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Improvement of Functional Properties of Glutens Extracted from Two Iranian Wheat Varieties (*Sardari and Mahdavi*) Employing Chemical and Enzymatic Modifications

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ABSTRACT

Proteins are not only considered as a nutritional source but also are responsible for a number of functional properties such as solubility, emulsification, foaming capacity and gel formation in food products. However, insolubility of the proteins (as can be seen in gluten) and therefore their insufficient functional properties have created some limitations for their incorporation in formulated foods. Studies have indicated that chemical and/or enzymatic modifications are potential ways to improve proteins functionality. In this study gluten was extracted from two wheat varieties, Sardary and Mahdavi, and then modified by deamidation, acylation (succuinylation and acetylation) as well as enzymatic hydrolysis. Functional properties such as solubility measured as nitrogen solubility (NSI), emulsifying capacity (EC), foaming capacity (FC) and stability (FS) as well as the electrophoresis patterns of native glutens were studied and compared with those of chemically- and/or enzymatically-treated samples. Most modifications significantly increased NSI from 20 to more than 70% and EC up to 35%. A great increase was also evidenced in foaming capacity that increased from 80 ml of foam volume in native samples to about 180 ml of foam in all the treated samples; however, only deamidated samples had stable foam over the time. Electrophoresis patterns indicated that, among chemical modifications, deamidation caused limited hydrolysis of gluten polypeptides while enzymatic hydrolysis led to a pronounced reduction in the molecular weight of wheat gluten polypeptides (less than 15 KD) of both varieties.

Keywords: Acylation, Deamidation, Electrophoresis, Functional properties, Modification.

INTRODUCTION

Wheat gluten is widely used in the baking industry to improve the functional properties of dough (e.g. enhancing the strength of flours for bread making). It is a typical insoluble protein, a characteristic that makes it suitable for bread making. The insolubility of proteins however sets limits for it's utilization in formulated food systems and so the solubilizotion of gluten has been attempted to extend its usefulness in the food industry (Kato *et al.*, 1991; Mimouni *et al*., 1994; Babiker *et al*.**,** 1996). The insoluble nature of gluten has been mainly attributed to its amino acid composition. Large amounts of nonpolar amino acid residues such as prolin and leucine tend to be involved in hydrophobic bonding and the presence of a considerable amount of amide groups in the side chains of amide type amino acids, such as glutamine in gluten, plays an important role in stabilizing the protein structure and in promoting the association of gliadin and glutenin molecules through hydrogen bonding (Schofield and Booth, 1983). Much research has focused on chemical or enzymatic modifications of gluten have resulted in enhancement of its solubility, foaming, and emulsifying properties (Linares *et al*., 2000;

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Drago and Gonzalez, 2001; Popineau *et al*., 2002; Webb *et al*., 2002). Protease digestion is the most promising way to solubilise gluten effectively. Drago and Gonzalez (2001) reported that mild hydrolysis of wheat gluten by fungal protease improved solubility and that gluten hydrolysate exhibited good foaming capacity at pHs 6.5 and 9.

Studies on the deamidation of food proteins have also become of great interest for the food industry since this technique provides an effective way to improve the functional properties of proteins by increasing the number of their negative charges (Finley, 1975; Shih, 1987; Shih and Kalmar, 1987). Even low levels of deamidation (e.g., 2-6%) could result in a significant improvement in protein functional properties (Hamada and Marshal, 1989). Deamidation of wheat gluten appears to be of particular interest because of its relatively high glutamine content, approximately one-third of the total amino acids (Schofield and Booth, 1983). Wu *et al.* (1976) found a significant improvement in the emulsifying properties of gluten by mild Acid hydrolysis. Kato *et al.* (1989) reported that proteolytic deamidation of gluten was effective for the improvement of its functional properties.

