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Incorporation of dietary fiber on the cookie dough. Effects on thermal properties and water availability

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Abstract

The present work it was analyzed the water mobility by characterizing the thermo-gravimetric analysis of the cookie dough with the incorporation of dietary fibers. The fibers analyzed were: inulin (IN), oat fiber (OF), hi amylose maize starch (RSII) and phosphate distarch-phosphate (RSIV). Four tests were performed: thermo-gravimetric analysis (TGA), pasting profile analysis using water and a solution of 50% sucrose as a solvent, and the study of the ultrastructure of the flour and fiber by SEM and particle size distribution. Changes in the thermo-gravimetric profile were explained by the pasting profile of composites and the ultrastructure of the wheat flour and fibers. OF and IN incorporation changed substantially dough water loss profile and rate. The addition of fibers produced a decrease in the starch pasting profile and inulin showed the highest reduction since less water was available for the hydration of wheat flour starch granules.

Keywords: cookie dough, dietary fiber, TGA, RVA, SEM, particle size

1. INTRODUCTION

New global trends in food have led consumers to search for food that directly contributes to their health (Siró et al., 2008). The variations in feeding patterns generated a new development area in food and nutrition science. For years, there has been scientific evidence that cereals have the potential to improve health beyond the provision of nutrients and that their consumption can substantially reduce the risk of chronic diet-related diseases (David Topping, 2007). However, the most commonly marketed bakery is made from refined grains. These, unlike whole grains, lack micronutrients, some proteins and dietary fiber (DF).

One of the strategies to increase the nutritional value of food elaborated from refined cereals is the incorporation of DF in its formulation. These non-degradable compounds, present in plant structures, reach the large intestine intact, where they can be substrates of the bacterial enzymes of the intestinal flora, causing different physiological effects in the host: on intestinal functioning, cholesterolemia, glycemia and insulinemia (Roberfroid 1999).

From the point of view of its physical properties, and more specifically, its degree of solubility in water, DF can be classified into soluble and insoluble fiber. This distinction is of particular importance since it largely determines its physiological properties (Topping 1991). Soluble fiber is easily fermentable by bacterial flora, while insoluble fiber is fermented slowly.

Inulin (IN) is considered a dietary fiber since only one enzyme, the inulinase, is able to hydrolyze it. This enzyme is not part of the human digestive track. Chemically, inulin consists of linear D-fructose polymers, linked by β-(2-1) linkage, with a polymerization degree ranging between 10 and 25.

Oat insoluble fiber (OF) is associated with great water retention capacity and basically comprises β-glucans, a linear polymer of glucose, important to maintain healthy colonic functions and reduce constipation (Manthey et al 1999). Resistant starch (RS) is a portion of the starch not digestible by human enzymes, producing longer satiety and controlling glucose release (Englyst et al 1999; Nugent 2005). Since the recent recognition of its nutritional properties, many studies have been carried out demonstrating that they show similar physiological function to those of dietary fibers (Asp 1994). Its beneficial effects on human health are related to the long time lag between intake and digestion (5-7 h), while normal starch is metabolized immediately, increasing glycaemia and insulinemia (Raben et al., 1994).

There are four types of resistant starches; however, only two are used as flour replacement in food products. RSII (resistant starch type II) resists enzyme digestion due to its certain granular form, while RSIV (resistant starch type IV) is a chemically modified starch, forming bonds other than α-(1-4) o α-(1-6) (Sajilata et al 2006).

Despite the widely described beneficial effect of dietary fiber intake, its incorporation produces structural changes on food. Particularly, cookies have a very complex structure, where each ingredient of the formulation plays a fundamental role. The small amount of water, together with high fat and sugar content, prevents gluten network development, undesirable in this kind of products. In spite of being in low amount, water has a complex function since it affects the nature of interactions between ingredients, determines the polymer conformational state and contributes to dough structure, modifying its rheological behavior. During the baking process, cookie dough dries up and produces changes in interactions, which are governed by the affinity of each component for water.

In any moment of the process, some water occupies inter-phase regions and can be supposed to move freely and generate a vapor phase with a given partial pressure; the rest of the water has to pass through phase boundaries and reach the inter-phase region to contribute to the overall water activity of the system (D Fessas & Schiraldi, 2001). When high water affinity ingredients are added to the formulation (as dietary fiber), water redistributes, generating changes in dough rheological properties and altering the location of water within the matrix. Variation in cookie formulation generates not only technological troubles during manufacturing, but also changes in texture, flavor and quality parameters that could lead to loss of acceptability by consumers (Maache-Rezzoug et al 1998).

In our previous work, we studied the effect of the incorporation of DF of different characteristics on cookie dough (rheology and spread rate) and its relationship with quality parameters of the baked product (width/thickness factor, cookie texture and surface characteristic) (Blanco Canalis et al 2016). It is generally accepted that a good cookie quality is related to tender final product, and large piece diameter and uniform surface-cracking pattern (Delcour & Hosene, 2010). It was found that DF incorporation affected dough characteristics and cookie quality and the extent of this effect largely depended on the type of fiber and the level of substitution. Oat fiber significantly increased dough consistency and negatively affected cookie quality. Resistant starches slightly affected the viscoelastic behavior of the dough and the quality of the baked product, while inulin incorporation slightly increased dough consistency but improved the effect on the quality of the cookies. Another investigation was carried to study the relationship of dough behavior during baking and cookie quality to proton mobility upon addition of inulin and oat fiber (Serial et al 2016). Authors found that the mobile part of the cookie dough was characterized by four proton populations with different mobility: related water bound to solid components (such as starch, proteins and other hydrophilic components), less bound water and populations with higher mobility were related to dough fat. It was observed that the intensity of these populations changed with dough temperature and that the population of less bound water split into two at around 50 °C for the control dough. Inulin incorporation decreased this

split temperature, while oat fiber significantly increased it. This availability of water fraction with higher mobility was related to the dough structure set time during baking and thus to cookie quality. This work confirmed that, in addition to the low water content that characterized the short dough cookie system, there were water populations structured which changed during the baking process.

It is important to note that, in addition to the effect of each fiber on dough and cookie quality, their incorporation brings about a nutritional improvement since two servings of the cookies represent between 13% and 29% of daily fiber reference value (considering a nutrient reference value of DF of 25 g day^{-1} and one cookie serving as 30 g)(Blanco Canalis et al. 2016).

