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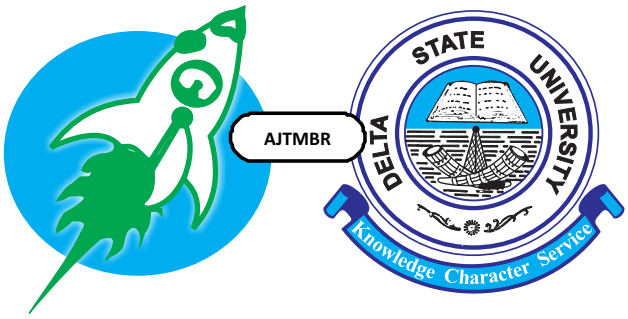

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The African Journal of Tropical Medicine and Biomedical Research is a multidisciplinary and international journal published by the College of Health Sciences, Delta State University of Abraka, Nigeria. It provides a forum for Authors working in Africa to share their research findings on all aspects of Tropical Medicine and Biomedical Sciences and to disseminate innovative, relevant and useful information on tropical medicine and biomedical sciences throughout the continent. The journal will publish original research articles, reviews, editorials, commentaries, short reports, case reports and letters to the editor. Articles are welcome in all branches of medicine and dentistry including basic sciences (Anatomy, Biochemistry, Physiology, Pharmacology, Psychology, Nursing etc) and clinical sciences (Internal Medicine, Surgery, Obstetrics and Gynaecology, Dental surgery, Child Health, Laboratory Sciences, Radiology, Community Medicine, etc). Articles are also welcome from social science researchers that document the intermediating and background social factors influencing health in countries of Africa. Priority will be given to publication of articles that describe the application of the principles of primary health care in the prevention and treatment of diseases.

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Original Articles

The effect of yeast *(Saccharomyces cerevisiae)* fermentation on amino acid composition of hot water extract of *Ficus capensis* leaf

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ABSTRACT

Introduction: In this study, the amino acid composition of cold and hot water extracts of *Ficus capensis* leaf was examined, along with the impact of yeast fermentation on the amino acid composition of the hot water extract. Although the hot water extract of *Ficus capensis* is commonly consumed as a beverage locally, there is currently a lack of documented literature regarding the amino acid composition of the wine produced from this extract.

Materials and Methods: Cold and hot water extracts of *Ficus capensis* leaf was extracted using homogenization and decoction method respectively. Ficus capensis wine was produced from hot water must prepared using 0.8 g/l yeast, 22 °brix, at pH 4.5 and fermented for 9 days. The amino acid composition of the cold water, hot water and wine extracts of *Ficus capensis* leaf were assessed using High Performance Liquid Chromatography.

Results: The result shows the presence of amino acids in the cold water, hot water and wine extract of *Ficus capensis.* However the wine contained more amino acids with methionine an essential amino acid having the highest concentration.

Conclusion: Fermentation of the hot water extract should be encouraged since it improved the amino acid content of the wine produced and would serve as an additional raw material for wine industry. Moderate consumption of *Ficus capensis* wine could potentially be beneficial to health and as well as combat the problem of malnutrition.

Keywords: *Ficus capensis,* cold water extract, hot water extract, *Saccharomyces cerevisiae,* malnutrition, amino acid.

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1.0 Introduction

Over three billion people worldwide are affected by malnutrition with tragic consequences and it is a major concern in developing countries in African[1]. The number of nutritional stunted and wasted children increased from 50.4 to 58.5

million between the years 2000 to 2016 [2]. Despite the guidelines set up by WHO for treatment of health workers to battle nutrient deficiency, the need for the deterrence of malnutrition is desired[2]. The use of wild indigenous fruits and vegetables as well as increasing the diversity of foods consumed helps to ingest more of the essential nutrients, improve food security and could combat malnutrition[3].

Proteins are composed of amino acids which are the building blocks of hormones (insulin, growth hormone, and glucagon) and physiologically active peptide glutathione[4]. Protein and enzyme synthesis in tissues is aided by amino acids. The body cannot generate essential amino acids, thus we must consume them through diet, but non-essential amino acids can be produced by the body [5]. They are essential to our diet since a lack of them causes a reduction in the production of protein, which finally results in disease conditions[6].

