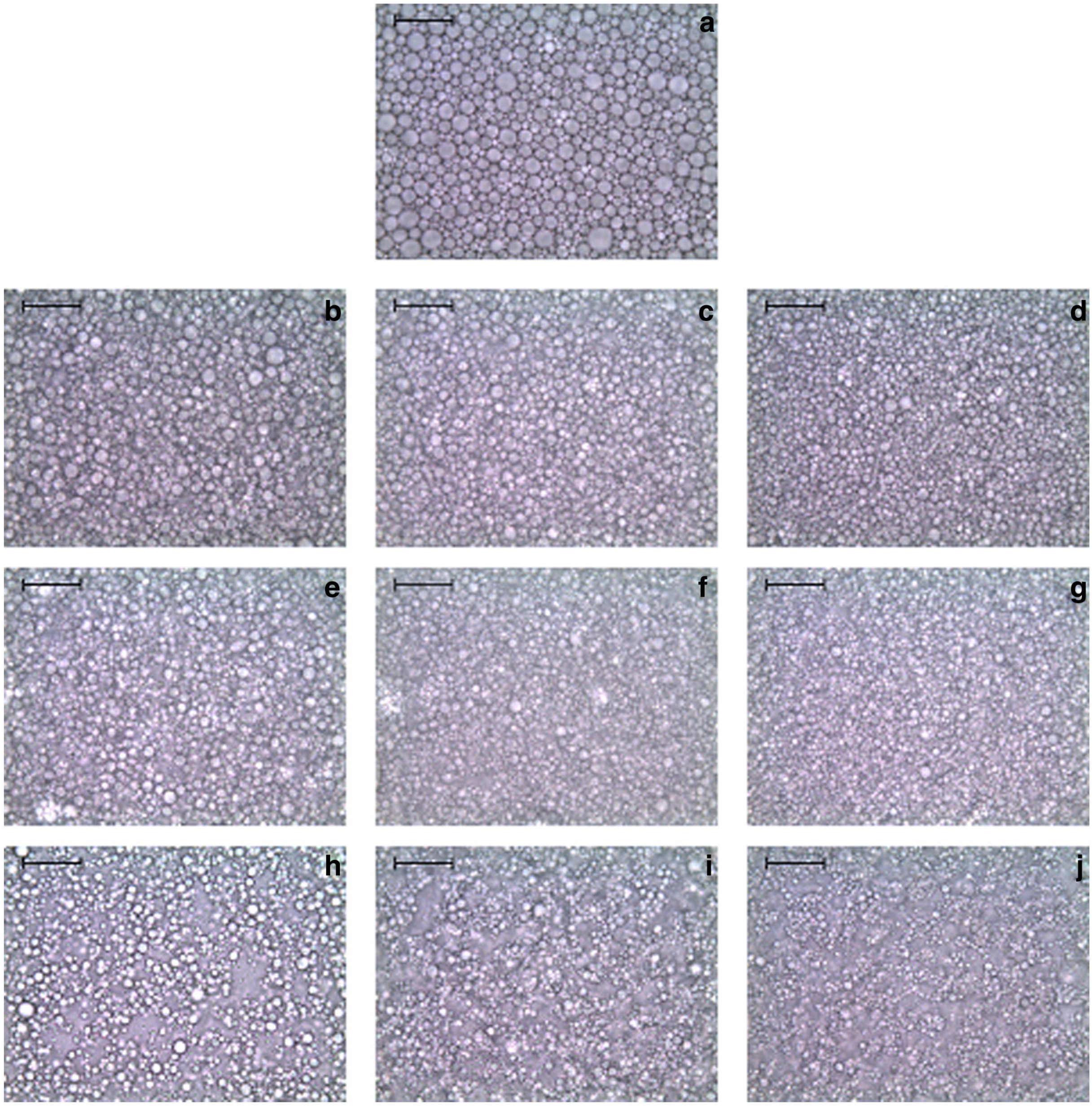

Fig. 1. Micrographs taken by optical microscope of full-fat and reduced-fat mayonnaises. The bar at the upper left corner of each photograph indicates a size of 20 μm. (a) Full-fat; (b) 30% Subst. and 1:4 Ratio; (c) 30% Subst. and 1:3.5 Ratio; (d) 30% Subst. and 1:3 Ratio; (e) 50% Subst. and 1:4 Ratio; (f) 50% Subst. and 1:3.5 Ratio; (g) 50% Subst. and 1:3 Ratio; (h) 70% Subst. and 1:4 Ratio; (i) 70% Subst. and 1:3.5 Ratio; (j) 70% Subst. and 1:3 Ratio.

Table 1
Parameters of the oil droplets of the full-fat and reduced-fat mayonnaises.

Samples	No of droplets	Mean area (μm^2)	Total area of droplets (μm^2)
Full-fat	918a	5.91f	5361.91c
30% Subst.			
Ratio 1:3	1530cde	2.97cde	4500.84bc
Ratio 1:3.5	1282bc	3.48de	4454.07bc
Ratio 1:4	1210b	3.71e	4451.85bc
50% Subst.			
Ratio 1:3	1704ef	2.51bcd	4231.43b
Ratio 1:3.5	1690ef	2.32bc	3870.75b
Ratio 1:4	1432bcd	2.91cde	4166.95b
70% Subst.			
Ratio 1:3	1813f	0.81a	1462.01a
Ratio 1:3.5	1679def	1.01a	1772.68a
Ratio 1:4	1686def	1.45ab	2481.87a

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. Ratio = extruded flour: water proportion.

with those obtained in other emulsions containing gums and/or modified starch (Dolz, Hernández, Delegido, Alfaro, & Muñoz, 2007; Raymundo et al., 2002).

3.3. Steady flow behaviour

To characterise the flow properties of mayonnaise, Ostwald the Waele model was used (Table 2). In the reduced-fat mayonnaises, the decrease of water content in the flour paste seemed to increase the consistency index. Generally, K value also increased with increasing level of oil replacement for 1:3 and 1:3.5 ratios, although no differences were shown between full-fat mayonnaise and reduced-fat mayonnaises of 30% and 50% oil substitution in the 1:3 ratio and 70% in the 1:3.5 ratio. This might suggest an increase in the viscosity of the continuous phase, since a higher K value in the emulsion indicates a more pronounced viscous characteristic, which corresponds to a stronger network structure (Ma & Boye, 2013). Starch polymers can interact with water

Table 2
Carreau and Ostwald the Waele parameters of the full-fat and reduced-fat mayonnaises.

Samples	η_o (Pa·s)	Υ_c (s^{-1})	s	K (Pa·s^n)	n	Relative thixotropic area
Full-fat	4166cd	0.0085ab	0.405abc	78.63de	0.213bc	24.63a
30% Subst.						
1:3 Ratio	2933abcd	0.0102abc	0.383a	73.13cd	0.220bc	29.08abc
1:3.5 Ratio	1284ab	0.0208de	0.394ab	52.52b	0.241d	25.80ab
1:4 Ratio	736a	0.0412g	0.439de	42.25a	0.239d	24.97a
50% Subst.						
1:3 Ratio	5101d	0.0113abc	0.437de	86.79e	0.207ab	39.54cd
1:3.5 Ratio	2508abcd	0.0160cd	0.417bcd	67.22c	0.214bc	32.66bcd
1:4 Ratio	946ab	0.0322f	0.433cde	39.65a	0.227cd	34.88bcd
70% Subst.						
1:3 Ratio	11150e	0.0061a	0.420bcde	111.73f	0.197a	36.97cd
1:3.5 Ratio	3551bcd	0.0134bc	0.445de	76.56cd	0.206ab	37.18cd
1:4 Ratio	1341abc	0.0233e	0.447e	43.13ab	0.224c	40.70e

Means values followed by the same letter in each column are not significantly different at $p \leq 0.05$. Ratio = extruded flour: water proportion. η_o = limiting viscosity, Υ_c = critical shear rate, s = slope of the region, K = consistency index; n = flow behaviour index.

because of its hydrophilic nature and form entangled network increasing the viscosity (Mason & Hoseney, 1986; Teklehaimanot et al., 2013). Furthermore, protein unfolding as a consequence of extrusion process (Martínez et al., 2014) could also have an influence on the water absorption and therefore the viscosity.

All mayonnaise samples exhibited shear-thinning behaviour, since their flow behaviour index was less than one. A decreased n means an increase in shear-thinning character, indicating that more mutual entanglements were formed in the emulsion (Ma & Boye, 2013). All mayonnaises had very low n values and thus, a pronounced pseudoplasticity. In general, significant differences between the reduced-fat and the full-fat mayonnaise were not found, only an increase in the n value for the higher water content between extreme water ratios being observed. According to these results, a low water content of the extruded flour paste positively influenced the flow parameters of the mayonnaises, since it increased K, leading to a strong structure, and slightly increased the shear-thinning behaviour of the emulsion. These results are in agreement with those previously reported by Mun et al. (2009) who replaced the oil content by a paste of modified rice starch and xanthan gum. The shear-thinning behaviour is beneficial for the organoleptic properties of emulsions, in terms of both flavour release and mouth feel (Ma & Boye, 2013), as in the improvement of mixing capability and flowability (Gamonpilas et al., 2011).

Time dependency of the mayonnaises was evaluated by determining the relative thixotropic area (Table 2). Thus, the larger the thixotropic area, the lower the rheological stability, since it is related to an ability to rebuild the damaged structure more rapidly after the removal of shear forces (Laca et al., 2010). All mayonnaises were found to have thixotropic behaviour. Full-fat mayonnaise and reduced-fat mayonnaises of 30% showed the lowest values of the thixotropic area. Generally, a higher fat replacement tended to increase the thixotropic behaviour, but significant differences were slightly observed among the different reduced-fat mayonnaises. Regarding water content of the pastes, differences were only observed for the highest level of oil replacement. These results might be explained by the ability of the extruded flour to slow down the movement of droplets particles, indicating that, after removal of shear force, more time was necessary to rebuild the original structure.

3.4. Viscoelastic behaviour and Bohlin parameters

The viscoelastic behaviour of mayonnaises is displayed in Figs. 2 and 3. All mayonnaises were more elastic (G′) than viscous (G″) in the frequency range studied (Fig. 2). Typically, it was expected that mayonnaises with greater oil content would show a higher value of G′ and G″ modules as a result of the higher packing of droplets in the emulsion (Lee et al., 2013; Ma & Barbosa-Canovas, 1995). However, in this study the oil content reduction slightly influenced the modules, whereas the

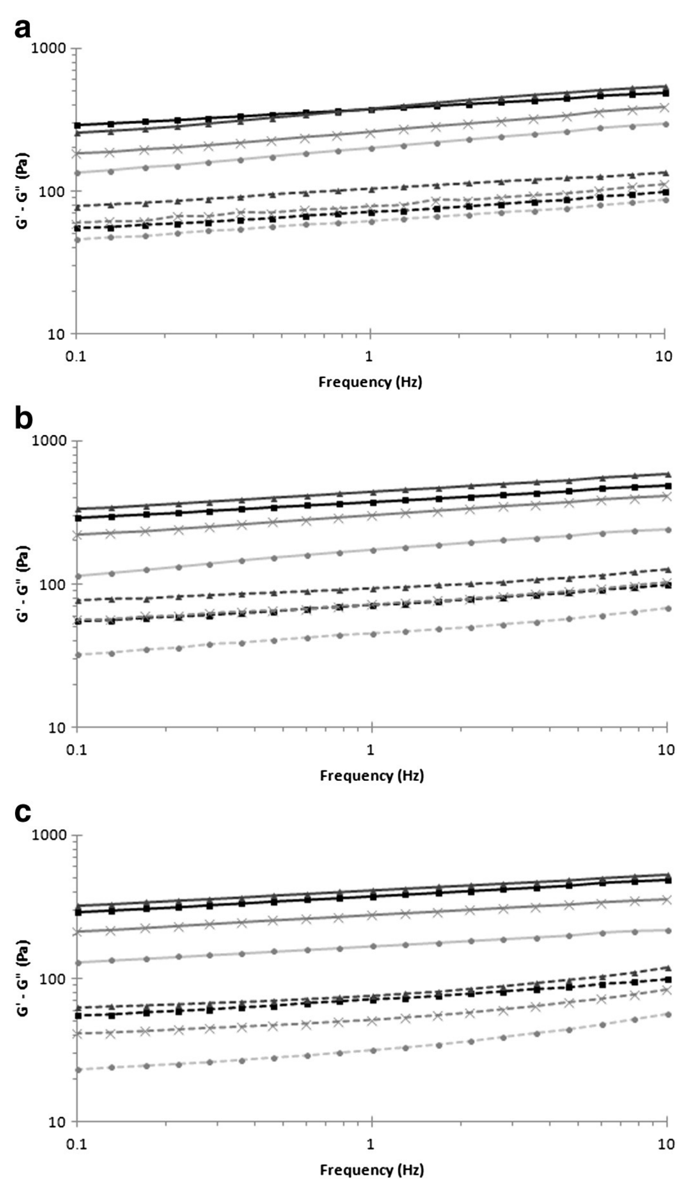

Fig. 2. Variation of storage (G′) and loss (G″) modulus with frequency of mayonnaise samples. a. Full-fat and reduced-fat of 30% oil substitution mayonnaises; b. full-fat and reduced-fat of 50% oil substitution mayonnaises; c. full-fat and reduced-fat of 70% oil substitution mayonnaises. G′ (continuous line) and G″ (broken line) modulus of full-fat (square), 1:3 Ratio (triangle), 1:3.5 Ratio (cross) and 1:4 Ratio (circle) samples.

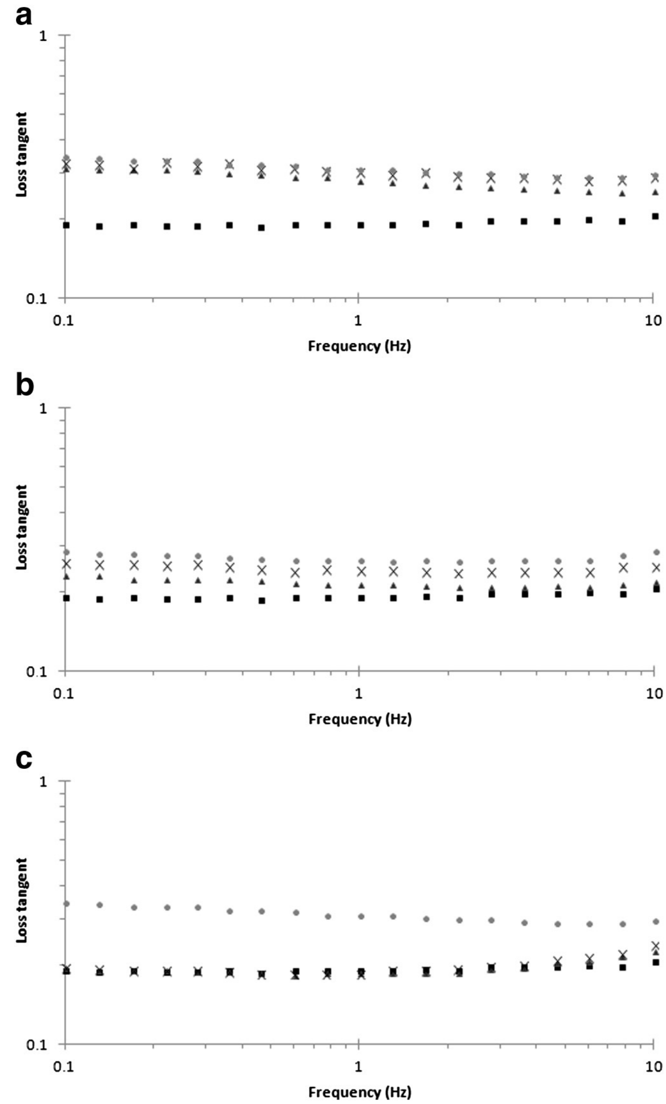

Fig. 3. Variation of loss tangent (tan δ) with frequency of mayonnaise samples a. Full-fat and reduced-fat of 30% oil substitution mayonnaises; b. full-fat and reduced-fat of 50% oil substitution mayonnaises; c. full-fat and reduced-fat of 70% oil substitution mayonnaises. Loss tangent of full-fat (square), 1:3 Ratio (triangle), 1:3.5 Ratio (cross) and 1:4 Ratio (circle) samples.

flour–water ratio of the paste seemed to be the main factor affecting such modules. For the three levels of substitution studied, G′ and G″ increased with decreasing water concentration of the paste. This increase might suggest a greater interaction between the structural components of the emulsion as a result of the pre-gelatinised starch present in the extruded flour. In fact, a similar trend was found by Bortnowska, Balejko, Tokarczyk, Romanowska-Osuch, and Krzeminska (2014), using a pre-gelatinised waxy maize starch as fat replacer in another oil-in-water emulsion.

Loss tangent, which indicates whether properties, elastic or viscous, predominate in the emulsion (Ma & Barbosa-Canovas, 1995), is shown in Fig. 3. In the frequency range studied, these emulsions behave like a solid (weak gel), with a tan δ greater than 0.1, typical of dressings and mayonnaises (Li et al., 2014). Loss tangent of the reduced-fat mayonnaises declined as the level of oil substitution was increased, showing a greater elastic character (Manoi & Rizvi, 2009; Zaidel, Chronakis, & Meyer, 2013). Moreover, differences respect to the full-fat sample became less noticeable as oil replacement was increased. On the other hand, concerning the extruded flour–water ratio, the loss factor diminished for the 30% and 50% levels of oil replacement as did water content of the paste, with no significant differences for the 70% level. In fact, Bortnowska et al. (2014) found that the higher the content of pre-gelatinised waxy maize starch in the reduced-fat emulsion, the lower the loss factor value. According to the reduced-fat mayonnaise microscopy, the smaller the droplet size, the lower the loss factor, which is also in accordance with results reported by Worrasinchai et al. (2006). Nonetheless, that hypothesis does not fit with the full-fat mayonnaise microscopy, and may be explained by the fact that the differences in the loss factor can also be influenced by the predominance of the continuous or dispersed phase (Ma & Barbosa-Canovas, 1995).

The A and z parameters of the Bohlin equation obtained from the oscillatory test are listed in Table 3. Low values of A and z together show a tendency of dispersed droplets to coalesce when the system undergoes mechanical stress, and can be correlated with emulsion stability (Peressini et al., 1998). Parameter A increased as the amount of water descended in the extruded flour paste, whatever the level of substitution, and thus the flour–water ratio in this type of sauce had a great influence on this parameter. Parameter A indicates the strength of the interactions and was positively correlated ($r = 0.85$, $p < 0.001$) with the consistency index of the flow test, a parameter which also reported a stronger structural network. Nevertheless, z value, which indicates the number of interactions, was mainly influenced by the level of substitution. Thus, as the level of substitution increased, the value of z was increased for all flour–water ratios. Meanwhile, for each substitution level, differences were hardly observed depending on water content in the paste. Surprisingly, the mayonnaises with lower oil replacement were those more different to the full-fat ones. This might be owed to a change in the structural model of these mayonnaises which reduces stability in the early stages but which is recovered as the oil replacement by the flour-water paste is increased. Actually, considering this parameter, the reduced-fat mayonnaises of 70% were the most similar to the full-fat. Moreover, a significant correlation of z with the loss factor at 1 Hz ($r = -0.92$, $p < 0.001$) was found. In general, based on the high values of A and z together, the samples which seemed to be less stable were those of the 1:3.5 and 1:4 ratios. On the contrary, A and z values increased for the 1:3 ratio as did the level of substitution. That suggested a more complex structure and higher level of interactions among emulsion components (Manoi & Rizvi, 2009) as well as a higher ability to maintain the original structure and prevent the destabilisation of the emulsion (Zaidel et al., 2013).

3.5. Emulsion stability and freeze–thaw stability

The most likely form of spoilage of mayonnaises is the breakdown of the emulsion because of a combination of the creaming and

Table 3
Bohlin's parameters A and z and freeze–thaw stability of the mayonnaises.

Samples	A (Pa.s$^{1/z}$)	z	Freeze–thaw stability (%)
Full-fat	384.40d	8.79de	24.16a
30% Subst.			
1:3 Ratio	389.23de	5.97a	63.14b
1:3.5 Ratio	274.95c	6.00a	65.93bc
1:4 Ratio	211.30b	5.80a	68.345c
50% Subst.			
1:3 Ratio	453.15f	8.15cd	79.01d
1:3.5 Ratio	310.38c	7.28bc	80.31de
1:4 Ratio	176.50ab	6.37ab	83.54e
70% Subst.			
1:3 Ratio	422.10ef	9.31e	97.99f
1:3.5 Ratio	283.25c	8.91de	98.63f
1:4 Ratio	172.23a	8.66de	98.69f

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. Ratio = extruded flour: water proportion. A = proportional coefficient, z = coordination number.

coalescence phenomenon of the oil droplets (Depree & Savage, 2001). Nevertheless, the study of the emulsion stability did not lead to the separation of oil in any of the mayonnaises studied, since stability was 100% for all the mayonnaises (data not shown). The great stability in terms of oil separation could be caused by the use of whole egg in the formulation. Thus, the additional emulsifying role of the white proteins (Kiosseoglou & Sherman, 1983) would improve that of the lipoproteins in the yolk. Furthermore, since full-fat mayonnaise was stable, it is logical that the mayonnaise keep on being so stable with the reduction in oil concentration, as the oil droplets were reduced in size and the extruded flour may promote a less droplet movement. Thus, creaming is retarded because the droplets are incapable of moving in the high viscosity of the gel-network formed by polysaccharides (Manoi & Rizvi, 2009). In fact, an increase in stability with oil replacement was found in other studies of oil-in-water emulsions formulated solely with egg yolk, due to the addition of substances with an emulsifier or thickener capacity (Bortnowska et al., 2014; Mun et al., 2009; Nikzade et al., 2012).

Oil-in-water emulsions are prone to destabilisation in the freeze–thaw process (Magnusson, Rosén, & Nilsson, 2011), because of the large volume expansion of the aqueous phase during crystallisation (Ghosh & Coupland, 2008). This forces the oil droplets still closer together (Saito et al., 1999) and it is possible that the ice crystals formed during freezing will break the oil droplets and alter their interfacial membranes. This makes them more susceptible to coalescence and phase separation after thawing (Thanasukarn, Pongsawatmanit, & McClements, 2004). In this research, full-fat mayonnaise exhibited the lowest freeze–thaw stability, whereas the stability was increased with the level of oil substitution (Table 3). Generally, the flour–water ratio had less effect on freeze–thaw stability. An increase in stability was only observed when the water content was increased between 1:3 and 1:4 ratios of the 30% and 50% level of substitution. Teklehaimanot et al. (2013) also found an increase of freeze stability when the fat was replaced by a chemically modified starch paste. This phenomenon could be attributed to the hydrophilic nature of the paste, which may stabilise water during crystallisation and maintain separated the oil droplets, protecting against possible coalescence. Magnusson et al. (2011) reported that stability to the freeze–thaw process may be increased by altering the crystallisation of the aqueous phase. This presumably took place when adding the extruded flour, whose pregelatinised starch trapped the free water and diminished the number of crystals, and, thus, volume expansion of the aqueous phase. According to these results, the replacement of oil by the extruded flour paste helps in retention of oil, preventing its separation, and making possible the potential application of mayonnaise as an ingredient in frozen products, since many frozen ready-meals contain emulsion-based sauces (Degner, Chung, Schlegel, Hutkins, & McClements, 2014).

4. Conclusions

From the results of the present work, it can be concluded that oil replacement by extruded flour paste had a pronounced effect on the microstructure, freeze–thaw stability and rheological properties of mayonnaises. Substitution of part of the oil gradually increased the number and reduced the size of the oil droplets. Regarding rheological behaviour, as water content of the paste was reduced, an increase in both consistency index and limiting viscosity in flow analysis and an increase in elasticity and Bolin's parameters in oscillatory measurement were observed. Nevertheless, thixotropic behaviour increased as did level of oil replacement. All mayonnaises showed great emulsion stability. Moreover, fat replacement by extruded flour produces mayonnaises that were more stable in terms of freezing. These results suggested that if the flour–water ratio of paste is controlled, extruded flour can be used as an effective oil replacer in oil-in-water emulsions.

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ORIGINAL ARTICLE

Effect of extruded wheat flour as a fat replacer on batter characteristics and cake quality

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Abstract The effects of three levels of fat replacement (1/3, 2/3, and 3/3) by extruded flour paste and the effects of the presence of emulsifier on layer cake batter characteristics and final cake quality were studied. Replacement of oil by extruded flour paste modified the batter density and microscopy, reducing the number of air bubbles and increasing their size, while emulsifier incorporation facilitated air entrapment in batter. Emulsifier addition also increased the elastic and viscous moduli of the batter, while oil reduction resulted in a less structured batter. Emulsifier incorporation leads to good quality cakes, minimizing the negative effect of oil reduction, maintaining the volume and reducing the hardness of cakes. Furthermore, consumer acceptability of the reduced fat cakes was improved by the addition of emulsifier. Thus, the results confirmed the positive effect of partial oil substitution (up to 2/3) by extruded flour paste on the quality of reduced fat cakes when emulsifier was incorporated.

Keywords Layer cake · Extruded flour · Emulsifier · Reduced fat · Acceptability

Highlights Extruded flour paste and emulsifier were used as oil replacer in cakes
Oil replacement reduces the number and increases the size of air bubbles in batters
Oil reduction decreases viscoelastic properties, leading to a less structured batter
Emulsifier addition results in good quality cakes minimizing oil reduction effect
Emulsifier incorporation improved consumer acceptability of the reduced fat cakes

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Introduction

Fat plays an essential role in the processing of shortening cakes, such as layer cakes or pound cakes, and similar products such as muffins and cake doughnuts. In these products fats are responsible for the incorporation of air in the batter in the form of small bubbles. The formation of a structure which incorporates the air in the form of small bubbles will improve the stability of the batter (Wilderjans et al. 2013) while minimizing the coalescence phenomena and the movement of the bubbles to the surface, thus increasing the volume of the cakes and allowing a finer and more uniform grain (Stauffer 1990). Fats also improve the eating quality of cakes as they help to create a more tender texture and extend the shelf life of cakes by slowing the rate of staling through their influence on the retrogradation of starch (Gómez 2008). Nevertheless, an excessive fat intake has been linked to obesity (Bray et al. 2004) and cardiovascular problems, as well as several types of cancer (Rothstein 2006). For this reason, companies and researchers have attempted to minimize the fat and oil content of products like cakes.

Most attempts to reduce the fat content of cakes and muffins have focused on their substitution by several carbohydrate fat replacers such as inulin (Rodríguez-García et al. 2012, 2014a, b; Zahn et al. 2010), β-glucan (Kalinga and Mishra 2009; Lee et al. 2005), cocoa fibre (Martínez-Cervera et al. 2011), modified corn starch (Chung et al. 2010), and potato maltodextrins, polidextrose or crystalline cellulose (Kamel and Rasper 1988; Khalil 1998). The use of protein fat replacers (Conforti 1998; Psimouli and Oreopoulou 2013) and chia gel (Borneo et al. 2010) has also been proposed. Occasionally, emulsifiers or enzymes have also been used, together with fat replacers, in order to improve the quality of these cakes (Conforti 1998; Kamel and Rasper 1988; Rodríguez-García et al. 2014a).

Hydrothermal treatments of flours can gelatinize the starch present in them, depending on their moisture, temperature, and time of treatment. The flours with a high degree of pregelatinization have a greater thickening power in cold water than the traditional flours, and a higher water absorption and retention capacity (Hagenimana et al. 2006; Martínez et al. 2014a), which may help to provide a more tender and less dry texture in cakes with reduced fat. Actually, pregelatinized starches and extruded flours have been successfully proposed to partially replace the oil in oil-in-water emulsions (Lee et al. 2013; Román et al. 2015). Extruded flours have also been used to improve performance in baking, since they allow the quantity of water in the formulation and in the final wheat bread to be increased (Martínez et al. 2013) or the quality of gluten-free breads to be improved (Martínez et al. 2014b).

The aim of this research is to study the effect of pregelatinized extruded flour as an oil replacer (oil substitutions of 1/3, 2/3, and 3/3) and emulsifier addition on the development of reduced fat cakes. The characteristics of the batter in terms of batter density, microscopy, and dynamic rheology were determined. The quality of the cakes was also evaluated with regard to specific volume, weight loss, crumb structure and texture, and they were consumer-tested to determine the acceptability of reduced fat cakes.

Materials and methods

Materials

Wheat flour (10.9 g/100 g moisture; 8.98 g/100 g protein; 81.30 μm mean particle size) was supplied by Harinera Castellana S.A. (Valladolid, Spain). Extruded wheat flour was provided by Molendum Ingredients (Zamora, Spain), which performed the extrusion treatment using a Bühler Basf single screw extruder (Bühler S.A., Uzwil, Switzerland). The extrusion conditions were carried out to ensure starch gelatinization. The length-to diameter (L/D) ratio for the extruder was 20:1. Wheat flour was extruded at a maximum barrel temperature of 160 °C and a feed moisture content of 50 L/h, a feed rate of 500 kg/h, and a screw speed of 340 rpm. The extruded product was dried by convection till it reached 11.2 % moisture. Then, it was ground with a compression roller to a particle size below 200 μm. Sugar, sunflower oil, milk, and liquid pasteurized egg were purchased from the local market. The baking powder was “25×1”, and the emulsifier was “SuperMixo T500”, both supplied by Puratos (Gerona, Spain).

Methods

Flour characterization

Flours were analysed following AACC (2010) methods for water hydration capacity (AACC, 56.30-01) and protein (AACC, 46-30.01) which was carried out with a Leco TruSpec®N (St. Joseph, MI, USA). The flour particle size was measured using a particle size analyser with laser diffraction (Heros & Rodos, Sympatec, Clausthal-Zellerfeld, Germany) following AACC method 55-40.01.

Cake making

Layer cakes were elaborated using the following formula: 350 g flour, 315 g sugar, 210 g milk, 175 g liquid pasteurized egg, 175 g sunflower oil, and 10.5 g baking powder. A single-bowl mixing procedure was used to prepare the batter. All ingredients were mixed for 10 min (speed 4 for 1 min and speed 6 for 9 min) using a KitchenAid Professional mixer (Kitchen Aid, St. Joseph, Michigan, USA). Cakes were also made by replacing the sunflower oil (1/3, 2/3, and 3/3) with a flour paste of a mixture of extruded flour and water (1:4 flour-water ratio). Furthermore, all cakes were formulated with and without emulsifier (4.5 g) addition. Cake batter (185 g) was placed in disposable oil-coated aluminium pans (109×159×38 mm) and baked at 190 °C for 25 min. After baking, the cakes were removed from the pans and left to cool for 60 min before being placed in sealed plastic bags to prevent drying. Cakes were kept at 20 °C in a chamber until further analysis. Each formulation was prepared in duplicate.

Batter characterization

All the analysis performed for the batter characterization was carried out in duplicate after mixing the batters. Batter density at 20 °C was determined by an Elcometer 1800 pycnometer (Manchester, UK). To visualize bubbles, a drop of batter was placed on a glass slide and overlaid with a cover lip, trying to avoid the inclusion of exogenous air bubbles. The slides were compressed under a 1 kg weight to create a layer of batter of uniform thickness. The batter samples were examined at 40 times magnification using a DM750 microscope (Leica Microsystems, Wetzlar, Germany) equipped with an EC3 video camera, and images were captured using LAS-EZ software (Leica Microsystems, Wetzlar, Germany). Images of each batter were analysed in a 2048×1536 pixel format with Image J software (National Institute of Health, Bethesda, MD, USA). The mean area and number of air bubbles for each area were measured after adjusting the threshold.

The viscoelastic properties of batters were determined using a Haake RheoStress1 rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P unit.

