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Effect of Non-Thermal Alkaline (Lime) Soaking on Amino Acid Profile of Maize (*Zea mays* L.): A Modified Nixtamalization Approach

Asnawi Baso[1,2], Rimbawan[1]*, Lilik Kustiyah[1]

[1] Nutrition Science Study Program, Faculty of Medicine and Nutrition, IPB University, Indonesia
[2] Gerak Makmur Health Center, South Buton, Southeast Sulawesi, Indonesia

Corresponding Author Email: rimbawan@apps.ipb.ac.id

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ABSTRACT

Maize (*Zea mays* L.) is one of the major sources of dietary energy and protein for humans; however, its protein quality is limited due to deficiencies in essential amino acids, particularly lysine and tryptophan. Traditional lime-based processing (nixtamalization) has long been applied in Mexico for tortilla production and shares similarities with local maize-processing practices on Buton Island, Indonesia, which also utilize lime solutions. This study applied a modified nixtamalization approach, in which maize kernels were soaked in a lime solution under non-thermal conditions, followed by water-based heating. The objective of this study was to evaluate the effects of the treatment on the amino acid profile and amino acid scores of maize protein, with water soaking used as the control. Amino acid profiles were analyzed using high-performance liquid chromatography (HPLC), and amino acid scores were calculated based on FAO/WHO reference patterns for children aged ≥3 years and adults. The results showed that the relative ratios of lysine and tryptophan were higher than those of the control (1.39 and 1.09, respectively), whereas arginine exhibited a markedly lower ratio (0.19). This non-thermal alkaline treatment reflects changes in the amino acid profile of maize protein, although it was accompanied by a reduction in total protein content. However, the implications of these changes in the amino acid composition for the biological quality of protein still require further verification through digestibility-based measurements. This lime-based approach inspired by local practices may represent an alternative processing strategy for modulating the amino acid profile of maize, with indications of alterations in essential amino acid composition and increased amino acid scores

Key Messages:

- This study introduces a modified nixtamalization approach using non-thermal lime soaking followed by water heating as a mild-processing method to modify maize protein.
- The non-thermal alkaline treatment selectively altered the amino acid profile and indicated relative increases certain essential amino acids, suggesting its potential application for improving the protein quality of maize-based traditional foods

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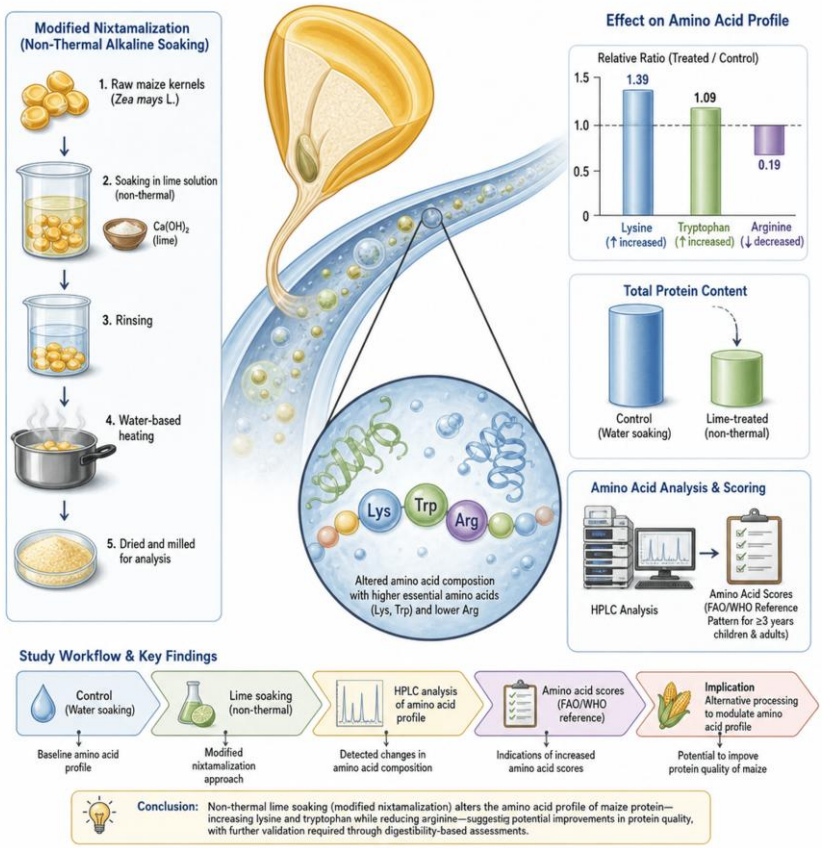
GRAPHICAL ABSTRACT