In order to analyze deeper the effect of the incorporation of fibers, the present work aimed at evaluating the thermo-gravimetric behavior of the cookie dough with the incorporation of DF during heating and its relationship with the pasting profile and ultrastructure of the fibers. Cookie is a product with limited water content, which makes very difficult to follow the thermal behavior, so different approaches were done to get information and to relate them with structural changes.

2. MATERIALS AND METHODS

2.1. Materials

Commercial wheat flour (protein=10.4% (db), moisture=11.9%, ash=0.62% (db), wet gluten=23.4%, alveographic properties: W (dough baking strength)=260, P (tenacity)=130, L (extensibility)=45, and Falling number=545 s) was used. Inulin (Orafti ®HP) was obtained from Orafti Food Ingredients (Belgium) with DP greater than 23. Resistant starch type II (Hi maize 260, National Starch) and resistant starch type IV (Novelose 480, National Starch) were supplied by Gelfix S.A. (Buenos Aires, Argentina). RSII is a high amylose maize starch and RSIV is defined as a phosphated distarch phosphate, a cross-linked high amylose maize starch. Oat fiber was supplied by Saporiti S.A. (Buenos Aires, Argentina) (Canadian Harvest®Oat Fibers 200/58 series, USA).

The fiber and wheat flour used were analyzed for their moisture (AACC Method 44-01 2001).

2.2. Dough thermo-gravimetric analysis (TGA)

Thermo-gravimetric analysis was performed on cookie doughs. Short dough were prepared according to Blanco Canalis et al. (2017). The ingredients used were flour (45 g), caster sugar (27 g) “Gloria”, vegetable shortening (20.2 g) “Dánica”, powdered skim milk (2.25 g) “La Serenísima”, NaHCO3 (0.50 g) “Alicante”, NaCl (0.42 g) “Celusal”, and 8.5 mL of water. Fiber and starch samples were incorporated in two levels: 6 and 12 g (wheat flour substitution), corresponding to 6% and 12%, respectively.

Dough samples (~10 mg) were heated in aluminum pans from 25 to 120 °C, using a heating profile of 4 °C/minute: Samples were analyzed in triplicate. Mass loss was determined as the difference between initial and final weight and expressed as a percentage. The resulting TGA trace of mass loss (%) was then analyzed for its first derivative, representing the rate of mass loss (Derivative Thermo-Gravimetry (DTG) (%/°C) (SigmaPlot 10.0 USA/Canada). With the aim to separate the overlapping events that represent different phenomena, DTG curves were fitted to a sum of Gaussian functions. The thermograms were baseline and the Gaussian peaks were initially added around chosen peak centers and the final location and area of the Gaussians were determined by automatic fitting to get the best fit to the data (Peakfit 4.0 software). Peak area was expressed as a percentage of the total area under the curve. Adjustments with regression coefficient (r) greater than 0.99 were considered.

2.3. Pasting profile

A rapid visco-analyzer (RVA) instrument (RVA series 4500, Perten instruments, USA) was used to prepare the samples and follow the apparent viscosity profile of the samples as a function of temperature and time. To carry out the assay, 3.5 g of wheat flour or flour + fiber (14% moisture basis) were suspended in 25 g of distilled water and placed into the aluminum canisters. Fibers were incorporated as wheat flour (WF) replacement in two levels (keeping the same flour:fiber ratio used in cookie dough): low level 3.03:0.47 g WF:g fiber ratio (13.33 % of fiber) and high level 2.79:0.91 g WF:g fiber ratio (26,67 % of fiber).
RVA Standard 1 Method (Palavecino, Penci, Caldrón-Dominguez, & Ribotta, 2016) was applied as follows: dispersions were stirred at 960 rpm for 10 s followed by constant stirring at 160 rpm until the end of the assay, with the temperature being maintained at 50°C for 1min, increased to 95°C at min 5 and maintained for 2.5 min, cooled to 50°C in 3 min, and finally held at 50°C for 2 min.
For complete characterization of the mixtures, two types of tests were performed:

- Using water as a solvent, the obtained pasting parameters included: pasting temperature (PT, temperature at moment of increase in viscosity), peak viscosity (PV, maximum hot-paste viscosity), trough viscosity (TV, lowest viscosity when maintained at 95°C) and final viscosity (FV, viscosity at the end of the assay). The parameters calculated were breakdown (BD=PV–TV) and setback (SB=FV–TV) using Thermocline for Windows© software (V 3.15, Perten Instruments, Australia).
- Using a solution of sucrose 50 % as a solvent (Kweon et al. 2009). From these viscograms two parameters were obtained: peak viscosity (PV) and final viscosity (FV).

Each analysis was done in duplicate.

2.4. Scanning Electron Microscopy (SEM)

The ultrastructure of fiber and wheat flour particles was studied through SEM. Dry fibers and wheat flour were sprinkled onto double-sided tape attached to the specimen stubs and coated with a thin layer of copper (30 nm thickness) through a cathodic spray coating system. For the observations, a FE-SEM Σigma electronic scanning microscope was used under high vacuum conditions (10-4 Pa) at an acceleration voltage of 5 kV. The photographs were taken using automatic image capture software. Images were obtained with magnifications between 100x and 3000x.

2.5. Particle size determination

Particle size was determined from particle size distributions generated using laser light diffraction Horiba (LA 960, Irvine, California). Distributions were made in triplicate for each sample, using 12-15 g sample weight for dry powder (~6-8 % moisture) particle size distribution and 1-2 g in an aqueous suspension for hydrated particle size distribution. An air flow of 0.40 MPa was used and the feeder speed was 75 for the dry assay.

2.6. Statistical analysis

The data obtained were statistically treated by variance analysis, while means were compared by Fisher's LSD test at a significance level of 0.05. These tests were carried out using INFOSTAT statistical software (Universidad Nacional de Córdoba, Argentina).

3. RESULTS AND DISCUSSION

3.1. Dough thermo-gravimetric analysis (TGA)

Weight loss curves were obtained at 4 °C/min by TGA measurement. In general, as materials are heated, they can lose weight from simple processes such as drying, or from chemical reactions that liberate gasses. In this particular system, weight loss was related only to reduction of water by evaporation, since in this temperature range no thermal decomposition of dough ingredients was described.

