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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network



RESEARCH ARTICLE

BIOSCIENCE RESEARCH, 2019 16(2): 1377-1390.

OPEN ACCESS

Enzymatic treatment of gliadins triggers anaphylactic reaction in a murine model of wheat allergy: *in vivo* and *ex vivo* study

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Enzymatic hydrolysis of wheat proteins is an effective mean to improve various functional proprieties. This study examines the effect of enzymatic hydrolysis of gliadins and the ability of these hydrolysates to elicit anaphylactic reactions in mice allergic to native gliadins (NG). Two groups of female Balb/c mice were established: naïve group sensitized with aluminum hydroxide (Alum) and NG group sensitized with 10 µg of NG. NG-specific IgG and IgE antibodies level were determined in serum by ELISA. Symptom scores, body temperature and vascular leakage were determined after *in vivo* challenge to NG and hydrolyzed gliadins. Jejuna were used for histological analysis and for the assessment of local anaphylactic response by an *ex vivo* study in Ussing chamber (measurement of electrophysiological parameters; short-circuit current (Isc) (µA/cm²) and conductance (G) (mmho/cm²)). NG-sensitized mice secreted higher levels of NG-specific IgG and IgE antibodies and showed significantly higher Isc (µA/cm²) and G (mmho/cm²) values as well as alterations of the intestinal barrier and villous atrophy. *In vivo* IP challenge of NG-sensitized mice with corolase 7089 hydrolysates caused less severe clinical manifestations than those observed after challenge with peptic and tryptic hydrolysates, the latter being similar to those observed with NG. The same results were observed for body temperature and vascular permeability. NG and digestive enzymes hydrolysates can elicit severe anaphylaxis symptoms, whereas, the industrial enzyme hydrolysates induce less severe symptoms.

Keywords: wheat allergy; gliadins; enzymatic hydrolysis; anaphylaxis; Ussing chamber

INTRODUCTION

Wheat is one of the most harvested cereals and an important part of the human diet in the world. In 2017, Algerian wheat production exceeded three million tons (FAOSTAT, 2018).

Wheat proteins are implicated in different types of adverse reactions (respiratory, food and contact allergies). Food-induced allergenic reactions may be responsible for different clinical symptoms that can be characterized by mild urticaria, diarrhea or anaphylactic shock. Food allergy is an important manifestation of Type I allergic reaction mediated primarily by

immunoglobulin E (IgE). Specific IgE can be induced by ingesting food allergens, the binding of these allergens by specific IgE on effector cells, such as basophils and mast cells, leads to mediators release (i.e., histamine), and a subsequent cascade of pathologic events (Wang et al., 2014). The world prevalence of the food allergy to wheat proteins has increased during the past decade; the rate of sensitization is 0.4-1.3% in children and 0.2-0.9% among adults (Prandi et al., 2013).

Several wheat allergens have been identified in different fractions of wheat grain, amongst

which gliadins. They are monomeric and ethanol-soluble proteins (Gourbeyre et al., 2012). According to their mobility in polyacrylamide gels, gliadins are classified into α - and β -, γ -, and ω -gliadins, which represent 44-60%, 30-45% and 6-20% respectively of total gliadins (Wieser et al., 1994; Wieser, 2007; Banc et al., 2009; Barak et al., 2015).

Food processing can potentially affect two aspects of the allergenic proprieties of proteins: 1) the integrity of epitopes recognized by IgG or IgE antibodies; 2) the ability of food proteins to induce allergic sensitization (Verhoeckx et al., 2015). The types of processing that have been implicated in influencing allergenic proprieties are: heating (thermal processing), fermentation including endogenous enzymatic hydrolysis, enzymatic and acid hydrolysis, physical treatments (such as high pressure processing or extrusion), the use of preservatives, changes in pH, or combinations of any two or more of these (Thomas et al., 2007; Mills and Mackie, 2008; EFSA, 2014).

Enzymatic hydrolysis is a commonly used method in the modification of proteins structure in order to enhance their functional proprieties (Corredig and Dalgleish, 1997) and to decrease their antigenicity by hydrolyzing them into small peptides (Li et al., 2016). Animal digestive proteases such as trypsin and α -chymotrypsin are widely used to hydrolyze vegetal proteins (Yu et al., 2011; Li et al., 2013; Yu et al., 2013). Several studies showed that enzymatic hydrolysis with alcalase and papain can reduce allergenicity to gliadins (Li et al., 2016). Most reports concluded that the use of acid hydrolyzed wheat gluten may induce allergenicity in wheat tolerant people, including also allergenicity to traditional wheat products (Verhoeckx et al., 2015).

In fact the number of studies indicating that acid-hydrolyzed wheat protein (HWP) induces immediate hypersensitivity by skin contact and/or food ingestion has increased (Leduc et al., 2003; Snégaroff et al., 2006; Laurière et al., 2007; Bouchez-Mahiout et al., 2010; Chinuki et al. 2011; Fukutomi et al., 2011). While the use of enzymatic proteases turned out to be of great interest into reducing gliadins proteins allergenicity but the effect of this treatment on gliadins allergy symptoms such as anaphylaxis has not been fully investigated.

Therefore the aim of the present work is to study the elicitation of anaphylaxis chock (*in vivo* and *ex vivo*) by enzymatic hydrolyzed gliadins using murine model of sensitization to native gliadins (Balb/c mice).

MATERIALS AND METHODS

Sequential Extraction of gliadins

Crude gliadins were extracted from Algerian hard wheat flour (*Triticum durum*) using the sequential procedure developed by Osborne et al., (1907) as adapted by Nicolas et al., (1998). Briefly, the albumin-globulin fraction of the flour was removed by several washings with saline buffer; the flour was then suspended in 70% ethanol for 1 h at room temperature to solubilize the gliadins. After centrifugation (20 000 g for 20 min at 4°C), the supernatant (whole native gliadins extract), was collected and freeze-dried. The concentration of proteins was determined by the Bicinchoninic Acid (BCA) method, using analytic grade bovine serum albumin (BSA) as standard.

