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Influence of *Psyllium*, sugar beet fibre and water on gluten-free dough properties and bread quality

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ABSTRACT

Celiac patients generally have a low intake of protein and fibre attributed to their gluten-free (GF) diet. To satisfy the increasing demand for healthier products, this research focused on the effects of the supplementation of *Psyllium* (P) and sugar beet fibre (SB) on the mixing and leavening behaviour of gluten-free doughs. Four doughs, having different consistencies that made them suitable to be poured into moulds or to be shaped, and their corresponding breads were evaluated. The results obtained suggested that a lower consistency is preferred to assure good dough performances during leavening, in particular when ingredients having a high water affinity are included into the recipe. Both P and SB improved the workability of the doughs, but P played a central role on GF bread development, thanks to its film forming ability, and evidenced a more effective antistaling effect, thanks to its high water binding capacity.

1. Introduction

Celiac disease (CD) is one of the most common lifelong disorders on a worldwide basis. It is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. At present, the only available treatment for celiac people is the strict adherence to a gluten-free (GF) diet, which means a permanent withdrawal of gluten from daily foods. With the increasing incidence of CD, a rising demand for GF goods broke out; at the same time, a higher quality and a prolonged shelf-life of GF products are expected by the consumers. In fact, nowadays, the quality of the GF products available on the market does not fulfil consumers' expectations, being these products often characterized by a low nutritional value and an unsatisfactory sensory quality, particularly when compared to their wheat counterparts. Therefore, even if many studies have been performed in the last decades on this topic, more investigations are necessary.

Bread is the most challenging among GF foods. When compared to their wheat based counterparts, GF breads are characterized by a heterogeneous recipe, usually made of a combination of rice (Arendt, Morrissey, Moore, & Dal Bello, 2008; Gujral & Rosell, 2004; Marco & Rosell, 2008) and corn (Brites, Trigo, Santos, Collar, & Rosell, 2010; Renzetti, Dal Bello, & Arendt, 2008) starch and flour, as well as proteins, fibres, fats, hydrocolloids, and eventually specific enzymes. In GF bread production, the first crucial point is to supply for the absence of the viscoelastic gluten network that makes the whole breadmaking process problematic, and penalizes the sensorial quality of the final product. Doughs lacking in gluten, in fact, show limited abilities of gas expansion and retention during leavening, that inevitably lead to bread with a reduced volume and a low crumb softness (Gallagher, Gormley, & Arendt, 2004; Mariotti, Lucisano, Pagani, & Ng, 2009).

Nowadays, to provide for the lack of gluten and to simulate its viscoelastic behaviour, the addition of hydrocolloids having a strategic role in making the GF dough workable and improving

Abbreviations: A, formulation containing 2.5% *Psyllium* and 0.5% sugar beet fibre; AMY, maltogenic amylase; ANOVA, analysis of variance; B, formulation containing 1.5% *Psyllium* and 1.5% sugar beet fibre; BD, breakdown; BU, Brabender Unit; B_{A200}, bread obtained from D_{A200}; B_{A500}, bread obtained from D_{A500}; B_{B200}, bread obtained from D_{B200}; B_{B500}, bread obtained from D_{B500}; CD, celiac disease; $CO_{2\text{-RET}}$, CO_2 retained by the dough; $CO_{2\text{-REL}}$, CO_2 released by the dough; $CO_{2\text{-TOT}}$, total CO_2 production; CS, corn starch; D_{A200}, dough obtained from M_A, with a consistency of 200BU; D_{A500}, dough obtained from M_A, with a consistency of 500BU; D_{B200}, dough obtained from M_B, with a consistency of 200BU; D_{B500}, dough obtained from M_B, with a consistency of 500BU; DS, damaged starch; FV, final viscosity; GF, gluten-free; GG, guar gum; Hf, final dough height; Hm, maximum dough development; HPMC, hydroxypropylmethylcellulose; LB, locust bean gum; LSD, least significant differences; M, GF baking mixtures: M_0 (CS + RS + RF + RP), M_1 (M_0 + HPMC + LB + GG), M_A (M_1 + 2.5%P + 0.5%SB), M_B (M_1 + 1.5%P + 1.5%SB); MVA, Brabender® Micro-Visco-Amylograph; PT, pasting temperature; PV, peak viscosity; P, *Psyllium* fibre; Rc, gas retention coefficient; RF, rice flour; RP, rice protein; RS, rice starch; Sb, setback; SB, sugar beet fibre; TS, total starch; Tx, time of dough porosity appearance; WA, amount of water required to reach the desired dough consistency; WBC, water binding capacity.

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at the same time the texture of the final GF product (Guarda, Rosell, Benedito, & Galotto, 2004; Kobylañski, Perez, & Pilosof, 2004; Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007), has become quite common. The most effective gums and hydrocolloids have been identified with hydroxypropylmethylcellulose (HPMC), locust bean gum, guar gum, carrageenan and xanthan gum (Gallagher et al., 2004; Lazaridou et al., 2007). Mariotti et al. (2009) investigated the role of *Psyllium* flour – differently combined with corn starch, amaranth flour and pea isolate – on the rheological properties and the ultrastructure of GF doughs. The same authors evidenced how *Psyllium* generally enhanced the physical properties of the doughs, due to the film-like structure formed during kneading, and how it appeared promising in terms of final bread technological and nutritional quality. *Psyllium* seed husks have the highest known level (about 70%) of soluble fibre, and this fibre is a polymer of arabinose, galactose, galacturonic acid, and rhamnose (Nelson, 2001).

Since celiac patients generally have a low intake of protein and fibre (Thompson, 2000), the enrichment of GF bread with vegetal and animal proteins, as well as with dietary fibre, is a winning way to increase the nutritional and sensorial quality of the final product. In wheat bread, the main source of fibrous materials is generally wheat bran. Nevertheless, the design of fibre-enriched traditional baked goods has always come up against consumers resistance to accept breads with reduced loaf volume and hard crumb accompanied by particular flavours (Rosell, Santos, & Collar, 2010). Filipovic, Djuric, and Gyura (2007) thus suggested to incorporate other fibres, such as sugar beet fibre, characterized by low phytate content and by a better water retention capacity in comparison to wheat bran. Sugar beet fibre is the beet pulp remaining after water extraction of the sugar from the sliced beet tuber; the isolated fibre is about 73% total dietary fibre, about one-third of which is soluble fibre, mainly pectin.

Generally, the higher the presence of fibre in a dough the higher the amount of water required to obtain a workable dough (Mariotti et al., 2009). Water and flour are the most significant ingredients in a bread recipe, as they affect texture and crumb the most. Water, in particular, has essential and critical functions: it is necessary for solubilizing other ingredients, for hydrating proteins and carbohydrates, and for developing the protein network (Maache-Rezzoug, Bouvier, Allaf, & Patras, 1998). Water also plays an important role in the changes associated to starch occurring during breadmaking (*e.g.* gelatinization and retrogradation), and in assuring the quality and the shelf-life of the final bread (*e.g.* in terms of crumb softness and crust crispness) (Wagner, Lucas, Le Ray, & Trystram, 2007). In GF bread production the amount of water used to prepare the dough is frequently almost the same as (or higher than) the total amount of the dry ingredients included in the recipe; the aspect and consistency of the resulting dough is generally closer to that of a batter than to that of a conventional wheat flour dough (Mariotti, Pagani, & Lucisano, 2013). Critical is the shelf-life of GF breads, due to the presence into the recipe of a large amount of starches and flours from different origin that inevitably determines an increase of the staling rate of the product. Fibres may play a positive effect on quality parameters related to bread staling (such as crumb softness and springiness) by increasing the water absorption of the dough (Chen, Rubenthaler, Leung, & Baranoski, 1988; Wang, Rosell, & de Barber, 2002).

