



Research Article

Assessment of the Effect of Thermal and High-pressure Processing on the Proximate Composition of *Trichosanthes lobata*

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ABSTRACT

This study assessed the impact of thermal and high-pressure processing (HPP) on the proximate composition of *Trichosanthes lobata*. Fruits were collected from Kujama, Chikun LGA, Kaduna State, and authenticated at the Herbarium of the Botany Department, Ahmadu Bello University, Zaria. Proximate composition was determined using AOAC (2005) methods. Thermal processing at 80°C, 90°C, and 100°C significantly ($P \leq 0.05$) reduced moisture content, with samples at 90°C and 100°C showing no significant difference but lower values than 80°C. Crude protein declined across all thermally treated groups, lowest at 100°C. Fat and ash contents decreased insignificantly, while crude fiber reduced significantly. Carbohydrate content increased at 80°C and 90°C but declined slightly at 100°C, though not significantly different from the control. HPP at 250, 300, and 350 MPa also reduced moisture significantly, with 300 MPa yielding the highest reduction. Protein content remained unchanged at 250 MPa, declined slightly at 300 MPa, and increased at 350 MPa, though variations were not significant. Fat increased slightly at 250 and 300 MPa but decreased at 350 MPa. Ash declined insignificantly across HPP groups, while crude fiber reduced significantly. Carbohydrate content showed a notable increase under HPP. Overall, thermal processing caused greater nutrient losses, particularly in protein and fiber, whereas HPP preserved protein integrity and enhanced carbohydrates. The study highlights the comparative advantages of HPP over thermal methods in retaining nutritional quality of *T. lobata*, offering useful insights for optimizing processing techniques.

Keywords: Carbohydrate content; Crude protein; High-pressure processing (HPP); Moisture content; Proximate composition; Thermal processing

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INTRODUCTION

Food processing techniques play a critical role in enhancing the nutritional quality, safety, and shelf life of various agricultural products. However, these processes can significantly alter the proximate composition, including moisture content, protein, fat, fiber, ash, and carbohydrate levels, of food materials (Adebo et al., 2018). One such underutilized but nutritionally significant plant is *Trichosanthes lobata*, a member of the Cucurbitaceae family, known for its rich phytochemical and nutrient profile (Li et al., 2020). Understanding the impact of different processing methods on its composition is crucial for optimizing its

use in food and pharmaceutical industries. Thermal processing, including boiling, steaming, and roasting, is widely used to enhance digestibility, deactivate anti-nutritional factors, and improve food safety (Rasane et al., 2015). However, it may also lead to nutrient losses due to heat-induced degradation and leaching. On the other hand, high-pressure processing (HPP) is a non-thermal preservation technique that maintains food quality while inactivating pathogens and enzymes through the application of high hydrostatic pressure (Oey et al., 2008). Compared to conventional thermal methods, HPP has been reported to retain better

nutrient profiles and improve bioavailability in various plant-based foods (Tiwari *et al.*, 2009).

Despite its potential, limited studies have been conducted on the effects of thermal and high-pressure processing on *Trichosanthes lobata*. Given the importance of this plant as a functional food ingredient, assessing how these processing techniques influence its proximate composition is essential. This study aims to evaluate the impact of thermal and high-pressure treatments on the macronutrient content of *Trichosanthes lobata*, providing insights into the optimal processing methods that preserve its nutritional integrity.

MATERIALS AND METHODS

Collection of Fruit Sample

Fruit samples of *Trichosanthes lobata* were collected from Kujama in Chiukun 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.

Aqueous Extraction

One hundred and fifty grams (150g) of powdered fruit materials were added to 500mL of distilled water and left to stand for 48hrs. Afterwards, filtered using muslin cloth and concentrated under a reduced pressure using a rotator evaporator to obtain the crude aqueous extract. The process was repeated until all the soluble compounds have been extracted as was judged by loss of colour of the filtrate. The extract was then suction filtered and concentrated under a reduced pressure using a rotary evaporator.

