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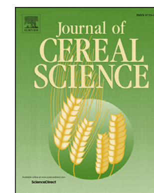

Gluten degradation in wheat flour with *Aspergillus niger* prolyl-endopeptidase to prepare a gluten-reduced bread supplemented with an amaranth blend

Nina G. Heredia-Sandoval [a], Ana M. Calderón de la Barca [b], Alma R. Islas-Rubio [a,*]

[a] Coordinación de Tecnología de Alimentos de Origen Vegetal, Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a La Victoria km 0.6, C.P. 83304, Hermosillo, Sonora, Mexico

[b] Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a La Victoria km 0.6, C.P. 83304, Hermosillo, Sonora, Mexico

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A B S T R A C T

Celiac disease and other-gluten related disorders are characterized by an inadequate immune reaction against dietary gluten. Some strategies have been developed for gluten modification, to reduce or avoid the immune response. The aim of this study was to modify gluten proteins in wheat flour using the *Aspergillus niger* prolyl-endopeptidase (AnPEP) to elaborate bread supplemented with amaranth blends, evaluating its immunoreactivity and technological quality. The reaction conditions: enzyme (dilutions 1:50, 1:100 and 1:500), temperature (35 or 40 °C), substrate (WF) (10% or 20%, w/v), time (1–8 h), gluten content by R5-ELISA, and the protein composition by SE-HPLC were analyzed. For bread-making, 60% of modified WF and 40% of amaranth blends (20:80, 30:70, 40:60; raw: popped, respectively), and two fermentation times (52 and 90 min) were tested. Specific volume and gluten content were evaluated. The best reaction conditions were (1:50) AnPEP, 20% WF, and 8 h incubation at 35 °C. The bread supplemented with the 20:80 amaranth blend showed a higher specific volume and after a 52 min fermentation had 99% less immunogenic gluten than the wheat bread. The use of the amaranth blend in combination with WF modified by AnPEP is an effective way to obtain gluten-reduced breads with acceptable quality.

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1. Introduction

Celiac disease (CD) and other-gluten related disorders are characterized by an inadequate immune reaction against dietary gluten proteins. The only effective treatment available is the gluten withdrawal of the diet (Villafuerte-Galvez et al., 2015). The demand of gluten-free products has shown a strong growth over the last years, due to the increasing prevalence of gluten-related disorders and interest among consumers to avoid or reduce gluten consumption, for medical reasons or the belief that gluten-free foods are a healthy option (Mintel, 2014; Packaged Facts, 2015). Currently, a large number of gluten-free food products are available, but CD patients miss aroma, taste, and texture of wheat bread (Montserrat et al., 2015); additionally, they contain additives which can increase symptoms related to intestinal absorption. Therefore, some strategies have been developed to modify gluten, to reduce or avoid the immune response, maintaining part of its properties for bakery.

A potential strategy for modifying gluten is the use of prolyl-endopeptidases from different sources such as fungi, bacteria, and germinated cereal grains. In contrast to gastrointestinal enzymes, these peptidases have the ability to cleave proline-rich gluten sequences, favoring the degradation of gluten peptides into non-immunogenic residues (Wieser and Koehler, 2012; Schwalb et al., 2012). The use of the endopeptidase from *Aspergillus niger* has been studied in different gluten-containing materials, such a wheat-bran and bread drink ("Kwas"), with an effective gluten degradation (<20 mg/kg) (Walter et al., 2014). However, the gluten degradation adversely affects the gluten technological properties, especially in baked goods; therefore, some ingredients could be

Abbreviations used: AnPEP, *Aspergillus niger* prolyl endopeptidase; TP, total polymeric protein; EP, Extractable polymeric protein; UP, unextractable polymeric protein; PPF, polymeric protein in the flour; UPF, unextractable polymeric protein in the flour.

* Corresponding author.
E-mail address: aislas@ciad.mx (A.R. Islas-Rubio).

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added to counteract these problems (Capriles and Arêas, 2014). In order to improve the rheological behavior, raw and popped amaranth have been successfully incorporated to gluten-free formulations and for partial or full substitution of wheat flour of the mixed doughs for breads, pastas, and cookies (Islas-Rubio et al., 2014; Fiorda et al., 2013; Calderón de la Barca et al., 2010). Furthermore, the addition of amaranth to wheat flour dough could enhance the nutritional properties of the supplemented product, due to its excellent nutritional quality: high quality proteins, lipids, and higher content of minerals and dietary fiber than cereal grains (Caselato-Sousa and Amaya-Farfán, 2012).

The objective of this study was to enzymatically modify the protein in wheat flour using an *Aspergillus niger* prolyl-endopeptidase (AnPEP) to elaborate bread supplemented with a raw and popped amaranth blend, evaluate the breads' immune-reactivity and technological quality.

