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A study on development of Gluten free pasta and its biochemical and immunological validation

S. Susanna, P. Prabhasankar*

Flour Milling, Baking and Confectionery Technology Department, CSIR-Central Food Technological Research Institute, Mysore 570020, India

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ABSTRACT

The present study is aimed at the development of Gluten free pasta with enriched protein content, and evaluation of product quality and its allergenicity. The pasta was developed using high protein flours such as Soya flour, Channa flour, Sorghum flour and Whey Protein Concentrate (WPC) along with gums. Prepared pasta was analysed for its quality characteristics and was subjected to immunological tests such as ELISA and Dot-Blot. Cooking quality of the pasta revealed that Gluten free pasta had a little higher cooking loss than that of *Triticum durum* pasta (control) and with addition of gums the starch loss was decreased. Gluten free Pasta was comparable to control in all other quality parameters. The amylose content of all pasta ranged from 2.14 to 3.1 g/100 g which was lower than the control (3.9 g/100 g). It also showed less starch digestibility and high protein digestibility. SDS-PAGE pattern showed distinct protein profile of Gluten free blends where bands corresponding to wheat allergen profile were not observed. Dot-Blot and ELISA confirmed that antibodies developed against gliadin did not recognize these proteins. Hence it can be concluded that the developed Gluten free pasta with high protein can be consumed by individuals who exhibit allergic symptoms to wheat gluten.

1. Introduction

Gluten is the main structure forming protein in the flour and responsible for the elastic characteristics of the dough (Gallagher, Gormley, & Arendt, 2004). The reason for this is the extensive network of intermolecular disulfide bonds arising from cysteine residues of glutenins (Cornell & Hoveling, 1998). On a dry matter basis, 100 g of gluten contain 75–86 g of protein, 10 g of starch and 5 g of fat (Cornell & Hoveling, 1998; Gallagher et al., 2004) held strongly within the gluten protein matrix. Gluten comprises the protein fractions glutenins and gliadins. The glutenin is a rough rubbery mass when fully hydrated, while gliadin produces viscous, fluid mass on hydration. Hence gluten exhibits combined properties of these two components (Gallagher et al., 2004). These gluten proteins are also known as wheat prolamins. Glaidins are monomeric proteins, accounting for about 30 g/100 g of the total proteins (of the wheat kernel) and can be classified into α, β, γ and ω-gliadins. Glutenins are very high molecular weight polymeric proteins in which, the high molecular subunits (HMG) and low molecular subunits (LMG) are linked together by disulfide (SS) bonds (Zorzi, Curioni, Simonato, Giannattasio, & Pasini, 2007).

Some individuals exhibit immune sensitivity to both soluble and insoluble fractions of the gluten proteins. Gluten enteropathy or Celiac disease (CD) is caused by an inappropriate immune response to dietary wheat gluten or similar proteins of barley or rye (Olexova, Dovicovicova, Svec, Siekel, & Kuchta, 2004). This is a genetic disorder characterized by small bowel villous atrophy and crypt hyperplasia. In symptomatic patients a strict Gluten free diet is highly effective in alleviating symptoms and may prevent long-term complication (Gallagher et al., 2004; Mustalahti et al., 2002). Persons with CD are unable to consume some of the most common products including breads, baked goods and other food products made with wheat flour (Moore, Schober, Dockery, & Arendt, 2004). Foods that are not included in Gluten free diets are: 1. Any bread, cereal or other food made with wheat, rye, barley, triticale, dinkel, kamut and oat flour or ingredients; 2. Any processed foods that contain wheat and gluten derivatives as thickeners and fillers; 3. Medications that use gluten as pill or tablet binders (Gallagher et al., 2004).

As the prevalence of CD is increasing in worldwide, in recent years there has been increase in consumer interest in wheat free foods driven part by an increasing awareness of the CD (Moore et al., 2004). The formulation of Gluten free (GF) bakery products presents a formidable challenge to food technologists. In recent years,

* Corresponding author. Tel.: +91 821 2517730; fax: +91 821 2517233.
E-mail addresses: prabhasankar@lycos.com, psankar@cftri.res.in (P. Prabhasankar).

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researchers have been working significantly more on development of Gluten free products involving a diverse approach which has included the use of starches, dairy products, hydrocolloids, non-gluten proteins, prebiotics and combination thereof, to replace gluten and to improve quality of the products. Many studies have been performed to develop and investigate the product quality of GF foods. Few studies have shown the application of soy flour, corn starch, buckwheat flour in the manufacture of Gluten free breads (Gallagher et al., 2004; Moore et al., 2004). Similarly incorporation of dairy ingredients such as sodium caseinate, milk protein isolate has long been established in the baking industry. Arendt, O′ Briend, Schober, Gormely, and Gallagher (2002) studied the effects of rice, corn, soya, millet, buckwheat and potato starch, in combination with different fat sources on the formulation of Gluten free biscuits. Nowadays Gluten free bakery products have become available in the market, prepared out of non-wheat flours such as rice, maize, soya, guar and amaranth. Haung, Knight, and Goad (2001) studied the different levels of gums on the quality of GF pasta. Similarly, Wang, Bherud, Sosulski, and Tyler (1999) studied the use of only pea flour on the quality of GF pasta. This special category of foods, which are designated as "Gluten free", should meet specific requirements regarding the content of the gluten. The current standards of the Codex Alimentary Commission for Gluten free diet go back to 1981. The report of the Codex Alimentarius (2003) indicates that the consumption of prolamines by celiac patients should not exceed 10 mg per day. However contamination of these food products by gluten containing cereals may take place at the stage of flour production or at the stage of production of the final product (Olexova et al., 2004). Recent research is focused on the development of immunological methods with high sensitivity and specificity (Collin, Thorell, Kaukinen, & Maki, 2004). In this regard many researchers have developed ELISA tests to detect gluten content in Gluten free foods. Chirdo, Anon, and Fossti (1995) used a polyclonal serum obtained against total gliadins, which recognize α, β, γ and ω-gliadins and the HMG glutenin subunits of wheat as well as the prolamins of barley, rye and triticale. Scientists have developed sandwich ELISA based on a cocktail of antibodies using a combination of two monoclonal antibodies raised against secalins and gliadins. Similarly a more original protocol using a MAb against a peptide derived from hydrolysis of α-gliadin by pepsin and trypsin and corresponding to its N-terminal sequence is also developed (Papini, Nicolas, & Popineau, 1999).