Figure 1a shows average curves of dough with and without fiber incorporation at the highest substitution level (12%). In all cases, 6% samples showed intermediate curves between the control and 12% dough (Figure 1S, of the Supplementary Material). Thermograms showed a continuous rise of weight loss from 25 to 120 °C. Control, RSII and RSIV dough samples exhibited a pronounced inflection around 85 °C, from which an increase in the water evaporation rate was produced. Inulin and oat fiber dough showed different curve shapes, with continuous growth, no inflection and a slope decrease from 95 °C.

The water content of the final dough samples were between 14.3 % and 14.7 % and no significant differences were found among samples. Weight loss percentage of samples was calculated at the end of the assay (Table I). Values ranged between 10.32% and 14.14%. OF and IN showed lower values, while RSII and RSIV presented higher weight loss than that found in the control dough.

Additionally, we determined the temperature at which samples lost 50% of water. Significant differences were found only for IN, OF and RSII with 12% replacement; weight losses in the first part of the baking-simulated process were higher for IN and OF and lower for RSII, as compared with the control dough.

The first derivative (DTG) was obtained from TGA curve data, which gives information about sample weight loss rate. Each curve resulted from the combination of different peaks. Control, RSII and RSIV samples showed similar curve shapes, with a maximum between 85 and 95 °C and a smaller peak around 40 °C. IN and OF curves showed the same peak around 40 °C, although curve shapes changed significantly from 50 °C (Figure 1).

Fessas and Schiraldi (2000, 2001) studied the TGA profile of wheat dough and suggested that water in the dough would mainly be in two states: free water to diffuse through a medium, whose viscosity increases with increasing T due to drying and transformations affecting starch and gluten; water tightly bound to the gluten network and able to flash off only at rather higher temperatures. These authors also suggested that below 45 C°, dough water simply increases its vapor tension and mobility at increasing energy; at 45 °C and above, water swells starch granules, solvates amylose and amylopectin molecules and sustains gluten cross-linking, playing also the role of plasticizer between polymer chains; it becomes less free to move away (more tightly bound), thus requiring higher temperatures to flash off.

The dough samples analyzed show unique DTG profiles, resulting from a combination of different peaks and determined by the water state into the matrix. Cookie dough is a complex and heterogeneous system where water molecules exist in a number of states, corresponding to water that is bound and associated to different sites within the different components. It is expected that water in a cookie dough is shared between different phases (such as starch, gluten and sucrose) and occupies the inter-phase regions. When the dough is heated up, separated phases are still present in the system and water accordingly rearranges its partition over the phases. Dough components are responsible for trapping water until it is released as a consequence of heating. Due to their proportion in the dough recipe and their hydrophilic properties, starch, gluten and sucrose were suggested as the main component trapping water. Highly hydrophilic components, such as fibers and starches, have to be considered when present.The deconvolution of DTG samples allowed studying these different water states and associating water distribution to dough components. Deconvolution by Gaussian functions showed that the overall signal might be reproduced as the sum of four main contributions. Adjusted

model curves show r^2 values greater than 0.998. Peak associated to control dough had maximum peak values around 38, 66, 88 and 97 °C (Figure 1b).

RSII and RSIV dough showed similar relative areas of the peaks and peak maximum temperatures to those found in the control sample. However, it was observed that the third peak area of RSII 12% was higher than in the rest of the samples and the fourth peak area of both resistant starches were lower than that in the control dough.

Replacement of flour by IN and OF notably changed the relative weight of different peaks, i.e., water distribution among the system components. IN and OF caused an increase in the relative area of the first peak and a decrease in the area of the third peak ($p<0.05$). Regarding dough OF, the fourth peak occurred at a temperature of almost 20 ºC below that of the control sample ($p<0.05$). Inulin incorporation at 12% also generated a reduction in the area of the fourth peak ($p<0.05$).

Roozendaal, Abu-hardan and Frazier (2012) performed thermo-gravimetric analysis of wheat flour suspensions and associated flour dough components to the peak obtained by DTG curve deconvolution. The first peak, whose maximum was around 40 °C, was attributed to excess water, i.e., less bound water that evaporated easier. These authors related two peaks (~60 °C and ~70 °C) to water associated with starch, corresponding to less and more bind water, respectively. In the present work, only one peak was found at 65 °C, attributed to water retained by this component. Fessas and Schiraldi (2000) also found a single peak for the starch, working with wheat flour-water mixtures. This event shows that water associated with polysaccharides are held by weak bonding, which largely comprises the hydrogen bonds of polysaccharides, also including its ability to form junction zones (Chaplin 2003). Further investigation by Orlowska, Utzig and Randzio (2009) using NMR and TGA showed that within the single peak of a wheat starch-water suspension, less and more bound water phases do exist, despite overlapping each other, shown as a result as one peak in the TGA.

In other studies with dough elaborated from wheat flour and water (Fessas abd Schiraldi 2000; 2005; Roozendaal et al 2012), it was found that the water that evaporated at a higher temperature was associated with dough proteins.

Starch, being a polysaccharide, holds water through hydrogen bonding between the amylose and amylopectin branches and inter amylopectin helices (Orlowska et al 2009). These helices have the ability to form junction zones in which large amounts of water can be stored (Chaplin 2003). This water is released easier against mechanical stress or temperature increase (Lazaridou and Biliaderis 2007). Flour protein (gluten), on the other hand, is bound to water tightly by hydrogen bonds via glutamine residues (Belton et al 1998). This portion of water will therefore resist an extended period of time (Durchschlag and Zipper 2001).

Figure 1 shows that the third and fourth peaks overlapped greatly and thus both peaks were attributed to water associated with dough proteins. The third peak was related to the water less bound to protein, while the fourth was related to protein-bound water. These results are consistent with those reported by Roozendaal, Abu-hardan and Frazier (2012), which also found two peaks around 80-85 °C and 90-95 °C.