Enzymatic hydrolysis

Crude gliadins were dissolved at 10 mg/mL in 70% ethanol solution, and then diluted at 2 mg/mL in correspondent buffers.

Peptic hydrolysis (PH)

Crude gliadins were diluted in 50 mM citrate buffer (pH 2.2) to the final concentration of 2mg/mL. Pepsin ((EC 3.4.23.1) 3200–4500 units/mg of protein) (1% w/w) was added and the mixture was incubated at 37°C for 180 min. The enzymatic reaction was stopped by increasing the pH to 7.0 with 5N NaOH.

Tryptic hydrolysis (TH)

Crude gliadins were diluted in 50 mM phosphate buffer (pH 8.0). The suspension was incubated at 37°C with trypsin ((EC 3.4.21.4) ≥ 10000 units/mg of protein) (2% w/w). After 180 min, the reaction was stopped by adding 1N HCl.

Corolase 7089 hydrolysis (C7089H)

For corolase 7089 treatment ((EC 3.4.24.28) 840 units/g of protein), 50 mM phosphate buffer (pH 8.0) was used. Sample containing enzyme (0.5% v/v) was incubated for 180 min at their optimum temperature (60°C). The hydrolysis process was terminated by heating the sample at 90°C for 20 min then cooled at room temperature immediately in cold water bath.

For each enzymatic hydrolysis, aliquots were collected at different times.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to Laemmli (1970) using 12% and 15% acrylamide separating gel and 5% acrylamide stacking gel. The samples were treated with β -mercaptoethanol. The gel sheets were stained with Coomassie brilliant blue R-250.

Determination of the degree of hydrolysis (DH)

The degrees of hydrolysis of gliadins were measured by the O-Phthaldialdehyde (OPA) method (Frister et al., 1988).

50 μ l of each sample was mixed with 1 mL of the OPA reagent. The reagent is composed of 50 mL of 0.1 M borate buffer (pH 9.3), 1.25 mL of 20% (w/v) SDS solution, 100 mg of N,N-dimethyl-2-mercaptoethylammonium chloride (DMMAC) and 40 mg of OPA dissolved in 1 mL methanol. The mixture was allowed to stand for 2 min before measurement of the absorbance at 340 nm. The number of amino groups was determined with reference of the L-Leucine standard curve (concentrations between 0.5 and 2.5 mM). The increase in amino groups between native and hydrolyzed gliadins was attributed to proteolysis and DH was calculated by the following equation: $DH(\%) = \frac{h}{h_{tot}} \times 100$

Where h_{tot} is the total number of peptide bonds per protein equivalent, and h is the number of hydrolyzed bonds. The h_{tot} factor was 8.3 (based on gluten) according to Adler-Nissen (1986). Three measurements were performed for each sample.

Sensitization of mice

Four-weeks-old Balb/c female mice from Pasteur Institute of Algeria were fed a gluten free semi-synthetic diet. This diet was essentially composed of corn starch, casein, sucrose, mineral and vitamin mixes and was prepared at Laboratory of Physiology of Nutrition and Food Safety (LPNSA, University Oran 1, Algeria). The mice were housed in filtered cages under standard specific pathogen-free husbandry conditions and were acclimatized for one week before starting the experiments.

The protocol of mice sensitization were performed as described by Bodinier et al., (2009).

Before use, freeze-dried NG were rendered soluble in 70% ethanol at 5 mg/mL and then slowly diluted at 0.1 mg/mL in sterile phosphate-buffered saline (PBS). Two groups of mice ($n = 10$ per group) were established. The first group (naïve) was sensitized with 2 mg aluminium

hydroxide (Alum) diluted in PBS, the second group was sensitized with 10 μ g NG adsorbed on 2 mg Alum. Intra-peritoneal (IP) sensitizations were performed at days 0, 10, 20 and 30 (0.2 mL per mouse).

Assessment of NG-Specific IgG and IgE

Blood samples were obtained one week after the last sensitization, sera were then collected.

The efficiency of mice sensitization was evaluated by the assessment of NG-specific IgG and IgE using an enzyme-linked immunosorbent assay (ELISA) as described by Guendouz et al., (2017) with slight modifications.

Briefly, soluble NG in 70% ethanol (1 mg/mL) were slowly diluted in PBS at 10 μ g/mL and then used to coat 96-well microtiter plates (MaxisorpNunc) then incubated overnight at 4°C. The plates are blocked with 3% BSA (Bovine Serum Albumin).

Assessment of systemic anaphylactic signs

The severity of anaphylaxis was evaluated by measuring the scoring clinical signs 30 minutes after mice *in vivo* challenge with NG and different hydrolysates at 1 mg without adjuvant.

Clinical signs were graded by a score scale adapted from those of Li et al., (1999) and Perrier et al., (2010) as follows: 0= no signs; 1=scratching and rubbing around the snout and head; 2= puffiness around the eyes and snout, diarrhea, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate; 3= wheezing, labored respiration, and cyanosis around the mouth and the tail; 4= no activity after prodding, or tremor and convulsions; and 5= death.

Measurement of rectal temperature

Rectal temperatures were measured 30 minutes after challenge using a thermal probe.

Assessment of intestinal anaphylaxis: *Ex vivo* experiments in Ussing chamber

At the end of the experiment, mice were anesthetized and intestinal segments were taken out. The jejunum segment was then opened, washed and mounted as sheets in Ussing chambers (exposed area 0.2 cm²). Both sides were filled with 5 ml of Ringer solution (in mM: Na⁺ 140 mM, K⁺ 5.2 mM, Ca⁺⁺ 1.2 mM, Mg⁺⁺ 1.2 mM, Cl⁻ 120 mM, 25 mM, HPO₄ 2.4 mM, H₂PO₄ 0.4 mM), maintained at 37°C and continuously oxygenated (95 % O₂, 5 % CO₂). After an equilibration period, 80 μ g of NG, PH, TH and

C7089H or in some cases ovalbumin (OVA) as a nonspecific antigen control were added to the serosal side of the tissue. In order to evaluate the tissue viability, glucose was added at a concentration of 10 mM to the luminal bathing medium at the end of each experiment (glucose stimulates Na⁺ absorption via SGLT1 which would increase I_{sc}) (Gar et al., 2015). Electrophysiological parameters, short current circuit I_{sc} (μA/cm²) and tissue conductance G (mmho/cm²) were than measured.

