Mimicking gluten functionality with β -conglycinin concentrate: evaluation in gluten free yeast-leavened breads

Johanan Espinosa-Ramírez^{1,2}, Raquel Garzon¹, Sergio O. Serna-Saldivar², Cristina M. Rosell^{1*}

¹ Institute of Agrochemistry and Food Technology (IATA-CSIC), C/ Agustin Escardino, 7, Paterna 46980, Valencia, Spain.

² Centro de Investigacion y Desarrollo de Proteinas (CIDPRO) and Centro de Biotecnología FEMSA. Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey, Av. Eugenio Garza Sada 2501 Sur, C.P. 64849 Monterrey, N. L., Mexico.

*Corresponding author e-mail: crosell@iata.csic.es. Phone number +34 963900022. Fax number: +34 963636301

Keywords: β -conglycinin, soy proteins, gluten-free bread, corn starch, structure, hydration

18 **Abstract**

19 Fractionation of soy proteins have proved to produce protein concentrates with viscoelastic
20 properties. In the present study, a β -conglycinin concentrate (β CC) obtained by a pH
21 fractionation of soy flour was tested as structuring agent in gluten-free yeast-leavened bread
22 model. A lean formulation with β CC and corn starch was used to produce gluten-free breads
23 with two hydration conditions and three levels of protein (5%, 10% and 15%). Vital gluten
24 was used to compare the functionality of β CC protein and its performance for breadmaking.
25 Breads were characterized in moisture, color, textural parameters and image analysis. β CC
26 presented lower hydration properties and higher emulsifying activity compared to vital
27 gluten. Blends β CC:starch had higher water binding capacity compared to vital gluten
28 blends. The hydration conditions tested affected the moisture, color and cell density of
29 breads. Breads produced with β CC presented higher 2D area and height and presented
30 higher crumb softness and cohesiveness, and did not present significant differences in
31 springiness and resilience compared to vital gluten breads. The image analysis of crumbs
32 showed higher cell density but lower porosity and mean cell areas in β CC breads. Thus,
33 β CC proved to have potential as a structuring agent in gluten-free breads.

1. Introduction

In the market trends, there is an increasing demand for gluten-free products. This augmented interest is associated to the better diagnoses of celiac disease and also because many novel consumers relate gluten-free products with health. Subsequently, the research to develop gluten free-products that resemble gluten-containing foods has increased (Taylor & Rosell, 2016).

Gluten proteins are the wheat storage proteins and represent about 80% to 85% of total proteins in wheat kernels. These proteins are responsible for the unique viscoelastic properties of wheat flour dough, which is their most important attribute from the technological point of view (Veraverbeke & Delcour, 2002). Gluten is formed by two fractions: gliadins and glutenins. Gliadins are monomeric proteins and contribute mainly to the viscosity and extensibility of the dough system, whereas glutenins are polymeric proteins which are both cohesive and elastic, and are responsible for imparting dough strength and elasticity (Wieser, 2007). After hydration of wheat flour and kneading, gluten proteins form a cohesive and viscoelastic network, capable of holding gas produced during fermentation and rise in oven. This leads to the typical structure of bread after baking and the desired texture, making wheat the most used grain for breadmaking (Veraverbeke & Delcour, 2002).

The substitution of gluten represents a technological challenge, especially in yeast or chemically-leavened bread products. Several studies have evaluated diverse raw materials in order to substitute and mimic the viscoelastic properties of gluten in gluten-free bread products (Taylor & Rosell, 2016). Among these materials, the most common compounds used as structuring agents are hydrocolloids, which enhance the elastic and viscous properties of the gluten-free doughs (Marco & Rosell, 2008; Matos Segura & Rosell, 2011;

Mir, Shah, Naik, & Zargar, 2016), although without mimicking completely gluten functionality. According to the chemical composition of commercial gluten-free breads analyzed by Matos Segura and Rosell (2011), these products were in general starchy based foods with low protein content. In fact, some approaches have been focused on the protein enrichment for enhancing their nutritional value (Krupa-Kozak, Baczek, & Rosell, 2013; Marco & Rosell, 2008). Later studies were focused in the addition of proteins as a structure and texture-forming agent to mimic the characteristics that gluten provides to bread and improve the texture, physical and sensorial properties of gluten-free breads (Aprodu, Alexandra Badiu, & Banu, 2016; Smith, Bean, Herald, & Aramouni, 2012; Ziobro, Witczak, Juszczak, & Korus, 2013). However, up to date only α -zeins, a fraction of the prolamins from corn (Lawton, 1992; Schober, Bean, Tilley, Smith, & Ioerger, 2011) and caroubin, found in the carob germ flour (Feillet & Roulland, 1998) presented gluten-like viscoelastic features but under specific conditions. Zeins proved to form a viscoelastic dough with corn starch but only when kneading was performed above its transition temperature (35°C) (Lawton, 1992), and with poor bread making performance (Andersson, Öhgren, Johansson, Kniola, & Stading, 2011). Likewise, carob germ flour led to the production of adequate gluten-free doughs and breads, but without completely imitating gluten functionality, being required the inclusion of hydroxypropyl methylcellulose (HPMC) (Smith et al., 2012). Therefore, up to date, gluten has not been fully replaced by any other protein. More studies are needed to find proteins that could produce gluten free breads resembling to those breads containing gluten.