The aim of this research was the investigation of the effects that different amounts of *Psyllium* fibre (a thickening agent and a source of fibre), sugar beet fibre (a fibre source), and water have on GF dough and bread properties, in order to: (*i*) evaluate the influence of the presence of different fibres on the rheological properties of the dough; (*ii*) study the importance of GF dough consistency for its workability and for the technological quality of the final product; (*iii*) increase the nutritional quality and the shelf-life of the GF breads.

2. Materials and methods

2.1. Raw materials

The raw materials used to prepare the GF mixtures were the followings: corn starch (CS; Roquette Frères, France), rice flour (RF; Beneo-Remy NV, Belgium), rice starch (RS; Beneo-Remy NV, Belgium), rice protein (RP; Beneo-Remy NV, Belgium), *Psyllium* fibre (P; Indian Psyllium seed husk; Roeper GmbH, Germany), sugar beet fibre (SB; Danisco Sugar AB, Sweden), hydroxypropylmethylcellulose (HPMC; UNIVAR S.p.A., Italy), locust bean gum (LB; Caremoli S.p.A., Italy), guar gum (GG; Caremoli S.p.A., Italy), maltogenic amylase (AMY; Novozymes, Switzerland).

2.1.1. Chemical–physical characterization

The chemical-physical properties of the main ingredients (CS, RS, RF, RP, P and SB) were evaluated. The moisture content was determined according to the Official Standard Method AACC 44-15A (2000). The total nitrogen content was evaluated according to the Official Standard Method AOAC 920.87 (1999), and the protein content was calculated adopting 6.25 as conversion factor. The amounts of total starch (TS) and damaged starch (DS) were determined using the “Total Starch Assay Kit” and the “Starch Damage Assay Kit” (Megazyme International Ireland Ltd., Bray Business Park, Bray, Co. Wicklow, Ireland), respectively. All these evaluations were made at least in duplicate ($n \geq 2$).

2.1.2. Pasting properties

The pasting properties of the main ingredients (CS, RS, RF, RP, P and SB) were investigated using a Brabender® Micro-Visco-Amylograph (MVA; Brabender OHG, Duisburg, Germany) (Mariotti, Zardi, Lucisano, & Pagani, 2005; Shuey & Tipples, 1980). Fifteen grams of raw materials were dispersed in 100 mL of distilled water, scaling both flour and water weight on 14% sample moisture basis. The suspensions were subjected (stirring at 250 min^{-1} and using a 300 cm·g_f cartridge) to the following standard temperature profile: heating from 30 °C up to 95 °C, holding at 95 °C for 30 min, cooling from 95 °C to 50 °C, holding at 50 °C for 30 min and cooling to 30 °C. A heating/cooling rate of 3 °C/min was applied. The following indices were considered: pasting temperature (PT, °C; temperature at which an initial increase in viscosity occurs), peak viscosity (PV, Brabender Units, BU; maximum viscosity achieved during the heating cycle), breakdown (BD, BU; index of viscosity decrease during the holding period, corresponding to the peak viscosity minus the minimum viscosity reached after the holding period at 95 °C); final viscosity (FV, BU; paste viscosity achieved at the end of the cooling cycle), and setback (Sb, BU; index of the viscosity increase during cooling, corresponding to the difference between FV and the minimum viscosity reached after the holding period at 95 °C).

The MVA was also used to test the pasting properties of some mixtures (M), paying attention to maintain the same ratios among the different ingredients as those present in the main recipe (see Section 2.2.1). In particular, the followings were tested: M_0 (CS + RS + RF + RP); M_1 (M_0 + HPMC + LB + GG); M_A (M_1 + 2.5%P + 0.5%SB); M_B (M_1 + 1.5%P + 1.5%SB) (Fig. 1). In this case, 10 g of each mixture were dispersed in 100 mL of distilled water, scaling both mixture and water weight on 14% sample moisture basis. The same standard temperature profile described before was applied, and the same indices were considered.

All these determinations were made at least in duplicate ($n \geq 2$).

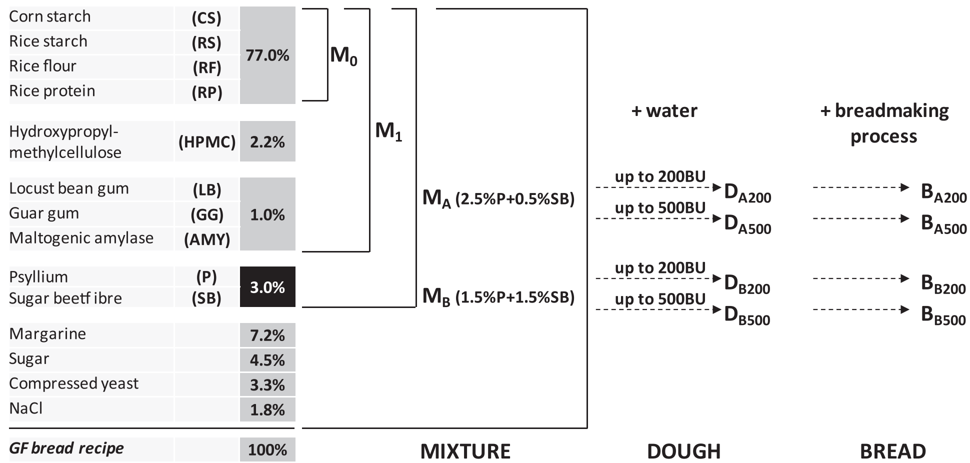


Fig. 1. Schematic overview of the gluten-free mixtures, doughs and breads investigated.

2.1.3. *Water binding capacity*

The water binding capacity (WBC) of the two fibres investigated, P and SB, was evaluated, according to the procedure described by Medcalf and Gilles (1965), opportunely adjusted: a proper amount of sample (0.2 g for P and 0.5 g for SB) was added to 50 mL distilled water in a 50 mL centrifuge tube; the tube was capped, the dispersion was shaken for 60 min at 25 °C, and then centrifuged for 10 min at 2200 × *g*, 25 °C; after the removal of the supernatant, the sediment was weighed, and the amount of water held by the sample was calculated by subtracting the initial weight of the sample. WBC was expressed as a percentage, referring to the initial weight of the sample (gH_2O/100 g sample db). Results are the average of at least 3 replicates ($n \geq 3$).

