Journal of Cereal Science 59 (2014) 79–87

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Manufacture and characterization of pasta made with wheat flour rendered gluten-free using fungal proteases and selected sourdough lactic acid bacteria

José Antonio Curiel [a], Rossana Coda [a,b], Antonio Limitone [c], Kati Katina [b], Mari Raulio [b], Giammaria Giuliani [c], Carlo Giuseppe Rizzello [a,*], Marco Gobbetti [a]

[a] Department of Soil, Plant and Food Sciences, University of Bari, 70126 Bari, Italy
[b] VTT, Technical Research Centre of Finland, 02150 Espoo, Finland
[c] Giuliani S.p.A., Milano 20129, Italy

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form
11 September 2013
Accepted 25 September 2013

Keywords:
Wheat flour
Pasta
Gluten-free
Sourdough

ABSTRACT

Wheat flour, which was rendered gluten-free by sourdough lactic acid bacteria fermentation and fungal proteases, was used for manufacturing experimental gluten-free pasta (E-GFp), according to a traditional process with low temperature drying cycle. Chemical, technological, structural, nutritional and sensory features were characterized and compared with those of commercial gluten-free (C-GFp) and durum wheat pasta (C-DWp). As shown through immunological analyses, the residual concentration of gluten of the hydrolyzed wheat flour was below 10 ppm. E-GFp showed rapid water uptake and shorter optimal cooking time compared to the other pastas. Despite the absence of the gluten network, the supplementation with pre-gelatinized rice flour allowed structural properties of E-GFp, which were comparable to those of C-GFp. The *in vitro* protein digestibility of E-GFp resulted the highest. Probably due to proteolysis during sourdough fermentation; chemical scores, essential amino acid profile, biological value and nutritional index of E-GFp were higher than those of C-DWp. The hydrolysis index (HI) of E-GFp was ca. 30% lower than that found for C-GFp. As shown by sensory analysis, the characteristic of E-GFp were acceptable. The manufacture of E-GFp should be promising to expand the choice of gluten-free foods, which combine sensory and nutritional properties.

1. Introduction

Celiac disease (CD) (gluten-sensitive enteropathy) is a chronic gastrointestinal disorder, where ingestion of gluten leads to damage of the small intestinal mucosa by an autoimmune mechanism in genetically susceptible individuals (Tye-Din and Anderson, 2008). Epidemiology of CD is increasing (ca. 1% of Europeans and North Americans) (Tye-Din and Anderson, 2008), as well as the prevalence of CD in elderly people is becoming evident. During the last decades, cereal food technology changed considerably so as to modify the dietary habits of entire populations, previously naïve to massive gluten exposure. Gluten is an unusual protein, since it is consumed in relatively large amounts, is partially resistant to digestion by gastric, pancreatic and brush border enzymes at the level of the human small intestine, and when absorbed, it is susceptible to post-translational modification (deamidation) by mucosal transglutaminase (Tye-Din and Anderson, 2008). Proline mainly imparts resistance to digestion, as many proteases are unable to cleave peptide bonds located at the N- and C-termini of this amino acid (Shan et al., 2002). Gluten-free diet (GFD) is effective and safe, and at present it is the only suitable treatment for CD. Despite the proven benefits of GFD, it may be exceedingly difficult to completely avoid gluten-containing foods, and effective adherence to the GFD is estimated to be only 45–80% (Leffler et al., 2008). Pasta is one of the most consumed foods in the world, and its appeal amongst consumers has made this food an important element of every diet, including GFD. Unfortunately, gluten-forming proteins are fundamental for the manufacture of pasta, most appropriately made from durum wheat (Mariotti et al., 2011). Durum wheat proteins are characterized by typical viscoelastic behavior, which allows good networking of the matrix, optimal dough formation during mixing and extrusion phases, as well as the most appreciated quality

Abbreviations: BV, Biological Value; CD, Celiac Disease; CS, Chemical Score; C-DWp, Durum Wheat pasta; C-GFp, Control Gluten-free pasta; DY, Dough yield; EAA, Essential Amino Acid; EAAI, Essential Amino Acids Index; E-GFp, Experimental Gluten-free pasta; GF, Gluten-free; GFD, Gluten-free diet; GI, Glycemic Index; HI, Hydrolysis Index; NI, Nutritional Index; OCT, Optimal cooking time; PER, Protein Efficiency Ratio; TPA, Texture Profile Analysis; TTA, Titratable acidity; WB, Wheat flour bread.