Acylation of amino acid residues with acetic anhydride and succinic anhydride have been the most common chemical modifications used in food protein applications. Under the condition prevailing in most foods the *e* -amino group of lysine is principal site of acylation (Kinsella and Shetty, 1979). Although lysine content is not high in wheat gluten, it is high enough effectively to carry out modifications (Lens *et al*., 1999). Alterations in the functional properties of succinylated and often more slightly acetylated proteins have commonly included higher solubility, improved emulsifying, foaming abilities and enhanced hydration (Franzen and Kinsella, 1976; Ponampalam *et al*., 1988; Wanasundra and Shahidi, 1997).

The objective of the present study was to investigate the effect of acid deamidation, succinylation, acetylation and enzymatic hydrolysis on the functional properties of the weak gluten obtained from two Iranian wheat varieties.

MATERIALS AND METHODS

Materials

Acetic anhydride and succinic anhydride were purchased from Merck, the alcalase enzyme (2.4 F LG) was a gift from Novozyme and the molecular weight standard proteins were obtained from Fermentas. Native gluten samples were extracted from wheat flours (Sardary and Mahdavi varieties).

Chemical and Enzymatic Modifications

Deamidation was carried out as described by Mimouni *et al*. (1994). A dispersion of gluten (25 mg ml^{-1}) in 0.1 m HCl was stirred at 70°C for two hours. The reaction was stopped by cooling the samples quickly in an ice bath, followed by neutralization (pH=7) with NaOH. The suspension was dialyzed with frequent changing of distilled water at 4°C for 48 hours then freeze-dried. Succinylation was performed using the procedure explained by Ma *et al.* (1986). A 5% (w/v) of aqueous gluten suspension was adjusted to pH 8.0 with 2N NaOH, and solid succinic anhydride was added while stirring and maintaining pH constantly until all anhydride was reacted as indicated by no more changes in pH. The total amount of anhydride added was 20g/100g gluten. The reaction mixture was dialyzed with frequent changing of distilled water at 4°C for 48 hours and then it was freeze-dried. Acetylation of gluten was carried out according to the method described by Franzen and Kinsella (1976). A saturated aqueous sodium acetate solution (120 ml) was prepared at 25°C by dissolving sodium acetate (55g) in distilled water (120 ml). Gluten (2g) was added to this solution and acetic anhydride was added in 0.2 ml increments to reach a total volume of 2.4 ml over a period of one

hour. The solution was dialyzed in distilled water at 4°C for 48 hours, and the acetylated gluten was recovered by lyophilization. Enzymic hydrolysis was fulfilled according to procedure of Mimouni *et al.* (1994). Gluten was dispersed (25 mg ml^{-1}) in 0.1 M sodium phosphate buffers (pH 8). A hydrolysis reaction was performed at pH 7.9 for alcalase (0.6 mAnson mg^{-1}). During the dispersion, which required vigorous agitation, the mixture was readjusted to the desired pH. The enzyme was added when dispersion was completed. The mixture was stirred for the appropriate reaction time, during which the pH was maintained in the desired range. The enzyme/substrate ratio was 1:33 (w/w), and the hydrolysis was carried out at 30°C. Hydrolysis was stopped by heating at 100°C for 2 minutes.

Chemical Analyses

The native and modified glutens were analyzed for total nitrogen and moisture content using AACC (2003) procedures.

Electrophoresis

SDS slab polyacrylamide gel electrophoresis was carried out following the method of Sambrook and Russel (2001) using 12% acryl amide separating gel and 5% stacking gel. Gluten samples (1 mg) were dissolved in a 170 ml sample buffer. The molecular weights of the polypeptides were estimated by comparing mobilities with those of standard proteins run in the same gel.

