Microscopic and Thermal Characteristics of Experimental Models of Starch, Gliadins, Glutenins and Gluten from Semolina

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Durum wheat semolina is the preferred and most often used raw material for the production of dried pasta. The high quality of pasta is attributed to its specific structure, obtained after successive structural changes of the two main semolina components, starch and proteins, gliadins and glutenins, in presence of water.

The present study was conducted to examine the contributions of gliadins, glutenins and starch to the structure and functionality of gluten by means of scanning electron microscopy (SEM) and of differential scanning calorimetry (DSC).

Isolated samples of the main semolina components (starch, gliadins, glutenins) and gluten were considered. Experimental models were then prepared from defined binary mixtures of starch and proteins (gliadins or gluteins or gluten) and from respective doughs with water (50 % w/w). SEM of surface morphology provided stereoscopic images with high magnification. Semolina starch was composed of small spherical B-type granules (average diameter 2-3 μm) and larger lenticular A-type granules (average diameter 30 μm). The micrographs from starch - gliadins dough showed a distinct film surrounding the small and large starch granules. In the starch - glutenins doughs, most of the starch granules appeared naked, it seems that the proteins are unable to surround all of the starch granules. A possible explanation for this is that the lack of gliadins can be affecting the formation of the film structure in fact gliadins may be involved in the development of the structure of the gluten film networks through covalent and non-covalent bonding with other gluten proteins. DSC allowed the observation of all phenomena that involves the heat exchange in models: protein denaturation and starch gelatinization.

1. Introduction

Dried pasta represents a basic food worldwide. It is prepared from dough obtained by mixing water with semolina from durum wheat (*Triticum turgidum* L. var. durum). The properties of dough are those from hydration of gluten proteins and starch granules, these latter as filler packing. In particular, the technological properties of the proteins are ascribed to the presence of gluten, which is formed by storage proteins of the endosperm - the alcohol soluble gliadins and the alcohol insoluble glutenins (Singh, et al., 2011).The structure of pasta has been described as a compact matrix with starch granules entrapped in a coagulated protein network (Bruneel et al., 2010). Indeed the structure and the most desirable characteristics of high quality pasta products are related to the interactions between starch (approximately 70 %) and proteins (12 -15 % db) of durum wheat semolina in the presence of water (Güler et al., 2002). Furthermore, both starch and gluten are frequently used as additives in the food industry, and thus interactions between wheat proteins and starch might be of importance for the quality of food products. Numerous authors report that protein extract and gluten interact differently with starch (Lindahl and Eliasson, 1986) and influence its gelatinization parameters (Delcour et al., 2000) and water behavior (Mohamed and Rayas-Duarte, 2003). Therefore, the understanding

of interactions among gluten, gluten protein, starch and water is crucial to improve the process involving gluten and starch as constituents of raw matter or as coadjuvant ingredients to improve food properties.

The purpose of this work was to examine the contributions of gliadins, glutenins and starch to the dough structure and functionality by means of scanning electron microscopy (SEM) and of differential scanning calorimetry (DSC) methods.

2. Materials and Methods

2.1 Materials

Chapter 2 Protein of durum wheat semolina (De Cecco®, Italy) were fractionated into three main fractions according to the procedure in Weiser et al. (1998). The experimental protein fractions (gliadins, glutenins and gluten), which were prepared and extracted using Dual Glutomatic (Glutomatic 2200, Perten Instruments), were used to formulate model batches (gliadin + starch; glutenin +starch; gluten + starch). Starch and food biopolymers (gliadins, glutenins and gluten) were mixed in the ratio starch to biopolymer of 4:1 (w/w). Doughs of model batches were prepared by means of a Brabender Farinograph (O. H. Duisburg, Germany) using deionized water at 50 % (w/w).

1.1 Microstructural analysis (SEM)

Samples were dried at the critical point and coated with gold particles in an automated critical point drier (model SCD 050, Leica Vienna). The microstructure of the samples was observed and photographed in a LEO EVO 40 SEM (Zeiss, Germany) scanning electron microscope at a magnification of x 2000 with accelerating voltage of 20 kV.

1.2 Thermal analysis (DSC)

The gelatinization properties of the samples were assessed using Differential Scanning Calorimeter (Q200, TA Instruments, Milan, Italy). The calorimeter was calibrated using indium as standard. The samples of 6 mg each were hydrated in the Tzero aluminium hermetic pans at about 50 % moisture. The pan was closed with a lid, weighed and kept at room temperature for 4 hour. All samples were heated from 30 °C to 90 °C at 10 °C min^{-1} using an empty pan as the reference. The gelatinization properties are reported as the onset temperature (To), peak temperature (Tp) and end temperature (Te) and gelatinization enthalpy (ΔH). Average values of five measurements were calculated for each sample.

