Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour.

Concha Collar^{1*}, Paola Conte^{1,2}, Costantino Fadda², Antonio Piga²

¹Cereals and Cereal-based Products. Food Science Department. Instituto de Agroquímica y Tecnología de Alimentos (CSIC). Avda. Catedrático Agustín Escardino, 7. 46980 Paterna. SPAIN.

²Dipartimento di Agraria. Sezione di Scienze e Tecnologie Ambientali e Alimentari. Università degli Studi di Sassari. Viale Italia, 39. 07100 Sassari. ITALY

*Corresponding author. Tel.: +34 963 90 00 22; Fax: +34 963 63 63 01

E-mail address: ccollar@iata.csic.es

Keywords: gluten-free, dough, starch, flour, additive, viscoelasticity

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Abstract

The capability of different gluten-free (GF) basic formulations made of flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make machinable and visco-elastic GF-doughs in absence/presence of single hydrocolloids (guar gum, locust bean and psyllium fibre), proteins (milk and egg white) and surfactants (neutral, anionic, and vegetable oil) have been investigated. Macroscopic (high deformation) and macromolecular (small deformation) mechanical, visco-metric (gelatinization, pasting, gelling) and thermal (gelatinization, melting, retrogradation) approaches were performed on the different matrices in order to a) identify similarities and differences in GF-doughs in terms of a small number of rheological and thermal analytical parameters according to the formulations, and b) to assess single and interactive effects of basic ingredients and additives on GF-dough performance to achieve GF-flat breads. Larger values for the static and dynamic mechanical characteristics and higher viscometric profiles during both cooking and cooling corresponded to doughs formulated with guar gum and Psyllium fibre added to rice flour/starch and rice flour/corn starch/chickpea flour, while surfactant- and protein-formulated GF-doughs added to rice flour/starch/amaranth flour based GF-doughs exhibited intermediate and lower values for the mechanical parameters and poorer visco-metric profiles. In addition, additive-free formulations exhibited higher values for the temperature of both gelatinization and retrogradation and lower enthalpies for the thermal transitions. Single addition of 10% of either chickpea flour or amaranth flour to rice flour/starch blends provided a large GF-dough hardening effect in presence of corn starch and an intermediate effect in presence of cassava starch (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and amaranth flours, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of corn starch and cassava starch, respectively.

1. Introduction

Research, development and innovation in gluten-free (GF) products constitute areas of increasing interest to meet cereal-based goods requirements of coeliac and wheat intolerant patients. Flat breads are the oldest and most well-known bread type worldwide (*pita, arepa, tortilla, chapati, roti, injera*), made from either gluten-forming (wheat) or non-gluten-forming (corn, sorghum, teff) cereals in regions of Central America, South Europe, Scandinavia, South Africa, the Middle East and part of China (Mohammadi et al., 2014). In some Mediterranean regions, flat breads are made of durum wheat to provide specialty baked goods like *spianata* in Sardinia, a major Mediterranean island. Durum wheat breads are not compatible with gluten-intolerant patients, and Sardinia has a significant prevalence of coeliac disease (124 per 100,000) over the population (Sardu et al., 2012).

Proper replacement of gluten-forming cereals by non gluten-forming systems in baked goods is still a major challenge particularly in the achievement of sensory and nutritionally balanced leavened baked goods, despite the accumulating knowledge on physical, chemical and technological principles of GF-matrices (Schober, 2009). Complex formulations involving the incorporation of starches of different origin, dairy proteins, other non-gluten proteins, gums, hydrocolloids, and their combinations, into a GF flour base (mostly rice and corn flour) are often used to simulate the viscoelastic properties of lacking gluten (Mariotti et al., 2009), and may result in variable success regarding structure, mouthfeel, acceptability and shelf-life of the finished GF-products. The incorporation of dairy and egg proteins has long been established in the baking industry, and has proven to significantly affect viscoelasticity of GF-systems (Ronda et al., 2014). Legumes can also be a good supplement for cereal-based foods added either in flour or concentrated/isolated forms since they substantially increase the protein content and complement the nutritional value of cereal proteins (Angioloni & Collar, 2012). Pseudocereals such as buckwheat, quinoa and amaranth can also be useful for nutritional improvement of breads with no significant impairment of the final bread quality when added at low amounts (Collar & Angioloni, 2014).

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Gums and hydrocolloids are either a good source of soluble dietary fibre (Angioloni & Collar, 2011) or essential structuring ingredients in GF bread formulations for improving the texture, the volume, and the keepability of the final products (Ronda et al., 2013). In breadmaking applications, a careful selection of structural ingredients with suitable physico-chemical properties preventing permanent disruption of the protein matrix that encompasses excessive weakening of the protein/starch networks, is a pre-requisite to obtain processable doughs, particularly for GF systems lacking the endogenous viscoelastic biopolymer. To date, the main approach for the development of GF breads has been the addition of structural macropolymers such as hydroxypropylmethylcellulose to mimic gluten viscoelastic properties (Ahlborn et al., 2005). Other hydrocolloids of vegetal origin such as galactomannans and high ester pectin (Angioloni & Collar, 2008), and more recently, *Psyllium* fibre (Mariotti et al., 2009) have shown to provide either a reinforced hydrated flour-fibre structure with promoted values for storage and loss moduli (locust bean gum), or an enhancement of the physical properties of the doughs due to the film-like structure that it was able to form (*Psyllium* fibre). In addition, a health promoting effect associated to the cholesterol-lowering effect and insulin sensitivity improvement capacity of *Psyllium* fibre (You et al., 2003), has been stated.

This study is aimed at exploring the capability of different GF-basic formulations made of different flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make processable and visco-elastic GF-doughs in absence/presence of single hydrocolloids (guar gum, locust bean and *psyllium* fibre), proteins (milk and egg white) and surfactants (neutral, anionic, and vegetable oil). Macroscopic (high deformation) and macromolecular (small deformation) mechanical, and visco-metric (gelatinization, pasting, gelling) and thermal (gelatinization, melting, retrogradation) approaches were performed on the different matrices in order to a) identify similarities and differences in GF-doughs in terms of a small number of rheological and thermal analytical parameters according to the formulations, and b) to assess single and interactive effects of basic ingredients and additives on GF-dough performance to achieve GF-flat breads.

2. Materials and methods

2.1. Materials

Commercial flours, starches, proteins, dietary fibres, surfactants and oils were used. Rice flour (RF), corn starch (CS), cassava starch (CaS), milk proteins (MP), guar gum (GG), diacetyl tartaric acid ester of mono- and diglycerides (DATA), Psyllium fiber (PF) and locust bean gum (LB) were from Chimab Campodarsego (PD, Italy). Amaranth flour (AF), egg white proteins (EP), and chickpea flour (CF) were from Molini Bongiovanni S.p.A. - Cambiano (TO, Italy). Sodium stearyl-2-lactylate (SSL) was from DuPont™ Danisco®, and sunflower oil (SF) was from Carapelli Firenze (Italy).

