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Physicochemical Changes in Gluten Following Oxalic Acid–Induced Deamidation: FTIR, HPLC, and Solubility Analysis

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Abstract

Gluten, a wheat-derived plant protein used in bread-making, animal protein replacement, and biodegradable packaging, suffers from water insolubility and allergenicity. Deamidation using oxalic acid addresses these limitations. Gluten was deamidated with 0.034, 0.082, and 0.133 M oxalic acid at 80°C for 30 minutes, followed by neutralization, dialysis, and freeze-drying. Physicochemical properties were evaluated using fourier-transform infrared (FTIR) spectroscopy, high-performance liquid chromatography (HPLC), and solubility tests. FTIR analysis showed the primary structure remained intact (amide I at 1580–1659 cm^{-1}; amide II at 1500–1600 cm^{-1}; amide III at 1200–1400 cm^{-1}). Secondary structure analysis revealed concentration-dependent changes in peak intensity: 0.034 M increased β-turn intensity (1665–1689 cm^{-1}), while 0.082 M and 0.133 M decreased α-helix (1580–1659 cm^{-1}) and β-sheet (1612–1643 cm^{-1}) intensities, indicating successful deamidation. HPLC analysis confirmed asparagine conversion to aspartic acid (from 8,406.54 to 464,537.33 mg/kg) and increased essential amino acids (valine, methionine, phenylalanine, isoleucine, leucine, lysine). Glutamic acid and cystine were undetected, consistent with FTIR-observed β-sheet reduction due to disulfide bond cleavage. Solubility improved markedly at 0.082 M and 0.133 M, correlating with reduced ordered secondary structures and increased polar amino acids. Oxalic acid-mediated deamidation enhances gluten solubility and nutritional profile while preserving structural integrity, with FTIR and HPLC providing complementary evidence of deamidation success. Deamidated gluten shows promise as a functional ingredient for food applications requiring improved solubility and nutrition.

Keyword: *Gluten, deamidation, oxalic acid, FTIR, HPLC.*

Abstrak

Gluten, protein nabati dari gandum yang digunakan dalam pembuatan roti, pengganti protein hewani, dan kemasan pangan biodegradable, memiliki kelemahan berupa kelarutan air yang rendah dan potensi alergenisitas pada sebagian orang. Deamidasi menggunakan asam oksalat mengatasi keterbatasan ini. Gluten dideamidasi dengan asam oksalat 0,034; 0,082; dan 0,133 M pada suhu 80°C selama 30 menit, dilanjutkan dengan netralisasi, dialisis, dan pengeringan beku. Sifat fisikokimia dievaluasi menggunakan spektroskopi fourier-transform infrared (FTIR), high-performance liquid chromatography (HPLC), dan uji kelarutan. Analisis FTIR menunjukkan struktur primer tetap utuh (amida I pada 1580–1659 cm^{-1}; amida II pada 1500–1600 cm^{-1}; amida III pada 1200–1400 cm^{-1}). Analisis struktur sekunder mengungkapkan perubahan intensitas puncak yang bergantung pada konsentrasi: 0,034 M meningkatkan intensitas β-turn (1665–1689 cm^{-1}), sedangkan 0,082 M dan 0,133 M menurunkan intensitas α-helix (1580–1659 cm^{-1}) dan β-sheet (1612–1643 cm^{-1}), mengindikasikan keberhasilan proses deamidasi. Analisis HPLC mengonfirmasi konversi asparagin menjadi asam aspartat (dari 8.406,54 menjadi 464.537,33 mg/kg) dan peningkatan asam amino esensial (valin, metionin, fenilalanin, isoleusin, leusin, lisin). Asam glutamat dan sistin tidak terdeteksi, konsisten dengan penurunan intensitas β-sheet pada FTIR akibat pemutusan ikatan disulfida. Kelarutan meningkat secara signifikan pada 0,082 M dan 0,133 M, berkorelasi dengan penurunan struktur sekunder teratur dan peningkatan asam amino polar. Deamidasi yang dimediasi asam oksalat meningkatkan kelarutan gluten dan profil nutrisi sambil mempertahankan integritas struktural, dengan FTIR dan HPLC memberikan bukti komplementer atas keberhasilan deamidasi. Gluten terdeamidasi menunjukkan potensi sebagai bahan fungsional untuk aplikasi pangan yang memerlukan kelarutan dan nilai gizi yang lebih baik.

Kata Kunci: Gluten, deamidasi, asam oksalat, FTIR, HPLC.