The I_{sc} (μA/cm²) represents the sum of net ion flux (mainly Na⁺, Cl⁻, and HCO₃⁻) across the epithelium in the absence of an electrochemical gradient. Transepithelial electrical conductance (G (mmho/cm²)) is an index of the epithelial integrity and is calculated according to Ohm's law. The increase in I_{sc} (ΔI_{sc}) and G (ΔG) is the difference between the peak value after challenge and the base-line value (Haddi et al., 2018).

Vascular leakage study

To assess the anaphylactic vascular permeability, five mice from each group received 100 μL of 0.5% Evan's Blue Dye through tail vein injection immediately before the IP challenge. Footpads of mice were examined for signs of vascular leakage (visible bluing) within 1h after dye/antigen administration and photographed (Chen et al., 2011).

Histopathological analysis

Segments of small intestine (jejunum) were embedded in paraffin after fixation in 10% neutral-buffered formaldehyde. Five-micrometer sections were stained with hematoxylin and eosin (H&E). The morphology of the small intestine was examined by light microscopy.

Villus Length

As a marker of inflammation, villus length was measured at six points for each sample using an optical microscope equipped with a micrometer and expressed in micrometer (μm).

Statistical analysis

All statistical analyses were performed using GraphPad Prism 5.01 (GraphPad Software Inc, San Diego, CA, USA).

Data were analyzed by ANOVA followed by Bonferroni's multiple comparison test, except for IgE, IgG and villus length, for which the Mann

Whitney test was used. Values were expressed as means ± SEM.

A *p* values of < 0.05 were considered to be statistically significant.

RESULTS

Degree of Hydrolysis

Degree of hydrolysis is defined as the percentage of cleaved peptide bonds in a gliadins hydrolysates. In the present study, the OPA method was used. The presence of SDS in the OPA solution served to inactivate the enzyme and ensured a full exposure of amino groups.

The amounts of free amino groups produced from gliadins during PH, TH and C7089H hydrolysis determined by reaction with OPA at different times (0, 10, 30, 60, 90, 120, 150 and 180 min) are shown in Figure 1.

DH values varied from 0% to 36% after 180 min of incubation, depending upon the proteases used. At T 0 min the gliadins showed an average DH value of 1.37 ± 0.11%, 1.67 ± 0.02% and 5.72 ± 0.14% (corolase 7089, trypsin and pepsin protease respectively).

The DH protease-treated gliadins obtained after 180 min of hydrolysis with the different proteases was 27.22 ± 1.10 % for pepsin, 8.95 ± 0.07 % for trypsin and 36.53 ± 0.99 % for corolase 7089. The DH increased rapidly in the first 10 minutes (23.81 ± 1.39 %) for corolase 7089. Corolase 7089 gave the highest DH values and more efficient hydrolysis than pepsin and trypsin (gastrointestinal proteases).

Characterization of hydrolyzed gliadins

The electrophoresis profile of C7089H represented in figure 2.A and figure 2.B shows components with molecular weight (MW) of 14.2 kDa, indicating an effective degradation of gliadins at each time of the enzymatic hydrolysis process.

For TH (figure 2.C), multiple protein bands with MW between 65.00 and 14.2 kDa were observed. These bands present a similar profile to the control (T0 line 2).

For gliadins treatment with pepsin (figure 2.D), protein bands with MW between 65.0 and 14.2 kDa were observed in all hydrolysates at different times but their intensities at T150 min (line 8) and T180 min (line 9) were significantly lower compared with control (line 2).

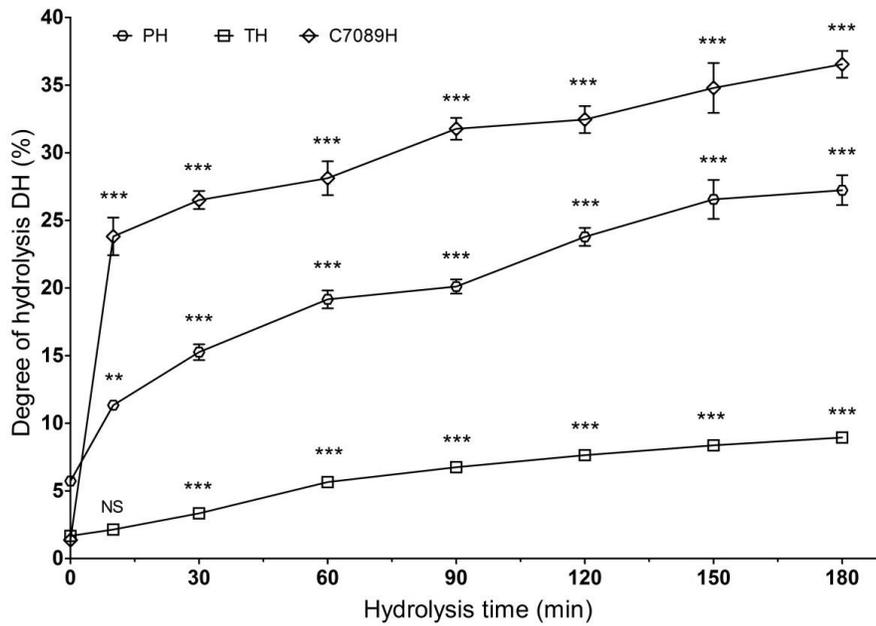


Figure 1: Enzymatic hydrolysis of wheat gliadins with different proteases (pepsin, trypsin and corolase 7089).