N-Hexane Extraction

Another 150g of the powdered fruit was extracted with 500mL of n-hexane using sox let apparatus. The process was repeated until all the soluble compounds have been extracted as was judged by loss of colour of the filtrate. The extract was then suction filtered, concentrated under a reduced pressure using a rotary evaporator.

Thermal Processing

Trichosanthes lobata samples were boiled at varying temperatures of 70°C, 80°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).

Determination of Proximate Composition

Ash Content Determination

The term ash refers to the residue left after the combustion of the oven dried sample and is a measure of the total mineral content. Determination of ash content was done according to (AOAC, 2005). The four crucibles were preheated in a muffle furnace at about 525°C. Each crucible was cooled in a desiccator and weighed. Approximately 1g of each sample was weighed into the different crucibles. The crucibles and their contents were transferred into the muffle furnace at 525°C and allowed to stay for 1hour. The weights of the crucible contents were taken and recorded.

Percentage ash was calculated using the expression below

$$\% \text{ Ash} = (\text{weight of ash (g)} \times 100) / (\text{Weight of dry sample})$$

Determination of Moisture

The method employed for the determination of moisture content of the sample was based on the measurement of the loss in weight due to drying at a temperature of about 105°C as described by (AOAC, 1990). Four watch glasses were washed and dried in an oven at about 105°C after which they were cooled and weighed empty. About 2.0g of each sample was weighed into their respective watch glasses. The watch glasses and their contents were dried in an air circulated oven at about 105°C to a constant weight. The watch glasses and their contents were cooled in a desiccator and reweighed.

The percentage moisture content of each sample was calculated using the expression:

$$\% \text{ Moisture} = (\text{loss of weight on drying (g)} \times 100) / (\text{Initial sample weight (g)})$$

Determination of Crude Lipid Content

The lipid content of each sample, was determined by a similar procedure to the one described by (AOAC, 1990). A clean dry round bottom flask containing anti bumping granules was used. Exactly 210 cm³ of petroleum ether (60 - 80°C) was poured into the flask fitted with Sox let extraction unit. The weighed sample was transferred into a thimble already fixed into the Sox let extraction unit. Cold water was put into circulation. The heating mantle was switched on and the heating rate adjusted until the solvent was refluxed at a steady rate. Extraction was carried out for 8hours. The sample was removed and dried to a constant weight in an oven, cooled in a desiccator and reweighed and the percentage crude lipid content was determined thus;

% lipid = (weight of lipid extracted x 100)/weight of dry sample.

Where the weight of lipid extracted was the loss in weight of the sample after extraction dried in an oven and cooled in a desiccator.

Determination of Crude Fiber

Crude fiber was determined by the method of (AOAC, 1990). Two grams of grounded sample was placed in a round bottom flask. 100ml of 0.25M H₂SO₄ was added and mixture was boiled under reflux for 30minutes. The insoluble matter was washed several times with hot water until it was acid free (C1). It was transferred into a flask containing 100ml of 0.312M NaOH solution. The mixture was boiled again under reflux for 30 minutes and filtered under suction; the insoluble residue was washed with hot water until it was base free (C2). It was dried to a constant weight in an incinerator in a furnace at 550°C for 2 hours. The furnace was put off and allowed to cool down. The samples were removed and cooled in a desiccator and weighed (C3). The crude fibre content was calculated as loss of weight in ashing. Weight of original sample was used as W.

$$\% \text{ crude fibre} = (C2 - C3 \times 100)/W$$

Determination of Crude Protein

Proteins are major compounds containing nitrogen. They include amino acids, purines, ammonium salts, and vitamin B1. Nitrogen is used as an index termed crude protein as distinct from true protein. The Kjedahl method of AOAC (1990) was used for the crude protein determination. Exactly 20g of each sample was weighed into 100ml Kjedahl flask and a few anti bumping granules were added. 1g of the mixed catalyst (CuSO₄ and K₂SO₄ in the ratio 8:1 respectively) and 15ml of concentrated sulphuric acid was beaded. 10ml of the digest was pipetted into Markham semi micro nitrogen steel tube. 10ml of 45% NaOH solution was added cautiously. The sample was steam- distilled liberating ammonia into a 100ml containing 10ml of 4% boric acid and a drop of methyl blue indicator until the colour changed from pink to green. 30ml of sample volume was collected. The content was titrated with 0.1M HCl. The end point was indicated by a colour change from green to pink and the volume (v) of the acid for each distillate was noted. Percentage nitrogen per sample was calculated using the expression below