Measurements were conducted at 20 °C using a serrated titanium plate-to-plate system (60 mm diameter) and a gap distance of 1 mm. Batter surface exposed to air between the plates was covered with Pancreac vaseline oil (Panreac Química SA, Castellar del Vallés, Spain) to prevent moisture loss. Before conducting any rheological measurement, the samples were allowed to rest in the measurement position for 5 min as an equilibration time, which is the time necessary to allow the stresses induced during sample mixing and loading to relax. This time was selected following previous time sweeps carried out within the linear region (0.3 Pa) at 1 Hz during 30 min at 20 °C. Then, the linear viscoelastic region was determined by performing a stress sweep (0.01–10 Pa) at a frequency of 1 Hz. Subsequently, a dynamic frequency sweep was executed using the shear stress values within the linear viscoelastic region, over a frequency range between 10 and 0.05 Hz. Analysis was monitored with RheoWin 4 Job Manager software and the rheological parameters were calculated using the Rheowin 4 Data Manager software (Thermo Fisher Scientific, Schwerte, Germany) by performing a regression analysis to adjust the graphs to a power-law model. Thus, in order to characterize the different batters, the storage (G′) and loss (G″) moduli at 1Hz and their dependency against frequency (ω) were calculated by fitting the following equations:

$$G' = G'(1Hz) \cdot \omega^{a}$$
$$G'' = G''(1Hz) \cdot \omega^{b}$$

where a and b coefficients indicate the frequency dependence of G′ and G″ respectively.

Cake quality

Quality determinations were made 24 h after baking. Cake volume was determined with the Volscan Profiler volume analyser (Stable Mycrosystems, Surrey, UK). The cake-specific volume was calculated as the ratio between the volume of the cake and its weight. Weight loss was calculated as the ratio between weight of batter in the mould and the weight of the cake after baking, expressed as a percentage. Measurements were run in duplicate (two cakes from each batter). Slices of cakes were scanned in an hp Scanjet G3110 (Palo Alto, CA, USA). The scanned images were treated using ImageJ software (National Institute of Health, Bethesda, MD, USA). The image was split in colour channels, the contrast was enhanced and, finally, the image was binarised after applying a threshold. Crumb texture was determined using a TA-XT2 texture analyser (Stable Microsystems, Surrey, UK) with the 'Texture Expert' software. A 25 mm diameter cylindrical aluminium probe was used in a Texture Profile Analysis (TPA) double compression test to penetrate to 50 % depth, with a test speed of 2 mm/s, and a 30-s delay between the first and second compression. Hardness (N), cohesiveness, and springiness were calculated from the TPA graphic. In order to determine cake staling, hardness was also determined after 7 days of storage in plastic bags at 20 °C, and the difference in hardness (ΔHardness) was determined between days 1 and 7. Measurements were made of two central slices (20 mm thickness) from two cakes of each batter.

Consumer testing

Hedonic sensory evaluation of cakes was conducted with 102 cake-usual consumer volunteers from 16 to 65 years of age. Consumer tests were carried out at the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain) in individual booths. Cakes were evaluated on the basis of the acceptability of their appearance, odour, taste, texture, and overall acceptability on a nine-point hedonic scale. The scale of values ranged from *dislike extremely* (score 1) to *like extremely* (score 9). Samples were analysed 1 day after baking. One whole cake and a central slice were presented for appearance evaluation and the rest were divided into 2-cm-wide portions. The samples were presented on white plastic dishes coded with four-digit random numbers and served in random order. Water was available for rinsing. Sensorial evaluation was only made for four cakes: control and 2/3 substitution, both with and without emulsifier.

Statistical analysis

Differences between the cakes were studied by analysis of variance (ANOVA). Fisher's least significant differences (LSD) method was used to describe means with 95 % confidence intervals. The statistical analysis was performed with Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA).

Results and discussion

Batter characteristics

Table 1 shows the values of the batter characteristics. For the batters without emulsifier, the reduction in the oil content only affected the density, significantly increasing its value 0.05 g/cm^3, when the level of substitution was 100 %. This trend could be motivated by the absence of oil in the batter, since oil is placed around the air bubbles maintaining the stability (Stauffer 1990). Moreover, the incorporation of emulsifier reduced the density in all cases, and therefore facilitated air entrapment during batter mixing (Khalil 1998; Kumari et al. 2011). In fact, the microscopy results (Table 1) confirmed that

Table 1 Batter characteristics of control and reduced fat cakes

	Batter density (g/cm^3)	Bubbles mean area (μm^2)	No. of bubbles (no./cm^2)	G′ (Pa)	a	G″ (Pa)	b
Control	1.08d	652.05a	61.21bc	133.15bcd	0.380c	101.34bc	0.463bcd
Control+E	1.01c	849.02ab	174.16e	164.85e	0.390c	119.15d	0.484d
RF1/3	1.10de	1294.84b	25.18a	124.85bc	0.385c	90.56b	0.479d
RF1/3+E	0.99bc	852.76ab	107.56d	153.10de	0.384c	107.95 cd	0.484d
RF2/3	1.11de	1325.66b	24.41a	111.20ab	0.337b	73.14a	0.446bc
RF2/3+E	0.98b	774.76ab	116.57d	143.40cde	0.348b	94.09b	0.443b
RF3/3	1.13e	1885.32c	29.34a	100.60a	0.341b	62.78a	0.472 cd
RF3/3+E	0.89a	859.19ab	86.70 cd	196.70f	0.307a	112.30 cd	0.409a

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. RF1/3, RF2/3, and RF3/3=reduced fat cakes with 1/3, 2/3, and 3/3 oil substitution, respectively; E=cakes containing emulsifier. G′ and G″ represent storage and loss moduli at 1Hz. a and b coefficients indicate frequency dependence of the storage and loss moduli dependence, respectively

emulsifier addition increased the volume of incorporated air by maintaining the mean size of the bubbles while increasing the number of bubbles. On the other hand, as the level of oil substitution in cakes with no emulsifier was increased, the bubbles were smaller in number but larger in size. Psimouli and Oreopoulou (2013) and Rodríguez-García et al. (2012) also observed a smaller size of bubbles in cakes prepared with shortening and oil, respectively, compared to those with a fat replacer. These authors attributed this effect to the ability of oil to stabilize bubbles by forming a film in the air-matrix interface. Furthermore, Lee et al. (2005), when preparing cakes with lower amounts of shortening replaced by β-glucan amylodextrins, also found that the number of entrapped air bubbles decreased. The emulsifier performs the function of stabilize the bubbles in the case of batters with less oil content, thus improving the structure. It is known that structures with smaller and more uniform bubbles are more stable, and these bubbles tend to remain in the final cake to a greater extent (Stauffer 1990). Consequently, it is expected that the batters with emulsifier will lead to a higher volume in the final cake.

Concerning the viscoelastic properties of the batter, in the frequency range tested the so-called plateau relaxation zone was observed for all the samples. The plateau region is characterized by physical entanglements in the polymeric materials (Ferry 1980). All batters showed a higher elastic modulus than viscous, indicating the solid nature, with both moduli depending on frequency but following a different pattern (Calero et al. 2013) as the different values of a and b coefficients indicated. The trend observed with the a coefficient for both cakes with and without the addition of emulsifier, and in the b coefficient for cakes with emulsifier, is to decrease from 2/3 replacement, indicating a less frequency-dependent modulus. Although the same trend is observed for both coefficients, higher values are exhibited for the b coefficient, which might suggest higher frequency dependence of G″ compared to G′ counterparts. For each of the levels of fat replacement, G′ and G″ significantly increased with the addition of emulsifier. Rodríguez-García et al. (2014a) and Sahi and Alava (2003) also found an increase in the moduli of low fat cakes with emulsifier and other fat replacers. Rodríguez-García et al. (2014a) suggested a stronger structure promoted by the water-binding capacity of the emulsifier. Nonetheless, the greater air incorporation and the distribution of this air in the form of fine bubbles generated by the emulsifier may also affect the rheological measurements of the batters. In fact, it has been found that the viscoelastic properties are also dependent on the concentration of air trapped during mixing (Kalinga and Mishra 2009). In this research, G′ and G″ moduli were correlated ($p<0.01$) to the mean area and the number of bubbles; thus, the smaller the area and the higher the number of the bubbles, the higher the elastic and viscous properties of the batters. The decrease in the oil content for the batters without emulsifier resulted in a progressive decrease in the values of G′ and G″, indicating a less structured batter, possibly due to the reduced aeration of the batter or to the extra water added with the extruded flour. According to these results, Rodríguez-García et al. (2014a), found a decrease of the moduli when the oil was replaced by inulin and water, which they attributed to a dilution effect. However, Lee et al. (2005) found the opposite results, since the storage modulus was higher for the cakes with decreasing amounts of shortening replaced by β-glucan amylodextrins. The more elastic behaviour of the reduced fat cakes can be attributed to the different fat replacer used and to the fact that they did not add extra water for the replacement.

Cake properties

Volume is an important physical attribute affecting consumer preference and acceptance. Thus, maintaining the volume has been recognized as one of the challenges of producing baked products with reduced fat (Min et al. 2010). The specific volume of cakes without emulsifier (Table 2) tended to decrease as the oil was replaced by the extruded flour paste. However, the specific volume did not show significant differences from the control sample with up to 2/3 oil substitution. Other authors have also reported that as oil substitution was increased (40–50 %), cakes yielded less volume when using other fat replacers (Borneo et al. 2010; Min et al. 2010; Rodríguez-García et al. 2014a, b). These authors attributed the results to the less air retention during baking, which means that the product is less aerated and denser (Borneo et al. 2010). On the other hand, emulsifier addition minimizes the reduction in volume, since no differences among cakes with emulsifier were found, nor with the control one. Some authors have previously reported an improvement in the volume of the cakes with the addition of emulsifiers (Khalil 1998; Kumari et al. 2011). These results indicate that the cause of the reduction in the specific volume may be due to the stability of the batters, as reported by Rodríguez-García et al. (2014b). This stability is reduced as the oil content is minimized, since oil tends to be around the bubbles, stabilizing them and maintaining more air during baking. At the same time, reducing the viscous and elastic properties ($p<0.001$) of the batter with the addition of extruded flour paste can also help to increase the loss of stability, as this is directly proportional to the viscosity of the material surrounding the bubbles (Stauffer 1990). However, the inclusion of the emulsifier helps to stabilize the batter due to a better distribution of air bubbles, as shown in the microscopy results. Actually, in the present research greater volume was obtained in the samples with higher batter aeration, since specific volume is inversely correlated to batter density and the mean area of the bubbles ($r=-0.72$; $p<0.001$). Khalil (1998) and Psimouli and Oreopoulou (2013) also found this negative relationship between cake volume and batter specific gravity in low fat cakes with other carbohydrate replacers.

Concerning weight loss, an increase in weight loss was observed when the emulsifier was added, but this increase was only significant in cakes with the highest substitution. Nonetheless, no clear trend was observed with regards to the oil substitution. Generally, the greater the amount of water content in the formulation, the more water evaporates during baking (de la Hera et al. 2014). Therefore, for reduced fat cakes, in which extra water has been added in the formula, greater baking losses as a consequence of the evaporation of water would be expected. Thus, the reduction in the oil content and its replacement by an extruded flour paste would promote a higher weight loss. Nevertheless, the greater the surface in contact with air in the oven or the higher the specific volume of the bakery product, the higher the water evaporation during baking, leading to a high weight loss (de la Hera et al. 2014). Hence, the decrease in the specific volume for the reduced fat cakes without the addition of emulsifier could counteract the effect of the higher water content. It is also important to consider the high water absorption capacity of the pregelatinized starches. In fact, Hesso et al. (2014) found that the substitution of native flour by a pregelatinized starch increased the water content of cakes. A relationship between a smaller volume and lower weight loss has been found by other authors in studies on cakes/muffins with reduced fat content (Rodríguez-García et al. 2014b; Zahn et al. 2010). However, with the addition of emulsifier, and thus, when minimizing the loss of volume, it is observed that the cakes with more water (higher level of substitution) are those which have greater weight loss.

Figure 1 shows the binarised images of the cell structure of the crumb. As fat content was replaced, the crumb showed a great number and bigger in size continuous air channels (c and d), indicating more coalescence of bubbles during baking. Rodríguez-García et al. (2014a) reported that low G′, G″ and complex viscosity during baking enhanced bubble migration and loss of air cells before the sponge batter set, which is in agreement with the rheological results previously found.

Table 2 Specific volume, weight loss and textural properties of control and reduced fat cakes

	Specific volume (cm^3/g)	Weight loss (g/100 g)	Hardness (N)	Cohesiveness	Springiness	ΔHardness
Control	2.20bc	9.51a	6.09c	0.637c	0.913d	50.46a
Control+E	2.08b	9.69ab	4.39a	0.625bc	0.879bc	59.55a
RF1/3	2.09b	10.24abc	6.31c	0.600ab	0.918d	42.07a
RF1/3+E	2.18bc	10.96c	4.80ab	0.606abc	0.897 cd	61.41a
RF2/3	1.89a	9.64a	7.29d	0.592a	0.886bc	30.94a
RF2/3+E	2.17bc	10.41abc	5.19b	0.612abc	0.860ab	27.19a
RF3/3	1.77a	10.80bc	8.33e	0.611abc	0.874abc	59.28a
RF3/3+E	2.27c	12.93d	4.19a	0.624abc	0.851a	69.22a

Mean values followed by the same letter in each column are not significantly different at $p\leq0.05$. RF1/3, RF2/3, and RF3/3=reduced fat cakes with 1/3, 2/3, and 3/3 oil substitution, respectively; E=cakes containing emulsifier

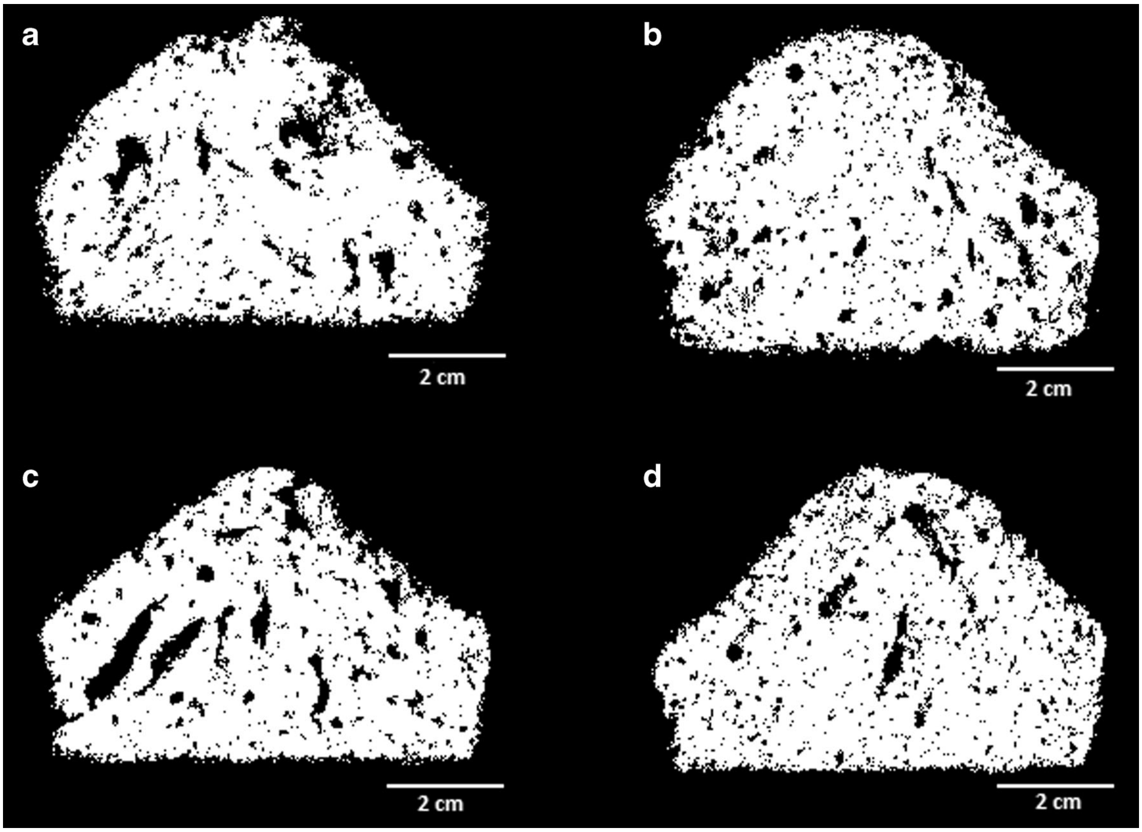

Fig. 1 Binarised images of scanned crumbs with scale bars, 2 cm. **a**. Control, **b**. Control+E, **c**. RF2/3 and **d**. RF2/3+E. *RF* reduced fat cake with 2/3 oil substitution, *E* cakes containing emulsifier

Furthermore, the addition of emulsifier leads to cakes with an improved crumb structure of finer and more uniform grain (b and d), in which the large channels were less visible. According to Sahi and Alava (2003) emulsifiers can make the bubbles decrease in size with a more uniform appearance, whereas the less presence of channels indicated that less air escaped during baking.

Table 2 also shows the values of the texture for the cakes studied. The reduction in the oil content increased the hardness of reduced fat cakes with no emulsifier compared to the control, which is related to a drop in the specific volume ($r=-0.67$; $p<0.001$). In fact, Chung et al. (2010) found that fat reduction in muffins caused an increase in hardness, which was associated with a decrease in both air incorporation and cake-specific volume. With the addition of emulsifier, the hardness was reduced for all the cakes studied. Instead of volume differences, this effect may be due to the fact that the hardness of the cakes was highly sensitive to the air bubble size distribution, as a distribution in small areas resulted in a significantly softer cake (Rodríguez-García et al. 2012), this distribution in small areas was also shown in the structure of the crumb. Thus, the way in which the air is retained into the batter during whipping and baking process would affect crumb hardness. These results are in agreement with those obtained by Khalil (1998) and Kumari et al. (2011). The cohesiveness of the cakes hardly varies with changes in the oil content or with the addition of emulsifier. Nevertheless, springiness seems to be reduced in cakes with no emulsifier as the oil content is reduced, although no significant differences were observed between the control and 1/3 oil substitution (with the highest value) or with the cakes with higher substitution (2/3 and 3/3). Martínez-Cervera et al. (2011) also observed that the springiness values fell significantly as soluble cocoa fibre increasingly replaced the fat in muffins, reflecting a more compact crumb structure. Zahn et al. (2010) suggested that the increase in elasticity is indicative of the increased strength of the bonds in the three-dimensional crumb network. On the contrary, the incorporation of the emulsifier reduced the elasticity of the control cake, but no significant differences between the cakes with partial or total replacement of oil were observed. Furthermore, the incorporation of the emulsifier also reduced the changes in elasticity as the oil was replaced in the formula. Sahi and Alava (2003) also reported that the addition of emulsifier diminished springiness in sponge cakes since emulsifiers reduce the strength of interactions between the fractions of flour in bakery systems and can lead to a rather crumbly product when applied in excess.

Regarding staling, a greater increase in hardness with the course of time would be expected as the oil was reduced since fatty acids minimize starch retrogradation (Zhou et al. 2007), reducing staling. However, no significant differences were observed between any of the elaborated cakes. This indicates that extruded flour may also reduce staling, counteracting the effect of the elimination of oil. In fact, Hesso et al. (2014) found that pregelatinized starches used as flour substitutes have a positive effect on retarding the staling of microcakes (degassed baked batters).

Sensorial evaluation

The results of the sensory evaluation of full-fat cakes, with or without emulsifier, and those with a 2/3 of substitution of oil are shown in Table 3. It can be observed that the cake with 2/3 oil substitution and no emulsifier presented a lower valuation in all the parameters compared to the control, which could be related to the volume, texture, and colour differences, as well as other aspects not analysed here such as the juiciness or flavour that provides the oil. Taking into account consumer observations, this cake was considered undercooked, and tasted like uncooked flour. However, the presence of emulsifier, which had no effect on the sensory acceptability of control cakes, significantly improved the appearance and overall acceptability of the cakes with reduced fat content, even reaching the score of the control cake in appearance. These changes can be caused by the effect of emulsifiers on the volume of the cakes. Basically, it is possible to obtain cakes with reduced oil content, with a good consumer acceptability (greater than 5 on a scale of 1 to 9), when substituting this ingredient by an extruded flour. In these cakes, the incorporation of emulsifier helped to increase their evaluation and leaded to cakes that did not differ from the control in their appearance, and only varied one point in their overall acceptability.

Conclusion

Oil replacement by an extruded flour paste helps to minimize the sensory changes of reduced fat cakes, but modified batter density and microscopy, by reducing the number of bubbles and increasing their size, and also reducing the viscoelastic properties. These changes produced the decrease in the volume of the final cake and a worse crumb appearance and a harder texture. Nevertheless, the addition of emulsifier minimized these differences. Emulsifier maintained the physical characteristics of the product, such as the batter properties and cake volume, and reduced its hardness while improving the consumer acceptability of the reduced fat cakes compared to the control. Thus, this study has shown that the substitution of oil within the cake formulation by an extruded flour paste up to 2/3 replacement and with the addition of emulsifier can be an effective fat replacer.

Table 3 Consumer acceptability of sensorial-tested cakes

	Appearance	Odour	Taste	Texture	Overall acceptability
Control	7.0b	6.9b	7.1b	6,9b	7.2c
Control+E	7.1b	7.0b	7.1b	7.2b	7.3c
RF2/3	5.9a	6.0a	5.7a	5.8a	5.8a
RF2/3+E	7.1b	5.8a	6.0a	6.2a	6.2b

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. RF2/3=reduced fat cakes with 2/3 oil substitution; E=cakes containing emulsifier

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CHAPTER 2

Exploiting the potential of extruded flours as viscosity enhancer in food applications

* Román, L., Pico, J., Antolín, B., Martinez, M. M., & Gómez, M. (2018). Extruded flour improves batter pick-up, coating crispness and aroma profile. *Food Chemistry*, 260, 106-114.

* Román, L., Reguilón, M. P., & Gómez, M. (2018). Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source. *Journal of Food Engineering*, *219*, 93-100.

This chapter includes two scientific articles published in SCI journals addressing the use of extruded flours as cold viscosity enhancers, in sauces and batters, with a view to obtaining "instant" sauces and fried batters with crunchier texture and improved coating. The two related publications, addressed in this chapter in the same order as below mentioned, are the following:

1. Román, L., Pico, J., Antolín, B., Martinez, M. M., & Gómez, M. (2018). Extruded flour improves batter pick-up, coating crispness and aroma profile. *Food Chemistry,* 260, 106-114.
2. Román, L., Reguilón, M. P., & Gómez, M. (2018). Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source. *Journal of Food Engineering,* 219, 93-100.

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Extruded flour improves batter pick-up, coating crispness and aroma profile

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ABSTRACT

Fried battered foods are widely consumed worldwide. In this study, the influence of the replacement of native wheat flour by extruded flours (7.5 and 15%) subjected to different extrusion severities on chicken nuggets was assessed. Microstructure, pick-up, moisture and fat content, texture, colour, volatile profile, and consumer acceptability were evaluated. Extruded flour replacement resulted in batters with improved pick-up with increasing percentage and severity of extrusion treatment. Extruded flour also contributed to a moisture increase, while oil remained practically unchanged. Textural properties highlighted a higher crispness of batters made with high-severity treatment extruded flours. Volatile compounds analyses revealed lower amount of lipid oxidation (lower rancidity notes) and higher furfuryl alcohol content (pleasant aroma and darker crust) in fried batters containing extruded flour. Consumers testing showed that batters made with intermediate-severity treatment extruded flour presented the best acceptability. These results confirm that extruded flour inclusion improves the quality of deep-fried batters.

1. Introduction

Fried battered foods are prominent in the diet of consumers all over the world. In the past, fried battered products have typically been prepared and consumed at home, but in more recent decades they have become very popular in the market of prepared food (Sanz, Salvador, & Fiszman, 2004b). Their growing demand is due to the convenience with which they can now be stored frozen at home and finished in a quick way for immediate consumption (Martínez, Sanz, & Gómez, 2015). A batter can be defined as a liquid dough, basically made of flour, water, leavening agent, and other minor ingredients, into which a product is dipped prior to cooking, normally by frying (Fiszman & Salvador, 2003).

Batter coatings provide a crunchy texture as well as pleasant flavour, and good appearance to foods, and they act as a barrier against the loss of moisture which, in turn, protects the natural juices of foods (Dogan, Sahin, & Sumnu, 2005b; Mohamed, Hamid, & Hamid, 1998). Therefore, batter coatings ensure that the final product is tender and juicy on the inside and crispy on the outside (Fiszman & Salvador, 2003). According to Loewe (1990), batter quality is based on the uniformity and thickness of the coating and its adhesion to the product, along with its general appearance, colour, crunchiness, and taste. In attempts to attain high quality batters, gums and hydrocolloids have been added to the formulations to improve some of these properties (Albert et al., 2009; Primo-Martín et al., 2010; Sanz, Salvador, & Fiszman, 2004a,2004b). The use of hydrocolloids is mainly related to their ability to absorb and retain water, acting as thickeners or regulators of viscosity. Hydrocolloids also contribute to batter properties by improving adhesion to the food substrate and stability to the freeze-thaw process, ultimately enhancing the textural attributes, maintaining freshness and reducing oil absorption during frying (Duxburry, 1998; Varela & Fiszman, 2011). With a similar aim, pregelatinised starches have been included in batter formulation, increasing coating pick-up (Altunakar, Sahin, & Sumnu, 2004; Mohamed et al., 1998).

Hydrothermal treatments of flours, such as extrusion, combining high temperatures and mechanical shearing at relatively low moisture levels and resident times can cause starch gelatinisation. Other than starch pregelatinisation, extrusion also leads to starch fragmentation, protein denaturation, enzyme (in)activation and Maillard reactions, whose extent is dependent on the severity of the extrusion process (Camire, Camire, & Krumhar, 1990). The intensity of the extrusion treatment, which is related to temperature, moisture content and screw speed, modify flour behaviour by changing its hydration as well as its thermal and pasting properties (Martínez, Rosell, & Gómez, 2014). In this way, extruded flours with a higher degree of pregelatinisation have greater thickening power in cold temperatures than traditional flours,

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along with higher water absorption and retention capacities (Hagenimana, Ding, & Fang, 2006; Martínez et al., 2014), yielding similar functionality to that of the hydrocolloids or pregelatinised starches. In fact, the use of extruded flours has been proposed to modify the rheology of batter systems (Martínez et al., 2015), demonstrating that the results depend highly on the severity of the extrusion conditions. A further advantage of extruded flours is that extrusion is a physical treatment that allows for the alteration of flour functionality while still maintaining a clean label (Jacobs & Delcour, 1998). Additionally, the process to obtain pregelatinised flour is more economic with a lower environmental impact compared to that of starches (Eckhoff & Watson, 2009). Despite the fact that pregelatinised starches have been used to improve the quality of battered products, to the best of our knowledge, the effect of the addition of extruded flours with different pregelatinisation degrees into these products has never been assessed. Furthermore, although batters coatings are thought to provide the covered food matrix with a pleasant flavour and aroma, no studies on the analysis of the volatile compounds of fried batter products have been performed so far. Moreover, considering that the kind of flour employed strongly influences the volatile profile in other thermally processed foods, such as breads (Pico, Tapia, Bernal, & Gómez, 2017), it is expected that the type of flour used in the elaboration of fried batter products would also influence its final aroma. In addition, it is worth mentioning that, in this work, different thermally modified flours are used in the batter formula, during which processing Maillard and caramelisation reactions are expected to occur (Camire et al., 1990), which can, in turn, further modify the volatile profile of the fried product.

In this study, the influence of partial wheat flour replacement by extruded wheat flours subjected to different extrusion conditions (mild, intermediate, and high) on the quality of batter coatings was evaluated. Wheat flour in the batters was partially replaced by extruded flour at 7.5 and 15% substitution levels and the microstructure, pick-up, textural properties, and colour of the resulting fried, battered nuggets were analysed. In addition, it is important to mention that in this study, for the first time, volatile compounds in batter systems before and after frying are evaluated. A consumer test was also carried out to evaluate the sensory acceptability of the different coatings.

2. Materials and methods

2.1. Materials

Native wheat flour (moisture and protein contents of 11.73 g/100 g and 11.20 g/100 g, respectively) was supplied by Harinera Castellana (Medina del Campo, Valladolid, Spain). Extruded modified wheat flours (flours 1, 2 and 3) were provided by Molendum Ingredients (Zamora, Spain), who performed the extrusion treatment using a Bühler Basf single screw extruder (Bühler S.A., Uzwil, Switzerland). The length-to-diameter (L/D) ratio for the extruder was 20:1. Initial wheat flour was subjected to different extrusion treatments, which included modifications in barrel temperature, feed rate and moisture content of the mass feed. Three types of extruded flours, numbered from 1 to 3 indicating the severity of the extrusion treatment (1, mild; 2, intermediate and 3, high), were obtained. Both flours 1 and 2 were extruded at a maximum barrel temperature of 120 °C and a screw speed of 397 rpm. However, flour 1 was extruded with a feed rate of 700 kg/h and a feed moisture content of 60 L/h, while flour 2 was extruded with a feed rate of 400 kg/h and a feed moisture content of 60 L/h. Flour 3 was extruded at a maximum barrel temperature of 160 °C and a feed moisture content of 50 L/h, with a feed rate of 500 kg/h and with a screw speed of 340 rpm. Then extruded flours were ground with a compression roller to a particle size below 200 μm.

Industrially made and rectangular shaped chicken pieces (30 × 50 × 10 mm) used as the food matrix were kindly provided by Productos Florida (Almazora, Castellón, Spain). Sodium bicarbonate (Manuel Riesgo, S.A., Madrid, Spain) and sodium pyrophosphate (Adín S.A. Paterna, Spain) were used as leavening agents. Sunflower oil and salt were purchased in the local market.

For the volatile profiles characterisation, 2-acetyl-1-pyrroline (2-ACPY, number 16 in Table 2) was purchased from Eptes (Vevey, Switzerland) and the other pure standards labelled from 1 to 15 and from 17 to 43 in Table 2 were obtained from Sigma-Aldrich (Steinheim, Germany). Dichloromethane was obtained from Scharlab (Barcelona, Spain) and methanol from VWR International (Fontenay-sous-Bois, France). Argon, nitrogen and helium were acquired from Carburos Metálicos (Barcelona, Spain).

2.2. Methods

2.2.1. Batter preparation and frying

The batter formulations and preparation was the same as that reported in Martínez et al. (2015). Briefly, the batter formulation was composed of native wheat flour (control) or a mixture of native wheat flour and extruded flour (91.40 g/100 g), sodium pyrophosphate (1.78 g/100 g), sodium bicarbonate (1.32 g/100 g), and salt (5.50 g/100 g). The levels of wheat extruded flour replacement in the flour mixture were 7.5 and 15 g/100 g. Batters were prepared with the three different types of wheat extruded flour (denoted as flour 1, 2, and 3). The pre-blended powders were mixed with water (20 °C) in a Kenwood Major Classic mixer (Kenwood Ltd, UK) at second speed for 2 min. The water/dry mix proportion was always 1.2:1. Batters containing extruded flours were labelled according to the type of extruded flour included (Flour 1, 2 or 3) and the percentage of wheat flour replacement (7.5 or 15%). Apparent viscosity of the batters at 20 °C was determined according to Martínez et al. (2015). Briefly, apparent viscosity versus shear rate data was recorded using a rheometer (Haake RheoStress 1, Thermo Fischer Scientific, Scheverte, Germany) with a titanium parallel plate geometry sensor PP60 Ti (60 mm diameter, and 1 mm gap). The test was programmed to increase the shear rate from 1 to 100 s-1 for 100 s after a resting time of 5 min. Measurements were made in duplicate and the obtained curves are depicted in Supplementary material 1. Viscoelastic and thermal properties of the 7 raw batters were also studied in a previous study (Martínez et al., 2015). The individual chicken pieces were pre-dusted with wheat flour, immersed in the batter, and allowed to drip for 30 s. Immediately afterwards, four battered chicken pieces were fried at a time in a deep fat fryer Taurus Profesional-3 (Taurus, Lleida, Spain) at 190 ± 2 °C for 3.5 min. The fryer was filled with fresh sunflower oil and preheated at 190 ± 2 °C for 1 h before frying. Fried chicken pieces were removed from the fryer and let to cool down on a tray covered with tissue paper for 10 min before further analysis. Each of the 7 batters (elaborations) was made in duplicate.

