MODIFICACIÓN FÍSICA DE ALMIDONES ENRIQUECIDOS CON PROTEÍNAS

PHYSICAL MODIFICATION OF PROTEIN-ENRICHED STARCHES

TRABAJO FIN DE MÁSTER

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RESUMEN

El tratamiento microondas (MW) es un método físico utilizado para modificar y mejorar las propiedades funcionales de los almidones. Esta investigación evaluó los efectos de la irradiación MW sobre almidones de patata, maíz y arroz humedecidos al 30% y enriquecidos con 0 o 5% de proteína (caseinato de calcio o soja). La capacidad de absorción de agua (WAC), el índice de absorción de agua (WAI), el índice de solubilidad de agua (WSI) y el poder de hinchamiento (SP) fueron utilizados para estudiar los cambios en las propiedades funcionales de las muestras. También se evaluó el contenido de amilosa, las propiedades de empastado y las propiedades viscoelásticas de los geles de los almidones nativos y tratados. Los resultados mostraron que el origen del almidón influyó considerablemente en sus propiedades, siendo el almidón nativo de arroz el que mostró los valores WAC, WAI, SP y WSI más altos. Los cambios en el WAC, SP, WAI y en los parámetros de viscosidad demostraron que el MW produjo cambios en la estructura original de los gránulos de almidón y en sus interacciones moleculares. La patata sufrió los cambios más marcados en las propiedades reológicas y de empastado. Además del enriquecimiento nutricional de los almidones, la incorporación de proteínas aumentó el WAC, WAI, SP y WSI, disminuyó la viscosidad de los geles y aumentó la estabilidad.

Palabras clave: Almidón. Arroz. Maíz. Patata. Propiedades funcionales. Reología. Microondas

ABSTRACT

The microwave treatment (MW) is a physical method used to modify and improve the functional properties of starches. This research evaluated the effects of MW irradiation on potato, maize and rice starches moistened to 30% and enriched with 0 or 5% protein (calcium caseinate or soy). Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) were used to study changes in functional properties of samples. The amylose content, the pasting properties and the viscoelastic properties of the gels made from native and modified starches were also evaluated. The results showed that the origin of the starch had a notable influence on its properties, being the native rice starch the one that showed the highest WAC, WAI, SP and WSI values. The changes in WAC, SP, WAI and viscosity parameters showed that MW caused changes in the original structure of the starch granules and in their molecular interactions. Potato suffered the most remarkable changes in rheology and pasting properties. Apart from enriching the starches, the inclusion of proteins increased WAC, WAI, SP and WSI, decreased the viscosity of gels and increased the stability.

Keywords: Functional properties. Maize. Microwave. Rheology. Rice. Starch. Potato.

1. INTRODUCTION

Celiac disease is a chronic inflammatory disease of the small intestine that is triggered and maintained by the intake of gluten-containing cereals such as wheat, barley or rye. It is characterized by an autoimmune response in genetically susceptible individuals, which results in this chronic inflammation and the progressive disappearance of the microvilli (Matthias et al., 2011). Gluten-free (GF) products are a growing sector in the food industry. The increasing number of people identified with gluten intolerance who demand GF foodstuffs with a good nutritional and organoleptic quality is one of the reasons why the development of these products is emerging. On the other hand, the group of people interested in GF products also includes those looking for nonallergenic ingredients. In addition, this kind of products can function as prototypes for the development of other products addressed to specific vulnerable groups of population with special nutritional needs such as diabetics (Villanueva et al., 2015).

Gluten is stored with starch in the endosperm of various grains and is composed of 75% storage proteins on a dry weight basis, called prolamins and glutenins (Shewry et al., 2002). The consumption of gliadins, a kind of prolamins, causes celiac disease in gluten intolerant individuals. In spite of this, gluten has a very important role in products for their singular viscoelastic properties where gas is occluded and contained in the liquid phase during dough development from the flour, water and other ingredients (Ashraf et al., 2012). Thus, the removal of gluten from food products has a significant impact on their structure, texture and sensory attributes (Witczak et al., 2016). So, despite there are several GF products nowadays in the market, baked products from gluten-free ingredients have normally poor physicochemical and sensory quality, lack fibre, vitamins and nutrients, which results in a worsening effect on the already nutritionally unbalanced diet of celiac sufferers (Ronda et al., 2013).

Starch is the main ingredient of such GF formulations and the primary source of stored energy in cereal grains. It is formed of granules of variable size and is composed mainly of two different polymer fractions named amylose and amylopectin. It may also contain proteins, lipids, water and very small amounts of other compounds such as phosphorus, magnesium, and calcium (Lewicka et al., 2015). The amylose and amylopectin in starch are not free in nature, but make up a semicrystalline aggregate organized in the form of granules. Amylose is an essentially linear polymer of α-1-4-linked D-glucopyranose with a small number of branches and with a helical structure where the interior of the helix contains hydrogen atoms and the exterior contains hydroxyl groups. The presence of these hydrogen atoms in the interior of the helix makes amylose hydrophobic and enables it to form complexes with free fatty acids, component glycerides of fatty acids, iodine and some alcohols (Zavareze & Dias, 2011). On the other hand, amylopectin is a very large, highly branched molecule consisting of much shorter chains of α-1-4-linked glucose segments connected by α-1-6-linked branch points (Peroni et al., 2006). Amylopectin chains are organized radially within the granule with their non-reducing terminal ends oriented towards the surface, and are arranged with alternating crystalline areas (as a double helix) and amorphous areas (with regions of branching points). The functionality of starch is due to the ratio of amylose and amylopectin as well as their physical organization in the granular structure (Zavareze & Dias, 2011).

Native form of starch has limited applications in industry and is often modified by physical, chemical or enzymatic processes to provide it with specific functional properties. Physically modified starch is considered a natural material and a highly safe ingredient, safer than chemically modified starch. Besides, the presence of physically modified starch in food is not limited by legislation, which is considered an outstanding advantage compared to chemical and enzymatic modified starches (Klein et al., 2013).

Microwave (MW) radiation is a suitable method to modify starches physically and to improve their functional properties. Microwave is an electromagnetic radiation in the range from 3000MHz to 30GHz. During this process, part of the microwave energy delivered directly to the material is reflected, part is transmitted through the surface and, of this latter quantity, part it is absorbed. The proportions of energy, which fit in these three categories, have been defined in terms of the dielectric properties (Brasoveanu & Nemtanu, 2014).

In general, microwave propagation in materials depends on the dielectric and the magnetic properties of the medium, but starch has no magnetic component and responds only to the electric field of microwaves (Brasoveanu & Nemtanu, 2014). So, the main mechanism of microwave absorption in starch is the reorientation of polar molecules with respect to the imposed oscillating electric field. The fast change in the orientation produces heat by molecular friction, causing a bulk heating in all the sample and a faster heating rate in comparison to the conventional heating (Bilbao-Sainz et al., 2007; Román et al., 2015).