2.2. *GF doughs*

2.2.1. *GF formulations*

The basic GF recipe (97% of the total mass) was composed as follow: 77% of a mixture of CS, RF, RS and RP; 7.2% margarine; 4.5% sugar; 3.3% compressed yeast; 2.2% HPMC; 1.8% NaCl; 1% of a mixture of LB, GG, and AMY (values are expressed as the percentage of the different ingredients on the total recipe weight basis). The remaining 3% of the total mass was made of the two fibres (P and SB) combined as follows: (A) 2.5%P + 0.5%SB; (B) 1.5%P + 1.5%SB. The investigated GF mixtures, selected on the basis of preliminary trials, are reported in Fig. 1.

2.2.2. *GF dough production and farinographic evaluation*

In accordance with Gujral and Rosell (2004) and Lazaridou et al. (2007), that studied doughs based on rice flour with or without hydrocolloids addition, the GF dough mixing properties (*e.g.* water absorption and dough consistency) were examined with a Brabender Farinograph (Brabender OHG, Germany), setting the temperature at 30 °C. Powders (263 g) were pre-mixed for 5 min, and the remaining ingredients were added within the next 5 min in the following order: yeast (previously suspended in an aliquot of water), AMY, margarine, and NaCl (previously suspended in another aliquot of water). The remaining amount of water was carefully added in order to reach the desired consistency, as explained later on, and kneading was carried out for 15 min. In particular, two different consistencies were considered, in order to make the GF dough suitable to be poured into moulds (200BU) or to be shaped by hand or with an industrial forming machine (500BU). Starting from the basic GF recipe (Section 2.2.1), four different GF doughs were thus investigated (Fig. 1): D_{A200} (dough obtained from M_A; 200BU; moisture content: 52.7 g/100 g; dietary fibre: 2.44 g/100 g, on dough basis), D_{A500} (dough obtained from M_A; 500BU; moisture content: 44.7 g/100 g; dietary fibre: 2.85 g/100 g), D_{B200} (dough obtained from M_B; 200BU; moisture content: 49.7 g/100 g; dietary fibre: 1.82 g/100 g), D_{B500} (dough obtained from M_B; 500BU; moisture content: 44.4 g/100 g; dietary fibre: 2.01 g/100 g). The amount of water required for each dough to reach the desired consistency was defined as water absorption (WA; %).

2.2.3. *Rheofermentographic test*

Dough development during leavening, and its gas production and retention, were investigated by means of a Chopin Rheofermentometer F3 (Chopin; Villeneuve-La-Garenne, Cedex, France). The instrument, originally developed to test the proofing performances of wheat doughs, can also be used for the evaluation of mixtures containing flours other than wheat and even for GF matrices, after the setting up of specific methods (Mariotti, Lucisano, & Pagani, 2006; Mariotti et al., 2013). Starting from these findings, in this study the rheofermentographic test was performed for 1 h at 30 °C on a 315 g portion of the GF dough, by placing only the weight support (254 g) of the instrument on the sample, without adding any extra weight. The following indices were taken from the resulting curves: dough maximum height (Hm; mm), dough final height (Hf; mm), time of dough porosity appearance (Tx; min), total gas production ($CO_{2\text{-TOT}}$; mL), CO_2 retained by the dough ($CO_{2\text{-RET}}$; mL), CO_2 released by the dough ($CO_{2\text{-REL}}$; mL), and gas retention coefficient (Rc, %).

2.2.4. *Image analysis*

Just after dough production, 6 aliquots (10 g each) were recovered from the GF dough produced in the Brabender Farinograph. They were moulded in a spherical shape, put into 6 Petri dishes, and leavened in a climatic chamber up to 1 h at 30 °C, contemporarily to the progress of the Rheofermentographic test. At the beginning of the test, and then every 10 min, the images of the Petri dishes were scanned full scale in 256 grey level at 300 dpi with a HP SCANJET 8300 (Hewlett-Packard Development Company, Palo Alto, CA, USA) scanner. They were saved in TIFF format and processed using a dedicated software (Image Pro-Plus 4.5.1.29, Media Cybernetics Inc, MD, USA). The following indices were considered: dough diameter (mm) and area (mm^2) increases during leavening ($n = 6$, for each sampling time).

2.3. GF breads

2.3.1. GF bread making process

The breadmaking process was performed as reported by Mariotti et al. (2013), with some adjustments due to the different raw materials used in the current recipe. The Hobart N-50 mixer, equipped with a flat beater (Hobart Corporation, Troy, Ohio, USA), was used to produce the dough. As generally known, in a complex recipe the order in which the ingredients are added is important for the final dough homogeneity. For this reason the same succession of ingredients reported at Section 2.2.2 was maintained. All the powders (789 g) were pre-mixed for 5 min at 60 rpm, yeast (previously suspended in an aliquot of water), AMY, margarine, NaCl (previously dissolved in another aliquot water), and the remaining water (the amount required to reach the previously determined WA values – see Section 2.2.2), were added. All the ingredients were added within the first 4 min of mixing, and then kneaded for 4 min more, at 60 rpm. At the end of this period, the mixing was interrupted, the dough manually scraped from the surface of the bowl, mixed again for 2 min at 124 rpm and, finally, for 5 min at 60 rpm. The whole process lasted 20 min (5 min of pre-mixing *plus* 15 min of kneading), as for the Farinographic test. At the end of the kneading period, the dough was collected, divided into 10 aliquots (150 g each) that were directly 'poured' into the baking moulds (in the case of D_{A200} and D_{B200}) or 'modelled' and then positioned into the baking moulds (in the case of D_{A500} and D_{B500}). The doughs were leavened at 30 °C and 80% RH for 35 min (Haereus Vötsch, mod. HC0020; Frommern, Germany), and then baked in an oven (Lotus mod. P4; Treviso, Italy) for 30 min at 230 °C (bottom) and 200 °C (top). At the end of baking, the samples were allowed to cool down to room temperature for 1 h, before being removed from their moulds. The GF breads thus obtained (B_{A200}, B_{A500}, B_{B200}, B_{B500}) (Fig. 1) were evaluated as described below.

2.3.2. GF bread characterization

Fresh GF breads were characterized for weight (g), height (mm), and specific volume (mL/g). Loaf volume was determined by means of the rapeseed displacement method. Samples weight losses during baking were also calculated ((dough weight–bread weight) × 100/dough weight; %). Results are the average of 10 replicates (n = 10).

As reported by Mariotti et al. (2013), bread samples were then packaged in paper bags (to simulate a domestic shelf-life) and stored at controlled conditions (20 °C, 60% RH) up to 72 h. Two loaves for each formulation, at each storage time, were weighed (to evaluate the weight loss during storage, %), then transversely sliced to obtain uniform 25 mm slices, and further characterized.

Bread crust and crumb colour was measured using a sensitive computer controlled tristimulus colour analyzer Chroma Meter II Reflectance (Minolta; Osaka, Japan). Colour was expressed in the CIELAB space, as L^* (lightness; from 0 = black to 100 = white), a^* (+a = redness, −a = greenness) and b^* (+b = yellowness, −b = blueness). At least 10 replicates were performed for each bread recipe ($n \geq 10$).