Qi et al. (2011) fractionated soy proteins by extracting them at different pH values and obtained fractions with elastic, extensible and sticky properties, which the authors

highlighted as a potential protein to substitute wheat gluten. These authors named these fractions as soy protein elastomers due to their viscoelastic properties, especially when the first precipitation was performed at pH 5.4. These elastomers presented only in β -conglycinin (7S globulin) proteins with a subunit distribution of α' , α and β of 22.5 %, 45.3 % and 18.6 %, respectively. Its stronger viscoelastic behavior was related to the larger amount of aggregates formed by the high molecular weight subunits α' and α . These authors suggested the potential of this β -conglycinin concentrate (β CC) to substitute gluten in gluten-free breadmaking. However, there are no studies that evaluate the building capacity of these proteins in yeast-leavened gluten-free systems. Besides, since the extraction method proposed by Qi et al. (2011) could be adapted to a food-grade industrial process, the evaluation of β CC as an ingredient to mimic gluten could be interesting.

The objective of this study was to explore the ability of freeze-dried β CC to imitate gluten viscoelastic functionality in breadmaking processes. With that aim a lean model system with corn native starch was proposed to compare β CC and gluten functionality when included in gluten-free yeast-leavened breads.

2. Materials and methods

2.1 Materials

β -conglycinin concentrate (β CC) was obtained according to Qi et al. (2011) with several modifications. Briefly, defatted soy flour (Ragasa, México) was dispersed in distilled water in a proportion 1:10 and the pH was adjusted to 9.5 using 4 N NaOH. After 2 h of continuous stirring at 25 °C, the pH was adjusted to 5.4 using 4 N HCl and centrifuged at

6,000 g for 10 min. The pH of the supernatant was adjusted to 4.8 with HCl 4 N and centrifuged at 6,000 g for 10 min. After fractionation, the precipitate was lyophilized at 0.036 mbar and -50 °C for the condenser temperature (Freeze Dryer 4.5, LABCONCO, Kansas City, MO) and milled using a coffee mill. The protein content of the lyophilized β CC was 93.4% in dry basis (d.b.) (N*6.25).

Vital gluten (84.4% protein, d.b.) was donated by Puratos (Groot-Bijgaarden, Belgium). The regular corn starch was purchased from Daesang Corporation (Dongdaemun, South Korea).

2.2 Protein functional properties

Water holding capacity (WHC) and swelling volume (SV) were determined according to Cornejo and Rosell (2015) and expressed as g water/g sample or ml/g sample, respectively. Water binding capacity (WBC), defined as the amount of water retained by the sample after centrifugation was determined according to AACC (1999) and expressed as g water/g sample. The WBC of starch:proteins blends was also determined. Vital gluten or β CC were mixed with starch in concentrations 0%, 5%, 10% and 15% (w/w). These concentrations were selected in order to cover the typical gluten concentration in different varieties of wheat breads and pastries (Traynham, Myers, Carriquiry, & Johnson, 2007; Veraverbeke & Delcour, 2002). β CC was mixed in the same proportions with starch in order to compare with gluten. Five replicates were made for each sample. The moisture content of the hydrated starch:protein blends was determined according to AACC (1999). Values were means of three replicates.

Water retention index was calculated as a percentage of the ratio of the WBC divided by the WHC of each protein to represent the force of the protein to bind water molecules.

The oil binding capacity (OBC) was determined according to Paredes-Lopez et al. (1991) and expressed as g oil/g sample. Five replicates were made for each sample.

For the emulsifying activity index (EAI) and emulsifying stability index (ESI), the turbidimetric method reported by Pearce and Kinsella (1978) was followed. Three replicates of each sample were performed. EAI was expressed as m^2/g and ESI in hours (h).

Foaming properties (volume and stability) were determined according to Martínez et al. (2014) with slight modifications. A suspension of water (15 ml) and protein (0.6 g) was whipped in an Ultra Turrax (IKA T18 basic, Wilmington, NC) at 14,000 rpm for one min at room temperature. The foam volumes at 30 s and 20 min were recorded and used to calculate the foam capacity (FC) and foam stability (FS) as follows:

$$\text{FC} = (ifv/tsv) * 100 \quad (1)$$

$$\text{FS} = (ffv/tsv) * 100 \quad (2)$$

Where *ifv* is the initial foam volume at 30 s, *ffv* is the foam volume after 20 min and *tsv* is the total suspension volume. Results were the average of three determinations.

2.3 Breadmaking process

For the breadmaking process, starch:protein blends (95:5, 90:10 and 85:5) were used as model flours. The baker's formulation, expressed in flour basis was: 1.5% salt, 1% sugar and 1% dry yeast. To determine the water role in the breadmaking performance, two

hydration levels were selected. The first was obtained from the WBC value of the starch:protein blends and was termed “optimum”. The second hydration named “consistency” was the water absorption needed to match the same consistency of the 90% starch and 10% vital gluten dough hydrated at its optimum WBC (0.77 g water/g flour). The consistency of the blends was related with the spread area reached in 5 min when 4 g of dough were set in a flat surface.