1.3 Statistical analysis

Duncan's multiple comparison test (SPSS v17.0) at the 95 % confidence level ($p \leq 0.05$) was used to compare mean differences of samples.

2. Results and Discussion

2.1 Microstructural analysis

The microscopic observations of initial microstructures and their changes after mixing with water (50% w/w) were conducted on the starch (S) and binary mixtures of starch and proteins such as gliadins (GD), glutenins (GT) and gluten (GLU), using SEM.

The SEM images of durum starch and its dough are shown in Figure 1. Starch granules are round, spherical or polygonal in shape with smooth surfaces and wide distribution of sizes. Dimensional results of semolina starch (Figure 1a) showed granules with small (< 10 µm, B- type) and large (> 20 µm, A- type) elements, in accordance with Svihus et al. (2005) and with Teo and Small (2012).
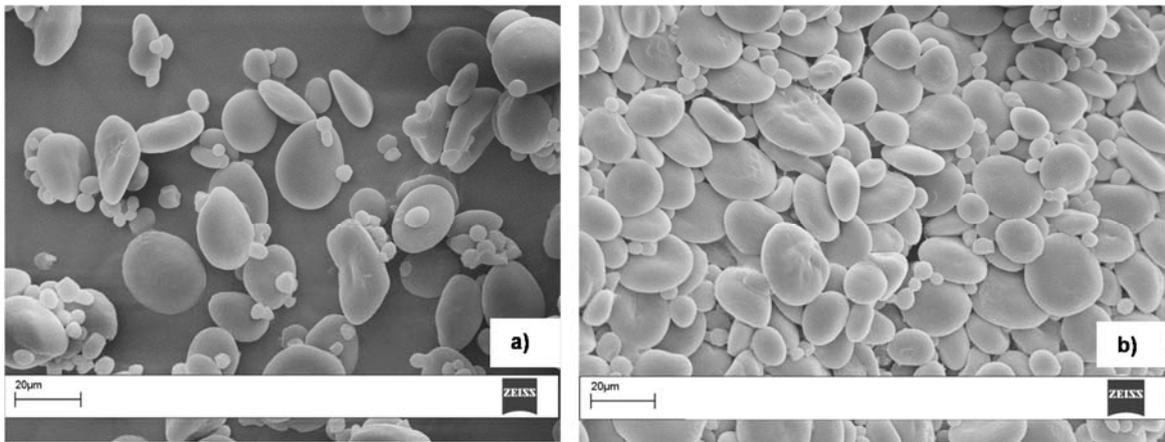
Figure 1: Scanning electron micrographs of a) starch (2.000 K) b) dough of starch with water (50 % w/w).

The starch granules did not lose their granular structure after mixing with water (Figure 1b). In the presence of water, the starch molecular chains confer the granules a much more detailed structure in which they can be seen as dissolving structures surrounded by a network of carbohydrate branches. Partially gelatinized starch can also be seen here (Figure 1b).

Figure 2 shows the effect of hydration on the mixtures of starch - GD (Figure 2a,b), starch - GT (Figure 2c,d) and starch - GLU (Figure 2e,f).

The initial microstructures of starch - GD (Figure 2a), starch - GT (Figure 2c) and starch - GLU (Figure 2e) were mainly characterized by starch granules of various sizes B- type (average diameter 2-3 µm) and A- type (average diameter 10 µm) granules. It was observed that surface of all samples was changed after mixing with water. The SEM micrograph of starch - GD doughs (Figure 2b) showed a distinct film surrounding the small and large starch granules. Starch granules were uniformly embedded in the open GD matrix, consistent with a ``space-filling'' role. In the starch - GT doughs (Figure 2d), instead most of the oval-shaped and circular starch granules appeared naked, it seems that the proteins are unable to surround the starch granules and starch - GT doughs had less extensive protein framework. A possible explanation for this difference could be attributed to the fact that GD are globular proteins which may be involved in the development of the structure of the gluten film networks through covalent and non-covalent bonding with other gluten proteins and behave mainly as a viscous liquid when hydrated and confer extensibility (Khatkar et al., 2002). Khatkar et al. (2013) reported also that the compactness of the gluten structure reduced considerably with the addition of GD leading to the formation of a more open weak GLU network. On the other hand, GT are elastic proteins (cysteine residues) which are capable of forming inter and intrachain disulfide bonds leading to the formation of highly networked protein structure and their addition results in a more elastic dough in comparison with GLU and GD additions (Edwards et al., 2003).