Data are expressed as means \pm SEM (** $p < 0.01$; *** $p < 0.001$).

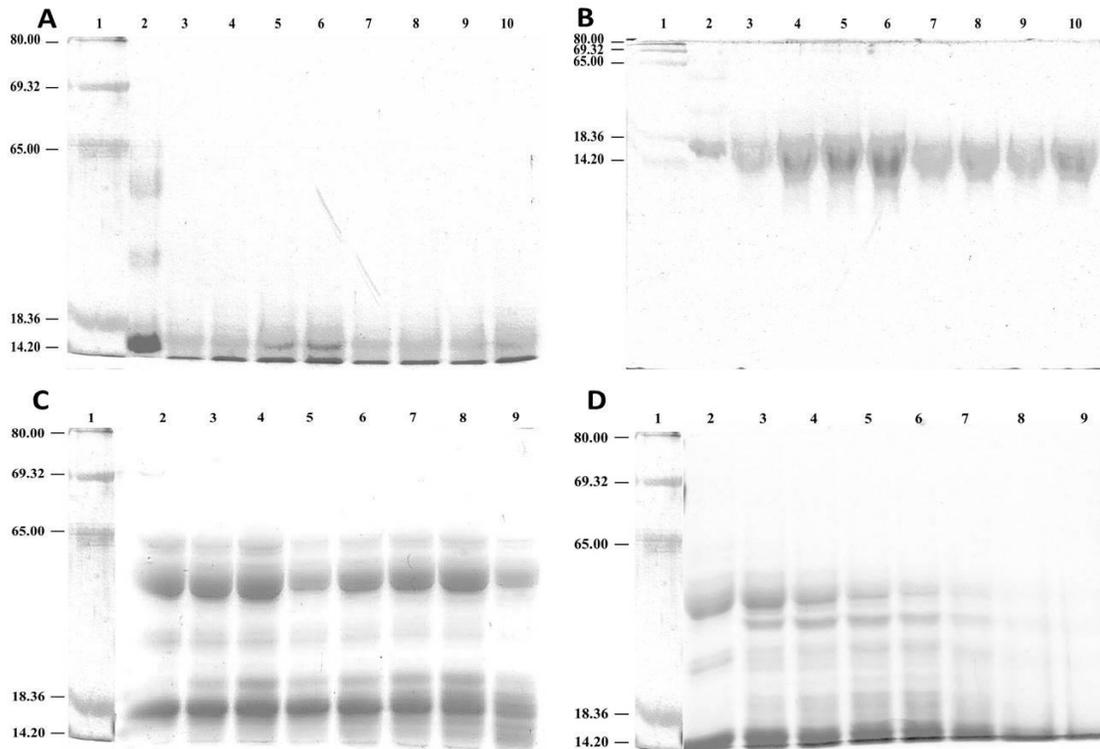


Figure 2: Reducing SDS-PAGE pattern of gliadins proteins subjected to different proteases at different times.

A: Corolase 7089 hydrolysis (12%) ,B: Corolase 7089 hydrolysis (15%) ,C: Tryptic hydrolysis (12%)

D: Peptic hydrolysis (12%)

1: Molecular Marker; 2: 0 min; 3: 10 min; 4: 30 min; 5: 60min; 6: 90min; 7: 120 min; 8: 150min; 9: 180 min.

Assessment of NG-specific IgG and IgE

Most of the food allergic reactions are mediated via immunoglobulin IgE and therefore, an attempt has been made to estimate the levels of specific IgE in the mice sera. As shown in figure 3, mice IP sensitized to NG produced significantly higher amounts of IgG and IgE than naïve mice IP sensitized to alum (there are no significant differences between naïve mice sensitized to alum and unsensitized mice –data not shown-). The production of NG specific IgG and IgE observed in sensitized mice ($p < 0.001$) as compared to naïve mice, indicates initiation of specific response to NG.

Assessment of systemic anaphylactic signs and body temperature

One week after the last IP sensitization, mice were challenged with IP injection of NG, PH, TH and C7089H. Severe symptoms were observed in NG sensitized mice challenged with native and hydrolyzed gliadins ($p < 0.001$). Naïve mice challenged with different gliadins displayed no symptoms (figure 4). The body temperature of IP challenged Balb/c mice was significantly decreased in experimental groups compared to their naïve groups ($p < 0.001$) (figure 5).

These results indicate a relationship between the decrease in body temperature and systemic anaphylaxis scores after elicitation of immediate hypersensitivity reactions.

NG mice challenged with PH and TH showed no significant difference in body temperature drops and clinical scores compared to NG mice challenged with NG.

This body temperature drop was significantly increased in NG mice challenged with C7089H compared to NG ($p < 0.01$) and PH ($p < 0.001$).

Assessment of intestinal anaphylaxis: *Ex vivo* experiments in Ussing chamber

To verify that a murine model of allergy could develop a local anaphylactic reaction, intestine tissues of naïve and sensitized mice were challenged *ex vivo* with NG, PH, TH and C7089H.

Compared to naïve mice, after serosal challenge with NG and different gliadins hydrolysates, an increased *Isc* was observed in jejunums of sensitized mice and challenged with NG, PH, TH and C7089H respectively (ΔI_{sc} :

11.03±0.52 vs 28.83±0.71; 11.13±0.44 vs 24.94±1.02; 11.08±0.40 vs 21.42±0.77; 10.90±0.45 vs 19.10±0.50 ($\mu A/cm^2$) ($p < 0.001$) (figure 6.A). The increased *Isc* response was correlated with increased tissue conductance which is a measure of the integrity of tight junctions. The conductance (ΔG) values change from 3.85 ± 0.36 to 10.19 ± 0.23 for NG, 3.75 ± 0.22 to 8.56 ± 0.28 for PH, 2.79 ± 0.14 to 8.01 ± 0.32 for TH and 2.64 ± 0.18 to 7.74 ± 0.23 for C7089H ($mmho/cm^2$) ($p < 0.001$) (figure 6.B).