$$\% \text{ Nitrogen} = (M \times v \times 14 \times 100 \times 100) / \text{Weight of sample} \times 1000 \times 10$$

Where, M = Molarity of HCl, 14 = Atomic weight of nitrogen, 100 = Total volume of digest, 100 = % conversion, 10=Volume of the digest taken, 1000 = Conversion to litre.

The crude protein was calculated as

$$\% \text{ Protein} = 6.25 \times \% \text{ nitrogen.}$$

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≤ 0.05 were taken to be statistically different.

RESULTS

Effect of Thermal Processing on the Proximate Composition of *T. lobata*

Table 1 shows the proximate composition of thermally processed *T. lobata* fruit. The moisture content of the thermally processed samples decreased significantly (P≤0.05) compared to the unprocessed sample (control). Whereas the samples processed at 90°C and 100°C had no significant difference in their moisture contents but decreased significantly when compared to the sample processed at 80°C. For the crude protein content, the different temperature processed groups decreased considerably in comparison to their unprocessed counterpart. Similarly, the sample treated with 100°C showed the least value of crude protein which varied significantly with the other groups. There was decrease in the fat and ash contents of all the treated groups, though insignificant to the control group. The crude fibre composition of the treated samples depreciated significantly when compared to the unprocessed group. For the carbohydrate components, the samples processed at 80°C and 90°C appreciated in value while that of 100°C depreciated though not significant from the control group.

Effect of High-Pressure Processing on the Proximate Composition of *T. lobata*

High pressure processing (HPP) of *T. lobata* fruit significantly (P≤ 0.05) reduced the moisture content of the samples relative to the control (unprocessed) group with 300Mpa producing the highest reduction of moisture Table 2. Crude protein constituent of the group processed with 250Mpa was the same as that of the control. That of 300Mpa declined slightly while 350Mpa processing resulted in an increase in crude protein content though there was no significant difference in the variations noticed in the crude protein of all the groups. For the fat content, HPP (250Mpa and 300Mpa) resulted in a slight increase of the fat content compared to the control while 350Mpa resulted in slight decrease. We also observed an insignificant decline in the ash content of all the treated groups relative to the control. Crude fibre content of the samples, following HPP decreased significantly when compared to the unprocessed group. For the carbohydrate constituent,

HPP brought about a considerable increase in this parameter.

Table 1. Effect of Thermal Processing on the Proximate Composition of *T. lobata*

Parameters (%)	Unprocessed	Thermally Processed		
		(80°C)	(90°C)	(100°C)
Moisture content	90.46 ^c ±0.78	58.19 ^b ±0.31	57.84 ^{ab} ±0.31	57.78 ^{ab} ±0.19
Crude protein	3.49 ^b ±0.01	3.20 ^{ab} ±0.26	3.17 ^{ab} ±0.59	2.91 ^a ±0.21
Fat content	1.36 ^a ±0.08	1.32 ^a ±0.07	1.34 ^a ±0.08	1.32 ^a ±0.03
Ash content	9.50 ^a ±0.50	8.80 ^a ±0.75	8.75 ^a ±0.75	8.94 ^a ±0.90
Crude fibre	17.26 ^c ±0.43	16.37 ^{ab} ±0.38	16.11 ^a ±0.10	16.56 ^{ab} ±0.40
Carbohydrate	1.14 ^a ±0.36	1.34 ^a ±0.32	1.35 ^a ±0.39	0.76 ^a ±0.18

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 Proximate Composition of *T. lobata*

