

Effect of heat, pH and shear on digestibility and antigenic characteristics of wheat gluten

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Abstract Processing-induced conformational changes may affect digestibility and antigenic potential of food proteins. In vitro gastrointestinal digestibility of gluten proteins was established after treatment at various pH (3 or 7), temperature (room temperature or 100 °C) and shear (0 or 1500 s⁻¹). Electrophoretic patterns (SDS-PAGE) of the resulting hydrolysates and antigenicity (ELISA and immunoblotting) of gliadin fraction of the proteins were also studied. Digestibility was positively correlated with α -helix/ β -sheet ratio. Lower antigenic reaction shown by digested hydrolysate of gluten samples treated at pH 3 and room temperature was ascribed to its acidic deamidation and improved digestibility. Further heating to 100 °C slightly increased antigenicity. In contrast, hydrolysate of gluten at pH 7 and room temperature exhibited highest antigenicity, attributed to partial resistance of α - β -gliadin to digestion and appearance of some new potentially antigenic polypeptides. However, heating at 100 °C caused heat-induced protein aggregation, consequently lowered digestibility and availability of antigenic components resulting in a minimum (60 % reduction) antigenicity. Overall, shear had no effect on digestibility and antigenicity irrespective of pH and temperature. Thus, antigenic potential of gliadins can be minimized by selecting appropriate parameters during processing.

Keywords Antigenicity · Digestibility · Gluten · Heat · Shear · pH

Introduction

Wheat is a staple food in many parts of the world which is consumed as various products. Extensive food use of wheat is attributed to viscoelastic properties of its gluten protein. Gluten is a major seed storage protein comprising 80 % of the total wheat protein. It consists of alcohol-soluble monomeric gliadins (α - β -, γ - and ω -gliadins) and polymeric glutenin. Glutenin consists of a high molecular weight glutenin subunit (HMW-GS) and a low molecular weight glutenin subunit (LMW-GS), which are interlinked by disulphide (SS) bonds. Due to their unique physico-chemical and nutritional properties, availability and low cost, gluten is used not only in cereal products but also in other foods such as sausages, ice cream and even some drugs [1]. Despite its wide use in food products, gluten creates adverse immune reactions in some individuals. The most prevalent condition is coeliac disease, which is an autoimmune disorder of small intestine triggered by ingestion of gliadin and characterized by mucosal inflammation, villous atrophy and consequent malabsorption of various nutrients [2]. Some IgE-mediated reactions such as baker's asthma, exercise-induced anaphylaxis and atopic dermatitis are also caused by gluten [3].

Such allergic reactions to food occur either by primary contact of food protein antigens to components of the immune system and initial sensitization or through later repetitive exposure to the same antigens and elicitation of allergic reaction. Since most of the immune sensitization to antigens takes place via gastrointestinal tract, digestibility of food protein antigens and their permeability into the immune system are key factors that may affect their antigenic potential [4]. A characteristic feature of most food antigens is their resistance to gastrointestinal digestibility allowing the preservation of sufficient intact epitopes

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to bind with a specific antibody. Therefore, understanding digestibility of food protein antigens and their post-digestion antigenic potential is fundamental to managing food allergenicity.

Foods undergo various processing to produce diversified end products that affect conformation of food proteins and, in turn, their susceptibility to gastrointestinal digestion [5]. Processing may increase [6, 7] or decrease [8] digestive susceptibility of food proteins which may, in turn, affect antigenic consequences. Wheat is always consumed after some degree of processing, e.g. baking, cooking, extrusion, and it is important to know how these processes affect the digestibility and antigenicity of gluten proteins. Some studies [9, 10] have shown that *in vitro* digestion of unheated bread dough showed almost complete disappearance of IgE-binding protein which persisted in bread crumb and crust. Heat-induced covalent protein aggregation during baking rendered the proteins less susceptible to proteolysis, thereby allowing passage of large IgE-reactive fragments through gastrointestinal tract where they could elicit more allergic reaction. Another important processing factor is pH, which can significantly affect the physico-chemical properties of gluten [11, 12] in products such as sourdough bread (pH < 5) [13]. Such variations in pH may affect digestive stability and consequently antigenicity of gluten proteins. Shear is another processing factor usually embedded in processes such as mixing, centrifugation and ultra-filtration that can potentially affect the physico-chemical properties of gluten [14]. However, shear alone may have no effect on the conformational and antigenic features of gluten proteins, but could have an influence in combination with particular pH and temperature [15]. To the best of our knowledge, no study has focused on the effect of different pH and shear in combination with different temperatures on the digestibility as well as antigenic potential of digested gluten proteins and, therefore, this work was aimed at addressing this gap.