2.2. Methods

Dough making of GF samples

GF doughs were prepared by using 6 different basic formulations coded A-F according to the following quali and quantitative composition on a 100 g solid basis: A - Rice flour (50%) + corn starch (50%), B - Rice flour (50%) + cassava starch (50%), C - Rice flour (45%) + corn starch (45%) + chickpea flour (10%), D - Rice flour (45%) + cassava starch (45%) + chickpea flour (10%), E - Rice flour (30%) + corn starch (30%) + amaranth flour (40%), F - Rice flour (30%) + cassava starch (30%) + amaranth flour (40%). Individual/single proteins, dietary fibres, surfactants and oils were added to each basic formulation (g/ 100 g solid basis) at 2 levels of addition (low /high) as it follows: GG (1/2), LB (1/2), PF (1/2), MP (5/10), EP (5/10), DATA (0.5/1.0), SSL (0.5/1.0), and SF (4/8). A total of 102 different GF doughs resulted from basic and 2 level additive-containing formulations. Solids (100 g), and water (70% for A and B, 61% for C and D, 58% for E and F basis) optimized according experimental trials to obtain non-sticky non-slack doughs, were mixed using a Kitchen-Aid Artisan mixer (5KSM150PS, Kitchen Aid, St. Joseph, MI) with a dough hook (K45DH) for 2 min at speed 2, and 2 min at speed 4.

Chemical and nutritional composition of GF ingredients

Chemical and nutritional composition of flours, starches, hydrocolloids, proteins and surfactants was provided by the manufacturers (Table 1). Amylose/ amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method developed by Yun and Matheson (1990) that uses an ethanol pre-treatment step to remove lipids prior to analysis.

Dough rheological measurements

a) Large-deformation mechanical tests

Dough machinability was assessed by texture profile analysis (TPA) in a TA-XTplus texture analyser (Stable Micro Systems, Godalming, UK) using a 5 cm diameter probe, a 75 s waiting period and 60% compression as described previously (Collar et al., 1999). The resistance to penetration was assessed with penetration tests according to Sciarini et al. (2012). Dough was compressed until the probe (P/5.5 mm diameter) disrupted the dough surface structure, penetrating into the sample, at 15 mm/s. The force value corresponding to the intersection of the two straight lines defined in the curve was set as the penetration force. Stress relaxation tests were accomplished according to Singh et al. (2006), and modified by Fois et al. (2012). % relaxation was calculated as the force registered after 35 s, divided by the maximum registered force in percentage.

b) Small-deformation tests

Fundamental dough rheology of GF-doughs was assessed by dynamic oscillation tests on an RS1 controlled stress rheometer equipped with a Phoenix II circulating bath (Haake, Karlsruhe, Germany) using a 60 mm serrated plate–plate geometry with a 1 mm gap between plates (Angioloni & Collar, 2009). The upper plate was lowered and the excess of sample was trimmed off. The exposed surface was covered with a thin layer of mineral oil to prevent moisture loss during testing. Samples were rested for 10 min after

loading prior to testing, to allow sample relaxation. Strain sweep tests were run to identify the linear viscoelastic region. Oscillatory measurements of storage modulus (G'), loss modulus (G''), and phase angle (δ) were performed at 25 °C within a frequency range from 0.1 to 10 Hz. All measurements were made in triplicate. Values for dynamic moduli were registered at $\lambda=1$ Hz and quoted G'_1 and G''_1 .

Viscometric Properties

Pasting profiles (gelatinisation, pasting, and setback properties) of formulated flour/starch blends were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC Standard method 162. The pasting temperature (in °C; when viscosity first increases by at least 25 cP over a 20-s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus holding strength or trough viscosity), viscosity at 95 °C, viscosity at the end of the 95 °C holding period, viscosity at 50 °C, final viscosity (end of test after cooling to 50 °C and holding at this temperature), setback (final viscosity minus peak viscosity) and total setback (final viscosity minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software (Collar 2003). For each visco-metric measurement, two replicates were made.

Thermal Properties

Thermal properties regarding starch gelatinization and retrogradation of formulated GF-doughs containing the higher level of the different additives were assessed in a Differential Scanning Calorimeter Perkin-Elmer DSC-7 according to the method of León et al (1997), with some modifications as previously reported by Andreu et al (1999) and Santos et al (2008).

Starch gelatinization. Dough samples were prepared by mixing all solid ingredients and 70% of water. For DSC analysis, 50–70 mg samples were weighed in large volume pre-weighed, sealed stainless-

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3 169 steel pans. An empty pan was used as a reference. Simulation of the temperature profile in the center of the
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5 170 bread crumb during baking was done in the calorimeter under the following scanning conditions: samples
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8 171 were kept at 30°C for 2 min, then heated from 30 to 110°C at a rate of 11.7°C/min, kept at 110°C for 5 min,
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10 172 and finally cooled from 110 to 30°C at a rate of 50°C/min. Gelatinized samples were stored at 22°C for 6
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12 days. Thermal transitions of starch samples were defined as T_o (onset), T_p (peak of gelatinization), and T_c
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14 (conclusion); the enthalpy associated with starch gelatinization was defined as ΔH_g .
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17 175 *Starch retrogradation.* Stored gelatinized dough samples were submitted to a second DSC scan to
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19 176 analyze starch retrogradation. Scanning conditions included keeping sample pans at 25°C for 1 min, and
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21 177 then heating from 25 to 130°C at a rate of 10°C/min. The enthalpy of amylopectin/~~amylose~~ retrogradation
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23 (ΔH_r) was calculated. All samples were analyzed in duplicate.
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27 179 Enthalpies were calculated from the area under the curves defined after scanning. Gelatinization
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29 180 and ~~retrogradation~~ enthalpies (ΔH) were expressed in J/g of dry sample. Each formulation was analysed
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36 183 **Statistical analysis**
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38 184 Multivariate analysis of variance and factor analysis were applied to data by using Statgraphics V.7.1
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40 185 program (Bitstream, Cambridge, MN). Multiple range test (Fisher's least significant differences, LSD) for
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48 188 **3. Results and discussion**
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50 189 3.1. GF-sample classification
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52 190 Classification of GF-samples on the basis of their distinctive and significant responses in terms of
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54 191 dynamic and static rheological performance, viscometric profile and thermal behaviour was achieved by
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doughs. The purpose of the analysis is to obtain a small number of factors which account for most of the variability in the 30 variables. Factor analysis grouped GF dough functional parameters into four different factors that explained 84.62% of the cumulative variance (VE), since 4 factors had eigenvalues greater than or equal to 1.0. The first three factors explained 76.28% of the variability of the results (Table 2). Factor 1 (36.18% VE) included dynamic and static rheological properties, while factor 2 (23.62% VE) grouped flour pasting and gelling characteristics, and factor 3 (16.48% VE) accounted for the thermal features during gelatinization and retrogradation (Table 2). Factor 1 correlated positively with storage modulus, loss modulus, penetration force, % of stress relaxation, hardness, cohesiveness, resilience, and springiness. Factor 2 correlated positively with the visco-metric characteristics during cooking –peak viscosity and holding strength- and cooling -viscosity at 50 °C and total setback-. Factor 3 showed positive dependence of T_p retrogradation and T_p gelatinization, while depended negatively on ΔH of both gelatinization and retrogradation thermal processes (Table 2). Plots of scores of factor 1 vs factor 2 and factor 1 vs factor 3 illustrating sample location in the scatterplot, are depicted in Fig. 1. Separation of samples along the x axis was observed according to factor 1, allowing to clearly differentiate GF-doughs formulated with hydrocolloids, that located in the positive zone of the x axis, from the rest of the samples (Fig. 1). These samples exhibited higher values for the static and dynamic mechanical characteristics in terms of higher mechanical spectra (G' and G''), texture profile, resistance to penetration and % of residual stress. In a descending order, surfactant- and protein-formulated GF-doughs with intermediate and lower values of the already mentioned characteristics, respectively, locate in the middle and in the negative zone of the x axis. Highest values for variables in factor 1 were observed for doughs formulated with GG and PF and bases E and F that contain amaranth flour AF, while lowest values corresponded to doughs with MP and EP and bases A and B containing rice flour and starch. Classification of samples according to factor 2 differentiated matrices with different basic formulation in such a way that A, C and B bases showing higher viscometric profiles during both cooking and cooling located in the positive zone of the y axe, while D, E and F based