I. INTRODUCTION

Gluten is an abundant source of vegetable protein and has potential for use in the food industry. Gluten is found in many cereal plants, such as wheat, rye and barley. In processed food products, gluten is also contained in wheat flour, which is a processed wheat product. Based on its chemical structure, gluten consists of gliadin monomer components and glutenin polymers. Judging from its amino acid composition, gluten contains a lot of the amino acids glutamine, proline, valine, leucine, isoleucine and phenylalanine, but contains little of the essential amino acids lysine, methionine and tryptophan [1].

Gluten has many benefits, especially in the food sector. Gluten can be used in the bread making process, a source of vegetable protein for vegetarians, and in the development of biodegradable food packaging. In the bread making process, the

gliadin content in gluten plays a role in the viscosity of the dough, while glutenin plays a role in the elasticity and strength of the dough [2]. Gluten can form a network that holds bread dough together and makes the dough rise by trapping gas bubbles, giving bread a unique texture and consistency. Apart from that, gluten also has cohesive and viscoelastic properties so that it can be used in making biodegradable food packaging materials or edible films [3,4]. The viscoelastic properties of gluten are also used in making plant-based meat as an alternative source of animal protein [5].

However, gluten has several disadvantages regarding its use in the food sector. Gluten has a high content of the amino acids glutamine and asparagine, making gluten insoluble in water. This characteristic causes the use of gluten in food processing to be limited because food processing involves a lot of water [6]. Apart from that, gluten cannot be consumed by all groups. In individuals who are allergic to gluten, gluten absorbed from the intestines into the blood will cause a severe hypersensitivity reaction due to the formation of immunoglobulin E (IgE) antibodies [7,8]. The formation of IgE antibodies occurs because several sequences of glutamine palindrome residues in gluten (especially gliadin) form epitopes that can interact with IgE [9]. To overcome this weakness, one way that can be done is by deamidating gluten.

Deamidation of gluten is the process of changing the structure of gluten by removing the amide group in the side chains of glutamine and asparagine residues to form charged amino acids (glutamic acid and aspartic acid) through the release of ammonia [10]. Through the deamidation reaction, the epitope on gluten that is recognized by IgE antibodies can be changed so as to reduce the allergenicity of gluten [11]. The deamidation reaction causes a decrease in the number of NH_2 groups and an increase in charged carboxyl groups, thereby increasing the electrostatic repulsion between gluten molecules. Thus, the solubility of gluten in water also increases [10]. Gluten deamidation can be done chemically or enzymatically. However, enzymatic gluten deamidation requires high costs in terms of the type of enzyme and operating conditions used.

Chemically, deamidation is carried out by breaking amide bonds with the help of an acid or base. The use of strong bases at high concentrations for the deamidation process often causes racemization of amino acids and produces the toxic peptide lysinoalanine (LAL) [12]. On the other hand, research on gluten deamidation using acids has been widely carried out. Gluten deamidation research used HCl with varying concentrations of 0.055 M; 0.137 M; and 0.220 M for 15 minutes and a temperature of 121°C, with the resulting degree of

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deamidation being 65.04%; 64.64%; and 35.08% [6]. However, deamidation under very acidic conditions can in some cases cause certain problems, including uncontrolled hydrolysis, potentially producing carcinogenic compounds as side products (such as mono-, dichloropropanol, and monochloropropanodiol), and isomerization of certain amino acids [10]. Other acid alternatives that can be used are organic acids, such as acetic acid, citric acid, malic acid, and tartaric acid [13-15]. Deamidation research used acetic acid (CH_3COOH) with varying concentrations of 0.034 M; 0.082 M; and 0.133 M at 121°C for 5 minutes produced deamidation degrees of 59.60%, respectively; 63.10%; and 65.04% [6]. The deamidation process uses citric acid with a concentration of 3.93×10^{-5} M; 3.14×10^{-3} M; and 2.36×10^{-2} M at 70°C for 120 minutes producing a degree of deamidation of 25% each; 40%; and 55% [16]. Organic acids are a type of weak acid that is safer to use in the deamidation process because it can minimize hydrolysis. Although these studies demonstrated the effectiveness of several organic acids for gluten deamidation, the application of oxalic acid as a deamidation agent has not been systematically investigated. Oxalic acid is a weak organic acid that occurs naturally in various plant-derived foods and has been reported to possess preservative properties under certain conditions [17]. While acetic and citric acid deamidation have been characterized, no study has compared oxalic acid's efficacy under similar conditions or examined its unique effects on gluten's amino acid profile, particularly regarding essential amino acid retention and aspartic acid formation. Additionally, existing studies primarily focus on deamidation degree and solubility, with limited comprehensive analysis linking FTIR structural changes to specific amino acid composition changes via HPLC.