Materials and methods

Preparation and selection of samples

Extracted gluten from plain wheat flour [16] was freeze-dried and ground to powder using a mortar and a pestle and sieved (no. 50) to obtain a fine powder. Exactly 8 % (w/w) gluten dispersion (concentration chosen on the basis of average gluten content in wheat flour [17]) was prepared in a citric acid-phosphate buffer at different pH values (3, 5 or 7) and then treated at different shear rates (500, 1000 or 1500 s⁻¹) and temperatures (80, 90 or 100 °C) in a pressure cell (CC25/PR-150) of a rheometer (Physica MCR 301 series, Anton Paar, GmbH, Graz, Austria) as described

previously [18]. Gluten dispersion in Milli-Q water (pH 6.6) without any treatment (shear and heat) served as a control sample. Selected treatments (pH 3, room temperature and 0 s⁻¹; pH 3, 100 °C and 0 s⁻¹; pH 7, room temperature and 0 s⁻¹; pH 7, 100 °C and 0 s⁻¹; and pH 7, 100 °C and 1500 s⁻¹) were chosen based on the results of our reported work [15]. These conditions of high and low antigenicity were selected for further investigation into their digestibility and consequent impact on antigenicity.

Quantification of α -helix and β -sheet ratio (α/β)

Fourier transform infrared spectroscopy (FTIR) of both treated and control samples was performed by using a FTIR spectrometer (IRAffinity-1, Shimadzu Corp., Kyoto, Japan) as described previously [15]. Briefly, gluten powder (4 mg) was mixed with spectroscopy-quality KBr (60 mg) and homogenized with mortar and pestle. The mixture was pressed into pellets (1- to 2-mm-thick films) with a 10-ton hydraulic press. FTIR spectra of the pellets were collected between the wavelengths of 400–4000 cm⁻¹, with 20 scans at a resolution of 4 cm⁻¹ using Happ-Genzel apodization. After smoothing the spectra (7 points), the bands for α -helix and β -sheet in amide I region (1600–1700 cm⁻¹) were identified [19, 20] and quantification of α -helix/ β -sheet was achieved by estimating respective peak areas from the peak table of the spectra obtained using IRsolution software (Shimadzu Corp.).

Simulation of gastrointestinal digestion

In vitro digestion of all gluten samples was performed as described before [9] with some modifications. Briefly, exactly 120 mg of sample was suspended in 8 ml of 0.2 N HCl (pH 2.2) containing 0.05 mg/ml of pepsin. One hour after peptic digestion at 37 °C in a shaking water bath, mixture of 2.30 ml of 1 M boric acid and 0.5 N NaOH containing pancreatin (0.25 mg/ml) was added. The pH of the mixture was finally adjusted to 7.6 with 1 M NaOH. After 3 h of pancreatic digestion, the hydrolysate thus obtained was heated in boiling water bath for 10 min to inactivate the enzyme and then centrifuged (3000g) in a bench-top centrifuge (RT7, Sorvall, Newtown, CT). The resultant supernatant was stored at -80 °C for further analysis.

Determination of degree of hydrolysis

The degree of hydrolysis (DH) of the digested gluten samples was determined as the ratio of the percentage of 10 % trichloroacetic acid (TCA)-soluble nitrogen to total nitrogen in the sample [21]. To 5 ml of hydrolysate removed at the end of pancreatic digestion, an equal volume of 20 % TCA was added so as to obtain 10 % TCA-soluble and