The rearrangement of intermolecular structure established by microwaves leads to changes in the water absorption ability, solubility and swelling power as well as in gelatinization parameters, syneresis and paste viscosity (Brasoveanu & Nemtanu, 2014). Because of that, the industrial application of microwaves has had a great interest on the chemical and physicochemical properties of cereal starches and, thus, numerous researches have been reported in the last decade. There are many factors related both to starch (type, water content, density, dielectric properties, etc.) and microwave processing (frequency, power, and time of exposure) that play an important function in dictating the response of granular starch to microwaves (Brasoveanu & Nemtanu, 2014). The structural characteristics of starches depend on this botanical source and determine the behavior of starches faced with MW treatment. Researchers have found that pasting and functional properties of starches are affected by amylose and phosphorous content, size distribution of starch granules and branch chain length of amylopectin (Peroni et al., 2006). Zavareze et al. (2010), Anderson & Guraya (2006) and Luo et al. (2006) investigated the effect of microwave on rice and maize starches with different amylose/amylopectin rates and observed that this treatment affects the pasting properties of high-amylose starches more intensely than in those with lower amylose content. Besides, Vermeylen et al. (2006) concluded that, in general, higher treatment temperatures and moisture contents caused the largest changes in starches. Some researchers have reported that peak viscosity of rice and potato starch samples decreased as temperature and microwave power level applied increased (Nadiah et al., 2015; Pinkrova & Hubackova, 2003), while the gelatinization temperature increased with these conditions (Vermeylen et al., 2006; Nadiah et al., 2015; Yang et al., 2017; Luo et al., 2006).

Beyond physico-chemical and functional considerations, gluten-free foods are often considered nutritionally poorer than equivalent wheat-derived foods. Different researches have studied the fortification of these GF products with proteins, not only for improving their nutritional values, but also to change their physico-chemical properties (Gallagher et al., 2003). Some studies have shown that starch-protein interactions affect the rheological, pasting, gelatinization, textural and physico-chemical properties of food system (Matos et al., 2014; van Riemsdijk et al., 2011; Gallagher et al., 2003; Marco & Rosell, 2008). Ronda et al. (2011) observed that using different starch sources (rice, corn, potato and wheat) with soy protein isolated at different percentages (0%, 10%, and 20%) affects the rheological properties of batters and the volume of baking products. The presence of protein made consistency, viscous and elastic moduli and adhesive energy values of batters increase and batter density decrease. Ronda et al. (2014) also reported that the supplementation of gluten-free doughs with proteins from vegetal sources (pea protein and soy protein isolates) led to higher viscoelastic moduli and steady viscosities, lower tan δ (loss tangent at a frequency of 1 Hz), instantaneous and retarded elastic compliances, being magnified with protein dose. While the incorporation of proteins from animal sources (egg albumin and calcium caseinate) resulted in different viscoelastic behaviors depending on the protein type, dosage and acidification, especially for casein. Proteins from vegetable sources led to breads with lower-specific volume and harder crumb, being these effects magnified with protein dose and reduced with acid addition (Villanueva et al., 2015).

In conclusion, fortification with proteins can improve the nutritional quality of GF products. However, the interaction between starch and protein has effects on the functional and rheological properties of products, so it is a critical issue in these formulations and should be more studied. On the other hand, MW treatment can improve functional properties of starches. However, a lot of studies show that, despite the advantages of microwave radiation, this process is joined by some limitations such as non-uniform temperature during microwave heating, complex and high cost specialized equipment and difficult optimization of processing parameters. So, the optimization of these variables and a better understanding of the changes which happens during MW treatment would enable to implement it in the food industry. Therefore, the effect of combining microwave treatment and protein addition on the rheological and functional properties of starches will be studied in this investigation.

2. OBJECTIVE

The aim of the present research was the physical modification of potato, maize and rice starches enriched with protein by microwave treatment to improve its application in gluten-free products.

The effect of microwave radiation (640s) to moistened (30%) potato, maize and rice starches enriched with 0 or 5% of protein isolate (Calcium Caseinate or Soy protein isolate) was evaluated on the functional and pasting properties, and the rheological properties of the gels prepared from the microwaved treated blends .

3. MATERIALS AND METHODS

3.1. Materials

Rice starch (9,9% moisture, amylose <10%, proteins 0,5% and 0,2% ash), maize starch (11,6% moisture, amylose 25%, proteins 0,35%, 0,1% ash) and potato starch (19,1% moisture, amylose 22%, proteins 0,06%, 0,4% ash) used in this research were procured by Ferrer Alimentación S.A. (Barcelona, Spain).

Soy Protein Isolated Supro 500-E IP was provided from Ferrer Alimentación S.A. (Barcelona, Spain) and Calcium Caseinate from Armor proteins (Saint-Brice-en-Coglès, France).

Rice flour from an *Indica* variety (13% moisture content, protein >6,5%, ash <0,9%, fat < 1% and gluten < 10 ppm) was supplied by Herba Ricemills SLU (Tarragona, Spain) and was used to predict the temperature evolution in the system during MW treatment.

The commercial assay kit Megazyme amylose/amylopectin (Wicklow, Ireland) was used to measure the amylose content of samples.

3.2. Methods

3.2.1. Temperature evolution test.

Moistened rice flours (30%) were used to carry out some preliminary tests in order to know the temperature evolution based on the duration of the microwave treatment. MW radiation was applied to samples in cycles of 20 s intervals combined with downtimes of 40 s. The temperature achieved by the starches after being treated with different durations of MW treatment (from 20s to 640s) was analyzed using Testoterm temperature strips (Testo, lugar, Spain). This flour was used as sample model because its protein content was similar to the exogenous protein added to starch (5%).

3.2.2. *Preparation of samples*

Samples were prepared with different starches (maize, rice and potato) and two types of proteins (Calcium Caseinate and Soy protein isolated). The protein content in the mixture starch-protein was either 0 or 5 g/100 g. When protein was added, the components were mixed during 15 min in order to get homogeneous.

First, the initial moisture content of samples was analyzed twice according to the official method 44-19 (AACC, 1995) by a Cobos Precision balance AX-200 (Barcelona, Spain) and a BINDER heater (Tulttlingen, Spain). The amount of water required to achieve the desired moisture (30%) was sprayed slowly while it was mixed in a Bear Teddy Mixer (Bear 5L Teddy, Swansea, UK) for 15 min. Samples were allowed to stand 24 h at 4 ± 2ºC in order to equilibrate the moisture content. Once the amount of moisture was correct, the starch or flour (100 g) was kept in closed polyamide-polypropylene bags provided by Comercial Bolsera Castellana (Burgos, Spain) which were hermetically closed by heat sealing PFS-400 in order to maintain the moisture content constant during storing at -28ºC.

3.2.3. Microwave irradiation of starch samples

Hydrated samples of starch-protein (100 g, 30% moisture content) were defrost at 25ºC during 15 min before being heated in a microwave oven SHARP R-342 (Osaka, Japan) at a frequency of 2450 MHz. Samples were introduced into a cylindrical polyethylene container (2L) at atmospheric pressure (with a drilled thread cap). The container was revolved constantly to ensure a uniform energy and temperature distribution on the samples during treatment. The MW power (900 W) was applied in cycles of 20/40 s during a total time of 32min. Samples were subsequently left cooling for 5 min and then, crushed in a laboratory mortar to get a uniform size. The moisture contents of the samples after the treatment were determined twice following the AACC 44–19 method. Samples were stored in plastic tubes at room temperature until further analyses.

3.2.4. Functional properties

Water absorption capacity (WAC), water absorption index (WAI), water solubility index (WSI) and swelling power (SP) were determined as technological functional properties of starch-protein samples (0 or 5 g/100 g), with or without MW treatment. Each sample was analyzed at least in triplicate.

Water absorption capacity (WAC) of the samples was determined by the centrifugation method described by Abebe et al. (2015). Two grams of sample were dispersed in 20 ml of distilled water in 50ml centrifuge tubes. The dispersions were hold at room temperature for 30 min with occasionally vortexed (Heidolph Reax, Schwabach, Germany) and followed by centrifugation for 30 min at 3000 x g (Thermo Fisher Scientific, Waltham, USA). The supernatant was removed and weighed and results were expressed as grams of water per grams of dry matter of sample.

