



## Analytical Methods

# Improvement of gluten-free bread properties by the incorporation of bovine plasma proteins and different saccharides into the matrix



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## ABSTRACT

The aim of this work was to improve the quality of gluten-free bread, incorporating plasma bovine proteins concentrated by ultrafiltration and freeze-dried with saccharides (inulin and sucrose). The influence of these compounds on textural properties and final bread quality was assessed. The textural studies revealed that with the addition of proteins and inulin, homogeneous and smaller air cells were achieved improving the textural properties while the bread hardness was comparable with breads with gluten. The volume of gluten-free breads increased with increasing proteins and inulin concentrations, reaching a maximum at a protein concentration of 3.5% (w/w). The addition of the enhancers improved moisture retention of the loaves after cooking and an increase of lightness of crumb with respect to the control was observed. The sensory analysis found no statistically significant difference in sensory attributes evaluated with respect to the control, so these ingredients do not negatively affect the organoleptic properties of bread.

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## 1. Introduction

In the production of breads, gluten is essential to form the strong protein network required for retention of gas produced during fermentation, and the desired volume and structure of the breads (Demirkesen, Mert, Sumnu, & Sahin, 2010). However, there is an increasing interest in gluten-free products with an increase in numbers of celiac patients. Celiac disease is a disorder of the intestine caused by the intake of gluten as reviewed by Marsh (1992) and Fasano and Catassi (2001). Gluten ingestion causes inflammation of the small intestine, leading to the mal-absorption of important nutrients including iron, folate, calcium and fat-soluble vitamins, and culminates in intestinal mucosal damage (Holtmeier & Caspary, 2006). Gliadin has been determined to be the pathogenic factor responsible meaning the only effective method of treatment has been strict avoidance of gluten, which, in time, allows mucosal recovery (Fasano & Catassi, 2001; Holtmeier & Caspary, 2006).

Gluten is the main structure-forming protein in flour, and is responsible for the elastic characteristics of dough contributing to the appearance and crumb structure of many baked products.

Thus, its removal causes problems for bakers and, currently, many gluten-free products available in the market are of low quality, exhibiting poor mouth-feel and flavor (Gallagher, Gormley, & Arendt, 2004; Torbica, Hadnadev, & Dapcevic, 2010). Rice flour is one of the most suitable cereal flours for preparing gluten-free products because of its bland taste, white colour, ease of digestion and hypoallergenic. It also has very low levels of protein, sodium, fat, fibre and high amount of easily digested carbohydrates (Demirkesen et al., 2010). However, the relatively small amounts of protein mean it is difficult to obtain an acceptable yeast-leavened product, such as bread, because of the absence of the network necessary to hold carbon dioxide produced during proofing (Blanco, Ronda, Pérez, & Pando, 2011). Bread has a short shelf-life mostly due to the loss of softness, moisture and flavor. The absence of gluten often results in a liquid batter rather than dough, producing bread with a crumbly texture, poor colour and other post-baking quality defects. Bread dough without gluten can only retain gas if another hydrocolloid replaces the gluten (Torbica et al., 2010) and it is necessary to use emulsifiers, enzymes or dairy products, together with rice flour, to achieve the desired viscoelastic mixture (Demirkesen et al., 2010).

There is, therefore, an urgent need to investigate potential bread-making ingredients, additives and technological aids to develop high-quality gluten-free products at a reasonable price (Blanco et al., 2011). Thus, in recent years, the incorporation of

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starches, dairy proteins and hydrocolloids in gluten-free flour (rice, and corn flour) have been investigated in order to mimic the properties of gluten and improve structure, mouth-feel, acceptability and shelf-life (Lazaridou, Duta, Papageorgiou, Belc, & Biliaderis, 2007; Blanco et al., 2011). But, the supplementation of gluten-free bread dough with additives is difficult because its structure is weaker than wheat bread dough, which contains gluten. The hydrocolloids used as a substitute for gluten seems to be the best alternative for gas retention and provide similar rheological properties to wheat dough (Blanco et al., 2011; Demirkesen et al., 2010). Hydrocolloids are also able to modify starch gelatinization, and to extend the overall quality of the product over time (Rosell, Rojas, & de Barber, 2001). It is known that proteins are good hydrocolloids and they have been used in different formulations, such as whey protein concentrate in unleavened flat bread (parotta) (Indrani, Prabhasankar, Rajiv, & Venkateswara Rao, 2007); soybean flour in gluten-free bread (Ribotta et al., 2004); soy protein isolate, pea protein isolate, egg white protein and casein in rice based gluten free muffins (Matos, Sanz, & Rosell, 2014). Traditionally, bovine plasma protein has not been used in bakery products because plasma proteins have poor sensory qualities. In previous studies, a plasma protein concentrate was obtained by ultrafiltration and freeze-drying, using polysaccharides as a protective agent. The powdered product was easy to use and improved functional and sensory properties (Rodríguez Furlán, Pérez Padilla, & Campderrós, 2010b).

