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Suitability of enzymatic hydrolyzates of extracted gluten from fresh pasta by-product used as bread improvers

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ABSTRACT

Gluten extracted from fresh pasta by-products (*PG*) was enzymatically hydrolyzed by two different commercial proteases (Alcalase 2.4 L and Pancreatin) to different degrees of hydrolysis (DH 2.0, 4.0 and 8.0%). Commercial gluten (*CG*) was used as reference. The evaluation of functional properties of hydrolyzates from pasta gluten (*PGH*) and commercial gluten (*CGH*) showed that Pancreatin hydrolyzates had the highest emulsifying capacities. Regarding the foaming activity, all hydrolyzates performed better than unhydrolyzed gluten. *PGH* and *CGH* were added to wheat flour (1%) and their effects on dough rheology were studied. Most hydrolyzates with DH 8% increased dough thermal stability and elasticity during mixing, accelerated the denaturation rate of the protein network, and delayed the gelatinization speed of starch as the temperature increased. Texture profiles and specific volumes of breads from low quality wheat flour with added Pancreatin hydrolyzates (DH 8%) were comparable to those of breads from high quality flour. This showed the potential suitability of *PGH* and *CGH* as bread improvers.

1. Introduction

Plant proteins are an economic and versatile substitute for animal proteins as functional ingredients in food products. Wheat gluten is an abundant plant protein source (Wang et al., 2006). Wheat gluten includes two main components, glutenins and gliadins. They are highly polymorphic polypeptides, consisting of more than 60 different molecular weight species ranging in Mr (relative molecular mass) from 30,000 to 90,000 kDa (Shewry et al., 1992; Payne et al., 1987). Gluten is insoluble in near-neutral pH and is viscoelastic when hydrated. The expanding utilization of wheat gluten in the food and non-food industries (Wang et al., 2006; Larré et al., 2000) has been limited by lack of some desirable functional properties, such as solubility and emulsifying ability. Moreover, celiac disease and gluten intolerances are also viewed very critically in the technological use of wheat gluten and especially that most gastroenterologists recommend a strict gluten-free diet in all patients diagnosed with celiac disease (Tursi et al., 2009).

It is possible to enlarge the field of gluten applications through chemical or enzymatic modifications. The use of proteolytic enzymes is an efficient protein modification method (Kong et al., 2007a; Kammoun et al., 2003). By controlling the reaction conditions during the enzymatic hydrolysis, it is possible to obtain hydrolyzates having different characteristics. Different proteases have been studied, such as alcalase, pepsin, trypsin, papain, pancreatin, neutrase and protamex. (Kong et al., 2007a,b; Mimouni et al., 1999). Enzymatic modification of wheat gluten has resulted in the enhancement of its solubility, foaming and emulsifying properties (Kammoun et al., 2003; Popineau et al., 2002; Linarès et al., 2000; Mimouni et al., 1999), which was responsible, in part, for certain textural and sensory properties of foods (Kim et al., 2004). Very mild enzymatic hydrolysis of wheat gluten is normally used in bakeries to soften hard wheat gluten (Drogo and Gonzalez, 2000). Another reason for gluten hydrolysis is the search for peptides with biological activity such as lowering the blood pressure (Asoodeh et al., 2014; Gottardi et al., 2014) and in the research of celiac disease (Wieser et al., 1984).

In the food industry, wheat gluten is used as an additive to improve the baking quality of flour (Ellouze-Ghorbel et al., 2010b);

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however this expanding utilization has been limited by lack of some desirable functional properties, such as solubility and emulsifying ability (Wang et al., 2006). Some studies have shown the possibility of using limited enzymatic proteolysis as a processing technology to prepare peptides which are different from the parent proteins and may possess new functional properties (emulsifying and foaming capacities) (Kong et al., 2007b). Due to their functionality, gluten hydrolyzates could be used as bread improvers. In fact, the effect of emulsifiers on the functional properties of wheat bread has been reported in the literature (Mnif et al., 2012; Ellouze Ghorbel et al., 2010a; Ribotta et al., 2004; Aziz et al., 2003). Different emulsifiers could be used to improve bread volume, crumb texture (Mnif et al., 2012; Kohajdova et al., 2009), and dough rheological proprieties such as dough strengtheners and crumb softeners (Ribotta et al., 2004; Aziz et al., 2003).

In Tunisia, the durum wheat pasta processing industries generate an enormous amount of fresh pasta leading to ecological problems when deposited in the environment as waste. Instead, suitable functionality would value it as an important economic source of wheat gluten (Ellouze-Ghorbel et al., 2010b).

Thus the aim of this work is to study the effect of enzyme hydrolysis on the functionality of gluten extracted from fresh pasta, to investigate the effect of gluten hydrolyzates on the rheology of a low breadmaking quality flour and to exploit the suitability of these enzymatic hydrolyzates as bread improvers.

2. Materials and methods

2.1. Materials

2.1.1. Flours

Commercial Tunisian soft wheat flour with low breadmaking quality (FLBM) of 13.2% moisture, 0.4% ash content and 9.8% protein (N × 5.7) was used. The alveographic parameters were $W = 156.85 \cdot 10^{-4}$ J and $P/L = 0.91$. The same batch of flour was applied throughout the optimization study. A premium quality Canadian wheat flour with high breadmaking performance (FHBM) ($W = 285.10^{-4}$ J; $P/L = 0.97$) was used in this work as reference (13.68% moisture, 0.5% ash and 12% proteins [$N \times 5.7$]).