Water absorption index (WAI) and water solubility index (WSI) were measured with slight modification of the method used by Abebe et al. (2015). Sample (2.5 g, w_0) was dispersed in 30 ml of distilled water in tared centrifuge tubes. After, it was cooked at 90ºC in a water bath for 10 min, cooled to room temperature and centrifuged at 4000 x g for 10 min. The supernatant was poured into a pre-weighed evaporating dish to determine its solid content and the sediment was weighed (w_{ss}). The weight of dry solids was recovered by evaporating the supernatant overnight at 110ºC (w_{ds}). WAC, WAI, WSI and swelling power (SP) were calculated from the following equations:

$$WAC\ \left(\frac{g}{g}\right) = \frac{g\ of\ water}{g\ of\ sample}$$

$$WAI\ \left(\frac{g}{g}\right) = \frac{w_{ss}}{w_0}$$

$$WSI\ \left(\frac{g}{100g}\right) = \frac{w_{ds}}{w_0} \times\ 100$$

$$SP\left(\frac{g}{g}\right) = \frac{w_{ss}}{(w_0 - w_{ds})}$$

3.2.5. Amylose content

The amylose content of samples was determined using the commercial assay kit Megazyme amylose/amylopectin according to the procedure provided by the manufacturer. Absorbance values were measured at 510nm using a UV/VIS Spectrometer (PerkinElmer Lambda 25, Singapore). The amylose content (g / 100g starch) was estimated according with the calculation described by Gibson et al. (1997). Each sample was analyzed twice.

3.2.6. Thermoviscous test: pasting properties

Viscometric profiles of samples without and with MW treatment were obtained with a Kinexus Pro+ rheometer (Malvern Instruments Ltd, UK) supplied with starch pasting cell and controlled by rSpace software using the Standard 2 method 76-21.01 (AACC, 2010) with small modifications. Rice and corn samples (3g, 14% moisture basis) and potato samples (2g, 14% moisture basis) were transferred into the canister where 25 mL± 0.1 mL of distilled water was added. Each sample was analyzed twice.

This method involves putting down the sample to a heating up to 95ºC, maintaining it at this temperature during 5 min and cooling it to 50ºC. Parameters calculated from the pasting profiles are shown in figure 1: peak viscosity (PV), trough viscosity (TV), breakdown (BR= PV-TV), final viscosity (FV), setback (ST=FV-TV), pasting temperature (PT) and Peak time (when peak viscosity occurred).

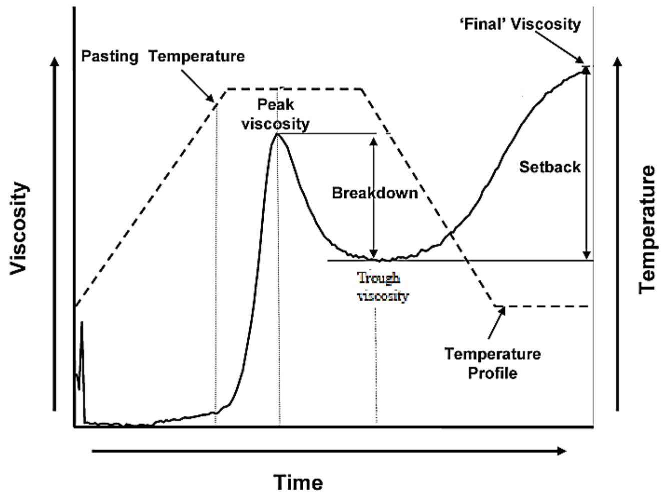

Figure 1. *Representative pasting cycle curve, showing definition of pasting parameters*

3.2.7. Oscillatory tests

Dynamic oscillatory tests of the gels obtained from the thermoviscous analysis were carried out with a Kinexus Pro+ rheometer (Malvern Instruments Ltd, UK) with parallel plate geometry (40 mm diameter) of serrated surface and with 1 mm gap. The gel was placed between the plates, the surplus of sample was removed and it was rested for 5 min to allow relaxation and to stabilize temperature at 25 ºC thanks to a Peltier that controlled the heat conditions.

Stress sweeps were carried out twice from 0,1 to 500 Pa at constant frequency of 1 Hz. This test allowed establishing the linear viscoelastic region (LVR) by the localization of $\tau_{max,}$ which indicated the decrease of G' and G'' moduli and the increase of tan δ. Frequency sweeps was done twice from 1 to 10 Hz in the linear viscoelastic region (at a constant value of 1 Pa). Frequency sweep data were fitted to the next potential equations described by Ronda et al. (2013):

$$G'(w) = G'_1 \cdot w^a$$

$$G''(w) = G''_1 \cdot w^b$$

$$\tan\delta\,(w) = \frac{G''(w)}{G'(w)} = \left(\frac{G''}{G'}\right)_1 \cdot\ w^{(b-a)} = (\tan\delta)_1 \cdot\ w^c$$

The coefficients G'_1, G''_1, and $(\tan\delta)_1$, represent the elastic and viscous moduli and the loss tangent at a frequency of 1 Hz. Fittings were done in the frequency range (1-10 Hz), where a linear double logarithm curve was systematically obtained. The *a, b* and *c* exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency, ω.

3.2.8. Statistical analysis

Statgraphics Centurion XVI (Statpoint Technologies, Warrenton, USA) was used for multiple analysis of variance (MANOVA) and Fisher's least significant difference test (LSD) was used to evaluated significant differences ($p<0.05$) among samples.

Eighteen samples were carried out. The studied factors in this research were: (1) starch type (three levels): maize, rice and potato; (2) protein type (two levels): Calcium Caseinate and Soy protein isolated; (3) protein dose (two levels): 0 or 5%; and (4) MW treatment (two levels): with or without MW treatment.

4. RESULTS AND DISCUSSION

4.1. Temperature evolution in the system during the microwave treatment

The evolution of the temperature of the 30% moistened rice flour during the microwave treatment is shown in figure 2. Rice flour was used as a reference matrix in this test to predict the temperature evolution of the moistened (30%) starch-protein mixtures treated by microwave radiation since previous studies had demonstrated that the water content of the flour has the main effect on MW radiation absorptivity (Carrillo, 2016). As it can be seen in Fig. 2, during the first 80s of treatment the energy absorbed by the sample served to change its temperature, meanwhile at 80s the flour attained a small plateau of constant temperature, where the heat absorbed by the flour served to boil the small amount of free water in the sample. Afterwards, the temperature increased again to attain a new plateau after 4 – 5 min of MW radiation, where the temperature was kept until the end of the treatment. The maximum temperature achieved by the sample was 150 ± 10ºC. The last and long plateau of constant temperature probably corresponded to a period in which the heat absorbed by the sample (not too much, as the water amount was really low) was equal to the heat lost by it towards the surroundings, that increased with the temperature of the sample. In a previous work, where the sample was treated inside an hermetic container, a single (long) temperature plateau was obtained below 100ºC, with water acting as "protector" of the flour constituents, which was used to explain the low impact of the MW treatment on flour functional properties (Pérez-Quirce et al., 2016).

The largest differences in starch happen with long times of MW treatment (Román et al., 2015). Therefore, this research was focused on studying the effects of starches with the longest time gotten: 640s of MW treatment.