GF-doughs exhibiting poorer visco-metric profiles were placed in the negative zone of the y axe of the sample scatterplot (Fig.1). Factor 3 clearly discriminated additive-free GF-doughs that accounted for the higher temperatures and lower enthalpies for both gelatinization and retrogradation thermal transitions.

3.2. Fundamental and empirical rheological properties of formulated GF-doughs

It has been widely recognised that dough should convene certain mechanical requests to produce good-quality bread. Those requirements concern a proper combination of small and large rheological properties and viscometric and thermal response during breadmaking steps. Suitable rheological trends to perform high-quality baked goods have been closely linked to dough formula. Changes in dough technological properties by using non-wheat/non-gluten raw materials may result in different processing performance and associated production problems linked with slack or excessively stiff dough, leading to bread of poorer quality (Collar, 2008).

In dynamic oscillation tests, the frequency sweep shows how the viscous and elastic behavior of the material changes with the rate of application of strain or stress, while the amplitude of the signal is held constant. Mechanical spectra of GF-doughs (plots not shown) significantly depended on both the basic formulation (flours/starches) (Table 3) and the presence and dose of main tested additives (Table 4). For major formulations in the whole range of frequencies, G' was greater than G'' giving to dynamic mechanical loss tangent ($\tan\delta = G''/G'$) values smaller than unity suggesting a solid elastic-like behavior of the GF doughs as found earlier by others (Lazaridou et al., 2007; Mariotti et al., 2009; Samutsri & Supphantharika, 2012). Effect of basic formulation on dynamic moduli and loss tangent (Table 4) evidenced significant changes in G' and $\tan\delta$ according to flour(s)/starch(es) composition.

High G_1' generally reflects a more rigid and stiff material whose $\tan\delta$ is small. The presence of CF (C, D vs A, B) and AF (E, F vs A, B) in the basic recipe determined higher values of G_1' and lower values of

tan δ_1 . Strengthening effects were more pronounced for CF in presence of CS ($G_1' = 59243$ Pa) and for AF in presence of CaS ($G_1' = 36820$ Pa). Replacement of CS by CaS in a basic formula (B vs A) significantly weakened the dough giving the highest values for tan δ_1 (0.750 vs 0.496). Additive incorporation into basic formulas provided significant effects in both elastic and viscous components of GF-samples, particularly for hydrocolloids and proteins, effects being opposite and concentration dependent (Table 4). An increase in both G_1' and G_1'' ~~as an indicator of the fluid nature of the composite (BoMiller, 2011)~~ was observed for GG, LB and PF formulated GF-doughs, especially for PF containing matrices as found earlier (Mariotti et al., 2009), and probably associated to a synergistic interaction between starch and hydrocolloid polymer molecules to form a co-polymer network (Chen et al., 2009). Protein incorporation strongly decreased the values of dynamic moduli, the extent being dependent on the protein concentration, and greater for G' than for G_1'' (Table 4). As a result, tan δ_1 values tend to increase. In a previous work (Ronda et al., 2014), doughs enriched with albumin at 5 and 10% of addition exhibited a lower mechanical spectra profiles than unsupplemented protein-samples, regardless the dose of addition and the absence/presence of acid. With few exceptions, effects of basic formulation followed a similar pattern on static mechanical properties (Table 4). Basic formulations flour/starch A and B exhibited the poorest textural quality in terms of resistance to penetration (0.16-0.18N), residual stress after compression (8.13-6.30N), resistance to indentation (2.34-2.60N), and cohesiveness (0.081-0.087), irrespective of the starch source (CS in A, CaS in B). Addition of 10% CF to RF/CS blends provided a large GF-dough strengthening effect in presence of CS (C) and an intermediate structuring effect in presence of CaS (D). AF encompassed similar intermediate reinforcement of GF-dough regardless the source of starch (E, F) (Table 3). Effects of different additives (data not shown) were significant in some cases but of very small extent, especially when compared to the effect of basic dough formulation.

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263 3.3. Visco-metric and thermal properties of formulated GF-doughs

264 In starch blends, both additive and non-additive visco-metric and thermal behaviors have been
265 described according to intrinsic properties such as gelatinization temperature, swelling power, carbohydrate
266 leaching during swelling, and granule size of the individual starches in the blend (Waterschoot et al.,
267 2014b). In more heterogeneous matrices such as flour/starch blends from different sources in
268 absence/presence of single dietary fibres, proteins, and surfactants, single (Tables 3-5) and interactive
269 effects (Figure 2) were both observed regarding visco-metric and thermal properties.