It is important to highlight that the incorporation of inulin increased the area of the first peak, attributable to the less bound water, and, consequently, reduced the areas of the peaks related to the more bound water. Oat fiber also presented a greater area of peak 1 than control dough. In a previous work it was reported that inulin and oat fiber retained more water (3.75 and 2.94 g water/g solid, respectively) than wheat flour (1.25 g water/g solid) and both starches (1.46 g water/g solid for RSII and 1.39 g water/g solid for RSIV) (Blanco Canalis et al 2016). Despite this, IN and OF seemed to retain water less tight as compared with wheat flour, since it is released in the first stage of the heating process. It is suggested that the IN and OF incorporation reduced amount of water available for protein hydration, allowing faster water evaporation.

IN and OF have the ability to form physically cross-linked gels whose three-dimensional structure is stabilized mainly by multiple inter- and intrachain hydrogen bonds in the junction zones of the polymeric network. . In these bond areas a large amount of water is enclosed and released easier against a ~~mechanical stress such as~~ temperature increase (Peressini and Sensidoni 2009).This is why these fibers showed the greater first peak area. Peressini and Sensidoni (2009) suggested that when wheat flour is partially replaced by inulin, more water is retained by this oligosaccharide which, in turn, could reduce starch swelling. In the present work water related to starch peak showed no difference in relation to control in area and peak maximum temperature. Conversely, both peak areas related to proteins were affected.

RSII produced a redistribution of the water related to proteins, where the highest incorporation level showed an increase of the third peak area of almost 70% and a reduction of more than 80% of the fourth peak, relative to the control curve (Figure 1e). Oat fiber showed the opposite effect, since it decreased the third peak area and increased the fourth (Figure 1d).

3.2. Pasting profile

In order to know how fiber incorporation affects the swelling and gelatinization of the starch of the wheat flour, the pasting profiles of the slurries were studied through RVA. This test allows analyzing the behavior of a mixture in a heating / cooling cycle, recording the viscosity of the solution. The paste formed by the flour and water mixture after heating is considered a composite material, where a continuous phase (mainly consisting of a network of amylose and dispersed proteins) is mixed with particles (swollen starch granules). The characteristics of this system depend on numerous factors, including the rheological properties of the continuous phase, the volume fraction, the deformability of particles and the interactions between the

dispersed and continuous phases (Copeland et al 2009; Fustier et al 2009).

Wheat flour replacement by dietary fiber affected starch pasting properties. In all the samples analyzed, a decrease in viscosity parameters (PV, BD, SB and PT) was observed, proportional to the substitution level (Table II). These changes are caused by the fact that less starch granules are available to gelatinize. Simultaneously, DF retains more water than wheat flour (Blanco Canalis et al 2016), hence, there is less water available, which would hinder swelling of starch granules (Rocha Parra et al 2015; Symons and Brennan 2004). This could explain the decrease observed in the peak viscosity of the pastes. The pasting temperature values significantly increased with the maximum level of incorporation for all fibers and for WF:IN_l and WR:$RSII_l$.

It is notorious that the pasting profile of WF:IN_h was higher than that of the rest of the fibers (Table II, Figure 2a). This differential behavior of inulin could be related to the particular molecular conformation of this fiber which provides it with the ability to form gels in the presence of excess of water, under certain conditions. Kim, Faqih and Wang (2001) studied exhaustively the factors that affect the formation of gels of this polysaccharide. They observed that this phenomenon occurs under two conditions: when a paste of inulin and water undergoes shear stress or when the paste is heated. In the RVA assay these two conditions are given together, i.e., samples are sheared as they heat. Therefore it can be suggested that WF + IN and water mixtures showed higher viscosity parameters than the rest of the samples due to the formation of inulin gel.

It should be noted that wheat replacement by RSII and RSIV also resulted in a decrease in the viscosity profile. Although these fibers are starches, they typically do not exhibit significant swelling or gelatinization at these temperatures under the conditions of this assay and do not present the characteristic thickening effect of the native starch (Cummings et al 1996; Nugent 2005; Ratnayake and Jackson 2008).

PV is related to the starch ability of binding water. Although inulin and oat fiber retain more water than wheat flour, the highest value of this parameter was for WF (2901 cp), since it had the highest starch proportion and water availability. DF incorporation significantly decreased this parameter, with values ranging between 2340 cp (WF:$RSIV_l$) and 1108 cp (WF:$RSII_h$).

The higher the BD value, the lesser the paste resistance. Again, as expected, the highest value was observed in WF (1080 cp), since DF incorporation decreased starch swelling, increasing paste resistance.

SB is a measure of the gelation capacity of the paste. All samples except WF:OF_l (1411 cp) and WF:$RSIV_l$ (1485 cp) showed values lower than those found in wheat flour (1562 cp).

In the formulation of cookie dough, sugar is the major ingredient after flour, and sucrose is the most common sugar used in baking. Sugars are plasticizers of the biopolymers of wheat flour; yet, concentrated aqueous

sugar solutions act as antiplasticizers, when compared with water alone (Slade & Levine, 1987, 1994). As a result, gluten development during dough mixing and starch gelatinization/pasting during cookie baking is delayed or prevented. Slade and Levine (1994) attribute this fact to a decreased mobility of the system rather than to a decreased amount of water available to hydrate the flour.

Sugar is the second major ingredient, after wheat flour, and its presence limits the availability of water to hydrate other particles, since it acts as an antiplasticizer of polymers (compared to water alone) when it is in high concentration. Kweon et al (2009) performed RVA profiles using 50 % (p/p) solution of four different sugars to relate them to cookie quality and to the behavior of dough during baking. In order to explore the behavior of the mixture of wheat flour and fibers, extra RVA assays were carried out using 50% sucrose solution. The shape of curves was highly different from those obtained using water as solvent, showing retardation onset of starch pasting compared with those in water. When 50% sucrose solution was used as solvent, paste viscosity started to increase at around 280 seconds, when temperature equipment had already reached the maximum (95 °C). Afterwards, a pronounced increase in paste viscosity was observed, until temperature decreased and the slope of viscosity increased. Around 600 seconds a small increase of this slope took place again, reaching maximum viscosity. These results agree with those observed by Kweon et al (2009), who obtained curves with the same general shape with sucrose solution. From these curves two parameters were obtained: peak viscosity (considered as the maximum viscosity at which pastes arrive during heating) and final viscosity (Table II).