TCA-insoluble fractions. After standing for 1 h, the mixture was centrifuged at 3000g using a bench-top centrifuge (RT7, Sorvall) at room temperature (20 °C). The supernatant was then analysed to determine nitrogen content by Kjeldahl method [22]. The DH was calculated as:

$$\text{DH} = \frac{(\text{Soluble nitrogen in 10 \% TCA in hydrolysate} - \text{soluble nitrogen in 10 \% TCA in sample without hydrolysate}) \times 100 \%}{\text{Total nitrogen in dispersion}}$$

Enzyme-linked immunosorbent assay

Antigenicity of the hydrolysates obtained was established by sandwich enzyme-linked immunosorbent assay (ELISA) using Ridascreen® Gliadin ELISA kit (R-Biopharm AG Darmstadt, Germany). An aliquot of 100 µl of supernatant from the hydrolysates was diluted with 1 ml of sample diluent. Exactly 100 µl of diluted samples was added to each separate duplicate well pre-coated with gliadin antibody. After 30-min incubation at room temperature (20 °C), the liquid was poured out and washed three times with a washing buffer (R-Biopharm AG Darmstadt, Germany) to remove unbound antigens. The plate was then tapped against absorbent paper to ensure complete removal of liquid from wells. After that, exactly 100 µl of diluted horse radish peroxidase (HRP)-conjugated detecting antibody was added to each well and incubated for 30 min at room temperature. Thereafter, the plate was washed three times and again tapped upside down against absorbent paper. Precisely, 50 µl of the substrate and 50 µl of the chromogen [3,3', 5,5'-tetramethylbenzidine (TMB)] were added to each well followed by 30-min incubation at room temperature in dark to allow for colour development. Finally, 100 µl of stop reagent (1 N H₂SO₄) was added to each well, and the absorbance was measured at 450 nm using a microplate reader (iMark™, Bio-Rad Laboratories, NSW, Australia). The antigenicity was calculated from the standard curve and expressed as equivalent of mg of gliadin/kg of sample.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Reducing SDS-PAGE of hydrolysates from various treated gluten samples, control as well as an unhydrolysed gluten standard was employed in a study of the electrophoretic patterns. Protein fractions from supernatants of each hydrolysates were extracted by chloroform-methanol precipitation method [23]. Briefly, 100 µl of protein samples was mixed with 400 µl of methanol in a 1.5-ml Eppendorf tube and vortexed and then 100 µl of chloroform and 300 µl of distilled water were added and additionally vortexed. After centrifugation at 14,000g, the aqueous supernatant was

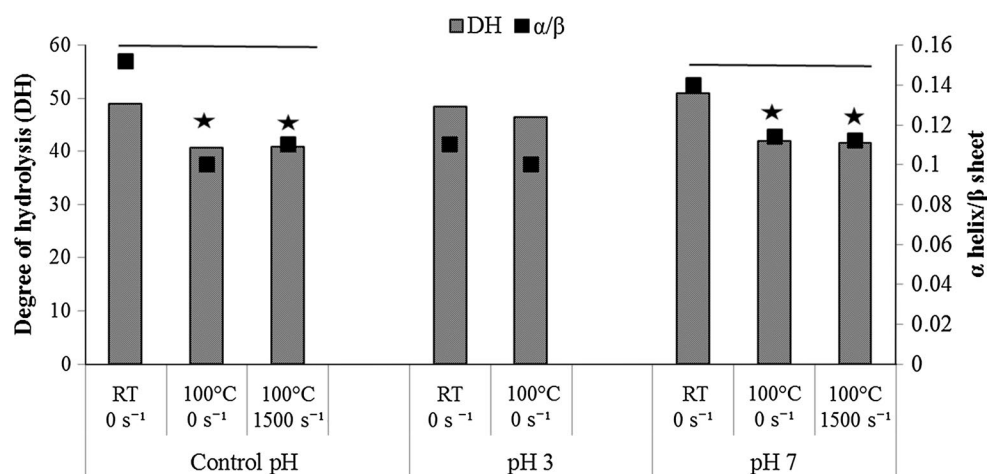
discarded. Again 400 µl of methanol was added, mixed thoroughly and centrifuged at 14,000g. Finally, the methanol was discarded leaving the protein pellets at the bottom. The pellets were air-dried and solubilized in 20 µl of Tris-Tricine sample buffer (200 mM Tris-HCl, pH 6.8,