270 RVA visco-metric profiles of single and associated basic ingredients and additive-formulated GF-
271 doughs are depicted in Fig. 2 for bases A and F. Single effects of qualitative levels (A-F) of basic formula
272 (Table 3) and quantitative additive levels (Table 4) were identified. During gelatinization and pasting, higher
273 RVA profiles were reached in base A, intermediate viscosity values were observed in B, C, and D bases,
274 while the lower values were attained in E and F bases (Table 3). This means that replacement of CS by
275 CaS and/or partial replacement of any of both starches by either CF or AF hinders blended starch granules
276 swelling during the process of gelatinization due to water competition, and composite starch polymer
277 molecules (primarily amylose molecules) easily leach from the swollen granules (Shi et al., 1991), and thus,
278 lower peak viscosity was reached. The process of pasting that follows gelatinization occurs with continued
279 heating of starch granules in the presence of excess water and involves considerable continued granule
280 swelling and leaching of starch polymer (primarily amylose) molecules. During the 95°C hold, the more
281 fragile swollen granules easily disintegrate under the shear conditions of the instrument, and the viscosity
282 decreases to a lower holding strength (Table 3), being the degree of fragmentation dependent on the shear
283 rate, shear time, and nature of the starch granules. Single effects of additives on the cooking cycle
284 viscosities (Table 4) revealed a general concentration-dependent increase in peak viscosity, holding
285 strength and viscosity of hot paste provided by hydrocolloids, EP and SSL, and some decrease in the
286 pasting temperature particularly for LB, PF, DATA and SF. During gelling/cooling, hot pastes, especially of

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3 287 amylose-containing starches, begin to cool, and become more elastic developing different solid properties,
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5 288 i.e., gelation occurs (BeMiller, 2011). The transition from a viscous liquid to a gel, is called setback; the
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8 289 molecular process that produces setback is known as retrogradation (Atwell et al., 1988), that is a non-
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10 290 equilibrium, polymer crystallization process. At higher amylose concentrations, which are the case in this
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12 291 study (amylose/amylopectin ratio: 17/83 CS, 7/93 CaS), a gel formation takes place. The first (short-term)
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14 292 phase of retrogradation occurs as the paste cools and involves network formation (entanglements and/or
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16 293 junction zone formation) between amylose molecules (Silverio et al., 1996), forming an elastic gel. Some
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18 294 amylopectin entanglements may be involved, but primarily retrogradation of amylopectin is a much slower
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20 295 process that may proceed for several weeks (Silverio et al., 1996), depending on the storage temperature.
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22 296 In this work, effects on gelling visco-metric properties of the different bases (Table 3) were much more
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24 297 prominent than those provided by additives (Table 4). Bases A and C exhibited the highest gelling profiles,
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26 298 while B and E showed intermediate behaviour, and D and F provided the lowest viscosity values during
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28 299 gelling (Table 3). CaS instead of CS decreased moderately the extent of retrogradation of the blend, of the
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30 300 same order that AF did in presence of CS. CF and AF significantly decreased retrogradation in presence of
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32 301 CaS. A relatively high cold paste viscosity can result from increased interactions between leached
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34 302 molecules and/or swollen granules of the different starches (Puncha-arnon et al., 2008), whereas a
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36 303 relatively low cold paste viscosity can be explained by a reduction in swelling power and thus carbohydrate
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38 304 leaching of one starch by the other (Waterschoot et al., 2014b). Concerning effects of additives, all the
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40 305 tested hydrocolloids, proteins and surfactants except SF promoted the RVA viscosity profiles during cooling,
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42 306 being effects concentration dependent (Table 4).
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50 307 It has been alluded that the addition of a hydrocolloid to a starch paste or gel makes an already
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52 308 complex system even more complex. It can be assumed that cooked starch–hydrocolloid systems are
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56 310 or polymer networks of varying rheological properties and that the contributions of the dispersed and
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continuous phases to the properties of the overall system vary with factors such as relative concentrations of starch and hydrocolloid, preparation conditions, and interactions between and/or compatibilities of the various polymer molecules present (BeMiller, 2011). Similar or even higher complexity can be applied to other additives such as surfactants or ingredients like proteins, when added to a blended starches and/or composite flour/starch systems. In fact, interactive effects base x additive were observed for many viscometric measurements. Fig. 2 illustrates RVA profiles of GF-doughs formulated with bases A (a) and F (b) containing hydrocolloids (GG, LB and PF), proteins (MP, EP), and surfactants (DATA, SSL, SF) at low (0) and high (1) level of addition. As it can be seen, in general, effects of additives were significant in promoting viscosity levels for the base A (RF+CS) exhibiting a high RVA curve, particularly for hydrocolloids and proteins, while poor effects were provided by the same additives/doses when added to base F (RF+CaS+AF) showing a lower RVA profile. Exceptions accounted for LB, EP and SSL that moderately increased RVA curves during both pasting and gelling with increased concentration. For all other bases (data not shown), B, C and bases with intermediate RVA profile behaved like base A, while E base with low RVA profile did like base F.

An aspect of the use of additives in this study that should be considered is, that apart from the complexity of flour composition, dietary fibres contain, in addition to the 81–88% polysaccharide, 2.5–5% protein which could influence behaviors of the starch-based matrix with which it is used (Table 1). Analogously, proteins from egg and milk (79-84%) contain 7.6-9.3% carbohydrates and up to 5.3% fat.

DSC thermal profiles of single and associated basic ingredients and additive-formulated GF-doughs at higher dose of addition were performed. Since effects of additives were not significant ($p>0.05$) in any of the thermal parameter determined, effects of individual basic ingredients (flours and starches) and qualitative levels (A-F) of basic formulations were studied (Table 5).

Heating starch in excess water ($>1:2$ starch:water) above the gelatinisation temperature disrupts the molecular order of the granules and melts the crystallites, but when relatively less water ($<1:2$ starch:water)

is available, gelatinisation is partly postponed to higher temperatures (Delcour and Hoseneey, 2010), and a biphasic thermal transition takes place (Andreu et al., 1999). The main endotherm occurs essentially at constant temperature but a progressive shift of the second endotherm temperature towards higher values occurs when the water content decreases. The second endotherm represents that portion of the sample that did not gelatinize during the first heating, and the shift of the peak temperature is attributed to the heterogeneity of the starch granules (Biliaderis et al., 1980). Simulation of the baking process in calorimeter pans led to a biphasic endotherm for starch gelatinization as a consequence of the limited water content of GF-doughs (41%). The first endotherm, corresponding to the gelatinization of the amorphous phase of the starch appeared between 71.09°C (CaS) and 87.08°C (RF) and had an enthalpy of 2.94-7.95 J/g dry weight (d.wt.). The second endotherm, corresponding to melting of the more stable crystalline structures was quantitative only in CF, CS, and CaS, appeared at 87.86-98.39°C with enthalpies ranging from 1.84 J/g d.wt. to 5.23 J/g d.wt. Gelatinisation onset (T_o), peak (T_p) and conclusion (T_c) temperatures of the different starches and flours used in the different basic formulations in restricted water (1:0.7 starch/flour:water) followed a general decreasing order: RF>AF>CF>CS>CaS, while gelatinization enthalpies (ΔH) were AF>CS>CaS>RF>CF (Table 5). For RF and AF, T_o and T_c for gelatinization defined a wide interval for gelatinization (23-24°C) and a high T_p , suggesting overlapping of gelatinization and melting in only one broad peak. Retrogradation is the process of crystallisation of AP molecules in a starch paste (Delcour & Hoseneey, 2010). Besides storage temperature, also the starch-to-water ratio has an important effect on retrogradation. Water content should neither be too high (>80%) nor too low (<30%) to allow retrogradation (Zeleznek & Hoseneey, 1986). After 6 days of storage of gelatinized samples, retrogradation was detected only in RF, CF and CaS, with melting of amylopectin crystals at T_p 59–65°C and at melting enthalpy at 2.3-6.4 J/g (Table 5).