INTRODUCTION

Maize (*Zea mays* L.) is one of the major staple foods in many countries, including Indonesia, serving as an important source of energy and protein. However, maize proteins are characterized by a limited content of essential amino acids, particularly lysine and tryptophan (1), which contributes to the low protein quality of maize-based food products. These amino acids are recognized as the primary limiting amino acids in maize-based diets; therefore, enhancing their availability has important implications for improving protein quality and overall nutritional value (2).

Various processing approaches have been explored to improve the protein quality of maize, including fermentation, germination, and nixtamalization. Among these, nixtamalization is known to be effective in modifying protein structure and enhancing mineral and amino acid availability. Nixtamalization involves cooking maize in an alkaline solution, typically with calcium hydroxide ($Ca(OH)_2$), followed by steeping. This traditional process, long practiced in Central America for tortilla production, has been reported to improve mineral bioavailability while reducing mycotoxins and antinutritional factors in maize (3)

Interestingly, a similar alkaline-based maize processing technique has been observed in Indonesia, particularly in Buton Island, Southeast Sulawesi. In this traditional method, maize kernels are soaked in lime water under non-thermal conditions and subsequently boiled in plain water without additional alkali. Although not identical to conventional nixtamalization, this local practice represents a natural adaptation of the process that may produce comparable effects on protein structure. However, the effects of nixtamalization on amino acid profiles—especially lysine and tryptophan—remain controversial in the literature.

Previous studies have reported inconsistent findings regarding the impact of nixtamalization on lysine and tryptophan. Sunico et al. observed increased levels of both amino acids in nixtamalized Quality Protein Maize (QPM), whereas Gallego-Castillo et al. found improved lysine retention but decreased tryptophan content (4,5). Conversely, Ramírez-Jiménez et al. reported that traditional nixtamalization

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reduced both amino acids, while Rojas-Molina et al. observed decreases of 36% and 38.7% in lysine and tryptophan, respectively, after five hours of lime soaking (2,6). Although most studies employed similar alkali concentrations (1% w/w), variations in heating temperature and duration have been shown to result in significant differences. This indicates that the intensity of thermal treatment under alkaline conditions is one of the key factor influencing amino acid profile changes, likely due to Maillard reactions and protein denaturation processes that are sensitive to heat and alkaline environments.

These variations may also be associated with shifts in protein fractions during nixtamalization. Mt. Pleasant reported that while the total protein content of nixtamalized maize decreased compared to raw maize, the amino acid composition improved, primarily due to an increased proportion of lysine associated with decreased zein solubility (7). In contrast, Rojas-Molina et al. found reductions in lysine and tryptophan linked to losses of soluble protein fractions such as albumins and globulins during the process, suggesting that protein degradation under thermal and alkaline conditions can reduce the availability of essential amino acids. Furthermore, Rojas-Molina et al. demonstrated that albumin and globulin fractions significantly declined in both thermally processed non-alkaline and thermally processed alkaline maize, with the most pronounced losses occurring under thermal-alkaline treatment (6). Therefore, alternative approaches that explore non-thermal conditions during the alkalization stage are needed to minimize the degradation of amino acids sensitive to thermal-alkaline processing, particularly lysine and tryptophan.