2.2.2. Microstructure

Two fried batters of control batter and batters made with 15% of extruded flours were separated from the nuggets and cut into 2 × 2 cm pieces. Photographs of the external surface of the coating were taken with a Stereoscopic Zoom Microscope Nikon SMZ-1500 (Nikon, Tokyo, Japan) for observation of the coating microstructure.

2.2.3. Batter pick-up determination

In batter-coated products, the term pick-up is generally used to denote the amount of batter adhered to the piece of food. The pick-up will be a determining factor in the yield and the quality of the final product (Albert et al., 2009). Therefore, the amount of batter adhered to the chicken nugget (g/100 g) was considered the batter pick-up value, calculated as:

$$\text{Batter pick-up} = \frac{B}{FP} \times 100$$

where B is the weight of batter coating the food matrix after cooking and FP is the weight of the whole fried product (chicken nugget and coating), after cooking (Baixauli, Sanz, Salvador, & Fiszman, 2003). Determinations were made on four nuggets per each elaboration.

2.2.4. Moisture and fat content

For moisture and fat determination, the coating was removed from the food matrix. Moisture content was determined according to approved method 44–15.02 (AACC, 2015). For fat determination, the dried sample was subsequently ground using a Super Junior S coffee grinder (Moulinex, Ecully, France). Crude fat content [g/100 g, in a dry basis (db)] was determined by extraction with petroleum ether using an extractor system Ankom XT10 (Ankom Technology Inc., Macedon, NY) following official procedure Am 5-04 (AOCS, 2005). Moisture and fat contents were determined in triplicate.

2.2.5. Colour determination

Colour was measured using a Minolta CN-508i spectrophotometer (Minolta Co., Ltd, Japan) with the D65 standard illuminant and the 2° standard observer; the results were expressed in the CIE $L^*a^*b^*$ colour space. Colour measurements were made on the external surface of the fried battered product. Two measurements were made on the crust of four batters from each elaboration.

2.2.6. Instrumental texture analysis

Four nuggets per each elaboration were measured to obtain mechanical data. A TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK) was used with a 25 kg load cell to evaluate the texture of the fried coatings 10 min after frying. A penetrometry test consisting of a single compression was performed using a P/6 aluminum cylinder probe. The test settings were: test speed 1 mm/s, trigger force 5 g, distance 10 mm. Force (N) vs. displacement (mm) curves were plotted.

2.2.7. Volatile compounds analysis

2.2.7.1. Preparation of standard solutions. 2-ACPY solutions were prepared in dichloromethane, as 2-ACPY is only stable in dichloromethane and ethyl acetate. It was necessary to work in every moment under inert atmosphere of argon due to the lack of stability of the compound to the oxygen and moisture. For this reason, dichloromethane was dried in a SDS PS-MD-5 purification system from Düperthal Sicherheitstechnik (Karlstein am Main, Germany).

For the other 42 volatile compounds marked from 1 to 15 and from 17 to 43 in Table 2, working solutions of each volatile compound were prepared in methanol. All the solutions were stored in a freezer at -21 °C.

2.2.7.2. Volatile compounds analysis by SPME-GC/QTOF. Both the unfried (UB) and fried batters (FB) were frozen with liquid nitrogen and ground in an Ika grinder model M20 (Staufen, Germany) for 10 s, achieving a powder.

The solid-phase microextraction (SPME) conditions were previously optimised and validated by the research group for the analysis of volatile compounds in bread crust (Pico, Antolín, Román, Gómez, & Bernal, 2018). 0.75 g (± 0.0050 g) of each batter powder (unfried or fried) was weighed into a 20 mL vial. The selected fibre was 50/30 µm DVB/CAR/PDMS (Sigma Aldrich, Gillingham, UK). The sample was incubated for 5 min at 60 °C (without the fibre) and then extracted for 51 min at 60 °C, without agitation. After that, the fibre was inserted into the GC injector port for thermal desorption during 5 min at 270 °C, with an injection volume of 1µL. Finally, the fibre was conditioned for 30 min at 270 °C after each analysis. Each sample was analysed in triplicate.

GC/QTOF analysis conditions were the same as used in Pico, del Nozal, Bernal, and Gómez (2017) All the volatile compounds were identified by comparison of their retention times and accurate mass spectra (with four decimal places) with standards as well as using their Mass Spectra Library (NIST MS Search 2.2 & MS Interpreter).

2.2.8. Consumer testing

Hedonic sensory evaluation of battered products was conducted with 94 volunteers, who were regular battered products eaters between 16 and 65 years of age and from various socioeconomic backgrounds. Consumer tests were carried out at the Sensory Science Laboratory of the Agricultural Engineering College at the University of Valladolid, Palencia (Spain) in individual booths. Although complete nugget pieces were provided to the consumers, they were only asked (both oral and written instructions) to evaluate the chicken nuggets based on their coatings. Batters were evaluated on the basis of acceptance of their appearance, odour, taste, texture, and overall liking on a nine-point hedonic scale. The scale of values ranged from “extremely like” to “extremely dislike” corresponding with highest and lowest scores of “9” and “1”, respectively. Samples were presented on white plastic dishes coded with four-digit random numbers and served in random order. Water was available for rinsing. Samples were tested 10 min after being fried. Since sensory evaluation was carried out with non-trained panelists, and then, a high number of samples can be excessive and saturate consumer’s perception, only four samples were chosen: control sample and the three batters with 15% of replacement by extruded flour. The samples with the highest level of replacement were chosen based on the greater differences in their batter psychochemical characteristics compared to the control.

2.2.9. Statistical analyses

Differences between the batters were studied by analysis of variance (ANOVA). Fisher’s least significant differences (LSD) method was used to describe means with 95% confidence intervals. The statistical analysis was performed with Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA). To assess the variation of the volatile profiles from unfried and fried batters regarding the use of native or extruded flours, a PCA was conducted with the peak areas average of each batter sample (unfried or fried) prepared by duplicate and analysed by triplicate ($n = 6$). The PCA was performed with the software LatentiX version 2.00 (Latent5, Copenhagen, Denmark), with data standardised prior to the analysis.

3. Results and discussion

3.1. Batter pick-up determination

Coating pick-up is an important quality parameter of battered products in the food industry, with values varying between 30 and 50 g/100 g. In this study, pick-up values (30.95–46.39 g/100 g) increased with increases in both the intensity of extrusion treatment of the wheat flour (flour 1 < flour 2 < flour 3) and the level of replacement of extruded flour in the batter (Table 1). No significant differences in pick-up were observed between control batter and batters made with extruded flour 1, the one subjected to the mildest extrusion treatment. However, higher pick-up values (i.e., greater thickness of the batter layer) were obtained for batters made with 15% extruded flour 2 and, especially, for 7.5 and 15% of extruded flour 3 (subjected to the most severe extrusion treatment), with higher pick-up observed with higher replacement level. It is known that coating pick-up is positively correlated with batter viscosity in the sense that as viscosity rises more batter remains adhered to the sample (Dogan, Sahin, & Sumnu, 2005a; Fiszman & Salvador, 2003). In this study, increasing extruded flour amount and severity of extrusion treatment in the batter formula resulted in an increase in the apparent viscosity of the batters, as seen in Supplementary material 1. Thus, the higher pick-up values are in agreement with the higher apparent viscosity of the batters as well as with the higher consistency index found by Martínez et al. (2015) in raw batters when using the same extruded wheat flours. These results are explained by the presence of pregelatinised starch in extruded flours, whose content increases with the severity of the extrusion treatment (flour 3 > flour 2 > flour 1) [Martínez et al., 2014; 2015].

Table 1
Values of batter pick-up, moisture, and fat content and colour parameters of coatings made with different substitution levels and types of extruded flours.

Sample	Pick-up (g/100 g)	Moisture content (g/100 g)	Fat content (g/100 g, db)	L*	a*	b*
Control	31.49a ± 0.76	14.56a ± 0.12	15.11a ± 0.26	55.25d ± 1.80	5.24a ± 1.33	24.16b ± 1.54
Flour 1 (7.5%)	30.95a ± 2.68	12.42a ± 2.38	14.63 a ± 0.82	53.11cd ± 2.80	6.59ab ± 2.01	20.19a ± 1.71
Flour 2 (7.5%)	32.87ab ± 2.24	15.50ab ± 0.76	15.49 a ± 0.28	51.79bcd ± 3.40	9.47c ± 0.59	20.59a ± 2.46
Flour 3 (7.5%)	41.69c ± 3.05	22.90d ± 0.89	14.41 a ± 0.45	48.35b ± 5.94	9.40c ± 1.10	19.96a ± 3.69
Flour 1 (15%)	32.22ab ± 1.31	18.51bc ± 0.01	16.01 a ± 0.05	52.99cd ± 3.39	7.63b ± 2.34	20.59a ± 1.86
Flour 2 (15%)	36.73b ± 1.91	21.55cd ± 0.30	15.36 a ± 0.86	50.87bc ± 3.28	9.46c ± 1.34	18.80 a ± 4.52
Flour 3 (15%)	46.39d ± 1.89	26.99e ± 0.52	14.62 a ± 0.24	43.72a ± 4.62	9.59c ± 1.61	17.77 a ± 3.77

Values ± standard deviation followed by the same letters within each parameter indicate no significant differences ($p \leq 0.05$).

In this way, a greater content of pregelatinised starch would contribute to a higher water absorption capacity and viscosity of the more severely extruded wheat flours, as seen by Martínez et al. (2014), yielding higher values of coating pick-up.

3.2. Moisture and fat content

Moisture and fat content for the different batter formulations are shown in Table 1. With regard to moisture retention, no differences were observed in moisture content between the 7.5% level of extruded flours subjected to mild treatments (flour 1 and flour 2) and the control. However, for the rest of the extruded flours, higher moisture content was observed with increasing severity of extrusion treatment and with increasing level of replacement in the most pregelatinised flour (flour 3). Again, this higher moisture content would be related to the superior water retention capacity of the more severely extruded flours (i.e., less free water able to evaporate during frying) leading to better barrier properties. Similarly, Altunakar et al. (2004), using different starches for batter formulation, reported that the highest pick-up and moisture values were found with pregelatinised tapioca starch, relating this event to its higher water binding capacity and film forming ability. Another plausible explanation for the moisture content could also be related to differences between the external structure of the batters. Formation of bubbles and vented holes was observed on the outer surface of all types of fried batters. Notably, batter solely containing native wheat flour seemed to show higher number of gas cells and more deeper and vented holes, which may indicate a greater ease in steam migration and gas escape during frying (Supplementary material 2). Thus, higher water evaporation is associated with larger number of voids, cracks, and crevices on the batter surface (Dana & Saguy, 2006).

Regarding fat content, whose values ranged from 14.62 to 16.01 g/100 g, no significant differences were found for the oil content of the different batters, During the frying process, oil absorption occurs as moisture is evaporated from the food (Dana & Saguy, 2006). Then, the higher moisture retention in batters with higher percentage of extruded flour should have been associated with lower fat content in the fried batter. It is noteworthy that, despite the fact that some authors have found a reduction in oil absorption content in the presence of hydrocolloids due to the increase in moisture retention (Sanz et al., 2004a,2004b), values of fat content with the hydrocolloid incorporation were similar to those of this study (~15–16 g/100 g, db). Furthermore, Primo-Martín et al. (2010) found that the relationship between moisture and oil content was not always held true, indicating that other mechanisms, apart from water replacement, are responsible for the oil absorption phenomenon. Oil uptake is a surface phenomenon, and its uptake and distribution are determined by the crust microstructure, ultimately leading to the restriction of oil absorption to the immediate crust and product surface (Dana & Saguy, 2006). In agreement with this, Varela, Salvador, and Fiszman (2008) measured the oil content in the crust and core of battered chicken nuggets and reported that oil content in the core did not significantly change. Dana & Saguy (2006) already reported that one of the approaches to reduce oil absorption after frying is to wipe the product surface with absorbent paper. Therefore, a plausible mechanism is that oil may have migrated from inside the crust after frying to its exterior towards the absorbent paper used to keep the fried nuggets before further analysis rather than inside the crust towards the nugget core. This oil migration would be more prone to occur in the control sample, since the greater number of deep and vented external holes (see Supplementary material 2) and the thinner crust (poor physical barrier to oil migration) could facilitate superficial oil loss after frying. Conversely, the higher coating pick up, which means enhanced crust formation and efficient coverage of the surface, might have controlled the diffusion of oil into and out of the product when more pregelatinised extruded flour was used in the batter formulation.

3.3. Colour determination

The colour of a battered product is directly related to its external appearance, and, therefore to its acceptance by consumers. Colour parameters of the different batters are shown in Table 1. All crusts presented the typical dark golden colour (see Supplementary material 2) caused by Maillard reactions and sugar caramelisation at high temperatures (Loewe, 1990). In general, the use of extruded flours in the formulation brought about darker (lower L^* values) and more reddish coatings (higher a^* values) with minimal differences in the yellowish (b^* values). Results also indicated a darkening of the crust (lower L^* and higher a^* values) with increasing both the percentage of the extruded flour and the intensity of its treatment. This occurrence should be attributed to starch dextrinisation during extrusion processing, resulting in the formation of reducing sugars that will participate in Maillard and sugar caramelisation. In fact, Martínez et al. (2014) reported that free sugar content increased in parallel with the extrusion severity, although a minimum intensity was necessary for promoting those changes, as the scarce differences in L^* values between control sample and mildly extruded flour confirmed. Furthermore, not only free sugars are produced during extrusion cooking but also the process conditions used (high temperatures in combination with shear strain and low water content) are known to favour the Maillard reaction between reducing sugars and proteins (Camire et al., 1990). This may have also accounted for a darker colour of the extruded flour, and, in turn, of the coating made with this flour. Therefore, it seems that the level of reducing sugars in batter formulations contributed to Maillard reactions on the crust during frying, and, in turn, on the greater colour development of batters containing extruded flours.

3.4. Instrumental texture analysis

The texture profiles of the different batters are depicted in Fig. 1a and b. In these figures, it can be seen that the curve of control sample, presenting a low jagged profile with few peak forces, had a similar shape to those batters containing flour 1. Whereas a highly jagged profile characterised by many force peaks and drops was found for batters containing flour 2 and 3 (intermediate and high extrusion level), especially when they were presented in a higher percentage in the formula (Fig. 1b). This more jagged profile is associated with numerous

Table 2
Volatile compounds studied in the different unfried (UB) and fried batters (FB). Control batters were elaborated with native flour, while the rest of batters were prepared with 15% of extruded flours of different extrusion levels (Flour 1 = mild, Flour 2 = intermediate, Flour 3 = high).

	UB control	UB Flour 1	UB Flour 2	UB Flour 3	FB control	FB Flour 1	FB Flour 2	FB Flour 3
2,3-Butanedione (1)	0.710a ± 0.00652	1.22d ± 0.0282	1.03c ± 0.0217	1.44e ± 0.0412	0.984c ± 0.00253	1.24d ± 0.0783	0.973c ± 0.0106	0.881b ± 0.00426
Hexanal (2)	1.93ab ± 0.0110	6.60e ± 0.312	5.36d ± 0.0396	11.5f ± 1.37	1.65a ± 0.0179	2.66c ± 0.0184	2.45bc ± 0.00930	5.13d ± 0.0423
2-Methyl-1-propanol (3)	0.456a ± 0.0185	0.731d ± 0.0104	0.586b ± 0.00930	1.56 g ± 0.0398	0.842e ± 0.0098	0.993f ± 0.00963	0.685c ± 0.00329	0.714d ± 0.0139
1-Methylpyrrol (4)	0.319ab ± 0.0112	5.46e ± 0.171	2.50d ± 0.0714	5.30e ± 0.343	0.643c ± 0.0266	0.420b ± 0.0114	0.387ab ± 0.00989	0.213a ± 0.0159
R-Limonene (5)	0.757a ± 0.00594	6.62f ± 0.0306	6.45f ± 0.00188	3.95d ± 0.419	2.54c ± 0.0586	4.75e ± 0.269	4.65e ± 0.292	1.84b ± 0.0439
Pyrazine (6)	0.172a ± 0.00589	0.745 g ± 0.00412	0.676 h ± 0.0267	0.498e ± 0.0271	0.397e ± 0.0096	0.430d ± 0.0139	0.607f ± 0.0309	0.275b ± 0.0285
2-Methyl-1-butanol (7)	2.61d ± 0.106	2.43cd ± 0.0250	0.700a ± 0.0184	1.69bc ± 0.0385	3.92f ± 0.276	2.69d ± 0.244	2.53d ± 0.0593	1.07ab ± 1.23
3-Methyl-1-butanol (8)	2.75ab ± 0.0242	3.82b ± 0.0963	1.87a ± 0.172	4.45b ± 0.161	56.9cd ± 1.31	22.4d ± 1.35	20.2c ± 0.478	35.9e ± 1.27
1-Pentanol (9)	0.787a ± 0.00728	5.19f ± 0.169	4.30e ± 0.203	6.43 g ± 0.234	1.36d ± 0.0781	1.02b ± 0.0302	1.22c ± 0.0191	1.48d ± 0.00911
2-Methylpyrazine (10)	0.347a ± 0.00799	0.407a ± 0.0101	0.478a ± 0.000271	0.425a ± 0.0217	7.66f ± 0.176	5.11c ± 0.0473	5.12c ± 0.0954	1.75b ± 0.0280
Acetoin (11)	0.724a ± 0.0155	0.882b ± 0.00982	0.865b ± 0.0187	1.48d ± 0.0175	3.23 g ± 0.0790	1.69e ± 0.0430	1.65e ± 0.0990	1.13c ± 0.113
2-Octanone (12)	1.03bc ± 0.0288	0.967b ± 0.00735	0.518a ± 0.0242	1.23c ± 0.0227	6.99f ± 0.250	3.76d ± 0.0186	3.91e ± 0.0643	4.62f ± 0.0416
2,5-Dimethylpyrazine (13)	0.176a ± 0.0135	0.282a ± 0.0218	0.254a ± 0.00475	0.412b ± 0.00567	3.06d ± 0.0892	2.51e ± 0.110	2.32d ± 0.0615	1.00c ± 0.0215
2,6-Dimethylpyrazine (14)	0.253a ± 0.0110	0.420a ± 0.00143	0.420a ± 0.0191	0.641b ± 0.0114	2.03g ± 0.0498	2.06d ± 0.177	2.26e ± 0.105	0.999c ± 0.00441
2-Ethylpyrazine (15)	0.157a ± 0.00743	0.207b ± 0.00250	0.216b ± 0.00191	0.346c ± 0.00696	1.95f ± 0.0250	1.58e ± 0.0234	1.66f ± 0.0274	0.670d ± 0.00122
2-Acetyl-1-pyrroline (16)	0.344a ± 0.0254	0.609a ± 0.0175	0.663a ± 0.0191	1.42b ± 0.013	14.1f ± 0.427	4.16c ± 0.000882	5.32d ± 0.237	6.72e ± 0.165
2,3-Dimethylpyrazine (17)	0.194a ± 0.0228	0.277b ± 0.0118	0.303b ± 0.00620	0.342c ± 0.0118	1.40a ± 0.0260	0.526d ± 0.00140	0.527d ± 0.0298	0.732e ± 0.0160
1-Hexanol (18)	2.41b ± 0.197	3.60c ± 0.178	3.27c ± 0.112	9.94d ± 0.825	1.11f ± 0.0804	0.938a ± 0.0420	0.911a ± 0.0260	0.737a ± 0.0337
Nonanal (19)	1.29a ± 0.00560	1.35a ± 0.00940	1.66b ± 0.0264	2.04c ± 0.116	6.28f ± 0.0882	3.31d ± 0.108	3.34d ± 0.115	4.04e ± 0.0346
2,3,5-trimethylpyrazine (20)	0.169a ± 0.00325	0.205b ± 0.0143	0.198b ± 0.0125	0.332e ± 0.0025	0.978f ± 0.0146	0.964f ± 0.00318	0.969f ± 0.0251	0.284c ± 0.0188
2-Ethyl-3-methylpyrazine (21)	0.418c ± 0.00864	0.276a ± 0.00605	0.298ab ± 0.0121	0.403c ± 0.00924	0.794f ± 0.00116	0.625d ± 0.0331	0.748e ± 0.0187	0.315b ± 0.0348
Ethyl octanoate (22)	1.33b ± 0.0339	0.979a ± 0.0199	4.19e ± 0.0675	1.96d ± 0.0949	4.65h ± 0.0471	5.30g ± 0.172	8.65h ± 0.00180	1.63c ± 0.0358
1-Octen-3-ol (23)	1.08a ± 0.0447	2.81b ± 0.0425	3.34c ± 0.0104	4.90e ± 0.157	8.24e ± 0.00626	4.28d ± 0.0172	5.05f ± 0.162	5.50g ± 0.0542
Acetic acid (24)	0.160a ± 0.0110	0.243ab ± 0.0174	0.221a ± 0.00156	0.371b ± 0.00854	5.56a ± 0.0637	4.83d ± 0.0258	8.10f ± 0.148	2.90c ± 0.00257
Furfural (25)	0.232a ± 0.02	0.433a ± 0.05	0.342a ± 0.05	0.548a ± 0.07	3.18b ± 0.159	4.05c ± 0.0888	6.30d ± 0.103	16.8e ± 0.385
2-Ethyl-1-hexanol (26)	1.10a ± 0.0134	2.54b ± 0.0571	3.38c ± 0.0991	4.94f ± 0.118	8.45e ± 0.0139	4.98f ± 0.234	4.51e ± 0.221	4.43d ± 0.108
Benzaldehyde (27)	0.353a ± 0.000883	0.628b ± 0.0107	0.751c ± 0.00503	1.92d ± 0.0158	2.01f ± 0.0623	2.92g ± 0.0271	2.80f ± 0.0687	3.65h ± 0.0137
2-(*E*)-Nonenal (28)	0.208a ± 0.00694	0.185a ± 0.00266	0.193a ± 0.0187	0.259b ± 0.00476	0.584de ± 0.0210	0.379d ± 0.0277	0.323c ± 0.0153	0.478e ± 0.00278
5-Methyl-2-furaldehyde (29)	0.108a ± 0.00241	0.183b ± 0.000330	0.134a ± 0.00458	0.447e ± 0.0187	0.413a ± 0.000337	0.333c ± 0.00682	0.386e ± 0.00322	0.755f ± 0.0745
Butyrolactone (30)	0.0466a ± 0.00126	0.143ab ± 0.00435	0.257bc ± 0.00891	0.353c ± 0.00993	4.62f ± 0.0483	5.77b ± 0.137	7.28c ± 0.0207	7.98d ± 0.0226
2-Acetylpyrazine (31)	0.780a ± 0.00777	0.527a ± 0.0144	1.58ab ± 0.159	2.11b ± 0.0637	26.4d ± 1.469	8.32d ± 0.0628	7.22c ± 0.0500	10.2e ± 0.149
Butyric acid (32)	0.428ab ± 0.0109	0.407ab ± 0.0122	0.485b ± 0.0149	0.216a ± 0.0139	4.39e ± 0.0481	2.66c ± 0.144	4.37d ± 0.124	6.41e ± 0.264
Phenylacetaldehyde (33)	1.08b ± 0.0163	0.846a ± 0.0192	1.07b ± 0.00729	1.56c ± 0.0794	4.29d ± 0.0117	5.18f ± 0.0222	5.47g ± 0.0289	2.84d ± 0.0775
Furfuryl alcohol (34)	2.15a ± 0.140	1.18a ± 0.00488	1.21a ± 0.0124	1.03a ± 0.00545	58.1b ± 0.901	67.5c ± 1.07	73.9d ± 1.44	80.7e ± 0.0484
2-Methylbutanoic acid (35)	0.284ab ± 0.0165	0.460b ± 0.0260	0.162a ± 0.0172	0.351ab ± 0.0206	3.02d ± 0.162	2.54c ± 0.0738	3.77e ± 0.120	3.44f ± 0.137
3-Methylbutanoic acid (36)	0.439ab ± 0.00923	0.492b ± 0.00893	0.251a ± 0.00766	0.286a ± 0.0139	2.71d ± 0.165	2.59c ± 0.0992	3.97f ± 0.0612	3.63e ± 0.177
2,4-(*E,E*)-Decadienal (37)	0.982a ± 0.0317	0.213a ± 0.0165	0.780a ± 0.0207	1.00a ± 0.0180	120e ± 1.09	8.06b ± 0.138	103c ± 3.90	108d ± 2.76
Hexanoic acid (38)	0.161a ± 0.00711	0.207a ± 0.0118	0.429b ± 0.00881	0.529b ± 0.00984	1.81c ± 0.0110	2.49d ± 0.0844	3.63e ± 0.0463	3.64e ± 0.167
Benzyl alcohol (39)	0.351d ± 0.00281	0.322bc ± 0.00619	0.375e ± 0.0154	0.690f ± 0.00701	0.315ab ± 0.0147	0.301a ± 0.0161	0.391e ± 0.00659	0.343cd ± 0.0182
Phenylethyl alcohol (40)	0.449f ± 0.0145	0.420e ± 0.00746	0.520g ± 0.00537	0.724h ± 0.0163	0.351c ± 0.00728	0.292a ± 0.00543	0.367d ± 0.0101	0.318b ± 0.00956
2-Acetylpyrrol (41)	0.147b ± 0.00956	0.0603a ± 0.00134	0.0877ab ± 0.00261	0.0983ab ± 0.000406	0.840c ± 0.00791	1.29d ± 0.0110	1.90f ± 0.0784	1.62e ± 0.0510
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (42)	0.0504b ± 0.00168	0.0192a ± 0.000391	0.0431b ± 0.000222	0.0339ab ± 0.000943	0.504d ± 0.0145	0.441c ± 0.00686	0.746f ± 0.0131	0.506d ± 0.0125
4-Vinylguaiacol (43)	0.0932b ± 0.000454	0.0727a ± 0.00118	0.0945d ± 0.00230	0.150c ± 0.00290	0.262d ± 0.00502	0.630f ± 0.00709	0.581e ± 0.0121	0.709g ± 0.0237

Values ± standard deviation followed by the same letters for each volatile compound indicate no significant differences ($p \leq 0.05$). The numbers between brackets after the names of the volatile compounds indicate the numeration followed in the PCA of the Fig. 2.

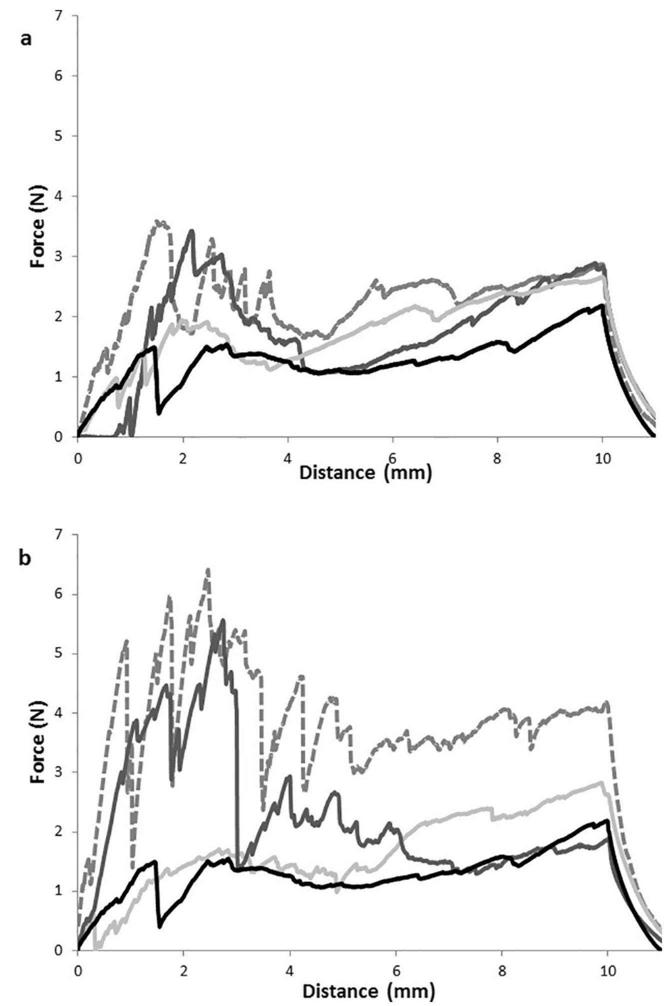

Fig. 1. Texture profile of wheat batters made with 7.5% (a) and 15% (b) replacement by extruded flour. Control sample (black line), Flour 1 (light grey line), Flour 2 (dark grey line) and Flour 3 (dashed grey line).

fracture events, and, then, it describes the behaviour of a crispy or crunchy product (Albert et al., 2009).

It would be expected that the higher moisture content of batter with extruded flours would decrease the crispness (less number of peaks). In fact, previous works have already related a higher moisture content of the crust to a decreased perception of crispness (Baixauli et al., 2003; Primo-Martín et al., 2010). However, samples with similar moisture contents in the crust can have very distinct crispness characteristics since crispness would depend on the water distribution within the crust, a more difficult parameter to measure in these products with a sandwich-like structure (Varela et al., 2008).

In is worth noting that the penetration curves presented two regions: the jagged zone, which was observed in the first part of the profile, and a second half, which hardly presented any fracture events. This could be attributed to the fact that the water distribution in the crust is not homogeneous, which may also help explain why moisture loss does not seem to be correlated with crispness. In fact, the moisture loss is more extreme in the outer layers, which is in agreement with the jagged profile observed here, while the internal regions of the batter are moist and tough rather than crisp (Luyten, Plijter, & Van Vliet, 2004; Varela et al., 2008). Therefore, it could be hypothesised that the higher moisture content with increasing percentages of severely treated extruded flour is mainly due to higher moisture retention in the inner part of their thicker crust (see pick-up values, Table 1); where moisture is more protected from evaporation as a result of the good film forming ability and viscosity of extruded flours. Furthermore, since crusts of battered products have a porous morphology, consisting of films of solid material surrounding air cells (Primo-Martín et al., 2010), the fracture behaviour depends on the distribution and homogeneity of the solid material and on the amount and structure of the air cells (Gibson & Ashby, 1988). Additionally, the greater barrier properties of these viscous extruded flours could have also contributed to enhanced retention of the gas produced by the leavening agents in the batters, bringing about a more aerated/expanded coating, and, thus, explaining the highly jagged profile of the batters. In agreement with this affirmation, Altunakar et al. (2004) reported that starch addition, especially when pregelatinised, enhanced gas entrapment inside the fried product, increasing the volume of the coating and improving texture, due to its greater pick-up and film forming ability.

On the other hand, higher peak force values were obtained for more severely treated extruded flours (flour 2 and 3), especially for 15% level of replacement. Conversely, flour 1 (7.5 and 15% level), with mild extrusion treatment, hardly showed differences in the height of peak force compared to the control sample, with values being only slightly higher at the end of the curve. In this case, the higher force values indicated more resistance to penetration in a less fragile covering (i.e., the presence of a harder coating), which may be associated with the higher pick-up and, possibly greater thickness of batters made with increasing percentages of extruded flours.