The crumb porosity was investigated through Image Analysis. The images of 2 slices of each experimental bread (n = 20, for each GF recipe) were scanned full scale in 256 grey level at 300 dpi with a HP SCANJET 8300 scanner (Hewlett-Packard Development Company, Palo Alto, CA, USA), and processed using a dedicated software (Image Pro-Plus v. 4.5.1.29/XP; Media Cybernetics Inc., MD, USA). The objects (holes) were identified, counted and classified into 5 classes on the basis of their size: $0.2 \leq x < 0.5$ mm^2; $0.5 \leq x < 1$ mm^2; $1 \leq x < 5$ mm^2; $5 \leq x < 10$ mm^2 and $x \geq 10$ mm^2. The following two parameters were then considered, for each class: holes number (%; expressed as the percentage of holes having a certain size divided by the total number of holes present in the slice), and holes area (%; expressed as the percentage contribution of the holes having a certain size to the total alveolate area of the slice). The crumb porosity (%; expressed as the total alveolate area of the slice divided by the total surface of the slice) was determined, too.

Variations in bread crumb softness were assessed by means of a compression test performed with a TA-HD*plus* Texture Analyzer (Stable Micro Systems, Surrey, UK), equipped with a 500 N load cell. The Texture Exponent TEE32 V 3.0.4.0 Software (Stable Micro System, UK) was used to control the instrument and for data elaboration. Bread slices (3 from each sample) were compressed up to 40% deformation, using a 36 mm diameter cylindrical probe, at a compression speed of 1.7 mm/s. The following parameters were evaluated: crumb hardness (N; load at 25% deformation) and Young's Modulus (N/mm^2; slope of the first linear trait of the stress *vs.* strain compression curve). At least six replicates ($n \geq 6$) were performed for each bread recipe, at each storage time.

During storage, the loss of water, expressed both in terms of slice and crumb moisture (g/100 g) and bread weight loss (%; difference between the fresh bread weight and the weight of the bread stored for prefixed times, divided by the initial weight), was evaluated, too.

2.4. Statistical analysis

Data correlation and analysis of variance (ANOVA) were performed by STATGRAPHIC®Plus for Windows 5.1. ANOVA was carried out using the Least Significant Differences (LSD) test to compare sample means; differences were considered significant at $P < 0.05$.

3. Results and discussion

The GF bread formulation adopted in the current study includes many ingredients that can be normally found in the products actually available on the market. However, due to the specific aims of the project, the attention was paid specifically on two of them (P and SB) and on water, which is crucial for the workability of the dough (especially at an industrial level) and for the shelf-life of the final product.

3.1. Chemical–physical properties of the raw materials

The chemical composition of the main raw materials used in the GF recipes is reported in Table 1. The moisture content ranged from 6.9 ± 0.3 g/100 g for RP to 11.5 ± 0.3 g/100 g for RF. RS and CS exhibited a total starch content higher than 90 g/100 g db, whereas it was 85.2 ± 1.9 g/100 g db for RF. On the other hand, as expected, RP was characterized by 87.4 ± 2.3 g/100 g db of protein and only 4.6 ± 0.2 g/100 g db of starch.

As reported by Mariotti et al. (2005), DS is one of the parameters that mostly affect flours water absorption and the gelatinization and retrogradation phenomena, therefore its level is crucial for predicting flours behaviour during the process and the shelf-life of baked goods. The starchy raw materials used in this study exhibited significantly ($P < 0.05$) different DS levels. DS was very low (0.8 ± 0.1 g/100 g db) for CS, a positive characteristic since bread crumb firmness seems to be increased with increasing damaged starch content (Leon, Barrera, Perez, Ribotta, & Rosell, 2006). On the contrary, it was very high (12.0 ± 0.4 g/100 g db) for RS, as a result of a very intense milling and grinding process. The value found for RF was in the middle (5.8 ± 0.3 g/100 g db), and it is quite close to those levels usually found for wheat flours.

The two fibres (P and SB) showed a similar moisture, but significantly ($P < 0.05$) different protein contents (3.7 ± 0.1 g/100 g db *vs.* 9.3 ± 0.3 g/100 g db, respectively). They differed greatly in terms of water binding capacity (WBC, 48.29 ± 2.77g H_2O/g db for P and

Table 1
Chemical composition of the rice flour, rice starch, corn starch and rice protein used in the GF recipes.

Sample	Moisture (g/100 g)	Protein (g/100 g db)	TS (g/100 g db)	DS (g/100 g db)	DS/TS (%)
RF	11.5 ± 0.3^c	7.9 ± 0.1^a	85.2 ± 1.9^b	5.8 ± 0.3^b	6.7
RS	10.4 ± 0.1^b	nd	90.7 ± 1.5^c	12.0 ± 0.4^c	13.2
CS	11.1 ± 0.1^c	nd	98.0 ± 1.3^d	0.8 ± 0.1^a	0.8
RP	6.9 ± 0.3^a	87.4 ± 2.3^b	4.6 ± 0.2^a	nd	nd

Abbreviations: RF, rice flour; RS, rice starch; CS, corn starch; RP, rice protein; TS, total starch; DS, damaged starch; nd, not determined.
Note: Values followed by different letters, in the same column, are significantly different ($P < 0.05$).

8.27 ± 0.48 gH_2O/g db for SB), which is the amount of water the system retains after being subjected to centrifugation. These values were also much higher than those found for rice bran (Cappa, Lucisano, & Mariotti, 2013). Furthermore, at the testing concentration used, P was able to form a gel, whereas SB originated a weak-slurry, thus indicating the higher strong ability of P to absorb and retain water. As the processing of food usually involves the use of some type of physical stress, the WBC of its ingredients is actually a very important issue; however, it must be also considered that, in a complex system, other factors such as pH, ionic strength, fibre concentration, as well as the presence of other water-binding ingredients (*e.g.* sugars, starches, *etc.*), can influence this index.

3.2. Pasting properties of the raw materials and mixtures

In Fig. 2, the pasting properties of the starchy raw materials (RF, RS, CS) and those of the GF mixtures investigated (M_0, M_1, M_A, M_B), are reported, both in terms of MVA profiles and MVA indices. When starch is the main component, the slurry increases its viscosity as the temperature raises, due to starch swelling and rupturing and to the release of amylose outside the granules. The swelling is slight at the beginning, then rapid and, in a final stage, a maximum value is reached (Tester & Morrison, 1990); when starch rupture becomes prominent, a decrease of viscosity is observed; on cooling, a further viscosity increase is experienced as the hot paste turns into gel. All these steps are representative of the gelatinization and retrogradation tendency of the starchy samples. CS exhibited the highest pasting temperature (PT), peak viscosity (PV), breakdown (BD), setback (Sb), and final viscosity (FV). This behaviour is certainly due to the high total starch and low damaged starch content of this sample, in comparison to RS and RF. CS thus seems to be able to form a strong gel during cooling, but highly sensible to retrogradation, suggesting a fast staling rate of the final GF bread. Even if RS had a very high total starch content, more than the 13% of it was damaged, so it exhibited significantly ($P < 0.05$) lower PV, BD, FV and Sb values, in comparison to CS. These values, in fact, were more close to those of RF than to those of CS.