Parameters (%)	Unprocessed	High Pressure Processed		
		(250Mpa)	(300Mpa)	(350Mpa)
Moisture content	90.46 ^d ±0.78	57.79 ^{bc} ±0.20	57.20 ^b ±0.63	55.93 ^a ±0.28
Crude protein	3.49 ^a ±0.01	3.49 ^a ±0.01	3.48 ^a ±0.03	3.50 ^a ±0.03
Fat content	1.36 ^a ±0.08	1.37 ^a ±0.15	1.37 ^a ±0.10	1.27 ^a ±0.03
Ash content	9.50 ^a ±0.50	8.95 ^a ±0.11	9.00 ^a ±0.03	9.00 ^a ±0.05
Crude fibre	17.26 ^c ±0.43	16.46 ^{ab} ±0.30	16.69 ^b ±0.04	16.85 ^{bc} ±0.17
Carbohydrate	1.14 ^a ±0.36	5.46 ^b ±0.52	5.40 ^b ±0.56	5.64 ^b ±0.26

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

DISCUSSION

The essence of analyzing the proximate composition of food materials is to give information on their basic chemical composition, such as moisture, ash, crude fat, protein and carbohydrate. The findings showed the proximate composition of *T. lobata* fruit. Thermal and high pressure processing significantly (P≤ 0.05) reduced the moisture content of all the treated samples. The finding of Onyeike *et al.* (2015) supported ours that the moisture content of African walnut flour was significantly decreased at all levels of cooking. Moisture content is of significance as it is one of the determinants of shelf-life of processed foods (Agunbiade *et al.*, 2013). Moisture content is an index of water activity.

Proteins are very essential component of human diet required for the replacement of worn-out tissue, supply of energy and required amount of amino acids. Their deficiency results in poor growth, muscle wasting, oedema, abnormal swelling of the body and collection of fluid in the body of children (Schubert and DeLuca, 2010). Thermal processing gave rise to significant (P≤ 0.05) decline in the protein composition of all the samples. The decrease in crude protein content of *T. lobata* due to thermal processing could be attributed to leaching of nutrients into the cooking water and may also have resulted from Maillard reaction (Wei *et al.*, 2009, Adeyeye, 2010) or protein denaturation. (Adeniyen *et al.*, 2013). Tsado *et al.* (2015), reported

that all the processing methods evaluated including boiling caused reduction in the protein contents of *vernonia amygdalina*.

The fat content of the thermally processed samples slightly depreciated in value compared to the control. This may have been occasioned by heat application which melted the fat causing a reduction in their levels within the sample (Tsado *et al.*, 2015). Similarly, high pressure processing at 350Mpa showed a decline in the fat, generating a loss more than that of 100° C. On the other hand, 250 and 300Mpa resulted in a statistically non-significant (p≥0.05) increase in the fat composition of the samples.

Ash content is a measure of the nutritional value of food which is regarded as the reflection of the total amount of inorganic compounds such as mineral contents preserved in food materials (Agrilasa, 2007). The ash contents of the thermally and high pressure processed samples depreciated relative to the control group in an insignificant (p≥0.05) manner. Onyeike *et al.* (2015) also reported a decline in the ash content of samples cooked at different temperatures.

Crude fibre is the amount of indigestible sugars present in a food sample which has the physiological role of adding bulk to stool, and thus contribute to the maintenance of internal distensions for a normal peristaltic movement (Akinyele and Oloruntoba, 2013). By facilitating peristalsis, dietary fibre helps to reduce

many gastrointestinal diseases, serum cholesterol, risk of coronary heart disease, colon and breast cancer and hypertension (Ganong, 2003).

Fibre in vegetables increases glucose tolerance and insulin sensitivity (Chaudary and Verma, 2011). It also functions as a serum cholesterol reducing agent and this prevents the risk of coronary heart disease and/or hypertension (Araya-Farias *et al.*, 2011).