In order to verify the gliadins-specific response, the tissues were challenged with non-specific protein (OVA), no changes in the *Isc* were observed (11.22 ± 0.18 vs 10.90 ± 0.16). This suggests an impairment of intercellular junctions of intestinal epithelium and implies that a local anaphylactic reaction occurs when gliadins interacts as an allergen with the intestinal immune system of gliadins-sensitized mice.

All mice sensitized with NG and challenged with PH, TH and C7089H presented significant decreased ΔI_{sc} values compared to ones challenged with NG ($p < 0.001$).

The deposit of glucose in the mucosal side induced an increased in the *Isc* values indicating tissue viability (data not shown).

Vascular leakage after IP challenge

Increased vascular permeability induced by vasoactive mediators such as histamine is a hallmark of systemic anaphylaxis.

Figure 7 shows that extensive Evan's Blue Dye extravasation was evident in footpads of NG sensitized mice and challenged with native and different gliadins hydrolysates, while naïve mice (sensitized with alum) showed uncolored footpads.

Intestinal pathology and villus length

Morphological changes in the epithelium due to the immunologic reaction were underscored by histological results demonstrating villus atrophy, increased mucosal inflammatory cells in jejunum, lymphocytes infiltration as well as goblet cells and crypt hyperplasia in sensitized mice (figure 8). The villus length of sensitized mice was significantly higher than that of naïve mice with values of 62.76 ± 1.72 μm vs 12.37 ± 1.81 μm respectively (figure 9).

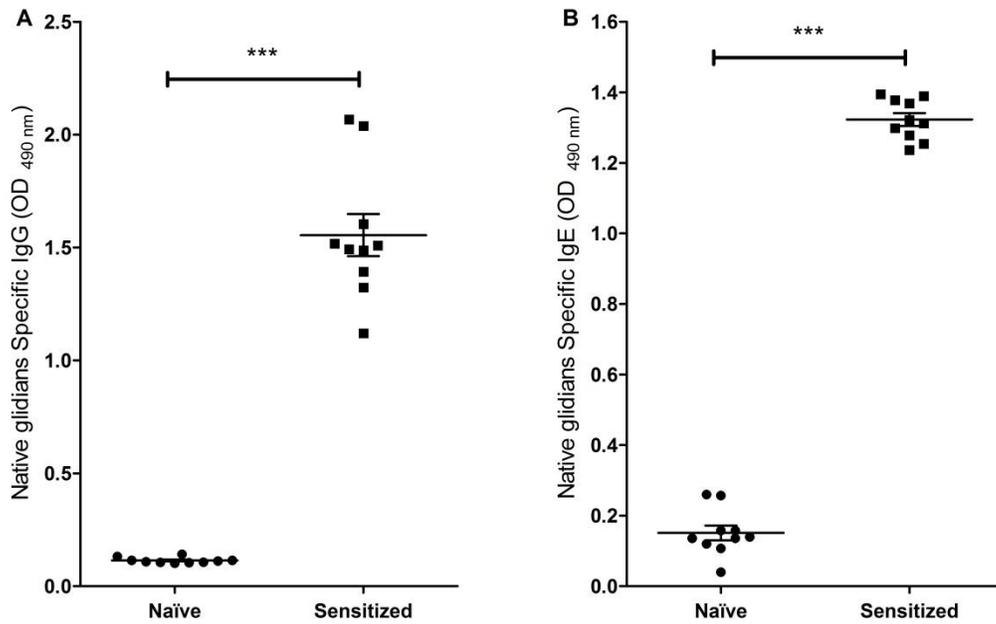


Figure 3: Native gliadins specific IgG (A) and IgE (B) induced in naïve and sensitized mice.
 Data are expressed as means ± SEM (***p*<0.001).
 (n= 10 mice *per* group).

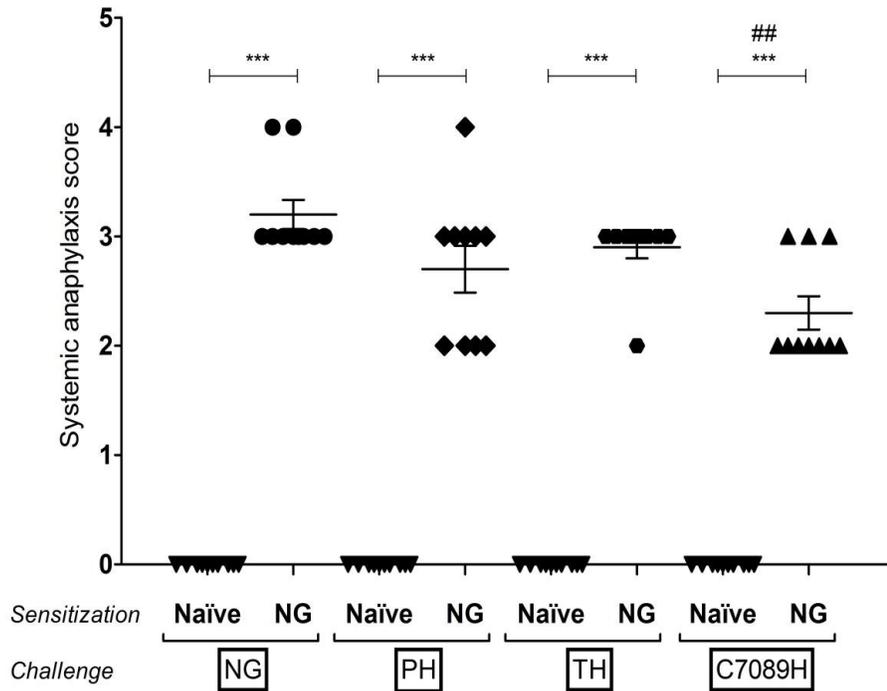


Figure 4: Systemic anaphylactic responses after IP challenge in naïve and sensitized mice.
 Data are expressed as means ± SEM
 *** *p* < 0.001 significance of difference between naïve vs NG;
 ## *p* < 0.01 significance of difference between NG vs PH, TH and C7089H.
 (n= 10 mice *per* group)