Ficus capensis also known as *Ficus sur* belongs to the family moracae[7]. It is an ever green plant that is grown in the wide. The hot water extract of *Ficus capensis* leaf is consumed locally as beverage. The leaves have been shown to contain high moisture, carbohydrates, moderate protein, ash and low concentration of fat and fibre [8]. It is also rich in carotenoids and vitamin A while the B and E are in reasonable amounts[8, 9]. The aqueous leaves extract also contain Vitamin C and flavonoid which are effective antioxidants[10, 11]. The leaf contains alkaloids, flavonoids, glycosides, reducing sugars, tannins, terpenoids and saponins[10, 12, 43]. This research studies the extent at which fermentation with *Saccharomyces cerevisae* would improve the amino acids content of the hot water extract.

2.0 Materials and Methods

Preliminary operations such as cleaning, sorting was done to remove extraneous materials from the *F. capensis* leaf collected from a local- bush in Abraka, Ethiope East Local Government Area of Delta State. It was identified and authenticated by a taxonomist, Dr. H.A. Akinnibosun, at the Division of Botany, University of Benin, Nigeria. A voucher specimen (UBH-f331) was given and kept for reference purposes in the herbarium.

2.1 Preparation of F. Capensis Cold and Hot Water Extracts and Must

The plant tissue homogenization technique was applied for the preparation of cold water leaf samples of *Ficus capensis*. About 100 g *F. capensis* leaf was weighed, and crushed to a smooth paste using mortar and pestle. Before filtering, about one liter of deionized water was added and shaken vigorously at 36.8^{O}C for 5 – 10 minutes. The centrifugation of filtrate was done for sample clarification[13].

The hot water extract was prepared by decoction technique by heating 150 g of the fresh leaf in 1litre of water for 15 - 20 minutes at 100 degrees Celsius and thereafter left to cool down[13]. The water was filtered from the leaves, 250 g/liter of table sugar was added to the juice to adjust the soluble solids from 1.0 to 22 °Brix. Must was sterilised in an autoclave at 121°C for 20 min and pH adjusted at 4.5. About 1 g of SO_2 in the form of sodium metabisulphite was added to the must to prevent the growth of bacteria, wild yeast and as well stops fermentation before the addition of the starter culture.

2.1.1 Starter Culture Preparation

The process of Ogodo *et al.,* (13) with slight modification was adopted. Exactly 0.8 g/l of commercial baker's yeast (*Saccharomyces cerevisiae*) was mixed with 200 ml of *F. capensis* hot water sample and was stilled vigorously. About 2 g potassium phosphate, ammonium sulphate and Magnesium sulphate each were dissolved in

100ml of distilled water and poured to the mixture. The mixture was permitted to stand for three hours before adding to the must for fermentation to begin.

2.1.2 Fermentation of Must to Wine

An aspirator was used to collect the extract from fermenting vessel. It was covered for 15 to 20 minutes to allow the yeast population to grow. To control oxidation, the fermenting vessel was covered with a safety lock containing 200 ppm sodium metabisulphite at the lid. The primary fermentation was permitted to stand for four days, during this time the must was stirred every 24 hours, and aliquots were collected for analysis. After fermentation, the wines were racked with minimal air exposure and clarified. The yeast lees were removed from the fermenting must to prevent further fermentation and were racked at room temperature immediately after the gas evolution stopped. The second racking was done after the addition of bentonite slurry to aid clarification[14,15,16].

2.1.3 Clarification of Young Wine

This was accomplished with bentonite. 250 g of bentonite was dissolved in 1 litre of boiling water and thoroughly mixed to form a gel. After 24 hours, 50 g of the gel-like bentonite was added to the wine with vigorous stirring using a sterile glass rod to dissolve properly. This was permitted for a one-month period. Muslin cloth was used to filter the wine[14]. The residues were removed, and the filtrate was allowed to mature for approximately 90 days.

2.2 Chromatographic Analysis of Amino Acids Compounds in the Cold Water, Hot water and Wine extracts of F. capensis

The mobile phase contains 1% aqueous acetic acid solution (Solvent A) and acetonitrile (Solvent B), and the flow rate was set to 2 ml/min. The column temperature was set to 28^0C, and the sample injection volume was 5 µL. The proportion of solvent B to solvent A was varied to perform gradient elution. In 55 minutes, the mobile phase composition was returned to its initial state of solvent B: solvent A: 10: 90, and it was left to stand for another 10 minutes before another sample injection. Per sample, the total analysis time was 65 minutes. According to the compounds studied, the HPLC chromatograms were detected using a photo diode array UV detector at three different wavelengths: 272, 280, and 310 nm. Compounds were identified based on their retention time and by spiking with the standards under the same conditions. Sample quantification was completed by measuring the integrated peak area and the content was calculated.