2 % SDS, 40 % glycerol, 0.04 % Coomassie Brilliant Blue G-250, 2 % β-mercaptoethanol). Precisely, 1 mg of gluten was suspended in 200 µl sample buffer and served as a standard. Exactly, 20 µl of samples, gluten standard and molecular weight marker were loaded onto a 16.5 % Mini-PROTEAN® Tris-Tricine Gel (Bio-Rad Laboratories, NSW, and Australia). Electrophoresis was performed at a constant voltage of 100 V for 100 min. Then, the gels were immersed overnight in the staining solution composed of 0.025 % Coomassie brilliant blue, 40 % methanol, 7 % acetic acid and 50 % distilled water. Thereafter, the gel was destained with a destaining solution containing 40 % methanol, 7 % acetic acid and 53 % distilled water for 2 h followed by immersing in distilled water until the background became clear. Gel images were taken using a ChemiDoc imager (Chemidoc MP, Bio-Rad Laboratories, NSW, Australia).

Immunoblot analysis

Immunoblotting of different hydrolysates was carried out following a previously reported method [24] with some modifications. After electrophoresis, the proteins were electrotransferred onto a nitrocellulose membrane (0.2 µm) using a Mini Trans-Blot® cell (Bio-Rad Laboratories, NSW, Australia) and a Tris-glycine transfer buffer system (25 mM Tris, 192 mM glycine and 20 % (v/v) methanol, pH 8.3). The membrane was washed three times with Tris-buffered saline (20 mM Tris, 500 mM NaCl, pH 7.4) and then incubated in a blocking solution (1 % casein) for 1 h at room temperature (20 °C). The washed and treated membrane was transferred to a diluted solution (1:1000 in the blocking buffer) of an anti-gliadin primary antibody (IgG) (Antibodies-online GmbH, Georgia, USA) for 1 h at room temperature. Thereafter, the blot was washed five times with Tris-buffered saline containing 0.1 % Tween 20 (TTBS) with gentle agitation. The membrane was then incubated in secondary antibody (goat anti-rabbit IgG) (Biorbyt Ltd, Cambridgeshire, UK) conjugated with horseradish peroxidase (HRP) at a dilution of 1:3000 in TTBS for 1 h at room temperature. The blot was then washed five times in TTBS. Antibody complexes captured by the immobilized target protein were detected by

Fig. 1 Degree of hydrolysis (DH) of control and treated gluten samples. The values in this figure are the mean of triplicates. The lines above signify the differences (asterisk) at $P > 0.05$. RT room temperature ($\sim 20^\circ\text{C}$)



enhanced chemiluminescence detection system using Clarity™ Western ECL Blotting Substrate (Bio-Rad Laboratories, NSW, Australia). Images were taken using a Chemi-Doc imager (ChemiDoc MP, Bio-Rad Laboratories, NSW, Australia).

Statistical analysis

The experiment was designed as a randomized block, full factorial design with pH as the main plot and shear and temperature as the subplots. This block was replicated with three subsampling resulting in at least six independent observations ($n \geq 6$). The results of the various determinations were analysed using the general linear model (GLM) procedure of SAS (SAS, 1996). The level of significance was set at $P = 0.05$.