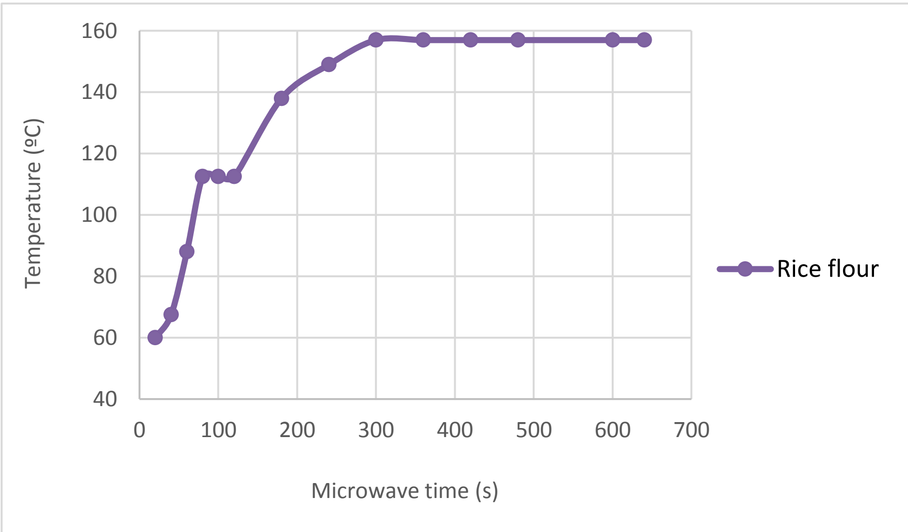

Figure 2. *Time-temperature profiles of 30% moistened rice flour during microwave treatment*

4.2. Amylose content

Amylose content is an important characteristic that affects many significant physical, chemical and functional properties. Amylose and total starch content of the starch samples with and without MW treatment are presented in table 1. The different values of these parameters among native starches could be due to different factors such as their botanical sources, soil type and climatic conditions during grain growth (Singh et al., 2006).

Table 1.: *Amylose content (g/100 g starch) of native and modified starches*

Starch	MW treatment	Amylose content (g/100g starch)
Rice	Native	15.81 ab
	Modified	17.06 b
Maize	Native	23.28 d
	Modified	24.24 d
Potato	Native	19.93 c
	Modified	15.37 a
Standard error		0.52

Values with a letter in common in the same column are not significantly different ($p > 0.05$).

Maize starch had the highest amylose content, followed by potato and rice starch. Besides, MW treatment reduced the amylose content of potato starch from 19,93% to 15,37% upon irradiation (Table 1).

Various effects of amylose content after a heat-moisture treatment have been reported. Some researchers observed that potato starch subjected to a heat-moisture treatment at 110ºC for 3 hours (Tsakama et al., 2011) or at 100ºC for 16h (Gunaratne & Hoover, 2002) did not cause significant changes in amylose content. While the effect of MW heating on potato starch in water suspension at 50 and 60ºC increased it significantly (Nadiah et al., 2015). Zhao et al. (2007) and Sun et al. (2013) showed an increase in the amylose content of rice after a microwave treatment. All these authors used staining by iodine-amylose inclusion complexed to measure amylose. However, in this study amylose content was determined by a more reliable method based on separation of precipitated amylopectin, due to its high molecular weight (Gibson et al., 1997). However, irradiation produces interactions between amylose and/or amylopectin chains (Zavareze et al., 2010) what can make starch chains react more or less readily with iodine or, in this case, disappear or not during the phase of precipitation by concanavalin-A, leading either to an underestimation or to an overestimation of the amylose content (Jane et al., 1999; Polesi et al., 2016).

4.3. Functional properties

Water absorption capacity

Water absorption capacity (WAC) represents the ability of a product to associate with water in limited moisture condition (Singh, 2001). This characteristic is very important in viscous foods such as sauces, soups or baked products in which good protein-water interaction is required, so getting a higher WAC value can make these starches more suitable ingredients for gluten-free formulations (Abebe et al., 2015).

Functional properties are summarized in table 2. The origin of the starch did not show a significant ($p>0.05$) effect on the water absorption capacity of samples. The little fluctuation in this parameter among types of starch could be due to variation in starch structure, internal forces accountable for granule structure, the degree of association to form covalent and hydrogen bonds between starch chains and the degree of availability of water binding sites (Gani et al., 2017)

It is known that the addiction of proteins is used to improve WAC in food systems for their hydrophilic constituents such as the polar or charged side chains (Chinma et al., 2013). In this experiment, the introduction of soy protein (5%) in native starches had a decisive impact on WAC, increasing it between 23% and 42%, while the inclusion of casein did not have significant effects ($p>0.05$) on this parameter. The protein conformation and the content of lipid and protein are the main causes of this behavior (Cornejo & Rosell, 2015). Polar amino acids have been shown to be primary sites for water interaction of proteins, so the higher availability of these amino acids in soy protein increases WAC (Li et al., 2010). Similar conclusions have been reported by Chinma et al. 2013, who observed an increase in WAC with the addition of isolated soy protein in cassava starch.

On the other hand, microwave heating is an interesting physical treatment to change the WAC value since significant differences between starches ($p\leq0.05$) with and without treatment were observed (Table 2). The degradation of starch owing to MW treatment could produce simpler molecules such as dextrin, maltose and other sugars which have greater affinity for water (Shah et al., 2016; Greer & Stewart, 1959; Chinma et al., 2013). So according to other authors (Shah et al., 2016; Chinma et al., 2013), lowest WAC was observed in native starch and highest WAC in those starches treated with 640s of MW.

Water absorption index and swelling power

When starch is heated with excess of water, its crystalline structure is collapsed due to the damage of hydrogen bonds and water molecules form hydrogen bonds with the exposed hydroxyl groups of amylose and amylopectin producing a swelling of the starch granule during gelatinization (Ratnayakea et al., 2002). Water absorption index (WAI) measures the volume occupied by the gelatinized starch, denatured protein and other components after this swelling, maintaining the integrity of starch in aqueous dispersion (Abebe et al., 2015) and swelling power (SP) is an indication of water absorption of the granules (Loos et al., 1981). These parameters depend on the interaction between starch chains within the amorphous and crystalline domains, and are influenced by amylose and amylopectin content, their molecular weight distribution, length and degree of branching, phosphate groups and conformation of the molecules (Ratnayakea et al., 2002).

Significant differences in SP and WAI were observed among native rice, potato and maize starch (Table 2), being rice's SP and WAI values higher than those of maize and the latter higher than potato's. The low value of swelling power in potato can be due to formation of stable amylose– phosphate groups complexes (Kong et al., 2015) and to weak internal organization that is resulted from negatively charged phosphate groups within the potato starch granules (Singh et al., 2006).

The addition of protein to native rice and maize starch had not any important influence in these properties, while showed a significant effect on potato starch, increasing its SP and WAI values. The insertion of proteins reduces amylose content due to decrease in starch amount and, since amylose acts as a dilutor and a swelling inhibitor, protein addition improves SP and WAI values of the mixtures (Chinma et al., 2013). Nevertheless, the high amount of lipids in maize and rice counteracts this behavior (Peroni et al., 2006) and the inclusion did not have any influence in SP and WAI

MW treatment had significant effects on SP and WAI values. These parameters decreased by 38% in rice and by 17% in maize starch with MW treatment compared to native starch, while they increased by 66% in treated potato starch. This result meets with previous research that says high amylose content inhibits SP of cereal starch (Singh et al., 2006; Cornejo & Rosell, 2015; Kong et al., 2015). Authors such as Nadiah et al. (2015) and Chinma et al. (2013) also observed a significant increase in SP for tuber's starches after heating. This increase of WAI and SP in treated potato starch could be due to the reduction in its amylose content after the treatment and the dissociation of phosphate groups and amylose complex through high temperatures reached by MW treatment (Thomas & Atwell, 1999).