Calculation
Total and Individual

3.0 RESULTS

Table 1: Amino acids profile of cold and hot water leaf extracts of *Ficus capensis*

Amino acids	CS (ppm)	HS (ppm)
Alanine	9.45	10.18
Asparagine	2.33	2.71
Cysteine	2.93	3.2
Glutamine	0.45	0.5
Glycine	0.00	0.43
Histidine	2.2	2.62
Leucine	10.03	10.35
Lysine	0.54	0.52
Trptophan	0.42	0.37
Tyrosine	0.00	0.33
Total AA	11.11	12.23

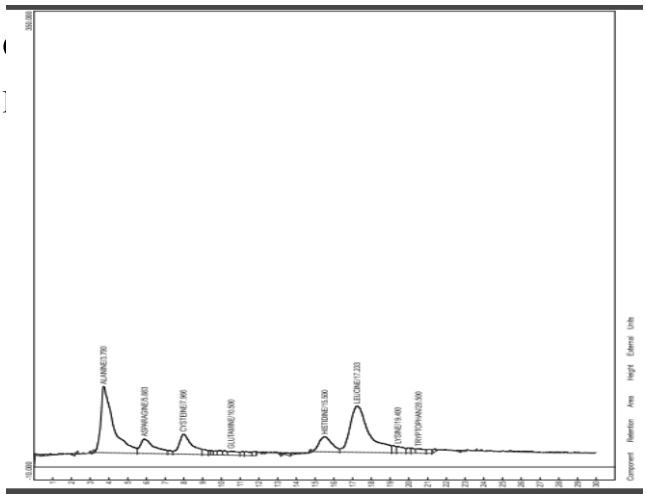

Figure 1: Chromatogram of amino acids composition of cold water leaf extract of *Ficus capensis*

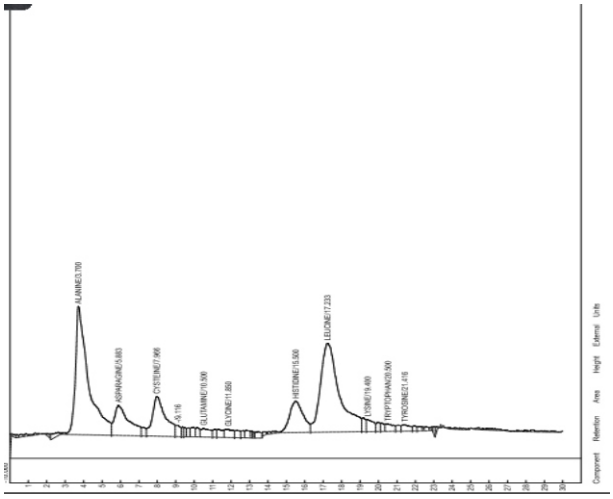

Figure 2: Chromatogram of amino acids composition of hot water leaf extract of *Ficus capensis*

Table 2: Amino acids profile of *F. capensis* wine

Amino acids	Wine (ppm)
Proline	0.93
Glutamic acid	0.28
Isoluecine	0.34
Glycine	0.04
Arginine	0.04
Valine	0.08
Aspartatic acid	0.05
Ornithine	0.07
Glutamine	0.04
Threonine	0.04
Leucine	0.39
Methione	1.15
Serine	0.05
Alanine	0.07
Phenylalanine	0.07
Gamma-aminobutyric	0.04