Figure 2b shows that fiber incorporation decreased WF viscosity profile, without substantially changing the curve general shape. The lowest viscosity was presented by IN, followed by RSIV, RSII and OF.

It is important to note that the peak viscosity values of IN compared to other OF and RS values showed an opposite behavior as a consequence of the solvent used (Table II): water as solvent ($WF:RSII_h$, $WF:OF_h$, $WF:RSIV_h$)<$WF:IN_h$<WF and 50% sucrose solution was used as solvent $WF:IN_h$< ($WF:RSIV_h$, $WF:RSII_h$, $WF:OF_h$)<WF. In addition, differences between WF and fiber samples were higher in the sucrose solution assay than those when water was used as solvent. This evidences an important competition for water between flour and fibers.

Final viscosity resulted around 30% higher than peak viscosity. Comparing the profiles using water as solvent, sucrose solution increased pasting temperature about 15 °C in all samples, i.e., a delay in starch gelatinization was produced. These results agreed with the informed by Slade and Levine (1994) and Kweon et al (2009).

3.3 Scanning Electron Microscopy (SEM) and particle size distribution

It is evident that the incorporation of each of the fibers in the cookie dough modifies the characteristics of the system in different ways. The behavior of each DF is probably determined by the size, shape and distribution of the particles. In order to explain the differences observed in the previous trials, the particle size distribution of each sample was evaluated and the results supported by SEM images.

Figure 3 shows the microphotographs of WF and different fibers. The shape and spatial arrangement of the sample particles can be observed. Wheat flour micrographs showed the presence of some irregular pieces of broken endosperm as aggregates of protein matrix embedding groups of cellular components, mainly starch granules (Figure 3a). In the image of inulin, particles of about 100 µm were observed, forming small aggregates with a homogeneous spatial distribution (Figure 3b). Oat fiber showed irregular and squamous particles, where large chunks could be distinguished among smaller ones, with a heterogeneous aggregation (Figure 3c). Both resistant starch samples showed spherical particle aggregates with a diameter of around 10 µm. RSII presented a homogeneous distribution (Figure 3d), while RSIV showed large particle aggregates (Figure 3e).

Figure 4 shows the particle size distributions of dry powders and hydrated particles (aqueous suspension of WF and dietary fiber samples). Dry wheat flour presented a bimodal distribution, ranging between 10 and 110 µm with peaks around 26 µm and 55 µm, largely overlapping. This confirmed the observation by SEM. These particle sizes corresponded to starch granules and endosperm fractions, respectively. When hydrated, WF showed a single wide curve, with a maximum around 39 µm. Particle size oscillated between ~5 and ~200 µm (Figure 4a). This indicates that some particles were soluble in water or disintegrated in water.

Dry inulin presented a homogenous distribution ranging between 30 and 1000 µm, with a single peak around 187 µm. Hydrated inulin curves resulted notoriously different, where a single peak in 7.67 µm could be observed and the curve width ranged between ~5 and ~20 µm (Figure 4b). This differential behavior was attributed to the ability of inulin to form microcrystals in solution, whose diameter is smaller than that of the extended molecule (André et al 1996; André et al 1996). These results agreed with those informed by Cooper and Carter (1986), who affirmed that inulin normally crystallizes in water taking ovoid shape with diameters between 1 and 10 µm.

Oat fiber particle size, as observed in the microphotographs, exhibited a bimodal distribution with a peak in ~14 µm and another one wider with a maximum around 150 µm, in a large range between 5 and 300 µm. These peaks correspond to the scales and fiber aggregates, respectively, observed in the SEM image (Figure 4c). The distribution of oat fiber in solution was similar to that of dry fiber, but showed a greater proportion of larger particle. The plot presented a bimodal shape, with a curve width between ~5 and ~500 µm and peaks around ~30 µm and ~150 µm. This similarity was due to the low solubility of the oat fiber in water.

Both resistant starches showed a bimodal curve and particle size ranged between ~5 and ~300 μm. However, curve shape was highly different. RSII presented a single peak around ~14 μm and a narrower one in ~230 μm (Figure 4d), while RSIV showed two overlapped peaks at ~23 and ~50 μm and a smaller one around ~250 μm (Figure 4e). The peaks related to the high diameters of both starches correspond to particle aggregates, observed in the microphotographs.

Hydrated RSII and RSIV presented curve shapes similar to each other, where a single peak of ~25 μm and an amplitude between ~5 and ~80 μm could be observed. This distribution is attributed to the breaking of the aggregates and there only stayed individual granules that hydrated and swelled.

From these results it is evident that wheat flour and fiber particle size and distribution depend on hydration extent. The water retained by each fiber determines the mobility of the system and, therefore, the availability of water for hydration of wheat flour starch.

In previous studies the improving effect of the inulin incorporation on cookie dough was demonstrated (Blanco Canalis et al 2017; Blanco Canalis et al 2016). Inulin behavior seemed to be related to the proton mobility and availability of the water in the system (Serial et al 2016). From the results obtained in the present study it can be added that the improving effect of inulin lies in their ability to form microcrystals in solution. These structures retained water inside, decreasing system mobility and preventing starch hydration. However, this water is released earlier in the baking process when compared to a control dough (no fiber), increasing mobility and allowing cookie expansion.

It should be noted that inulin and oat fiber showed a similar TGA behavior; however, the effect on cookie quality is very different. Both, inulin and oat fiber retain more water than wheat flour. However, their solubility is very dissimilar: IN is ~33 times more soluble in water than OF (M. Blanco Canalis et al., 2016). Because of this, the behavior of both fibers in the dough is markedly different: inulin contributes to the liquid phase of the system, softening the dough and allowing greater expansion during baking, resulting in higher cookie quality. On the other hand, oat fiber retains the water absorbed, increasing dough consistency.

The incorporation of both starches (RSII and RSIV) resulted in a change of the properties of dough and slurries. As stated before, these starches typically do not gelatinize in the temperature range analyzed, thus these changed are mainly due a diluent effect when these fibers were added as flour replacement.

4. CONCLUSIONS

In this work we studied the effect of the incorporation of four different DFs on the thermo-gravimetric behavior of the cookie dough. The differences in dough behavior could be explained analyzing the pasting profile of the mixtures of wheat flour and DFs and studying the ultrastructure of both DF and wheat flour through SEM and dry and hydrated particle size distribution.