Water solubility index

Water solubility index (WSI) of a sample relates to the amount of soluble solids and is often used as an indicator of degradation of starch molecules and dextrinization.

WSI of rice starch was higher than maize and the latter higher than potato's. Abebe et al. (2015) related the high value of WSI in tef flours to the presence of high soluble matter content. Besides, the addition of protein increased WSI of samples but MW treatment decreased this parameter significantly in maize and potato starches (table 2).

Table 2.: *Functional properties of the starches referred to dry matter*

Starch	Protein	MW treatment	Moisture		WAC (g/g)		WAI(g/g)		WSI(g/100g)		SP(g/g)	
Rice	without	Native	11.19	f	1.15	ab	13.42	jk	2.77	b	13.76	ij
		Modified	5.13	ab	1.57	d	7.29	ab	2.97	bc	7.50	ab
	casein	Native	11.18	f	1.04	a	12.98	j	4.55	d	13.52	i
		Modified	6.71	cd	0.91	a	9.00	fg	6.92	f	9.62	f
	soy	Native	10.86	f	1.41	bcd	13.77	k	2.94	b	14.14	j
		Modified	5.73	b	1.49	cd	7.57	bc	3.08	bc	7.80	bc
Maize	without	Native	10.92	f	0.93	a	10.72	i	5.59	e	11.28	g
		Modified	5.37	ab	1.51	cd	7.93	cd	2.88	b	8.15	c
	casein	Native	10.79	f	0.89	a	11.09	i	7.64	g	11.90	h
		Modified	7.33	d	1.45	cd	7.66	bc	5.76	e	8.10	c
	soy	Native	10.61	f	1.28	bc	10.83	i	9.18	h	11.80	gh
		Modified	6.53	c	1.67	d	8.05	cd	3.31	bc	8.31	c
Potato	without	Native	14.90	g	0.91	a	6.75	a	3.57	c	6.96	a
		Modified	6.67	cd	1.98	e	9.50	h	0.99	a	9.59	f
	casein	Native	14.57	g	0.91	a	7.44	b	6.52	f	7.88	bc
		Modified	9.16	e	1.28	bc	8.72	ef	3.12	bc	8.97	de
	soy	Native	14.39	g	1.29	bc	8.39	de	6.40	f	8.88	d
		Modified	4.85	a	3.01	f	9.37	gh	1.15	a	9.47	ef
Standard error			0.27		0.10		0.20		0.23		0.21	
Starch			***		***		***		***		***	
Protein			***		***		*		***		**	
Mw treatment			***		***		***		***		***	
Starch* protein			***		***		**		***		**	
Protein * Mw treatment			***		**		ns		***		**	
Starch * Mw treatment			***		***		***		***		*	

Data are expressed in dry basis. Values with a letter in common in the same column are not significantly different (p > 0.05).
*Level of significance: * p<0,05; ** p<0,01; *** p<0,001; ns: no significant (p > 0.05).*
WAC = water absorption capacity, WAI = water absorption index, WSI = water solubility index and SP = swelling power.

4.4. Thermoviscous test: pasting properties

Pasting viscosity profiles of rice, potato and maize starches, both native and microwave-treated, with and without proteins are shown in Fig. 3, 4 and 5, respectively. The main results derived from them are summarized in table 3. Microwave promoted intense changes in the pasting profile of samples, which are affected, at the same time, by starch granule size, amylose, lipid and phosphorous content (Jane et al., 1999).

Starch is insoluble in water but, when it is heated in the presence of sufficient moisture, different structural changes in its granules can be detected: gelatinization, pasting and retrogradation. When samples are heated up in excess of water, the crystalline organization of the granules is disrupted and amylose leaches from the granules. The leaching of amylose and the rise of swelling because of the absorption of water produce an increase of the viscosity of the medium, which is known as gelatinization. Additional heating destructs the granules by disrupting hydrogen bindings between polymer chains, what causes the dispersion of amylose and amylopectin and the loss of viscosity (pasting). Finally, when the sample is cooled down, the viscosity increases again gradually (retrogradation) due to the re-association of the solubilized starch polymers in a firmly packed structure (Schirmer et al., 2015). Retrogradation is a continuous process occurring over an extended period. The association of amylose takes place in a short time while amylopectin requires around 6-9 days.

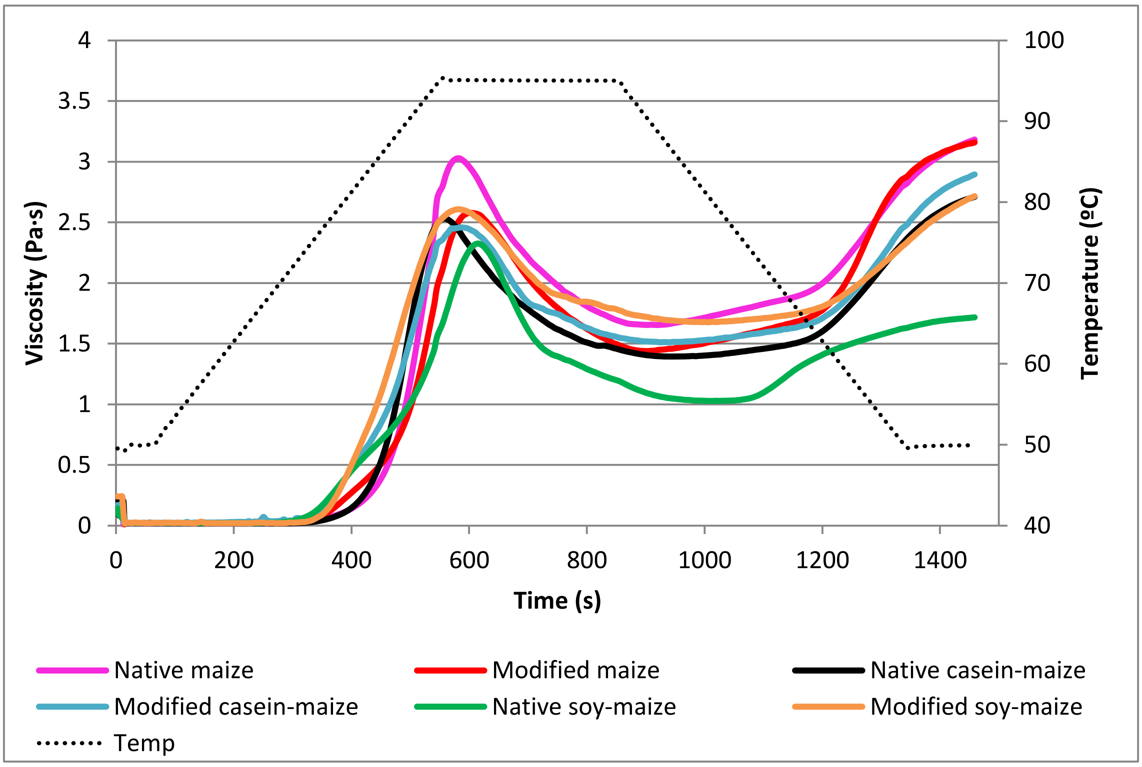

Figure 3. *Pasting viscosity profile of native and modified maize starch with and without protein*