Breadmaking was carried out following the procedure described by Garzon et al. (2017) using a mini-scale system. Doughs were kneaded at 100 rpm for 90 s in a stirrer with a turbine accessory (IKA Eurostar 40, Staufen, Germany). After kneading, 2 g of dough were set in greased glass pans (diameter, 1.8 cm; height, 3 cm) and proofed for 40 min at 35 °C and 65% relative humidity. After fermentation, 100 µL of distilled water were added to the surface of the proofed dough to avoid dough surface dryness and were baked in an oven at 130 °C for 10 min. Two batches were run for each protein and hydration conditions.

2.4 Bread characterization

The breads obtained were characterized in moisture, color and texture. Moisture was determined in two steps according to AACC (1999). Results were means of 4 replicates.

The color of the crumb was determined using a colorimeter (Chroma Meter CR-400/410, Konica Minolta, Tokyo, Japan) after white calibration ($L^* = 96.9$, $a^* = -0.04$, $b^* = 1.84$).

Results are means of 4 replicates and expressed using the CIE- $L^*a^*b^*$ scale where L^* indicates lightness, a^* redness to greenness, and b^* yellowness to blueness.

The texture parameters were determined in a Texture Analyzer TA-XT2i (Stable Micro Systems, Surrey, UK) using a texture profile analysis (TPA) double compression test. Bread slices of 10 mm thickness were compressed to 50% of their original height at a test speed of 1 mm s⁻¹ with a stainless steel probe of 25 mm diameter. Texture was assayed in four slices of each batch.

Internal bread crumb structure was evaluated by digital image analysis. Eight breads of each formulation were selected and cut in the middle with a scalpel. Images of the longitudinal section breads were captured at 1200 dpi in a scanner HP Scanjet G3110 (Hewlett- Packard, USA). Images were saved in tiff format and analyzed by Fiji Image J software (Schindelin et al., 2012). The image analysis procedure consisted in: find the edge of the sample to crop the section to analyze, modify the contrast to emphasize the difference between cells and bread crumb, and finally, apply a pre-established algorithm (otsu) to establish the image threshold. To study the whole morphogeometric characteristics of breads, 2D area (mm²) and bread perimeter (mm) were calculated. For the bread crumb structure analysis cell density (u/cm²), crumb porosity (%) and mean cell area (mm²) were annotated.

2.5 Statistical analysis

Analysis of variance (ANOVA) were used for the statistical analysis of the results. Means were compared using Tukey's test. Analyses were performed using Minitab 17 statistical software and 95% confidence.

3. Results

3.1 Functional properties of soy protein elastomer and vital gluten

The functionality of the single proteins was studied to identify their performance and potential use in food systems (Traynham et al., 2007). In general, the β CC functional properties (Table 1) differed from those that have been reported for soy protein isolates. Commercial soy protein isolates presented WBC values ranging from 3.0 to 5.7 g/g (Foh, Wenshui, Amadou, & Jiang, 2012; Idouraine, Yensen, & Weber, 1991; Paredes-Lopez et al., 1991). These values were up to 5.8 times higher than the value obtained for the β CC. Also, the foaming capacity and the emulsifying activity values of the β CC were lower compared to those obtained for commercial soy protein isolates (Foh et al., 2012; Idouraine et al., 1991; Paredes-Lopez et al., 1991). However, the OBC was similar to that reported for soy isolates (Idouraine et al., 1991; Paredes-Lopez et al., 1991). According to Arrese et al. (1991), the soy protein isolates contain approximately 37% and 47% of 7S and 11S proteins, respectively. However, according to Qi et al. (2011) the β CC did not contain 11S proteins. Thus, the higher proportion of the 7S fraction led to the observed changes in functionality. This was consistent with previous findings where the gelling functionality of soy protein isolates differed depending on their subunit composition (Bainy, Corredig, Poysa, Woodrow, & Tosh, 2010).

When functional properties of the β CC and vital gluten were compared, significant differences were found in all the parameters tested (Table 1). Hydration properties, including WBC, WHC and SV were significantly lower for the β CC, compared to gluten. According to the water retention index, the β CC held mainly water that was intimately bound by the protein. In contrast, gluten held a higher proportion of unbound water. Binding of water

molecules during breadmaking is important to avoid the diffusion of water into the crust which leads to firmer crumbs (Houben, Höchstötter, & Becker, 2012).

The β CC presented higher values for OBC and EAI compared to vital gluten (Table 1). However, the ESI and foaming properties were lower for the β CC. Proteins with adequate foaming and emulsifying properties contribute to the stabilization of gas bubbles into the dough and thus create a good crumb structure (Houben et al., 2012). High OBC values allows the stabilization of high fat products and therefore is a property required in the ingredients of bakery products such as muffins (Singh, Kaur, & Singh, 2016).