Results and discussion

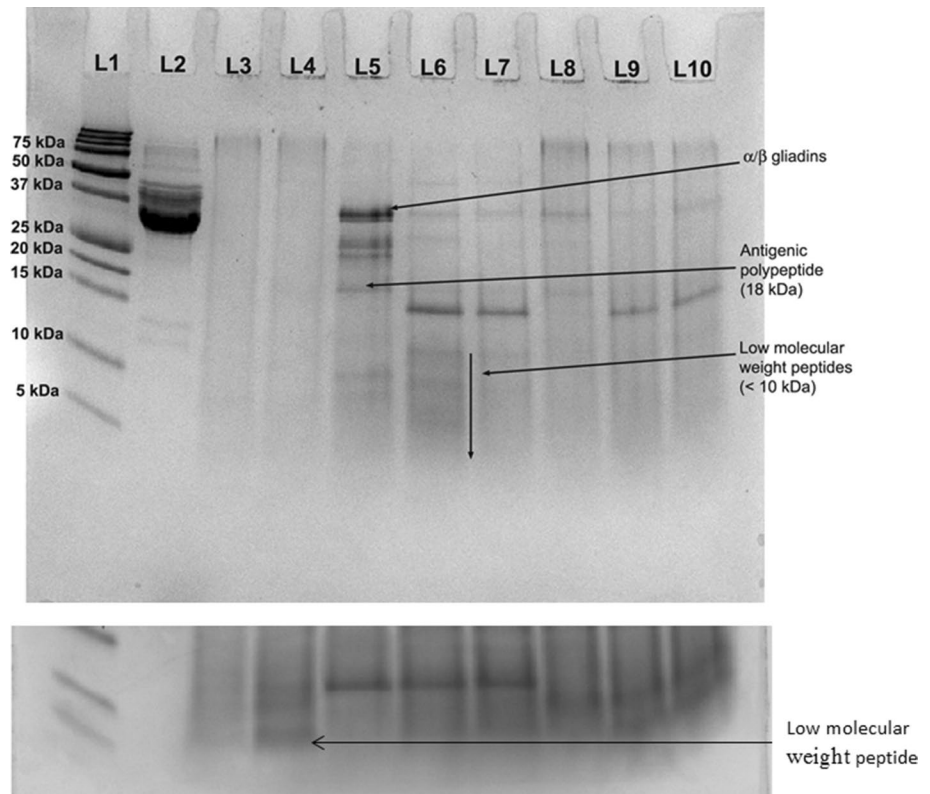
Digestibility of gluten treated at different pH, shear and heat

In vitro digestion of both control and treated gluten samples was performed to study the effect of various pH, temperature and shear on the extent of proteolysis of gluten proteins. Degree of hydrolysis (DH) was measured as 10 % TCA-soluble nitrogen content, which consisted of partially digested gliadins as well as small and/or large polypeptides released as products of enzymatic breakdown. Temperature significantly ($P < 0.05$) affected DH of gluten, whereas pH and shear had no remarkable effect (Fig. 1). At pH 7, room temperature and 0 s^{-1} gluten had as high DH as in control sample, and heating to 100°C caused significant ($P < 0.05$) reduction in DH, which could be attributed to heat-induced physico-chemical changes [9, 15, 25]. One such a change involves variations in conformations of the

secondary structure (α -helix and β -sheet) of the protein. Quantification of α -helix and β -sheet components from the FTIR spectra showed that at both, control pH (6.6) and pH 7, and room temperature, gluten had a higher α -helix content. Increasing the temperature to 100°C brought about a decrease in α -helix content at the expense of the β -sheet conformation (reduction of α/β ratio) suggesting a positive correlation between DH and α/β (Fig. 1). This could be attributed to the comparatively more efficient access by digestive enzymes to flexible helical conformation of gluten than the β -sheet structure. Similar findings have also been reported previously [26]. Such decreased accessibility of digestive enzymes to the β -sheet conformation can also be ascribed to the presence of β -branched amino acids (threonine, valine and isoleucine) in gluten, which are not preferable sites of action for trypsin [27]. Another possible reason for reduced digestibility at high temperature could be due to lowered surface hydrophobicity, as was reported in our earlier work [15], which are the active sites for peptic and chymotryptic digestion [28]. Moreover, at high temperature gluten proteins undergo disulphide-bonded aggregation as evident in our previous study [15], which provides the protein with a more compact structure that could have rendered it partly inaccessible to digestive enzymes [25, 29].

On the other hand, DH of sample at pH 3, room temperature and 0 s^{-1} was as high as control (Fig. 1) although the α/β ratio was not as high. Such higher DH at pH 3 without a substantial change of α/β ratio could be ascribed mainly to the effect of deamidation. It is well known [15, 30, 31] that gluten undergoes acidic deamidation at low pH with a concomitant partial hydrolysis of peptide bonds and such a deamidation results in improved digestibility [26, 32]. However, further increase of temperature to 100°C caused slight decrease ($P > 0.05$) of DH that could be associated with covalent cross-linking among the gluten proteins that in turn reduced its accessibility to digestive enzymes.