3.5. Volatile compounds analysis

43 volatile compounds were determined (Table 2), belonging to most of the chemical classes reported in bakery products (Birch, Petersen, & Hansen, 2014; Pico, Bernal, & Gómez, 2015). For a first exploration of the impact of the use of native or extruded flours (15% replacement) in the elaboration of the batter, a bidimensional PCA was performed (Fig. 2). Regarding the PC1 (60.92% of the variance), there was a clear separation between unfried batters (positive component) and fried batters (negative component). The main reason was that the fried batters presented higher abundance of the volatile compounds, with the highest content of 33 of the 43 studied volatile compounds. On one hand, the high temperatures applied during frying encouraged the generation of higher abundances of volatile compounds from Maillard reactions (Loewe, 1990), such as 2-methylpyrazine, 2-ethylpyrazine, dimethylpyrazines, 2,3,5-trimethylpyrazine, 2-ethyl-3-methylpyrazine, 2-acetylpyrroline as well as 2-acetylpyrrol or furan derivatives, including furfural, 5-methylfurfural, furfuryl alcohol or 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (see Table 2). These high temperatures also promoted caramelisation reactions (Loewe, 1990), which led to the generation of more furfural and 5-methylfurfural from pentose degradation (Ait Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008). Acetoin, acetic acid, benzaldehyde, benzyl alcohol or phenylacetaldehyde were volatile compounds that could have been generated from several origins, being one of them Maillard, and they were also higher in the fried batters than in the corresponding unfried batters. Concretely, acetoin, acetic acid, phenylacetaldehyde can be also generated by fermentation, benzyl alcohol from lipids oxidation and benzaldehyde from both fermentation and lipids oxidation (Pico et al., 2015). The incidence of volatile compounds from fermentation without added yeast is not surprising, since these volatile compounds have been reported in raw flours (Pico, Tapia et al., 2017). Moreover, the small amount of endogenous yeasts, the small amount of free sugars as well as the presence of endogenous α-amylases in flours, can lead to a soft fermentation during mixing even in absence of exogenous yeasts (Martínez-Anaya, 1996). On the other hand, the use of oil for frying justified the higher abundances of volatile compounds from lipids oxidation (LOx), such as nonanal, 1-octen-3-ol, 2-ethyl-1-hexanol, 2-(*E*)-nonenal or 2,4-(*E*,*E*)-decadienal (see Table 2). Nevertheless, LOx and volatile compounds from Maillard reactions of low boiling point (i.e. hexanal, pentanol, 1-hexanol from LOx and 2,3-butanedione, 1-

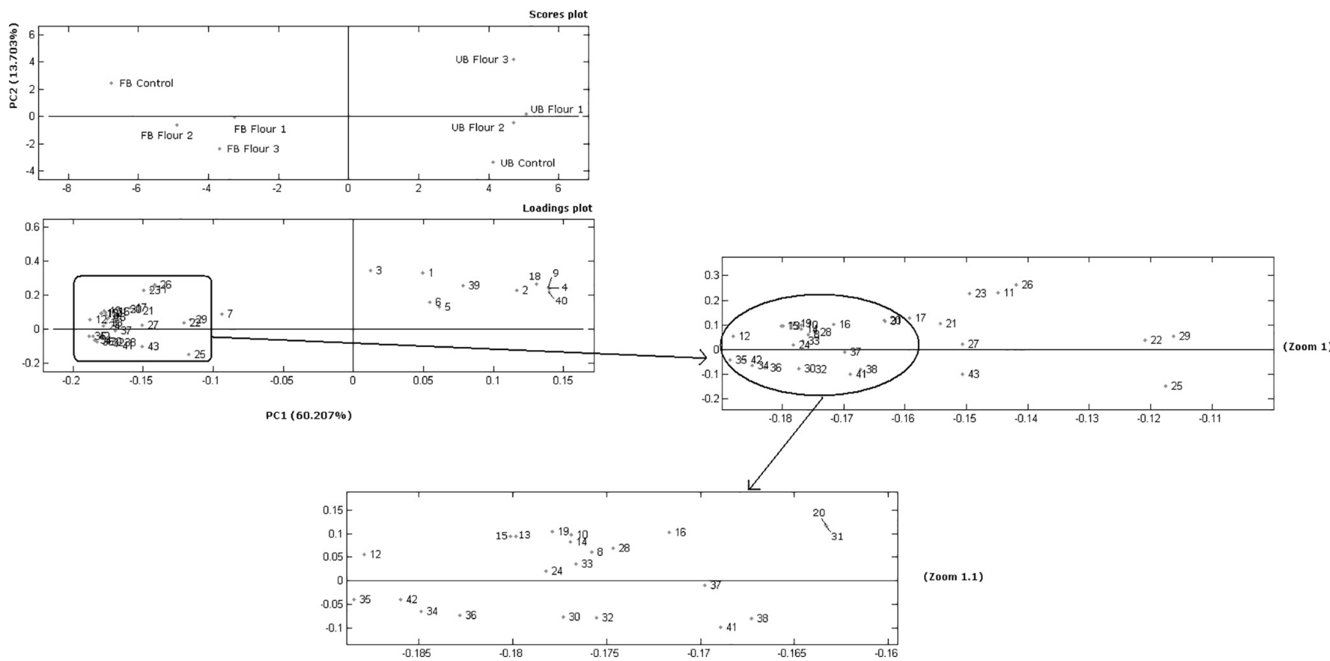

Fig. 2. PCA of the 43 volatile compounds studied in the different unfried (UB) and fried batters (FB). Control batters were elaborated with native flour, while the rest of batters were prepared with 15% of extruded flours of different extrusion levels (Flour 1 = mild, Flour 2 = intermediate, Flour 3 = high).

methylpyrrol or pirazine from Maillard) were in lower amount in the fried batters, which can be explained by an evaporation during frying.

Regarding the PC2, there was a clear separation between the control sample and the sample made with the most severely extruded flour (flour 3, both for the unfried and fried batters, not being observable a clear trend for the batters containing flours 1 and 2 (due to their proximity to x axis). In the case of the unfried batters, the LOx and volatile compounds from Maillard reactions mentioned above were, generally, in higher concentration in the unfried batter made with extruded flour 3 (Table 2). This can be explained by the extrusion process, which caused severe starch dextrinisation and protein denaturation (Yaylayan, Fichtali, & van de Voort, 1992), with the subsequent formation of reducing sugars and free amino acids, respectively, that will participate in sugar caramelisation and/or Maillard (Camire et al., 1990), as was explained above. Moreover, the high temperatures produced during extrusion process (especially in flour 3; see 2.1 section) not only encouraged the higher generation of volatiles from Maillard reactions and caramelisation, but also the higher generation of LOx, due to the cleavage of the hydroperoxides produced during the mixing by the lipoxygenases (Camire et al., 1990; Pico et al., 2015). In relation to the fried batters, the opposite effect was observed, and it is the control sample the one with higher content in LOx and volatile compounds from Maillard reactions and caramelisation (Table 2). As mentioned above, during the extrusion process there is a starch dextrinisation that could somehow change the interaction between the volatile compounds and the matrix, hindering the release of volatile compounds during SPME analyses. In fact, even in low amounts, cyclodextrins have been reported during extrusion process of wheat flour (Román, Dura, Martínez, Rosell, & Gómez, 2016), which are known to be encapsulators of flavours (Astray, Gonzalez-Barreiro, Mejuro, Rial-Otero, & Simal-Gándara, 2009) forming stable inclusion complexes (Astray, Mejuto, Morales, Rial-Otero, & Simal-Gándara, 2010). Moreover, as it was observed in the fat content analyses (Section 3.2), the fried batter with 15% of flour 3 absorbed slightly less fat than the control sample due to starch pregelatinisation during extrusion (although with small differences), explaining the lower content of hexanal, pentanol, 1-hexanol, nonanal, 1-octen-3-ol, 2-ethyl-1-hexanol, 2-(*E*)-nonenal and 2,4-(*E*,*E*)-decadienal in batter made with flour 3. However, when comparing the LOx between the different fried batters containing extruded flours, flour 3 presented the highest amount of all the LOx, which is justified by the highest content in LOx of the corresponding unfried batter (Table 2). Concerning to the Maillard reaction volatile compounds, it would be expected that the highest content of pyrazines in the control sample would lead to a darker colour of the fried control batter. Pyrazines have been reported as important Maillard compounds in bread crust (Paraskevopoulou, Chrysanthou, & Koutidou, 2012), that should contribute to its colour (Cho & Peterson, 2010). However, it was fried batter containing extruded flour, which presented the darkest colour (Table 1), due to the starch dextrinisation and the release of free sugars. In view of these results, the content of the furan derivatives (furfural, 5-methylfurfural, furfuryl alcohol) was higher in fried batter with flour 3, which can be generated by Maillard reactions but also by caramelisation (Ait Ameur et al., 2008) and, hence, can also contribute to the colour of the heated food (Hofmann, 1998). Concretely, furfuryl alcohol has been reported to polymerise in acidic conditions to aliphatic polymers that give a brown colouration to the bread (Okaru & Lachenmeier, 2017).

In conclusion, the extrusion process resulted in fried batters that should present lower fatty and rancid notes (Pico et al., 2015) due to their lower amount of LOx and fat absorption. There was also a decrease in the content of pyrazines of the fried batter with extruded flour, thus, its darkest colour should be due to the highest content in furfuryl alcohol from Maillard reactions and caramelisation.

3.6. Consumer testing

Sensory evaluation of control batter and batters made with 15% extruded flour incorporation are presented in Table 3. The nuggets coated with batters containing severely treated extruded flours (flour 2 and 3) were found to have a significantly better appearance than the control, which can be related to their darker colour and greater thickness of the coating (higher batter pick-up). Regarding the odour and taste of the batters, although volatile compounds analyses demonstrated aroma differences between batters these differences were not enough to promote significant changes in consumer perceptions since no significant differences were found for these parameters in any of the samples. Considering the texture, although batters made with extruded flours did not present significant differences from the control, the batter

Table 3
Effect of extruded flours on sensorial properties of battered nuggets.

Sample	Appearance	Odour	Texture	Taste	Overall acceptability
Control	5.6a ± 1.5	6.4a ± 1.4	6.6ab ± 1.5	6.7a ± 1.4	6.5a ± 1.3
Flour 1 (15%)	6.0ab ± 1.5	6.4a ± 1.4	6.8ab ± 1.5	6.9a ± 1.3	6.7ab ± 1.2
Flour 2 (15%)	6.7c ± 1.4	6.5a ± 1.4	6.9b ± 1.4	7.0a ± 1.4	7.0b ± 1.3
Flour 3 (15%)	6.4bc ± 1.7	6.5a ± 1.2	6.4a ± 1.5	6.7a ± 1.4	6.6a ± 1.3

Values ± standard deviation followed by the same letters within each parameter indicate no significant differences ($p \leq 0.05$). 15% indicates the level of extruded flour replacement in wheat flour based batters.

made with 15% of flour 2 showed a higher score than that of flour 3. These results can be related to the greater thickness of the batter made with the most severely treated extruded flour (flour 3) and to the different textural profiles of these batters. Thus, the good crispiness of batter made with flour 3 may have been masked by the higher force required to break the crust in the mouth leading to a worse evaluation of this batter compared to that containing flour 2.

All these differences may contribute to the fact that the batter with incorporation of flour 2, with intermediate extrusion treatment, was the most highly rated by panelists in terms of overall acceptability, albeit it did not significant differences with flour 1. However, it should be noted that all batters were highly rated based on the overall acceptability and the highest differences were less than 0.5 points on a scale of 1 to 9. More specifically, although significant, differences between batters made with flour 2 and flour 3 were less than 0.4, and therefore, on an industrial scale, the use of fully gelatinised flour 3 may be preferable in order to achieve a higher pick-up at the expense of a slight loss of organoleptic quality.

4. Conclusions

Results demonstrated that it is possible to use extruded flours in batter formula to obtain batters of quality that satisfy industrial needs. More specifically, partial replacement of native wheat flour by wheat extruded flours in batter formula yielded higher coating pick-up, better external appearance, and good crispy textural properties. Regarding the volatile profiles of the fried batters, the extrusion leads to a decrease in the rancid volatile compounds from lipids oxidation compared to the control sample. In addition, batters made with extruded flour showed good consumer acceptability, which, in some cases, was better than in the control sample.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.03.136.

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Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source

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ABSTRACT

Physically modified extruded flours are suitable ingredients for cold preparation of products such as creams and sauces. The rheological and textural properties and stability of model sauces prepared with wheat and rice extruded flours with three different particle size fractions were assessed. All tested sauces showed non-Newtonian, shear-thinning and thixotropic fluid characteristics. Sauces made with rice extruded flours, with lower protein and amylose contents, presented lower shear-thinning behaviour, consistency index and yield stress than wheat based sauces. Rice sauces also displayed a less compact microstructure and lower viscoelastic character but a higher resistance to freeze-thaw process. Regarding particle size, the finest flours showed lower values of consistency index, yield stress, and G′ and G″ moduli but led to higher syneresis. Overall, results proved that pregelatinised extruded flours can be used in sauces applications without heating, but their properties greatly depend on the cereal type and its particle size.

1. Introduction

Sauces are a product of high added value and represent an important part of the human diet. Essentially, the formulation of sauces includes three main ingredients: water (or milk), starch (or flour) and oil. Among the different ingredients, starch plays a key role in the rheological and textural properties obtained. Therefore, in recent years, different studies have been focused on investigating the influence of the starch type in the microstructure and rheological properties of sauces (Arocas et al., 2009a, c), the sensorial properties (Arocas et al., 2010b), or even, in their interaction with hydrocolloids (Arocas et al., 2009b), proteins (Guardeño et al., 2012; Quiles et al., 2012) and other ingredients (Arocas et al., 2010a). Furthermore, during the last decade, the hectic lifestyle that people in western countries generally has led to a growing interest in convenience foods or foods that are quick and easy to prepare. These prepared products are frequently accompanied by sauces, in which starch plays an important functionality.

In terms of preparation, sauces need to be cooked to develop their viscous properties, based on the gelatinization of starch granules. To ease the preparation and remove or reduce this heating stage, pregelatinised starches and flours can be used. Hydrothermal treatments such as extrusion or drum-drying foster the gelatinization of the starch when this is subjected to enough heat and moisture, enabling the attainment of products with higher water absorption capacity and thickening power in cold temperatures (Chiu and Solarek, 2009; Doublier et al., 1986; Hagenimana et al., 2006; Martínez et al., 2014b). Therefore, pregelatinised flours represent an alternative to starchy products allowing the preparation of sauces with no need for heating. Drum-drying treated flours have higher viscosities and less starch solubilisation than extruded flours (Doublier et al., 1986). However, extrusion is a very versatile and relatively low cost process, with less environmental impact and less space requirements than drum-drying, in such a way that this technique is being widely used in the food industry. Added to starch gelatinization, a denaturation of proteins (Camire et al., 1990) and the formation of amylose-lipid complexes (Hagenimana et al., 2006) is also promoted, which will affect the rheological properties of the pastes. Furthermore, the particle size and the type of cereal used can also influence the properties of the extruded flours (Martínez et al., 2014a, b). In those works it was demonstrated that as extrusion intensity increased (i.e. barrel temperature and moisture content), higher gelatinization of starch was produced, but a minimum barrel temperature and feed moisture content were necessary to break the starch structure and induce gelatinization phenomena. When those processing

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requirements were met, an important increase in cold water absorption capacity and swelling power of the flours was found, being those values generally higher when using coarser flours (132–200 μm). In fact, due to this ability to absorb water at low temperatures, pregelatinised extruded flours have been used to improve the hydration in bread doughs (Martínez et al., 2013), as well as fat replacements in mayonnaises (Román et al., 2015a) and cakes (Román et al., 2015b).

The rheological behaviour of emulsions, such as sauces, is a critical characteristic that must be studied as it is closely related to the sensory attributes, quality, and processing properties of a food product (McClements, 2005). Most importantly, it provides fundamental insights into the structural organization and interaction between the components within the emulsion (McClements, 2005). Moreover, the stability towards freezing is also an important aspect to consider since many convenience foods containing sauces are preserved this way so as to extend their shelf life. In general, these sauce properties have been studied on different native or modified starches. Nevertheless, to the best of our knowledge, there are no studies based on the use of pregelatinised extruded flours for sauce development.

The objective of this study was to assess the effect of extruded flours of different particle sizes on the rheological characteristics and stability towards freezing of model sauces with no need for heating. To achieve this aim, two types of extruded flours were selected, wheat flour, as it is the most commonly used cereal in developed countries, and rice flour, the most commonly used gluten-free flour.

2. Materials and methods

2.1. Materials

The extruded rice and wheat flours used in this study commercially available as Ooc 190 and Toc 190, respectively, were provided by Molendum Ingredients (Zamora, Spain). The extrusion conditions were chosen according to the manufacturer expertise in order to ensure starch gelatinization, and DSC analyses of the flours were carried out to confirm that gelatinization was complete. In this way, when flour samples were analysed, a flat DSC curve with no endothermic peak in the range of starch gelatinization was observed, indicating that gelatinization was complete. The extrusion treatment was carried out in a single screw extruder Bühler Basf (Bühler S.A., Uzwil, Switzerland). The diameter of the extruder was 140 mm, the length to diameter (L/D) ratio of the extruder was 20:1 and the screw speed was 340 rpm. The diameter of the die hole and the number of holes used was 8 mm and 18 holes, respectively. The flours were extruded at a maximum barrel temperature of 160 °C, a feed rate of 500 kg/h and a feed moisture content of 25%. The extruded products were dried by convection air until reaching 10% moisture and then ground with a compression roller (Bühler S.A., Uzwil, Switzerland) to a particle size below 200 μm. Each of the flours were sieved and separated into three fractions according to the amount of particles remaining on each sieve. The different particle sizes obtained were as follows: fine (less than 80 μm), intermediate (80–130 μm) and coarse (130–200 μm). The sieving process was performed with an Automatic Bühler MLI300B Sieve (Braunschweig, Germany) for 15 min. Each of the fractions was stored separately in sealed plastic bags until their analysis and use. Particle size of the flours was measured in duplicate with a laser diffraction particle size analyser (Mastersizer 3000, Malvern Instruments, Ltd., Worcestershire, UK) equipped with an Aero S unit. The mean diameter of equivalent volume D (4,3), which indicates the central point of the volume distribution of the particles, was recorded. For wheat extruded flours, D (4,3) values were 72.4 μm, 146.0 μm and 279.5 μm for fine, intermediate and coarse fractions, respectively. D (4,3) values of rice flours were 78.5 μm, 151.0 μm and 213.5 μm for fine, intermediate and coarse fractions, respectively.

2.2. Methods

2.2.1. Flour characterisation

The characterisation of the extruded flours was carried out before the preparation of the sauces. Flours were analysed following AACC Methods (AACC, 2012) for moisture, method 44–16.01 and protein method 46–30.01 with a Leco TruSpec device (Leco, St. Joseph, MI, USA). Amylose-amylopectin content was measured using Concanavalin A precipitation method (K-AMYL, Megazyme International, Wicklow, Ireland). Determinations were carried out in duplicate.

Water binding capacity (WBC) defined as the amount of water retained by the flour after it has been subjected to centrifugation was measured as described by method 56.30 (AACC, 2012). After centrifugation, the supernatant was decanted into an evaporating dish and the residue of the eppendorf tube was weighed (Wr). The weight of dry solids in the supernatant was recovered by evaporating the supernatant at 105 °C till constant weight. Water solubility index (WSI) was calculated as the amount of dry solids recovered in the supernatants divided by the initial sample weight in percentage (g/100 g). Determinations were carried out in triplicate.

Pasting properties of flours were analysed using the standard method (AACC, 2012), (AACC 61–02.01) with a Rapid Visco Analyser (RVA-4) (Newport Scientific Pty Ltd., Warriewood, Australia) controlled by Thermocline software (Newport Scientific Pty. Limited, Warriewood, Australia) for Windows. All flours were run in duplicate.

2.2.2. Sauces preparation

Model sauces were prepared using flour (16.78%), sunflower oil (2.68%) and water up to 100%. It is noteworthy than extruded flours have less thickening power than the native ones after cooking (Martínez et al., 2014a, b), so it has been necessary to increase the flour content of the sauces to obtain a texture similar to conventional sauces. All the ingredients were placed in a food processor (Thermomix TM31, Wuppertal, Germany), and mixed at 1100 rpm and 30 °C for 10 min. The resulting sauces were placed in glass beakers, covered with plastic film. Two sauces were prepared for each of the particle sizes of the two flours studied. In order to be able to study freshly prepared sauces, measurements were performed within the same day.

2.2.3. Sauces characterisation

2.2.3.1. Optical microscope observation. Sauces were examined with a DM750 microscope (Leica Microsystems, Wetzlar, Germany) with 20× times magnification, fitted with an EC3 video camera. Images were captured with LAS-EZ software (Leica Microsystems, Wetzlar, Germany). First of all, the sample was placed on a glass microscope slide and covered with a cover slip. The slides were compressed under a 1 kg weight for 2 min to create a sample layer of uniform thickness. Micrographs were performed on the freshly prepared sauces at least twice in two random points of each sample.

2.2.3.2. Rheological analysis. Rheological properties of sauces were determined with a Thermo Scientific Haake RheoStress1 rheometer (Thermo Fisher Scientific, Schwerte, Germany) and a Phoenix II P1-C25P unit to control the temperature, which was fixed at 30 °C for all analyses. All tests were monitored with RheoWin 4 Job Manager software and rheological parameters were calculated with Rheowin 4 Data Manager software (Thermo Fisher Scientific, Schwerte, Germany). All rheological measurements were performed twice on each freshly prepared sauce.

2.2.3.2.1. Flow behaviour. The flow behaviour measurements of sauces were carried out in control rate mode with a concentric cylinder system (Z34 DIN Ti). The shear rate was increased linearly from 1 to 100 s^{-1} for 200 s (up curve), then this shear rate (100 s^{-1}) was maintained for 150 s and was finally reduced to 1 s^{-1} in a further period of 200 s (down curve). This measurement is useful since it simulates the forces that food products (such as sauces) might experience during preparation and consumption, such as stirring, pouring, and mastication (Chung et al., 2013). Furthermore, this shear rate is within the range typically used to simulate oral conditions (McClements, 2005). The data of the up curve was adjusted to the Herschel-Bulkley equation since it was the best fit to the experimental data (R^2 equal to or higher than 0.99):

$$\sigma = \sigma_o + K(\dot{\gamma})^n$$

Where σ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency index ($Pa \cdot s^n$), σ_o is the critical shear stress or yield stress (Pa) and n is the flow behaviour index. Moreover, the "relative thixotropic area" was calculated as the area between the rising and falling curves divided by the area under the up curve, which allows a correct comparison of the rate of internal structural breakdown in systems with different viscosities (Dolz et al., 2000).

2.2.3.2.2. Viscoelastic behaviour. The viscoelastic properties of sauces were measured with serrated titanium parallel plate with a diameter of 60 mm and a gap distance between plates of 1 mm. The sample surface exposed to air between the plates was covered with Panreac vaseline oil (Panreac Química SA, Castellar del Vallés, Spain) to prevent moisture loss. In order to determine the minimum resting time allowed to aid structure recovery and temperature equilibration in further analysis, a time sweep within the linear viscoelastic region (at a stress value within the linear viscoelastic region from the stress sweep) for 1800 s was carried out. After the time sweep, a resting time of 600 s was selected for all rheological tests. The linear viscoelastic region was determined by performing a stress sweep (0.01–10 Pa) at a frequency of 1 Hz. Subsequently, a dynamic frequency sweep was executed using a shear stress value within the linear viscoelastic region (previously established by the stress sweep), over a frequency range between 10 and 0.1 Hz. Viscoelastic parameters, such as storage modulus (G') and loss modulus (G''), were acquired. G' represents the energy stored in the material or that which is recoverable per cycle of deformation while G'' is a measure of the energy which is lost as viscous dissipation per cycle of deformation.

2.2.3.3. Syneresis. Syneresis was assessed on the freshly prepared sauces on the same day and after storing the samples at −18 °C for 24 h following the method of Heyman et al. (2010) with modifications. Samples of 15.00 g (W_0) of sauce were placed in falcon centrifuge tubes (50 ml) and centrifuged for 15 min at 4000 × g. The separated liquid was then removed and weighed (W_1). Syneresis was expressed as weight percent of the decanted liquid phase.

$$\text{Syneresis} = (W_1/W_0) \times 100$$

To perform the measurement after freezing, samples were gradually thawed at room temperature during 2 h. Syneresis was carried out in duplicate for each of the sauces.

2.2.4. Statistical analysis

The obtained data was subjected to a one-way analysis of variance (ANOVA) using the LSD Fisher test ($p < 0.05$). All analyses were performed with Statgraphics Centurion XVI software (StatPoint Technologies Inc., Warrenton, USA).

3. Results and discussion

3.1. Flour characterisation

Moisture, protein and amylose content of the different extruded flours are displayed in Table 1. In terms of moisture content, barely any significant differences were observed between the different flours studied and among the different particle size fractions of the rice samples, but for wheat flours slightly higher values of moisture content were detected for the finest particle sizes compared to the coarsest fraction. These small differences are due to the fact that the flours were previously dried to levels of 10% moisture content after being extruded.

Regarding amylose content, wheat flours presented more amylose than rice flours for each of the particle size fractions studied. Despite amylose content depends on the different varieties, amylose content is usually higher in wheat starches compared to rice starches (Waterschoot et al., 2015). Meanwhile, the intermediate particle size of both types of flour presented the lower values of amylose content. On the contrary, the protein content was significantly different for each type of flour and its particle size. In general, the protein content is higher for wheat flours compared to rice flours (Delcour and Hoseney, 2010). It is important to highlight the high protein content of the finest fraction of wheat flour (21.04 g/100 g) in comparison to the rest of the flours. The finest fraction of rice flour also presented the highest values of protein compared to the other two particle sizes. de la Hera et al. (2013) and Kim and Shin (2014), when assessing the protein content of the rice flour, obtained opposite results, reporting an increase in the protein content when the particle size was increased. However, the flours studied in those articles were not subjected to extrusion treatment. During extrusion treatment the gelatinization and breakage of the starch granules as well as protein denaturation takes place, and the flour components are mixed together. In addition, flours were remilled after extrusion, thus, being able to alter the distribution of these components as a function of particle size. In this way, both protein denaturation during extrusion and further milling may have contributed to the increase in the amount of protein in the finest fractions. This fact may suggest that the weaker fractions of the extruded product, and therefore, the particles which break into smaller fragments, are those with a higher protein content and lower starch content.

Regarding water binding capacity of the flours, no great differences were observed between the extruded flours. Nevertheless, fine wheat presented the significantly lowest WBC value compared to the rest, which is likely to be attributed to its high protein content. Although proteins exhibit greater water absorption capacity than native starches (Manley, 2000), the extrusion process increases flours capacity to absorb water up to more than 500% due to starch gelatinization (Martínez et al., 2014a). Therefore, higher protein content, and consequently lower starch content in this type of flours will reduce its water absorption capacity. WSI indicated that wheat extruded fractions were less soluble in water than rice counterparts which would suggest that rice flour was more degraded during extrusion treatment. In agreement with these results, Chinnaswamy and Hanna (1990) found that the more degraded samples after extrusion demonstrated higher solubility values and that water solubility increased with decreasing amylose contents.

Table 1
Composition, water binding capacity and solubility of extruded flours.

Sample	Moisture (g/100 g)	Amylose (g/100 g)	Protein (g/100 g)	WBC (g/g)	WSI (g/100 g)
Fine wheat	9.76b ± 0.11	28.11d ± 0.31	21.04f ± 0.04	5.10a ± 0.04	19.94c±0.30
Intermediate wheat	9.15ab ± 0.08	24.30c ± 0.42	7.00a ± 0.05	6.11bc ± 0.03	12.92b ± 0.38
Coarse wheat	9.05a ± 0.10	27.63d ± 0.41	8.84d ± 0.09	6.44c ± 0.00	7.83a ± 0.88
Fine rice	9.85b ± 0.08	20.55ab ± 1.50	9.33e ± 0.02	6.07b ± 0.06	20.92cd ± 0.40
Intermediate rice	9.62ab ± 0.05	17.86a ± 0.04	7.54c ± 0.01	6.14bc ± 0.16	21.87cd ± 0.70
Coarse rice	9.57ab ± 0.47	22.50bc ± 1.40	7.40b ± 0.05	6.38bc ± 0.20	22.82d ± 1.33

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. WBC= Water binding capacity, WSI= Water solubility index. Fine, intermediate and coarse fractions correspond to particle sizes of 0–80 µm, 80–130 µm and 130–200 µm, respectively.

3.2. Pasting behaviour of flours

The pasting behaviour of the flours studied is depicted in Fig. 1a and b. All the samples exhibited very similar pasting profiles, with a cold peak viscosity before heating as a result of the pregelatinisation that occurred during extrusion process. These pasting profiles were similar to those obtained in other studies with extruded flours (Hagenimana et al., 2006; Martínez et al., 2014a, b). Extruded flours, as opposed to native flours, have the ability to absorb water and thicken at low temperature (Wolf, 2010). The peak viscosity provides a measure of the thickening power of the starch (Sanz et al., 2016). In general, wheat flours presented higher viscosity peaks than rice flours, which could be related to the higher solubility in water of rice extruded flours and also to the different starch structure of both flours. For both wheat and rice flours, the time needed to reach the peak viscosity was inversely related to the particle size, since fine flours needed less time to reach the peak than intermediate or coarse flours. This fact indicates that with great ease the fine particle sizes are hydrated, being associated with their higher surface to volume ratio. Fine flours also presented lower peak values compared to the other particle sizes, which may be to do with their higher protein content, and hence, lower starch content, being responsible for the peak viscosity. Furthermore, in the case of wheat extruded flours it is noteworthy that the cold peak viscosity was significantly higher for the intermediate fraction which could also be linked to its lower protein content (i.e. more pregelatinised starch able to absorb water) compared to the other particle size fractions.

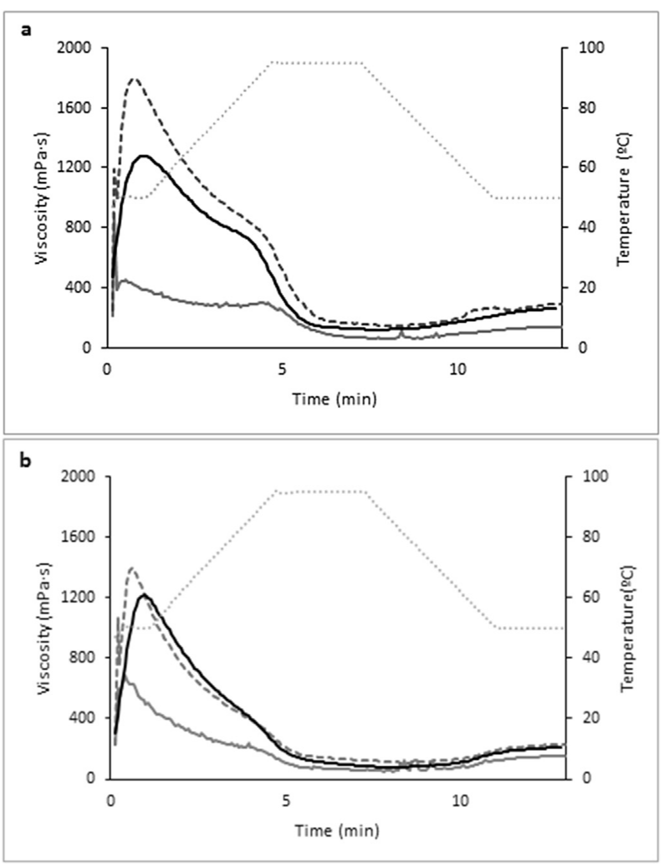


Fig. 1. Pasting profiles of wheat (a) and rice (b) flours with coarse (black continuous line), intermediate (grey broken line) and fine (grey continuous line) particle sizes used in sauces making. Pointed line indicates the temperature profile. Fine, intermediate and coarse fractions correspond to particle sizes of 0–80 µm, 80–130 µm and 130–200 µm, respectively.