As pointed out very recently (Waterschoot et al., 2014b), limited research has been done on the gelatinization properties of blends in concentrated starch–water systems (35–65% water content) although

such systems are of particular practical relevance. Contrary to the behavior in excess water, in limited water conditions, the starch granules from starch and flour compete for the available water. In this study, blended flour/starch bases A-F followed a general behaviour regarding the temperatures of thermal transitions (Table 5). Higher values of T_0 , T_p and T_c of gelatinization, melting and retrogradation were observed in bases E and F, while lower values were provided by base B, and intermediate values were assigned to bases A, C and D. This means that CaS significantly decreased the temperature of thermal transitions in presence of RF when compared with CS. Results are in line with the lower T_0 , T_p and T_c of gelatinization stated for CaS when compared to CS (Gomand et al., 2010). In blended starches, the one with the lowest gelatinization temperature gelatinizes first and leaves less water for gelatinization of the other starch, resulting that gelatinization of the latter occurs at higher temperatures (Liu and Lelièvre, 1992). However, probably not only differences in gelatinization temperature, but also in granule size and rate of water absorption impact the gelatinization properties. In other studies, CS and CaS starches have been described to have granules with somewhat similar dimensions (5–20 μm for maize starch and 3–32 μm for cassava starch), but cassava starch has round or truncated granules while maize starch granules are polygonal (Jane et al., 1994). In this study, the water solubility Index is greater for CaS (11.78%) than for CS (0.4%), leaching more amylose and amylopectin outside the granules (Waterschoot et al., 2014a). Moreover, addition of CF increased the transition temperatures in blends RF-CaS, and did not affect those of RF-CS. The presence of AF significantly promoted the temperature at which gelatinization, melting and retrogradation take place, regardless the nature of the starch blended with RF. Enthalpies of gelatinization-peak 1 and peak 2), melting and retrogradation ranged 1.78-2.74J/g, 2.01-3.80J/g, and 3.55-4.06J/g, respectively (Table 5), and no relevant differences (even statistically significant) within bases were observed. Gelatinisation-onset (T_0), peak (T_p) and conclusion (T_c) temperatures of the different starches and flours used in the different basic formulations in restricted water (1:0.7 starch/flour:water) followed a general decreasing order: RF>AF>CF>CS>CaS, while gelatinization enthalpies (ΔH) were

~~AF>CS>CaS>RF>CF (Table 5)~~. For RF and AF, T₀ and T_c for gelatinization defined a wide interval for gelatinization (23-24°C) and a high T_p, suggesting overlapping of gelatinization and melting in only one broad peak.

4. Conclusions

The ability of rice flour-based GF formulations to provide machinable and visco-elastic GF-doughs to make specialty flat breads, depended primarily on both the type of starch (corn and cassava) and the additional flour (amaranth and chickpea) of the basic blends, and in second place on the additional ingredients -proteins (milk and egg white)- and additives -hydrocolloids (guar gum, locust bean and psyllium fibre). Basic formulations rice flour/starch exhibited the poorest textural quality in terms of macroscopic mechanical properties but the higher visco-metric profile, irrespective of the starch source. Single addition of 10% of either chickpea flour or amaranth flour to rice flour/starch blends provided a large GF-dough strengthening effect in presence of corn starch and an intermediate structuring effect in presence of cassava starch (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and amaranth flours, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of corn starch and cassava starch, respectively. Replacement of corn starch by cassava starch in a basic formula significantly weakened the dough, whereas an increase in both dynamic moduli as an indicator of the fluid nature of the composite was observed for hydrocolloid formulated GF-doughs, especially for psyllium fibre containing GF-doughs, probably associated to a synergistic interaction between starch and hydrocolloid polymer molecules to form a co-polymer network. Protein incorporation strongly decreased the values of dynamic moduli, the extent being dependent on the protein concentration. During gelatinization and pasting, replacement of corn starch by cassava starch and/or partial replacement of any of both starches by either chickpea or amaranth flour hinders blended starch granules swelling during the process of gelatinization

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3 407 due to water competition, and lower peak viscosity and extent of retrogradation were reached. Cassava
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5 408 starch significantly decreased the temperature of thermal transitions in presence of rice flour when
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8 409 compared with corn starch. The presence of amaranth flour significantly promoted the temperature at which
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10 410 gelatinization, melting and retrogradation take place, regardless the nature of the starch blended with rice
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12 411 flour.

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15 412 According to obtained results, a proper balance of visco-elastic, visco-metric and thermal GF-dough
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17 413 properties is reached by matrices formulated with bases A -Rice flour (50%) + corn starch (50%)- and C -
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19 414 Rice flour (45%) + corn starch (45%) + chickpea flour (10%)- in presence of 2% of hydrocolloids, particularly
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21 415 Psyllium fibre. This formulation is encouraged to make GF-breads with promoted protein and fibre contents,
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23 416 from machinable and moderately visco-elastic doughs.
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29 418 **Acknowledgements** The authors acknowledge the financial support of Regione Autonoma della Sardegna,
30
31 419 Legge 7, project title «Ottimizzazione della formulazione e della tecnologia di processo per la produzione di
32
33 420 prodotti da forno gluten-free fermentati e non fermentati» and Spanish institutions Consejo Superior de
34
35 421 Investigaciones Científicas (CSIC) and Ministerio de Economía y Competitividad (Project AGL2011-22669).
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41 423 **5. References**
42
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44
45 425 Ahlborn, G., Pike, O., Hendrix, S., Hess, W., & Huber, C. (2005). Sensory, mechanical, and microscopic
46
47 426 evaluation of staling in low-protein and gluten-free breads. *Cereal Chemistry*, 82, 328-335.
48
49
50 427 Andreu, P., Collar, C. & Martínez-Anaya, M. A. (1999). Thermal properties of doughs formulated with
51
52 428 enzymes and starters. *European Food Research and Technology*, 209, 286-293.
53
54
55 429 Angioloni, A. & Collar, C. (2008). Functional response of diluted dough matrixes in high-fibre systems: a
56
57 430 viscometric and rheological approach. *Food Research International*, 41, 803-812.
58
59
60