Starch granules of starch - GLU doughs (Figure 2f) were embedded in and covered with an amorphous protein matrix. Starch granules of various sizes, protein matrix and adhesive protein areas attached to starch granule surfaces can be observed. The surface of the starch - GLU doughs (Figure 2f) appeared as a fibrillar network, but, a complete development of a gluten network, as would be the case in bread dough (Romano et al., 2013), was not found. A GLU fibrillar network that surrounding the starch granules after hydration has been reported in the literature (Cunin et al., 1995). Our results confirm the deep changes which starch and proteins of semolina undergo during hydration.
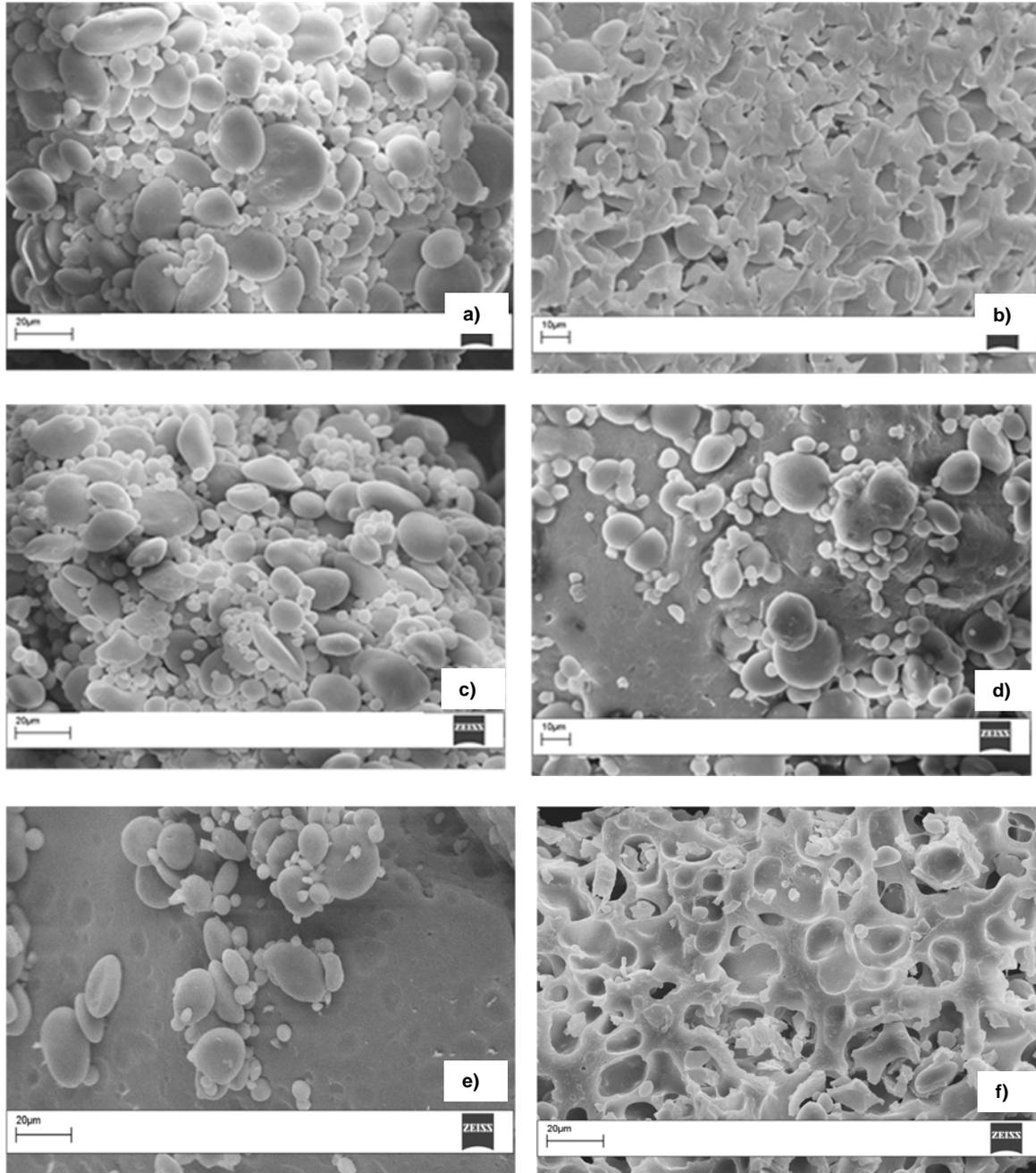
Figure 2: Scanning electron micrographs (2.000 K) of mixtures of : a) starch - GD; b) dough of starch - GD with water (50 % w/w); c) starch - GT; d) dough of starch - GT with water (50 % w/w); e) starch - GLU b) dough of starch - GLU with water (50 % w/w).

