LWT - Food Science and Technology 53 (2013) 346–354

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Protein enrichment and its effects on gluten-free bread characteristics

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ARTICLE INFO

Article history:
Received 1 September 2010
Received in revised form
30 January 2013
Accepted 4 February 2013

Keywords:
Rice
Rheology
Bread
Proteins
Transglutaminase

ABSTRACT

Protein-enriched, rice-based flours, composed of casein and albumin protein isolates as well as transglutaminase for reinforcing protein networks, were designed for optimising a gluten-free formulation suitable for breadmaking. Experimental design resulted in composite protein-enriched blends with different pasting, mechanical and surface-related textural properties. The presence of transglutaminase resulted in a significant decrease of all the protein fractions, which suggested protein crosslinking. Protein isolates significantly ($p < 0.05$) modified the gelatinisation and gelling behaviour of the flour, reducing both its peak viscosity and final viscosity. The textural properties were primarily affected by the addition of casein and transglutaminase. The design allowed the determination of the optimum formulation for obtaining the highest specific volume of gluten-free baked product with the lowest crumb hardness by combining transglutaminase (1.35 U of enzyme/g of rice flour protein), egg albumin (0.67 g/100 g of flour) and casein (0.67 g/100 g of flour). The use of albumin and casein protein isolates and transglutaminase constitutes a promising approach to producing protein-enriched blends for making fermented, gluten-free products.

1. Introduction

Celiac disease is one of the most common lifelong disorders worldwide, with an estimated mean prevalence of 1% in the general population (Catassi & Fasano, 2008). Individuals who have this disease (celiacs) are unable to consume some of the most common products on the market, including breads and other food products made with wheat flour. Bread is a highly consumed product and usually contributes significantly to caloric intake; however, gluten-free bread suitable for consumption by coeliacs is difficult to find because its supply is restricted to speciality shops.

Recently, rice flour has been widely proposed as an alternative for making gluten-free breads due to its hypoallergenic proteins, soft taste and white colour (Clerici, Airoldi, & El-Dash, 2009; Rosell, Brites, Pérez, & Gularte, 2007); however, rice-based bread has low volume and hard crumb (Gujral, Guardiola, Carbonell, & Rosell, 2003; Gujral & Rosell, 2004a,2004b). The use of other cereal sources in breadmaking can increase the variability of products and can also improve nutritional characteristics (Flander, Salmenkallio-Marttila, Suortti, & Autio, 2007; Renzetti, Courtin, Delcour, & Arendt, 2010); however, gluten-free baked products are only acceptable when their sensory characteristics are similar to those of wheat flour yeast bread (Gallagher, Gormley, & Arendt, 2004; Marco & Rosell, 2008a). Gluten-free breads are usually characterised by low quality and exhibit dry, crumbling crumb, poor mouthfeel and dry flavour (Gallagher, Gormley, & Arendt, 2003a, 2004).

To improve the quality of gluten-free breads, ingredients like modified starch, hydrocolloids, enzymes and proteins of different sources have been proposed (Gujral et al., 2003; Gujral & Rosell, 2004a,2004b; Moore, Heinbockel, Dockery, Ulmer, & Arendt, 2006). A common practice in food processing is the incorporation of protein ingredients in product formulation to increase product qualities such as flavour, texture and storage stability (Bonet, Bonet, Blaszczak, & Rosell, 2006). The addition of dairy and egg ingredients in breadmaking has been widely used because their proteins are highly functional and can be readily incorporated into the dough. These ingredients can be used in bread for both nutritional and functional benefits. The use of dairy powder in gluten-free baked product formulations has resulted in improved volume as well as better appearance and sensory aspects of the loaves (Gallagher, Kundel, Gormley, & Arendt, 2003b). According to Stathopoulos (2008), the most used ingredients in gluten-free baked product formulations are caseinates, skim milk powder, dry milk, whey

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0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.02.005

protein concentrate and milk protein isolate. Demirkesen, Mert, Sumnu & Serpil Sahin (2010) also reported that it is necessary to use a gum, emulsifier, enzyme or dairy product together with rice flour for the desired viscoelastic mixture.

Nevertheless, proteins often do not meet the requirements for food processing, and additional modifications are necessary. The use of enzymes as processing aids in breadmaking is a frequent method for improving functional properties to promote networks and improve dough baking characteristics (Bonet et al., 2006; Gujral & Rosell, 2004a,2004b; Marco & Rosell, 2008a; Renzetti, Bello, & Arendt, 2008). An enzyme that has received extensive attention for its ability to crosslink proteins is transglutaminase. This enzyme catalyses an acyl–transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins (Motoki & Seguro, 1998). Compared to other cereals, rice has high lysine content, so it potentially has more substrate for enzyme action. According to Renzetti et al. (2008), rice and wheat are the best substrates for this enzyme action when comparing different flours.

Gerrard and Sutton (2005) and Cabrera-Chávez and Calderón de la Barca (2008) published a research into the molecular mechanism of coeliac disease that suggests the disturbing possibility that transglutaminase in baked products may act upon gliadin proteins in dough to generate the epitope associated with the coeliac response and considered not safe the use of transglutaminase in cereal processing. But, the researchers have not found anything about this mechanism in rice-based products, only in wheat, barley, rye and oats.