After the cold peak was reached, a decrease in viscosity was produced when heating followed by a slight increase in viscosity during cooling. The retrogradation trend was scarcely observed during cooling, somewhat fostered during cooling after previous extrusion. Doublier et al. (1986) had already correlated the reduction in final viscosity and setback with the fragmentation of the amylose chains during extrusion, which might lose the ability to associate and retrograde during cooling. Furthermore, this slight increase in viscosity during the cooling stage should be mostly associated with the increase in viscosity linked to temperature reduction (Sanz et al., 2016). The viscosity profile after the peak was similar for all the rice flours, with no differences with regards to particle size. Conversely, for wheat flours, a lower viscosity profile was evident for the fine particle size, which may be related to its remarkably high protein content and then, low starch content, leading to a poorer thickening ability.

3.3. Microstructure of sauces

The microstructure of the sauces showed in Fig. 2 consisted of a network which was mainly composed of protein and the starch components forming the continuous phase, and some oil droplets immersed in the network. Similar structures were found in the microstructure of all the sauces regardless of the particle size used. Regarding the type of flour used for sauce making, it seemed that wheat sauce particles (a, b, c) were more aggregated forming a tightly packed network structure whereas the particles in rice sauces (d, e, f) seemed more dispersed giving rise to a less cohesive structure with some voids and apparently lower number of small oil droplets (see arrows). Guardeño et al. (2012) reported similar results for sauces made with the same amount of oil content. These authors observed that rice starch based sauces showed a greater degree of dispersion in the continuous phase, less capacity to swell and lower tendency to aggregate than corn starch sauces. Therefore, this less aggregated structure of rice sauces is expected to bring about a lower viscosity.

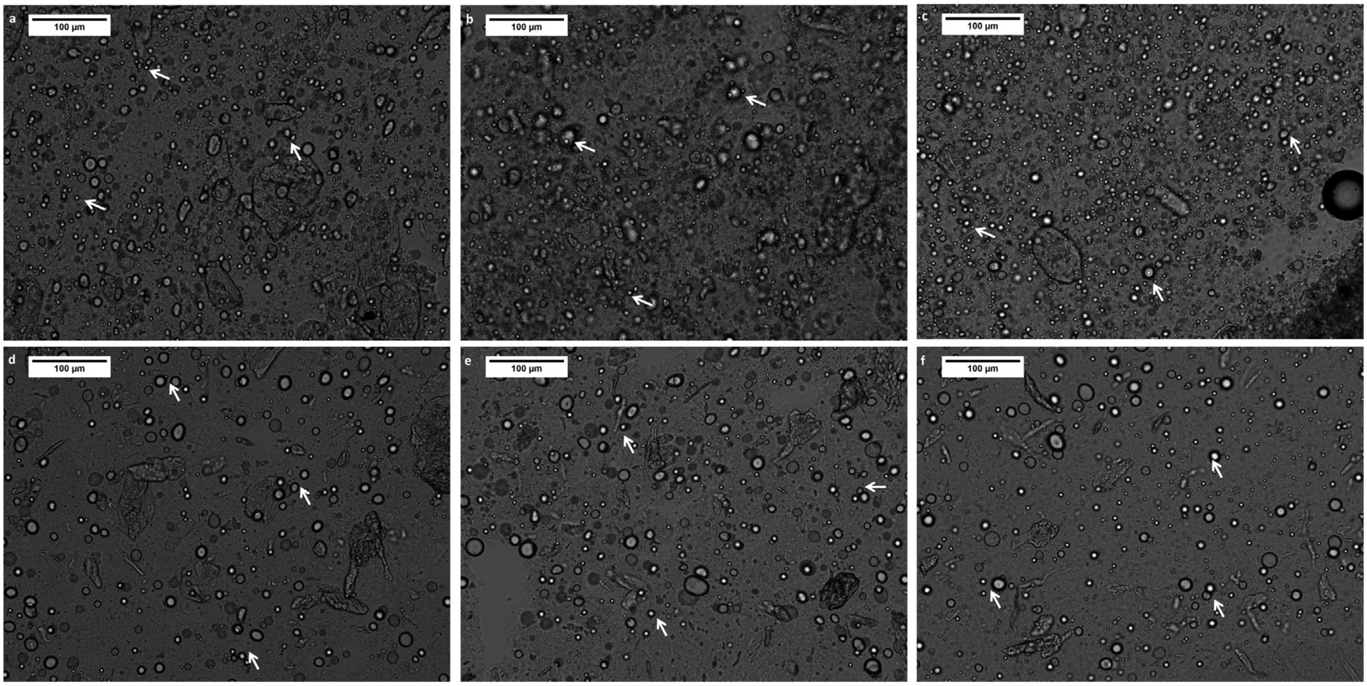


Fig. 2. Microphotographs of sauces made with wheat (a, b, c) and rice (d, e, f) flours with fine (a, d), intermediate (b, e) and coarse (c, f) particle sizes. The bar in the upper right side of each picture indicates a 100 µm size. White arrows indicate oil droplets. Fine, intermediate and coarse fractions correspond to particle sizes of 0–80 µm, 80–130 µm and 130–200 µm, respectively.

3.4. Flow behaviour

Herschel-Bulkley model was used to characterise the flow behaviour of sauces (Table 2). All the sauces exhibited non-ideal plastic behaviour, with a critical shear stress below which no flow was observed. Above this critical shear stress, all the sauces exhibited a non-Newtonian shear-thinning behaviour ($n < 1$) in which the viscosity decreases as shear rate increases. Shear-thinning behaviour is characteristic for starch gels and more specifically for starch based sauces (Heyman et al., 2010; Mandala et al., 2004). The observed shear-thinning behaviour of sauces may be attributed to structural breakdown and evolution of the particle's alignment in the direction of flow followed by the decrease in physical interactions between adjacent polymer chains (Hosseini-Parvar et al., 2010). A greater shear-thinning behaviour was found with wheat sauces, with lower values of n as the particle size of the flour decreased. However, no differences were observed for the different particle sizes of rice flours. An increase in shear-thinning character indicates that more mutual entanglements were formed in the emulsion (Ma and Boye, 2013). A strong negative correlation ($r = -0.850$; $p < 0.001$) was found between this parameter and amylose content of the samples. The increase in shear-thinning character with increasing amylose content was generally attributed to an increase in entanglements between amylose chains, since the highly branched amylopectin was not expected to form effective entanglements (Willett et al., 1995). This theory is confirmed by the micrographs of the sauces previously explained, where wheat sauces, with higher amylose content, demonstrated a more compact structure.

The consistency coefficient K can be considered as a measure of the resistance to flow (Heyman et al., 2010). Consistency index was significantly higher for wheat sauces compared to rice ones, indicating a stronger structure of the sauce. In actual fact, other authors (Guardeño et al., 2012; Quiles et al., 2012) have related the increase in viscosity and consistency of sauces with the number and interaction or aggregation of the sauce components, in such a way that larger aggregates can be associated with higher viscosities. Mandala et al. (2004) also stressed that the resistance to shearing forces is increased as macromolecules' entanglements increase. Regarding the particle size of the sauces, the finest flours led to the lowest values of K, but significant differences were only observed between the fine and the two coarsest fractions of wheat sauces.

The yield stress of a product is defined as the stress above which a material starts to flow. All sauces demonstrated yield stress (σ_o), which is perceived to be particularly important for salad dressings because it allows the retention of their ability to keep non-fluid on

Table 2
Parameters of Herschel-Bulkley equation, relative thixotropic area and freeze-thaw syneresis for the studied sauces.

Sample	K ($Pa \cdot s^n$)	n	σ_o (Pa)	Thixotropic area (%)	Freeze-thaw syneresis (%)
Fine wheat	16.43b ± 1.73	0.380a ± 0.021	61.22d ± 1.38	34.46b ± 1.05	22.55d ± 3.64
Intermediate wheat	26.67c ± 2.58	0.388ab ± 0.021	121.85f ± 2.28	43.19b ± 4.17	14.14c ± 3.31
Coarse wheat	22.48c ± 1.13	0.414b ± 0.008	109.83e ± 8.03	39.64b ± 3.79	19.14d ± 1.34
Fine rice	4.92a ± 0.43	0.578c ± 0.017	16.74a ± 1.73	18.85a ± 1.01	6.27b ± 0.94
Intermediate rice	6.37a ± 0.28	0.583c ± 0.010	26.42b ± 0.93	19.24a ± 0.97	2.13a ± 0.43
Coarse rice	7.96a ± 0.39	0.564c ± 0.008	32.74c ± 1.28	21.48a ± 1.58	2.15a ± 0.56

Mean values followed by the same letter in each column are not significantly different at $p \leq 0.05$. K = consistency index, n = flow behaviour index, σ_o = critical shear stress. Fine, intermediate and coarse fractions correspond to particle sizes of 0–80 µm, 80–130 µm and 130–200 µm, respectively.

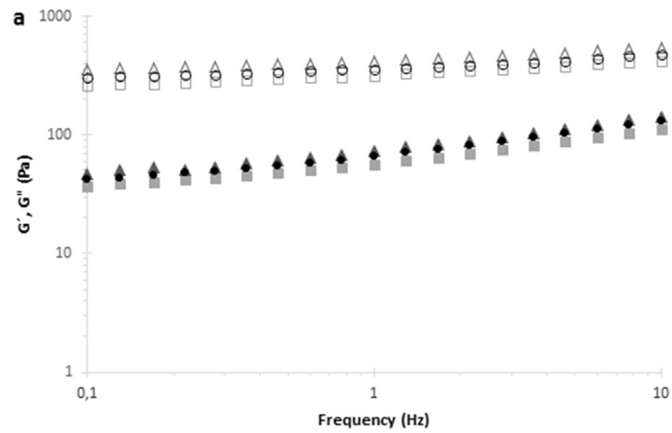
the salad surface (Liu et al., 2007). Yield stress or critical shear stress was also significantly higher for each of the particle sizes of the wheat sauces in comparison to rice sauces, following the trend observed by the consistency index. For other low-fat sauce model systems, Chung et al. (2013) found that suspensions containing thermally processed corn starch exhibited a critical shear stress around 25 Pa, similar to those obtained for rice extruded flours. Taking into account the different particle sizes, rice based sauces revealed that the yield stress significantly increased as the particle size increased also, suggesting that the intermolecular interactions in these samples might be stronger. Similar behaviour was observed for wheat sauces, with the finest particle size presenting the lowest value, but in this case the intermediate fraction presented the highest value. Therefore, it seems that finest particle sizes of the flours gave rise to weaker sauces, as the lowest values for consistency index and yield stress demonstrated. According to these results, Quemada (1988) pointed out that large particles occupy more space during aggregation than small particles, due to less efficient particle packing which leads to a higher volume occupation by these large particles, and they also give rise to an increase in flow resistance.

Time dependency of the sauces was evaluated by determining the relative thixotropic area. The results retrieved for the thixotropic area highlight that wheat samples displayed a greater thixotropic behaviour compared to rice samples, with no differences depending on the particle size. The lower value of relative thixotropic area of rice sauces indicates a more stable structure in response to agitation (Sanz et al., 2016), being much less sensitive to shearing. These results might be explained by the different microstructure of the sauces, indicating that, after removal of shear force, more time was necessary to rebuild the original structure of the more complex wheat sauces.

3.5. Viscoelastic behaviour

Oscillatory spectra of all the studied sauces were very similar to those obtained for other heated white sauces (Arocas et al., 2009c; Mandala et al., 2004). The mechanical spectra in Fig. 3a and Fig. 3b showed the existence of solid-like gels, with values of G′ higher than those of G″ (see also Supplementary material 1) and with both moduli, but mainly G″, depending on the frequency throughout the frequency range measured. Heyman et al. (2010) also reported that G″ always appeared to be more frequency dependent than G′ in all the sauces studied, a typical trait of weak gels.

Rice sauces presented lower values for both G′ and G″ moduli compared to wheat based sauces, and a greater dependence on frequency. Sanz et al. (2016) related this behaviour to the poorer thickening ability of the starch described in the pasting properties. In this way, the lower values of the moduli for rice sauces are coherent with the lower cold peak viscosity observed in the pasting profile. Then, a poorer thickening power was found for rice flours in comparison to wheat flours. Furthermore, rice sauces revealed a clearly less elastic behaviour than wheat ones, with values of G′ only slightly higher than those of G″. The smaller differences between the two moduli in rice sauces may indicate they were closer to the crossover, after which the liquid-like behaviour starts to be predominant, also denoting a less rigid structure. Karaman et al. (2013) reported that amylopectin fraction softens the structured behaviour, giving rise to lower values of G′ compared to those which can be found in systems composed of starches with a greater amylose content. In this way, the greater amylose content in wheat flours than in rice flours most likely could have contributed to their greater elastic properties, since statistical correlations at 99.9% level were found for amylose and G′ and G″ ($r = 0.788$ and $r = 0.765$, respectively). Lu et al. (2009) also highlighted that gels with higher



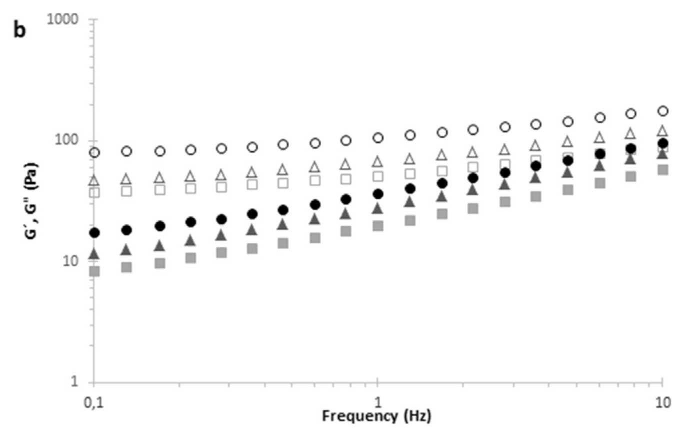


Fig. 3. Storage (unfilled symbols) and loss (filled symbols) moduli as a function of frequency for wheat (a) and rice (b) sauces made with coarse (circle), intermediate (triangle) and fine (square) particle sizes. Fine, intermediate and coarse fractions correspond to particle sizes of 0–80 μm, 80–130 μm and 130–200 μm, respectively.

amylose content showed more independence from frequency, and the storage modulus became higher for higher amylose content samples.

In terms of particle size, for both moduli there were no great differences among the different fractions of wheat sauces, indicating the higher protein content did not affect the finest flour. On the contrary, for rice flours, as particle size increased the values of the moduli also increased, suggesting more interaction among the structural components of the sauces. This trend is also in concordance with what was reported by Sanz et al. (2016) since a greater thickening ability was found for coarser particle sizes.

3.6. Syneresis of sauces

One of the main phenomena observed after the freeze/thaw process is the appearance of syneresis, or released water, from the sauce structure which is, actually, a very negative quality factor. This water release negatively affects some texture attributes and a certain lack of homogeneity or a grainy mouth feel could be found (Ferrero and Zaritzky, 2000; Navarro et al., 1997). No water release was observed in any of the freshly prepared sauces (data not shown) suggesting a great initial stability of the sauces which can be associated with the high water absorption capacity of the pregelatinised starch presented in the extruded flours (Wolf, 2010). On the contrary, syneresis occurred after freeze/thaw process for all the sauces studied (Table 2). These results are consistent with those reported by Arocas et al. (2009a) in which no syneresis was found on fresh white sauces, but water was indeed released after freeze-thaw process. Rice flours led to higher freeze-thaw stability of their sauces, with values of syneresis significantly lower than those of wheat sauces for each of the particle sizes. Meanwhile, the finest particle size presented the highest values of syneresis for both types of flours studied. Rice sauces presented no significant differences among the two coarsest particle sizes, with the lowest water release. Nevertheless, for wheat sauces, the significantly lower values of syneresis were obtained for the intermediate particle size. Therefore, it seems that the larger volume occupation of coarser particles or the more reduced superficial area may have helped to avoid water release. Arocas et al. (2009a) related the syneresis of the sauces to the viscoelastic properties of the starches used in their study, being the syneresis greater for the starches with higher G′ and G″ values. This could explain the greater syneresis found for wheat sauces compared to rice ones, but not the differences between the different particle sizes, which would confirm that the internal structure of the sauces also play an important role on the stability.

4. Conclusions

This study assessed the suitability of physically gelatinised extruded flours for the making of sauces with no need of heating. The results showed that it is possible to obtain model sauces with similar rheological properties to those obtained in other sauces which were heated to develop their viscous properties. Nevertheless, it is noteworthy that the finest particle sizes are not preferable for sauce making due to their weaker rheological behaviour and higher syneresis. Results also suggested that wheat flours give rise to sauces with a more tightly packed network structure, while a more disperse and less cohesive structure is shown when using rice flours. These different structures greatly influenced the rheological properties of the sauces, leading to higher consistency and viscoelastic character for wheat based sauces, which will be reflected as an increase in the sensory consistency and pumping requirements. On the other hand, rice flours are less sensitive to shearing and release less water after freeze-thaw process.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfoodeng.2017.09.024.

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Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source

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Supplementary material

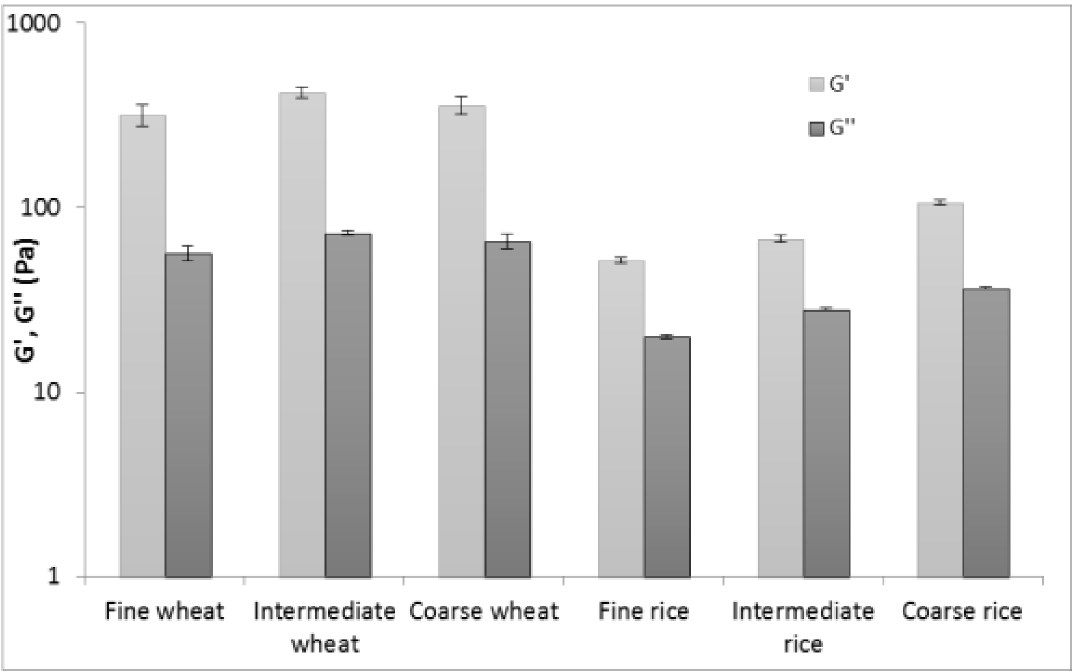

Fig S1. Effect of extruded flours on storage (G') and loss (G'') moduli of studied sauces. Storage and loss moduli were obtained at 1 Hz frequency within the linear viscoelastic region

CHAPTER 3

Exploiting the potential of extruded flours to reduce gel hardening in starch-based foods

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Shear scission through extrusion diminishes inter-molecular interactions of starch molecules during storage

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Abstract

The starch retrogradation behavior of native and extruded maize, wheat and rice flours were compared to better understand the efficacy of extrusion to decrease the propensity of different starches to form inter-molecular interactions. In all flour pastes, the increase of the storage modulus (G') during a 7-day storage (representing inter-molecular interactions) was diminished with extrusion, albeit this effect was not detectable in rice due to the low gel consistency. Furthermore, thermo-rheological analyses revealed two distinct mechanisms depending on starch composition and structure. In maize and wheat flours, AM fragmentation through extrusion (detected through HPSEC) decreased the residual G' at 85 ^{o}C of 7-day stored gels, which represents AM-AM physical junction zones that build gel structure. On the other hand, the extrusion of rice flour, which contains starch with lower AM ratio, resulted in a marked AP fragmentation that caused a decrease in relative G' drop, representing inter-molecular interactions involving AP.

Keywords: Extrusion; flour; starch; retrogradation; texture; amylopectin

1 Introduction

Retrogradation has been characterized as the process by which dispersed starch molecules in an aqueous medium begin to re-associate and form three-dimensional network structures. Some studies of gelled mixtures of amylose (AM) and amylopectin (AP) have used the idea of phase separation of AM and AP, indicating that mixture behavior was based on simple combination of the retrogradation ability of each component (Kalichevsky & Ring, 1987; Leloup, Colonna & Buleon, 1991). On the other hand, others authors have suggested that interactions between AM and AP could contribute to the gel properties of their mixtures (Boltz & Thompson, 1999; Jane & Chen, 1992; Klucinec & Thompson, 1999, 2002; Parovuori, Manelius, Suortti, Bertoft & Autio, 1997). More specifically, Klucinec and Thompson (2002) described starch gels as a combination of internal elements, terminal elements, network linkages, and terminal linkages, based on the model for elastic networks of vulcanized rubber reported by Flory (1953), who recognized that some chains may not contribute to the elasticity of a gel network. In this way, only internal elements are responsible for gel structure (and its mechanical properties), which may be formed through the development of AM-AM, AM-AP, and AP-AP inter-molecular associations, also known as physical junction zones (Klucinec & Thompson, 2002). Klucinec and Thompson (1999) reported that these inter-molecular interactions can be categorized in two types, namely inter-molecular double helices (type I junction zones) or inter-molecular aggregation of intra-molecular double helices (type II junction zones). The density and stability of the inter-molecular junction zones determine the mechanical properties of the network structure of starch gels, and therefore, of starch-based foods. In this way, the extent of starch gelation extensively controls the quality of many food products, such as bread staling, custard syneresis, rice stickiness, etc.

Gelation depends on factors such as the AM ratio (described as AM/AP ratio), AM and AP size and AP fine structure. In particular, weaker gels (less dense/abundant AM-AP and AP-AP inter-molecular interactions with adjacent molecules) were attained with: 1) AP with shorter branches (Jane & Chen, 1992; Klucinec & Thompson, 2002; Kohyama, Matsuki, Yasui & Sasaki, 2004; Matalanis, Campanella & Hamaker, 2009); 2) larger AP molecules and AP molecules with shorter external chains or a higher proportion of these short chains (Klucinec & Thompson, 2002) and; 3) AM of either short (530 DP) or long length (1500 DP) (Jane & Chen, 1992). However, the effect of a size reduction of AM and AP preserving AP fine structure (smaller starch molecules from the same plant source) on the mechanical properties of starch gels is not clear and may vary among starches.

Extrusion cooking is a physical process combining high temperature with a large amount of mechanical energy, which can be used to promote structural changes on starch, including gelatinization, melting and fragmentation (Hagenimana, Ding & Fang, 2006; Martinez, Calviño, Rosell & Gómez, 2014; Martinez, Rosell & Gómez, 2014). Despite the fact that some studies have investigated the effect of extrusion on starch molecular degradation (Li, Hasjim, Xie, Halley & Gilbert, 2014b; Liu, Halley & Gilbert, 2010; Zhang et al., 2015), to the best of our knowledge there is no research conducted on the propensity of fragmented molecules during extrusion to form inter-molecular interactions during storage (retrogradation). In this study, we hypothesized that the starch fragmentation during the extrusion at a fixed specific mechanical energy (SME) would depend on the starch source, resulting in noticeable differences in inter-molecular aggregation during storage, and therefore, to different outcomes in gel development. This would also give fundamental understanding about some of the reasons for the lower bread staling found in breads made with extruded flours (Martinez, Marcos & Gomez, 2013).

In this work, three flours, wheat, maize and rice, were industrially extruded (SME ~ 300 kJ/kg) to obtain commercially available extruded flours with fragmented starch from different origins (as measured by HPSEC). Native and extruded flours were analyzed for gelatinization, retrogradation and gelation properties over 7 days storage using differential scanning calorimetry (DSC) and small amplitude oscillatory shear rheometry (SAOS).

2 Materials and Methods

2.1 Materials

Native maize (32% AM content and 5.4% protein content), rice (20% AM content and 7.4% protein content), and wheat (26% AM content and 9.0% protein content), flours were supplied by Molendum Ingredients (Zamora, Spain). Extrusion of flours was industrially carried out by Molendum Ingredients in a twin screw extruder Bühler Basf (Bühler S.A., Uzwil, Switzerland). Three commercially available extruded flours from maize (ZOC 190), rice (OOC 190) and wheat (TOC 190) were obtained for this study. The length to diameter (L/D) ratio for the extruder was 20:1. The three flours were extruded to achieve a similar specific mechanical energy (SME) of ~ 300 kJ/kg. Maize flour was extruded at a maximum barrel temperature of 160 °C, a feed rate of 500 kg/h and a screw speed of 453 rpm, with a further water addition of 12%. Rice flour was extruded at a maximum barrel temperature of 140ºC with a feed-rate of 500 kg/h, a screw speed of 340 rpm and feed moisture content of 25 %. Wheat flour was extruded at a maximum barrel temperature of 160 °C, a feed rate of 500 kg/h, and a screw speed of 340 rpm and feed moisture content of 10 %. After extrusion, the product was dried by convection air and then ground with a compression roller to a particle size below 200 µm. AM content was measured in duplicate using Concanavalin A precipitation method (K-AMYL, Megazyme International, Wicklow, Ireland). Protein content was measured according to AACC 46–30.01 method (AACC, 2015) with a Leco TruSpec device (Leco, St. Joseph, MI, USA). The pasting profiles of native and extruded flours (supplementary material 1) was obtained following the standard method 61-02.01 (AACC, 2015) with a Rapid Visco Analyser (RVA-4) (Perten Instruments Australia, Macquarie Park, Australia).

2.2 Methods

2.2.1 Size-exclusion chromatography

For molecular size distribution of starch, protein was first removed with protease and sodium bisulfite solution (Tran et al., 2011). The treated flour was then dissolved in DMSO-0.5% (w/w) LiBr solution (DMSO/LiBr), and remaining non-starch polysaccharide components then removed by precipitating starch using 6 volumes of ethanol followed by centrifugation at 4000 g for 10 min. The precipitated starch was collected and re-dissolved in DMSO/LiBr at 80 ºC overnight. The molecular size distribution of cereal starches was analyzed in triplicate using an HPLC system (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector (RID, RID-10A, Shimadzu, Kyoto, Japan) following the protocol given by Tran et al. (2011). Size separation of branched starch molecules was carried out using GRAM 30 and GRAM 3000 columns (PSS) at 80 ºC. A series of pullulan standards with molecular weights ranging from 342 to 2.35×10^6 were used for calibration, allowing elution volume to be converted to hydrodynamic volume (V_h, or the corresponding radius, R_h) using the Mark–Houwink equation (Vilaplana & Gilbert, 2010). The size distribution was plotted as SEC weight distribution, $w(\log V_h)$, derived from RID signals, against hydrodynamic radius, R_h.

2.2.2 Thermal properties of native and retrograded starches

Analyses were performed in a differential scanning calorimeter Q-2000 (TA instruments, Crawley, UK) equipped with a refrigerated cooling system (RCS 40). Prior to sample measurements, the calibration for enthalpy and temperature was completed using indium (T_{onset} = 155.74 ºC and ΔH =28.69 J/g). Tzero high volume DSC pans (TA-Instruments) were employed. An empty pan was used as reference and dry nitrogen at a flow rate of 50 mL/min, was used as the purge gas. Flour (12 mg) was loaded into the aluminum pan and distilled water (20 µL) was added with the help of a micro syringe. Samples were hermetically sealed and allowed to equilibrate for 60 min at 30ºC before heating in the DSC oven. Samples were heated from 30 to 100 ºC at 10 ºC/min. Pans were rapidly cooled down to room temperature and then subjected to the following temperature cycle: 4 ºC for 24 h followed by 30 ºC for 24 h. This cycle was repeated for 7 days. After 7 days samples were rescanned from 20ºC to 180ºC at 5ºC/min. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), temperature range (T_c-T_o), as well as the enthalpy (ΔH) (expressed as J/g of sample in dry basis) of starch gelatinization (day 1) and retrogradation (day 7) were determined. All samples were run in triplicate.

2.2.3 Small amplitude oscillatory shear (SAOS) measurements of flour gels

Flour gels (20 % w/w) were made by dispersing 5g of starch into 23g of distilled water and running the standard cycle in the Rapid Visco Analyser (RVA-4C). Starch gels were formed (5 disks of 1.8 cm of diameter and 0.25 mm of high) following Matalanis et al. (2009) procedure. After 1 hour cooling, a single disk was placed inside a closed cell controlled stress rheometer (DH3 Rheometer, TA Instruments, New Cartle, DE) with 100 grit medium sand paper on the bottom and surface of 2.0 cm diameter parallel plate geometry to avoid slippage effect. The gap used was 2 mm. The remaining disks were placed in small plastic containers and perfectly sealed with a moisture barrier film (Parafilm, Pechiney Packaging, Chicago, IL, USA). Disk storage was performed following the same temperature cycle as previously mentioned for the thermal analysis.

Gels stored for 1 hour and 7 days were subjected to a temperature sweep from 25 to 85 ºC at 5 ºC/min at a frequency of 1 Hz with 4% strain was performed. Initial strain sweeps showed that 4% strain was within the linear viscoelastic range for the tested samples. The storage modulus (G') was calculated from the strain response of the material relative to the initial stress input as the sample was heated. G' represents the solid-like or elastic component of a material. ΔG' after 7 days of storage was calculated as G' at 25 ºC minus the corresponding G' measured at 85 ºC. Relative G' drop was calculated as G' drop divided by G' at 25 ^{o}C and multiplied by 100. Coefficient of variation among triplicate measurements was generally 15% or less.

2.2.4 Statistical analysis

Differences among results were studied by analysis of variance (one-way ANOVA). Fisher's least significant difference (LSD) was used to describe means with 95% confidence intervals. The statistical analysis was performed with the Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA).

3 Results and discussion

3.1 Starch molecular size of native and extruded flours

The molecular size distribution of starch polymers was analyzed by size exclusion chromatography (SEC) in order to quantify the molecular degradation during extrusion processing. All SEC weight distributions were normalized to yield the same height of the highest peak, facilitating qualitative comparison and interpretation (Fig. 1). SEC weight distribution of starch from native and extruded flours exhibited two distinct peaks for AM and AP molecules separated at R_h ~20-40 nm. The average hydrodynamic radius for AM (R_{hAM}) and AP (R_{hAP}) molecules is also summarized in Table 1. For native flours, R_{hAM} and R_{hAP} indicated that AM and AP molecules in wheat flour were significantly larger in size that those of maize and rice flours. In contrast, maize flour presented the smallest AM and AP molecules, although no significant differences were found for R_{hAP} in rice and maize.