2.2 Thermal analysis

Thermal analysis is a valuable tool for studying the effect of thermal processing on vegetable proteins (Ma, 1990) and the phase transition of starch (Laaksonen and Roost, 2000). It is well known that the cooking processes cause some structural changes on starch and gluten network of dough. In fact, during heating starch gelatinization process takes place that is the transition of insoluble starch granules to a solution composed of individual molecules (León et al., 2003). In order to evaluate thermal interactions between starch and protein fractions, doughs of starch and of starch - GD, starch - GLT, starch - GLU were studied by means of DSC.

All samples presented a single major endothermic transition, with the corresponding temperatures and enthalpies of the transition shown in Table 1.

Table 1: Thermal results[1] of the analyzed samples: ΔH, transition enthalpy, To, onset temperature; Tp, peak temperature; Te, end temperature

Sample	ΔH (J / g)	To (°C)	Tp (°C)	Te (°C)
starch - 50%water	2.40 ±0.3c	54.8 ±0.4c	59.4 ±0.3c	66.6 ±1.2c
starch- GD's dough	2.16 ±0.1b	50.9 ±1.2a	56.1 ±0.5a	60.5 ±0.7a
starch - GT's dough	2.26 ±0.3b,c	53.5 ±0.4b	57.5 ±0.6b	61.6 ±1.1a
starch - GLU's dough	1.72 ±0.1a	54.1 ±0.5b	57.1 ±0.3b	63.5 ±0.7b

Data are expressed as mean ± standard deviation (n=3), means followed by a different letter within a column are significantly different (p < 0.05)

The results showed that the DSC parameters transition enthalpy (ΔH), transition onset temperature (To), transition peak temperature (Tp) and transition end temperature (Te) varied significantly (p < 0.05) among the samples. The most pronounced transition was exhibited by the starch samples.

The endothermic peak of the starch with water corresponds to the gelatinization transition. According to literature, the range of gelatinization temperatures of wheat starch is: 51 – 79°C (Singh et al., 2003). Values obtained in this experiment fall within this range.

Gelatinization of starch is a cooperative process, such that structural relations between amorphous and crystalline regions within the starch granules are responsible for the sharpness of thermal transition and the temperature at which it occurs (Kruerger et al., 1987). In a heterogenous system such as our binary mixtures, enthalpy may be better designated as overall transition enthalpy encompassing all heat changes associated with components in the system capable of thermal transitions. Indeed the water content in a food system has great influence on the gelatinisation behaviour of starch and similarly water is a major factor determining the thermal stability of proteins (Eliasson, 1983a). Thus, the ΔH values obtained in this study represent a composite, comprising the balance of heat changes involved with gelatinization of starch, denaturation of proteins and the changes associated with protein- starch interactions. The values of binary mixtures of starch and food biopolymers were significantly lower (p < 0.05) than values reported for starch. Enthalpy (ΔH) values (Table 1) ranged between 1.7 to 2.3 J/g. In particular the lowest value of ΔH of doughs with starch – GLU may be due to a dilution effect on starch, starch - GLU interactions (Eliasson, 1983b) or competition of GLU and starch for water (Ottenhof and Farhat, 2004).

On the other hand, the low value of transition temperatures and ΔH of doughs with starch – GD may be attributed to the reduced thermal stability of gliadin which, as monomeric proteins, are considered to possess lower thermostability (Khatkar et al., 2013). Similarly, glutenins which are polymeric proteins showed higher thermostability than doughs of starch and GD and starch and GLU (Falcaò-Rodrigues et al., 2005).

3. Conclusions

Our results confirm the deep changes which starch and proteins of semolina undergo during hydration. The SEM observations of starch and protein fractions demonstrated a great diversity of microstructure and their evolutions during hydration. In particular, starch granules composed of small spherical B-type granules and larger lenticular A-type granules and they do not seem to lose their granular structure during hydration. The presence and type of protein fraction caused considerable effects on the microstructure of the doughs. The micrographs from starch - GD doughs showed a distinct film surrounding the small and large starch granules. In the starch - GT doughs, most of the starch granules appeared uncovered, it seems that the proteins were unable to surround the starch granules. DSC allowed the observation of all phenomena that involves the heat exchange in the dough models such as protein denaturation and starch gelatinization. The values of binary mixtures of starch and food biopolymers were significantly lower (p < 0.05) than the values reported for starch. Wheat proteins have a peak endotherm at temperatures ranging from 50 to 64°C, the GD being the most heat sensitive, followed by the GT. The latter are also the most ordered structure proteins based on the high enthalpy values.

This may be expected to have implications not only on extrusion process but for the performance of gluten in baked products. It is worth to highlight that controlling the components of the gluten fraction and the structure of the gluten network is essential for improving the processability of wheat dough and the quality of food products.

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