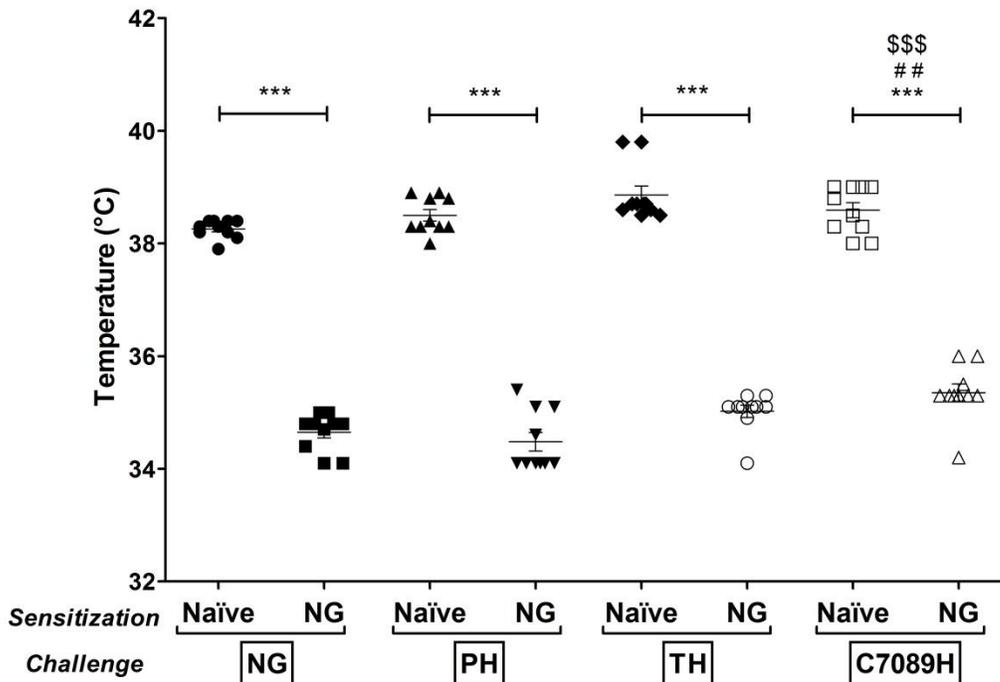


Figure 5: Rectal temperature after IP challenge in naïve and sensitized mice.

Data are expressed as means \pm SEM

*** $p < 0.001$ significance of difference between naïve vs NG;

$p < 0.01$ significance of difference between NG vs PH, TH and C7089H.

\$\$\$ $p < 0.001$ significance of difference between PH vs TH and C7089H.

($n = 10$ mice per group)

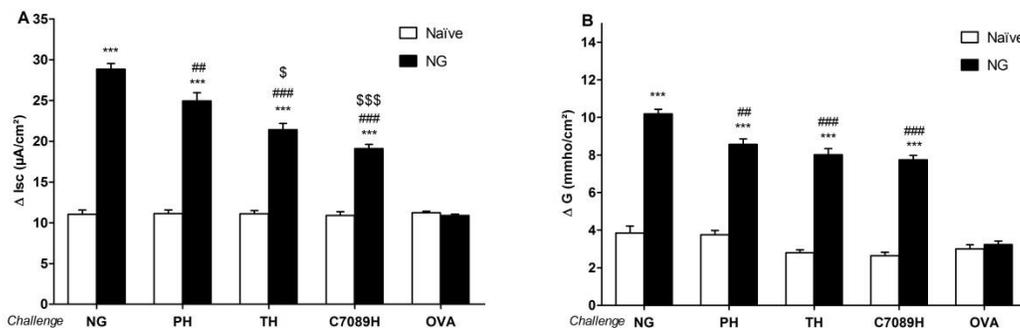


Figure 6: Effect of native and hydrolyzed gliadins on the short circuit current (Isc) (A) and conductance (G) (B) in Ussing chamber measured in mice jejunal fragments sensitized intraperitoneally with native gliadins.

Data are expressed as means \pm SEM

*** $p < 0.001$ significance of difference between naïve vs NG.

$p < 0.01$; ### $p < 0.01$ significance of difference between NG vs PH, TH and C7089H.

\$ $p < 0.05$; \$\$\$ $p < 0.001$ significance of difference between PH vs TH and C7089H.

($n = 10$ mice per group)

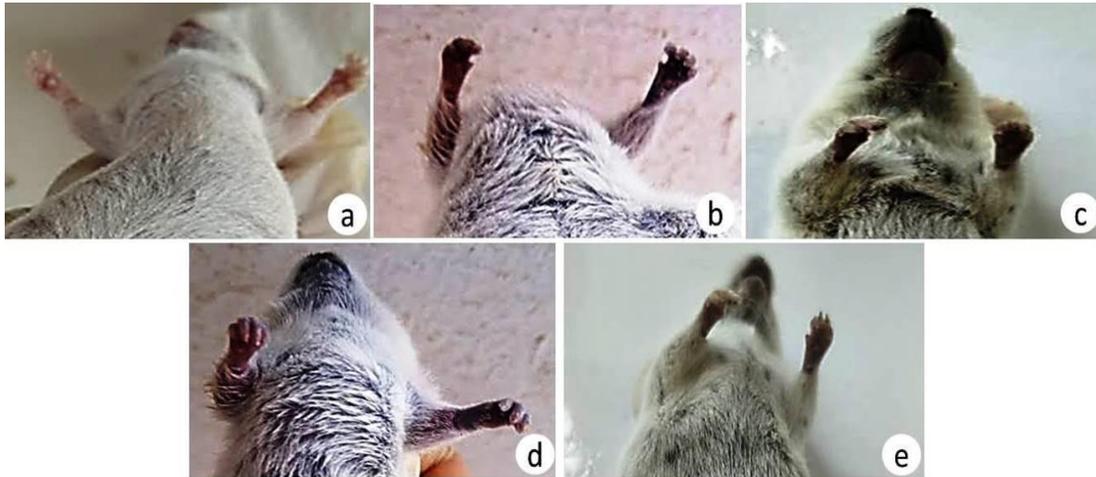


Figure 7: Gliadins antigen induced vascular leakage.

