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Research Article

Impact of Thermal and High-pressure Processing on the Amino Acid profile of *Trichosanthes lobata*

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ABSTRACT
This study investigated the impact of thermal and high-pressure processing (HPP) on the amino acid profile of *Trichosanthes lobata*, an underutilized plant with high nutritional potential. The objective was to evaluate how these food processing methods affect amino acid content, which directly influences the nutritional value of the plant. Samples of *T. lobata* were subjected to thermal treatment at 80°C, 90°C, and 100°C, and HPP at 250 MPa, 300 MPa, and 350 MPa. Amino acid concentrations were then analyzed and compared to unprocessed controls. Thermal processing resulted in a significant reduction ($P \leq 0.05$) in amino acid content across all temperatures, with decrease in key amino acids such as leucine, lysine, isoleucine, and glutamic acid. Essential amino acids were especially vulnerable, indicating loss of nutritional quality even at moderate temperatures (80°C). In contrast, HPP at 250 MPa and 300 MPa preserved amino acid levels effectively, with no significant difference from the unprocessed samples ($P > 0.05$). However, at 350 MPa, there was amino acid degradation suggesting a pressure threshold beyond which nutritional integrity is compromised. In conclusion, while thermal processing significantly diminishes the amino acid content of *T. lobata* moderate HPP (≤300 MPa) is a promising non-thermal alternative that preserves its protein quality. Despite its potential, limited studies have been conducted on the impact of thermal and high-pressure processing on *Trichosanthes lobata*. Given the importance of this plant as a functional food and medicinal ingredient, assessing how these processing techniques influence its amino acid profile is essential.

Keywords: Amino acid profile; High pressure processing (HPP); Nutritional quality; Thermal processing; *Trichosanthes lobata*

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INTRODUCTION

The plant *Trichosanthes lobata*, commonly known as kudzu or snake gourd, is a perennial vine native to East Asia and some part of Africa including South west and North central Nigeria, recognized for its medicinal properties and nutritional potential. The plant is traditionally used as bitter tonic, laxative, depurative, digestive, cardiotonic, anthelmintic and in the treatment of jaundice (Patil and Wadekar, 2017). It has been reported to contain various phytoconstituents such as cucurbita-5, 24-dienol, α, β carotene, Vitamin C and β-sitosterol and plays an important role in the Ayurvedic and Siddha system of medicine due to its various medicinal values like anti-HIV, Cardioprotective, anti-ulcer, antidiabetic, heteroprotective, anti-inflammatory and larvicidal effects (Sonwalkar *et al*., 2013). They are also low in calories, contains high fiber and rich in essential

amino acids (Olaide Aderibigbe *et al*.,2019). This unique plant with many nutritional and medicinal benefit is however not well known in this part of the world hence its underutilization. The amino acid profile of *T. lobata* is a critical determinant of its nutritional value, influencing its protein quality and potential health benefits. Understanding how processing methods affect this profile is essential for optimizing its use in food and pharmaceutical applications (Adu, *et al*., 2015).

Increase in health consciousness of food consumers has placed high demand for food with better quality such as improved safety, freshness, nutritional value and flavors (Zemser, 2015).

One way to make food available as farm-fresh state for later use in distant markets is to convert them into more stable form. Thus, the major emphasis of food processing is preservation or shelf-life extension by preventing undesirable changes in the wholesomeness, nutritive value and sensory qualities by controlling chemical, biochemical, physiological and microbiological activities (Daher *et al*., 2017).

New processing technologies to deliver products in compliance with the above requirements, while maintaining other main quality attributes such as nutritive and sensory properties has become imperative (Amsasekar *et al*., 2022).

Thermal processing, including methods such as boiling, drying, and roasting, is widely used to enhance the edibility and safety of plant-based foods. However, these processes can significantly impact the protein content and amino acid composition of the food material. For instance, a study on *Terminalia catappa* seeds revealed that thermal treatments led to a reduction in crude protein content and alterations in amino acid profiles, with essential amino acids ranging from 26.34% to 34.06% of the total amino acids. Notably, glutamic acid, aspartic acid, leucine, and lysine were predominant, while methionine and cysteine were identified as limiting amino acids (Adu, *et al*., 2015). Similarly, research on *Monodora myristica* seeds demonstrated that heat treatment reduced crude protein levels and affected amino acid concentrations, with glutamic acid, aspartic acid, leucine, and lysine being the most abundant (Agiriga and Muthulisi, 2018).

High-pressure processing (HPP) is an emerging non-thermal technology that inactivates microbial populations and enzymes while preserving the sensory and nutritional properties of foods. Studies have shown that HPP can influence the free amino acid content of various food products. For example, research on lamb cuts subjected to HPP indicated a significant increase in total free amino acids compared to untreated samples, suggesting enhanced proteolysis (Alahakoon, *et al*., 2020). Additionally, a review on aquatic foods highlighted that HPP effectively inactivates microbial populations and endogenous enzymes while retaining sensory and nutritional properties (Aubourg, 2018).