When a mixture of different ingredients is tested in the MVA, the phenomena that take place during the test are much more complex, as interactions occur. In particular, the following GF mixtures were considered: M_0, made only of the starchy ingredients (CS + RS + RF) and RP; M_1, in which a mixture of two hydrocolloids (LB + GG) was added; M_A, composed of M_1 plus 2.5%P and 0.5%SB; M_B, composed of M_1 plus 1.5%P and 1.5%SB. The lowest MVA indices were evidenced, as expected, by M_0, in which no hydrocolloid or fibre was present. The addition of LB and GG slightly moved up the MVA profile of M_1, in comparison to that of M_0, but no significant differences were evidenced in terms of MVA indices between the two mixtures. Gums such as GG and LB form colloidal dispersions in water, and they are among the most widely used as thickening agents to specifically increase the viscosity of the food. GG, in particular, belongs to the highest-viscosity plant gums and exhibits high viscosities at very low concentrations. In this case, the system was very diluted (10 g of GF mixture in 100 mL of water), so the effect of their presence was not as much evident as expected. However, the consequence of their addition was highlighted by the significantly ($P < 0.05$) lower pasting temperature of M_1 in comparison to that of M_0. In fact, the strong ability of GG and LB to absorb water at room temperature accelerated the viscosity increase of the system. This effect was much more evident when the two fibres, P and SB, were included in the GF mixture, too. In particular, 2.5% of P determined the highest MVA viscosity indices and the lowest PT, underling once more the strong ability of P to bind water. Above all, it appeared evident how the mixture of many ingredients in the same system, and in particular the presence of hydrocolloids and fibres, determined interactions among the various raw materials and strong competitions for water. It was quite evident the strong decrease of the MVA indices of all the GF mixtures in comparison to those of the pure starchy materials. Alvarez-Jubete, Auty, Arendt, and Gallagher (2010) reported that, for GF breads, the FV of a slurry is an indicator of the strength of the gel formed upon cooling and thus represents an important quality parameter, together with the PV of the batters that seem to have implication in relation to the final quality of the resultant product. Accordingly to them, MVA indices could be considered as important predictive quality parameters, in the GF bread sector, non only when the test is performed on each single raw materials, but in particular when it is carried out on mixtures that are as close as possible to the real ones used in the GF baking process.

3.3. GF doughs properties during mixing

M_A and M_B mixtures were deeply investigated in terms of dough properties, focusing on the concept of 'dough consistency'. In particular, two different consistencies were considered, in order to make the GF dough suitable to be poured into moulds (200BU) or to be shaped by hand or with an industrial forming machine (500BU). Four GF doughs were obtained, as described at Section 2.2.2, and they were evaluated both during mixing and leavening: D_{A200} (2.5%P + 0.5%SB; 200BU), D_{A500} (2.5%P + 0.5%SB; 500BU), D_{B200} (1.5%P + 1.5%SB; 200BU), D_{B500} (1.5%P + 1.5%SB; 500BU). Results are reported in Fig. 3.

During mixing, the water amount added to the mixture in order to obtain a coherent mass having a specific consistency strongly influences the subsequent workability of the dough itself. The main question is: which is the more suitable consistency? In the GF bread sector, two techniques are usually adopted: some prefer to produce a batter that will be poured into the moulds; some others prefer to obtain a more solid structure that can be modelled. Frequently, the decision depends both on the industrial plants available and on the formulated GF recipes. In this study, the same recipes were treated in order to obtain both the above mentioned structures: liquid-like and solid-like. By means of previous trials conducted by the authors on different GF mixtures, at various dough consistencies, it was possible to define 'liquid-like' a GF dough having a consistency of 200BU, and 'solid-like' a GF dough having a consistency of 500BU.

In the specific case of GF mixture M_A and M_B, the amount of water added (water absorption, WA) to reach this values ranged from 57.3% to 85.0% depending primarily on the dough consistency desired and, secondarily, on the amount and type of fibres added. In

(a) STARCHY RAW MATERIALS					
Sample	PT (°C)	PV (BU)	FV (BU)	BD (BU)	Sb (BU)
RF	65.3 ± 0.4^{b}	1118 ± 18^{a}	1036 ± 8^{b}	309 ± 54^{a}	616 ± 12^{b}
RS	62.6 ± 0.2^{a}	1144 ± 22^{a}	930 ± 19^{a}	259 ± 24^{a}	496 ± 23^{a}
CS	69.0 ± 0.1^{c}	1374 ± 15^{b}	1568 ± 9^{c}	400 ± 31^{b}	1062 ± 1^{c}

(b) GF MIXTURES					
Sample	PT (°C)	PV (BU)	FV (BU)	BD (BU)	Sb (BU)
M_0	74.1 ± 0.4^{d}	385 ± 5^{a}	595 ± 11^{a}	173 ± 2^{a}	383 ± 8^{a}
M_1	71.8 ± 0.2^{c}	407 ± 4^{a}	607 ± 18^{a}	184 ± 5^{a}	384 ± 17^{a}
M_A	69.6 ± 0.4^{a}	556 ± 22^{c}	781 ± 9^{c}	270 ± 29^{b}	495 ± 2^{c}
M_B	70.6 ± 0.1^{b}	462 ± 9^{b}	661 ± 21^{b}	248 ± 1^{b}	447 ± 13^{b}

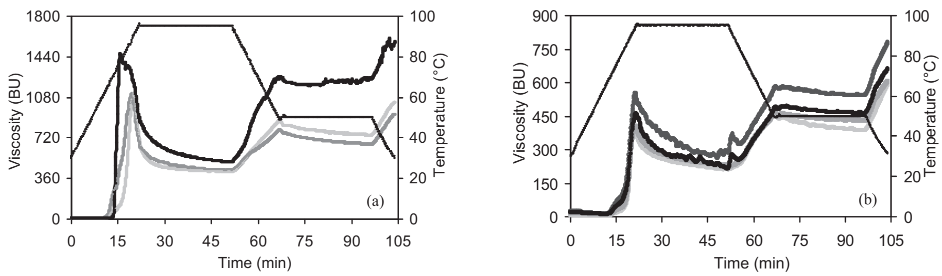


Fig. 2. Pasting properties of: (a) starchy raw materials, RF ( ), RS ( ), CS ( ), temperature profile ( ); (b) GF mixtures, M_0 ( ), M_1 ( ), M_A ( ), M_B ( ), temperature profile ( ).

particular, the higher the presence of P the highest the WA values, thanks to its high water binding capacity, especially for the 200BU doughs.

3.4. GF doughs properties during leavening

The leavening behaviour of the GF doughs was investigated by means of both the rheofermentographic test (in terms of dough volume development and gas production and retention) (Fig. 3) and Image Analysis (in terms of variations of the geometrical indices) (Fig. 4).