* Corresponding author. Tel.: +39 (0)80 5442948.
E-mail addresses: rizzello@agr.uniba.it, carlogiuseppe.rizzello@uniba.it (C. Giuseppe Rizzello).

0733-5210/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jcs.2013.09.011

attributes of cooked pasta (Mariotti et al., 2011). Substitution of a gluten network for making gluten-free (GF) products is one of the major challenges because of the essential structure-building role of gluten (Mariotti et al., 2011). Indeed, the use of novel ingredients or non-conventional technologies is requested to find substitutes with the same technological features of gluten. Recent researches consider different approaches, including grains different from wheat, starches, dairy products, gums and hydrocolloids, and their combinations (Mariotti et al., 2011; Mastromatteo et al., 2011). Since the last decade, several studies (Di Cagno et al., 2004; Rizzello et al., 2007) were carried out aiming at showing the capacity of proteolytic enzymes, mainly peptidases, of selected sourdough lactobacilli to degrade gluten during food processing. It was shown that selected sourdough lactobacilli, in combination with fungal proteases, decreased the residual concentration of gluten (*Triticum aestivum* and *Triticum durum* flours) to below 10 ppm during sourdough fermentation (De Angelis et al., 2010; Rizzello et al., 2007). Baked goods made with such hydrolyzed wheat flour were *in vivo* proven to be safe for CD patients during challenges lasting 60 days (Di Cagno et al., 2010; Greco et al., 2011).

This study aimed at using *T. aestivum* flour, which was rendered gluten-free using lactic acid bacteria fermentation and fungal proteases (Rizzello et al., 2007). The manufacture of pasta was according to a protocol, which included the use of pre-gelatinized rice flour and a low temperature drying cycle. The chemical, structural, sensory and nutritional properties of the GF pasta were characterized and compared both to gluten-containing and GF commercial pastas.

2. Experimental

2.1. Microorganisms and enzymes

Lactobacillus sanfranciscensis 7A, LS3, LS10, LS19, LS23, LS38 and LS47, *Lactobacillus alimentarius* 15M, *Lactobacillus brevis* 14G, and *Lactobacillus hilgardii* 51B were previously selected based on their peptidase activities, with particular reference to degradation of Pro-rich peptides (Di Cagno et al., 2004). Strains were propagated for 24 h at 30 °C in modified MRS broth (Oxoid, Basingstoke, Hampshire, United Kingdom), with the addition of fresh yeast extract (5%, v/v) and 28 mM maltose at the final pH of 5.6 (mMRS). When used for fermentation, cells of lactobacilli were cultivated until the late exponential phase of growth was reached (*ca.* 12 h). Fungal proteases (E1 and E2) from *Aspergillus oryzae* (500,000 haemoglobin units on the tyrosine basis/g) and *Aspergillus niger* (3000 specrophotometric acid protease units/g), routinely used as improvers in bakery industry, were purchased from BIO-CAT Inc. (Troy, VA).