Although the effects of nixtamalization on the amino acid profile of maize have been widely reported, the impact of alkaline soaking under non-thermal conditions on amino acid profiles and amino acid scores remains incompletely understood. Based on this background, the present study hypothesizes that soaking maize in a lime solution under non-thermal conditions may affect the amino acid profile and amino acid scores compared with water-soaked controls. Accordingly, this study aims to evaluate the effects of non-thermal alkaline soaking on the amino acid profile and amino acid scores of maize as an adaptation of local processing practices that are traditionally applied on Buton Island, Indonesia.

METHODS

Experimental Design

This study employed a one-factor experimental design to compare the effects of non-thermal alkaline soaking and water soaking (control) on the amino acid composition of maize. The experiment was conducted in March 2024 at the Integrated Laboratory and Advanced Research Unit, IPB University (Bogor, Indonesia).

Materials and Sample Preparation

Yellow maize kernels (local variety, 11.55% moisture content) were obtained from South Buton Regency, Southeast Sulawesi, Indonesia. Two treatments were applied:

1. Alkaline soaking (non-thermal): maize was soaked at room temperature (28 ± 2 °C) in a 5% (w/w) food-grade calcium hydroxide [$Ca(OH)_2$] solution for 12 h, followed by boiling in water (without lime) for 2 h.
2. Control: maize was soaked in water for 12 h and boiled for 2 h under the same conditions.

After boiling, samples were drained, homogenized using a blender, and stored in sealed containers prior to analysis. Untreated maize kernels (raw) were also analyzed as the baseline reference. The analysis was performed in single replicate due to resource limitations but under controlled laboratory conditions. The choice of 5% lime concentration was based on Febrianto et al., who reported that this level produces optimal texture and enhanced flavor in lime-treated maize products such as tortillas (8).

Protein and Amino Acid Analysis

Protein content was determined using the Kjeldahl method (AOAC 978.04). The nitrogen content obtained was converted to crude protein using a conversion factor of 6.25, as recommended by WHO (2013)(9). The protein values were then corrected for sample moisture and expressed on a dry weight basis (g/100 g dry matter). The amino acid profile was determined using a Shimadzu HPLC system (Model SCL-10A/CBM-20A) equipped with a Thermo Scientific ODS-2 Hypersil column (1 mL min^{-1} flow rate).

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Samples were hydrolyzed with 6 N HCl at 110 °C for 24 h, evaporated using a rotary evaporator, and reconstituted in 0.01 N HCl before derivatization with ortho-phthalaldehyde (OPA). For tryptophan determination, alkaline hydrolysis was carried out using 4.2 M NaOH at 110 °C for 16 h to prevent degradation. Pre-column derivatization with OPA was followed by fluorescence detection at an excitation wavelength of 340 nm. Eighteen amino acids were quantified based on calibration with standard amino acid solutions (0.5 μmol mL^{-1}). Amino acid contents were initially obtained as μmol per g of sample from the chromatographic data and converted to mass units using the molecular weight of each amino acid. Values were then expressed as percent weight by weight (% w/w) and corrected to a dry weight basis using the measured moisture content. For the calculation of amino acid scores, amino acid contents were further expressed as mg per g protein in accordance with FAO/WHO recommendations.

Amino Acid Scoring Pattern

Amino acid scoring is widely recognized as a reliable approach for assessing protein quality based on its amino acid composition. Currently, three reference amino acid patterns are commonly used in nutritional science, namely those proposed by the Food and Agriculture Organization (FAO), the Institute of Medicine (IOM), and the whole egg protein model (9). The Airlee Conference (1981) established that the most appropriate method for evaluating protein quality involves comparing the amino acid composition of foods with human amino acid requirements (10). In this study, the FAO/WHO reference pattern for individuals above three years of age was used, in accordance with the target consumer group of the product under evaluation, including adolescents and pregnant women who have higher protein requirements (11,12).

