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Impact of Fermentation on Amino Acid Profile of Cold and Hot Water Extracts of *Justicia carnea* Leaves

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Abstract

This study investigates the impact of fermentation on the amino acid profile of *Justicia carnea* leaves extracts and the fermented must of the hot water extract. The cold water extract (C) **(25^0C)**, hot water extract (H) at 100^0c and the fermented must (W) using bakers yeast (*Sacharamyces cerevisiae*) were analyzed with High-Performance Liquid Chromatography (HPLC) to determine changes in amino acid concentrations. The results show that fermentation significantly ($p<0.05$) alters the amino acid composition, with increases in key amino acids such as L-Proline, L-Serine, and L-Leucine relative to the hot water and cold water extracts. These findings highlight the potential of fermentation to enhance the amino acid profile of *J.carnea* water extract, a plant known for its medicinal properties.

Keywords

Justicia carnea, fermentation, amino acids, hot water extract, nutrition.

Introduction

Justicia carnea which is the largest genus of the family of plants known as *Acanthaceae*, is a medicinal plant known for its bioactive properties, some local names include Ewueje by the yorubas and ogwuobara by the Ibos. it is commonly known as jacobina, brazillian plume, etc (Onyeabo *et al*., 2017; Anarado *et al.*, 2021,). It is traditionally used for its potential in improving blood levels, obesity control, treatment of malaria, diabetes, blood pressure reduction and supporting overall health (Okwu and Josiah 2006; Olayinka & Adesina, 2020; Igbinovia *et al*., 2020; Anigboro *et al.*, 2021a; Anigboro et al., 2021b; Ajuru *et al*., 2021; Andrew *et a.*, 2024). Various studies have shown that *justicia carnea* is rich in phytochemicals and antioxidants, which includes; flavonoids, phenols, tannins, saponins alkaloids and cardiac glycosides (Akinmoladun *et al*., 2016; Adebayo *et al*. 2018; Ogunyemi *et al*., 2020). Amino acids, the building blocks of proteins, play a critical role in cellular function and health maintenance. They are involved in the regulation of key metabolic pathways and processes that are crucial for the growth and the maintenance of organisms. (Cesari *et al*., 2005; Wu, 2009).

Fermentation, a biochemical process involving microorganisms, is known to improve the bioavailability of nutrients in plant extracts by breaking down complex molecules. (Adams, 1990; Holzapfel 1995; Gaggia, et al.,2011; Rizzello *et al*.,2016). Fermentation has been shown to break down complex plant molecules, making nutrients more bio-available and potentially altering the amino acid profile (Azat *et al*., 2016).

Fermentation based processes have been of great interest for humans since the ancient times because of their potential application. This is because fermented food condiments enhance flavour and improve the protein content, essential amino acids and fatty acids of foods (Ojinnaka *et al*., 2011 Poutanen, *et al*., 2009 Çabuk, *et al*., 2018). The outcome of fermentation gives insights into enhancing the nutritional value of plant based products. By understanding these changes, we can explore new ways to enhance the nutritional and therapeutic value of the plant.

While *Justicia carnea* has been studied for its health benefits, limited researches on the effects of fermentation on its amino acid profile has not been observed. The aim of this study was to address this gap by examining how fermentation affects the amino acid concentration in *Justicia carnea* hot water extracts.

Materials and Methods:

Collection and Cleaning of Plant Leaf

Fresh *J.carnea* leaves were locally collected from a nearby bush in Abraka, Ethiope East Local Government Area of Delta State and authenticated by a taxonomist, Prof, Joseph Ebiguai of the Dept. of Botany, Faculty of biological Science, University of Calabar, with Voucher number: UC-BOT/038 The leaves were weighed and cleaned by rinsing in clean potable water.