Functional Properties

The nitrogen solubility index (NSI) was measured according to the AACC (2003) standard methods at pH 7. Emulsion capacity was determined using the method of Barber and Wartesen (1982) after slight modifications. A suspension of gluten (0.2% (w/v) of protein, pH 7; 150 ml) was placed in a jar container of Omni-Mixer fitted with electrodes to measure the resistance of an electrical current through the dispersion. The electrodes were placed opposite each other on the sides of the jar just above the blender blade. The electrodes were connected to a volt-ohm meter (VOM). The sample was blended for 30 seconds at high speed (20000 rpm). At the end of this time, sunflower oil was rapidly introduced into the blending dispersion through a hole in the jar via a burette at a rate of approximately 0.2 ml s^{-1}. The addition of oil was terminated when the electrical resistance of sample shown by VOM reached out of limit. A change in the VOM reading was considered the point of oil inversion in the emulsion since oil is unable to conduct an electrical current. Emulsification capacity was the amount of oil required by the protein dispersion to reach the phase inversion (or emulsion break point). The emulsification capacity was calculated as milliliters of oil emulsified per 0.1 g of protein. Foaming capacity and foam stability were determined by a slightly modified procedure of Puski (1975). The gluten suspension [1% (w/v) of protein, pH 7; 100 ml] was placed in the Omni-Mixer cup, attached to the Omni-Mixer, and stirred at 20,000 rpm for 6 minutes. The content was immediately transferred to a 250 ml graduated cylinder and the foam volume noted and defined as foaming capacity. After standing for 10, 30, 60 and 120 minutes, the residual foam volume was measured again, and the foam stability at each time calculated by the following equation:

$$\text{Foam stability\%} = \frac{\text{Residual foam volume}}{\text{Initial foam volume}} \times 100$$

Statistical Analysis

Experiments were performed in triplicate by factorial experiment in complete randomized design and the data were analyzed for comparison of significant differences between means by Duncan's test at the significance level of $p<0.05$.

RESULTS AND DISCUSSION

Chemical Composition

The results in Table 1 indicated that, among modified glutens, the protein content of hydrolyzed samples of the Sardari and Mahdavi varieties, significantly decreased compared to native ones that might be due to imperfect elimination of impurities from the solution of glutens and/or passing of low molecular weight polypeptides from the solution of hydrolyzed gluten in their purification stage by the dialyzing tube.

Gel Electrophoresis

Comparison of the electrophoretic pattern of chemically modified glutens with unmodified ones (Figure 1) revealed that the weak acidic condition used for deamidation had caused hydrolysis of the polypeptides.

Enzymatic hydrolysis had also caused a pronounced decrease in the molecular weight of polypeptides and increases in polypeptides with a molecular weight of 15

Table 1. Chemical analysis of native and modified glutens.[a]

Modification	Mahdavi variety		Sardari variety	
	Protein (% db) [b]	Moisture (%)	Protein (%db) [b]	Moisture (%)
Native	87.3[a] ± 0.59	4.3[a] ± 0.08	86.9[a] ± 1.6	4.7[a] ± 0.07
Deamidated	85.4[a] ± 0.29	4.8[a] ± 0.035	86.0[a] ± 0.6	5.3[b] ± 0.05
Succinylated	86.9[a] ± 0.59	5.1[a] ± 0.056	835[b] ± 1.2	4.8[a] ± 0.03
Acetylated	81.8[c] ± 0.59	4.5[a] ± 0.049	86.9[a] ± 1.2	4.2[a] ± 0.02
Hydrolyzed	82.9[c] ± 0.29	5.3[b] ± 0.056	82.9[b] ± 0.3	5.5[b] ± 0.03

[a] Average ± Standard deviation.

[b] N(%)× 5.7.

Values in the same column bearing different letters are significantly different ($p<0.05$).