Calculation of Amino Acid Concentration and Chemical Score

Amino acid concentration (μmol AA) was calculated using the following formula:

$$\text{AA concentration} = \frac{\text{Peak area (sample)}}{\text{Peak area (standard)}} \times 0.5\ (\mu\text{mol/mL}) \times 10\ (\text{mL dilution volume})$$

The amino acid percentage (% w/w) was computed as:

$$\text{AA \%} = \frac{\mu\text{mol AA} \times \text{Mr AA}}{\mu\text{g sample}} \times 100 \qquad (\text{Mr AA = molecular weight of amino acid})$$

The amino acid score was determined by comparing the content of each essential amino acid (EAA) with the reference amino acid pattern established by FAO/WHO (10):

$$\text{Amino Acid scores} = \frac{\text{EAA (mg/g protein)}}{\text{FAO/WHO reference pattern (mg/g protein)}}$$

RESULTS

Amino Acid Profile

Non-thermal treatment of maize using a lime solution, followed by heating in water without additional lime, resulted in discernible differences in protein content and amino acid profiles compared with the control. Total protein content showed a slight decrease from 11.67 g in the raw material to 10.87 g in the control and 10.54 g in the treated samples (on a dry weight basis), indicating that lime soaking exerted a modest effect on total protein reduction. Nevertheless, most amino acids exhibited increased concentrations in the treated samples. Among all detected amino acids, glutamic acid, proline, and leucine were the most abundant, whereas cysteine, methionine, and tryptophan were present at the lowest levels.

The relative ratios between the treated and control samples indicated an average increase of 1.08. Amino acids with relative ratios exceeding 1.10 included glutamic acid, proline, alanine, leucine, serine, valine, isoleucine, phenylalanine, and lysine. In contrast, glycine, histidine, and arginine exhibited ratios below 1.00. Taken together, these results indicate a consistent trend toward higher amino acid levels in the treated samples compared with the control.

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Table 1. Amino acid profile and relative ratio in treated and control maize samples

Parameter	Treatment	Control	Relative ratio
	(mg g^{-1} protein)		(T/C)
Asp	64,4	59,0	1,09
Thr	39,6	36,3	1,09
Ser	59,5	52,2	1,14
Glu	255,2	217,9	1,17
Pro	101,6	88,5	1,15
Gly	32,2	34,0	0,95
Ala	94,2	81,7	1,15
Val	42,1	36,3	1,16
Cys	12,4	11,3	1,09
Met	9,9	9,1	1,09
Ile	42,1	36,3	1,16
Leu	166,0	133,9	1,24
Tyr	27,3	25,0	1,09
Phe	59,5	49,9	1,19
His	49,6	52,2	0,95
Lys	34,7	25,0	1,39
Arg	19,8	104,4	0,19
Trp	2,5	2,3	1,09
Mean	-	-	1,08
Total Protein	10,54	10,87	-

Footnote: Values are expressed on a dry weight basis. Amino acid contents are expressed in mg g^{-1} protein, while total protein is expressed as % (w/w). Relative ratio = (treatment / control).

Amino Acid Score

Based on the data presented in Table 2, most essential amino acids in maize subjected to lime soaking treatment exhibited higher amino acid scores compared with the control. The most pronounced increase was observed for lysine, which increased from 52 to 72, whereas tryptophan, identified as the limiting amino acid, showed a modest increase from 34 to 38. Other amino acids, including threonine, valine, isoleucine, leucine, and tyrosine, exhibited variable increases, while histidine showed slightly lower values in the treated samples compared with the control. Nevertheless, sulfur-containing amino acids, lysine, and tryptophan remained below a score of 100 in both the control and treated samples.