And so far very few studies only, have reported on the immunological evaluation of the developed Gluten free pasta. With this background, the present study aims at the development of the Gluten free pasta with enriched protein content mainly covering evaluation of product quality and its allergenicity to test its suitability for consumption by CD patients.

2. Materials and methods

2.1. Raw material

Soy flour, Channa flour, Sorghum flour and Whey Protein Concentrate (WPC) were obtained from local market. Soy flour was defatted using Hexane and toasted at 70 °C for 2 h. Channa flour, Sorghum flour were also toasted at 70 °C for 2 h. Gliadin was procured from SIGMA Chemicals, USA. Molecular marker for SDS-PAGE, and Goat anti-rabbit IgG-alkaline phosphatase conjugate were procured from Bangalore Genie, India. All other chemicals used were of analytical grade.

2.2. Pasta processing

After initial trials the formulation was fixed as toasted Soy flour, Channa Flour, Sorghum flour and WPC with Xanthan gum, Guar Gum, and Hydroxypropylmethylcellulose (HPMC) as additives in different combination per 100 g of formulation. Control pasta was prepared with only *Triticum durum* flour (100 g). Details of the pasta formulation are provided in Table 1. Flour was mixed in Hobart mixer (model N-50, Ontario, Canada) using low speed of $0.46\times$ *g* with optimal 30 ml–40 ml of water/100 g of flour and mixed for 7–10 min. Mixed samples were extruded using La Moniferrino (Model Dolly, Asti, Italy) pasta extruder. The samples were dried at 70 °C for 3 h in Sakar Drier (Shirsat Electronics, Mumbai) and packed in polypropylene covers for further use.

2.3. Proximate analysis

Proximate compositions of all the flours used in the pasta preparation were evaluated using standard methods. Moisture and protein content were estimated by AACC approved method (2000).

2.4. Cooking quality

Optimization of cooking time and determination of cooking loss of pasta samples were determined according to AACC approved method (2000). Twenty-five grams of pasta samples were cooked with 250 ml of boiling water for its optimum cooking time. Optimum cooking time was considered when core of the pasta gets completely hydrated. Cooked pasta were drained and weighed when cooled. The drained gruel was measured and 20 ml of gruel in triplicates were transferred to pre-weighed glass petridish to estimate the cooking loss of the pasta.

2.5. Colour

Colour of the raw and cooked pasta was measured using Hunter Colour measurement (Colour measuring Labscan XE system, USA). Colour readings were expressed by Hunter values for L^*, a^* and b^*. L^* values measure black to white (0–100), a^* values measure redness when positive and b^* values measure yellowness when positive.

2.6. Determination of pasta firmness

Firmness of cooked pasta was measured according to Walsh, Youngs, and Gills (1970) using a universal texture measuring system (Lloyds Instruments, LR-5, Hampshire, UK). The cooked pasta strands were sheared at a 90° angle. The shear was performed at a cross-head speed of 50 mm/min and load cell of 10 kg. The gram force required to shear the pasta was measured in multiple determinations and the average value reported.

Table 1
Formulation for pasta processing.

Flours	Ix	IIH	IIIGG	IVXHG	VC	VI	NC1	NC2
Toasted soy flour (g)	29.85	29.85	29.85	29.77	30	31.08	29.62	39.50
Toasted channa flour (g)	34.82	34.82	34.82	34.74	35	36.29	34.60	24.70
Toasted sorghum flour (g)	29.85	29.85	29.85	29.77	30	31.08	29.62	29.62
Whey Protein Concentrate (g)	4.98	4.98	4.98	4.97	5	–	4.92	4.94
Xanthan gum (g)	0.5	–	–	0.25	–	1.55	0.5	0.5
Guar Gum (g)	–		0.5	0.25	–	–	0.24	0.24
HPMC(g)	–	0.5	–	0.25	–	–	0.5	0.5

Ix – Gluten Free (GF) pasta with Xanthan gum, IIH – GF pasta with HPMC (hydroxypropylmethylcellulose), IIIGG – GF pasta with Guar Gum, IVXHG – GF pasta with Xanthan gum, HPMC and Guar Gum, VC – GF pasta without any additive, VI – GF pasta without WPC, NC1 – new combination 1 and NC2 – new combination 2.