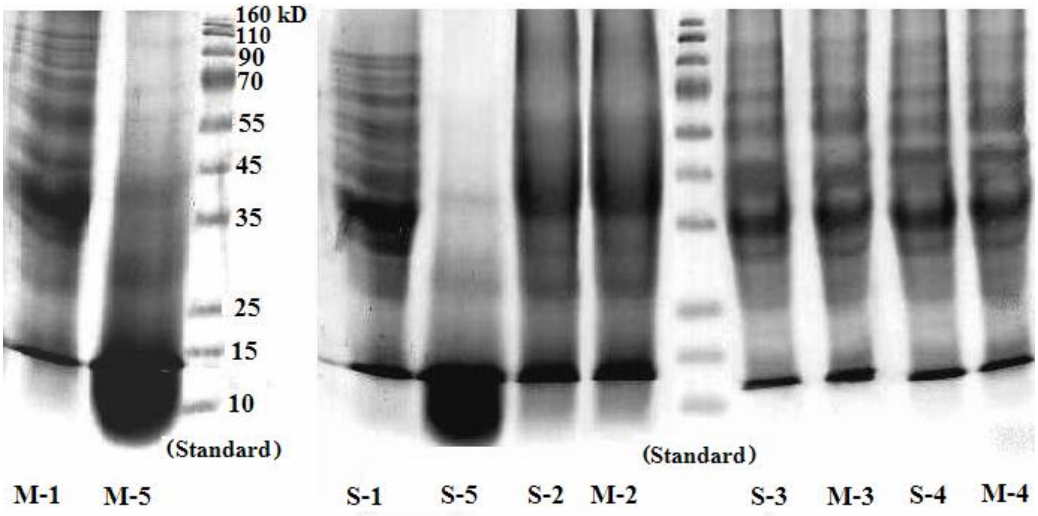

Figure 1. Electrophoretic patterns of native and modified glutens. (S) Sardari and (M) Mahdavi varieties; (1) Native; (2) Deamidated; (3) Succinylated; (4) Acetylated, (5) Anzymatic hydrolyzed.

to less than 10 KD. In general, succinylation increases net negative charge, changes conformation and so may be able to increase the propensity of proteins to be dissociated (Kinsella and Shetty, 1979) (Figure 1). Through comparison of electrophoretic patterns of acylated glutens and their native samples, no remarkable changes which show hydrolysis of polypeptides were observed.

Solubility

The solubility of modified glutens at pH 7 (deamidated, succinylated, acetylated and enzymatic hydrolyzed) as compared with native gluten in both varieties is shown in Figure 2. In spite of the kind of treatment, modified glutens had far greater solubility than native gluten ($p< 0.05$). Enzymatic hydrolysis had the most and acetylation had the least increasing effects on the solubility of gluten as compared with the native one in each variety. The increase in solubility at pH 7 of gluten following acid deamidation has been reported by Wu *et al.* (1976) and Mimouni *et al.* (1994). Taking into account that acid deamidation of gluten can cause partial hydrolysis of peptide bonds (Figure 1), solubilization of deamidated gluten was a result of both deamidation of gluten and rupture of few peptide linkages in gluten molecules. Much of the insolubile nature of gluten may arise from hydrogen bondings of numerous amide groups on the glutamine residues. However; deamidation disrupts these extensive hydrogen bandings and consequently increases solubility (Wu *et al.*, 1976; Lens *et al.*, 1999).

Barber and Wartesen (1982) noted that upon, succinylation of gluten, there was a significant increase in solubility at pHs 7 and 9. Succinylation can also cause the unfolding of protein due to electrostatic repulsions between the added anionic carboxyl groups and the neighboring native carboxyl groups, producing more protein-water interactions and less protein-protein interactions (Wanasundra and Shahidi, 1997). Upon acetylation, ammonium cations were replaced by a neutral acetyl group producing less electrostatic repulsion compared to succinylation (Franzen and Kinsella, 1976). The aqueous solubility of acetylated gluten in both varieties was therefore approximately an intermediate in magnitude between that of native and succinylated gluten (Figure 2).