Table 2. Amino Acid Scores of Treated and Control Maize Samples

Amino Acid	Treated	Control
Thr	159	145
Val	105	91
Met + Cys	97	89
Ile	140	121
Leu	272	220
Phe + Tyr	212	183
His	310	326
Lys	72	52
Trp	38	34

DISCUSSION

Treatment of maize using a modified nixtamalization approach involving non-thermal alkaline soaking followed by boiling resulted in a 9.69% reduction in protein content, whereas water soaking followed by boiling resulted in a 6.86% decrease relative to the initial level. This reduction is consistent with previous reports on conventional nixtamalization and simple boiling (13–15), which indicate that thermal denaturation is the primary cause of protein loss, while alkaline reactions also contribute to protein degradation. However, the percentage of reduction observed in this study was lower than that reported by Joshi et al. (15), indicating a lower overall loss of total protein compared with conventional

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nixtamalization. This finding aligns with previous reports that albumin and globulin fractions are thermolabile and sensitive to pH changes, and therefore are easily lost under nixtamalization conditions (6,16). Accordingly, a non-thermal alkaline approach may contribute to protein retention and potentially reduce the loss of protein fractions known to be sensitive to thermal–alkaline treatments.

Amino acid profile analysis indicated that soaking maize in lime solution at room temperature without prior heating can selectively modify amino acid content through chemical mechanisms influenced by alkaline conditions, rather than by the combined effects of thermal and alkaline treatments. This phenomenon suggests that relative changes in amino acids are not solely dependent on thermal processing but are also associated with protein solubility and structural stability under alkaline conditions. This observation aligns with findings by Gallego-Castillo et al. (5), who, despite studying conventional nixtamalization, reported differences in amino acid retention attributable to alkaline exposure. Conceptually, these relative changes may be interpreted as one possible consequence of limited leaching of soluble protein fractions and changes in the solubility of more stable fractions during treatment (17). These results indicate that the solubility dynamics of protein fractions likely play an important role in determining changes in amino acid composition during non-thermal alkaline processing, in which soaking conditions, including alkali concentration and temperature, selectively modulate protein fraction solubility.

The paradoxical relationship between the decrease in total protein content and the increase in relative amino acid concentrations can be explained by the redistribution among protein fractions. Alkaline and thermal treatments are known to reduce the solubility of more water-soluble fractions such as albumin and globulin, while the proportion of glutelin and zein tends to increase due to their greater structural stability under these conditions (2,6). Glutelin forms strong covalent and noncovalent networks that are resistant to degradation, whereas zein is highly hydrophobic with a predominance of nonpolar amino acids (18). Changes in fraction composition align with the increased concentrations of dominant amino acids from these fractions, including glutamate, leucine, proline, and alanine (19). Therefore, although total protein content decreased, this redistribution may improve the amino acid profile of maize, as indicated by the relative increase of several amino acids known to be associated with protein fractions that are more stable against processing treatments.

At the compositional level, non-thermal alkaline treatment increased the relative lysine ratio compared with the control (≈1.39), indicating that lysine exhibits comparatively higher resilience to the applied processing conditions. This observation is consistent with the findings of Gallego-Castillo et al. (5), who reported that the true retention of lysine in nixtamalized tortillas tended to be higher than that in raw kernels, although the difference was not statistically significant, suggesting that the majority of lysine is preserved during alkaline processing. Rojas-Molina et al. further reported a positive correlation between lysine content and the albumin–globulin protein fractions, highlighting the important role of these fractions in maintaining lysine availability during processing (6). Complementarily, Sethi and Chaudhary demonstrated that glutelin content correlates positively with lysine concentration, whereas zein shows a negative correlation, indicating that the redistribution of structural protein fractions during alkaline soaking and subsequent heating can modulate lysine retention (20). Collectively, the observed increase in the relative lysine ratio likely reflects a compensatory mechanism involving an enhanced contribution of lysine-rich protein fractions that remain stable under non-thermal alkaline conditions and boiling temperatures well below the optimal range for Maillard reactions (110–120 °C) (21).