Oat fiber and inulin incorporation changed substantially the dough water loss profile. Deconvolution of four DTGA peaks provided new insights into dough water distribution. Results suggested that IN and OF reduced the proportion of water related to protein and increased the less bound water fraction, leaving less water available to starch granules hydration of the wheat flour. The water retained by both IN and OF seemed less tight than the water bound to wheat flour, since it is released in the first stage of the heating process. The addition of all fibers produced a decrease in the wheat pasting profile using both water and sucrose solution as a solvent. These effects were related to a reduction in the amount of starch granules and water availability. However, inulin showed the most extreme effects, because of its ability to form gel, decreasing system mobility. The ultrastructure study showed than wheat flour and fiber distribution size substantially changed in solution, according to sample solubility. Particularly, inulin presented the ability to form microcrystals in solution, granting this fiber a different behavior.

From this study it could be determined that the improving effect of inulin incorporation in the cookie dough is related to the ability of this fiber to form microcrystals in solution, trapping water inside. This decreases system mobility and prevent starch hydration. However, this water is released early in the baking process, increasing mobility, which can allow further cookie expansion as was determined in previous study.

Acknowledgments

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Figure captions

Figure 1. Effect of fiber addition on the weight loss (a) and deconvolution of weight loss rate curve and areas of dough samples during heating (b,c,d,e,f).

Figure 2. Effect of fiber addition on the pasting profile using water (a) and 50% sucrose solution

Figure 3. Microphotographs of SEM.

Figure 4. Particle size distribution of dry and hydrated samples.

Table I. Effect of fiber addition on weight loss during dough heating.

Table II. Effect of fiber addition on pasting parameters

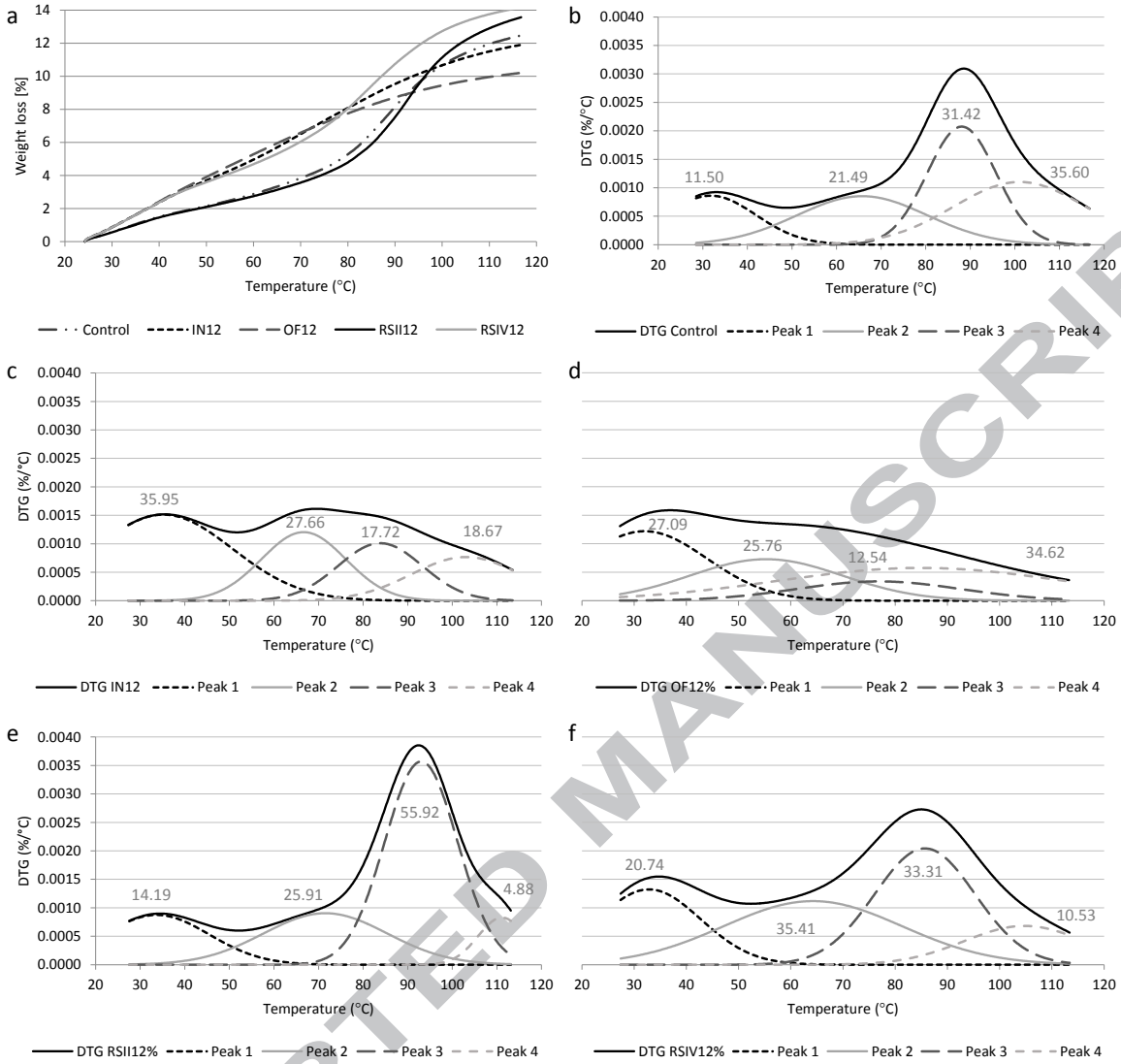

Figure 1. Effect of fiber addition on: a) Weight loss of cookie dough b) Deconvolution of weight loss rate Control dough; c) Deconvolution of weight loss rate Inulin 12%; d) Deconvolution of weight loss rate Oat fiber 12%; e) Deconvolution of weight loss rate RSII 12% and f) Deconvolution of weight loss rate RSIV 12%. Data in the figures correspond to the relative area of each peak (%).