- 1
2
3 431 Angioloni, A. & Collar, C. (2009). Small and large deformation viscoelastic behaviour of selected fibre
4
5 432 blends with gelling properties. *Food Hydrocolloids*, 23, 742-748.
6
7
8 433 Angioloni, A. & and Collar, C. (2011). Physicochemical and nutritional properties of reduced-caloric density
9
10 434 high-fibre breads. *LWT - Food Science and Technology*, 44, 747-758.
11
12 435 Angioloni, A. & Collar, C. (2012). High Legume-Wheat Matrices: an Alternative to Promote Bread Nutritional
13
14 436 Value Meeting Dough Viscoelastic Restrictions. *European Food Research and Technology* 234/2,
15
16 437 273-284.
17
18
19 438 Atwell, W. A., Hood, L. F., Lineback, D. R., Marston, E. V., & Zobel, H. F. (1988). The terminology and
20
21 439 methodology associated with basic starch phenomena. *Cereal Foods World*, 33, 306-311.
22
23
24 440 BeMiller, J. N. (2011). Pasting, paste, and gel properties of starch-hydrocolloid combinations. *Carbohydrate*
25
26 441 *Polymers*, 86, 386-423.
27
28
29 442 Biliaderis, C. G. , Maurice, T. J. & Vose, J. R. (1980). Starch gelatinization phenomena studied by
30
31 443 Differential Scanning Calorimetry. *Journal of Food Science*, 45, 1669-1674, 1680.
32
33
34 444 Chen, C.-H., Kuo, W.-S., & Lai, L.-S. (2009). Rheological and physical characterization of film-forming
35
36 445 solutions and edible films from tapioca starch/decolorized hsian-tsao leaf gum. *Food Hydrocolloids*, 23,
37
38 446 2132-2140.
39
40
41 447 Collar, C. (2003). Significance of viscosity profile of pasted and gelled formulated wheat doughs on bread
42
43 448 staling. *European Food Research and Technology* 216, 505-513.
44
45
46 449 Collar, C. (2008). Novel high fibre and whole grain breads. In B. Hamaker (Ed.), *Technology of functional*
47
48 450 *cereal products. Part 2: Technology of functional cereal products* (pp. 184-214). Abington Hall,
49
50 451 Abington, Cambridge CB21 6AH, England: Woodhead Publishing Limited.
51
52
53 452 Collar, C., Andreu, P., Martinez, J., & Armero, E. (1999). Optimization of hydrocolloid addition to improve
54
55 453 wheat bread dough functionality: a response surface methodology study. *Food Hydrocolloids*, 13, 467-
56
57 454 475.
58
59
60

1
2
3 455 Collar, C. & Angioloni, A. (2014). Pseudocereals and teff in complex breadmaking matrices: impact of lipid
4
5 456 dynamics on the bread functional and nutritional profiles. *Journal of Cereal Science*, 59, 145-154.
6
7
8 457 Delcour, J. A., Hoseney, R. C. (2010). (Eds.), *Principles of Cereal Science and Technology*, 3rd edn., AACC
9
10 458 International, Inc., St. Paul, MN.
11
12
13 459 Fois, S., Fadda, C., Tonelli, R., Sanna, M., Urgeghe, P. P., Roggio, T. & Catzeddu, P. (2012). Effects of the
14
15 460 fermentation process on gas-cell size two-dimensional distribution and rheological characteristics of
16
17 461 durum-wheat-based doughs. *Food Research International*, 49, 193-200.
18
19
20 462 Gomand, S. V., Lamberts, L., Derde, L. J., Goesaert, H. et al., (2010). Structural properties and
21
22 463 gelatinisation characteristics of potato and cassava starches and mutants thereof. *Food Hydrocolloids*,
23
24 464 24, 307–317.
25
26
27 465 Jane, J. L., Kasemsuwan, T., Leas, S., Zobel, H. & Robyt, J. F. (1994). Anthology of starch
28
29 466 granule morphology by scanning electron microscopy. *Starch/Stärke*, 46, 121–129.
30
31
32 467 Lazaridou, A., Duta, D., Papageorgiou, M., Belc, N., & Biliaderis, C. G. (2007). Effects of hydrocolloids on
33
34 468 dough rheology and bread quality parameters in gluten-free formulations. *Journal of Food Engineering*,
35
36 469 79, 1033–1047.
37
38
39 470 León, A., Duran, E., & Benedito de Barber, C. (1997). A new approach to study starch changes occurring in
40
41 471 dough baking process and during bread storage. *Zeitschrift Lebensm. Unters. Forschung*, 204, 116-
42
43 472 120.
44
45
46 473 Liu, H., & Lelievre, J. (1992). Differential scanning calorimetric and rheological study of the gelatinization of
47
48 474 starch granules embedded in a gel matrix. *Cereal Chemistry*, 69, 597–599.
49
50
51 475 Mariotti, M., Lucisano, M., Pagani, M. A., & Ng, P. K. W. (2009). The role of corn starch, amaranth flour, pea
52
53 476 isolate, and Psyllium flour on the rheological properties and the ultrastructure of gluten-free doughs.
54
55 477 *Food Research International*, 42, 963–975.
56
57
58
59
60

- 478 Mohammadi, M., Sadeghnia, N, Azizi, M, H., Neyestani, T. R. & Mortazavian, A. M. (2014). Development of
479 gluten-free flat bread using hydrocolloids: Xanthan and CMC. *Journal of Industrial and Engineering*
480 *Chemistry*, 20, 1812–1818.
- 481 Puncha-arnon, S., Pathipanawat, W., Puttanlek, C., Rungsardthong, V. & Uttapap, D. (2008). Effects of
482 relative granule size and gelatinization temperature on paste and gel properties of starch blends. *Food*
483 *Research International*, 41, 552–561.
- 484 Ronda, F., Pérez-Quirce, S., Angioloni, A. & Collar, C. (2013). Impact of viscous dietary fibres on the
485 viscoelastic behaviour of gluten-free formulated rice doughs: A fundamental and empirical rheological
486 approach. *Food Hydrocolloids*, 32, 252-262.
- 487 Ronda, F., Villanueva, M. & Collar, C. (2014). Influence of acidification on dough viscoelasticity of gluten-
488 free rice starch-based dough matrices enriched with exogenous protein. *LWT- Food Science and*
489 *Technology* DOI: 10.1016/j.lwt.2014.05.052, available online: 11-June-2014.
- 490 Samutsri, W. & Supphantharika, M. (2012). Effect of salts on pasting, thermal, and rheological properties of
491 rice starch in the presence of non-ionic and ionic hydrocolloids. *Carbohydrate Polymers*, 87, 1559–
492 1568.
- 493 Santos, E. , Rosell, C.M., & Collar, C. (2008). Gelatinisation and staling kinetics of high fiber-flour blends: a
494 calorimetric approach. *Cereal Chemistry*, 85, 455–463.
- 495 Sardu, C., Cocco, E., Mereu, A., Massa, R., Cuccu A, et al. (2012). Population Based Study of 12
496 Autoimmune Diseases in Sardinia, Italy: Prevalence and Comorbidity. *PLoS ONE* 7(3): e32487.
497 doi:10.1371/journal.pone.0032487.
- 498 Schober, T. J. (2009). Manufacture of Gluten-Free Specialty Breads and Confectionery Products. In:
499 *Gluten-Free Food Science and Technology*, Edited by Eimear Gallagher, Blackwell Publishing. P. 130-
500 180.