2.1.2. Glutens

Fresh pasta by-product gluten (*PG*) was extracted from durum wheat pasta by-product supplied by a Tunisian pasta producer (DIARI-SPIGA, Sfax-Tunisia) (Ellouze-Ghorbel et al., 2010b). *PG* was composed as follows: 8.4% ± 0.3 moisture, 80.9% ± 3.6 protein ($N \times 5.7$), 0.9% ± 0.3 starch, 2.90% ± 0.08 ash and 0.5% ± 0.1 lipid compared to a commercial soft wheat gluten (*CG*) (7.9% ± 0.4 moisture, 74.8% ± 2.6 protein ($N \times 5.7$), 11.8% ± 0.5 starch, 0.80% ± 0.06 ash and 1.4% ± 0.2 lipid) gifted from Roquette Company (Keokuk, IL).

2.2. Chemicals and reagents

All chemicals and reagents used in this work were food-grade or reagent-grade. Alcalase 2.4 L (25×10^5U/ml) donated by Novo Nordisk (France) and Pancreatin 8× USP GRADE (52×10^5 U/g) purchased from MP Biomedicals, Inc. (Illkirch, France) were used for gluten hydrolysis.

2.3. Extensive hydrolysis with Alcalase and Pancreatin

PG was extracted from fresh pasta by-products as described by Ellouze-Ghorbel et al. (2010b). Briefly, *PG* was hand washed from the fresh pasta by-product by NaCl solution (2%). *CG* was taken as reference. Both glutens *PG* and *CG* were suspended in 100 mmol/L Tris–HCl buffer, pH 8.5 (8%, w/v). The suspensions were incubated for 30 min at optimum temperature (60 °C for Alcalase, 37 °C for Pancreatin) under continuous stirring. The enzymatic hydrolyses were carried out with an enzyme to substrate ratio [E/S] of 6000 U/g for 6 h. The pH of the mixtures was kept constant by continuously adding a 4 mol/L NaOH solution to the reaction mixture. After the reaction period, the mixtures were cooled, the pH adjusted to 7.0 with 0.5 mol/L and 4 mol/L NaOH and heating at 95 °C for 10 min to inactivate the enzyme. Then the mixtures were centrifuged at 10,000 × g for 20 min at 4 °C. The supernatants were freeze-dried and stored at −20 °C.

2.4. Preparation of gluten hydrolyzates with defined degrees of hydrolysis

When the Alcalase or the Pancreatin hydrolysis had reached the set degree of hydrolysis (2.0%, 4.0% or 8.0%), it was terminated by adjusting the pH and heating at 95 °C for 10 min. Then the reaction mixtures were centrifuged and the supernatants were freeze-dried. Hydrolyzates of gluten extracted from fresh pasta by-product (*PGH*) and hydrolyzates of commercial gluten (*CGH*) were kept at −20 °C until needed.

2.5. Degree of hydrolysis

The degree of hydrolysis (DH), defined as the percent ratio of the number of peptide bonds broken (h) to the total number of peptide bonds per unit weight (h_{tot}), in each case, was calculated from the amount of base (NaOH) added to keep the pH constant during the hydrolysis (Adler-Nissen, 1986) as given below:

$$DH(\%) = \frac{h}{h_{tot}} \times 100 = \frac{B \times Nb}{MP} \times \frac{1}{\alpha} \times \frac{1}{h_{tot}} \times 100$$

where B is the amount of NaOH consumed (ml) to keep the pH constant during the reaction, Nb is the normality of the base, MP is the mass (g) of protein ($N \times 5.7$), h is the hydrolysis equivalents in meqv/g protein and h_{tot} is the total number of peptide bonds in the protein substrate (8.38 eqv/g gluten protein) and α is the average degree of dissociation of the α-NH2 groups released during hydrolysis expressed as:

$$\alpha = \frac{10^{\mathbf{pH}-\mathbf{pk}}}{1 + 10^{\mathbf{pH}-\mathbf{pk}}}$$

where pH and pk are the values at which the proteolysis was conducted. $1/\alpha$ was assumed to be 1.7 (Adler-Nissen, 1986).

2.6. Functional properties of PGH and CGH

2.6.1. Emulsifying capacity and emulsion stability

The emulsifying activity of *PGH* and *CGH* was determined by the turbidimetric method of http://www.sciencedirect.com/science/article/pii/S0308814606001300Kong et al. (2007a). To prepare the emulsions, 9 ml of soybean oil and 21 ml of 0.2% protein solution (pH 7.0) were shaken together and homogenized in a blender at 12,000 × g for 1 min at 20 °C. Emulsion (50 μl) was taken from the bottom of the container after different time intervals (0 and 30 min) and diluted with 5 ml of 0.1% SDS solution. The absorbance of diluted emulsions was then determined at 500 nm and indicated as turbidity. Emulsifying activity was determined from the absorbance measured immediately after emulsion formation. The emulsion activity index (EAI) was calculated as follows.