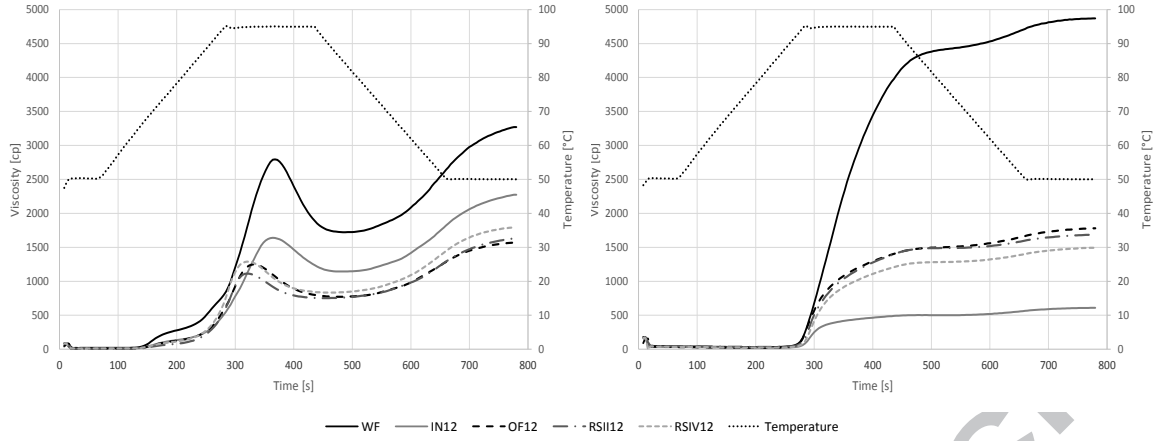

Figure 2. Effect of fiber addition on the pasting profile using water (a) and 50% sucrose solution (b) as a solvent. WF: wheat flour; IN: Inulin; OF: Oat fiber; RSII: Resistant starch II; RSIV: Resistant starch IV. 12 refer to fiber incorporation level.

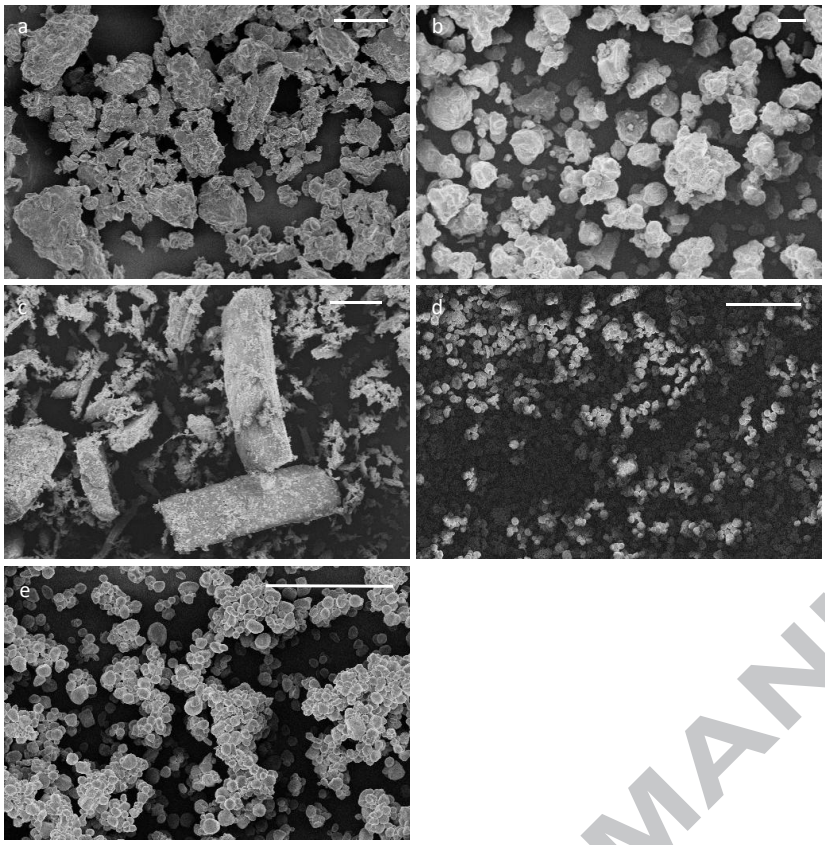

Figure 3. Microphotographs of SEM. a) wheat flour, magnification 150x; b) inulin, 80x; c) oat fiber, 144x; d) RSII, 200x and e) RSIV, 350x. Barr: 100 µm.