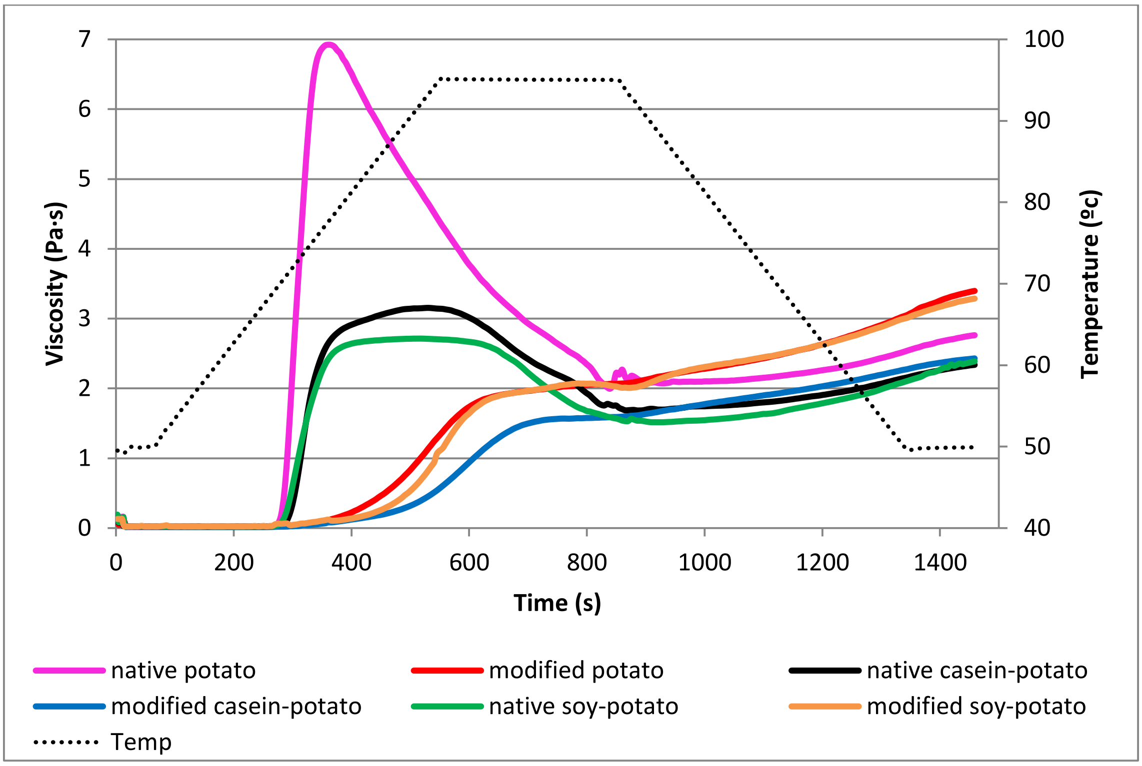

Figure 4. *Pasting viscosity profile of native and modified potato starch with and without proteins.*

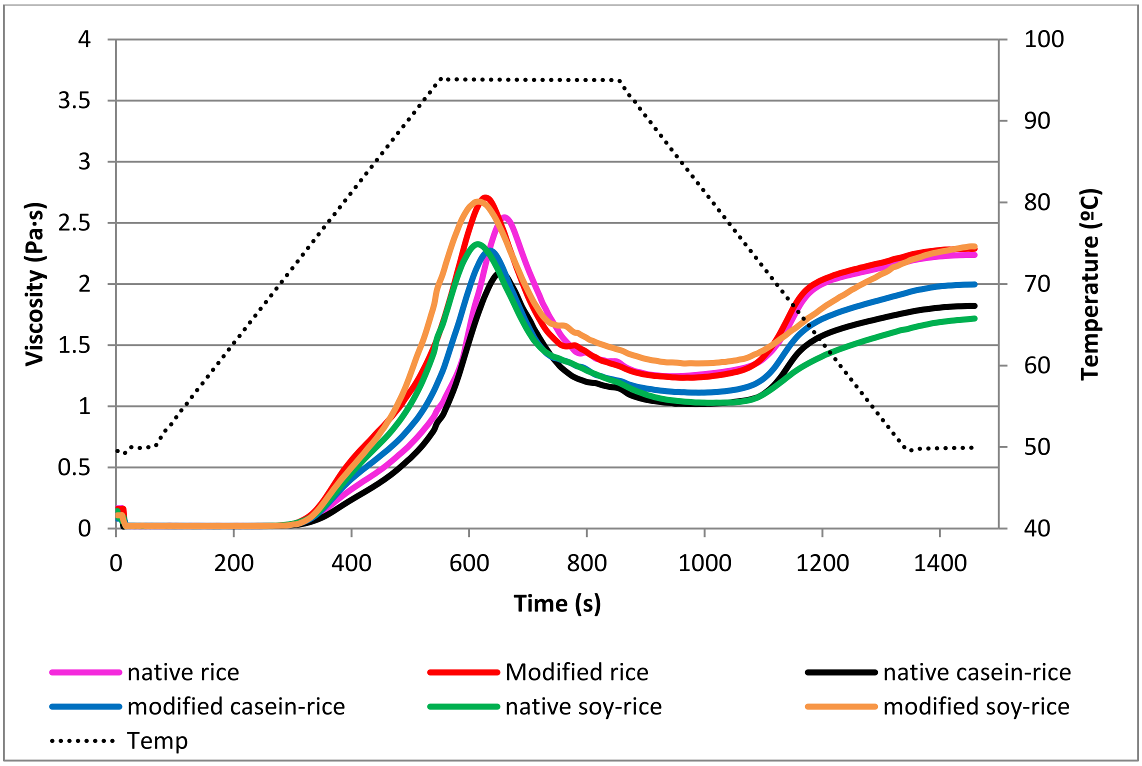

Figure 5. *Pasting viscosity profile of native and modified rice starch with and without proteins.*

The pasting curves obtained reflected the molecular events that happened in starch granules during the heating cycle and provided a means of comparing the behavior of potato, rice and maize starches during cooking.

Pasting temperature (PT) is related with the initiation of paste formation. After comparing the native starches, potato had the lowest PT (table 3) due to the characteristic absence of lipids and phospholipids of the tuber starches and its lower degree of crystallinity (Jane et al., 1999). This behaviour was reported by other authors, such as Kim et al. (1996) and Jane et al. (1999) who observed the low PT of potato starch compared to that of bean and cereal starches. On the other hand, MW treatment increased by 27% the PT of potato starch. Tsakama et al. (2011), Huang et al. (2015) and Nadiah et al. (2015) showed that the PT of potato and tapioca starches increased by MW heating. The reason of the rise of this parameter was the appearance of more strength forces and cross-links between chains within the starch granule during the treatment, what required a higher heating temperature for structural disintegration and paste formation (Zavareze & Dias, 2011).

The parameter that determines when the paste is formed is the peak time. Just as the differences in PT, potato starch showed the lowest peak time and MW treatment increased it significantly.