This study addresses these gaps by investigating oxalic acid-mediated gluten deamidation at 0.034 M, 0.082 M, and 0.133 M at 80°C for 30 minutes, with comprehensive characterization using FTIR spectroscopy, HPLC amino acid profiling, and solubility testing. This research provides novel insights into: (1) whether oxalic acid achieves comparable or superior deamidation compared to acetic and citric acid under milder conditions; (2) the specific effects on essential amino acid profiles, particularly the conversion of asparagine to aspartic acid; and (3) the correlation between FTIR-observed secondary structure changes and HPLC-quantified amino acid alterations. These findings will establish oxalic acid as a viable, safer alternative for gluten modification with enhanced functional and nutritional properties for food applications.

II. MATERIALS AND METHODS

2.1. Chemicals and Instrumentation

Chemicals used for research include commercial vital wheat gluten, oxalic acid dihydrate (Sigma), sodium hydroxide (Merck), hydrochloric acid (Sigma), lead (II) nitrate (Merck), and magnesium chloride (Merck).

The analytical instruments used in this study included an FTIR spectrophotometer (Prestige-21, Shimadzu Corporation, Kyoto, Japan) with DLATGS (deuterated triglycine sulfate doped with L-alanine) detector and a high-performance liquid chromatography (HPLC) system (1260 Infinity, Agilent Technologies, Waldbronn, Germany) with diode-array detector (DAD).

2.2. Gluten Deamidation

Gluten (moisture content < 7%) was deamidated using an oxalic acid solution with a ratio of 1:10 (w/v) at three concentration variations, namely 0.034 M; 0.082 M; and 0.133 M at 80°C for 30 minutes. The temperature is maintained using a thermostat and an oil bath. As a reactor, a four-neck flask is used which is paired with a stirrer motor, condenser and thermometer. The deamidation mixture is cooled to room temperature and neutralized to pH 7. This stage is carried out to neutralize the remaining acid used in the deamidation stage. The deamidated mixture was subjected to dialysis at 4°C for 48 hours until a deamidated gluten solution free of oxalate ions was obtained. Dialysis is carried out using a cellophane membrane and water medium, with the aim of separating the remaining unreacted ions so that only the deamidated gluten solution remains. The deamidated gluten solution was lyophilized using a freeze dryer.

2.3. Solubility Test

The solubility of native gluten and deamidated gluten (prepared using oxalic acid) was compared using a qualitative visual dissolution assay. A fixed mass of gluten was gradually added to distilled water under constant stirring, and the minimum volume required for complete dissolution was determined by visual observation. Tests were conducted at equivalent concentrations (0.082 M and 0.133 M). The dissolution endpoint was defined as a clear solution with no visible suspended particles.

2.4. Functional Group Analysis

FTIR analysis was performed to compare the functional groups and structural characteristics of gluten before and after deamidation. Samples were prepared using the potassium bromide (KBr) pellet method, in which the sample powder was thoroughly mixed with dry KBr and compressed into a transparent pellet under a pressure of 200 kg cm^{-2} for 15 s. Spectra were recorded over the wavenumber range of 4000–400 cm^{-1} at a

resolution of 4 cm^{-1}. The absorption intensity of the amide I region (1600–1660 cm^{-1}) was subsequently evaluated to assess structural changes in the gluten samples. Measurements were conducted under ambient laboratory conditions using an instrument operated within the manufacturer's recommended environmental range of 15–30°C and relative humidity below 70%.

2.5. Amino Acid Analysis

HPLC analysis was performed to determine changes in the amino acid composition of gluten before and after deamidation. Amino acid separation was carried out using Zorbax Eclipse C18 column (150 × 4.6 mm, 5 μm). The column temperature was maintained at 30°C and the injection volume was 20 μL. Separation was performed using a gradient elution program with 10 mM Na_2HPO_4 and 10 mM $Na_2B_4O_7$ (pH 8.2) as mobile phase A and acetonitrile:methanol:water (45:45:10, v:v:v) as mobile phase B, according to the manufacturer's amino acid analysis method. The flow rate was 1.5 mL min^{-1} and the total run time was 45 min. Amino acids were detected at 338 nm, while proline was detected at 264 nm after pre-column derivatization with OPA and FMOC reagents.

III. RESULTS AND DISCUSSION

3.1. Solubility of Deamidated Gluten

Based on qualitative visual observation, deamidated gluten demonstrated markedly improved solubility compared to native gluten at equivalent concentrations (0.082 M and 0.133 M). For the same mass of gluten, deamidated gluten fully dissolved in 5 mL of distilled water, whereas native gluten remained partially undissolved even after the addition of >100 mL of water. This represents a greater than 20-fold difference in the volume of solvent required for dissolution.