3.2 Effect of protein concentration on the hydration of starch:protein blends

The WBC of starch:protein blends was assayed to study the effect of the protein concentration in the hydration properties of these blends and to determine the optimum hydration for the production of breads, since it has been already stated the essential role of water in dough systems lacking gluten (Marco & Rosell, 2008). An increment in the WBC values was observed when enhancing the protein concentration (Fig. 1). The same trend was observed for both blends of starch with gluten or β CC, but starch: β CC blends presented higher WBC values. The type of protein and the protein concentration significantly increased the WBC values ($P<0.05$). An attenuated increment in the moisture content of hydrated blends was also observed when the protein content increased. An increment in the WBC values of the blends was expected since gluten and β CC presented higher WBC compared to native corn starch (Table 1, Fig. 1). Traynham et al. (2007) found that the amount of protein and its nature influenced the WBC of blends. They also observed higher

WBC values when more protein was available, which was attributed to higher protein–water interactions.

Therefore, even when the WBC of the β CC was lower compared to the vital gluten (Table 1), the WBC of the starch-protein blends was higher when β CC was used (Fig. 1).

Presumably, the higher retention index obtained for β CC was kept in the starch-protein blends; likewise, stronger interactions between starch and gluten might hinder water bound in starch-gluten systems. This could have led to a diminishing in the interaction of gluten with water, yielding lower WBC values in the starch:gluten blends.

3.3 Bread characterization

Water absorption is an important parameter in breadmaking since it affects the hydration of the main ingredients during kneading and it is involved in the starch gelatinization during baking (Aprodu et al., 2016). In the present study, two levels of water (Table 2) were evaluated for each starch:protein blend in order to assess the effect of the quantity of water in the features of breads. Optimum hydration was considered when proteins linked the maximum amount of water molecules, as determined the WBC. In the second case, constant consistency was used to compare all the bread doughs obtained with the different proteins.

When the hydration was optimum, the moisture content of breads was significantly higher, independently of the protein used, and augmented when higher amounts of gluten protein were used (Table 2). The moisture content of the bread containing β CC did not change when modifying the amount of protein. Previous studies also related a higher moisture in bread with higher water content in the formulation (De La Hera, Rosell, & Gomez, 2014).

The color of the crumb was significantly affected by the hydration level (except a^* parameter), the type of protein and its concentration (Table 2, Fig 2). Breads produced with optimum WBC hydration presented lighter crumbs and lower b^* values. This could be associated to the higher moisture of these breads. According to Özkan et al. (2003) the moisture content affected the reflectance color of dried samples and they observed a positive increment of the L^* values with the increment of moisture. When the protein used was β CC, the breads presented higher values of L^* and lower values for a^* and b^* . This was associated to the color of raw materials. The powder of β CC presented lighter color ($L^*=88.3 \pm 0.1$, $a^*=-1.2 \pm 0.0$, $b^*=14.1 \pm 0.6$) in comparison to that of vital gluten ($L^*=85.9 \pm 0.3$, $a^*=-0.4 \pm 0.0$, $b^*=13.8 \pm 0.4$). Moreover, the color of β CC breads was more affected by the color of the starch, which presented low values of a^* (-2.1 ± 0.0) and b^* (5.7 ± 0.0). Since low levels of sugars could be found in vital gluten (Veraverbeke & Delcour, 2002), non-enzymatic browning reactions could have occurred during baking, leading to the observed differences in the bread color parameters. Likewise, the L^* , a^* and b^* values were significantly affected by the protein concentration (Table 2). As the protein concentration increased, lightness (L^*) decreased whereas b^* and a^* values increased, although a^* values were only affected in gluten containing breads. This was expected since the starch was comparatively lighter ($L^*=96.5 \pm 0.0$). As the content of proteins increased, the color was increasingly noticed, especially in the case of gluten. Krupa-Kozak et al. (2013) also observed that the color of the crumb was influenced by the level of dairy proteins added in gluten-free formulations.

Morpho-geometric parameters of breads were evaluated by assessing the area and perimeter of the longitudinal cross section. Considering that breads were confined to pans, high

perimeter values were related to high volume. Breads produced with β CC had significantly higher areas and perimeters compared to those produced with vital gluten (Table 2, Figure 2). The increment in perimeter of β CC breads was attributed to their higher height, (Figure 2). Therefore, β CC produced breads with higher volumes compared to vital gluten, likely due to better gas retention during yeast proofing. Higher volumes are related to a higher retention of carbon dioxide during proofing and is considered one of the most relevant quality indicator in bread (Houben et al., 2012; Smith et al., 2012). The hydration and protein concentration did not cause a significant effect in the area and perimeter of breads (Table 2).