Thermal processing as well as high pressure significantly ($P \leq 0.05$) reduced the crude fibre contents of *T. lobata*. The decrease in crude fibre content during processing could be attributed to solubilization as increase in temperature leads to breakage of weak bonds between polysaccharide chains and glycosidic linkages in dietary fibre polysaccharides (Căpriță *et al.*, 2011). Most fibres are carbohydrates with varying degree of solubility in water. This could give rise to a decrease in the association between fibre molecules and/or a depolymerization of the fibre resulting in solubilization (FAO, 1998).

The carbohydrate constituent of *T. lobata* was not significantly ($P \geq 0.05$) affected by thermal processing. This is corroborated by Onyeike *et al.* (2015) who submitted that the total carbohydrate contents of the samples were not significantly affected by processing. During cooking, a hydrothermal processing that involves heat hydrolysis of carbohydrate, there is gelatinization of starch. This refers to the disruption of granular structure, hydration, swelling and solubilization of starch molecules (Ilelaboye *et al.*, 2013). Therefore, the flavor and nutritional quality of the food is usually unaffected, although microorganisms and enzymes may be inactivated (Tangwongchai *et al.*, 2000).

CONCLUSION

From the results obtained, it can be established that *Trichosanthes lobata* is highly nutritious because it is rich in macro and micro nutrients; it is safe for consumption because its antinutrients contents are low and it is richer in antioxidants when compared to *Solanum lycopersicum*. Results obtained also showed that high pressure processing of food is better than thermal processing in term of minimizing important nutrients depletion during processing. This research finding has provided information about the proximate compositions in *Trichosanthes lobata* fruit and it is safe for consumption. High pressure processing of food will preserve some nutrients usually lost or reduced during thermal processing. *Trichosanthes lobata* in large quantity, can be processed and packaged to serve as alternative to common tomatoes (*Solanum lycopersicum*) which does not thrive well enough during rainy season, this can also create self-employment for

young school leavers. The Method used in processing food (food preservation and packaging) is important if the nutrients will be retained thus High-pressure processing is better than thermal processing in processing of fruits and vegetables like tomatoes and related fruits. Further research work should be done to compare the microbial load after processing using thermal and Whigh-pressure processing methods since literature says high pressure processing eliminates pathogenic and spoiling microorganisms better than thermal processing. *Trichosanthes lobata* is said to retain its sweet taste (it does not taste sour) even after undergoing fermentation. Further research should be carried out to substantiate this claim scientifically.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

OMR managed data collection, Design of the study, writing of manuscript and review of manuscripts. A.Z managed the development of methodology and review of manuscripts. Y.O.S, managed the literature searches. D.J. data analysis, interpretation of data and wrote the first draft of the manuscript. All authors read and approved the final manuscript

REFERENCES

Adebo, O. A., Kayitesi, E., & Njobeh, P. B. (2018). Reduction of mycotoxins during fermentation of whole grain sorghum to whole grain ting. *Food Control*, 90, 48–52.

Adeniyi, O. O., Ibukun, E. O., Ogunbolude, Y., & Eseigbe, M. I. (2013). Effect of boiling on the nutritional composition and antioxidant properties of Beniseed sesame (*Sesamum indicum* L.). *Food Science and Quality Management*, 11, 39–48.

Adeyeye, E. I. (2010). Effect of cooking and roasting on the amino acid composition of raw groundnut (*Arachis hypogaea*) seeds. *Acta Scientiarum Polonorum, Technologia Alimentaria*, 9(2), 201–216.

Agilasa. (2007). *Wet ashing plant and feed analysis handbook* (2nd ed.). Agricultural Laboratory Association of Southern Africa.

Agunbiade, F. O., Adeosun, B. C., & Daramola, G. G. (2013). Nutritional properties and potential values of *Cordia sebestena* seed and seed oil. *The Journal of Food*, 38(3), 127–133.

Akinyele, B. J., & Oloruntoba, O. S. (2013). Comparative studies on *Citrullus vulgaris*, *Citrullus colocynthis* and *Cucumeropsis mannii* for "Ogiri" production. *British Microbiology Research Journal*, 3(1), 1–18.