This phenomenon occurs because deamidation converts the amide groups of glutamine and asparagine residues into carboxyl groups [6]. The conversion of neutral amide groups to negatively charged carboxylate groups increases electrostatic repulsion between protein molecules, causing the protein secondary structure to unfold and consequently improving gluten solubility. This finding is consistent with Wang *et al.* [15], who quantitatively demonstrated that acid deamidation increased wheat gluten solubility from approximately 5% to over 70% through the same mechanism.

Although solubility was evaluated by visual observation rather than quantitative spectrophotometric assays, such as the Bradford or Lowry methods, the observed difference (>20-fold) was sufficiently large to

be reliably distinguished through visual assessment. Qualitative screening is generally considered acceptable for detecting large-effect differences in preliminary studies. Nevertheless, future studies should incorporate quantitative protein concentration measurements to determine precise solubility values and strengthen the robustness of the findings.

3.2. Functional Groups Profile

FTIR characterization was performed on gluten samples before and after deamidation using oxalic acid concentrations of 0.034 M; 0.082 M and 0.133 M. Comparison of the FTIR spectra of all samples is shown in Figure 1, while the characteristic absorption bands and their corresponding functional groups are provided in Table 1.

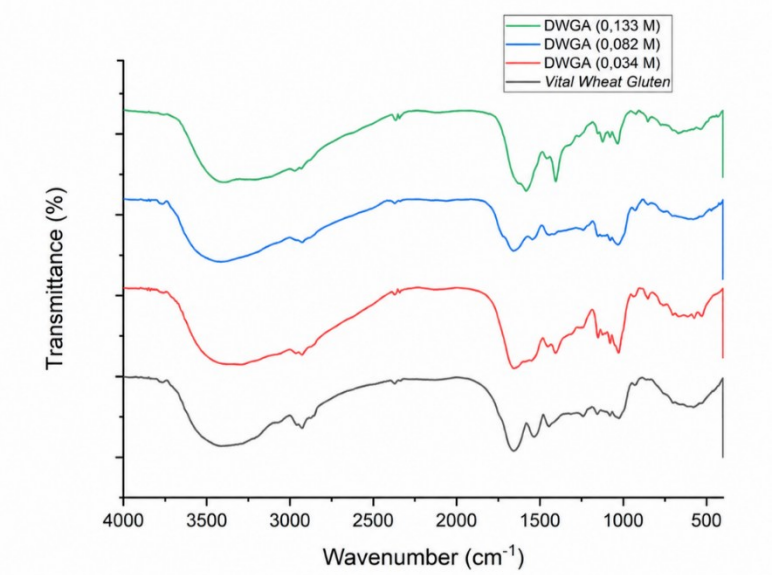

Figure 1. Gluten spectrum after and before Deamidation. DWGA refers to deamidated vital wheat gluten prepared using oxalic acid treatment.

Based on Figure 1 and Table 1, the FTIR spectra of native gluten and oxalic acid–deamidated gluten exhibited the typical absorption bands of proteins, particularly in the amide I, amide II, and amide III regions. The overall spectral patterns were broadly similar, indicating that the gluten backbone was preserved after deamidation and that the treatment primarily affected the molecular environment rather than causing extensive degradation of the protein structure. This interpretation is consistent with the known sensitivity of FTIR spectroscopy to protein conformation, especially in the amide I region, which is commonly used to evaluate secondary structural changes in gluten and

other food proteins [8]. The secondary structure of the protein consists of α-helix, β-sheet, and β-turns. In this study, the wave numbers for α-helix (1655 cm^{-1}), β-sheet (1612-1641 cm^{-1}), and β-turns (1665-1689 cm^{-1}), were used as references to determine the presence of α-helix, β-sheet, and β-turns in the results of gluten deamidation using oxalic acid

Table 1. Functional Group Composition of Gluten Before and After Deamidation.

	Wavenumber (cm^{-1})						
Variable	**N-H**	**C-H**	**Amide I**			**Amide II**	**Amide III**
			α-helix	**β-sheet**	**β-turns**	**NH bending CN**	**NH bending CN**
VWG	3410.15	2926.01	1658.78	1612-1641	1665-1689	1535.34	1240.23
DWGA-0.034	3388.93	2960.73	1656.85	1612-1641	1665-1689	1550.77	1246.02
DWGA-0.082	3390.86	2927.94	1656.85	1612-1642	1665-1689	1546.91	1242.16
DWGA-0.133	3408.22	2972.31	1583	1612-1643	1665-1689	-	1265.30

Based on Table 2, although there are slight variations in band intensity and shape between the native and deamidated samples, these differences are insufficient to determine changes in α-helix, β-sheet, β-turn, or random coil content, because the amide I region consists of overlapping vibrational bands from various structural motifs. Recent studies emphasize that quantitative secondary structure interpretation requires spectral enhancement, curve fitting, or amide I deconvolution, rather than visual comparison alone. In protein systems such as gluten, the choice of band assignment and matching procedure can substantially affect the estimated structural fraction [18].