Textural parameters of breads are summarized in Table 3, and crumb structure is displayed in Figure 2. The crumb texture was not affected by the hydration level at which the breads were produced ($P=0.242$). This result agrees with Aprodu et al. (2016) findings that reported no significant differences in the crumb hardness when gluten-free breads were produced with water absorptions between 65% and 105%. The type and concentration of protein did affect significantly ($P<0.05$) the hardness of breads (Table 3). β CC gave softer crumbs than gluten when optimum amount of water was used in breadmaking. Conversely, when breads were obtained from the same dough consistency, β CC gave softer crumbs when the lowest amount of protein was used. Differences in crumb hardness of gluten-free breads owing to the protein added were reported before (Aprodu et al., 2016; Krupa-Kozak et al., 2013). In addition, crumb hardness was significantly affected by protein concentration when doughs were blended to attain the same consistency, although the trend was dependent on the type of protein. In the presence of gluten, crumb hardness increased with the amount of proteins and the opposite trend was observed in the case of β CC; likely the interaction

between starch and gluten was different from the one between starch and β CC. The increment in hardness with higher non-gluten protein concentration was also reported by Krupa-Kozak et al. (2013) in gluten-free breads containing dairy proteins. Cohesiveness was significantly higher for β CC breads compared to those produced with gluten (Table 3). High cohesiveness is greatly appreciated owing to the lack of cohesiveness in gluten-free breads. Higher values of this property are desirable because it represents a higher cohesion of the material and lower disaggregation during mastication (Cornejo & Rosell, 2015). Cohesiveness also increased with increasing the protein concentration in the case of gluten, but this property was independent of the β CC concentration (Table 3). Chewiness is obtained by multiplying hardness, cohesiveness and springiness (Cornejo & Rosell, 2015), being predominantly affected by hardness. Therefore, chewiness followed the same trend observed with hardness (Table 3), but it was not significantly affected by the type of protein. High values of springiness and resilience are related to adequate crumb elasticity (De La Hera et al., 2014). When springiness and resilience were evaluated, no significant differences were found regardless the type and concentration of proteins, thus β CC induced similar values compared to gluten containing crumbs. In fact, both springiness and resilience were higher compared to values reported previously for gluten-free breads (Cornejo & Rosell, 2015; De La Hera et al., 2014; Krupa-Kozak et al., 2013; Shin et al., 2010). Structural features of the bread crumbs, responsible for the textural behavior, were also evaluated, since they are intimately related to viscoelastic properties and CO₂ holding ability of the doughs. In fact, type of protein significantly affected the structural features of the crumbs. Visually, the β CC breads showed a more uniform crumb structure than gluten containing breads that displayed more open air cells or loci (Fig. 2). Cell density represents

the number of cells in one cm^2 and was significantly affected by the protein type and the hydration applied during breadmaking. βCC breads had 10 times higher cell density compared to breads produced with gluten (Table 3). The cell density was only affected significantly by the hydration level in breads produced with 15% βCC , leading to lower values when hydration to reach the same consistency was applied.

In contrast, hydration insignificantly affected crumb porosity and mean cell area; both parameters were dependent on the protein type and their concentration. βCC breads presented very low cell areas, which ranged from 0.11 to 0.14 mm^2 . In contrast, gluten breads presented cell areas from 0.81 to 1.80 mm^2 . Resulting structural features might be related to the different emulsifying properties of the proteins (Table 1). Emulsifiers stabilize gas bubbles and reduce their surface tension (Houben et al., 2012). The higher emulsifying activity index of the βCC , compared to that found in vital gluten, could have led to the observed differences in the bread crumb (Fig. 2). These crumb characteristics could also be related to the lower hardness of βCC breads (Table 3).

4. Conclusions

The functionality of two different vegetable proteins, gluten and βCC , was evaluated in a very demanding process, a simulated yeast-leavened bread made with corn starch. The commercial vital gluten and the experimental soy-extracted proteins significantly differed in their hydration, emulsifying and foaming properties. In comparison to vital gluten, the βCC presented a relatively higher water retention index also observed when it was blended with corn starch. It is noteworthy to indicate that hydration of starch-protein systems, made of

gluten or β CC, did not significantly affect either the crumb textural features or 2D bread area, despite the generally recognized crucial role of the water in gluten free systems made with other proteins or hydrocolloids. The main differences observed between the two proteins were their effect on the crumb structure. β CC breads presented smaller cell areas and a higher cell density in comparison to gluten breads. These results highlight the potential use of the fractionated soy protein (β CC) as gluten replacer owing to its structuring performance in bread-making.

Acknowledgments

Authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project AGL2014-52928-C2-1-R), Generalitat Valenciana (Project Prometeo 2017/189), the European Regional Development Fund (FEDER) and the joint project between research group from IATA-CSIC and the research groups of Nutriomics and CIDPRO from Tecnológico de Monterrey. Johanan Espinosa-Ramírez acknowledges the support from CONACyT for the PhD scholarship (375643).

References

- AACC International, 1999. Method 56-30.01 Water Hydration Capacity of Protein Materials; Method 44-15.02 Moisture—Air-Oven Methods. In: Approved Methods of Analysis, 11th ed. AACC International, St. Paul, MN, 1999.
- Andersson, H., Öhgren, C., Johansson, D., Kniola, M., & Stading, M. (2011). Extensional

365 flow, viscoelasticity and baking performance of gluten-free zein-starch doughs
 366 supplemented with hydrocolloids. *Food Hydrocolloids*, 25, 1587–1595.

367 Aprodu, I., Alexandra Badiu, E., & Banu, I. (2016). Influence of protein and water addition
 368 on gluten-free dough properties and bread quality. *International Journal of Food*
 369 *Engineering*, 12(4), 355–363.