2. Materials and methods

2.1. Reaction conditions for AnPEP

A commercially available prolyl-endopeptidase preparation from *Aspergillus niger* (DSM Food Specialties B.V., Delft, The Netherlands) was used for the modification of gluten proteins. Initially, two dilutions of the AnPEP preparation (1:100 and 1:500), two concentrations of substrate (10% or 20%, w/v) and time of incubation (1–8 h) were probed at 40 °C with constant stirring (150 rpm). Two additional dilutions of AnPEP (1:50 and 1:100) with 20% substrate for 8 h at 35 °C, were tested. After incubation, each sample was centrifuged at 1250#*g* for 15 min and the gluten content was evaluated in the lyophilized pellets as described below.

2.2. Protein composition by SE-HPLC

Before SE-HPLC analysis, a total (TP), extractable (EP) and unextractable (UP) polymeric proteins were extracted from the freeze-dried modified flours as described by Batey et al. (1991). The protein extracts were analyzed using an Agilent 1100 Series (Agilent Technologies, Santa Clara, CA) by automatic injection and were fractionated using a Biosep-SEC-S4000 column (Phenomenex, Torrence, CA). Injection volume was 20 μL. The eluting solution was 50% acetonitrile in water with 0.05% (v/v) trifluroacetic acid and the flow rate was 0.5 mL/min. Solutes were detected at 214 nm.

The SE-HPLC profile was divided into #1, 2, and 3 peaks corresponding to polymeric protein (glutenin), gliadins, and albumins/globulins, respectively. The ratio of the area of #1 peak of the unextractable polymeric protein to the sum of #1 peak areas of the extractable and unextractable polymeric proteins, a measure of the relative size distribution of the polymeric protein, was calculated. The percentage of polymeric protein in the flour (PPF) was calculated by multiplying the protein content in the flour and the proportion of polymeric protein, while the percentage of unextractable polymeric protein in the flour (UPF) was calculated by multiplying the protein content in the flour and the unextractable polymeric protein (Batey et al., 1991).

2.3. Bread making

2.3.1. Raw: popped amaranth flours blends

Raw amaranth grain was obtained from a local producer (Invernadero de Tuyehualco, Tuyehualco, México) and the pooped amaranth was from a local market (Puebla, Mexico), manually cleaned. Each type of amaranth was ground and the flour was passed through a 40 mesh (0.425 mm sieve size), the moisture content was evaluated according to the AACC (2000). Three different ratios 40:60, 30:70, and 20:80 (raw:pooped, respectively) of amaranth flours were prepared (adjusted to 14% moisture).

2.3.2. Supplementation of modified wheat flour with Raw:Pooped amaranth blends

The pellet of the modified wheat flour obtained after centrifugation was used to prepare breads. Previously, the protein (Dumas method 46–30) (AACC, 2000) and starch content (Albalasmeh et al., 2013) in the supernatants, and the moisture content in the pellet was determined. The wet pellet was weighed to compose the 60% of wheat flour and the other 40% was supplemented with the amaranth flour blend. The ingredients were mixed in a mixograph (National Manufacturing Co., Lincoln, NE) for 2 min and 30 s. Then, the dough was molded and fermented (52 or 90 min at 85% relative humidity and 30 °C). After fermentation, the doughs were baked at 215 °C for 17 min.

2.3.3. Specific volume

Weight and loaf volume after baking were evaluated. The specific loaf volume was calculated by dividing loaf volume and weight (method 10-10B) (AACC, 2000). Specific volume was expressed in cm^3/g.

2.4. Quantification of reactive gluten

The gluten content was measured in the pellets as well as in loaves of bread by using the ELISA-R5 RIDASCREEN® Gliadin kit (R-Biopharm, Darmstadt, Germany), the assay was performed according to the manufacturer's instructions.

2.5. Statistical analysis

Differences between treatments were determined by one-way analysis of variance (ANOVA). Tukey-Kramer test for the multiple comparisons of means was used and the level of significance was $p < 0.05$. The NCSS statistical software, version 2007 was used for analysis.

3. Results and discussion

3.1. Reaction conditions for AnPEP

The reactive gluten content of the wheat flour (lyophilized pellet) after incubation without AnPEP, was up to 200,000 mg/kg (Table 1). The higher reduction of gluten respect to untreated flour at 40 °C, was with the addition of a higher concentration of AnPEP (1:100 vs. 1:500, v/v) with a significant decrease ($p < 0.05$) of 92 and 89% of the reactive gluten in the 10 and 20% (w/v) treated wheat flours, respectively. As there were no significant differences

Table 1
Effect of AnPEP incubation at two wheat flour concentrations on gluten content of freeze dried samples.*

Dried samples	Substrate (wheat flour) concentration (% w/v)		
	10**	20**	20***
Control (no AnPEP)	210 400[a]	234 000[a]	234 000[a]
AnPEP (1:500)	106 100[c]	125 200[c]	ND
AnPEP (1:100)	17 200[b]	25 400[b]	73 600[b]
AnPEP (1:50)	ND	ND	16 000[c]

*Values are expressed as mg/kg.
**8 h-Incubation at 40 °C.
***8 h-Incubation at 35 °C.
Mean value of duplicate determinations. Different letters in the same column indicate statistical significance ($p < 0.05$). ND = not determined.

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