The observed spectral variations may reflect limited conformational rearrangement induced by deamidation, including changes in hydrogen bonding and electrostatic interactions following conversion of glutamine and asparagine amide groups to carboxyl groups. At 0.034 M oxalic acid, the β-sheet–associated band intensity increased relative to pure gluten, whereas at 0.082 and 0.133 M, a decrease in β-sheet intensity was

observed. However, this observation was based on the raw FTIR spectrum and should therefore be regarded as qualitative. Because no formal deconvolution analysis was performed, the present data do not support definitive conclusions regarding the relative proportions of secondary-structure elements.

Table 2. Amide I absorption intensity

Sample	Amide I		
	α-helix	**β-sheet**	**β-turn**
VWG	0.5791	0.4936	0.5227
DWGA 0.034	0.7127	0.6574	0.6099
DWGA 0.082	0.3268	0.2966	0.2976
DWGA 0.133	0.4915	0.4125	0.2735

Importantly, recent gluten-focused studies have shown that deamidation can produce measurable changes in FTIR-derived secondary structure when the amide I region is properly analyzed. Quantitative FTIR studies have reported that deamidation alters the balance of structural motifs in gluten and may shift the contributions of β-sheet- and β-turn-related bands [8,19,20]. In addition, method-oriented studies have shown that reliable structural quantification requires careful treatment of the amide I and amide III regions, since peak overlap can obscure subtle conformational changes. Accordingly, the present FTIR results should be regarded as qualitative evidence of structural modification, while deconvolution of the amide I band and peak fitting would be needed for more rigorous interpretation.

Overall, the FTIR analysis confirms that deamidation did not eliminate the characteristic protein bands of gluten, but it may have induced subtle changes in the spectral profile consistent with altered molecular interactions. These findings support, but do not prove, the structural basis for the improved solubility observed in deamidated gluten. Future work should include amide I deconvolution and quantitative peak-area analysis to better resolve the secondary-structure transitions associated with oxalic acid deamidation.

3.3. Amino Acids Profile

In this study, amino acid analysis was carried out using RP-HPLC. This test was

carried out on deamidated gluten using 0.082 M oxalic acid, which was determined as the best concentration based on the decrease in intensity in the α-helix and β-sheet as an indication of deamidation. Based on the results obtained, there was an increase in the concentration of aspartic acid from the deamidation process using oxalic acid.

Based on Figure 2 and Table 3, we can see the comparison of aspartic acid concentration from gluten without deamidation and deamidated. There is an increase in the concentration of aspartic acid, where this increase indicates that the deamidation process has succeeded in converting asparagine into aspartic acid. The formation of aspartic acid is easier to form when compared to glutamic acid because the rate of asparagine deamidation is faster than glutamine [21]. Therefore, there is a significant increase in the concentration of aspartic acid.