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7
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47
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50
51
52
53
54
55
56
57
58
59
60

Sciarini, L.S., Ribotta, P.D., León, A.E. & Pérez G.T. (2012). Incorporation of several additives into gluten free breads: Effect on dough properties and bread quality. *Journal of Food Engineering*, 111, 590–597.

Shi, Y. C., Seib, P. A., & Lu, S. P. W. (1991). Leaching of amylose from wheat and corn starch. *Advances in Experimental Medicine and Biology*, 302, 667–686.

Silverio, J., Svensson, E., Eliasson, A.-C., & Olofsson, G. (1996). Isothermal microcalorimetric studies on starch retrogradation. *Journal of Thermal Analysis*, 47, 1179–1200.

Singh, H., Rockall, A., Martin, C. R., Chung, O. K., & Lookhart, G. L. (2006). The analysis of stress relaxation data of some viscoelastic foods using a texture analyzer. *Journal of Texture Studies*, 37, 383–392.

Waterschoot, J., Gomand, S. V., Fierens, E., & Delcour, J. A. (2014a). Production, structure, physicochemical and functional properties of maize, cassava, wheat, potato and rice starches. *Starch/Stärke*, 66, 1–16.

Waterschoot, J., Gomand, S. V., Fierens, E., & Delcour, J. A. (2014b). Starch blends and their physicochemical properties. *Starch/Stärke*, 66, 1–13.

You, L., Perret, J., Parker, T., & Allen, G. D. (2003). Enzymatic modification to improve the water-absorbing and gelling properties of Psyllium. *Food Chemistry*, 82, 243–248.

Yun, S. H. & Matheson, N. K. (1990). Estimation of amylose content of starches after precipitation of amylopectin with Concanavalin A. *Starch/Starke* 42, 302-305.

(a)

(b)

Figure 1.- Scatterplots of scores of factor 1 vs factor 2 (a) and factor 1 vs factor 3 (b) of GF-doughs formulated with bases A to F containing hydrocolloids (guar gum GG, locust bean gum LB and psyllium fibre PF), proteins (milk MP, egg white EP), and surfactants (diacetyl tartaric acid ester of mono- and diglycerides DATA, sodium stearyl-2-lactylate SSL, sunflower oil SF) at high level of addition.

Figure 2.- RVA curves of GF-doughs formulated with bases A (a) and F (b) containing hydrocolloids (guar gum GG, locust bean gum LB and psyllium fibre PF), proteins (milk MP, egg white EP), and surfactants (diacetyl tartaric acid ester of mono- and diglycerides DATA, sodium stearyl-2-lactylate SSL, sunflower oil SF) at low (0) and high (1) level of addition.

Table 1.- Proximate chemical and nutritional composition of gluten-free ingredients.

Ingredient	Moisture	Protein	Fat	Ash	Digestible Carbohydrates	Total Dietary Fibre
(g/ per 100 g ingredient, as is)						
Flours						
Rice	14	7.1	1.3	0.8	76.5	0.22
Amaranth	14.5	14.5	6.5	2.4	51	15
Chickpea	9.8	23	6.6	2.8	48.7	15
Starches						
Corn	12	0.3	0	0	88	0
Cassava	12.6	0.5	0.5	0.2	86	0.5
Proteins						
Egg white	2.73	84.39	0.1	3.47	9.31	0
Milk	4.8	79.2	5.3	3.2	7.6	0
Dietary Fibres						
Guar gum	7	5	0	1	0	88
Locust bean gum	10.0	5	1	1.1	0	83
Psyllium fibre	10	2.5	0.5	2	4	81 ¹
Surfactants						
DATA	2.3	0	100 ²	0.3	0	0
SSL	0.6	0	100 ²	9.7	0	0
Sunflower oil	0	0	92 ³	0	0	0

DATA diacetyl tartaric acid ester of mono- and diglycerides, SSL sodium stearyl-2-lactylate

(¹) 44 soluble fibre, 36 insoluble fibre

(²) 98% saturated fat

(³) 11.1% saturated fat

Table 2.- Factor Loading Matrix After Varimax Rotation in Factor Analysis.

	Factor 1	Factor 2	Factor 3	Factor 4
	(36.18%VE)	(23.62%VE)	(16.48%VE)	(8.34%VE)
Storage modulus, $\lambda=1$ Hz	0.9124	0.0425	0.0399	0.1337
Loss modulus, $\lambda=1$ Hz	0.9180	-0.0133	-0.0035	0.0985
Penetration force	0.8706	-0.0450	0.1383	0.0867
Stress Relaxation	0.8053	-0.0500	0.1236	0.0723
Hardness	0.9253	-0.1045	-0.0001	-0.0266
Cohesiveness	0.9516	-0.1010	-0.0357	0.0499
Resilience	0.8969	0.0212	-0.0406	0.0308
Springiness	0.8234	-0.0328	-0.1479	-0.0937
PastingTemperature	0.1046	0.2980	0.1618	0.8860
Peak Viscosity	-0.1484	0.9147	-0.1278	-0.2378
Holding Strength	-0.0721	0.9763	-0.0212	0.0398
Viscosity at 95°C	-0.0907	-0.0575	-0.1287	-0.9358
Viscosity at 50°C	-0.0345	0.8721	-0.0469	0.4019
Total Setback	0.0535	0.8358	-0.0468	0.4612
T _p _{gelatinization}	-0.0766	-0.2586	0.8710	0.1872
$\Delta H_{gelatinization}$	0.03486	-0.5961	-0.5352	-0.1546
T _p _{retrogradation}	-0.0192	0.0615	0.9616	-0.0620
$\Delta H_{retrogradation}$	-0.1324	0.0064	-0.8430	-0.1385

Table 3.- Single significant effects ($p < 0.05$) of qualitative levels (A-F) of basic formula on selected dynamic, textural, and visco-metric gluten-free doughs properties.