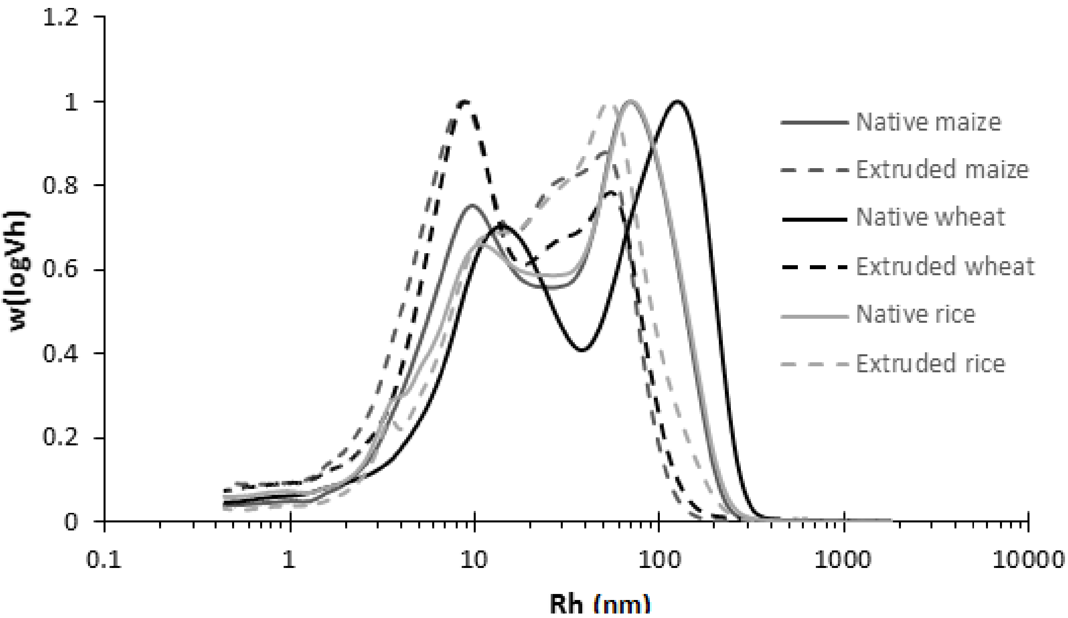

Figure 1. Weight molecular size distributions of starch from native and extruded flours.

A reduction in the molecular size of AP was observed for all extruded samples compared to their native counterparts, as denoted by the smaller R_h values and the shift of the whole AP peak to lower values (Fig. 1). Among all the cereal sources, the largest AP fragmentation was observed for extruded wheat flour (as shown also by the greatest shift of AP peak), which may indicate a more pronounced shear degradation of AP with initial larger molecular size. In fact, Liu et al. (2010) reported a more reduced degradation as the size of the polymer becomes smaller, implying a size-dependent degradation mechanism. Furthermore, for fragmented AP molecules, R_h

values at the peak maximum were similar for the three types of starches (~50 nm). This may suggest that no further size reduction of a population of AP molecules is attained beyond this size at these extrusion conditions (Li et al., 2014b), although there are also populations of smaller AP molecules as observed by the left-handed hump at R_h ~27 nm in the AP peak. Even though there was significant AP fragmentation in these studies, its fine structure is not altered. This occurrence was explained on the basis that large reductions in the whole starch molecular size can occur with the cleavage of only a few AP branches (Tran et al., 2011; Zhang et al., 2015).

Table 1. Hydrodynamic radius of amylose (R_{hAM}) and amylopectin (R_{hAP}) molecules of native and extruded flours.

Flour	Cereal	R_{hAM}	R_{hAP}
Native Flours	Wheat	13.79d	123.92c
	Maize	9.62b	68.39b
	Rice	11.14c	71.77b
Extruded Flours	Wheat	8.86a	54.02a
	Maize	8.85a	52.57a
	Rice	15.66e	53.53a

Values with different letters in the same column are significantly different with $p < 0.05$.

Regarding AM, extrusion only resulted in AM shear fragmentation in wheat and maize flours (Table 1), with a size reduction no greater than 5 nm. This is explained by the fact that AM is less likely to be degraded due to the greater flexibility of its molecules while shear degradation is more pronounced on the large molecular size and highly branched structure of AP (Liu et al., 2010). AM molecules of rice sample presented a non-defined peak that merged with a larger peak at higher R_h, which suggests that fragmented AP molecules co-eluted with AM. This mechanism has been previously suggested by other authors who hypothesized that the cleavage occurs in the inner part/close to the center of the AP molecule, resulting in degraded AP with hydrodynamic size comparable to AM (Liu et al., 2010; Roman, Gomez, Li, Hamaker & Martinez, 2017; Tran et al., 2011). The co-elution of AP with AM molecules in rice would indicate that a population of AP molecules is extensively fragmented during extrusion. This more sensitivity to shearing of AP molecules in rice may be due to a higher native crystallinity/molecular order that is more susceptible to shear fragmentation (Li et al., 2014b).

3.2 Thermal properties of native and retrograded flours

The amount of heat needed for the gelatinization of starch (and its transitions temperatures) gives useful information about the stability and amount of AP double helices present in the granular starch contained in cereal tissues, which can be measured through differential scanning calorimetry (DSC, Table 2). This endothermal peak was characterized by a higher onset (T_o) and conclusion (T_c) temperatures and a higher enthalpy in rice and maize flours. However, after extrusion, the endothermal peak disappeared in all cereal flours, indicating a complete starch gelatinization at the extrusion conditions used in this study.

Table 2. Gelatinization properties of native and extruded flours.

Sample	Cereal	T_o (ºC)	T_p (ºC)	T_c (ºC)	T_c - T_o (ºC)	ΔH_g (J/g)
Native Flours	Wheat	57.39a	63.82a	80.44a	23.05b	5.79a
	Maize	67.62c	73.40c	86.49c	18.87a	7.29b
	Rice	62.43b	69.72b	83.00b	20.57ab	7.46b
Extruded Flours	Wheat	n.d.	n.d.	n.d.	n.d.	n.d.
	Maize	n.d.	n.d.	n.d.	n.d.	n.d.
	Rice	n.d.	n.d.	n.d.	n.d.	n.d.

Values with different letters in the same column are significantly different with $p <0.05$. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinization, ΔH_g= enthalpy of gelatinization, nd, not detectable.

In fact, the pasting profiles of extruded flours (supplementary material 1) confirmed this complete starch gelatinization since only viscosity at cold temperatures was observed (instead of a viscosity peak at gelatinization temperatures typical from native starch/flours). An increase in the cold water absorption capacity of starchy materials due to the dual gelatinization-melting phenomena suffered during extrusion has already been reported by other authors (Hagenimana et al., 2006; Martinez et al., 2014). The RVA pasting curves of native counterparts also revealed an earlier pasting temperature (i.e. faster raise in viscosity when increasing the temperature) and a higher peak viscosity for maize flour, followed by rice and wheat, respectively. Li et al., (2014a) and Roman et al., (2017) reported a more delayed and restricted granular swelling in flours with higher protein content, which is in agreement with the protein content shown in this study (see materials section).

During storage of non-granular starch-based gels, AP branches tend to re-associate with other AM and AP chains (either from the same molecule or from a different one) through the formation of double helices or aggregates of double helices (Klucinec & Thompson, 1999). Since this leads to greater short-range molecular order, heat is necessary to melt these supramolecular structures into an amorphous phase, endotherm that can be detectable with DSC at around 55 ^{o}C (Matalanis et al., 2009). In this study, the thermal transitions and enthalpy of this endothermal peak in samples stored for 7 days, which corresponds to retrograded AP, are summarized in Table 3 and Fig. 2A. A reduction of the AM and AP size did not affect the propensity of double helix formation involving AP during storage in maize and rice flours, i.e. no differences in the double helical content involving AP was detected among samples. This would indicate that a reduction of 18 nm in R_h (or lower), which entails a 23 and 25 % size reduction in maize and rice, respectively (Table 1), causes no effect on AP double helical increase during storage (retrogradation). On the other hand, a reduction of 69 nm in R_h in wheat flour (56% size reduction) significantly increased the double helical content (three-fold increase) attained during storage. It is worth noting that these AP interactions may be of intra- and inter-molecular nature and, therefore, they also count with starch re-associations that do not contribute to the formation of physical junction zones responsible for gel structure and strength (Klucinec & Thompson, 2002). In other words, the retrogradation enthalpy is not necessarily an indication of gel strength/elasticity.

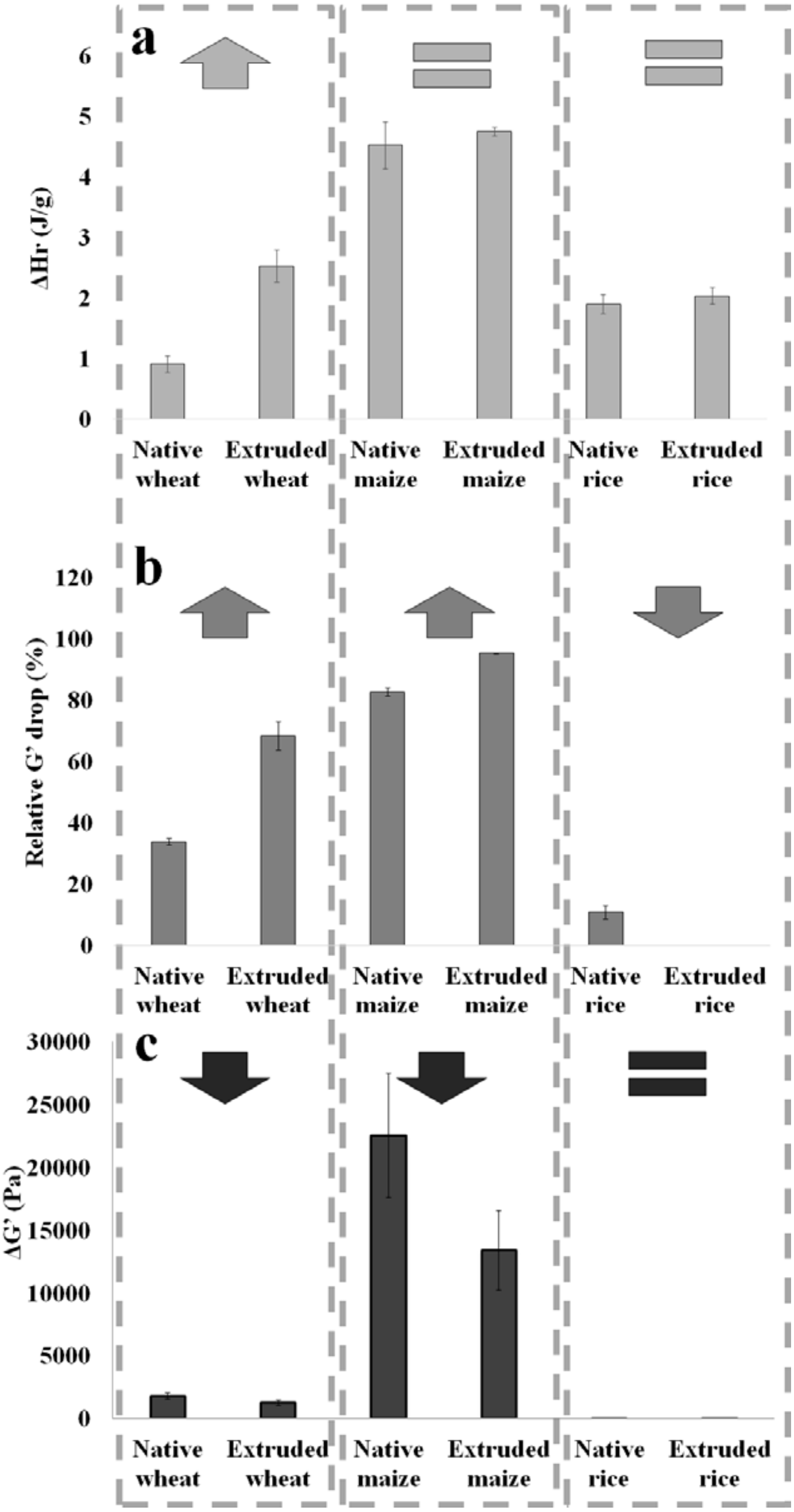

Figure 2. Retrogradation enthalpy (a) and relative G' drop (b) of flour-based gels after 7 days storage. G' increase (Pa) at 1 Hz and 25 ºC from day 1 to day 7 (ΔG') of flour-based gels (c).

Table 3. Thermal properties of retrograded amylopectin of native and extruded flours.

Sample	Cereal	T_o (ºC)	T_p (ºC)	T_c (ºC)	T_c - T_o (ºC)	ΔH_r (J/g)	$G'_{85\,ºC}$ (Pa)
Native Flours	Wheat	46.56b	54.36abc	61.60a	15.04a	1.08a	2923
	Maize	44.65ab	55.09bc	68.27d	23.63c	5.96c	3989c
	Rice	44.25a	54.18ab	62.93ab	18.68b	2.74b	323a
Extruded Flours	Wheat	44.54a	53.99ab	64.57c	20.03c	2.82b	569a
	Maize	44.80a	55.17c	70.52e	25.72d	6.01c	714a
	Rice	44.47a	54.50bc	64.25bc	19.78c	2.29ab	123a

Values with different letters in the same column are significantly different with p <0.05. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures of retrogradation, ΔH_r= enthalpy of retrogradation, G' $_{85ºC}$ (Pa) elastic modulus at 1 Hz and 85 ºC at day 7

In general, although slight differences were found in the transition temperatures of retrograded AP before and after extrusion, a noticeable wider temperature melting range was observed in extruded flours. This wider temperature range implies a larger amount of crystals of varying stability which are more heterogeneous in nature (Fredriksson, Silverio, Andersson, Eliasson & Åman, 1998). In fact, Klucinec and Thompson (1999) reported that the temperature range is related to double helical length, in such a way that a wider endotherm would suggest a wider range of double-helical lengths. Although the AP chain length distribution is not modified after extrusion (Li et al., 2014b; Liu et al., 2010), AP fragmentation may improve its mobility and orientation during storage resulting in a more heterogeneous short-range molecular order. Furthermore, the starch contained in maize flours, which possesses a population of AP short branches (A+B1 chains) longer than those in rice and wheat counterparts (Jane et al., 1999; Matalanis et al., 2009; Srichuwong, Sunarti, Mishimac, Isonoa & Hisamatsu, 2005), exhibited the widest temperature range, which also presented the highest enthalpy of retrogradation, i.e., highest number of double helix.

3.3 Dynamic oscillatory rheometry of retrograded flour based gels

When freshly prepared (cooled down for 1 h) starch gels were subjected to a temperature sweep from 25 to 85 ^{o}C, no thermal transition (loss of storage modulus, G') was observed in the temperature range of 40-60 °C, temperature range for melting of retrograded AP (data not shown). Furthermore, no significant differences in G' at 85 °C (Table 3) were found among 7 days aged gel samples, except for native wheat and maize samples, which showed higher G' values at those temperatures. This would indicate that in native wheat and maize, with higher non-fragmented AM content, AM-AM interactions are significantly contributing to build the structure of starch gels.

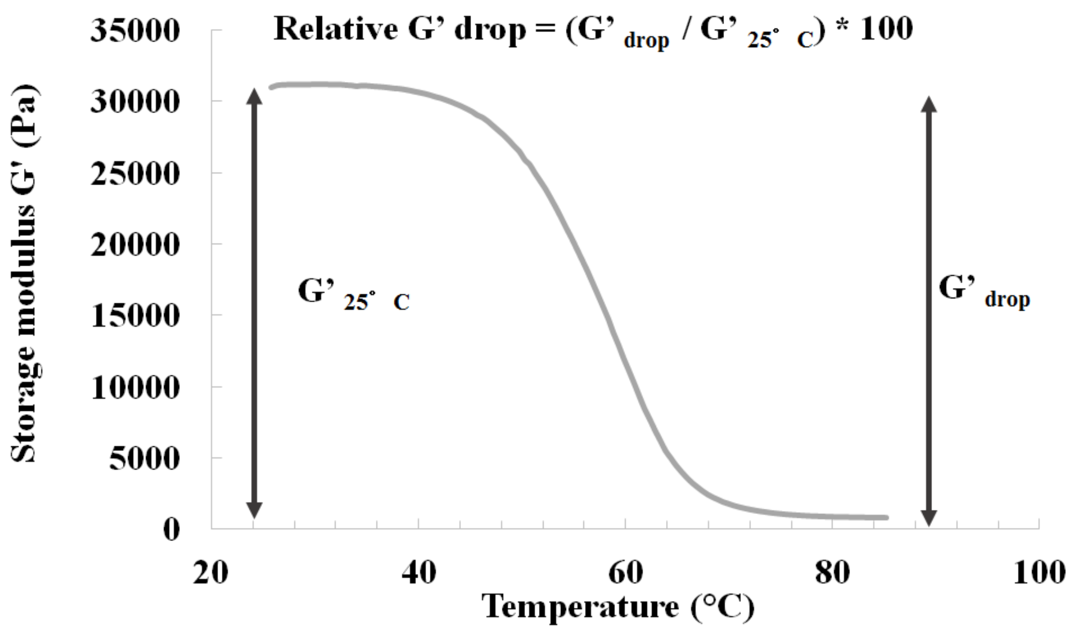

Figure 3. Evolution of the storage modulus (G') in a temperature sweep and representation of the calculation of G' drop.

A temperature sweep of retrograded samples stored for 7 days revealed a decrease of G' (Fig. 3), which is the result of loss, or melting, of inter-molecular associations involving AP molecules, i.e. AM-AP and AP-AP. Among flours, the highest loss in G' was observed for maize (Fig. 2B), which would be in agreement with the highest amount of total double helixes observed with DSC. However, it is noteworthy that, although the formation of intra-molecular double helices along external AP chains (A and B1 chains) increases enthalpic transitions of retrograded AP (Klucinec & Thompson, 2002), no correlation has been previously reported with G' loss. In fact, Klucinec and Thompson (2002) and Matalanis et al. (2009) noted that the formation of intra-molecular double helices has little impact on G' values for aged starch gels, since this type of re-association does not contribute to the network structure of a starch gel. Extruded maize and wheat samples displayed a higher relative G' drop than native counterparts, indicating a more prominent role of AM-AP and/or AP-AP inter-molecular interactions on forming gel structure. Klucinec & Thompson (1999) mentioned that the possibility of chains from different molecules to participate in the formation of physical junction zones would depend on whether they are near the periphery of the molecule, something that is related to its size. Therefore, fragmented AP chains as a result of extrusion, with smaller size and higher likelihood to come into proximity, seem to have an increased role on the formation of inter-molecular starch interactions. Interestingly, extrusion exhibited an opposite effect in rice, showing a significant decrease of the propensity of starch to form inter-molecular interactions during storage after extrusion. An explanation could be found based on the higher AP degradation in rice during extrusion, since noticeable AP co-elution with AM was observed (Fig. 1). Furthermore, AP content in rice samples is higher, therefore higher AP contribution to form gel structure could be expected. Since no significant differences were found in the total number of intra- and inter-molecular interactions involving AP after rice extrusion (Table 3, Fig. 2A), results would indicate a lower inter- to intra-molecular ratio of molecular interactions. Another explanation to this opposite behavior of extruded rice samples may be also related to the lower proportion of long chains (B2+B3) of rice AP compared to maize and wheat, since AP containing higher proportions of long B-chains has been reported to have an increased tendency to retrograde (Matalanis et al., 2009). This mechanistic understanding would support the attribution given by Martinez et al. (2013) to extruded rice flour for decreasing gluten-free bread hardness during storage (52 % reduction). Moreover, Roman, Reguilon and Gomez (2018) found that syneresis of sauces made with extruded rice flour (~3.5%) was significantly lower than that from extruded wheat flour (~18.6%).

Although the amount and stability of AP double helices have been many times related to the formation of gel structure, already in 1953, Flory recognized that some chains may not contribute to the elasticity of a gel network in vulcanized rubber, which was later extrapolated to starch gels by Klucinec and Thompson (1999). Therefore, the increase of the storage modulus (G') after 7-day storage was used as an indicator of gel development and, therefore, of the formation of physical junction zones. This should be considered as the main parameter that is related to the mechanical properties of the starch gel and ultimately to the food physical properties.

As retrogradation proceeds for 7 days, junctions in starch molecules extend significantly in length, and simultaneously additional inter-molecular bridges are likely established, which markedly increased G'. In wheat and maize samples, a lower G' development during storage was observed in samples after extrusion (Fig. 2C). This event was not detectable in rice because of the low G' development attained in its native and extruded form, although it could also be hypothesized that a reduction of G' development is also attained. This low consistency in rice could be based on its lower AM content and the less number of interactions involving AM. In fact, higher AM contents in the network are positively correlated with higher storage modulus (Kong, Kasapis, Bertoft & Corke, 2010), which agrees with the trend observed for the solid-like behavior of the three flours in this study.

Results indicated that the effect of the extrusion in weakening gel structure during storage depends on the starch composition and fine structure of the cereal source. In maize and wheat, although extrusion fragmented AP molecules and increased their likelihood to form inter-molecular physical junction zones, a reduction of the strength of gels made with extruded counterparts was observed. This would indicate that the reduction of AM size, and the resulting decrease of AM-AM interactions that contribute to gel structure, would mask the effect of a higher proportion of inter-molecular interactions involving AP. This explanation would also be supported by the higher residual G' values at 85 ^{o}C in native maize and wheat samples stored for 7 days (Table 3), which indicates that AM-AM interactions are significantly contributing to build the structure of gels made with native maize and wheat flours. In extruded rice, no AM fragmentation was found, and the lower gel consistency during storage could be based on the significant reduction of AP size attained during extrusion, which showed fewer inter-molecular interactions involving AP (Fig. 2C). A visual representation is depicted in Fig. 4.

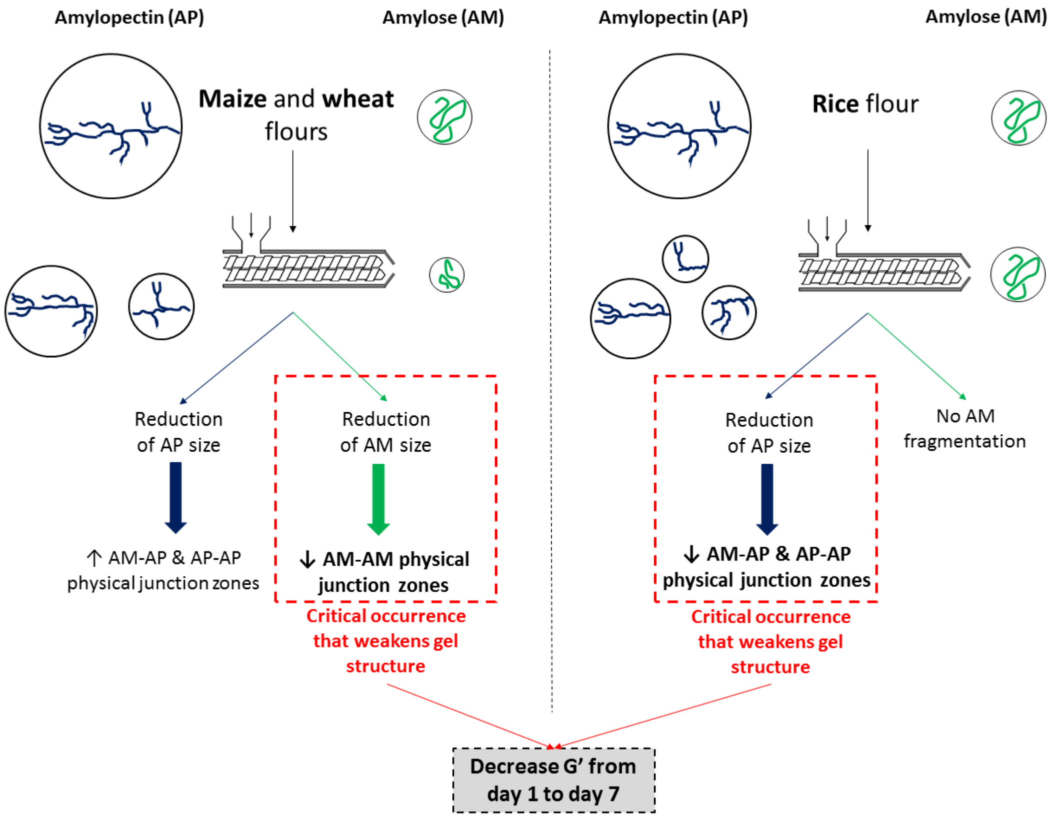

Figure 4. Effect of extrusion of maize, wheat and rice flours on amylose and amylopectin fragmentation and formation of physical junction zones during storage.

4 Conclusions

The density and stability of the inter-molecular junction zones determine the mechanical properties of the network structure of starch gels and, therefore, the physical and sensory quality of starch containing foods. In this work, extrusion processing of the most common cereal flours, namely wheat, maize and rice, is shown to be an effective way to reduce gel hardening during storage. Interestingly, results suggested two distinct predominant mechanisms, based on the starch composition and structure of the cereal source, that result in a weaker gel structure. In wheat and maize, the significant reduction of AM size diminished the number of AM-AM physical junction zones that build gel structure. On the other hand, the extrusion of rice flour, containing starch with lower AM ratio (higher AP ratio) and previously reported to have lower proportion of long chains, resulted in a marked AP fragmentation that caused a decrease in the ratio of inter- to intra-molecular interactions involving AP. Results suggest that extrusion is a useful technology to decrease starch retrogradation, although attention must be paid to the raw material used. This study gives important understanding at the molecular level for the optimization of flour functionality through extrusion in order to reduce retrogradation-controlled phenomena such as bread staling, sauce/pure syneresis and optimize rice stickiness, among others.

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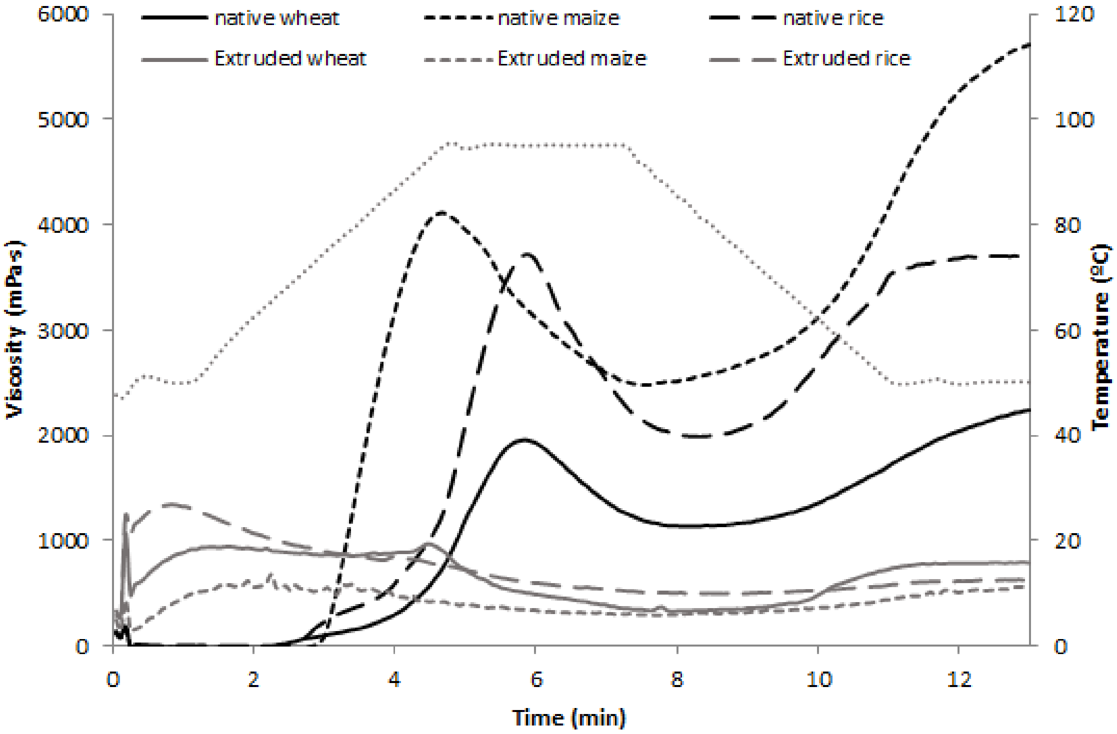
Supplementary material

Supplementary material 1. Pasting profiles of maize, wheat and rice flours before and after extrusion treatment



CHAPTER 4

Exploiting the potential of molecular shear fragmentation during extrusion to increase slowly digestible starch in gelatinized baked goods

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Banana starch and molecular shear fragmentation dramatically increase structurally driven slowly digestible starch in fully gelatinized bread crumb

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Abstract

This study shows the role of native (NB) and extruded (EB) banana starch, and a 1:1 native to extruded banana starch composite (MB), in slowing down the starch digestibility of bread crumb and crust. During extrusion, the molecular weight of banana starch was reduced from 2.75x10^8 to 4.48x10^6 g/mol (HPSEC-MALS-RI). Results showed an SDS increase from 1.09 % (control) to 4.2, 6.6, and 7.76 % in NB, MB and EB crumbs (fully gelatinized), respectively. DSC data attributed this occurrence to the formation of supramolecular structures upon storage involving amylopectin branches (especially those from fragmented amylopectin in EB). The hedonic sensory test showed no differences in overall liking between MB, EB and control, validating feasibility of including banana in the formulation. For the first time, this study shows a molecular size reduction as a strategy to manufacture selected starches that result in highly gelatinized baked products rich in structurally driven SDS.

Keywords: banana; extrusion; starch digestion; retrogradation; gluten-free bread; glycemic index

1 Introduction

Dietary glycemic carbohydrates that result in small excursions in postprandial plasma glucose and insulin concentrations are associated with improved insulin secretion and sensitivity and enhanced glycemic control (Vinoy, Laville, & Feskens, 2016). On the other hand, recurrent postmeal high blood glucose levels have been linked to an increased risk of cardiovascular events and type two diabetes mellitus (Ceriello & Colagiuri, 2008). Starch, the major polysaccharide related to postprandial glycaemia, is hydrolyzed by salivary and pancreatic α-amylase in the mouth and small intestine of humans, and then to glucose by the mucosal brush border α-glucosidases. The rate and extent to which starch is digested by these enzymes will greatly influence differences in the glycemic response of starch-based products.

In baked goods, the rate of starch digestibility has been correlated with the degree of starch gelatinization (Englyst, Vinoy, Englyst, & Lang, 2003). The amorphous structure of gelatinized starch results in a greater availability of α-amylase binding sites, which makes the substrate more susceptible to enzyme hydrolysis. Most of starches have been reported to gelatinize approximately in the range from 54 to 78 ^{o}C in excess of water (≥20% water) (Vamadevan, Bertoft, & Seetharaman, 2013). Therefore, considering that bread dough/crumb exhibits a moisture content always ≥ 35% (Martinez, Roman, & Gomez, 2018) and temperatures ≥ 70 ^{o}C during the course of baking (Thorvaldsson & Skjoldebran, 1998), an extensive starch gelatinization in high moisture baked goods, such as bread, is expected to occur. In fact, Primo-Martin, van Nieuwenhuijzen, Hamer, and van Vliet (2007) and Martinez et al. (2018) reported a complete starch gelatinization in wheat bread crumb. What is more, Martinez et al. (2018) reported a complete starch gelatinization in candeal bread, also known as Fabiola or Bregado, with only 45% dough hydration (close to the minimum amount of water possible to make bread). Since bread constitutes the highest proportion of carbohydrates (mainly starch) to the daily dietary intake (Cust et al., 2009), successful strategies that decrease the digestibility of fully gelatinized starch might have a remarkable impact at decreasing the prevalence of metabolic syndrome associated diseases in the entire world.

Retrogradation is the recrystallization process of glycan chains in gelatinized starch, during which dispersed starch molecules begin to re-associate through H-bonding. Albeit retrogradation has been reported as one reason for bread staling (Gray, & BeMiller 2003), it is also known to result in a reduction of the rate and extension of starch digestion, depending on the main constituent involved. Thus, amylose double helices are known to be enzymatically resistant and yield resistant starch (RS) [Patel et al., 2017], whereas retrograded amylopectin has been attributed to the formation of slowly digestible starch (SDS) [Cui & Oates, 1997; Zhang, Sofyan, M., & Hamaker, 2008]. Based on previous evidence indicating that cereal starches containing high proportions of amylopectin with long chains (Benmoussa, Moldenhauer, & Hamaker, 2007), or amylopectin with longer chains (Zhang, et al. 2008), result in a higher amount of slowly digestible starch in fully gelatinized material, Martinez, Li, Okoniewska, Mukherjee, Vellucci, and Hamaker (submitted) investigated starch molecular structure-digestibility relationships in fully gelatinized starches. These authors reported for the first time two potential factors that may result in slowly digestible supramolecular structures during storage, namely: 1) double helices between A and B1 chains of an average length ≥ 15.5 Glucose Units (perhaps involving internal long chains) that also are prone to forming intermolecular associations and; 2) interactions of starch molecules with lower molecular size that may be more mobile and easily aligned during retrogradation. In that study, the authors also demonstrated an increase of structurally-driven slowly digestible starch in a real intermediate moisture (~20%) baked food. However, the effect of starch molecules with both significantly small size and long amylopectin chains at resulting in structurally-driven slowly digestible starch has never been studied.