Fig. 2 Reducing SDS-PAGE pattern (after in vitro digestion) of gluten proteins subjected to different pH and temperatures and shear. *L1* molecular marker, *L2* undigested gluten standard, *L3* pH 3 room temp. 0 s^{-1} , *L4* pH 3 $100\text{ }^{\circ}\text{C}$ 0 s^{-1} , *L5* pH 7 room temp. 0 s^{-1} , *L6* pH 7 $100\text{ }^{\circ}\text{C}$ 0 s^{-1} , *L7* pH 7 $100\text{ }^{\circ}\text{C}$ 1500 s^{-1} , *L8* control, *L9* control pH $100\text{ }^{\circ}\text{C}$ 0 s^{-1} , *L10* control pH $100\text{ }^{\circ}\text{C}$ 1500 s^{-1}



Gel electrophoretic analysis

Electrophoretic patterns of hydrolysed gluten samples obtained by reducing SDS-PAGE (Fig. 2) varied as did the DH (Fig. 1) as a consequence of the various treatment conditions. Out of all main factors, pH and temperature influenced the gel electrophoresis patterns greatly, while shear did not have any effect. Undigested gluten (standard) showed typical bands for gluten proteins— α - β -gliadins (MW 28–35 kDa), γ -gliadins (MW 35 kDa), ω 1,2-gliadins (MW 45 kDa), ω -5gliadins (MW 55–60 kDa), HMW-GS (MW 65–90 kDa) and LMW-GS (MW 30–45 kDa). Similar to the control sample (Lane 8), hydrolysis of gluten at pH 7, room temperature ($20\text{ }^{\circ}\text{C}$) and 0 s^{-1} (Lane 5) produced polypeptides corresponding to 25, 18 kDa and some low molecular weight (<10 kDa) fragments. However, the S-rich prolamins, α - β -gliadins underwent a partial hydrolysis (Fig. 2), which could be attributed to creation of intramolecular disulphide bonds, which rendered their globular structure partly inaccessible to proteases. A similar finding has been reported in another study [9]. In contrast, ω -gliadins, lacking cysteine residues and intramolecular S–S bonds, were more susceptible to proteolysis and were completely hydrolysed as evident by the absence of corresponding bands in the gel (Fig. 2). However, electrophoretic patterns of the hydrolysates from heated samples at pH 7 (Lanes 6 and 7) and

control pH (Lanes 9 and 10) were considerably different to those obtained from the corresponding unheated samples (Fig. 2). Digestion of heat-treated samples resulted in the formation of another prominent band at 13 kDa representing an intermediate polypeptide, which was absent/very faint in unheated samples. These additional polypeptides could be a result of partial proteolysis of any of the gluten component, which could otherwise be completely hydrolysed into low molecular weight fractions (<10 kDa) as observed in unheated samples (Lane 5 for pH 7 and Lane 8 for control). Such an incomplete proteolysis of heated samples was also supported by their lower DH value (Fig. 1) and could be attributed to heat-induced conformational modifications such as covalent aggregation and Maillard reactions [10].

Interestingly, gel electrophoretic patterns of samples adjusted to pH 3 were different from those of pH 7 and control pH. Unheated samples at pH 3 underwent extensive hydrolysis to very low molecular weight peptides, which were not apparently retained in the gel. Moreover, contrasting to other pH, all the gliadins (including S-rich components) were completely hydrolysed in the samples at pH 3. Such an extensive hydrolysis that yielded very low molecular fragments in samples at pH 3 could be attributed not only to enzymatic breakdown but also to the effect of acidic deamidation. Our earlier work [15] showed that at low pH gliadins underwent acidic deamidation with partial

Table 1 Antigenicity of in vitro digested gluten treated at different temperature, pH and shear rate

pH	Temperature	Gliadin antigenicity equivalent (mg/kg)	
		Shear (s^{-1})	
		0	1500
Control (6.6)	RT	18.8 ^a	–
	100	11.47 ^b	10.63 ^a
3	RT	12.46 ^b	–
	100	16.12 ^c	–
7	RT	19.69 ^a	–
	100	8.90 ^d	9.32 ^b
SEM	0.44		

All values presented are the means of six independent observations ($n = 6$)

Significant differences ($P < 0.05$) between means across the same column are indicated by different letters

SEM pooled standard error of the mean, RT room temperature ($\sim 22^\circ\text{C}$)

hydrolysis of peptide bonds into very low molecular peptides. Deamidation is a post-translational modification of a protein resulting in conversion of glutamine (Gln) and asparagine (Asn) to glutamic acid (Glu) and aspartic acid (Asp), respectively, by the removal of the amide functional group [30, 33]. Thus, acidic deamidation and enzymatic breakdown synergistically degraded the gluten proteins almost completely to produce only very low molecular weight peptides.