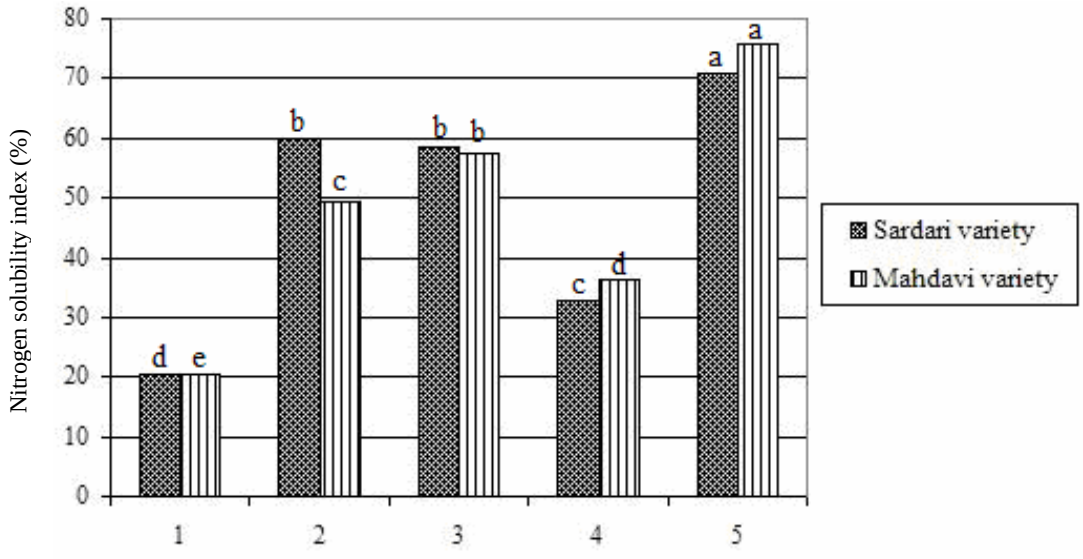

Figure 2. Comparison of the solubilities of native and modified glutens: (1) Native; (2) Deamidated; (3) Succinylated; (4) Acetylated, (5) Enzymatic hydrolyzed.
Bars with different superscripts have significant differences at $p<0.05$).

The increased solubility of wheat gluten through enzymatic hydrolysis has been extensively reported by Mimouni *et al.* (1994); Babiker *et al.* (1996), Popineau and Tebaudin (1991), Linares *et al.* (2000) and Drago and Gonzalez (2001). The enhanced solubility of hydrolysates is due to their smaller molecular size and the newly exposed ionizable amino and carboxyl groups that increase the hydrophilicity (Mahmoud, 1994).

Emulsifying and Foaming Properties

Figure 3 shows that the emulsifying properties at pH 7 of modified (deamidated, succinylated, acetylated and hydrolyzed) glutens were improved in both wheats ($p< 0.05$). Figure 4 shows that, upon all chemical and enzymatic modifications, there was a significant increase in foaming capacity at pH 7 in each variety of wheat. Figures 5 and 6 show the foam stability of native and

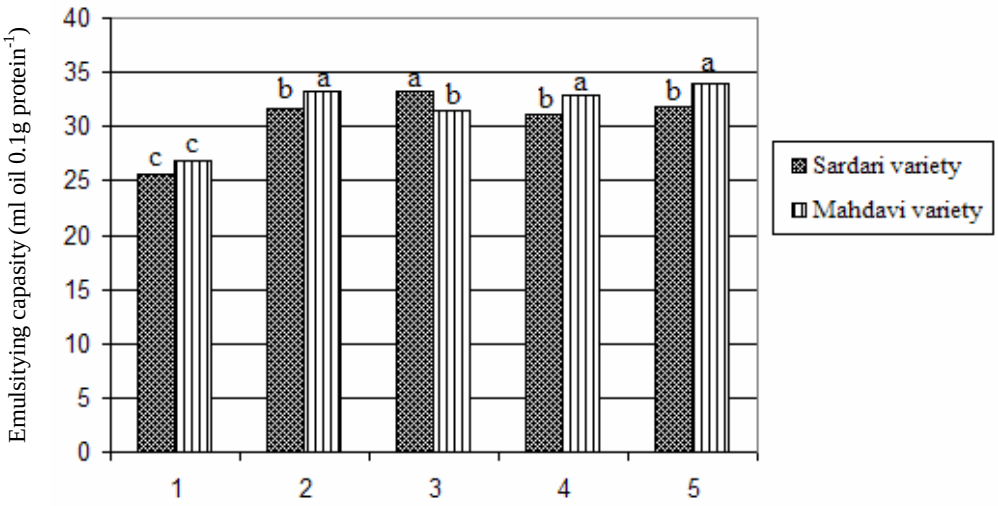

Figure 3. Emulsifying capacity of native and modified glutens at pH 7: (1) Native; (2) Deamidated; (3) Succinylated; (4) Scetylated, (5) Enzymatic hydrolyzed.
Bars with different superscripts have significant differences at $p<0.05$.