Photographs show mice footpads after Evan's Blue dye/antigen administration.

Marked vascular permeability was observed in sensitized mice after IP challenge with native and hydrolyzed gliadins (b,c,d,e). No vascular leakage was observed in alum sensitized mice (a); (b): NG; (c): PH; (d): TH; (e): C7089H. Results represent five mice from each group.

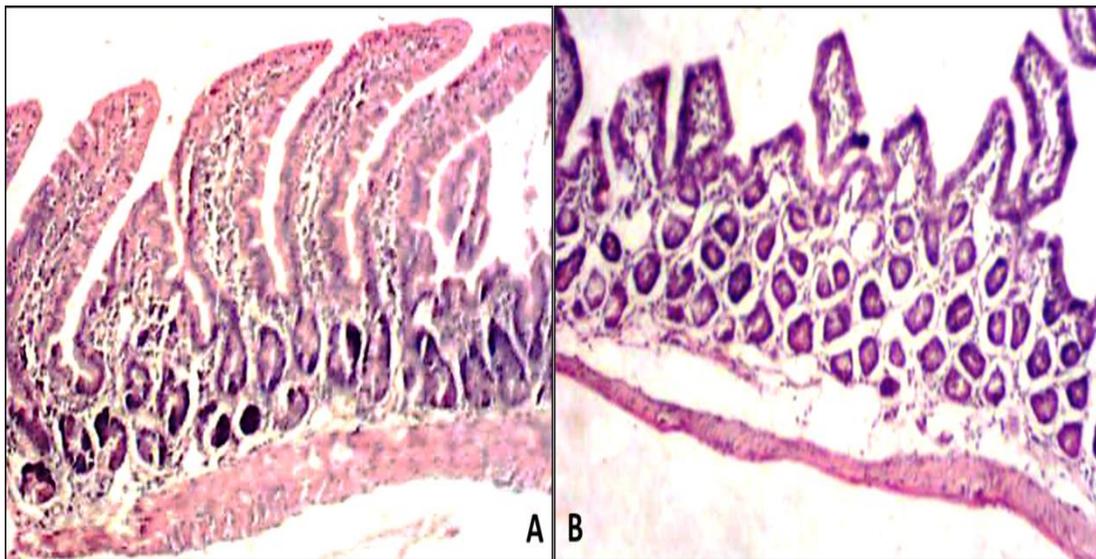


Figure 8: Representative histological sections of the jejunum from naïve mice (A) and sensitized mice (B) colored with hematoxylin and eosin observed with light microscopy (G×40).

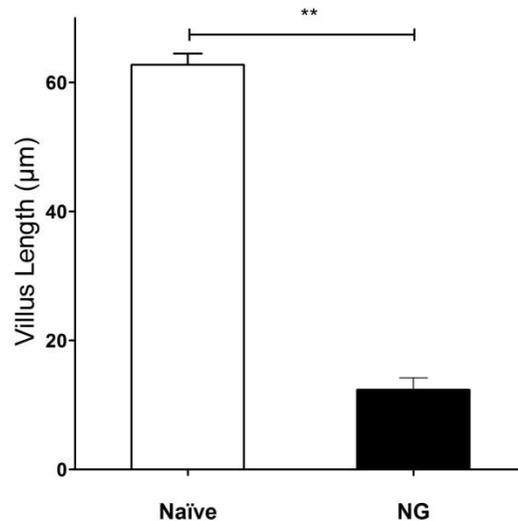


Figure 9: Evaluation of villus length in jejunum fragments of mice sensitized intraperitoneally with native gliadins compared with naïve mice.

Data are expressed as means \pm SEM (** $p < 0.01$).

Discussion

In the present study we used a murine model of anaphylaxis in which native gliadin-specific IgE was induced by intraperitoneal sensitization, and systemic anaphylaxis symptoms were provoked by IP challenge with native and hydrolyzed gliadins. The symptoms involved multiple target organs, including the skin, gastrointestinal tract and respiratory system (Li et al., 2000).

Firstly, our results showed that the degree of hydrolysis of native gliadins with corolase 7089 was more significantly higher compared with gastrointestinal proteases (pepsin and trypsin).

This was in agreement with (Kong et al., 2007) who demonstrated that treatment of wheat gluten with pepsin and trypsin has lower degree of hydrolysis compared to alcalase treatment. Corolase 7089 is a neutral protease that contains exclusively endopeptidase activity. It is obtained from *Bacillus subtilis* cultures. Because corolase 7089 is an endopeptidase enzyme and is less specific than pepsin and trypsin, we hypothesized that corolase 7089 could hydrolyze proteins more completely than animal digestive enzymes. Indeed, the results of Watanabe et al., (1994) confirmed that wheat allergens are sometimes characterized by their high stability against digestive enzymes, with their epitope structures remaining unchanged.

Secondly, our results demonstrated that NG-sensitized mice displayed higher concentrations of specific IgG and IgE as determined by ELISA method. These results corroborate those of

(Gourbeyre et al., 2012) who reported that mice sensitized with NG and deamidated gliadins (DG) displayed higher concentration of NG and DG specific IgE antibodies. However, levels of anti-DG IgE were higher. Authors concluded that wheat gliadins modified by deamidation are more efficient than native gliadins in inducing a Th2 response in Balb/c mice experimentally sensitized to wheat allergens.

The IgE-dependent allergic reaction is composed of two phases. The first is an inducing step, where the immune system of the host is sensitized by the allergen, resulting in production of specific IgE anti-allergen antibodies, which are then fixed by mast cells in target organs. The second step is a triggering phase mediated by the allergens binding to these IgE antibodies and stimulating mediator (histamine) release from mast cells (Yu et al., 2016).