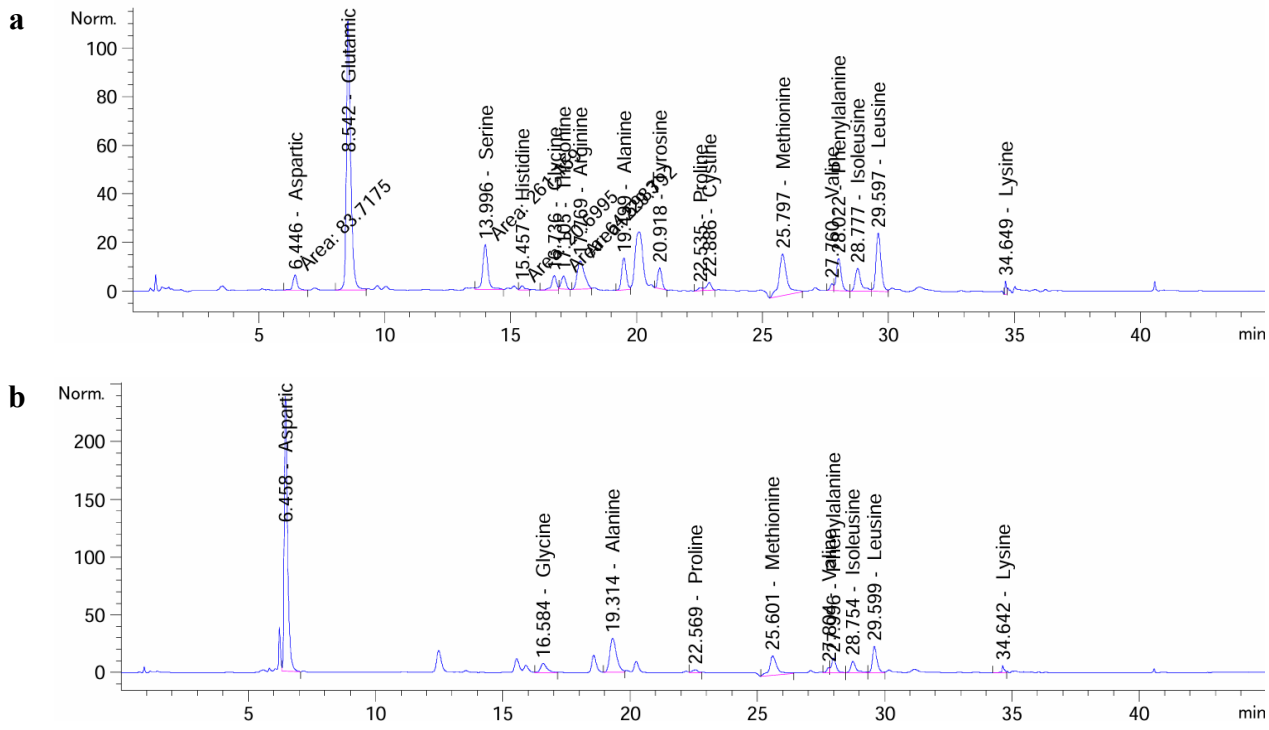

Figure 2. Gluten chromatogram (a) before and (b) after deamidation.

Table 3. Amino acid composition of gluten before and after deamidation (oxalic acid 0.082 M).

Amino Acid	Gluten Before Deamidation (mg/kg)	Gluten After Deamidation (mg/kg)
Aspartic acid	8406.54	464537.33
Glutamic acid	121778.19	*Not Detected*
Serine	14002.12	*Not Detected*
Histidine HCl Monohydrate	5026.56	*Not Detected*
Glycine	5263.43	21214.61
Threonine	4421.26	*Not Detected*
Arginine	13449.59	*Not Detected*
Alanine	9832.87	69845.88
Tyrosine	42808.91	*Not Detected*
Valine	1363.64	3885.79
Methionine	12236.78	24751.11
Phenylalanine	14159.14	26394.64
Isoleucine	10375.05	21004.13
Leucine	20643.14	37126.63
Lysine HCl	2961.59	12502.08
Proline	3851.25	22872.57
Cystine	89981.87	*Not Detected*

The absence of detectable glutamic acid in deamidated gluten is chemically plausible and can be explained by the specific reaction pathway of glutamine deamidation under acidic conditions. According to established biochemical principles, glutamine deamidation does not exclusively yield glutamic acid. It can also form pyroglutamic acid (5-oxoproline) through intramolecular cyclization, particularly when glutamine is located at the N-terminus or in specific peptide contexts [22,23].

In acidic conditions (such as 0.082 M oxalic acid used in this study), glutamine residues can undergo deamidation to form glutamic acid transiently, which may then rapidly cyclize to pyroglutamic acid [24]. This cyclization is favored under acidic and heated conditions and results in a stable lactam that is not detected as free glutamic acid by RP-HPLC. This explains why glutamic acid was "not detected" rather than simply present at low concentrations.

In deamidated gluten, the concentration of cystine in deamidated gluten was not detected. In the gluten structure, cystine plays an important role due to the presence of intermolecular disulfide bonds. The decrease in cystine concentration indicates the cleavage of disulfide bonds in deamidated gluten [15].

In this test, there was an increase in the concentration of several essential amino acids, such as valine, methionine, phenylalanine, isoleucine, leucine, and lysine HCl. The deamidation process is expected to maintain or increase the concentration of essential amino acids because these amino acids cannot be synthesized by the body and must be obtained through food or supporting supplements.

IV. CONCLUSION

Deamidation of vital wheat gluten using oxalic acid significantly enhances its functional properties while preserving its fundamental protein architecture. Visual solubility assessments demonstrated that deamidated gluten samples exhibited markedly improved solubility compared to native vital wheat gluten, with optimal results achieved at oxalic acid concentrations of 0.082 M and 0.133 M, despite identical sample mass and solvent conditions. FTIR spectroscopic analysis confirmed that the primary protein structure of gluten remained intact following deamidation, with characteristic amide I (1580–1659 cm^{-1}), amide II (1500–1600 cm^{-1}), and amide III (1200–1400 cm^{-1}) bands preserved. Notably, secondary structure analysis revealed concentration-dependent conformational changes: at 0.034 M oxalic acid, β-turn intensity increased, whereas at 0.082 M and 0.133 M, decreased α-helix (1580–1659 cm^{-1}) and β-sheet (1612–1643 cm^{-1}) intensities accompanied β-turn enhancement (1665–1689 cm^{-1}). These spectral shifts constitute definitive evidence of successful deamidation, reflecting partial unfolding of ordered secondary structures without global protein denaturation. RP-HPLC amino acid profiling further corroborated successful deamidation through a substantial increase in aspartic acid concentration (from 8,406.54 to 464,537.33 mg/kg), confirming the conversion of asparagine to aspartic acid. Importantly, deamidation enhanced the content of nutritionally critical essential amino acids, including valine, methionine, phenylalanine, isoleucine, leucine, and lysine HCl. The non-detection of glutamic acid and cystine in deamidated gluten is attributed to acid-catalyzed cyclization of glutamic acid to pyroglutamic acid and cleavage of intermolecular disulfide bonds, respectively, consistent with established