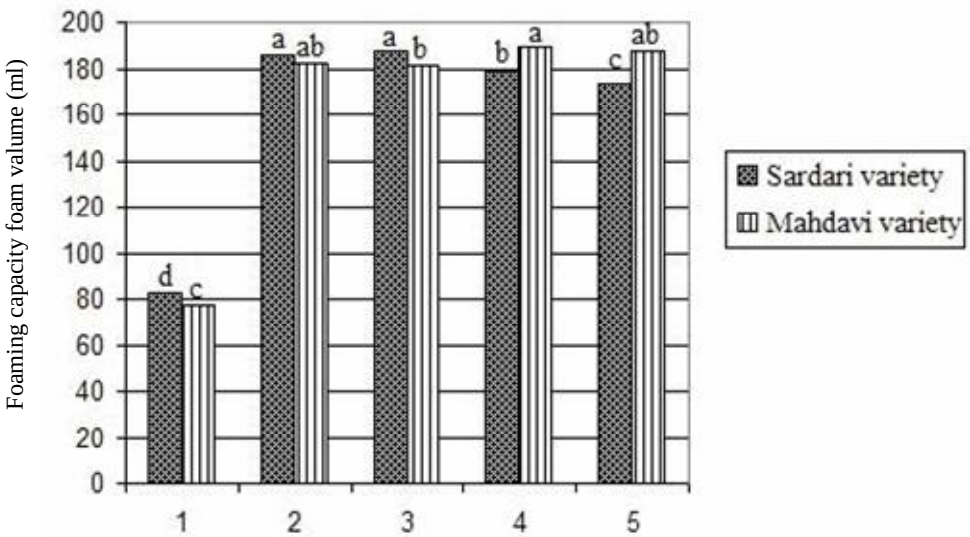

Figure 4. Foaming capacity of native and modified glutens at pH 7: (1) Native; (2) Deamidated; (3) Succinylated; (4) Acetylated, (5) Enzymatic hydrolyzed.
Bars with different superscripts have significant differences at $p<0.05$).

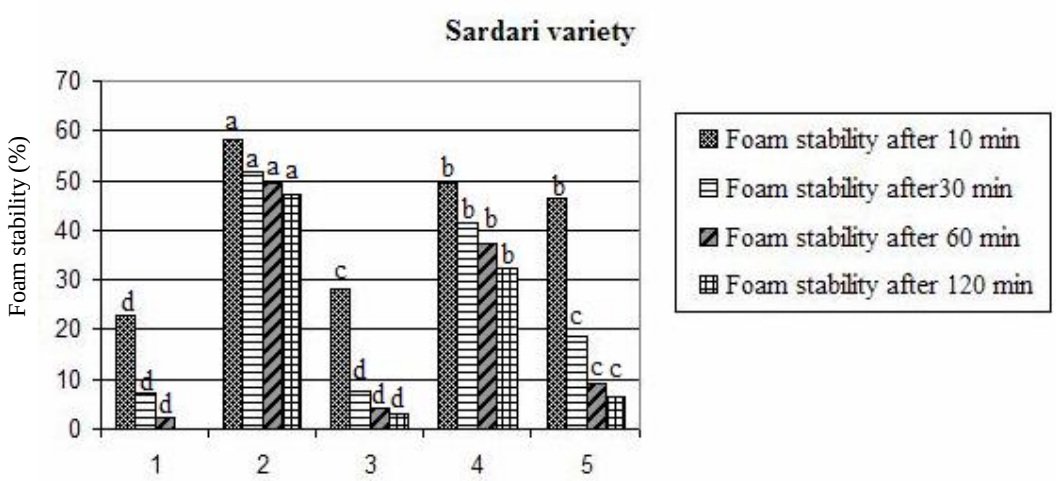

Figure 5. Foam stability of native and modified glutens at pH 7: (1) Native; (2) Deamidated; (3) Succinylated; (4) Acetylated, (5) Enzymatic hydrolyzed.
Bars with different superscripts have significant differences at $p<0.05$).