370 Arrese, E. L., Sorgentini, D. A., Wagner, J. R., & Añon, M. C. (1991). Electrophoretic,
 371 solubility, and functional-properties of commercial soy protein isolates. *Journal of*
 372 *Agricultural and Food Chemistry*, 39, 1029–1032.

373 Bainy, E. M., Corredig, M., Poysa, V., Woodrow, L., & Tosh, S. (2010). Assessment of the
 374 effects of soy protein isolates with different protein compositions on gluten
 375 thermosetting gelation. *Food Research International*, 43, 1684–1691.

376 Cornejo, F., & Rosell, C. M. (2015). Physicochemical properties of long rice grain varieties
 377 in relation to gluten free bread quality. *LWT - Food Science and Technology*, 62, 1203–
 378 1210.

379 De La Hera, E., Rosell, C. M., & Gomez, M. (2014). Effect of water content and flour
 380 particle size on gluten-free bread quality and digestibility. *Food Chemistry*, 151, 526–
 381 531.

382 Feillet, P., & Roulland, T. M. (1998). Caroubin: A gluten-like protein isolated from carob
 383 bean germ. *Cereal Chemistry*, 75(4), 488–492.

384 Foh, M. B. K., Wenshui, X., Amadou, I., & Jiang, Q. (2012). Influence of pH shift on
 385 functional properties of protein isolated of tilapia (*Oreochromis niloticus*) muscles and
 386 of soy protein isolate. *Food and Bioprocess Technology*, 5, 2192–2200.

387 Garzon, R., Rosell, C. M., Malvar, R. A., & Revilla, P. (2017). Diversity among maize
 388 populations from Spain and the United States for dough rheology and gluten-free
 389 breadmaking performance. *International Journal of Food Science and Technology*,
 390 52(4), 1000–1008.

391 Houben, A., Höchstötter, A., & Becker, T. (2012). Possibilities to increase the quality in
 392 gluten-free bread production: An overview. *European Food Research and Technology*,
 393 235, 195–208.

394 Idouraine, A., Yensen, S. B., & Weber, C. W. (1991). Tepary bean flour, albumin and
 395 globulin fractions functional properties compared with soy protein isolate. *Journal of*
 396 *Food Science*, 56(5), 1316–1318.

397 Krupa-Kozak, U., Baczek, N., & Rosell, C. M. (2013). Application of dairy proteins as
 398 technological and nutritional improvers of calcium-supplemented gluten-free bread.
 399 *Nutrients*, 5, 4503–4520.

400 Lawton, J. W. (1992). Viscoelastic of Zein-Starch Doughs. *Cereal Chemistry*, 69(4), 351–
 401 355.

402 Marco, C., & Rosell, C. M. (2008). Breadmaking performance of protein enriched, gluten-
 403 free breads. *European Food Research and Technology*, 227, 1205–1213.

404 Martínez, M. M., Rosell, C. M., & Gómez, M. (2014). Modification of wheat flour
 405 functionality and digestibility through different extrusion conditions. *Journal of Food*
 406 *Engineering*, 143, 74–79.

407 Matos Segura, M. E., & Rosell, C. M. (2011). Chemical composition and starch digestibility
 408 of different gluten-free breads. *Plant Foods for Human Nutrition*, 66, 224–230.

409 Mir, S. A., Shah, M. A., Naik, H. R., & Zargar, I. A. (2016). Influence of hydrocolloids on
 410 dough handling and technological properties of gluten-free breads. *Trends in Food*
 411 *Science and Technology*, 51, 49–57.

412 Özkan, M., Kirca, A., & Cemeroglu, B. (2003). Effect of moisture content on CIE color
 413 values in dried apricots. *European Food Research and Technology*, 216, 217–219.

414 Paredes-Lopez, O., Ordorica-Falomir, C., & Olivares-Vazquez, M. R. (1991). Chickpea
 415 protein isolates: Physicochemical, functional and nutritional characterization. *Journal*
 416 *of Food Science*, 56(3), 726–729.

417 Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a
 418 turbidimetric technique. *Journal of Agricultural and Food Chemistry*, 26(3), 716–723.

419 Qi, G., Venkateshan, K., Mo, X., Zhang, L., & Sun, X. S. (2011). Physicochemical
 420 properties of soy protein : Effects of subunit composition. *Journal of Agricultural and*
 421 *Food Chemistry*, 59, 9958–9964.

422 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ...
 423 Cardona, A. (2012). Fiji: An open source platform for biological image analysis.
 424 *Nature Methods*, 9(7), 676–682.

425 Schober, T. J., Bean, S. R., Tilley, M., Smith, B. M., & Ioerger, B. P. (2011). Impact of
 426 different isolation procedures on the functionality of zein and kafirin. *Journal of Cereal*
 427 *Science*, 54, 241–249.

428 Singh, J. P., Kaur, A., & Singh, N. (2016). Development of eggless gluten-free rice muffins
 429 utilizing black carrot dietary fibre concentrate and xanthan gum. *Journal of Food*
 430 *Science and Technology*, 53(2), 1269–1278.