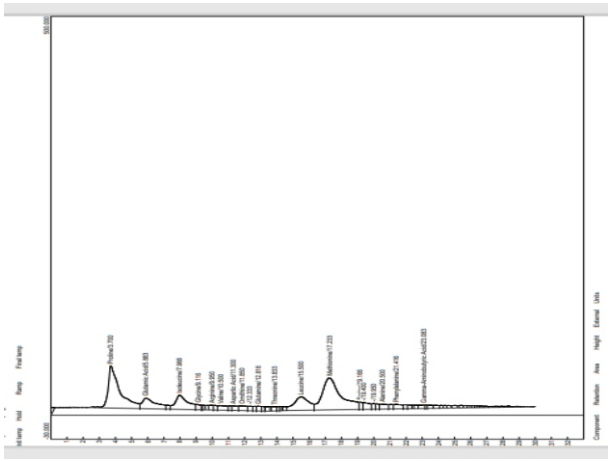

Figure 3: Chromatogram of amino acids composition of *F. capensis* wine

4.0 Discussion

The result of amino acids profile and the HPLC chromatograms of cold and hot water *F. capensis* leaf extracts are depicted in Table 1 and Figure 2 – 3 respectively. The amino acids occurred as follows: (leucine> alanine>cysteine>asparagine > histidine >lysine > glutamine >trptophan) in the cold water leaves extract while (leucine > alanine > cysteine > asparagine >histidine> lysine > glutamine > glycine > tryptophan > tyrosine) in the hot water leaf extract of *Ficus capensis*. Leucine (10.3ppm) and alanine (9.45 ppm) had the highest concentration while glutamine (0.45 ppm) and tryptophan (0.42 ppm) had the lowest concentration in the cold-water extract of the *F. capensis* leaf. Leucine (10.35 ppm) and alanine (10.18 ppm) had the highest concentration while tryptophan (0.37 ppm) and tyrosine (0.33 ppm) had the lowest concentration in the cold-water extract of the *Ficus capensis*. The total amino acids (TAA) concentration (12.23 ppm) of the hot water extract was shown to be higher in concentration than the TAA concentration (11.11 ppm) of the cold-water extract of *Ficus capensis*

This result of the amino acid profile of cold and hot water extract of *F. capensis leaf* shows that the extracts are rich in amino acid. Leaves of plants have been established scientifically as rich sources of amino acids[17,18]. The free amino acid in the cold and hot water extracts of *F. capensis* leaf (Table 1) revealed ten amino acids with alanine and leucine as the most abundant free amino acids present in the leaf. Amino acids are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules that participate in cell signaling, gene expression and homeostasis regulation[18], protein phosphorylation, synthesis of hormones and antioxidant capacity[19]. Also, amino acids participate in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia and cancer.[19]

Alanine is involved in hepatic autophagy, gluconeogenesis and transamination. Isoleucine, valine and leucine are essential substrate. Leucine regulates the protein turnover (mTOR signaling), gene expression and it's a source of psychic energy[20, 21, 22]. lysine helps to maintain intestinal integrity and health[21,24]. Deficiency of tryptophane causes weakening of mental abilities. Tryptophane eliminates depression and improves sleep. Methionine reduces excitation and anger[23,

[24]. Proline is a synergist of an inhibiting effect of gamma-aminobutyric acid.[25] Aspartic and glutamic acids are neurotransmitters involved in energetic shifts and mechanisms preventing the organism from the toxic ammonium[26,27]. Phenylalanine is known to improve memory and the ability for learning[28]. Threonine stimulates activity of the central nervous system[29-31].

The result of amino acids profile and the HPLC chromatograms of *F. capensis* wine are depicted in Table 2 and Figure 3 respectively. The amino acids occurred as follows: (methionine > proline > leucine > isoleucine > glutamic acid > valine > alanine > phenylalanine = ornithine > aspartic acid = serine > glycine > glutamine > gamma aminobutyric = threonine > arginine) in the wine extract. Methionine (1.146 ppm) had the highest concentration while arginine had the least (0.037 ppm) concentration in the wine. The total amino acids (TAA) concentration of the wine was (17.00 ppm). The *F. capensis* wine was found to contain more amino acids (Table 2). Changes in amino acids as a result of fermentation has been reported[32,33,34,35]. Wine has been reported to contain amino acids during post fermentation period[32, 33]. It is established that transfer of biologically active substances from grape berries to wine takes place in the process of wine-making[36]. The amino acid of *F. capensis* wine increased without increase in protein. The low concentration of amino acids in the wine could be as a result of amino acids being consumed by yeasts during alcoholic fermentation which might led to the yield of higher alcohols, aldehydes, esters, and other volatile compounds[37], thereby influencing the final wine aroma[38]. Wines with higher amounts of residual nitrogen have more risk of microbiological instability, with the possible formation of ethyl carbamate and biogenic amines, which are negative compounds for wine quality[39]. The increase in free amino acids in wine has traditionally been considered as a sign of the beginning of autolysis during the aging of sparkling wines[40, 41, 35]. The excretion of these amino acids to the wine could also be possibly the consequence of interactions within cellular metabolism, such as the reduction of intermediary compounds in their biosynthesis with the aim of re-oxidation of certain co-enzymes in order to maintain a normal redox balance[42, 35]. About 40% of the total nitrogen in wines is attributed to amino acids and yeasts excrete other amino acids at the end of fermentation which is released by yeast autolysis or produced by enzymatic degradation of fruit's protein[38,35].

CONCLUSION

This study had shown that the wine contain more amino acids than the cold and hot water extract though in lower concentration. Therefore moderate consumption of *F. capensis* wine would positively influence human health.

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Competing interest

There is no competing interests.

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