deamidation chemistry. Collectively, these findings demonstrate that oxalic acid-mediated deamidation effectively improves gluten solubility and essential amino acid profile while maintaining structural integrity, positioning deamidated gluten as a promising functional ingredient for food applications requiring enhanced solubility and nutritional value.

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REFERENCES

[1] H. Wieser, P. Koehler, and K. A. Scherf, "Chemistry of wheat gluten proteins: Qualitative composition," Cereal Chemistry, vol. 100, no. 1, pp. 23–35, 2023, doi: 10.1002/cche.10572.

[2] H. Kumagai and R. Urade, "Deamidation of gluten proteins as a tool for improving the properties of bread," in Flour and Breads and Their Fortification in Health and Disease Prevention, 2nd ed., V. R. Preedy and R. R. Watson, Eds. Academic Press, 2019, pp. 3–11. doi: 10.1016/B978-0-12-814639-2.00001-0.

[3] L. Day, "Wheat gluten: Production, properties and application," in Handbook of Food Proteins, G. O. Phillips and P. A. Williams, Eds. Woodhead Publishing, 2011, pp. 267–288. doi: 10.1533/9780857093639.267.

[4] N. S. Djenar, J. Suryadi, N. S. Nursaadah, and E. Putri, "Pengaruh penambahan minyak zaitun terhadap karakteristik dan reologi edible film berbahan dasar gluten," KOVALEN, vol. 8, no. 2, pp. 127–141, 2022, doi: 10.22487/kovalen.2022.v8.i2.15948.

[5] J. H. Chiang, S. M. Loveday, A. K. Hardacre, and M. E. Parker, "Effects of soy protein to wheat gluten ratio on the physicochemical properties of extruded meat analogues," Food Structure, vol. 19, p. 100102, 2019, doi: 10.1016/j.foostr.2018.11.002.

[6] L. Liao, M. Zhao, J. Ren, H. Zhao, C. Cui, and X. Hu, "Effect of acetic acid deamidation-induced modification on functional and nutritional properties and conformation of wheat gluten," Journal of the Science of Food and Agriculture, vol. 90, no. 3, pp. 409–417, 2010, doi: 10.1002/jsfa.3830.

[7] R. Abe, S. Shimizu, K. Yasuda, M. Sugai, Y. Okada, K. Chiba, M. Akao, H. Kumagai, and H. Kumagai, "Evaluation of reduced allergenicity of

deamidated gliadin in a mouse model of wheat-gliadin allergy using an antibody prepared by a peptide containing three epitopes," Journal of Agricultural and Food Chemistry, vol. 62, no. 13, pp. 2845–2852, 2014, doi: 10.1021/jf4034078.

[8] Y. H. Yong, S. Yamaguchi, and Y. Matsumura, "Effects of enzymatic deamidation by protein-glutaminase on structure and functional properties of wheat gluten," Journal of Agricultural and Food Chemistry, vol. 54, no. 16, pp. 6034–6040, 2006, doi: 10.1021/jf060344u.

[9] H. Kumagai, A. Suda, H. Sakurai, H. Kumagai, S. Arai, N. Inomata, and Z. Ikezawa, "Improvement of digestibility, reduction in allergenicity, and induction of oral tolerance of wheat gliadin by deamidation," Bioscience, Biotechnology, and Biochemistry, vol. 71, no. 4, pp. 977–985, 2007, doi: 10.1271/bbb.60645.

[10] X. Chen, W. Fu, Y. Luo, C. Cui, I. Suppavorasatit, and L. Liang, "Protein deamidation to produce processable ingredients and engineered colloids for emerging food applications," Comprehensive Reviews in Food Science and Food Safety, vol. 20, no. 4, pp. 3788–3817, 2021, doi: 10.1111/1541-4337.12759.