As regards the rheofermentographic test, D_{A200} and D_{B200} showed the maximum dough height development (Hm), almost three times higher than those exhibited by D_{A500} and D_{B500}. Despite the fact that a batter is not easy to be treated, from an industrial point of view, of course in presence of a higher amount of water, fibres and hydrocolloids can be better dispersed and they can perform their thickening action at the best, creating a structure able to sustain and develop itself during the leavening process. The

(a) MIXING PROPERTIES			(b) LEAVENING PROPERTIES							
Mixture	Consistency (BU)	WA (%)	Dough	Hm (mm)	Hf (mm)	Tx (min)	$CO_{2\text{-TOT}}$ (mL)	$CO_{2\text{-REL}}$ (mL)	$CO_{2\text{-RET}}$ (mL)	Rc (%)
M_A	200	85.0	D_{A200}	44.7	44.7	-	628	4	624	99.3
	500	58.1	D_{A500}	14.3	13.1	25	744	38	706	95.0
M_B	200	74.0	D_{B200}	43.1	43.1	-	614	4	609	99.3
	500	57.3	D_{B500}	6.2	5.6	25	691	32	659	95.4

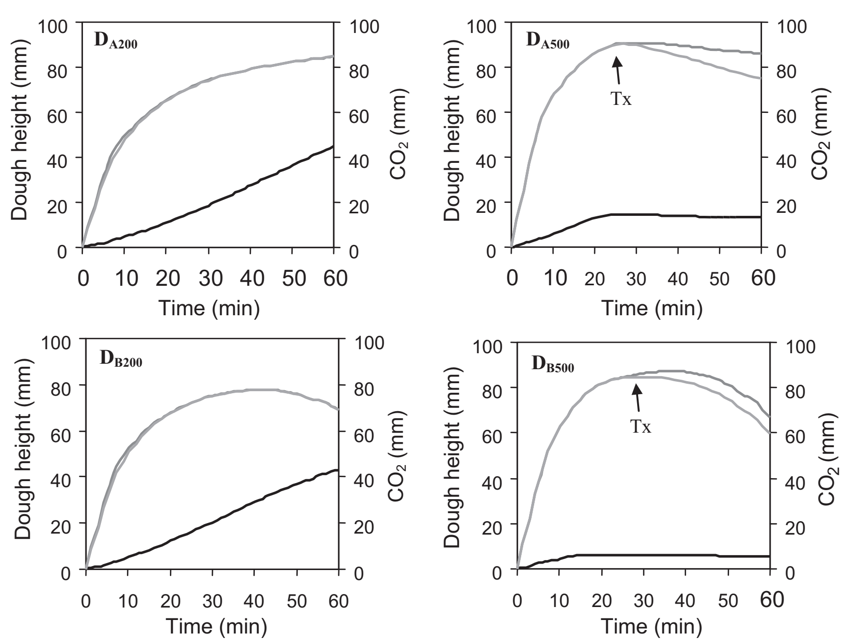


Fig. 3. Dough properties: (a) water amount required to reach the desired consistency; (b) leavening behaviour, Hm ( ), $CO_{2\text{-TOT}}$ ( ), $CO_{2\text{-REL}}$ ( ).

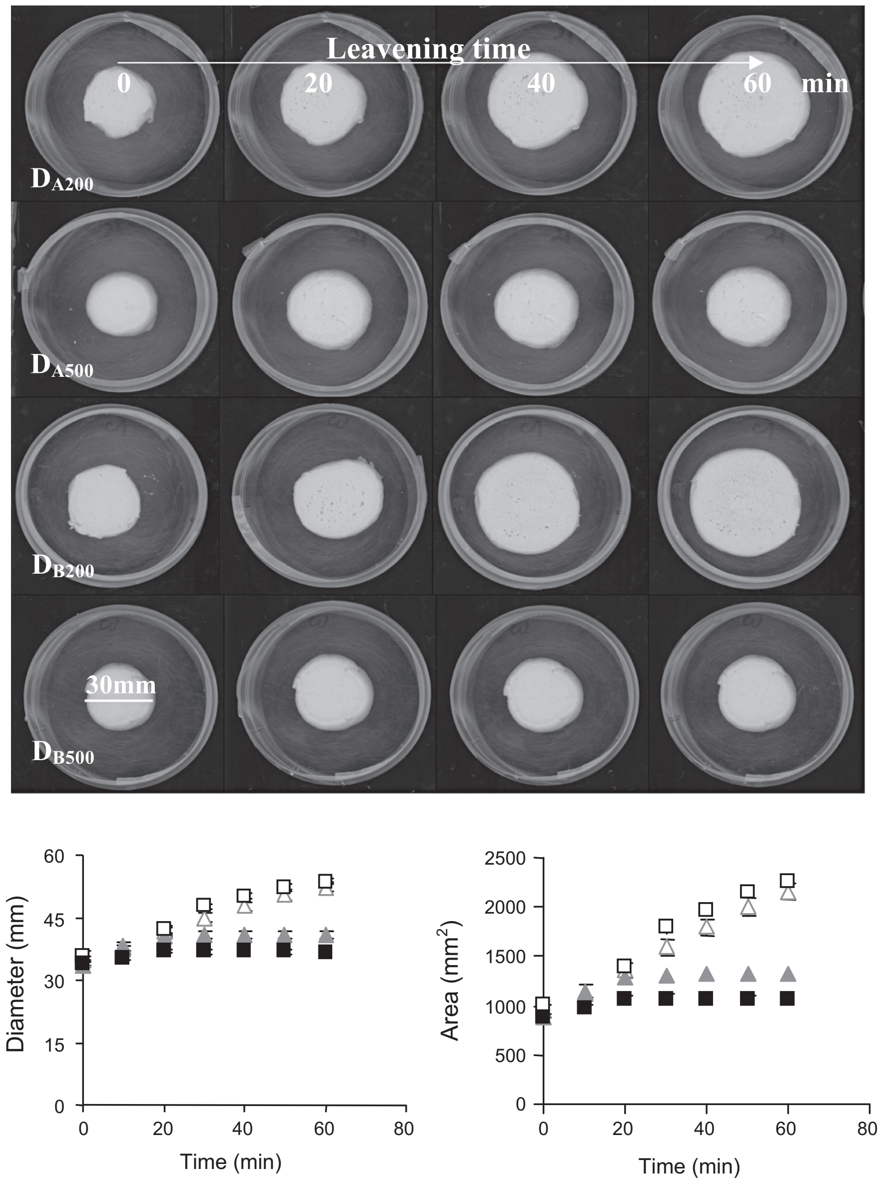
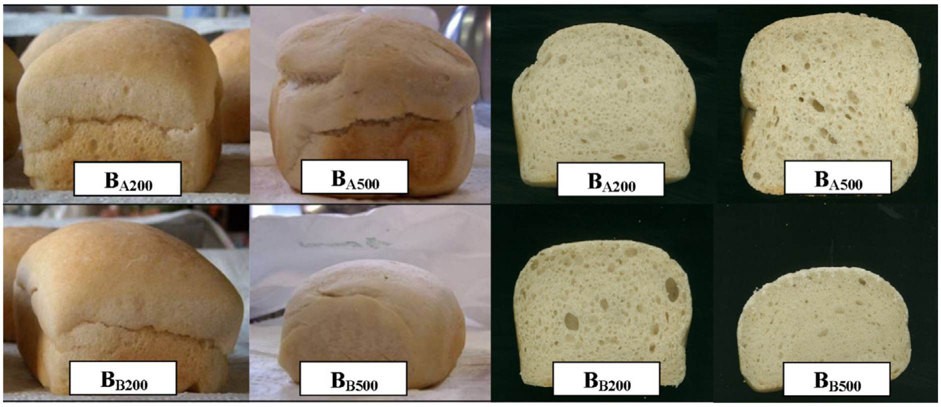