Due to consumers demand for high quality processed foods with minimal changes in nutritional and sensory properties, it has become important for alternative or novel processing technologies to be explored and implemented to provide safe, fresher-tasting, nutritive foods without the use of heat or chemical preservatives (Ravishankar, 2016).

While extensive research exists on the effects of thermal and high-pressure processing on the amino acid profiles of various plant and animal sources, there is a paucity of data specifically concerning *T. lobata*. Investigating how these processing methods affect the amino acid composition of *T. lobata* could provide valuable insights into optimizing its nutritional and functional properties for food and medicinal applications.

MATERIALS AND METHODS

Collection of Fruit Sample

Fruit samples of *Trichosanthes lobata* were collected from Kujama in Chikun LGA of Kaduna state and authenticated at the Herbarium of Botany Department, Ahmadu Bello University, Zaria.

Preparation of Plant Samples and Extraction

The fruits were rinsed with distilled water, vacuum-dried in the laboratory and made into powder by grinding.

Thermal Processing

Trichosanthes lobata samples were boiled at varying temperatures of 80°C, 90°C, and 100° C using regulated hot plate after which they were filtered. The boiled sample was dried in an oven at 55°c for 24h. after which, the sample was grinded in a Laboratory Bench Mill (Thomas-WILLKEY, Laboratory Model 4, Arthur H. Thomas Company, Philadelphia, PA, U.S.A.) and kept in a cool dry rubber container for subsequent analysis.

Non-thermal Processing

The freshly grinded samples were subjected to high pressure of 250Mpa, 300Mpa, and 350Mpa at room temperature for ten minutes. They were then vacuum-dried at 720mmHg for 48 hr. (ESRA *et al*, 2010).

Amino Acid Profiling

The Amino Acid profile in the known sample was determined using methods described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting of Sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 2.0g of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus (AOAC, 2006).

Nitrogen Determination:

A small amount (150mg) of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture (0.5g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added. The flask was then put in Kjeldahl digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

Percentage Nitrogen = ((a-b) x 0.01 x 14 x V x 100)**/**(W x C)

Where: a.=Titre value of the digested sample, b.=Titre value of blank sample, v. =Volume after dilution (100ml), W.= Weight of dried sample (mg), C.=Aliquot of the sample used (10ml) and 14.= Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7ml of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105^0C± 50C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6 N HCl during hydrolysis. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer

The amount loaded was 60 microliters. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Data Analysis

Results were presented as mean ± Standard Deviation. Differences in means were determined by ANOVA tool of the Statistical Package for Social Sciences (SPSS version 21). While Duncan's multiple range test was used to determine the extent of variation of the parameters. $P \leq 0.05$ were taken to be statistically different.

RESULTS

Effect of Thermal Processing on the Amino Acid Profile of *T. lobata*

The observed result in table 1 revealed that thermal processing at 80°C, 90°C, and 100°C significantly reduced the amino acid content in *T. lobata* across all measured amino acids. This reduction was statistically significant ($P \leq 0.05$), amino acids, such as leucine, lysine, isoleucine, phenylalanine, and glutamic acid, dropped by over 85–95% after thermal treatment. Leucine decreased from 6.92 mg/100g in unprocessed samples to about 0.64–0.65 mg/100g across all processing temperatures. Increasing temperature from 80°C to 100°C did not result in further significant losses for many amino acids. Essential amino acids like lysine, tryptophan, methionine, and threonine were significantly reduced, which could negatively impact the

nutritional value of the thermally processed *T. lobata*. Thus, thermal processing, even at moderate temperatures (80°C), causes a significant degradation of amino acids in *T. lobata*, leading to a sharp decline in its protein quality and nutritional value.

Effect of High-Pressure Processing on the Amino Acid Profile of *T. lobata*

High Pressure Processing (HPP) at 250 MPa and 300 MPa preserved the amino acid profile of *T. lobata* extremely well, showing no significant changes ($P \leq 0.05$) compared to the unprocessed sample. However, at 350 MPa, there was a sharp and significant decline in amino acid content across all measured compounds. Amino acid concentrations at 250 MPa and 300 MPa are statistically identical to unprocessed values indicating that Leucine: 6.92 (unprocessed), 7.01 (250 MPa), 6.92 (300 MPa) while Glutamic acid remains 10.13 mg/100g at all three levels. This demonstrates that moderate HPP does not degrade amino acids, maintaining the nutritional integrity of the sample. On the other hand at 350 MPa, amino acid levels drop dramatically, similar to the effects seen under thermal processing. Like Leucine drops from 6.92 mg/100g to 0.64 mg/100g and Lysine: 5.57 → 0.53 mg/100g. This suggests a threshold effect where HPP becomes damaging to amino acids at or beyond 350 MPa. Low to moderate HPP (≤300 MPa) is highly effective for preserving protein quality without the degradation observed in thermal processing. At ≥350 MPa, HPP leads to comparable amino acid losses as heat treatments, making it unsuitable for preserving amino acid content.