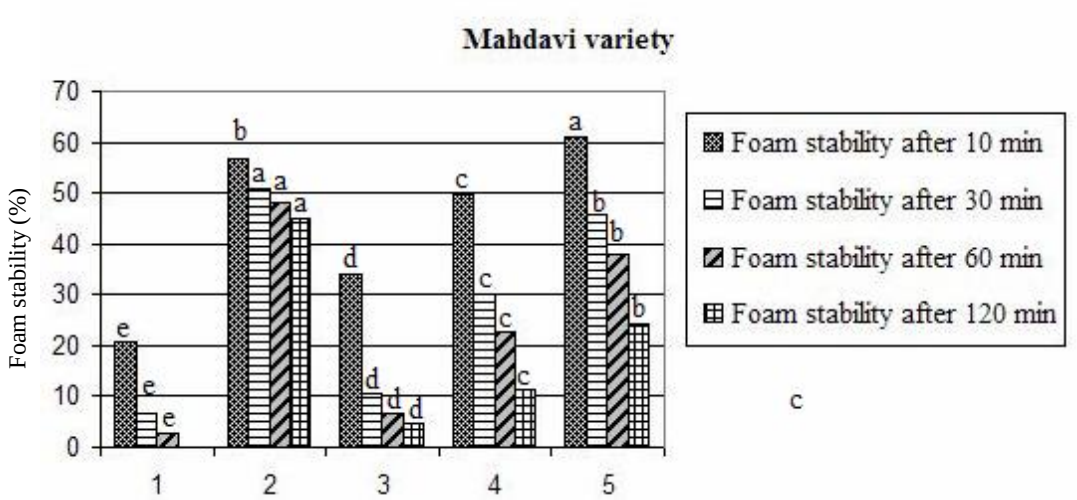

Figure 6. Foam stability of native and modified glutens at pH 7: (1) Native; (2) Deamidated; (3) Succinylated; (4) Acetylated, (5) Enzymatically hydrolyzed.
Bars with different superscripts have significant differences at $p< 0.05$).

modified glutens at the same pH but different holding times. All modifications in Mahdavi and all modifications except succinylation in Sardari had significantly increased foam stability of gluten at least in short time ($p< 0.05$). In both varieties deamidation and succinylation had the most and least increasing effects on foam stability, respectively.

Chemical and enzymatic deamidation have been shown to increase the charge density on proteins, causing changes in protein conformation due to electrostatic repulsion, and exposure of more hydrophobic residues to the protein surface (Wu *et al.*, 1976; Kato *et al.*, 1987). Matsudoumi *et al.* (1982) reported that the surface hydrophobicity increased in linear proportion to the degree of deamidation of gluten up to 40% deamida-

tion. Emulsifying properties were improved greatly by deamidation and were linearly correlated with surface hydrophobicity. Mimouni *et al.* (1994) noted great increases in the emulsifying capacity, foam capacity and stability at pH 7 of deamidated gluten compared to the native one.

Acetylation and succinylation increased the emulsifying property of gluten mostly due to increasing the effect of these modifications on gluten solubility. Barber and Wartesen (1982) showed that there were similarities between the emulsion capacity and nitrogen solubility profiles of acylated wheat gluten. In fact, an increase in protein solubility would encourage a rapid migration and adsorption at the water–oil interface. As in the case of emulsion properties, the whipping/foaming characteristics of acylated gluten are also directly related to its solubility (Canella *et al.*, 1979). Unfolding of the proteins during whipping, largely contributes to the formation of stable molecular layers in the air–water interface which imparts texture, stability and elasticity to the foam (Canella *et al.*, 1979). However, the weak foam stability of succinylated glutens observed in both varieties is probably related to repulsion between protein molecules and, thereby, reduces their ability to associate and form a stable film around the air cells of the foam (Wanasundra and Shahidi, 1997). The improvement observed in the emulsifying and foaming properties upon limited peptide bond hydrolysis could presumably be due to a decrease in molecular weight of the polypeptide and an increase in solubility of the hydrolyzed gluten, which may enhance the protein adsorption rate in the interface layer. Moreover, unfolding of the compact structure of protein and, thereby, exposure of interior hydrophobic protein enhanced their absorption at the interface. This may form a cohesive interfacial film, with the hydrophobic residues interacting with oil and/or air and hydrophilic residues with water (Nakai and Li-Chain, 1988; Mahmoud, 1994). Mahmoud *et al.* (1992) reported a strong correlation between the emulsifying activity and hydrophobicity of casein hydrolysates during extensive hydrolysis by porcine pancreatin. Popineau and Thebaudin (1991) noted that enzymatic hydrolysis of gluten with neutrease resulted in a large increase of its emulsifying activity.