AOAC International. (2005). *Official methods of analysis of AOAC International* (18th ed.). AOAC International.

Araya-Farias, M., Makhlof, J., & Ratti, C. (2011). Drying of Sea buckthorn (*Hippophae rhamnoides* L.) berry: Impact of dehydration methods on kinetics and quality. *Drying Technology*, 29(3), 351–359. <https://doi.org/xxxxxx> (add DOI if available)

Căpriă, A., Căpriă, R., Simulescu, V. O., & Drehe, R. M. (2011). The effect of temperature on soluble dietary fiber fraction in cereals. *Journal of Agroalimentary Processes and Technology*, 17(3), 214–217.

Chaudary, M., & Verma, S. K. (2011). Analysis of the physicochemical properties of the processed fruits and vegetables products. *International Journal of Pharmaceutical and Biosciences*, 2(4), 660–668.

Food and Agriculture Organization of the United Nations. (1998). *Effect of food processing on dietary carbohydrate: Carbohydrate in human nutrition* (FAO Food and Nutrition Paper No. 66). FAO.

Ganong, W. F. (2003). *Review of medical physiology* (21st ed., pp. 316–318). McGraw Hill.

Ilelaboye, N. O. A., Amoo, I. A., & Pikuda, O. O. (2013). Effect of cooking methods on proximate composition, gross energy and dietary fibre of some green leafy vegetables. *European Journal of Applied Engineering and Scientific Research*, 2(2), 41–47.

Li, X., Wang, T., Zhou, B., Gao, W., & Cao, J. (2020). Phytochemistry, pharmacology, and nutritional properties of *Trichosanthes* species: A review. *Molecules*, 25(12), 2754. <https://doi.org/10.3390/molecules25122754>

Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008). Effect of high-pressure processing on colour, texture, and flavour of fruit- and vegetable-based food products: A review. *Trends in Food Science & Technology*, 19(6), 320–328. <https://doi.org/10.1016/j.tifs.2008.03.008>

Onyeike, E. N., Anyalogbu, E. A., & Monanu, M. O. (2015). Effect of heat processing on the proximate composition and energy values of African walnut (*Plukenetia conophora*) and African elemi (*Canarium schweinfurthii*) consumed as masticatories in Nigeria. *International Journal of Scientific and Technology*, 4(8), 295–301.

Rasane, P., Jha, A., Sabikhi, L., Kumar, A., & Unnikrishnan, V. S. (2015). Nutritional advantages of oats and opportunities for its processing as value-added foods: A review. *Journal of Food Science and Technology*, 52(2), 662–675. <https://doi.org/10.1007/s13197-013-1039-6>

Schubert, L., & DeLuca, H. F. (2010). Hypophosphatemia is responsible for skeletal muscle weakness of vitamin D deficiency. *Archives of Biochemistry and Biophysics*, 500(2), 157–161. <https://doi.org/10.1016/j.abb.2010.03.003>

Tiwari, B. K., O'Donnell, C. P., Patras, A., Brunton, N., Cullen, P. J., & Rice, R. G. (2009). Effects of ozone processing on phytochemicals and associated benefits to human health: A review. *Trends in Food Science & Technology*, 20(12), 618–630. <https://doi.org/10.1016/j.tifs.2009.07.004>

Tsado, A. N., Lawal, B., Santali, E. S., Shaba, A. M., Chirama, D. N., Balarabe, M. M., Jiya, A. G., & Alkali, H. A. (2015). Effect of different processing methods on nutritional composition of bitter leaf (*Vernonia amygdalina*). *IOSR Journal of Pharmacy*, 5(6), 08–14.

Wei, Z. Y., Wang, J. S., Cao, Z. Y., Zhang, Z. Y., & Li, X. J. (2009). Effect of high temperature high humidity on contents of protein, amino acids and sugars in wheat grains. *Journal of Chinese Cereals and Oils Association*, 24(9), 5–11.