Antigenicity of hydrolysed gluten

Antigenic prospective of gliadins in the hydrolysed gluten samples was assessed by ELISA (Table 1) and immunoblotting (Fig. 3) against anti-gliadin antibody, which, in general, varied with different pH and temperature, while shear had no effect. Highest antigenic value determined by ELISA was recorded for hydrolysates from gluten samples at room temperature, 0 s^{-1} and pH 7 that was similar to the control (pH 6.6). Immunoblotting study (Fig. 3, Lanes 5 and 8) indicated that two components, viz. at 28–35 and 18 kDa molecular weight, bind with antibody, contributing to such high antigenic value. Components around 28–35 kDa corresponded to α/β -gliadins (S-rich prolamins), which persisted during in vitro digestion (Fig. 1). A polypeptide observed around 18 kDa likely resulted from hydrolysis of gliadins preserving sufficient epitopes to react with antibody. However, both these reactive components showed lower band intensity (Fig. 3, Lanes 6, 7, 9 and 10) (Table 2) for hydrolysates of gluten at 100°C , which resulted in a 60 % reduction of antigenic value (Table 1) in comparison with that at room temperature. At higher temperature (100°C), gliadins undergo covalent aggregation with glutenin as shown in our previous work [15] as well as other studies [34, 35] that might have caused lower availability of α/β -gliadins to react with the anti-gliadin antibody. Moreover, such heat-induced changes could have also lowered digestibility (Fig. 1) of gliadins resulting in a lower yield of the potentially antigenic polypeptide fraction (MW 18 kDa).

Fig. 3 Immunoblotting showing the binding of anti-gliadin antibodies to the reactive gliadin polypeptides obtained from enzymatically hydrolysed gluten. L1 molecular marker, L2 undigested gluten standard, L3 pH 3 room temp. 0 s^{-1} , L4 pH 3 100°C 0 s^{-1} , L5 pH 7 room temp. 0 s^{-1} , L6 pH 7 100°C 0 s^{-1} , L7 pH 7 100°C 1500 s^{-1} , L8 control, L9 control pH 100°C 0 s^{-1} , L10 control pH 100°C 1500 s^{-1}

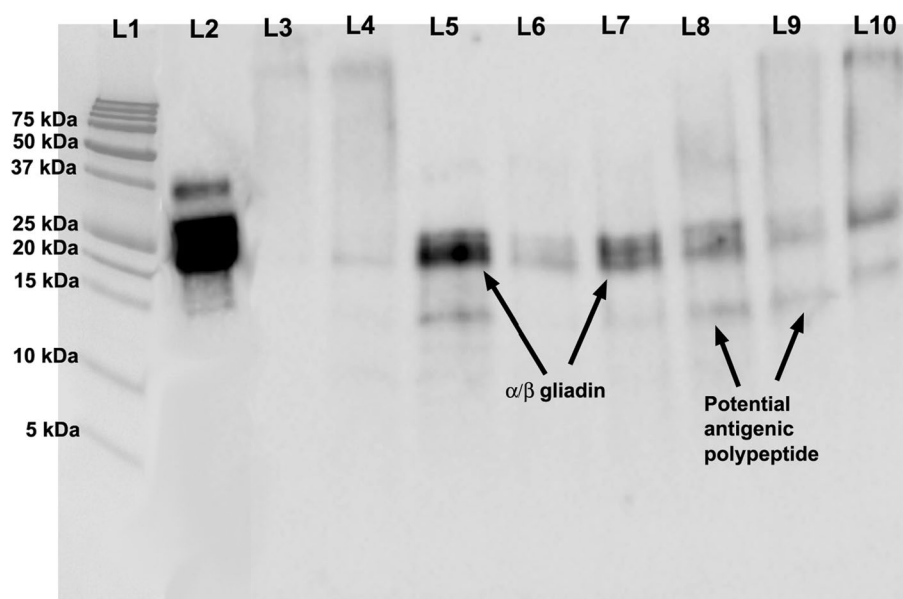


Table 2 Band intensities of potentially antigenic fractions from different gluten hydrolysates, due to binding with anti-gliadin antibody in immunoblotting represented in Fig. 2