Preparation of *Justiciacarnea* extracts

Cold extract preparation

Ten grams of *J.carnea leaves* were freshly harvested, weighed and washed with tap water respectively. The weighed leaves were then transferred into a mortar and crushed under cold ice. 1000 mL (1 litre) of distilled water was utilized for the homogenization, the extract was then filtered through cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman No. 1 filter paper. The resulting filtrate was then used for the study. Storage of filtrate was done in a refrigerator at 4^0C (Sahira and Catherine, 2015).

Hot extract preparation

The decoction of hot water (10%w/v) of the *J.carnea* was prepared by weighing a freshly washed 100 gram of the leaves and boiled with 100ml of distilled water for 10 mins under a mild heat condition. The extract was obtained from a two-step filtration employed by cheese cloth and Whatman No. 1 filter paper respectively. The filtrate obtained was stored in refrigerator at 4^0C. (Sahira and Catherine, 2015).

Fermentation of hot water extract

Fifteen litres (15 L) of the hot water extract was fermented for 9 days. The fermentation vessel was washed and sterilized with a weak bleach solution and rinsed with plenty of water. Aluminium hydrogen sulfite ($Al(HSO_3)_3$) was added in the ratio of 0.14 g/L to prevent the growth of wild strains of bacteria/ fungi. (Gutierrez, *et al*., 2017). The must for fermentation was prepared by adding sugar in the ratio of 10 g/L (making a total weight of 1.5 Kg.) to the hot water extract of *J. carnea*. and inoculated with a fermentation broth prepared from scratch as described below.

Preparation of Broth: A mineral salt (ammonium and magnesium) solution containing 0.6 g/L baker's yeast (*S. cerevisiae*), for nutrients 4.0 g/L ammonium tetraoxosulphate (vi), $(NH_4)_2SO_4$ and 2.0 g/L of magnesium tetraoxosulphate (vi), $MgSO_4$

was added and this was dissolved in 375mLs of the extract. This was then autoclaved at 121ºC for 15 min and allowed to cool.
This was mixed with the extract and sugar solution and allowed to ferment for 9 days. (Ogodo et. al., 2018)

The profiling of amino acids was carried out for the cold and hot water extracts, and the fermented must using the high-performance liquid chromatography (HPLC)

All results were expressed as mean and standard deviation using the SPSS Version 16 software. One way anova analysis was used for comparison using turkey post hoc tool.

Results and Discussion

Table 1 shows the amino acid concentrations of the cold water extract, hot water extract and the fermented must. (C, H and W).

Table 1: The variation in the amount of amino acids found in the extracts of J. carnea and the fermented must

Amino Acid	Cold	Hot	Fermented must
Aspartic acid	5.66 ±0.04[a]	8.58 ±0.01[b]	8.74±0.02[c]
Glutamic acid	1.94±0.04 [a]	1.02±0.40[b]	1.91±0.03[a]
Asparagine	0.23±0.02 [a]	0.39±0.01[b]	1.26±0.02[c]
Serine	2.70±0.04[a]	5.70±0.04[b]	8.08±0.04[c]
Glutamine	0.12±0.02 [a]	2.36±0.02[b]	2.58±0.04[c]
Histidine	0.26±0.03[a]	0.47±0.02[b]	0.68±0.03[c]
Glycine	1.44±0.04[a]	3.89±0.01[b]	3.92±0.03[c]
Threonine	1.04±0.02[a]	3.01±0.01[b]	3.06±0.02[c]
Arginine	5.62±0.02[a]	5.43±0.01[b]	5.55±0.03[c]
Alanine	3.50 ±0.01[a]	3.54±0.03[b]	3.79±0.02[c]
Tyrosine	1.05±0.03[a]	2.87±0.01[b]	2.92±0.02[b]
Cystine	0.09±0.01[a]	1.02±0.01[b]	1.06±0.02[c]
Valine	1.03±0.04[a]	3.90±0.02[b]	3.98±0.02[c]
Methionine	1.06±0.04[a]	2.25 ±0.03[b]	2.16±0.03[c]
Tryptophan	4.13±0.04[a]	0.82±0.06[b]	1.87±0.06[c]
Phenylalanine	0.42±0.03[a]	3.37±0.06[b]	5.41±0.07[c]
Isoleucine	3.13±0.03[a]	3.5 2±0.05[b]	4.59±0.09[c]
Leucine	1.16±0.07[a]	6.08±0.05[b]	7.09±0.07[c]
Lysine	4.27±0.05[a]	4.08±0.10[b]	4.14±0.04[b]
Proline	31.2±0.93[a]	60.0±0.38[b]	70.7±0.71[c]