Peak viscosity (PV) is the maximum viscosity reached before cooling and indicates the thickening power of the starch during cooking. Native potato starch had a very high PV because of its great phosphate monoester content and long brand chains (Jane et al., 1999; Biliaderis, 2009). The lower PV observed in cereal starches can be related to their high amount of lipids and phospholipids which can be linked with amylose and long chains of amylopectin, inhibiting amylose leaching (Mcpherson, 1999). A significant decrease in PV was observed in native starches as a result of the addition of protein (table 3). Ronda et al. (2014) observed that the reduction of starch because of the replacement with proteins caused lower values of pasting viscosities. These proteins can retain water from the starch granules and, thus, reduce initial starch granule swelling. On the other hand, the application of MW treatment reduced the PV of potato starch from 6,88 to 1,85 Pa·s. This result meets previous research such as Trancoso-Reyes et al. (2016), Huang et al. (2015), Varatharajan et al. (2010) and Collado & Corke (1999) who observed that MW treatment induced a decrease in the viscosity of potato flour or starch. This reduction could be due, at least partially, to the decrease of amylose content of potato starch from 19% to 15% during the MW treatment (Table X) since, in accordance with Varatharajan et al. (2010), the reduction of amylose leaching reduces the PV of the samples.

Another important parameter used to measure the stability of starches is the breakdown (BR) (Nadiah et al., 2015). Native potato starch showed higher BR values than maize and rice starch (table 3). Singh et al. (2003) observed that the different BR values among the origins of starches were related to the granule rigidity, lipid content and peak values, and concluded that maize and rice starches showed lower BR values because they had a high amount of lipids. On the other hand, the addition of protein decreased significantly the BR of samples, being the potato starch the most affected, with a reduction between 69 and 75%. This decrease in the BR of samples reflected an increase in the shear stability of treated starches. MW treatment did not have an influence neither on maize nor on rice starch. However, the value of BR of potato starch decreased significantly after MW and was close to zero, indicating the high stability of this microwaved-starch when exposed to heating and mechanical agitation. This behaviour was also observed by authors such as Jane et al., (1999), Huang et al. (2015), Nadiah et al. (2015), Tsakama et al. (2011) and Klein et al. (2013) who perceived a significant decrease in this parameter when potato and tapioca starches were heated. The tendency observed in the trough viscosity (TV) was the same seen for the BR.

Final viscosity (FV) indicates the ability of starch to form a viscous paste with cooling and is related to its gel hardness. This variable showed significant differences among native starches, being the gel of maize and potato starch harder than that of rice starch as was also reported by Mishra & Rai (2006) and clearly sensorial perceived when handling the gels. On the other hand, this parameter decreased in presence of either animal or vegetal protein as Ronda et al. (2014) observed. MW tended to increase FV of samples but it did not often show a significant effect.

On the other hand, setback viscosity (ST) is a measure of the degree of retrogradation of starch during the cooling phase. It is influenced mainly by the amylose content and amylose leaching since this polymer, in comparison to amylopectin, has a faster tendency to rearrange after gelatinization (Nadiah et al., 2015). Making a comparison of the native starches, maize showed the highest ST which could be related to its higher amylose content with respect to rice and potato starches. The addition of proteins did not affected the ST of native samples but MW treatment increased this parameter significantly in potato starch (from 0,78 to 1,59 Pa·s). MW produced interactions between polymer chains making the starch more stable and, thus, it required more heat for paste formation and increased the FV (Van Hung et al., 2017).

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Table 3.: *Pasting properties of native and modified starch samples*

Starch	Protein	MW	PV (Pa·s)		TV (Pa·s)		BR (Pa·s		FV (Pa·s)		ST (Pa·s)		Peak time (s)		PT(ºC)	
Rice	without	Native	2.55	fg	1.25	bc	1.30	ef	2.24	bcd	0.99	abcd	664.80	def	74.59	c
		Modified	2.73	g	1.23	bc	1.49	h	2.29	bcd	1.06	bcde	627.30	cde	73.89	c
	casein	Native	2.09	c	1.02	a	1.08	c	1.82	ab	0.80	ab	649.80	def	75.76	c
		Modified	2.27	cd	1.11	ab	1.16	cd	2.00	abc	0.88	abc	634.80	cdef	74.62	c
	soy	Native	2.32	de	1.03	a	1.30	ef	1.72	a	0.69	ab	617.55	cde	74.61	c
		Modified	2.68	g	1.35	cd	1.33	ef	2.31	cd	0.96	abcd	619.80	cde	74.30	c
Maize	without	Native	3.03	h	1.65	ghi	1.38	fg	3.18	gh	1.53	fgh	582.30	cde	78.13	c
		Modified	2.59	fg	1.44	def	1.15	cd	3.16	gh	1.72	h	599.80	cde	75.59	c
	casein	Native	2.53	efg	1.39	cde	1.14	cd	2.71	defg	1.32	defg	556.90	bcd	77.13	c
		Modified	2.46	def	1.51	efgh	0.95	b	2.90	fgh	1.38	efgh	432.85	ab	69.14	ab
	soy	Native	2.54	fg	1.45	def	1.10	cd	2.63	def	1.18	cdef	554.55	bcd	78.28	c
		Modified	2.61	fg	1.68	i	0.93	b	2.71	defg	1.04	abcde	584.80	cde	76.52	c
Potato	without	Native	6.88	i	2.06	j	4.82	i	2.84	efgh	0.78	ab	358.15	a	60.06	a
		Modified	1.85	b	2.11	j	0.00	a	3.70	i	1.59	gh	687.35	ef	76.19	c
	casein	Native	3.15	h	1.66	hi	1.49	gh	2.34	cd	0.67	a	518.25	bc	74.09	c
		Modified	1.60	a	1.59	fghi	0.00	a	2.43	cdef	0.84	abc	859.95	g	76.53	c
	soy	Native	2.72	g	1.50	defg	1.22	de	2.39	cde	0.89	abc	537.45	bcd	70.66	c
		Modified	1.92	bc	2.01	j	0.00	a	3.29	hi	1.28	defg	762.30	fg	76.05	c
Standard error			0.07		0.05		0.04		0.16		0.12		43.07		3.26	
Starch			*		***		ns		***		***		**		ns	
Protein			**		***		**		**		**		ns		ns	
Mw treatment			**		**		***		**		**		**		ns	
Starch * protein			**		**		*		ns		*		**		ns	
Protein * Mw treatment			**		***		*		ns		ns		ns		ns	
Starch * Mw treatment			***		ns		***		*		*		***		**	

Values with a letter in common in the same column are not significantly different (p > 0.05).
*Level of significance: * p<0,05;** p<0,01; *** p<0,001; ns: no significant (p > 0.05).*
PV=peak viscosity, TV=trough viscosity, BR=breakdown, FV=Final viscosity, ST=setback, PT=pasting temperature

4.5. Oscillatory tests

The viscoelastic properties of gel samples were studied by dynamic oscillatory tests applying a sinusoidal strain to the samples. The dynamic rheological characteristics of starch samples are shown in table 4.

Stress sweeps were carried out on varying the amplitude of the input signal from 0.1 to 500 Pa at a constant frequency of 1 Hz to determinate the limits of the linear viscoelastic region (LVR) by identifying a critical value of stress beyond which the dough structure was broken (τ_{max}) (Steffe, 1996). Afterwards, the gel samples were also subjected to controlled stress so that the stress amplitude was fixed and the deformation was measured versus frequency (frequency sweep). Frequency sweeps were conducted by increasing the frequency from 1 to 10 Hz in the LVR at constant amplitude of 1 Pa the input signal (stress or strain). This last test allowed knowing how the viscous and elastic behaviour of the material changed with the rate of application of strain or stress (Steffe, 1996).