Fig. 4. Variation of the geometrical indices (image analysis) of the different gluten-free doughs during leavening. *Samples identification:* D_{A200} (△), D_{A500} (▲), D_{B200} (□), D_{B500} (■).

same doughs, in fact, progressively increased in height during the whole leavening period, as can be noticed both from the curves and Hm and Hf values. They did not even exhibit a Tx (an index of dough porosity appearance), and their retention coefficient (R_C) was higher than 99%, thus indicating a higher ability in retaining into the dough the developed CO_2. On the contrary, D_{A500} and D_{B500}, even if more workable, slightly increased their height during leavening due to the high resistance to deformation (running it out during the first 15–25 min), exhibited a Tx, and were characterized by R_C close to 95%. No differences were observed, in terms of rheofermentographic indices, when P and SB percentages in the recipe were varied, indicating that: (*i*) water is the principal 'ingredient' that affects the leavening behaviour of the GF doughs investigated; (*ii*) even the 1.5% of P adopted in these recipes was enough to ensure the formation, in the GF dough, of the film-like structure that Mariotti et al. (2009) highlighted as a promising condition to obtain a good technological quality of the final bread.

By means of Image Analysis, accurate measurements of dough development during leavening at 30 °C into Petri dishes (Fig. 4), in terms of geometrical indices (diameter and area), were performed at prefixed times, as described at Section 2.2.4. Both diameter and area increased with analogous trends: the 'solid-like' doughs (D_{A500} and D_{B500}) grew mostly during the first 20–30 min, and did not increase any more till the end of the leavening phase; on the contrary, the 'liquid-like' doughs (D_{A200} and D_{B200}) exhibited a continuous increase of their geometrical dimensions during proofing, reaching a final percentage area increase equal to 128% and 124%, respectively, *vs.* 48% for D_{A500} and 8% for D_{B500}.

These trends were actually the same as those highlighted by the rheofermentographic test. In fact, significant correlations were found between the rheofermentographic dough development and the geometrical index 'area' ($r > 0.975$, $P < 0.0001$) and the geometrical index 'diameter' ($r > 0.981$, $P < 0.0001$), measured at the different leavening times (0, 10, 20, 30, 40, 50 and 60 min). Therefore, it can be stated that following the leavening of a dough in a Petri dish by means of Image Analysis can represent a valid tool for the on-line controls of the proofing process, too.



Variable	GF FRESH BREADS			
	B_{A200}	B_{A500}	B_{B200}	B_{B500}
Height (mm)	52.4 ± 1.1^{c}	61.3 ± 2.1^{d}	48.0 ± 1.3^{b}	39.4 ± 1.5^{a}
Volume (mL)	319.6 ± 21.9^{c}	360.9 ± 32.3^{d}	282.6 ± 16.3^{b}	243.2 ± 19.4^{a}
Specific volume (mL/g)	2.4 ± 0.17^{c}	2.7 ± 0.26^{d}	2.1 ± 0.12^{b}	1.8 ± 0.16^{a}
Baking weight loss (%)	12.7 ± 1.07^{c}	12.4 ± 0.63^{c}	11.2 ± 1.16^{b}	9.5 ± 1.3^{a}
Crumb moisture (g/100g)	52.8 ± 0.1^{d}	44.9 ± 0.1^{b}	50.1 ± 0.1^{c}	44.8 ± 0.1^{a}
Slice moisture (g/100g)	46.2 ± 0.3^{d}	37.5 ± 0.4^{a}	42.3 ± 0.3^{c}	38.5 ± 0.2^{b}
Crumb L*	76.5 ± 1.7^{b}	79.6 ± 2.2^{c}	74.3 ± 2.5^{a}	74.8 ± 1.3^{a}
Crumb a*	-1.4 ± 0.3^{a}	-1.4 ± 0.2^{a}	-1.3 ± 0.2^{a}	-1.1 ± 0.3^{b}
Crumb b*	12.5 ± 0.7^{a}	13.3 ± 0.6^{b}	13.9 ± 0.6^{c}	16.1 ± 0.6^{d}
Crust L*	66.0 ± 2.8^{a}	74.5 ± 4.4^{b}	74.3 ± 1.9^{b}	80.3 ± 2.3^{c}
Crust a*	5.2 ± 1.1^{d}	3.7 ± 1.7^{c}	0.2 ± 0.5^{b}	-0.7 ± 0.2^{a}
Crust b*	29.9 ± 1.7^{c}	22.4 ± 3.8^{b}	22.4 ± 2.5^{b}	15.5 ± 2.7^{a}
Hardness (N)	7.43 ± 0.82^{a}	12.14 ± 1.72^{b}	10.63 ± 0.59^{b}	nd
Young's Modulus (N/mm^2)	0.038 ± 0.007^{a}	0.067 ± 0.013^{c}	0.054 ± 0.004^{b}	nd
Crumb porosity (%)	26.48 ± 2.34^{c}	21.87 ± 3.45^{a}	25.87 ± 4.03^{bc}	23.81 ± 5.51^{ab}

Fig. 5. Gluten-free fresh bread characteristics.

3.5. GF bread properties

GF breads (B_{A200} and B_{B200}) obtained from the 'liquid-like' doughs, as expected, exhibited a higher crust and crumb moisture in comparison to samples B_{A500} and B_{B500} obtained from the 'solid-like' doughs (Fig. 5). In addition, they were also characterized by high heights, volumes and specific volumes, and by quite a good crumb softness. These findings are consistent with those obtained from the predictive test performed on the corresponding doughs. However, in contrast with these expectations, B_{A500}, that was characterized by a limited development of the dough during the leavening phase (Figs. 3 and 4), showed the highest height and specific volume after baking. This could be probably related to the high viscosity of the dough (D_{A500}), due to the presence of 2.5%P, that allowed the creation of the film-like structure fundamental to obtain a good technological quality of the final bread. Despite these interesting bread features, B_{A500} was characterized by the highest crumb hardness of the fresh product, due to the low crumb moisture (Fig. 5).

The use of hydrocolloids and high-fibre ingredients generally determines positive effects on the texture of breads and related products. The addition of these substances, in fact, in particular in GF breads, can enhance the softness of the crumb, thanks to fibres ability in binding water and holding it through the baking process. In particular, fibres that are high in soluble fibre (such as SB and P) can positively influence the softness of the crumb by helping to retain moisture and by increasing the perception of crumb moistness. However, if water is limited in the original mass, and many substances have to compete for it, both hydrocolloids and high-fibre ingredients cannot carry out their functionality at the best (as in the case of B_{A500}).

When both P and SB were present at 1.5% level, dough consistency was the most critical factor for product development. In fact, B_{B500} exhibited the worst performances both during proofing (Figs. 3 and 4) and baking (Fig. 5). B_{B200}, that was able to retain at a satisfactory level the CO_2 produced during leavening (Rc, 99.3%), was not able to maintain this performance during baking, increasing its dimensions as much as B_{A200}; however, it was characterized by a good fresh crumb softness.