Band	Band volume (intensity)								
	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10
α - β -Gliadin (MW 28–35 kDa)	14,692,500	0	104,000	3,534,208	674,848	2,090,192	1,392,848	472,320	788,928
Intermediate antigenic polypeptide (18 kDa)	0	0	0	376,112	0	129,424	319,824	300,088	232,768

L2, undigested gliadins standard; L3, pH 3 room temp. 0 s⁻¹; L4, pH 3 100 °C 0 s⁻¹; L5, pH 7 room temp. 0 s⁻¹; L6, pH 7 100 °C 0 s⁻¹; L7, pH 7 100 °C 1500 s⁻¹; L8, control pH room temp. 0 s⁻¹; L9, control pH 100 °C 0 s⁻¹; L10, control pH 100 °C 1500 s⁻¹

On the other hand, changes in antigenic potential of hydrolysates of gluten at pH 3 were different to those observed at other pH (Table 1) (Fig. 3). A digest from gluten at pH 3, room temperature and 0 s⁻¹ had a significantly ($P < 0.05$) lower antigenic value (Table 1) than that of the control sample. Such a lower antigenicity at pH 3 could be attributed to the absence of two antigenic components (α - β -gliadins, MW 28–35 kDa and a polypeptide, MW 18 kDa; Fig. 3, Lanes 3 and 4) which were present at both pH 7 and control pH (Fig. 3, Lanes 5 and 8). As discussed above, a synergistic effect of acidic deamidation and enzymatic breakdown at pH 3 might have caused an extensive degradation of gluten proteins into predominantly very low molecular weight fragments. These fragments may be too small to be retained in the gel during SDS-PAGE and subsequent transfer to the membrane for immunoblotting although they might have some epitopes to show positive reaction in ELISA. Similar phenomenon was reported in other studies [26] where it has been shown that deamidated gluten became more susceptible to pancreatic digestion and produced very low molecular weight peptides (<5 kDa). However, hydrolysate of heated (100 °C) gluten at pH 3 showed slightly higher ($P < 0.05$) antigenicity (Table 1). Such an increase could have originated from a residual antigenicity of smaller peptides (<5 kDa) (Fig. 2), resulting from limited hydrolysis of gliadins as evident by reduced DH (Fig. 1) due to heat-induced aggregation. However, such smaller peptides were absent in case of the unheated sample at pH 3. Moreover, such heat-induced aggregation left a small portion of α - β -gliadins undigested which exhibited binding reaction with antibodies during immunoblotting (Fig. 3).

Conclusions

Different processing conditions caused varied effects on physico-chemical properties of gluten, which consequently affected its digestive stability as well as antigenic potential of its gliadin fractions. The results indicated that pH and temperature influence the digestibility and thereby the antigenic characteristics of the resulting hydrolysates, while shear alone had no effect. At low pH (pH 3), gliadins

underwent acidic deamidation with enhanced hydrolysis into very low molecular weight fractions resulting in lower antigenicity. At high temperature (100 °C), due to protein aggregation, partial resistance of some gliadins to hydrolysis resulted in slight increase in antigenicity. Nevertheless, at neutral pH (pH 7), similar to control (pH 6.6), gluten had high digestibility yielding some potentially antigenic fractions that produced in maximum antigenicity values. Heating to 100 °C at both pH 7 and control pH (6.6) induced covalent protein aggregation that resulted in decreased digestibility and less availability of antigenic components and therefore minimum antigenicity. Thus, although no treatment can totally abolish the antigenic potential of gliadins in gluten post-digestion, heating samples at neutral pH (pH 7) to 100 °C could remarkably reduce the intensity of sensitization. However, the results need to be further investigated in vivo.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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