Table 1: Effect of Thermal Processing on the Amino Acid Profile of *T. lobata*

Amino Acids (mg/100g)	Unprocessed	Thermally Processed		
		(80°C)	(90°C)	(100°C)
Leucine	6.92^{b}±0.22	0.64^{a}±0.01	0.64^{a} ±0.01	0.65^{a}±0.01
Lysine	5.57^{b}±0.06	0.53^{a}±0.00	0.50^{a}±0.01	0.51^{a}±0.01
Isoleucine	3.45^{b}±0.04	0.20^{a}±0.00	0.15^{a}±0.00	0.18^{a}±0.00
Phenylalanine	3.90^{b}±0.10	0.20^{a}±0.00	0.20^{a} ±0.00	0.20^{a}±0.00
Tryptophan	0.79^{b}±0.01	0.05^{a}±0.00	0.05^{a}±0.00	0.05^{a}±0.00
Valine	1.58^{c}±0.01	0.11^{b}±0.00	0.11^{b}±0.00	0.10^{a}±0.00
Methionine	0.69^{b}±0.01	0.05^{a}±0.00	0.05^{a}±0.00	0.05^{a}±0.00
Proline	3.29^{b}±0.10	0.40^{a}±0.00	0.37^{a}±0.00	0.35^{a}±0.00
Arginine	5.67^{b}±0.01	0.51^{a}±0.00	0.50^{a}±0.00	0.50^{a}±0.00
Tyrosine	3.78^{c}±0.02	0.34^{b}±0.00	0.30^{a}±0.00	0.32^{ab}±0.00
Histidine	1.79^{d}±0.01	0.13^{c}±0.00	0.10^{a}±0.00	0.12^{b}±0.00
Cystine	0.73^{c}±0.02	0.12^{b}±0.00	0.10^{a}±0.00	0.12^{b}±0.00
Alanine	4.46^{b}±0.12	0.40^{a}±0.00	0.39^{a}±0.00	0.38^{a}±0.00
Glutamic acid	10.13^{b}±0.01	0.91^{a}±0.00	0.90^{a}±0.00	0.90^{a}±0.00
Glycine	3.29^{b}±0.10	0.24^{a}±0.00	0.24^{a}±0.00	0.24^{a}±0.00
Threonine	3.22^{b}±0.00	0.22^{a}±0.00	0.20^{a}±0.00	0.21^{a}±0.00
Serine	4.00^{b}±0.01	0.40^{a}±0.00	0.40^{a}±0.00	0.40^{a}±0.00
Aspartic acid	6.93^{b}±0.03	0.50^{a}±0.00	0.48^{a}±0.00	0.47^{a}±0.00

Values are Mean±SD (n=3). Values in the same row with different superscripts are significantly different (P≤ 0.05).

Table 2: Effect of High-Pressure Processing on the Amino Acid Profile of *T. lobata*

Amino Acids (mg/100g)	Unprocessed	High Pressure Processed		
		(250Mpa)	(300Mpa)	(350Mpa)
Leucine	6.92 ^{b}±0.22	7.01^{b}±0.01	6.92^{b}±0.21	0.64^{a}±0.01
Lysine	5.57^{b}±0.06	5.61^{b}±0.01	5.57^{b}±0.06	0.53^{a}±0.01
Isoleucine	3.45^{b}±0.04	3.42^{b}±0.04	3.45^{b}±0.04	0.20^{a}±0.00
Phenylalanine	3.90^{b}±0.10	4.00^{b}±0.06	3.90^{b}±0.10	0.20 ^{a}±0.00
Tryptophan	0.79^{b}±0.01	0.79^{b}±0.00	0.79^{b}±0.01	0.05 ^{a}±0.00
Valine	1.58^{b}±0.01	1.57^{b}±0.01	1.58^{b}±0.01	0.11^{a}±0.00
Methionine	0.69^{b}±0.01	0.69^{b}±0.00	0.69^{b}±0.01	0.05^{a}±0.00
Proline	3.29^{b}±0.10	3.23^{b}±0.03	3.29^{b}±0.10	0.40^{a}±0.00
Arginine	5.67^{b}±0.01	5.67^{b}±0.01	5.67^{b}±0.01	0.51^{a}±0.00
Tyrosine	3.78^{b}±0.02	3.77^{b}±0.01	3.78^{b}±0.02	0.34^{a}±0.00
Histidine	1.79^{b}±0.01	1.79^{b}±0.00	1.79^{b}±0.01	0.13^{a}±0.00
Cystine	0.73^{b}±0.02	0.72^{b}±0.01	0.73^{b}±0.02	0.12^{a}±0.00
Alanine	4.46^{b}±0.12	4.40^{b}±0.00	4.46^{b}±0.12	0.40^{a}±0.00
Glutamic acid	10.13^{b}±0.01	10.13^{b}±0.01	10.13^{b}±0.01	0.91^{a}±0.00
Glycine	3.29^{b}±0.10	3.21^{b}±0.10	3.29^{b}±0.02	0.24^{a}±0.00
Threonine	3.22^{b}±0.00	3.22^{b}±0.00	3.22^{b}±0.00	0.22^{a}±0.00
Serine	4.00^{b}±0.01	4.01^{b}±0.01	4.00^{b}±0.01	0.40^{a}±0.00
Aspartic acid	6.93^{b}±0.03	6.92^{b}±0.03	6.93^{b}±0.03	0.50^{a}±0.00