This experiment led to the definition of various material functions. The parameter G' (dynamic shear storage modulus) is a measure of the energy recovered per cycle of deformation and acts as an indicator of the solid or elastic character of the material; and G'' (loss modulus) is the value of the energy dissipated as heat per cycle of deformation and serves to know the viscous properties of the material (Biliaderis, 2009). In all samples, G' and G" increased over the frequency range for the test samples, being G' higher than G" what indicated that elasticity prevailed over viscosity (Xie et al., 2013; Ziobro & Witczak, 2016; Marco & Rosell, 2008; Matos et al., 2014). Among native starches, G' and G'' of maize samples were significantly higher than those for potato and rice starch. This can be explained by the higher amylose content of maize samples since, according to Case et al. (1998) the rigidity of different starches decreases as amylose content decreases.

On the other hand, the ANOVA study indicated that the addition of proteins decreased the elastic modulus of maize starches by 51-53%. Arimi et al. (2011) reported that the decrease of G' with the introduction of protein in cheese with maize starch was owing to the increase in water-protein ratio. During MW treatment, both moduli increased in maize and potato starch but did not do it in rice probably because of its low amount of amylose. The increases in G' and G" depends on the level of gelatinization and the development of a net relating to those chains leached from the starch granules during the microwave treatment (Xie et al., 2013).

Both moduli are related by tan δ, a parameter that measure the loss tangent or G''/G' ratio. Tan δ was very low for all samples (always < 0.4), which correspond with hard gels. Maize starch had the lowest value of the loss tangent because it was highly elastic (Steffe, 1996). On the other hand, the addition of protein (5%) did not show any significant effect on the tan δ of native starches. The level of protein addition could be low to influence in this parameter. Ronda et al. (2014) and Ronda et al. (2011) observed that supplementation of proteins affected GF dough viscosity, but did differently depending on the type and dose of protein: the higher the protein concentration in the batter, the clearer the effect of the protein. On the contrary, MW generally decreased tan δ values of gels compared to the respective non-modified gels, producing stronger gels (Gryszkin et al., 2014).

Stress sweep test also allowed knowing the maximum stress (τ_{max}) that samples could tolerate in the LVR conserving their structure. The decline of elastic modulus, G', of native starch gels started to occur at 108, 267 and more than 500 Pa for rice, potato and maize, respectively, indicating that starch samples structures had different resistances to the rupture by the action of stress (Ronda et al., 2013). Maize gels preserved their structure beyond the maximum stress tested (500 Pa) meaning that it had an extraordinary resistance and hardness. The addition of protein always weakened the rice gels, while they had opposite effect in potato gels depending on the type of protein: casein protein decreased τ_{max} of potato gels, while the addition of soy protein isolate increased it.

Microwave treatment increased the strength of rice gels with respect to the non-treated counterparts, although this effect was only significant in the soy protein-enriched rice gels. The effect of MW radiation on potato gels was stronger, being much more remarkable in presence of protein: τ_{max} decreased by 60% in gels without protein, 88% in casein protein-enriched gels and 72% in soy protein-enriched gels.

The values of the "a" and "b" exponents were always lower than 1, which means that G' and G'' hardly depended on the frequency (Ronda et al., 2013). Maize showed the lowest values of "a" and "b" exponents, what indicated that G' and G'' did not depend on the frequency and what confirmed that maize starch was capable of making a strong gel (Ronda et al., 2011; Case et al., 1998)

Table 4.: *Dynamic rheological characteristics of native and modified starch samples.*

Starch	Protein	MW treatment	G' (Pa)		a		G'' (Pa)		b		tan δ		c		τ_{max} (Pa)	
Rice	without	Native	51.56	a	0.17	ef	13.66	a	0.3	e	0.27	e	0.18	Ij	107.6	cd
		Modified	73.63	a	0.15	d	17.08	a	0.3	e	0.23	d	0.20	j	119.37	d
	casein	Native	41.25	a	0.18	fg	11.91	a	0.3	e	0.29	ef	0.18	Ij	68.53	b
		Modified	47.37	a	0.17	ef	12.66	a	0.3	e	0.27	e	0.18	ij	85.00	bc
	soy	Native	39.28	a	0.19	g	11.95	a	0.3	e	0.30	f	0.17	hi	54.89	ab
		Modified	88.15	a	0.14	d	18.87	a	0.3	e	0.22	d	0.20	j	104.70	cd
Maize	without	Native	781.99	e	0.03	a	41.93	bc	0.2	b	0.05	a	0.16	ghi	>500	
		Modified	1159.05	g	0.03	a	57.95	d	0.1	a	0.05	a	0.13	bcd	>500	
	casein	Native	561.89	d	0.04	a	32.88	b	0.2	b	0.06	a	0.16	ghi	>500	
		Modified	889.58	ef	0.04	a	53.99	d	0.1	a	0.06	a	0.11	abc	>500	
	soy	Native	543.86	d	0.04	a	35.53	b	0.2	b	0.07	a	0.16	fghi	>500	
		Modified	983.64	f	0.03	a	57.75	d	0.1	a	0.06	a	0.11	ab	>500	
Potato	without	Native	52.51	a	0.18	fg	16.06	a	0.2	d	0.31	f	0.11	abc	267.25	f
		Modified	420.76	cd	0.08	b	50.56	cd	0.2	bc	0.12	b	0.15	efg	101.10	cd
	casein	Native	45.86	a	0.18	fg	14.14	a	0.2	d	0.31	f	0.10	a	215.65	e
		Modified	170.87	ab	0.14	d	38.58	bc	0.2	d	0.23	d	0.14	def	25.62	a
	soy	Native	52.58	a	0.16	e	14.26	a	0.2	d	0.27	e	0.12	abc	304.00	g
		Modified	309.32	bc	0.10	c	47.93	cd	0.2	c	0.15	c	0.14	cde	84.43	bc
Standard error			52.19		0.00		4.05		0.01		0.01		0.01		11.01	
Starch			***		***		***		***		***		***		***	
Protein			**		**		ns		ns		**		ns		**	
Mw treatment			***		***		***		***		***		ns		***	
Starch * protein			*		ns		ns		ns		ns		ns		**	
Protein * Mw treatment			ns		*		ns		ns		*		ns		ns	
Starch * Mw treatment			***		***		***		*		***		***		***	

Values with a letter in common in the same column are not significantly different (p > 0.05).

*Level of significance: * p<0,05;** p<0,01; *** p<0,001; ns: no significant(p > 0.05).*

CONCLUSIONS

As the demand of gluten free (GF) products is increasing because of the growing number of diagnosed celiacs and the trend to eliminate gluten proteins from diets, it is necessary to improve both the nutritional quality and the functional properties of these foods. This research shows that MW treatments can be used successfully to modify starches in order to improve their properties and the quality of the final GF products.

The results showed that properties of starches varied depending on their origin, especially among cereal and tuber starches. WAI, SP and WSI were higher for native cereal starches than for native potato starch. The PV and BR of potato were higher than that of maize and rice starches, while the PT of potato starch was the lowest. FV depended on the amount of amylose of samples and, thus, maize and potato had higher FV than rice starch. The addition of protein increased WAC, WAI, SP, WSI and decreased peak viscosity and breakdown of samples. MW radiation influenced mainly the properties of potato starch, increasing its pasting temperature and setback, and decreasing its peak viscosity and breakdown. MW treatment increased significantly the consistency of potato and maize gels with and without protein. In the case of protein enriched-maize gels, MW treatment compensated the significant decrease that both proteins exerted on the viscoelastic moduli.

This research allows understanding changes occurring during MW treatments and can help to design and improve the quality of GF products. MW is a new inexpensive and safe method that can be used as an alternative to the chemical modification that is currently being used in a number of foodstuffs. Future studies should be undertaken to determine the sensory quality and consumers' acceptance of the GF foods made with this physically modified starches.

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