Enrichment of gluten-free bread with dietary fibres has also proved to be necessary since it has been reported that celiac patients have, generally, a low intake of fibres attributed to their gluten-free diet (Lazaridou et al., 2007). It is known that fibres increase calcium absorption, and promote the growth of intestinal bacteria (Griffin, Hicks, Heaney, & Abrams, 2003; Johnson, 2013). In this sense, the oligosaccharide inulin, which behaves as dietary fibre is considered a prebiotic (Rubel, Pérez, Genovese, & Manrique, 2014). This compound was employed in bread with gluten formulations by Pointot et al. (2010).

Therefore, formulations enriched in fibre such as inulin could be developed to improve the nutritional quality of gluten-free bread. Also, the incorporation of inulin may improve the final properties of the gluten-free bread (texture, volume, etc.) as a result of increased water holding capacity, emulsification, etc. (Rodríguez Furlán, Pérez Padilla, & Campderrós, 2010a). Previous studies (Skendi, Biliaderis, Papageorgiou, & Izydorczyk, 2010) demonstrated the addition of fibre to wheat flour had negative effects, specifically weakening the crumb cell structure by the dilution/weakening of the wheat gluten network and impairing gas retention, reducing the volume and changing the texture and appearance of the final product. During storage, bread becomes stale because structural deterioration takes place due to starch recrystallization and loss of moisture (Mandala, Karabela, & Kostaropoulos, 2007).

The resistance of the bread crumb to deformation is referred to as hardness, and is considered an important indication of staling. Because of the role of gluten in the prevention of staling, these problems are more prevalent in gluten-free breads. Mechanical compression tests showing the stress–strain relationship between cell wall elasticity, rigidity and susceptibility to fracture have been used to measure staleness in spongy bakery products (Ahlborn, Pike, Hendrix, Hess, & Huber, 2005). Evaluating the mechanical properties of bread crumb is important not only for staling/shelf-life, but also for assessing the effects of changes in dough ingredients and processing conditions.

In the literature, there are no studies investigating the effect of plasma bovine proteins in combination with saccharides on the properties of bread gluten-free breads. Therefore, the aim of the present study was to use bovine plasma proteins and saccharides

(sucrose and inulin) in gluten-free formulations and examine their effects on dough texture properties, as well as on quality parameters (volume, hardness and sensory analysis) on the end-product. The effect of staling during storage on quality attributes was also assessed. Furthermore, the influence of these hydrocolloids on the quality properties of gluten-free breads was evaluated using sensory, mechanical, and microscopic techniques.

## 2. Materials and methods

### 2.1. Raw materials

Enhancing agents used in the formulation of gluten-free bread were: bovine plasma protein unprocessed (P) and processed by ultrafiltration and freeze-drying operations (PUF) with the addition of sucrose (PUFS) or inulin (PUFI) as lyo-protective agents (Rodríguez Furlán et al., 2010a, 2010b). The compositions of these concentrates are described in Supplementary Table 1.

### 2.2. Bread formulations

The basic bread formula per 100 g of gluten-free flour (rice) was: 60.0 g water, 8 g sunflower oil, 1.5 g sugar, 1.3 g salt and 2.0 g powdered yeast species *Saccharomyces cerevisiae*. The enhancing agents (P; PUF; PUFS or PUFI) were added as a function of their protein content to reach concentrations of 0.5% (w/w), 1.5% (w/w), 2.5% (w/w) and 3.5% (w/w) in each sample. A control sample without the addition of enhancers was also baked.

### 2.3. Breadmaking process

The experiment was carried out following the method described by Lazaridou et al. (2007), Torbica et al. (2010) and Mandala et al. (2007) with several modifications.

Firstly, yeast was dissolved in water at  $35 \pm 1$  °C. This dispersion was added to dry ingredients and sunflower oil and then was mixed with a 5-speed mixer (average mixing speed was 100 rpm) (Santini, Argentina) for 5 min. Approximately 150 g of dough was poured into aluminium rectangular moulds (90 cm<sup>2</sup>). Samples were allowed to ferment for 60 min at 25 °C. Baking was carried out in an air electric oven at 200 °C for 20 min (convection/fan). After baking, the breads were removed from the moulds and cooled at room temperature for 30–40 min. Samples were packed in hermetically-sealed bags (Ziploc Brand) and stored at ambient temperature for 3 days.

Each formulation was replicated at least three times, and all the analyses were carried out independently in triplicate.

### 2.4. Bread quality evaluation

For the baking industry, the benefits expected of enhancers are improved dough handling including greater dough strength, water absorption, crumb structure, brightness of crumb, uniformity in cell size (increased), slicing characteristics of bread, symmetry, gas retention, oven-spring, loaf volume (increased), shelf-life of bread (longer) (Stampfli & Nerden, 1995).

To evaluate the effects of bovine plasma proteins and polysaccharides on gluten free formulations, the following studies were performed:

#### 2.4.1. Moisture content

Moisture content was measured by weighing samples before and after drying for 5 h at 103 °C in a lab dryer. The results are expressed as percent of water on a wet basis (w/w) (Fontanet, Davidou, Dacremont, & Le Meste, 1997).

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