2.2. Wheat flour fermentation

The main characteristics of the wheat (*T. aestivum* cv. Aubusson) flour, which was rendered GF according to the protocol of Rizzello et al. (2007), were as follows: moisture, 10.2%; protein, 11.1% of dry matter (d.m.); fat, 1.8% of d.m.; ash, 0.6% of. d.m.; and total carbohydrates, 76.5% of d.m. Wheat flour was obtained through a high milling process at the Tandoi factory (Corato, Bari, Italy). The median particle size of the wheat flour, analyzed by sieving, was 100 μm. Wheat flour, E1 and E2 (200 ppm final concentration), and tap water containing ca. 10^9 cfu/g (cell density in the dough) of each lactic acid bacterium were used to obtain a sourdough with dough yield (DY, dough weight × 100/flour weight) of 500. The sourdough was incubated at 30 °C for 48 h, under stirring conditions (200 rpm). After fermentation, sourdough was freeze-dried. After milling, the flour was analyzed and used for pasta making. Protein fractions were extracted from flour according to the method of Osborne, further modified by Weiss et al. (1993).

2.3. Immunological analyses

Immunological analyses were carried out by using R5 antibody-based sandwich and competitive ELISA. The R5-based sandwich ELISA was carried out with the Transia plate detection kit (Diffchamb, Västra, Frölunda, Sweden). The R5-based competitive ELISA was carried out at the gluten unit of the Centro National de Biotecnologia (Madrid, Spain).

2.4. Pasta making

The experimental GF pasta (E-GFp), containing wheat flour rendered gluten-free, was manufactured at Giuliani S.p.a industries (Milano, Italy), using a Storci (Collecchio, Italy) V70 pilot plant. Optimal dough moisture was chosen through the evaluation of firmness and cooking loss during several trials (data not shown). The following formula was used: pre-gelatinized rice flour (34.5%); wheat flour rendered gluten-free (34.5%) and tap water (31%). The main characteristics of the pre-gelatinized rice flour (Scotti, Pavia, Italy) were as follows: moisture, 12.2%; protein, 6.5% of d.m.; fat, 0.7% of d.m.; ash, 0.5% of. d.m.; total carbohydrates, 76.2% of d.m. Ingredients were mixed for 2 min, and the dough was left to hydrate for a further 8 min. The dough was then mixed for another 2 min and extruded through a teflon die n.1174, 153.0 × 50 mm (Capitanio S.n.c., Grandate, CO, Italy) with 21 holes. The extruded material was cut with a rotating knife for short pasta shapes to obtain smooth "macaroni". The die insert dimensions were 18.0 × 16.0 × 2.0 mm. For drying, E-GFp was arranged on frames (1.5 kg for frame) and treated according to the cycle (450 min) described in Fig. 1S. Finally, E-GFp was packed in plastic bags. A commercial GF pasta (C-GFp) made with maize and rice flours (added with mono- and di- glycerides as emulsifiers), and a commercial durum wheat pasta (C-DWp), having both the smooth macaroni shape, produced and distributed by two leading Italian brands were also analyzed.

2.5. Hydration test

Five grams of each pasta sample were placed in a beaker containing 100 mL of tap water (ratio pasta : water of 1:20), which was placed in a thermostatic bath at 25 °C. After 5, 10, 15, 30, 60 and 180 min of incubation, samples were removed from water, drained for 1 min, carefully blotted with tissue paper to remove superficial water, and weighed. The results were expressed as $((W_1 - W_0)/W_0)$ *100, where W_1 is the weight of the hydrated sample and W_0 is the weight of the dry sample (Marti et al., 2011).

2.6. Cooking time

The method of Schoenlechner et al. (2010) was used to determine the cooking time. Twenty-five grams of pasta were put into a beaker containing 300 ml of boiling water (without salt addition). Every minute, some macaroni pieces were taken out and pressed between two perspex plates. The optimal cooking time (OCT) corresponded to the disappearance of the white core.

2.7. Cooking loss and water absorption

Cooking loss was evaluated by determining the amount of solid losses into the cooking water (D'Egidio et al., 1990). Portions of 30 g of pasta were cooked in 300 mL of boiling tap water (ratio pasta : water of 1:10) without salt addition. Pasta samples were cooked for the optimum cooking time (OCT). After cooking, the volume of water was brought to the initial volume. Dry matter was determined on 25 mL of freeze-dried cooking water. The residue was