CONCLUSION

In summary, the results of this study indicate that deamidation, succinylation, acetylation and enzymatic hydrolysis can modify and significantly improve the functional properties at pH 7 of wheat gluten of Sardari and Mahdavi varieties, both of which contain weak glutens. The increase in solubility, ES, FS and FS at neutral pH render these modified glutens with improved functionality for their incorporation into products such as cakes, where these properties may be required.

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بهبود خواص عملکردی گلوتن استخراج شده از دو رقم گندم ایرانی (سرداری و مهدوی) با استفاده از تعدیل‌های شیمیایی و آنزیمی

ا. ح. صابری، م. کدیور و ج. کرامت

چکیده

پروتئین‌ها نه‌تنها یک منبع مهم تغذیه‌ای به‌شمار می‌آیند که عامل ایجاد بسیاری از خصوصیات عملکردی مطلوب در محصولات غذایی نیز محسوب می‌شوند. از جمله خواص عملکردی مهم پروتئین‌ها می‌توان به قابلیت حل‌شدن، خواص امولسیون کنندگی، تولید کف و ژل اشاره کرد. نامحلول بودن پروتئین‌ها محدودیت‌هایی را برای استفاده از آنها در سیستمهای غذایی فرموله شده ایجاد می‌کند، طوری‌که خصوصیات امولسیون کنندگی و تولید کف ناچیزی از خود نشان می‌دهند. گلوتن گندم یک نمونه از یک پروتئین نامحلول است. بررسی‌ها نشان می‌دهند که با انجام تعدیل‌های شیمیایی و آنزیمی، باعث بهبود خصوصیات عملکردی پروتئینها می‌شود. در این تحقیق گلوتن از دو رقم گندم سرداری و مهدوی استخراج شده و سپس تحت تیمارهای دآمیده کردن، آسیله کردن (شامل استیله و سوکسینیله‌کردن) و هیدرولیز آنزیمی قرار گرفت. در مرحله بعد خصوصیات عملکردی نظیر شاخص حلالیت نیتروژن، ظرفیت امولسیون‌کنندگی، ظرفیت تولید کف، پایداری کف و الگوی الکتروفورز گلوتن‌های طبیعی در مقایسه با نمونه‌های تیمار شده شیمیایی و آنزیمی بررسی شد. بیشتر اصلاحات انجام شده باعث افزایش معنا داری در میزان حلالیت گلوتن(از 20 به 70 درصد) و ظرفیت امولسیون‌کنندگی آن (35 درصد) گردیدند. همچنین افزایش قابل توجهی در ظرفیت تولید کف تمامی نمونه‌های تیمار شده (180 میلی لیتر) نسبت به نمونه‌های طبیعی گلوتن (80 میلی‌لیتر) مشاهده شد هر چند تنها نمونه‌های دآمیده شده کف پایداری را تولید کردند. بررسی نمودار الکتروفورز نشان داد که از بین تعدیلهای شیمیایی انجام شده، تنها دآمیده کردن موجب هیدرولیز جزئی پلی پپتیدهای گلوتن شده در حالی‌که هیدرولیز آنزیمی، کاهش چشم‌گیری را در وزن مولکولی پلی پپتیدهای گلوتن باعث شد.