A major task of modern technology is to generate new structures with characteristics that please the consumer while using a limited range of ingredients. Proteins are one of the main classes of molecules available for conferring textural attributes, and the cross-linking and aggregation of protein molecules has been cited as one of the most important mechanisms for engineering food structures with desirable mechanical properties (Gerrard, 2002).

Several studies have evaluated the influence of transglutaminase in gluten-free baked product formulations (Collar & Bollaín, 2004; Gujral & Rosell, 2004a,2004b; Marco, Pérez, León, & Rosell, 2008; Marco & Rosell, 2008c; Moore et al., 2006; Renzetti et al., 2008). However, relatively little work has been done to investigate the effect of interactions between transglutaminase, albumin and casein on the technological and textural properties of bread with rice flour. transglutaminase reacts differently with various protein sources and at different addition levels; therefore, the aim of this study was to design a protein-enriched, rice-based, gluten-free baked product in the presence of transglutaminase to favour the formation of a protein network that improves crumb textural properties. With this goal, an experimental design was used with different levels of transglutaminase and two different sources of proteins: albumin and casein.

2. Materials and methods

A rice cultivar (IRGA 417) was grown in southern Brazil in 2007. Samples were harvested at a 20 g/100 g moisture content, dried to 12 g/100 g in a pilot dryer (at 38 °C) (Stationary Model, MV-Máquinas Vitória, Pelotas, Brazil) and stored in a closed bag in a cold room maintained at 17 °C ± 3 and 65 g/100 g ± 5 relative humidity prior to milling. Rice kernels were dehusked and polished using a Zaccaria rice test-machine (Industrias Machina Zaccaria S/A, São Paulo, Brazil). Polished samples were then ground (Hammer Mill, Model 3100, Perten Instruments, Hägersten, Sweden) and sieved (sieve openings 70 μm). Flour was stored in plastic bags at 17 °C ± 3 and 65 g/100 g ± 5 relative humidity. The rice flour chemical composition were 12.9 g/100 g moisture, 7.3 g/100 g protein (by nitrogen determination using the Kjeldahl method – N × 5.95), 0.8 g/100 g ash (550 °C/5 h), 0.6 g/100 g crude fat (Soxhlet machine – solvent ether) using methods of AOAC (1995) and 31.6g/100 g amylase (Gilbert & Spragg, 1964).

The food grade microbial transglutaminase was Activa STG-M (27 U/g) from Ajinomoto Co. Bread. The improver was Soft'r Alpaga obtained from Puratos – São Paulo, Brazil, composed by corn starch, stearoyl-2-lactil sodium lactate, ascorbic acid and alpha amylase. The casein was from Synth (São Paulo, Brazil), and egg albumin was from Neo Nutri (Poços de Caldas, Brazil). Xanthan gum 200 (water relative density = 1/solution at 25 of 1.0068/20°; pH at 1% = 6–8; particle size 92% pass in a 0.075 mm) was from Arinos química, Brazil. The other ingredients for baked product production were obtained in a local market.

2.1. Experimental design

Rice flour was replaced by combinations of transglutaminase, albumin and casein following a central composite design of three factors (transglutaminase, albumin and casein), and a five-level pattern was used for sampling (Table 1). Design factors (quantitative independent factors) that were tested at five levels (−α, −1, 0, +1, +α) included transglutaminase (0–12 U/g of protein), albumin (0–6 g/100 g of flour) and casein (0–6 g/100 g of flour). The model resulted in 16 different combinations of composite flours.

2.2. Protein quantification

Protein fractions were extracted following a sequential extraction using various solvents according to a method described by Marco et al. (2008) with modifications. Dough was prepared by mixing 2 g of solid sample (according to the experimental design) with 2 ml of water and placed in a centrifuge tube. An albumin-globulin extraction was conducted by adding 10 ml of 5 ml/100 ml (w/v) NaCl to dough. The suspension was homogenised for 5 min in a shaker (Shaker Certomat MO – B. Braun Biotech International, Melsungen, Germany) and was centrifuged at 5500 g for 10 min. After albumin-globulin extraction, the alcohol-soluble fraction was extracted from the residue by adding 10 ml of 50 ml/100 ml (v/v) 1-propanol, following the same procedure as described for the albumin-globulins. Insoluble proteins were extracted with 10 ml of 0.1 mol equi/L NaOH containing 0.5 ml/100 ml (w/v) SDS and 0.6 ml/100 ml (v/v) β-mercaptoethanol (ME). Each extraction was repeated twice to increase the protein extraction. The supernatants were collected, and the protein contents in both the mixed flour and the fractions were determined following the micro-Kjeldahl method (n° 46-13 of AACC, 1995) using N × 5.95 as a protein conversion factor. The protein in the final residue was determined by subtracting the albumin-globulin, alcohol-soluble and insoluble fractions from 100%.

Table 1
Central composite design for sampling.

Factors	Levels				
	− α	−1	0	+1	+ α
Transglutaminase (U/g of rice flour protein)	0	1.35	6	10.65	12
Albumin (g/100 g of rice flour)	0	0.67	3	5.32	6
Casein (g/100 g of rice flour)	0	0.67	3	5.32	6

Concentrations are based on 100 g of flour.