Our results showed that in almost all of experimental mice, systemic anaphylaxis symptoms appear within 30 min after IP challenge with either native or hydrolyzed gliadins, these symptoms include scratching and rubbing around the nose and head, puffiness around the eyes and mouth, pilar erecti, reduced activity, and/or decreased activity with increased respiratory rate, wheezing, labored respiratory, and cyanosis around the mouth and the tail, as well as increased vascular leakage. However less intense symptoms were observed in mice challenged with corolase 7089, these results concord with the study of Gourbeyre et al., (2012) who showed that regarding the severity of clinical

signs following IP challenge with native and hydrolyzed gliadins, there were no significant differences between groups used for sensitization in the anaphylaxis scores. We noted also, that IP challenge of native and hydrolyzed gliadins induced a more pronounced temperature drop in sensitized mice. The ability of corolase 7089 to induce less intense clinical symptoms is confirmed by its ability to provoke less brutal temperature drops.

The results of Ussing chamber stimulation with native and hydrolyzed gliadins of jejunal fragments of the experimental group induced an increase in the ΔI_{sc} and ΔG values compared to the naïve group. This suggests an impairment of intercellular junctions of intestinal epithelium and implies that a local anaphylactic reaction occurs when food-proteins interact as an allergen with the intestinal immune system of food-proteins sensitized mice. This increase is probably due to a secretory response and might reflect local anaphylactic responses. The typical responses of the intestinal epithelium to allergens are an increase in watery diarrhea due to the stimulation of chloride secretion, and increased protein inward permeability from lumen to blood (Baron et al., 1988). Most studies concluded that a significant increase in I_{sc} was recorded after a basolateral food allergen challenge of intestine from allergen-sensitized mice (Negaoui et al., 2009; El Mecherfi et al., 2015; Guendouz et al., 2017). Same results were observed in infants jejunal responses to bovine Beta Lactoglobulin (β -Lg) during the active phase of cow's milk allergy (Saidi et al., 1995) and in mice jejunal fragments, where a significant increase in the I_{sc} value was registered due to electrogenic chloride secretion (Negaoui et al., 2009). These studies showed that the increase in the I_{sc} was associated with significant increases of the epithelium conductance. Several studies have reached similar conclusions regarding the effect of β -Lg on the epithelium conductance, concluding that immunization alters the tight junction and increases the paracellular permeability of the intestinal epithelium (Terpend et al., 1999; Negaoui et al., 2009; El Mecherfi et al., 2015; Grar et al., 2015). Conductance indicates the passive movement of ions across the epithelium mainly via paracellular pathway (Benjamin et al., 2000). The I_{sc} and conductance (G) measurements in Ussing chamber studies have shown that electrogenic Cl^- secretion is not only important to normal digestive physiology, but is also a marker of enterotoxic and inflammation-mediated

secretory diarrhea (Clarke, 2009).

Mast cells are key effector cells of type I allergic reactions as well as of other inflammatory processes (Hagenlocher and Lorentz, 2015). The role of mast cells in regulation of epithelial ion secretion has been well studied, and it has been clearly established that mast cell mediators such as histamine and prostaglandins can act via specific receptors on the intestinal epithelium to initiate chloride ion secretion (Crowe and Perdue, 1992); the driving force for water secretion leading to diarrhea.

In our study, stimulation of tissues of NG-sensitized mice with different hydrolysates reduced I_{sc} values compared to stimulation with NG. These findings could be explained by the fact that pepsin, trypsin and corolase 7089 might have cleaved some of the allergenic epitopes of NG. This could be explained by proteolytic destruction of the gliadin linear epitope(s) in the applied experimental conditions. Also, Abe et al., (2014) examined the permeability of Horseradish Peroxidase (HPR) through the jejunum of DG administered mice. The administration of untreated gliadins (UG) significantly enhanced the intestinal permeability, whereas that of DG was scarcely altered. They concluded that the oral administration of DG to gliadin-sensitized mice suppressed enhancement in intestinal permeability. In contrast, preliminary results of Bouchaud et al., (2016) presented in 4th Food Allergy and Anaphylaxis Meeting (FAAM 2016 Rome, Italy) showed an increase of intestinal permeability after oral gavage challenge with deamidated and hydrolyzed gliadins.

In celiac patients, most prominent electrophysiological increase was found in the biopsies with partial atrophy (Reims et al., 2002). Morphological changes in the epithelium due to the immunologic reaction were underscored by histological results demonstrating villus atrophy and crypt hyperplasia as well as a greater increase of mucosal inflammatory cells in jejunum. These are findings in agreement with several published studies which showed that food allergy in mice is characterized by villus atrophy and goblet cell hyperplasia, as well as infiltration of IgE-positive mast cells performing degranulation in the jejunum and increased histamine release (Asero et al., 2007; Pali-Schöll et al., 2008). In addition hand, Gliadin-induced enteropathy can alter or change the clinical features of small intestine jejunum sections morphology (Papista et al., 2012).

CONCLUSION

To our knowledge, there are no published reports on the effects of native and hydrolyzed gliadins on the electrophysiological intestinal parameters. In this study, we demonstrated that NG modified by enzymatic process with pepsin, trypsin and corolase 7089 is able to elicit systemic and intestinal anaphylaxis reactions, with the corolase 7089 hydrolysates as the most efficient treatment capable of attenuating these reactions. These findings provide useful information regarding the efficiency of enzymatic treatment to alter gliadins allergenicity.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

This work was supported by the Directorate General for Scientific Research and Technological Development (DGRSDT, MESRS, Algeria).

AUTHOR CONTRIBUTIONS

YB designed and performed the experiments, data analysis and also wrote the manuscript. YB and AH performed animal treatments and tissue collection, NM provided scientific advice, DS and OK designed experiments and revised the manuscript. All authors read and approved the final version.

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