Another important parameter for consumer acceptability is the colour of the product (Fig. 5): it can change in relation to the presence of fibres and depending on fibres types and amounts. The GF breads obtained in this research were characterized by a high lightness, a low redness, and *b** values ranging from 12.5 to 16.1 for crumb and from 15.5 to 29.9 for crust. In general, the formulations adopted allowed to obtain products that were satisfactory from this point of view, too, as they were not too 'pale' as the majority of GF breads actually available on the market, showing an appealing golden crust and white crumb appearance.

A further index of bread quality, investigated by Image Analysis, was the crumb porosity (Fig. 5). All the GF breads showed a porosity (slice area occupied by holes *vs.* total slice area) higher than 20%; in particular, B_{A200} and B_{B200} had the more alveolate crumb. This is in agreement with the fact that, even if D_{A200} and D_{B200} CO_2 production was lower than that of D_{A500} and D_{B500}, their retention coefficient was higher as well as their water content: the growth of gas bubbles, in fact, is not only a result of the generation of carbon dioxide during leavening, but also of water vapour deriving

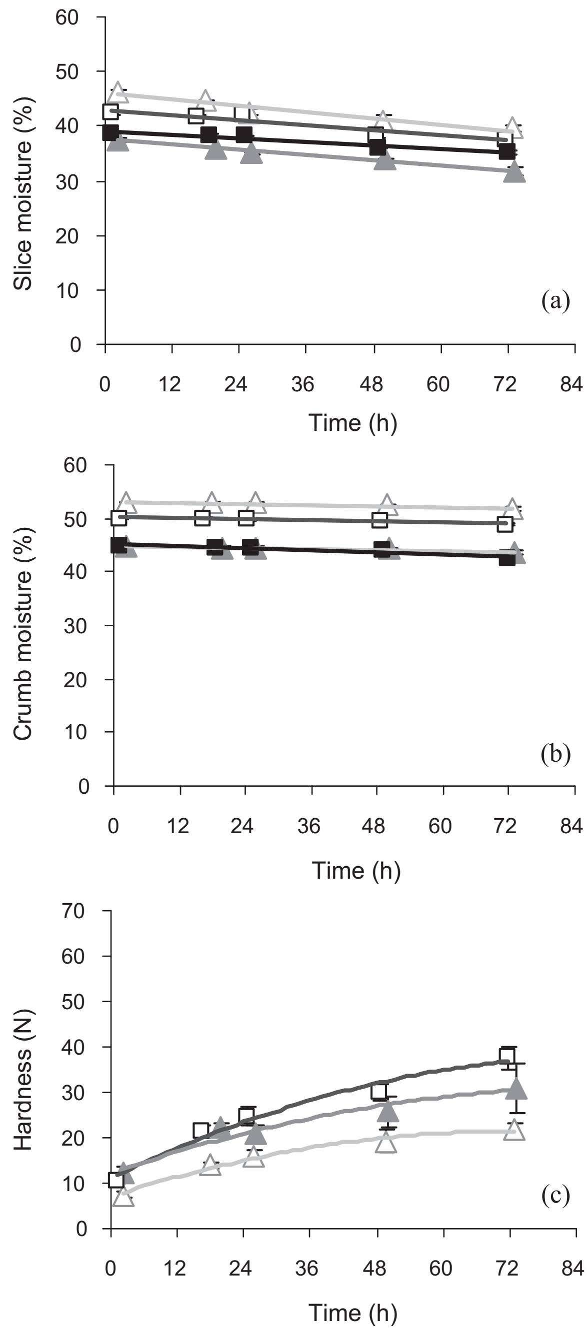


Fig. 6. Gluten-free bread properties during storage: (a) slice moisture, (b) crumb moisture, (c) crumb hardness. *Samples identification:* B_{A200}, bread obtained from D_{A200} (△); B_{A500}, bread obtained from D_{A500} (▲); B_{B200}, bread obtained from D_{B200} (□); D_{B500}, bread obtained from D_{B500} (■).

from the surrounding viscous doughs during baking. Furthermore, the B_{A200} and B_{B200} showed a similar distribution holes number in the different dimensional classes considered (Section 2.3.2): 37.5% and 38.1% of small size holes ($0.2 \leq x < 0.5\,mm^2$), 24.7% and 23.0% of medium size holes $0.5 \leq x < 1\,mm^2$, 32.8% and 33.9% of large size holes ($1 \leq x < 5\,mm^2$), and a total of 5% of bigger holes, respectively. On the contrary, B_{A500} and B_{B500}, characterized by a denser structure, exhibited a higher number of small size holes (45.7% and 47.8%, respectively) and a lower number of intermediate size holes (28.3% and 24.5%, respectively).

In Fig. 6, GF bread properties during storage are resumed. A limited moisture decrease (2–5%) in bread crumb was observed, whereas it was much more evident, as expected, in terms of bread slice (from 9% to 16%). In accordance with the findings of many authors (Bechtel & Meisner, 1954; Gallagher, Kunkel, Gormley, & Arendt, 2003; Mariotti et al., 2009; Platt & Powers, 1940), both hardness and Young's Modulus values highlighted that a higher dough water content (*i.e.* a lower dough consistency) is a prerequisite to maintain the sample softer during its shelf-life. Particularly, B_{A200} had a Young's Modulus of $0.038 \pm 0.01\,N/mm^2$ just after its production and of $0.147 \pm 0.02\,N/mm^2$ at the end of the storage period, while B_{A500}, characterized by a denser structure, had a Young's Modulus of $0.067 \pm 0.01\,N/mm^2$ after the production and of $0.210 \pm 0.06\,N/mm^2$ at the end of the storage period. Furthermore, the significant differences ($P < 0.05$) in crumb softness evidenced in the fresh products were maintained and became even more evident after 72 h of storage: in particular, B_{B200} staling rate was much higher not only than that of B_{A200} but also of B_{A500}, underling the higher anti-staling effect of P in comparison to SB fibre during storage.

4. Conclusions

The results obtained suggested that, in the GF bread sector, a lower consistency of the dough is preferred in order to assure good performances during leavening, in particular when ingredients having a high water affinity (*e.g.* hydrocolloids, fibres) are included in the recipe. In fact, the more 'solid-like' doughs (D_{A500} and D_{B500}) were characterized by a reduced development and gas retention during proofing, whereas the more 'liquid-like' doughs (D_{A200} and D_{B200}) showed better abilities.

As regards the supplementation of P and SB in GF products, in order to increase the technological and nutritional features of the final products, it can be concluded that both P and SB can improve the workability and the technological performances of GF doughs, but *Psyllium* plays a central role on bread development, both during proofing and baking. However, P supplementation has to be carefully modulated, as it could determine an excessive increase of bread hardness if a proper amount of water is not added to the recipe. In addition, a more effective antistaling effect of P *vs.* SB was evidenced after 3 days of storage presumably due to P higher water binding capacity.

To conclude, in general, both the amount of hydrocolloids and fibres and of water are crucial factors for the final quality of a GF bread, and they must be well balanced. Researchers working in the GF sector should take into account the importance of those features of the dough and of the final product in order to satisfy not only the consumers (satisfaction of the dietary, nutritional and sensorial requirements) but also the producers (plant/line equipment, handling/workability of the product) requirements.

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