The relatively high ratio of lysine may also be interpreted from the perspective of the chemical reactivity of its ε-amino functional group during alkaline soaking and subsequent heating. Under alkaline conditions, the ε-amino group of lysine is theoretically subject to an increased degree of deprotonation (pKa > 10), transitioning from a positively charged form ($-NH_3^+$) to a neutral form ($-NH_2$), thereby enhancing its nucleophilicity toward carbonyl groups. While this condition could theoretically increase the propensity of lysine to participate in Maillard reactions, the reaction rate under non-thermal treatment (28–30 °C) is expected to be extremely low, rendering lysine degradation via Maillard pathways minimal. When boiling is subsequently applied at 90–100 °C, Maillard reactions may be thermodynamically favored; however, the near-neutral pH (pH ≈ 7) and high water activity (Aw ≈ 1) of the system are known to effectively suppress the formation of Maillard complexes (21). These combined conditions provide a

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conceptual explanation for the observed stability of lysine and its relatively higher ratio compared with other amino acids, as non-enzymatic reactions that typically compromise lysine are kinetically constrained under the applied non-thermal alkaline and neutral-pH heating conditions. Accordingly, the increased relative lysine ratio observed after non-thermal alkaline soaking followed by thermal processing likely reflects a balance between the protective effects of alkaline exposure prior to heating and the limited progression of Maillard reactions during subsequent cooking.

Non-thermal alkaline treatment resulted in a decrease in the relative arginine ratio compared with the control (≈0.19), indicating that alkaline soaking conditions may influence the stability of arginine residues within the maize protein matrix. At the high pH generated by a 5% (w/w) lime solution, the guanidinium group of arginine ($H_2N–C(=N^+H_2)–NH–R$) may theoretically undergo partial deprotonation (with reported pKa values of arginine residues in proteins ranging from approximately 11.5 to 15, with an average of around 12), rendering a fraction of arginine residues more nucleophilic and potentially more reactive toward nucleophilic addition reactions (21). In addition, arginine, together with lysine and proline, is among the basic amino acid residues reported to be particularly susceptible to oxidative deamination and to contribute to protein carbonyl formation in food proteins (22). This susceptibility may be further enhanced when proteins undergo conformational loosening or partial unfolding under high-pH conditions, which increases the exposure of reactive residues to oxidative agents (23). The present findings are consistent with previous studies reporting arginine reductions of approximately 18.7% at 1% (w/w) lime concentration (24), as well as more pronounced decreases reaching up to 55.7% under alkaline treatment (25). Taken together, the observed decrease in the relative arginine ratio in this study likely reflects the combined effects of changes in guanidinium protonation state, oxidative deamination, and protein unfolding during non-thermal alkaline treatment, which collectively contribute to reduced arginine stability and retention relative to the control.

In general, the relative increase in lysine and the pronounced decrease in arginine can be attributed to the possible redistribution of protein fractions as well as to differences in the physicochemical properties of these two amino acids under non-thermal alkaline conditions followed by heating at neutral pH. The apparent stability of lysine seems to be associated with the relative resistance of the albumin–globulin fractions to degradation, together with the contribution of glutelin fractions, which have been reported to form strong covalent linkages and to remain relatively stable under non-thermal treatment. In contrast, arginine, which contains a highly basic guanidinium group, is more susceptible to partial deprotonation and oxidative deamination under alkaline conditions, thereby contributing to a marked reduction in arginine content. This phenomenon is consistent with the findings of Gallego-Castillo et al. (5), who reported that lysine remains relatively stable during the nixtamalization process, whereas other basic amino acids, such as arginine and histidine, are more prone to chemical modification, as observed in the present study. Accordingly, non-thermal alkaline treatment not only preserves lysine stability but also indicates a redistribution of essential amino acid composition during processing. Although arginine exhibited a relatively large reduction, the essential amino acids lysine and tryptophan, which are required across all age groups, were relatively preserved.