Banana starch has been reported to have amylopectin with small size (Yoo & Jane, 2002) and a low proportion of short chains (Zhang & Hamaker, 2012). It can be added into bakery products as purified starch or starch-predominant flour particles after simple liquid-solid extraction (Zhang, Whistler, BeMiller, & Hamaker, 2005) or simple processing involving drying and grinding (Segundo, Román, Gómez, & Martínez, 2017) of banana pulp, respectively. Thus, several studies have been done on the application of green banana flour for the production of bakery products (Agama-Acevedo, Islas-

Hernández, Pacheco-Vargas, Osorio-Díaz, & Bello-Pérez, 2012; Ho, Aziz, & Azahari, 2013; Juarez-Garcia, Agama-Acevedo, Sáyago-Ayerdi, Rodriguez-Ambriz, & Bello-Perez, 2006; Segundo et al., 2017). When banana bunches arrive at central collection stations, bananas too small for shipping are removed, along with those that have damaged or spoiled areas. These rejected bananas are normally disposed of improperly and their successful industrial use would significantly reduce fruit food waste. Based on the aforementioned mechanistic understanding provided by Martinez et al. (submitted), we hypothesized that: 1) banana starch, an untapped food by-product, would result in breads with a significant increase in slowly digestible starch and; 2) that the reduction of its molecular size through shear scission (by extrusion) would further slowdown the digestion rate of the starch glycemic fraction in breads. The objective of this study is, therefore, to identify the ultimate starch material that results in slowly digestible starch from fully gelatinized material. To do so, gluten-free breads served as an ideal high-moisture baked system (and therefore with extensive starch gelatinization) to investigate the effect of native and extruded banana starch on their *in vitro* starch digestion and physical and sensory properties. The starch digestion fractions and degree of starch gelatinization and retrogradation were analyzed in the crust and the crumb separately to provide insights about the spatial location and origin of those slowly digestible starch supramolecular assemblies.

2 Materials and Methods

2.1 Materials

Atlantic rice flour (9.58 % moisture, 7.43 % protein, 0.11 % RS) was provided by Molendum Ingredients (Zamora, Spain), Miwon maize starch (8.07 % moisture, non-detected protein, 0.76 % RS) was purchased from Daesang Co. (Seoul, Korea) and banana starch from green lady finger bananas (6.30 % moisture, 3.17 % protein, 42.21 % RS) was purchased from Natural Evolution (Walkamin, Queensland, Australia). Starchy materials (including extruded banana flour) were characterized according to AACC methods (AACC, 2015) for moisture (44-15.02), protein content (46-30.01) with a Leco TruSpec device (Leco, St. Joseph, MI, USA) and resistant starch content (32-40.01) using RS Megazyme assay kit. Particle size was measured with a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Instruments, Ltd., Worcestershire, UK). The mean diameter of equivalent volume d(4,3), which indicates the central point of the volume distribution of the particles, was recorded. The plot of the particle size distribution is included as Fig. 1A. Thermal properties were analyzed in triplicate as reported by Roman, Gomez, Li, Hamaker and Martinez (2017) using a differential scanning calorimeter Q-20 (TA instruments, Crawley, UK) equipped with a RCS 40 refrigerated cooling system (supplementary material 1 and 3). Furthermore, the pasting profile of the starchy materials (supplementary material 2) was obtained following the standard method 61-02.01 (AACC, 2015) with a Rapid Visco Analyser (RVA-4) (Perten Instruments Australia, Macquarie Park, Australia).

The ingredients used for bread making were VIVAPUR 4KM HPMC (Hydroxypropyl Methylcellulose, JRS, Rosenberg, Germany), Saf-Instant dry yeast (Lesaffre, Lille, France), salt (Union Salinera de España, Madrid, Spain), sucrose (Azucarera, AB, Madrid, Spain), Abrilsol sunflower oil (Ourense, Spain) and tap water. Amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger*, 300 U/mL, pepsin (EC 3.4.23.1) from porcine stomach mucosa, 800-2500 U/mg solid, pancreatin from porcine pancreas, 8 x USP Specification, and guar gum were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose assay reagents were from Megazyme International Ireland Ltd. (Wicklow, Ireland).

2.2 Methods

2.2.1 Extrusion process

The extrusion of banana starch (final moisture of 3.33 %) was performed in a Krupp Werner and Pfleiderer ZSK-25 twin screw extruder (Ramsey, NJ, USA) with co-rotating and closely intermeshing screws of 25 mm diameter and length to diameter (L/D) equal to 25. The extruder was equipped with 5 barrel sections heated to 40, 60, 90, 120 and 130 ± 1 ºC from the feeding to the die, respectively. The screw speed was maintained constant to 200 rpm. A combination of feeding, transporting, compression and mixing elements was used to provide a moderate-shear screw configuration that resulted in 330 KJ/Kg of specific mechanical energy (SME). Feed input flow rate was 5 Kg/h with a water addition to obtain a total moisture content of 22 %. Banana starch was extruded through two circular dies of 4 mm diameter (each). Extruded banana starch was dried overnight at 55 °C by convection air and then ground with a pin mill. After milling, extruded banana starch (3.29 % protein, 0.84 % RS) contained a mean particle size [d(4,3)] of 299 µm, which was similar to the d(4,3) of native banana (268 µm) due to the presence of particles formed by starch aggregates (Fig. 1).

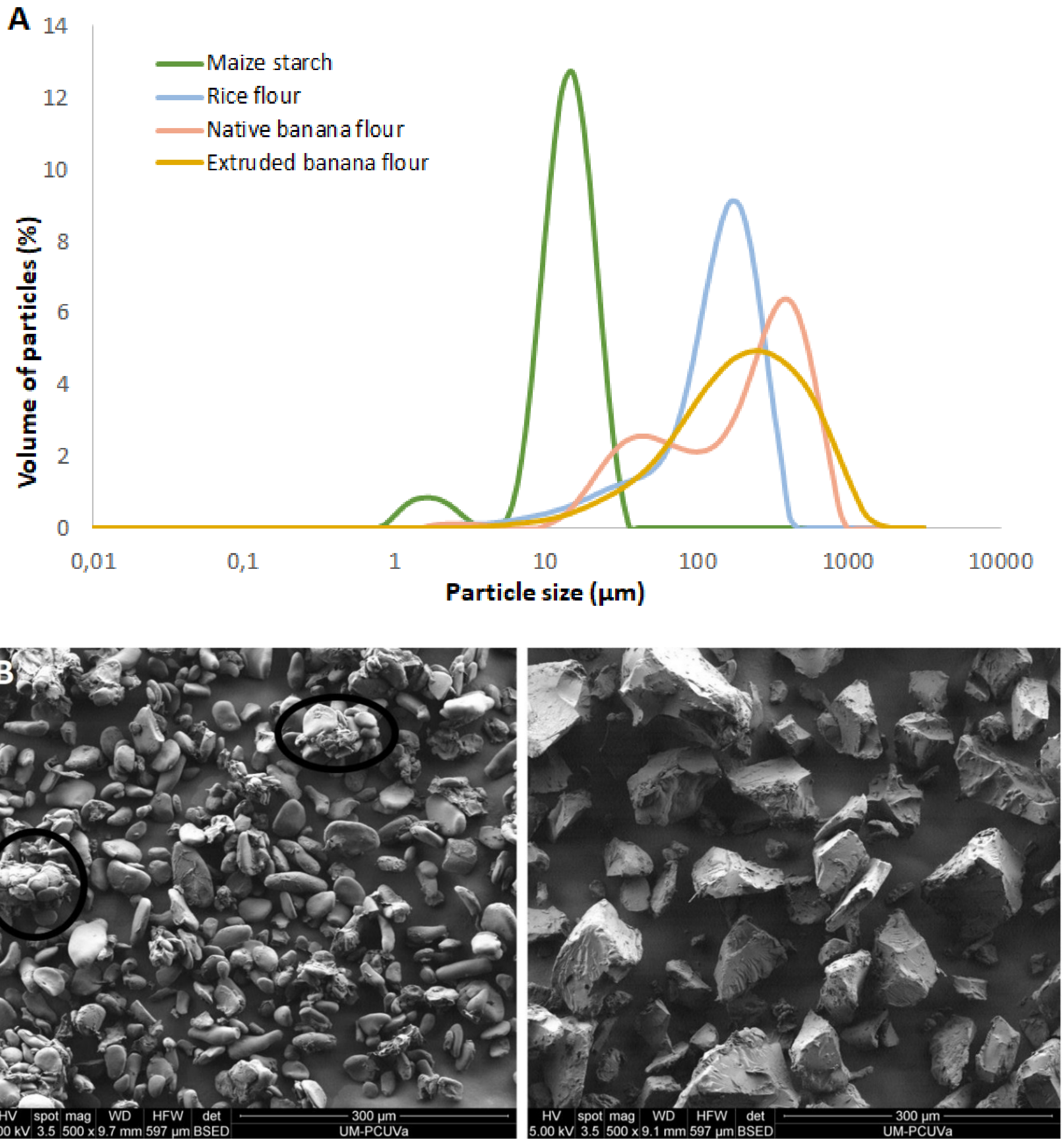

Figure 1. Particle size distribution (A) of starchy ingredients and SEM pictures (B) of native and extruded banana samples (from left to right). Black circles highlight aggregates of banana starch granules.

2.2.2 Native and extruded banana starch molecular structure

Starch fine structure of native banana starch was analyzed in triplicate using the method reported by Cave, Seabrook, Gidley, and Gilbert (2009) with minor modification as reported previously in Martinez et al. (submitted). Analyses were performed using a size exclusion chromatography (SEC) system (Agilent 1260 series, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector (RID, 1260 RID, Agilent, Agilent Technologies, Waldbronn, Germany). The length of short (A and B1) and long internal (B2, B3, etc.) amylopectin branches is denoted as X_{AP1} and X_{AP2}, respectively, and the molar of long to short amylopectin chains is represented as $h_{Ap2/Ap1}$. The amylose content of banana starch was determined from the SEC molecular size distribution of debranched starch as the ratio of the area under the curve (AUC) of amylose branches to the AUC of overall amylopectin and amylose branches. The chain length distribution of extruded banana was not analyzed since it has been reported not to be altered after extrusion (Zhang et al., 2015).

The molecular weight (Mw) of banana starch molecules was measured in triplicate using a multi-angle light scattering detector (MALS, Dawn Heleos, Wyatt Technology, Santa Barbara, CA, USA) containing a K5-cell. 20 µL of starch [previously dissolved in DMSO/LiBr as described in Martinez et al. (submitted) for the analysis of the size of fully branched starch] were injected into GRAM 30 and 3000 columns (PSS GmbH, Mainz, Germany) connected in series (0.3 mL/min at 80 ºC). Data was analyzed with ASTRA software (version 4.72.03, Wyatt Technology Corporation, Goleta, CA, USA) and using the Berry second order plot procedure. This procedure involves an extrapolation of the function $\sqrt{(K^{*}c/R_{\theta})}$ to zero scattering angle θ [as $\sin^2(\theta/2)$]. K^* is a constant depending on the wavelength of the light and refractive index increment of the starch and R_{θ} is the 'Rayeligh excess ratio', which is a measure of the ratio of the intensity of excess light scattered compared to pure solvent at an angle θ to that of the incident light intensity (Harding, Adams, & Gillis, 2016). The specific refractive index increment (dn/dc) was assumed to be 0.066 mL/g as usually reported for starch dissolved in DMSO (Zhong, Yokoyama, Wang, & Shoemaker, 2006) and the second viral coefficient (A_2) was assumed to be negligible (Yoo & Jane, 2002).

2.2.3 Dough preparation and bread-making

Breads were made with water (105 g/100 g flour-starch mixture), instant dry yeast (3 g/100 g), salt (2 g/100 g), oil (6 g/100 g), HPMC (2 g/100 g) and white sugar (5 g/100 g).

The base starchy source was comprised by a 1:1 mixture of maize starch and rice flour (control). Native banana starch (NB), extruded banana starch (EB) and a 1:1 mixture of native and extruded banana starches (MB) were used as a replacement of 20 % of the base starchy material. In all makings, water temperature was held between 20 and 22 °C for yeast dissolution before its incorporation. All the ingredients were mixed for 8 min at speed 2 with a flat beater (K45B) in a Kitchen Aid 5KSM150 mixer (Kitchen Aid, Michigan, USA). Subsequently, 150 g of dough were placed in small aluminum molds (109×159×38 mm) and fermented at 30 °C and 90 % relative humidity for 60 min. After fermentation, doughs were baked in an electric modular oven for 40 min at 190 °C. After baking, the loaves were demolded after 5 min and let to cool down for a total time of 60 min before their storage in sealed polyethylene bags at 20 ºC for 24 h. All the elaborations were prepared in duplicate.

2.2.4 Microstructural analysis of bread

Bread and banana starch photomicrographs were taken with Quanta 200FEI (Hillsboro, Oregon, USA) environmental scanning electron microscope (ESEM). Photomicrographs were taken in high vacuum mode. Crumb and crust samples were pictured from a parallel slant (lengthwise section showing the thickness of the crust), whereas crust was also imaged from a perpendicular slant (supplementary material 4 and 5). In order to better assess crumb macrostructure, bread slices were pictured with a Canon EOS 1300D (Tokyo, Japan).

2.2.5 Moisture content, starch thermal transitions and starch digestion fractions in bread crumb and crust

Crumb, taken from the center of a bread slice, and crust samples, obtained by scratching the bread surface, were analyzed for moisture content according to approved method 44-15.02 (AACC, 2015). The starch thermal transitions were measured as in Martinez et al. (2018), although results from the degree of starch gelatinization must be interpreted differently since, in the present study, a pre-gelatinized starch source is added into the recipe. DSC measures the reduction of the endothermal peak attributed to native amylopectin double helices between dough and crumb (or crust), which indicates the degree of starch gelatinization produced during baking (GD_B). If all the starch present in the baked good was in a native state, the degree of starch gelatinization during baking would be equal to the percent of gelatinized starch in the final product (g gelatinized starch/100 g starch). However, in this case, results must be interpreted as a reduction of native starch (or an increase of gelatinized starch) during baking, which does not necessarily represent the percent of gelatinized starch in the final product. DSC thermograms of the control dough and bread crumb and crust are reported as an example in supplementary material 3. The starch digestion fractions of crumb and crust samples were analyzed as in Martinez et al. (2018), who followed the procedure proposed by Englyst, Hudson and Englyst (2000) and the AACCI approved method 32-40.01 (AACC, 2015) with the RS assay kit from Megazyme (Megazyme International, Wicklow, Ireland) for slowly digestible and resistant starch fractions, respectively. Each sample was analyzed in triplicate.

2.2.6 Specific volume of breads and crumb texture

Bread volume was determined using a laser sensor with the Volscan Profiler (Stable Micro Systems, Godalming, UK). The volume measurements were performed on three loaves of each elaboration. The specific volume was calculated as the ratio of bread volume to its mass. Crumb texture was measured after 24h storage with a TA-XT2 texture analyzer (Stable Microsystems, Surrey, UK) equipped with the “Texture Expert” software. A 25-mm diameter cylindrical aluminum probe was used in a “Texture Profile Analysis” (TPA) double-compression test to penetrate up to 50% of the sample depth at a test speed of 5 mm/s, with a 30 s delay between the two compressions. Hardness (g), cohesiveness, springiness and resilience were calculated from the TPA curve. Texture analyses were performed on two 30 mm central slices from two loaves from each elaboration.

2.2.7 Consumer testing

Hedonic sensory evaluation of gluten-free breads was conducted with 83 volunteers, between 16-65 years of age and from various socioeconomic backgrounds. Consumer test was conducted at a sensory laboratory in individual booths. Breads were assessed for their appearance, odor, flavor, texture, and overall liking on a nine-point hedonic scale. The scale ranged from “extremely like” to “extremely dislike” corresponding with highest and lowest scores of “9” and “1”, respectively. Samples were presented on white plastic dishes coded with four-digit random numbers and served in random order. Water was available for rinsing. For the evaluation of bread appearance, a coded loaf of each bread type was presented. Samples were tested 24 h after baking.

2.2.8 Statistical analysis

Differences among results were studied by analysis of variance (one-way ANOVA). Fisher’s least significant difference (LSD) was used to describe means with 95% confidence intervals. The statistical analysis was performed with the Statgraphics Centurion XVI software (Statpoint Technologies, Inc., Warrenton, USA).

3 Results and Discussion

3.1 Moisture content, degree of starch gelatinization and amylopectin retrogradation in crumb and crust

The moisture content ranged from 45.87 to 49.85% and from 18.40 to 21.21% in crumb and crust samples, respectively (Table 1). It is well known that, during baking, faster moisture transfer phenomena occur at the bread surface resulting in the development of a drying zone (Luyts et al., 2013), also known as crust, which will be a barrier for further heat and moisture transfer (Thorvaldsson & Skjoldebran, 1998). In contrast, as soon as bread is removed from the oven, water rapidly migrates from the interior to the crust layer (Gorton, 2009). Results indicated a greater water migration from the crumb to the crust in EB, as seen by the significantly lower and higher moisture values in crumb and crust, respectively (Table 1). This might be attributed to two simultaneous factors: 1) a lower water retention capacity (loose bound water) of extruded banana starch (as suggested with the low pasting curve shown in supplementary material 2) due to the notable starch fragmentation (Fig. 2) and/or, 2) a lower bread specific volume that may facilitate a faster transfer of water to the crust (i.e., shorter path for water to reach the bread surface).

Table 1. Effect of native and extruded banana starch on physical properties of breads

	Control	Native Banana	Mixture Banana	Extruded Banana
Crumb moisture (g/100 g)	49.36b ± 0.12	49.85c ± 0.17	48.90b ± 0.01	45.87a ± 0.52
Crust moisture (g/100 g)	18.95a ± 0.01	18.96a ± 0.06	18.40 a ± 0.09	21.21b ± 0.76
Specific volume (mL/g)	4.29c ± 0.15	5.34d ± 0.13	3.57b ± 0.07	2.51a ± 0.03
Hardness (g)	622b ± 79	304a ± 18	1051c ± 146	3604d ± 558
Springiness	0.99a ± 0.02	0.99a ± 0.05	1.00a ± 0.00	1.02a ± 0.02
Cohesiveness	0.38b ± 0.02	0.45c ± 0.02	0.36ab ± 0.02	0.34a ± 0.03
Resilience	0.16b ± 0.01	0.21c ± 0.02	0.14a ± 0.01	0.16b ± 0.01

Values followed by the same letters within each parameter indicate no significant differences ($p \leq 0.05$).

The increase of starch gelatinization in crumb during baking (DG_B) was complete for all breads (Table 2). Conversely, DG_B was limited in the crust, ranging from 5.56 % in NB to 8.48 % in EB. The fast water evaporation suffered on the bread surface as the result of the high oven temperatures causes a rapid water depletion enough to hinder starch from gelatinization in the crust (Primo-Martín et al., 2007).

Table 2. Degree of starch gelatinization and amylopectin retrogradation of bread crumb and crust.

	Crumb		**Crust**	
	DG_B (%)	**ΔH_r (J/g)**	**DG_B (%)**	**ΔH_r (J/g)**
Control	100a ± 0.00	2.79a ± 0.13	7.16b ± 0.56	n.d.
Native Banana	100a ± 0.00	3.01a ± 0.08	5.56a ± 0.74	n.d.
Mixture Banana	100a ± 0.00	3.38b ± 0.07	7.47bc ± 0.55	n.d.
Extruded Banana	100a ± 0.00	3.47b ± 0.16	8.48c ± 0.99	n.d.

Values followed by the same letters within each parameter indicate no significant differences ($p \leq 0.05$). n.d. non detectable. ΔHr, enthalpy for retrograded amylopectin. DG_B, degree of starch gelatinization during baking.

Interestingly, NB crust displayed a lower DG_B than the control, which suggests that the higher onset temperature of starch gelatinization (T_o) of banana starch (71.74 ºC) compared to that from rice (67.57 ºC) and maize (62.12 ºC, supplementary material 1) is enough to result in a significant reduction of starch gelatinization in the crust (even with a 20 % replacement). On the other hand, the higher DG_B in EB crust could be explained by the higher moisture content in the crust over the course of baking due to the higher water migration from the crumb to the crust as discussed before. This would result in higher availability of water for the starch to gelatinize.

During cooling and storage, amylopectin branches of gelatinized starch tend to re-associate with other amylose and amylopectin chains through the formation of double helices or aggregates of double helices (Klucinec & Thompson, 2002). The melting of these structures can be detected by DSC (Martinez et al. submitted) and in this study, crumbs of breads stored for 1 day after baking exhibited the onset of this transition between 43 ± 1 ºC and 68 ± 1 ºC (supplementary material 3). The addition of banana resulted in an increase of the enthalpy for retrograded amylopectin in crumb (Table 2), although significant differences were only observed in EB and MB. HPSEC results revealed that banana amylopectin contains short chains (A and B1) and long internal chains (B2, B3, etc.) of an average length (X_{AP}) of 17.0 and 42.1 Glucose Units (GU), respectively, and a molar ratio of long to short chains ($h_{AP2/hAP1}$) of 0.73 (data not shown). These values are significantly higher than those from the maize and rice amylopectins reported by Martinez et al. (submitted), with X_{AP1}, X_{AP2} and $h_{AP2/hAP1}$ lower than 14.6 GU, 39.8 GU and 0.58. Longer amylopectin chains have been already reported to be more prone to form molecular interactions during retrogradation (Klucinec & Thompson, 2002). What is more, Zhang and Hamaker (2012) reported a higher retrogradation rate of cooked banana starch compared to maize and potato starches, which was associated with the higher proportion of long chains of banana amylopectin. Interestingly, a molecular size reduction of banana starch from 2.75×10^8 to 4.48×10^6 g/mol during extrusion (Fig. 2), led to a higher potential of extruded banana starch to form molecular interactions during retrogradation, which may be the result of a higher mobility and alignment properties.

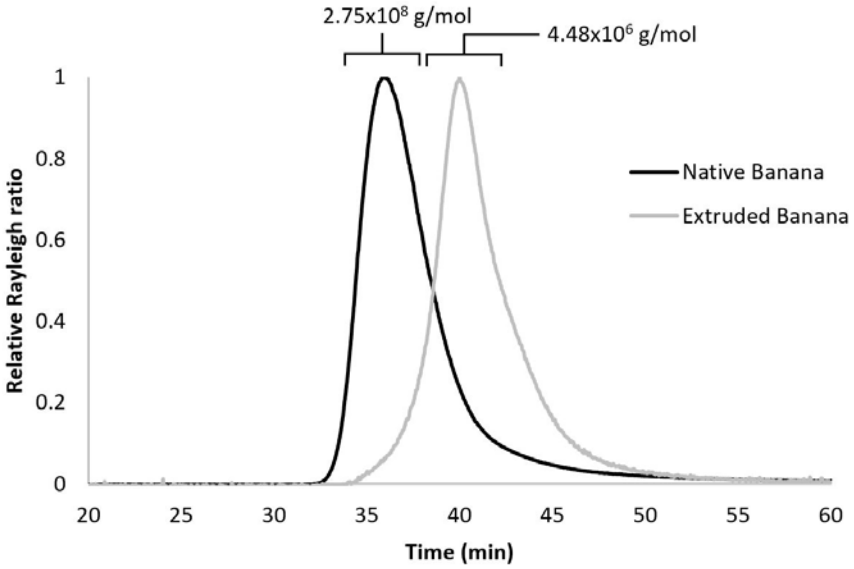

Figure 2. HPSEC-MALS chromatogram of native and extruded banana starch.

This occurrence was already observed by Martinez et al. (submitted) in acid-converted maize starch with extensive amylopectin hydrolysis. It is worth of mentions that no significant differences in amylose content between banana and maize starches were found (~30 %), although rice starch exhibited slightly lower amylose ratio (18%, data not shown).

Unlike crumb samples, no endothermic peak attributed to retrograded amylopectin was detected in crust samples, which is likely ascribed to the minimum amount of gelatinized starch in the crust.

3.2 Physical properties of breads

Crumb porosity and microstructure images (Fig. 3 and supplementary material 4) revealed slight differences in the crumb pore distribution of the breads. The gluten-free crumb consists of a porous and resilient HPMC-starch matrix that encloses, in honeycomb fashion, minute gas cells, making up the loaf volume. Control, NB and MB breads possessed a fine and closely-packed pore distribution, whereas EB bread crumb presented larger and heterogeneous cells. Carrillo-Navas, et al. (2016) found a more heterogeneous crumb microstructure of (larger and heterogeneous gas cells) of bread containing gelatinized flour. This occurrence could be explained based on the thicker cell walls observed through SEM in MB and EB crumbs (supplementary material 4), which has been previously reported to result in coarser cell structures (Gorton, 2009).

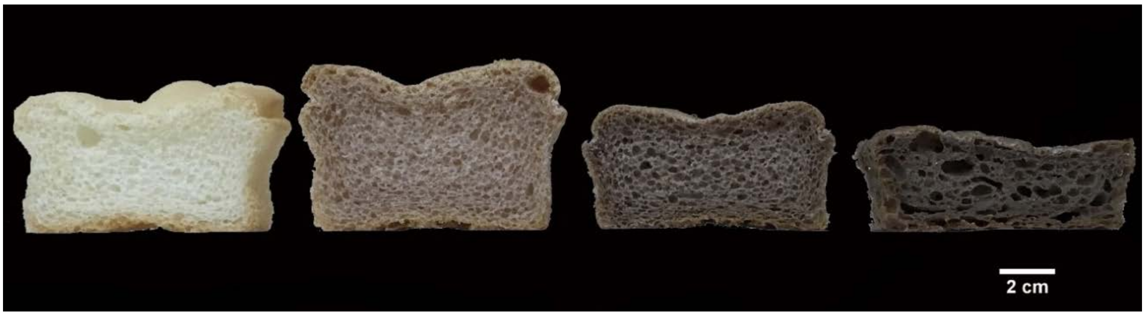

Figure 3. Pictures of central slices of bread. a) Control bread; b), bread made with 20% native banana starch (NB); c) bread made with 20% of a 1:1 mixture of native and extruded banana starch (MB) and d) bread made with 20% extruded banana starch (EB).

The specific volume of breads and textural properties of crumbs are shown in Table 1. Interestingly, the inclusion of native banana starch brought about breads with higher specific volume (Table 1 and Fig. 3), effect that was similarly reported by Sarawong, Gutiérrez, Berghofer, and Schoenlechner (2014) with inclusion of up to 25% of green plantain flour. It is well-known that during the first steps of baking, the expansion of gas cells embedded in the dough (i.e., oven rise) occurs until, when reaching a certain temperature (60-90 ºC), the hydrated starch gelatinizes leading to crumb setting (Le-Bail et al., 2011). Therefore, the higher onset of gelatinization (T_o) of banana starch (supplementary material 1) compared to the basic starch source (rice flour and maize starch) could give the dough extra time for expansion before crumb settling, as Gorton (2009) suggested to occur with other starches. This would also entail a greater integrity of banana starch granules during prolonged heating (Zhang et al., 2005), which would reinforce the continuous phase (starch-hydrocolloid matrix) surrounding the gas cells (Martinez & Gomez, 2017; Roman, de la Cal, Gomez, & Martinez, 2018). On the other hand, the drop in the specific volume with the addition of extruded banana starch could be due to the slightly higher cold viscosity property of gelatinized starch compared to native counterparts, as observed in the pasting profile (supplementary material 2). This event would increase dough consistency and limit the mobility of gases and other dough components and ultimately limit dough expansion (Carrillo-Navas et al., 2016). Interestingly, in this work we further showed that a 1:1 native to extruded banana starch composite would diminish the negative effects of the presence of gelatinized starch in the dough, attaining breads with slightly lower specific volume than control but greater than EB.

The texture parameters of bread are presented in Table 1. A higher hardness was found when decreasing the specific volume of the gluten-free breads. An inverse correlation of bread specific volume with its hardness has been reported previously in several studies (Martinez, et. al. 2018; Pongjaruvat, Methacanon, Seetapan, Fuongfuchat, & Gamonpilas, 2014). This higher resistance to crumb deformation has been related to the lower cell density (number of cells per area) and more compact structure in low volume breads. Furthermore, the lower moisture content of EB (Luyts et al., 2013) and the thicker cell walls of EB and MB crumbs (Carrillo-Navas et al., 2016; Martinez, Oliete, Román, & Gómez, 2014; Pongjaruvat et al., 2014) may have also accounted for its increased hardness. In general, an increase in hardness tends to be accompanied by a decrease in crumb elasticity and cohesiveness (Martínez, et al., 2018; Pongjaruvat et al., 2014). Although no significant differences were found for the springiness of any of crumb samples, more resilient and cohesive breads were evident when including native banana in the formula, which likewise could support the idea that banana starch help reinforce the dough matrix. Conversely, the inclusion of extruded banana starch resulted in less cohesive and resilient breads compared to the native banana counterpart, but with small differences with the control sample. In any case, the negative effect of extruded banana on crumb texture was minimized when mixed with its native counterpart.

3.3 Starch digestibility in bread crumb and crust

Starch digestion fractions corresponding to slowly digestible starch (SDS) and resistant starch (RS) were determined according to the Englyst time-based classification (Englyst, Kingman, & Cummings, 1992) and shown in Fig. 4 as bread basis (b. b.).

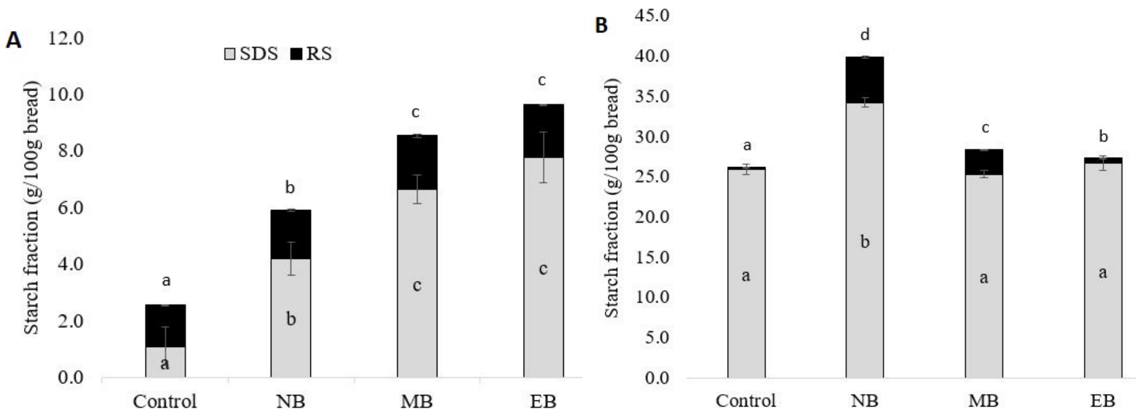

Figure 4. Slowly digestible starch (SDS) and resistant starch (RS) of bread crumb (A) and crust (B). a) Control bread; b), bread made with 20% native banana starch (NB); c) bread made with 20% of a 1:1 mixture of native and extruded banana starch (MB) and d) bread made with 20% extruded banana starch (EB).