Values are Mean±SD (n=3). Values in the same row with different superscripts are significantly different (P≤ 0.05).

DISCUSSION

This study showed that thermal processing significantly reduced all the essential amino acids investigated when compared to the unprocessed sample. The losses in the amino acids following varied temperature treatment could be attributed to their susceptibility to hot-water leaching or may have resulted from Maillard reaction or protein denaturation (Nwosu *et al.,* 2008). Our finding agrees with Chinyere and Obasi (2011) who reported that different processing treatments caused significant losses in the seventeen amino acids contents of selected leafy vegetable. Tsado *et al*.,2015 also reported that all the processing methods evaluated including boiling caused reduction in amino acid content of *Vernonia amygdalina*. High pressure processing at 250Mpa and 300Mpa (Table 2) produced no statistical difference in the essential amino acid composition of *Trichosanthes lobata* fruit especially Leucine which is present in very high amount in this fruit, this agrees with previous observations reported by Ade-Omowaye *et al*., 2015; and *James et al*., 2020 that Leucine is one of the most concentrated essential amino acid in some underutilized legumes while HPP at 350Mpa resulted in a significant *(P≤ 0.05)* decline in the amino acid profile relative to the control (unprocessed). Decline in all amino acids profile at a pressure of 350Mpa may be due to the high-pressure effects on the peptide bonds holding the amino acid chain together thereby breaking the bonds and causing leaching of the amino acid into the water that serves as the pressure medium. Amino acids degrade under heat but not under moderate HPP because high pressure processing unlike thermal processing functions at low temperature which will most likely not cause protein denaturation. This agrees with the findings of Adu *et al*., 2015 who discovered that processing methods that reduced moisture content had greater effect on improving essential amino acid content especially at very low temperature and high pressure. HPP is performed at room temperature, reducing energy consumption associated with heating and subsequent cooling therefore it does not significantly affect the flavour, nutritional value and functional properties of food (Daher *et al*.,2017). The foregoing, implies that the use of high-pressure processing within the range of 250-300Mpa or below could be much more beneficial in processing vegetables or fruits to retain or improve their amino acid profile.

CONCLUSION

The findings of this study demonstrate that thermal processing significantly degrades the essential amino acid content of *Trichosanthes lobata*, leading to a marked reduction in its nutritional quality because essential amino acids cannot be synthesized naturally it can only be obtained from food. Even at moderate temperatures (80°C), essential and non-essential amino acids were lost at rates exceeding 85%, indicating that heat treatment is detrimental to the protein integrity of the fruit. In contrast, High pressure processing (HPP) at 250 MPa and 300 MPa effectively preserved the essential amino acids, with no statistically significant differences compared to unprocessed samples. However, at 350 MPa, HPP induced substantial essential amino acid degradation, comparable to thermal effects. These results suggest that low to moderate HPP is a superior alternative to thermal methods for processing *T. lobata*, as it ensures that food products retain essential nutrients, maintains overall protein quality, freshness, organoleptic and nutritional qualities without the use of flavor-altering additives therefore processors in the food industry must use processing technologies capable of reducing additives while maintaining natural flavors and food quality this positions HPP as a promising technique for developing functional foods and nutraceuticals especially in Nigeria where food processing industries are yet to embrace HPP for processing food.

Further research work should be done to compare the microbial load after processing using thermal and high-pressure processing methods since literature says high pressure processing eliminates pathogenic and spoiling microorganisms better than thermal processing. Further research work should also be done to compare the amino acid profile of both fermented and unfermented fruits since the local farmers consume both.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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