Amino acid score analysis revealed that, in maize treated with a lime solution at room temperature, tryptophan emerged as the primary limiting amino acid, while lysine functioned as the secondary limiting amino acid. This pattern contrasts with the commonly reported amino acid limitation in maize, in which lysine is typically identified as the principal limiting amino acid (26,27). This treatment indicates a shift in amino acid composition that is likely associated with the redistribution of protein fractions during non-thermal alkaline processing, thereby increasing the relative availability of lysine and tryptophan. These findings are consistent with previous reports indicating that, following alkaline treatment, tryptophan frequently becomes the dominant limiting amino acid (28,29). This phenomenon has been linked to the relative stability of lysine and the susceptibility of tryptophan to thermo-alkaline conditions, which have been reported to reduce tryptophan content by up to 32% (15,28). However, the present study exhibits a distinct pattern, wherein tryptophan shows a relative increase, resulting in a higher chemical score. This divergence indicates that non-thermal alkaline treatment may suppress tryptophan degradation while preserving lysine, thereby rebalancing the proportions of essential amino

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acids and enhancing the chemical score that reflect changes in the relative amino acid composition of maize protein. Although protein quality was not directly assessed, the functional implications of the observed changes in essential amino acid composition and chemical scores may potentially have a positive impact on protein quality, as reported in previous studies on nixtamalization (2,17,30).

This study demonstrates the potential of non-thermal lime treatment as an efficient and culturally adaptive approach for modulating the compositional characteristics of maize protein. In addition to indicating an improved relative balance of essential amino acids, this approach provides a conceptual framework for the development of similar technologies in other cereals, thereby opening opportunities for food innovation based on nixtamalization-derived modifications that may help reduce protein component degradation during processing, while further validation of nutritional quality remains necessary.

This study has several limitations that should be considered when interpreting the findings. First, the data were generated from a single analytical replicate, which constrains the statistical power and limits the generalizability of the results. Second, the study did not include protein fractionation or structural characterization, so the mechanisms of protein redistribution and modification underlying the observed changes in amino acid profiles can only be inferred indirectly from the literature. Third, although changes in amino acid profiles and amino acid scores were documented, protein digestibility was not assessed; therefore, the implications of these changes for the biological quality of the protein cannot be directly confirmed.

CONCLUSION

This study demonstrates that soaking maize in a lime solution under non-thermal conditions is able to modulate the compositional characteristics of maize protein, as reflected by changes in amino acid profiles and amino acid scores compared with water-soaked controls. Although a moderate reduction in total protein content was observed, this treatment contributed to a redistribution of amino acids, as indicated by an increased chemical score of tryptophan as the limiting amino acid. These findings suggest that non-thermal alkaline treatment has the potential to maintain the compositional stability of amino acids within the maize protein matrix, without the involvement of thermal.

Future studies should be directed toward measuring lysine retention, with particular emphasis on reactive lysine as an indicator of nutritionally available lysine after processing. Variations in lime concentration and soaking duration should be evaluated to understand the causal relationship between the intensity of non thermal alkali treatment and the stability of amino acids. In addition, protein fractionation and structural analyses, along with in vitro and in vivo protein digestibility assessments, are required to establish mechanistic links between processing conditions, protein structural changes, and their potential implications for protein quality. Comparative studies between thermal and non-thermal alkaline treatments are also important to clarify the effects of processing conditions on the stability of lysine and other essential amino acids.

DECLARATION OF THE USE OF AI

The authors declare that no artificial intelligence (AI), AI-assisted technologies, or large language models (LLMs) were used in the conception of the study, data analysis, or the drafting, writing, and editing of this manuscript. The only exception is the graphical abstract, which was created using the design platform Illustrae (https://illustrae.co/). The authors take full responsibility for the content and accuracy of the graphical abstract and the entire manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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