[11] C. Villemin, O. Tranquet, V. Solé-Jamault, J. J. Smit, R. H. H. Pieters, S. Denery-Papini, and G. Bouchaud, "Deamidation and enzymatic hydrolysis of gliadins alter their processing by dendritic cells in vitro," Journal of Agricultural and Food Chemistry, vol. 68, no. 5, pp. 1447–1456, 2020, doi: 10.1021/acs.jafc.9b06075.

[12] J. Guan, R. Takai, K. Toraya, T. Ogawa, K. Muramoto, S. Mohri, D. Ishikawa, T. Fujii, H. Chi, and S. J. Cho, "Effects of alkaline deamidation on the chemical properties of rice bran protein," Food Science and Technology Research, vol. 23, no. 5, pp. 697–704, 2017, doi: 10.3136/fstr.23.697.

[13] W. He, W. Zhao, and R. Yang, "Effects of wheat gluten modified by deamidation-heating with three different acids on the microstructure of model oil-in-water emulsion and rheological–physical property of ice cream," Food Hydrocolloids, vol. 87, pp. 679–690, 2019, doi: 10.1016/j.foodhyd.2018.08.025.

[14] L. Liao, X. Y. Han, M. M. Zhao, L. Ni, Z. B. Liu, and W. Zhang, "Effect of native aggregation state of soluble

wheat gluten on deamidation behavior in a carboxylic acid/heat water solution," Journal of Cereal Science, vol. 72, pp. 1–9, 2016, doi: 10.1016/j.jcs.2016.09.011.

[15] Y. Wang, J. Gan, Y. Zhou, Y. Cheng, and S. Nirasawa, "Improving solubility and emulsifying property of wheat gluten by deamidation with four different acids: Effect of replacement of folded conformation by extended structure," Food Hydrocolloids, vol. 72, pp. 105–114, 2017, doi: 10.1016/j.foodhyd.2017.04.013.

[16] L. Liao, X. Han, L. P. Chen, L. Ni, Z. B. Liu, W. Zhang, and Q. Chen, "Comparative characterization of the deamidation of carboxylic acid deamidated wheat gluten by altering the processing conditions," Food Chemistry, vol. 210, pp. 520–529, 2016, doi: 10.1016/j.foodchem.2016.04.113.

[17] E. Betiku, A. M. Akintunde, and T. V. Ojumu, "Banana peels as a biobase catalyst for fatty acid methyl esters production using Napoleon's plume (Bauhinia monandra) seed oil: A process parameters optimization study," Energy, vol. 103, pp. 797–806, 2016, doi: 10.1016/j.energy.2016.02.13.

[18] L. Barnés-Calle, P. Gou, E. Fulladosa, and F. W. J. van den Berg, "Exploring the use of FTIR Amide I band deconvolution to investigate protein secondary structure and texturisation during high moisture extrusion," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 351, no. 127509, 2026, doi: 10.1016/j.saa.2026.127509.

[19] M. Fevzioglu, O. K. Ozturk, B. R. Hamaker, and O. H. Campanella, "Quantitative approach to study secondary structure of proteins by FT-IR spectroscopy, using a model wheat gluten system," International Journal of Biological Macromolecules, vol. 164, pp. 2753–2760, 2020, doi: 10.1016/j.ijbiomac.2020.07.299.

[20] Y. Dai, W. Zhong, X. Hu, X. Ji, Q. Sun, R. Wang, D. Yu, F. Wu, and L. Wang, "Conformational and functional changes from deamidation of wheat gluten with electrochemical treatment," Journal of the Science of Food and Agriculture, vol. 103, no. 11, pp. 5677–5686, 2023, doi: 10.1002/jsfa.12702.

[21] K. Kato, T. Nakayoshi, E. Kurimoto, and A. Oda, "Computational studies on the nonenzymatic deamidation mechanisms of glutamine residues," ACS Omega, vol. 4, pp. 3508–3513,

2019, doi: 10.1021/acsomega.8b03199.

[22] X. Li, C. Lin, and P. B. O'Connor, "Glutamine deamidation: Differentiation of glutamic acid and gamma-glutamic acid in peptides by electron capture dissociation," Analytical Chemistry, vol. 82, no. 9, pp. 3606–3615, 2010, doi: 10.1021/ac9028467.

[23] G. A. N. Gowda, Y. N. Gowda, and D. Raftery, "Massive glutamine cyclization to pyroglutamic acid in human serum discovered using NMR spectroscopy," Analytical Chemistry, vol. 87, no. 7, pp. 3800–3805, 2015, doi: 10.1021/ac504435b.

[24] L. M. Bersin, S. M. Patel, and E. M. Topp, "Effect of pH on the rate of pyroglutamate formation in solution and lyophilized solids," Molecular Pharmaceutics, vol. 18, no. 8, pp. 3116–3124, 2021, doi: 10.1021/acs.molpharmaceut.1c00338.