431 Smith, B. M., Bean, S. R., Herald, T. J., & Aramouni, F. M. (2012). Effect of HPMC on the
 432 quality of wheat-free bread made from carob germ flour-starch mixtures. *Journal of*
 433 *Food Science*, 77(6), C684–C689.

434 Taylor, J., & Rosell, C. (2016). Foreword to special issue on cereal based non-gluten dough
 435 systems. *Journal of Cereal Science*, 67, 1.

436 Traynham, T. L., Myers, D. J., Carriquiry, A. L., & Johnson, L. A. (2007). Evaluation of
 437 water-holding capacity for wheat-soy flour blends. *Journal of the American Oil*
 438 *Chemists' Society*, 84, 151–155.

439 Veraverbeke, W. S., & Delcour, J. A. (2002). Wheat protein composition and properties of
 440 wheat glutenin in relation to breadmaking functionality. *Critical Reviews in Food*
 441 *Science and Nutrition*, 42(3), 179–208.

442 Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24(2), 115–9.

443 Ziobro, R., Witczak, T., Juszczak, L., & Korus, J. (2013). Supplementation of gluten-free
 444 bread with non-gluten proteins. Effect on dough rheological properties and bread
 445 characteristic. *Food Hydrocolloids*, 32(2), 213–220.

446

448

449

454 **Fig. 1. Effect of the increasing amount of gluten or β CC proteins on the hydration**
 455 **properties of corn starch.** Legends: circles: moisture content (%); diamonds: water binding
 456 capacity (g water/g solid). Filled symbols are values of gluten:starch blends; open symbols
 457 are values of β CC:starch blends. Error bars represent the standard deviation of at least three
 458 replicates.

Fig. 2. Longitudinal section and crumb image analysis of starch:gluten and starch:β-conglycinin concentrate (βCC) yeast leavened breads. Breads were made with two different hydrations (optimum and at constant consistency). Percentages indicated the amount of proteins added to the starch:protein blends. Scale bar in the bread section was 5 mm and in the crumb image analysis 1 mm.

459 **Table 1. Functional properties of β -conglycinin concentrate (β CC) and vital gluten^a**

Functionality	β CC				Vital gluten			
WBC (g water/g sample)	0.98	±	0.03	b	1.45	±	0.02	a
WHC (g water/g sample)	1.60	±	0.21	b	3.92	±	0.11	a
Water retention index (%)	61.3				37.0			
SV (ml/g sample)	2.12	±	0.20	b	4.61	±	0.14	a
OBC (g oil/g sample)	1.10	±	0.02	a	0.82	±	0.03	b
EAI (m ² /g)	22.86	±	3.39	a	3.04	±	0.24	b
ESI (h)	1.22	±	0.01	b	1.33	±	0.04	a
Foaming capacity (%)	22.85	±	4.67	b	49.33	±	2.31	a
Foaming stability (%)	17.89	±	3.85	b	34.67	±	2.31	a

460 ^aValues are means and standard deviation of at least three replicates; means with different letters within the same parameter differ
461 significantly ($P < 0.05$). WBC= Water binding capacity; WHC= Water holding capacity; SV= Swelling volume; OBC= Oil binding
462 capacity; EAI= Emulsifying activity index; ESI= Emulsifying stability index.

463

464

465 **Table 2. Physical characteristics of starch:gluten and starch:β-conglycinin concentrate (βCC) yeast leavened breads**

Hydration	Protein	Protein concentration (%)	Water absorption (g water/100 g flour)	Moisture (%)	Color parameters			Bread 2D Area		Bread perimeter	
					<i>L</i> *	<i>a</i> *	<i>b</i> *	(mm ²)		(mm)	
Optimum	Gluten	5%	75	43.3 ± 0.7 d	81.4 ± 1.8 cd	-1.8 ± 0.2 cd	9.3 ± 0.8 de	348 ± 31	bc	96.7 ± 11.2	c
		10%	77	44.9 ± 0.5 c	78.5 ± 3.1 de	-1.6 ± 0.2 bc	12.8 ± 1.9 bc	333 ± 5	c	96.9 ± 7.8	c
		15%	78	45.0 ± 0.6 c	77.8 ± 1.5 e	-1.2 ± 0.2 a	15.1 ± 1.9 ab	329 ± 24	c	106.8 ± 14.0	bc
	βCC	5%	81	46.0 ± 0.1 ab	87.8 ± 0.6 a	-2.1 ± 0.0 ef	8.1 ± 0.2 e	405 ± 22	a	120.3 ± 7.9	ab
		10%	83	46.3 ± 0.2 ab	87.8 ± 1.0 a	-2.2 ± 0.1 ef	9.8 ± 0.6 de	411 ± 14	a	120.5 ± 8.1	ab
		15%	84	46.9 ± 0.2 a	83.7 ± 1.1 bc	-2.2 ± 0.1 f	11.1 ± 0.9 cd	424 ± 22	a	124.9 ± 9.4	ab
Consistency	Gluten	5%	72	42.5 ± 0.6 d	81.8 ± 1.4 cd	-1.9 ± 0.2 de	10.8 ± 2.0 cd	369 ± 13	b	108.7 ± 7.8	bc
		10%	77	44.9 ± 0.5 c	78.5 ± 3.1 de	-1.6 ± 0.2 bc	12.8 ± 1.9 bc	333 ± 5	c	96.9 ± 7.8	c
		15%	82	45.9 ± 0.3 bc	72.5 ± 1.1 f	-1.3 ± 0.2 ab	15.6 ± 1.7 a	337 ± 9	c	99.6 ± 12.3	c
	βCC	5%	71	42.3 ± 0.1 d	89.5 ± 1.4 a	-2.2 ± 0.1 f	9.1 ± 0.7 de	400 ± 11	a	120.6 ± 5.1	ab
		10%	70	42.4 ± 0.4 d	86.7 ± 0.8 ab	-2.3 ± 0.1 f	10.4 ± 0.5 c-e	424 ± 23	a	138.6 ± 21.2	a
		15%	68	42.6 ± 0.1 d	81.2 ± 1.6 cd	-2.4 ± 0.0 f	12.7 ± 0.5 bc	400 ± 22	a	129.2 ± 13.3	a
<i>P</i> -value											
Hydration			0.000	0.000	0.021	0.060	0.009	0.610		0.067	
Protein			0.408	0.964	0.000	0.000	0.000	0.000		0.000	
Protein concentration			0.005	0.003	0.000	0.012	0.000	0.354		0.507	