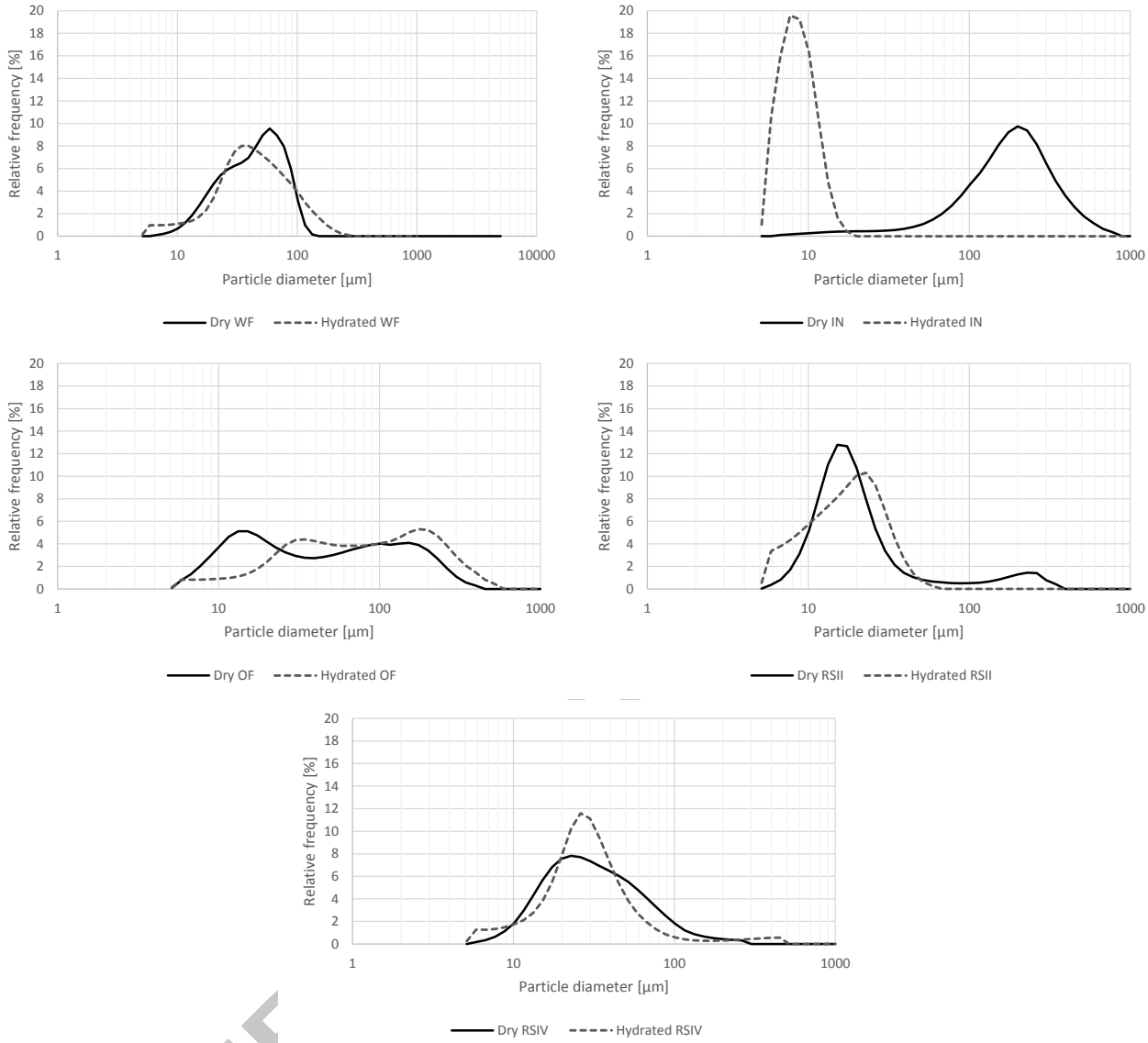

Figure 4. Particle size distribution of dry and hydrated samples. WF: wheat flour; IN: Inulin; OF: Oat fiber; RSII: Resistant starch II; RSIV: Resistant starch IV.

Sample	50% WL temperature [°C]				TWL [%]			
C	83.87	±	0.57	bc	12.46	±	0.39	bc
IN6	78.06	±	4.10	bc	11.92	±	0.61	c
IN12	67.23	±	5.58	de	11.94	±	0.15	c
OF6	83.94	±	1.23	bc	13.14	±	0.03	ab
OF12	59.00	±	5.11	e	10.23	±	0.48	d
RSII6	86.62	±	2.75	ab	13.73	±	0.05	a
RSII12	93.24	±	0.95	a	13.60	±	0.40	ab
RSIV6	85.15	±	0.10	ab	12.81	±	1.29	bc
RSIV12	75.52	±	8.79	cd	14.14	±	0.08	a

Table I. Effect of fiber addition on weight loss during dough heating. C: control; IN: Inulin; OF: Oat fiber; RSII: Resistant starch II; RSIV: Resistant starch IV. 6 and 12 refer to fiber incorporation level.
50% WL temperature: temperature at which samples lose 50% of water. TWL: percentage of total weight loss obtained at the end of the assay.
Values in the same column with common letter are not significantly different ($p>0.05$)

Table II. Effect of fiber addition on pasting parameters.

Sample	PV [cp]	BD [cp]	SB [cp]	PT [°C]	PV (sucrose) [cp]	FV (sucrose) [cp]
WF	2901 ± 35 ^{a}	1081 ± 60 ^{a}	1562 ± 53 ^{a}	67.8 ± 0.0 ^{c}	3772 ± 98 ^{a}	4870 ± 78 ^{a}
WF:IN$_{l}$	2000 ± 394 ^{bc}	683 ± 160 ^{c}	1300 ± 81 ^{bc}	73.0 ± 6.0 ^{ab}	1332 ± 52 ^{d}	1599 ± 45 ^{ef}
WF:IN$_{h}$	1641 ± 12 ^{cd}	497 ± 11 ^{d}	1130 ± 4 ^{cd}	87.1 ± 0.0 ^{a}	480 ± 4 ^{f}	609.5 ± 1 ^{g}
WF:OF$_{l}$	2327 ± 217 ^{b}	912 ± 61 ^{b}	1411 ± 26 ^{ab}	69.0 ± 1.0 ^{bc}	2430 ± 66 ^{b}	3115 ± 73 ^{b}
WF:OF$_{h}$	1238 ± 159 ^{de}	493 ± 62 ^{d}	776 ± 81 ^{f}	88.0 ± 2.0 ^{a}	1358 ± 30 ^{d}	1781 ± 40 ^{d}
WF:RSII$_{l}$	1915 ± 206 ^{c}	751 ± 52 ^{bc}	1255 ± 226 ^{bcd}	85.0 ± 2.0 ^{a}	2387 ± 98 ^{b}	3031 ± 105 ^{b}
WF:RSII$_{h}$	1108 ± 45 ^{e}	387 ± 34 ^{d}	836 ± 122 ^{ef}	86.7 ± 0.5 ^{a}	1348 ± 62 ^{d}	1688 ± 71 ^{de}
WF:RSIV$_{l}$	2340 ± 139 ^{b}	902 ± 78 ^{b}	1485 ± 59 ^{ab}	67.8 ± 0.0 ^{c}	2171 ± 1 ^{c}	2744 ± 1 ^{c}
WF:RSIV$_{h}$	1327 ± 76 ^{de}	473 ± 0 ^{d}	1044 ± 115 ^{de}	86.0 ± 1.0 ^{a}	1168 ± 15 ^{e}	1494 ± 21 ^{f}

PV: Peak viscosity; BD: breakdown; SB: setback; PT: pasting temperature; FV: final viscosity. WF: wheat flour; IN: Inulin; OF: Oat fiber; RSII: Resistant starch II; RSIV: Resistant starch IV. Subscript l: low WF:fiber ratio (13.33 % of fiber). Subscript h: low WF:fiber ratio (26.67 % of fiber). Values in the same column with common letter are not significantly different ($p>0.05$)