Control gluten-free bread crumb (Fig. 4A) exhibited low SDS content (1.09 %), which is in agreement with results from multiple studies with breads (Martinez et al. 2018; Lau, Soong, Zhou, & Henry, 2015) and may be expected due to the complete starch gelatinization in the crumb (Table 2). SDS significantly increased with the incorporation of banana starch and, especially, of extruded banana starch. Martinez et al. (submitted) recently reported that amylopectin with a short chain population of branches of an average length higher than 15.5 GU may result in the formation of slowly digestible supramolecular structures from fully gelatinized material during retrogradation. In the present work, banana amylopectin short branches exhibited an average length of 17.0 GU that were involved in molecular interactions during retrogradation, as indicated by the higher enthalpy of retrograded enthalpy in banana containing crumbs (Table 2). These supramolecular assemblies have been reported to be slowly digested by different authors (Zhang, et al. 2008; Martinez et al. submitted). In this work, banana amylopectin also showed a higher proportion of long chains ($h_{AP2/hAP1}$ = 0.73), structural feature that has been previously reported to result in faster retrogradation on cooling than other amylopectins and lead to retrograded starch less accessible to amylase digestion (Zhang and Hamaker 2012). Secondarily, other mechanical properties at macroscopic level, such as crumb porosity, hardness and resiliency, may have had an influence on starch bioaccesibility during *in vitro* digestion. Control and NB presented similar crumb porosity and specific volume and NB crumb was softer. Therefore, the higher cohesiveness of NB crumb would be the only contributor at reducing crumb breakdown during digestion, which would not coincide with the trend reported by Martinez et al. (2018) showing higher SDS in less cohesive bread crumb.

As hypothesized in the present study, extruded banana starch resulted in a further increase of SDS in the crumb. Interestingly, a fivefold improvement in SDS performance (from 1.09 to 7.76%) was attained with a 20 % replacement of the base starchy ingredient by extruded banana starch. Martinez et al. (submitted) reported that a molecular size reduction of amylopectin through acid-hydrolysis increased its propensity to re-associate during storage and resulted in slowly digestible supramolecular structures. Here, this occurrence is also demonstrated with shear molecular fragmentation during extrusion according to the higher enthalpy of retrograded amylopectin in MB and EB crumbs. Smaller amylopectin molecules could be more mobile and have better alignment properties during retrogradation. Added to that, MB and EB crumbs were harder and more compact, which may result in a higher resistance to physical breakdown during digestion and, therefore, in limitations of digestive enzymes to access the “entrapped” starch (Martinez, et al., 2018).

The amount of resistant starch in the crumb ranged from 1.45 to 1.89% (g/100 g bread), which should be considered low to promote any beneficial physiological implication. An enhancement in the RS content of the crumb was visible with the incorporation of banana starch, especially in MB and EB samples. Similarly, Sarawong et al. (2014) found a significant higher content of RS (up to ~2.3%) in gluten-free breads with the addition of 15-35% native banana flour. These authors related this event to the higher initial amounts of RS in the initial flour (RS2), although the degree of starch gelatinization and the spatial location of RS (crumb or crust) were not reported. In all our samples, since starch was completely gelatinized (Table 2), short-term retrogradation upon cooling and storage, mainly involving the re-association of amylose chains that are enzymatically resistant (RS3) [Patel et al., 2017], is expected to be the main precursor for the RS increase (Englyst, et al., 1992). In fact, banana starch presents an amylose content of 30 % (relatively higher than that of the rice-maize base mixture). The native banana starch provided by the manufacturer contained 42.21 % RS (in the form of RS2), however attention must be paid when this ingredient is incorporated into foods that will suffer hydrothermal processing with enough amount of water for starch gelatinization.

Crust samples presented significantly higher SDS (25.30-34.17 %) and relatively higher RS (0.26-5.66 %) than crumb counterparts, which agrees with previous works (Martinez, et al. 2018) and was attributed to the significantly lower degree of gelatinization of bread crust compared to crumb (Table 2). Surprisingly, only the inclusion of native banana starch gave rise to a significant increase in SDS of bread crust, which could derive from the higher amount of ungelatinized maize starch, an inherently perfect source of SDS (~50 %), resulting from the lower starch gelatinization in NB (Table 2). Added to that, part of the RSII of native banana starch, which is relative fragile under cooking conditions (even after 10 min cooking), could have been converted into RDS and SDS (Bi et al., 2017).

Results also showed that a 20 % replacement with native banana starch brought about the highest RS in the crust (5.66 %), which seems logical regarding the inherent higher resistance of banana starch in its native granular state (RS of 42.21%). It is worth of mentions that the inclusion of extruded banana starch also resulted in a small but significant increase in RS compared to control crust (from 0.26 to 0.72 %), which could comprise RS3 (retrograded amylose) and RS5 (complexation of banana amylose with free lipids during extrusion) inherently developed in the raw ingredient during extrusion and upon cooling (Camire, Camire, & Krumhar, 1990). In fact, extruded banana starch possessed higher RS than the combination of rice and maize flours.

3.4 Sensory evaluation

A hedonic sensory test was conducted to evaluate the feasibility of using banana starch as a nutritional improver from the carbohydrate standpoint (Table 3). Interestingly, the inclusion of native and extruded banana starch generally resulted in gluten-free breads with improved odor and flavor (the latter especially improved with extruded banana) compared with control bread. Visual appearance exhibited the greatest differences, highlighting a significantly lower rating with the use of extruded flour (EB), effect that was minimized when mixing this flour with the native counterpart (MB). We believe that it might be related to the lower specific volume of MB and EB compared to NB and control bread (as previously seen in Fig. 3 and Table 1), rather than to differences in color. In fact, some of the panelists indicated their preference for banana-based breads due to their darker color. Differences in crumb color (shown in Fig. 3) should be explained based on the darker brown color of banana starch compared with the whitish rice flour and maize starch (data not shown), as Segundo et al. (2017) already pointed out for wheat cakes enriched with banana flour. On the other hand, in bread crust, alongside the natural color of the starch material, the higher amounts of simple sugars in banana flour (Emaga, Andrianaivo, Wathelet, Tchango, & Paquot, 2007) also significantly darken crust color. Furthermore, starch dextrinization during extrusion results in a subsequent formation of reducing sugars (Camire, et al., 1990) that participate in Maillard and caramelization reactions on the hot bread surface during baking (Gorton, 2009). Therefore, a greater color of EB crusts should be expected. Regarding the consumer perception for bread texture, a negative correlation with crumb hardness instrumentally measured was evident (Table 1), which was discussed in section 3.2.

Table 3. Effect of native and extruded banana starch on sensory properties of breads

Sample	Appearance	Odor	Flavor	Texture	Overall liking
Control	6.6c ± 1.4	5.5a ± 1.6	4.9a ± 1.7	5.4b ± 1.8	5.5ab ± 1.5
Native Banana	6.5c ± 1.4	6.0b ± 1.4	5.3ab ± 1.6	5.8b ± 1.6	6.0b ± 1.4
Mixture Banana	5.9b ± 1.6	6.1b ± 1.4	5.1ab ± 1.6	5.3b ± 1.7	5.6ab ± 1.3
Extruded Banana	4.2a ± 2.0	6.2b ± 1.4	5.5b ± 1.6	4.7a ± 1.8	5.4a ± 1.5

Values followed by the same letters within each parameter indicate no significant differences ($p \leq 0.05$).

Despite the lower scores of EB for appearance and texture, interestingly some panelists expressed their preference for extruded banana containing breads due to their more pleasant flavor. In fact, EB bread was the best valuated in terms of flavor, which may have had an important weight at reducing the differences of overall liking. Specifically, differences for overall liking between EB and NB (the best-scored one) were not greater than 0.5. The enhancement in the scores for odor and flavor with the inclusion of 20%

banana starch are undoubtedly a positive indication of the feasibility of including banana in the formulation of gluten-free breads without declining their acceptability. In fact, in this study we attained breads with 30 % replacement by native banana starch and a 1:1 native to extruded banana starch composite with acceptable physical attributes. However, 30 % replacement was not chosen due to the flat bread (very low volume) with solely extruded banana starch. In this way, a further replacement level (at least 30 %) with native banana starch and 1:1 native to extruded banana starch composite is attainable and expected to promote an additional reduction of starch digestion rate.

Differences in bread appearance should be taken into account when including banana starch into the formulation, although this score can be modulated by increasing fermentation time or adjusting the water content (dough hydration) in the formulation in order to increase the specific volume of the loaves.

4 Conclusions

Diets containing a high proportion of foods with low glycemic response are associated with reduced risks of metabolic syndrome diseases. However, white bread still remains the consumers’ first choice and its crumb is formed by starch highly susceptible to enzyme digestion due to its gelatinization during hydrothermal processing. Here, we present a successful strategy to slow down the digestion rate of the fully gelatinized starch present in baked goods by the manipulation of the starch molecular structure. A and B1 chains of banana starch, with an average length of 17.0 GU, re-associated upon cooling (perhaps also with long internal amylopectin chains) forming slowly digestible supramolecular structures. This study also shows, for the first time, that the propensity of amylopectin molecules to form structurally driven SDS is further improved by a reduction of their molecular size by means of extrusion (a clean and cost-effective technology). Results of this work are also expected to contribute to the improvement of the sustainability of food systems and increasing local and global food availability. Banana pulp is an untapped food by-product and, in this work, we demonstrate its enormous nutritional potential with a successful application.

Acknowledgments

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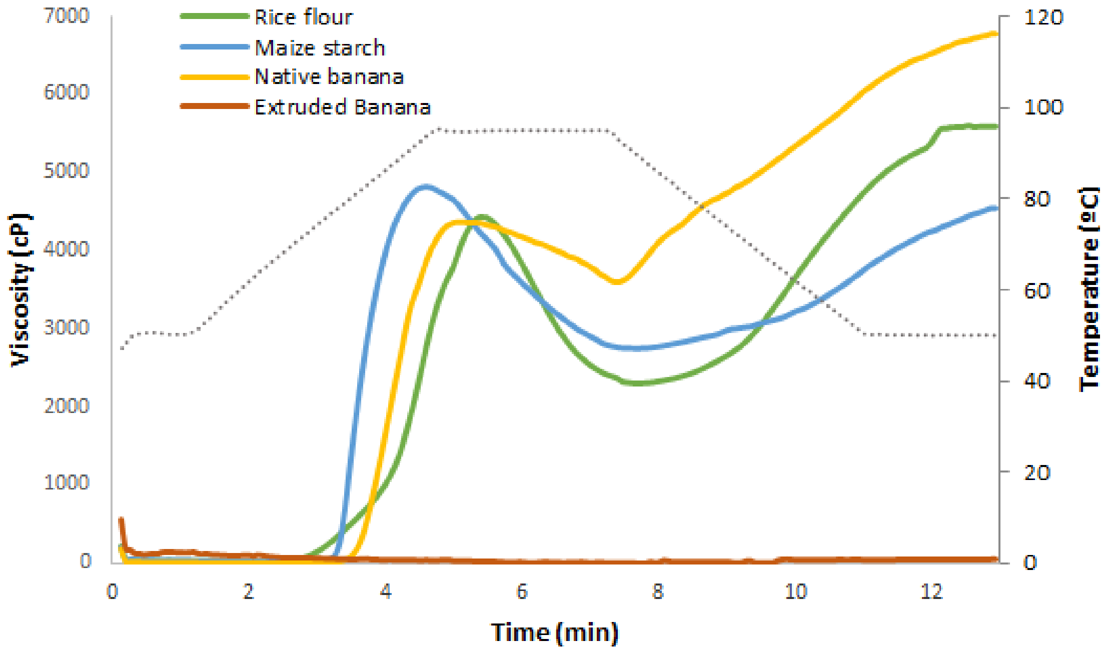
Supplementary material

Supplementary material 1. Gelatinization properties of starchy ingredients.

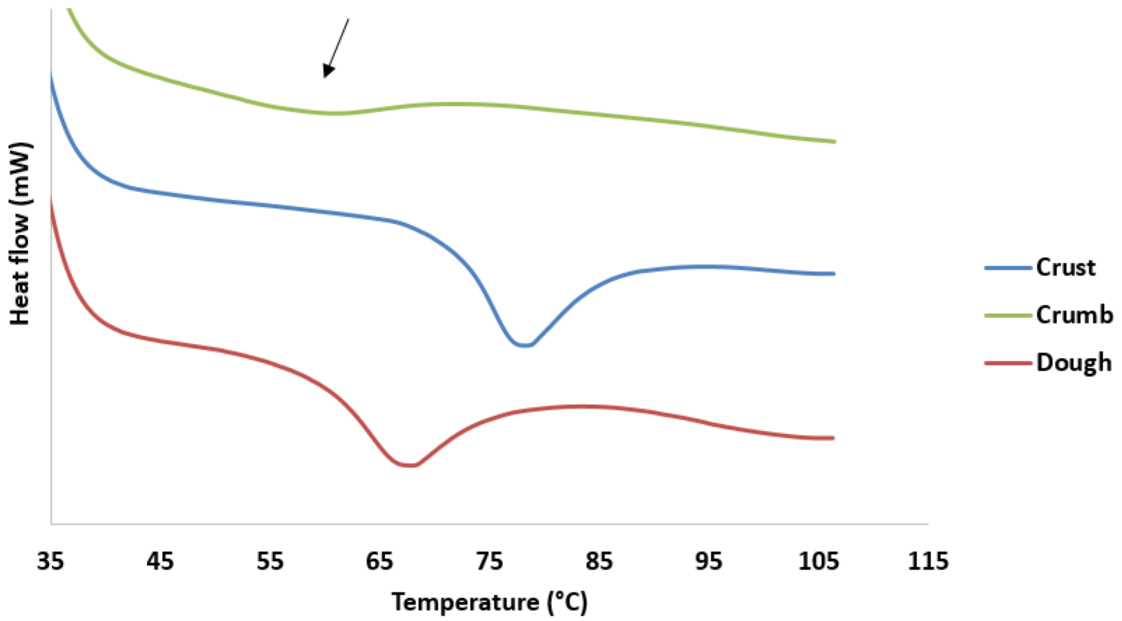
Sample	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH_g (J/g, db)
Rice flour	67.57 ± 0.02	73.72 ± 0.05	81.64 ± 0.20	11.91 ± 0.18
Maize starch	62.12 ± 0.14	69.87 ± 0.01	85.36 ± 0.60	7.39 ± 0.17
Native banana starch	71.74 ± 0.25	78.62 ± 0.03	87.69 ± 0.30	10.81 ± 0.19
Extruded banana starch	n.d.	n.d.	n.d.	n.d.

T_o, T_p and T_c indicates onset, peak and conclusion temperature of gelatinization; ΔH_g, enthalpy of gelatinization expressed in J/g dry solids, n.d. non detectable.

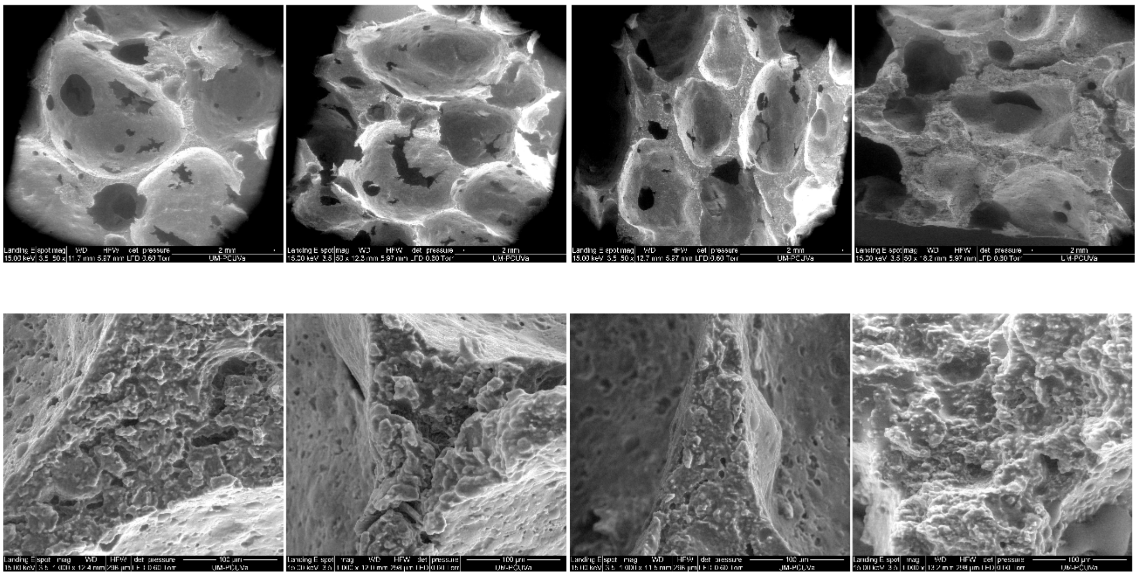
Supplementary material 2. Pasting profile of starches and flours used for bread making.



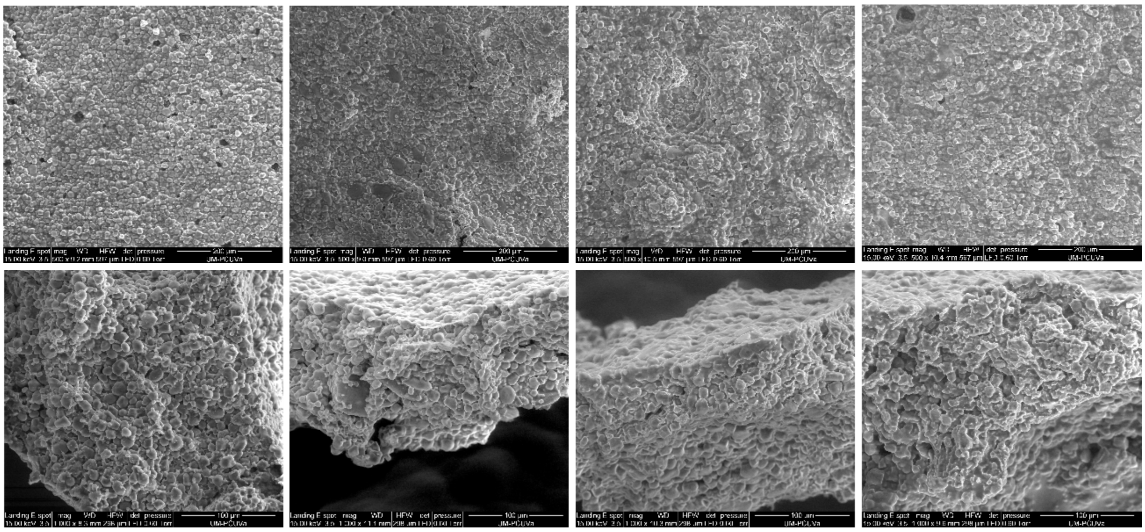
Supplementary material 3. DSC thermograms of crumb samples. Arrow point at endotherm corresponding to retrograded amylopectin.



Supplementary material 4. Micrographs of crumb sections of breads at low (first row) and high (second row) magnification corresponding to control, native banana, mixture banana and extruded banana (from left to right).



Supplementary material 5. Micrographs of crust surface (first row) and crust section (second row) corresponding to control, native banana, mixture banana and extruded banana (from left to right).



DISCUSSION OF THE RESULTS

9. DISCUSSION OF THE RESULTS

The main objective of this doctoral thesis was the development of novel plant-based products with improved physical, organoleptic and/or nutritional value by utilization of extruded flours as a functional ingredient. Thus, aspects such as the starch source, particle size or severity of the extrusion treatment of the extruded flours were assessed during this research work.

Based on the greater water binding capacity and thickening power of pregelatinized extruded flours, the potential of extruded flour to work as fat substitutes in food emulsions was evaluated. In mayonnaise-like emulsions, increasing levels of oil replacement by an extruded flour paste gradually increased the number and reduced the size of oil droplets and led to a greater elastic and thixotropic character of the oil-in-water emulsion. Nonetheless, extruded flour yielded mayonnaise-like emulsions with a great emulsion stability and higher stability in terms of freezing, which could be attributed to the hydrophilic nature of the pregelatinized extruded starch, stabilizing water during crystallization and maintaining separated the oil droplets. Differences in the rheological behavior were minimized when appropriate flour-water ratio of the extruded paste (1:3 flour/water) was chosen, obtaining more similar rheological properties to the full-fat one. Conversely, fat replacement in cake batters reduced the number of air bubbles and increased their size, leading to a less viscoelastic batter. This worse aeration and lower viscous and elastic properties of the batter could have given rise to a higher instability and coalescence, which resulted in a decreased volume of the cake and a harder, less elastic and cohesive crumb. Nonetheless, the negative effect of partial oil substitution (up to 2/3) by the extruded flour paste (1:4 flour/water) was minimized when emulsifier was incorporated due to an improved aeration. Thus, emulsifier addition helped obtained better scores in the consumer acceptability of the reduced fat cakes with 2/3 of oil substitution. It is noteworthy that water content in the extruded flour paste could be diminished to a 1:3 ratio in order to further achieve a more stable and viscoelastic cake batter, as already seen in mayonnaise emulsions.

The greater water absorption capacity of extruded flours can also be useful to optimize/enhance viscosity in cold solutions, which may be very interesting for certain food applications such as improvement of batter pick-up or the making of instant dishes. In batters for coating, replacement of native wheat flour by wheat flour subjected to increasing severities of extrusion treatment caused an important increase in the apparent viscosity of the batters, especially when extruded flour was subjected to more severe extrusion conditions (complete gelatinization). This higher cold-viscosity and presumably stickiness of depolymerized extruded starch led to an increased batter pick-up, indicating a better coverage of battered food. Partial replacement of native wheat flour by wheat extruded flours in batter formula also yielded higher moisture content due to higher water binding capacity and film forming ability of more pregelatinized flours while oil remained practically unchanged. A harder and crispier texture was also visible when higher levels of more severely treated extruded flour were incorporated. For the first time, volatile compounds in fried batter systems were analyzed. This analysis revealed that fried batters containing 15% of most severely treated extruded flour presented lower amount of lipid oxidation (lower rancidity notes) and higher furfuryl alcohol content (pleasant aroma and darker crust). In addition, fried batters made with mild and intermediate severely treated flours showed better consumer acceptability based on their more appealing appearance and crispier texture attributes. Thus, a 15% of wheat flour replacement by wheat flour subjected to different extrusion treatments demonstrated to be an effective tool to improve the physical and sensory quality of batters for coating.

On the other hand, for instant makings, pregelatinized extruded flours (extruded under severe extrusion conditions) can be used in sauces applications without heating with similar rheological properties to those obtained in sauces heated to develop their viscous properties. Nonetheless, these properties greatly depend on the cereal type and its particle size. In this sense, cold-prepared model sauces made with rice extruded flours, with lower protein and amylose contents, presented lower shear-thinning behavior, consistency index and yield stress than wheat based sauces, which also presented more tightly packed network structure. The higher consistency and viscoelastic character for wheat based sauces may be reflected as an increase in the sensory consistency and pumping requirements. Regarding particle size of the extruded flours, the finest particle sizes of wheat and rice extruded flours were not preferable for sauce making due to their weaker rheological behavior and higher syneresis. Conversely, extruded flours coming from rice seemed to be the more appropriate flours for cold-sauces making, based on their lower sensitivity to shearing and lower water release after freeze-thaw process.

Food texture is a critical quality parameter that depends on the retrogradation behavior of starch. In this thesis, it was hypothesized that starch molecular shear fragmentation during extrusion could decrease starch inter-molecular associations responsible for negatively affecting the mechanical properties (G' increase over time) of extruded flour gels. Therefore, contributing to diminish the extent of starch retrogradation phenomena in starch-based foods, and, increase their physical and sensory quality. Extrusion processing under the same specific mechanical energy (SME) of wheat, maize and rice flours was shown to be an effective way to reduce gel hardening during storage. Interestingly, results suggested two distinct predominant mechanisms, based on the starch composition and structure of the cereal source, which led to the weaker gel structure. In wheat and maize, the significant reduction of amylose (AM) size diminished the number of AM-AM physical junction zones that build gel structure. On the other hand, the extrusion of rice flour, containing starch with lower AM ratio [higher amylopectin (AP) ratio] and previously reported to have lower proportion of long chains, resulted in a marked AP fragmentation that caused a decrease in the ratio of inter- to intra-molecular interactions involving AP. The reduced starch retrogradation of flour gels after extrusion can give insights into retrogradation-controlled phenomena such as sauce/cream syneresis and gluten-free bread staling. In fact, the results of the lower syneresis found in extruded rice based sauces compared to wheat counterparts are explained by the aforementioned mechanism observed for retrograded extruded rice flour that resulted in decreased AP inter-molecular interactions.

Albeit retrogradation has been reported as one reason for bread staling it is also known to result in a reduction of the rate and extension of starch digestion and, hence, beneficial from a nutritional perspective. Previous evidence has shown that certain starches containing long A and B1 amylopectin chains, such as banana starch, and starches smaller in size are more prone to form structurally-driven slowly digestible starch in fully gelatinized material, such as a bread crumb. Based on these facts, it was hypothesized that a molecular size reduction by extrusion would further improve mobility and alignment properties of banana amylopectin molecules resulting in bread crumbs with significantly higher slowly digestible starch (SDS). Banana starch results showed a reduction of molecular weight of banana starch from 2.75×10^8 to 4.48×10^6 g/mol during extrusion due to shear fragmentation. Meanwhile, in gluten-free breads, a fivefold SDS increase in fully gelatinized bread crumb with only a 20 % of extruded banana inclusion was observed. DSC data attributed this occurrence to the formation of supramolecular structures upon storage involving amylopectin branches (A and B1 chains) especially those from fragmented amylopectin. Interestingly, results from the hedonic sensory test showed no differences in global acceptability between control and banana containing breads, validating feasibility of including banana in the formulation. Therefore, this work shows for the first time a successful application where the propensity of amylopectin molecules to form structurally driven SDS is further improved by a reduction of their molecular size by means of extrusion.

CONCLUSIONS

10. CONCLUSIONS

The main conclusion of this doctoral thesis is that it is possible to successfully use extruded flours as a functional ingredient to develop novel cereal based products with an improved physical, organoleptic and/or nutritional value, fulfilling in this way, the main objective of this doctoral thesis. More specifically, pregelatinized extruded flours were demonstrated a feasible ingredient for oil replacement, cold-viscosity or “instant” application, viscosity enhancer, reduction of retrogradation-related phenomena, and as a nutritional improver of baked goods from the carbohydrate standpoint. In addition, deeper knowledge was gain on the physicochemical properties of these extruded flours as well as on starch structure-function relationship, helping, in turn, find new end-uses of extruded flours, which may increase their market.

The most relevant conclusions obtained from each of the research works included in this thesis are described below.

- Fat replacement by extruded flour yields mayonnaise-like emulsions with a great emulsion stability and higher stability in terms of freezing, making possible the application of these mayonnaises in frozen ready-meals.
- Extruded flour can be used as an effective oil replacer in mayonnaises and cakes if the water amount of the extruded flour paste is controlled and if an emulsifier is included, respectively.
- Replacement of native wheat flour by wheat flour subjected to increasing severities of extrusion treatment resulted in a greater batter pick-up and more pleasant volatile compounds in fried batters.
- Fried batters made with extruded flour showed improved consumer acceptability, based on their better appearance and crispy texture attributes.
- The finest particle sizes of wheat and rice extruded flours are not preferable for sauce making due to their weaker rheological behavior and higher syneresis.
- Extruded flours coming from rice seem to be the more appropriate flours for cold-sauces making, based on their lower sensitivity to shearing and lower water release after freeze-thaw process.

- Extrusion processing under the same specific mechanical energy (SME) resulted in different starch fragmentation in maize, wheat and rice flours, which in all cases reduced the tendency of starch to form inter-molecular interactions contributing to gel development over time.
- The reduced starch retrogradation of flour gels after extrusion can give insights into retrogradation-controlled phenomena in starch-based products, such as sauce/cream syneresis and gluten-free bread staling.
- A fivefold slowly digestible starch (SDS) increase in fully gelatinized bread crumb was attained with only 20% substitution of the starchy material by extruded banana starch.
- The propensity of banana amylopectin molecules to form structurally driven SDS is further improved by a reduction of their molecular size by means of extrusion.
- The use of native and extruded banana starch in gluten-free makings offers an interesting alternative approach to nutritionally improve gluten-free breads without worsen consumer's acceptability.

As a whole, the findings of the present thesis could contribute to improve the quality of plant-based products, expanding the food choices available in the market. The physical modification of starchy materials by extrusion is consistent with new food megatrends towards natural products and offers the potential to change starch functionality at a low cost and in an environmentally friendly way, fulfilling in this way both consumer and industrial needs. Results of this work are also expected to contribute to the improvement of the sustainability of food systems and waste reduction while increasing food availability. Banana pulp is an untapped food by-product and, in this work, we demonstrate its enormous nutritional potential with a successful application in a baked good. Based on the latest results presented in this doctoral thesis, future work perspectives are oriented to deeper studies of the nutritional potential of banana extruded flours by its inclusion in other food products and by more *in vitro* and *in vivo* starch digestion studies.

ANNEX

ANNEX

This Annex includes other SCI publications in which the author of this thesis also contributed and that were not included in this doctoral thesis.

1. Román, L., Martínez, M. M., Rosell, C. M., & Gómez, M. (2015). Effect of microwave treatment on physicochemical properties of maize flour. *Food and Bioprocess Technology*, *8*, 1330-1335.

2. Román, L., Dura, Á., Martínez, M. M., Rosell, C. M., & Gómez, M. (2016). Combination of extrusion and cyclodextrin glucanotransferase treatment to modify wheat flours functionality. *Food Chemistry*, *199*, 287-295.

3. Barcenilla, B., Román, L., Martínez, C., Martínez, M. M., & Gómez, M. (2016). Effect of high pressure processing on batters and cakes properties. *Innovative Food Science & Emerging Technologies*, *33*, 94-99.

4. Dhen, N., Román, L., Rejeb, I. B., Martínez, M. M., Garogouri, M., & Gómez, M. (2016). Particle size distribution of soy flour affecting the quality of enriched gluten-free cakes. *LWT-Food Science and Technology, 66*, 179-185.

5. Román, L., González, A., Espina, T., & Gómez, M. (2017). Degree of roasting of carob flour affecting the properties of gluten-free cakes and cookies. *Journal of Food Science and Technology*, *54*, 2094-2103.

6. Segundo, C., Román, L., Lobo, M., Martinez, M. M., & Gómez, M. (2017). Ripe banana flour as a source of antioxidants in layer and sponge cakes. *Plant Foods for Human Nutrition*, *72*, 365-371.

7. Dhen, N., Rejeb, I. B., Martínez, M. M., Román, L., Gómez, M., & Gargouri, M. (2017). Effect of apricot kernels flour on pasting properties, pastes rheology and gels texture of enriched wheat flour. *European Food Research and Technology*, *243*, 419-428.

8. Román, L., Martínez, M. M., Rosell, C. M., & Gómez, M. (2017). Changes in physicochemical properties and in vitro starch digestion of native and extruded maize flours subjected to branching enzyme and maltogenic α-amylase treatment. *International Journal of Biological Macromolecules*, *101*, 326-333.

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10. Roman, L., Gomez, M., Li, C., Hamaker, B. R., & Martinez, M. M. (2017). Biophysical features of cereal endosperm that decrease starch digestibility. *Carbohydrate Polymers*, *165*, 180-188.

11. Martínez, M. M., Román, L., & Gómez, M. (2018). Implications of hydration depletion in the in vitro starch digestibility of white bread crumb and crust. *Food Chemistry*, *239*, 295-303.

12. Pico, J., Antolín, B., Román, L., Gómez, M., & Bernal, J. (2018). Analysis of volatile compounds in gluten-free bread crusts with an optimised and validated SPME-GC/QTOF methodology. *Food Research International*, *106*, 686-695.

13. Román, L., de la Cal, E., Gómez, M., & Martínez, M. M. (2018). Specific ratio of A-to B-type wheat starch granules improves the quality of gluten-free breads: Optimizing dough viscosity and pickering stabilization. *Food Hydrocolloids, 82*, 510-518.

14. Gómez, M., Román, L. (2018) Role of different polymers on the development of gluten-free baked goods. In Gutierrez, T. (Ed.) *Polymers in Food Applications*. Springer Nature. Expected publication date: Sep 2018.