466 Means with different letters in the same column are significantly different ($P < 0.05$).

Table 3. Textural parameters and crumb characteristics of starch:gluten and starch:β-conglycinin concentrate (βCC) yeast leavened breads

Hydration	Protein	Protein concentration (%)	Texture parameters					Cell density (u/cm ²)	Crumb porosity (%)	Mean cell area (mm ²)
			Hardness (g)	Springiness	Cohesiveness	Chewiness (g)	Resilience			
Optimum	Gluten	5%	371 ± 77 ab	0.97 ± 0.08 b	0.61 ± 0.20 bc	215 ± 70 cd	0.41 ± 0.16 ab	24 ± 7 c	37 ± 10 ab	1.67 ± 0.76 a
		10%	306 ± 46 bc	1.00 ± 0.01 a	0.62 ± 0.05 bc	189 ± 24 cd	0.42 ± 0.04 ab	23 ± 4 c	41 ± 5 a	1.80 ± 0.34 a
		15%	378 ± 47 ab	1.00 ± 0.00 a	0.74 ± 0.04 ab	280 ± 35 ab	0.50 ± 0.04 a	33 ± 15 c	24 ± 8 c	0.81 ± 0.37 b
	βCC	5%	234 ± 16 c	1.00 ± 0.00 a	0.74 ± 0.07 ab	172 ± 12 d	0.41 ± 0.06 ab	227 ± 47 a	25 ± 6 c	0.11 ± 0.01 c
		10%	245 ± 31 c	1.01 ± 0.01 a	0.73 ± 0.06 ab	178 ± 12 cd	0.39 ± 0.04 b	245 ± 30 a	28 ± 3 bc	0.11 ± 0.01 c
		15%	302 ± 16 bc	1.01 ± 0.01 a	0.77 ± 0.04 a	234 ± 16 bc	0.40 ± 0.03 ab	250 ± 46 a	28 ± 3 bc	0.12 ± 0.02 c
Consistency	Gluten	5%	372 ± 112 ab	1.00 ± 0.01 a	0.59 ± 0.08 c	219 ± 65 cd	0.39 ± 0.07 ab	22 ± 9 c	24 ± 9 c	1.19 ± 0.45 ab
		10%	306 ± 46 bc	1.00 ± 0.01 a	0.62 ± 0.05 bc	189 ± 24 cd	0.42 ± 0.04 ab	23 ± 4 c	41 ± 5 a	1.80 ± 0.34 a
		15%	259 ± 29 c	1.01 ± 0.01 a	0.65 ± 0.08 a-c	170 ± 25 d	0.41 ± 0.08 ab	17 ± 5 c	28 ± 12 bc	1.69 ± 0.69 a
	βCC	5%	262 ± 26 c	1.00 ± 0.01 a	0.78 ± 0.06 a	206 ± 22 cd	0.47 ± 0.04 ab	242 ± 21 a	28 ± 1 bc	0.12 ± 0.01 c
		10%	311 ± 54 bc	1.00 ± 0.00 a	0.72 ± 0.07 a-c	224 ± 41 b-d	0.39 ± 0.05 ab	240 ± 14 a	28 ± 2 bc	0.12 ± 0.01 c
		15%	432 ± 39 a	1.00 ± 0.01 a	0.77 ± 0.04 a	331 ± 20 a	0.42 ± 0.02 ab	183 ± 17 b	26 ± 3 bc	0.14 ± 0.01 c
<i>P</i> -value										
Hydration			0.242	0.306	0.522	0.258	0.777	0.025	0.527	0.391
Protein			0.023	0.159	0.000	0.185	0.289	0.000	0.001	0.000
Protein concentration (%)			0.021	0.166	0.010	0.000	0.399	0.205	0.000	0.044

Means with different letters in the same column are significantly different ($P < 0.05$).