Parameter	Unit	Overall mean	Level					
			A	B	C	D	E	F
Storage modulus G'_1	Pa	36668	31690b	20943a	59243d	31815b	39498c	36820c
Loss modulus G''_1	Pa	15706			ns			
$\tan \delta_1$		0.471	0.496c	0.750d	0.265a	0.494c	0.398b	0.427b
Penetration force	N	0,338	0,164a	0,182a	0,618c	0,372b	0,369b	0,321b
Stress Relaxation	%	11,97	8,13a	6,30a	20,39c	13,91b	11,96b	11,12b
Hardness	N	3,377	2,34a	2,60a	4,04b	3,54b	3,97b	3,77b
Cohesiveness		0,095	0,087a	0,081a	0,099b	0,091a	0,105b	0,107b
Resilience		0,043			ns			
Springiness		0,136			ns			
Pasting Tre.	°C	75,52			ns			
Peak viscosity	cP	5927	7913c	6183b	6569b	6271b	4195a	4432a
Holding strength	cP	3491	4891d	3002b	3830c	3761c	2707a	2753a
Viscosity at 95°C	cP	2700	1886b	3106d	1435a	5578	1789b	2407c
Viscosity at 50°C	cP	5363	8187f	5474d	6899e	2846a	4641c	4127b
Total Setback	cP	2904	4073d	2750c	4103d	2243b	2433b	1824a

Within rows, values with the same following letter do not differ significantly from each other ($p > 0.05$). ns: non significant.

Table 4.- Single significant effects (p<0.05) of additives on selected dynamic and visco-metric gluten-free doughs properties.

Parameter	Unit	Level	Factors							
			Guar gum	Locust bean	Psyllium	Milk protein	Egg protein	DATA	SSL	Sunflower oil
G'_1	Pa	0	34074a	32395a	28868a	41410c	41211c	ns	ns	40744b
		1	32535a	45051b	74131b	1325b	4192b			6397a
		2	79723b	92382c	116212c	880a	1003a			5806a
G''_1	Pa	0	9507a	9312a	8915a	11900b	11817c	ns	ns	11652b
		1	11525b	13070b	17578b	1061a	2240b			2755a
		2	26086c	27466c	28920c	666a	721a			2683a
Tan δ_1'		0	ns	ns	ns	0,287a	0,287a			0,286a
		1				0,801b	0,534b			0,431b
		2				0,756b	0,718c			0,462b
Pasting temperature	°C	0	ns	76.21c	76.70c	ns	ns	75.41b	73.50	75.80b
		1		75.41b	75.28b			75.88b	75.79	75.60b
		2		74.94a	74.59a			75.28a	77.28	75.17a
Peak viscosity	cP	0	5369a	5293a	5674a	6046c	5210a	5918a	5479a	ns
		1	5943b	5907b	5995b	5914b	5966b	5867a	6002b	
		2	6469c	6582c	6112b	5821a	6605c	5997b	6301c	
Holding strength	cP	0	3218a	3224a	3368a	3539b	3078a	3606b	3092a	3601b
		1	3512b	3485b	3505b	3467a	3553b	3435a	3492b	3467a
		2	3741c	3763c	3598b	3465a	3840c	3431a	3888c	3403a
Viscosity at 95°C	cP	0	2563a	2427a	2308a	2653a	2432a	2672a	2786b	2627a
		1	2700b	2656b	2777b	2742b	2747b	2661a	2617a	2707b
		2	2837c	3016c	3015c	2704b	2921c	2766b	2697a	2766c
Viscosity at 50°C	cP	0	5033a	5013a	4899a	5283a	4883a	ns	4557a	5497c
		1	5367b	5324b	5419b	5396b	5420b		5313b	5319b
		2	5687c	5750c	5770c	5409c	5784c		6217c	5271a
Total Setback	cP	0	2831a	2825a	2680a	2716a	2704a	2807a	2264a	2946b
		1	2898b	2896b	2954b	2968b	2890b	2917b	2846b	2891a
		2	2984c	2992c	3079c	3029c	3119c	2988c	3603c	2875a

For each variable, within columns, values with the same following letter do not differ significantly from each other (p> 0.05). Levels: 0 (absence), 1 (low addition), 2 (high addition). Ns: non significant.

Table 5.- Significant effects ($p<0.05$) of basic ingredients and qualitative bases of gluten-free basic formula on dough thermal properties.

	Ingredients					Bases						
Thermal transition	RF	CF	AF	CS	CaS	A	B	C	D	E	F	
Gelatinization, peak 1												
T ₀ (°C)	78.11±0.13e	70.37±0.4c4	73.27±0.62d	68.65±0.74b	64.01±1.09a	69.2±0.37b	65.78±0.12a	71.2±0.65c	68.26±0.90b	73.01±0.58d	70.96±0.08c	
T _p (°C)	87.08±0.41e	78.89±0.69c	82.01±0.41d	74.89±0.83b	71.09±0.69a	74.99±0.52b	72.65±0.14a	77.72±0.41c	75.87±1.66b	80.25±0.97d	81.03±1.79d	
T _c (°C)	101.52±0.52e	87.4±0.19c	97.69±0.38d	83.47±0.48b	78.39±0.14a	81.89±0.43	77.84±0.04a	82.42±0.07c	81.12±0.07b	87.57±0.00e	84.22±0.03d	
ΔH (J/g, d.b.)	5.07±0.12	2.94±0.03	7.95±0.08	7.15±0.31	6.46±0.3	2.38±0.16c	2.11±0.04b	2.15±0.01b	1.94±0.32a	2.74±0.21c	1.78±0.06a	
Gelatinization, peak 2												
T ₀ (°C)	nd	87.40±0.19c	nd	83.47±0.48b	78.39±0.14a	81.89±0.43b	77.84±0.04a	82.42±0.07c	81.12±0.07b	87.57±0.00e	84.22±0.03d	
T _p (°C)		98.39±0.42c		91.76±0.69b	87.86±1.24a	93.61±0.82	92.83±0.00a	95.27±0.14b	94.78±1.10b	95.37±0.28b	94.59±0.55b	
T _c (°C)		105.89±0.26c		100.8±0.11b	97.26±0.21a	103.33±0.06	103.96±0.39a	104.34±0.29a	106.21±0.38b	104.28±0.02a	103.85±0.97a	
ΔH (J/g, d.b.)		1.84±0.01a		3.17±0.07b	5.23±0.001c	2.75±0.14	3.80±0.01d	2.94±0.10b	3.22±0.10c	2.01±0.01a	2.05±0.44a	
Retrogradation												
T ₀ (°C)	44.01±0.62a	48.43±0.09c	nd	nd	46.47±0.16b	nd	43.19±1.08a	45.24±0.92b	46.18±0.62b	47.87±1.2b	46.97±0.33b	
T _p (°C)	62.18±0.02b	64.7±0.24c			58.78±0.59a		56.18±1.16a	57.53±0.71a	58.7±0.71a,b	59.02±0.21b	58.95±0.59b	
T _c (°C)	77.09±0.10b	80.73±3.26c			72.05±1.61a		74.42±0.02b	75.49±0.31b	75.11±0.68b	74.74±0.22b	73.1±0.65a	
ΔH (J/g, d.b.)	5.41±0.16b	2.31±0.02a			6.24±0.20c		4.06±0.001b	3.55±0.08a	3.67±0.03a	3.69±0.13a	3.58±0.01a	

For each variable, within rows, values with the same following letter do not differ significantly from each other ($p>0.05$). nd: non detected.