Fermentation has been noted to improve the quality of processed food (Ross *et al*., 2021) The result of this study indicates that fermentation significantly increased the concentration of an important amino acid, Proline. This amino acid, Proline is involved in collagen production, wound healing and immune function. This increase suggests that fermentation may enhance the synthesis or stability of proline in plant matrix which could contribute to the enhanced health benefits of fermented *Justicia carnea* extract (Wu *et al*., 2014).

Similarly, there are increases in the concentration of serine and leucine in the hot water extract (H) and fermented must (W). Serine is important for protein synthesis and phospholipid metabolism. leucineis a branched-chain amino acid that is essential for muscle protein synthesis (Zhao *et al*., 2018). These findings aligned with previous research showing that fermentation can boost the availability of essential amino acids in plant-based foods (Rizzello *et al*., 2016).

While most amino acids increased as a result of fermentation, L-Glutamic acid, which plays a key role in nitrogen metabolism and neurotransmission, showed a decrease in the hot extract H (1.02 mg/L)compared to the cold extract C (1.94 mg/L). This reduction may be due to the conversion of L-Glutamic acid into other metabolites as observed in other studies (Tamang *et al*., 2016).Also, lysine, tryptophan and arginine depict to be heat liable amino acids as it was observed in this study, astheir concentrations significantly decrease ($p<0.05$) in hot extract,the heat treatment during food processing aid in the softening of cell wall matrix of food materials and ensure the availability of nutrients. Heat treatment process in food processing affects heat liable compounds that are in cytosolic bounds . Thus, the amino acids of glutamate, lysine, tryptophan and arginine could have possibly been cytosolic bound amino acids in *J. carnea* leaves. With the exception of lysine, histidine and cysteine, the yeast cells can derive it nitrogen source from the rest seventeen (17) L-amino acids (Takagi, 2019). Changes in the biomolecules of must are largely influenced by the dynamism of yeast adaptive nature (cell physiology and metabolism) in circumventing the environmental and industrial growth conditions (Jach *et al*., 2022). The influence of these both factors are depicted by the shift in the organism's metabolic enzymes, membranes and cell wall composition, which consequently alter the proteome, lipidome (lipid components of the cell membrane) and carbohydrate composition of the cells (Zhou *et al*., 2022). Yeast driven fermentation process of wine production are geared towards desirable changes in composition of amino acids profiles, these changes influence changes in taste (glutamine, aspartate) and colour (aromatic amino acids) are largely influences by the availability of free amino acids in the fermenting bio-system (Tome *et al*., 2021). Fermentation enhances the probiotics composition and reduce the level of anti-nutrient content in food materials. This reduction in food anti-nutrients foster easily digestion and availability of food nutrients in the cells. Amino acids are the building blocks of protein plays specialized cellular function, the regulate neurological activities, genetic information transfer, innate immunity etc. and such the enhancements of this biological compounds efficiency ensure the fortification of the human body against disease conditions.

Conclusion

Food processing methods largely influence their nutritional composition, the changes in the concentration of the amino acids profile of cold extract, hot extract and fermented must of *J. carnea* clearly depicts that the heat treatment of *J. carnea* significantly caused decrease ($p<0.05$) in concentration of amino acids of glutamate, lysine, arginine and tryptophan. Although the heat treatment and fermentation enhanced other amino acids by causing their release from bounded cellular organ compartments and components. The application of fermentation to can use to enhance the amino acid composition of *J. carnea* extracts there bolstering its the therapeutic potentials.

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