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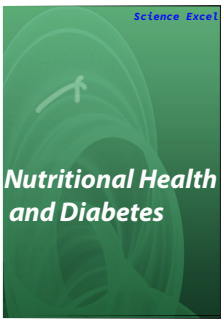

Determination of Proximate, Amino acids and Fatty acids Compositions of *Syzygium aromaticum* (Clove flower buds) in Ogbomoso Southwestern Nigeria

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Abstract

Syzygium aromaticum flower buds are one of the most important spices that are used as pepper soup ingredients in southwestern Nigeria. Many traditionalists have claimed that the flower buds relieved symptoms of diarrhea, gastric irritability and vomiting when added to diet or taken as tea. However, the nutritional properties are yet to be evaluated indepthly to confirm their claims. The reasons for this study. The Proximate compositions were determined using standard methods of analysis and amino acid composition was determined by spectrophotometrically while fatty acid content was determined by gas chromatography/mass spectrometry method.

The results of the proximate compositions revealed that the buds contained the following amount of nutrients: moisture 16.21 ± 0.01, ash 2.21 ± 0.01, crude fat 1.41 ± 0.05, crude protein 18.38 ± 0.02, carbohydrate 49.55 ± 0.05% and total energy value 284.31kcal/g. The amino acids composition results revealed that the buds contained essential amino acids in the following order: Leucine > phenylalanine > tryptophan > isoleucine > valine > lysine > threonine > methionine and non essential amino acids (arginine >glutamic acid >aspartic acid>alanine>glycine>serine>cystine>tyrosine).

The fatty acids compositions revealed the presence of both the saturated and unsaturated fatty acids but the most prominent were unsaturated fatty acids (oleic acid, 20.44% and linoleic acid, 15.33%) while others were present in minute quantity.

This study revealed that the *Syzygium aromaticum* flower buds could be a source of energy, nutrients, unsaturated fatty acids and amino acids which could be harmonized into the healthcare system to produce drugs, liniment, food supplements and animal feeds.

Introduction

Man dependence on plant is divinely originated and this is been discovered every day due to the advancement in science and technology which addresses the nutritional and medicinal properties embedded in plants. Therefore, there are many plant parts that are used as spices because of their nutritional, pharmaceutical and medicinal values. Spices are dried roots, seeds, fruits or flowers of plant which have many functions such as flavoring agent, food addictives, coloring agent, preservatives and medicine [1].

Syzygium aromaticum is a spice plant; the cloves are the flower buds of the tree Diego *et al*. [2] which is a native to Indonesia. The flower bud is the organ used as spice. It is commonly called clove in English language while the botanical names are; *Syzygium aromaticum*, *Eugenia aromatical*, *Caryophyllus aromatica*. It is called Kanafuru in Southwestern Nigeria (Yoruba), Albasa in Northern Nigeria (Hausa) and Kloovu in Southeastern Nigeria (Igbo). It is domicile in the Southwestern Nigeria [3].

Syzygium aromaticum (clove) tree is a slow growing long lived tree which can survive up to 100 yr and beyond. The oldest clove tree in Indonesia is 375yr old [4].

The *Syzygium aromaticum* flower buds are aromatic pink flowering buds which are dried until become brown and used as spicing and medicine. The buds are the commercial part

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Figure 1: *The raw Syzygium aromaticum flower buds*

Figure 2: *The ground powdered Syzygium aromaticum flower buds*

of the tree and are collected at the maturity stage by physical (manual) or chemical (using natural phyto hormone with the liberation of ethylene in the vegetable tissue) means [5].

The taxonomy of *Syzygium aromaticum* shows that its domain is *Eukaryota*, Kingdom is *Plantae*, subkingdom is *Viridaeplantae*, phylum is *Tracheophyta*, subphylum is *Euphyllophytina,* class is *Magnoliopsida*, family is *Myrtaceae*, genus is *Syzygium* and the specie of study is *aromaticum* [6]. The flower buds posses' fragrance and burning taste, the color is brown with powerful fragrant odor which is warm, pungent and slightly astringent [4].

Syzygium aromaticum flower buds are available throughout the year due to different harvest seasons in different countries, the tree can live up to 100 yr and above, requires heavy sunlight with high atmospheric temperature (25-35^{0}C), well distributed rainfall above 150 cm and high humidity above 70% [7].

The chemical compositions of the *Syzygium aromaticum* flower bud (clove) depends on the agro climatic conditions under which is being exposed, grown, processed and stored therefore one might not obtain the same result from the chemical evaluation of *Syzygium aromaticum* flower bud of the same species from different locations. *Syzygium aromaticum* flower bud also possess medicinal properties which manifest when the flower buds is taken as tea to relief stomach upsets, chills, impotence, also when chewing reliefs tooth pain. When the clove oil is used externally it relieves toothache, headache, cold, arthritis and rheumatism. It also heals ulcers, burns, bronchitis and asthma [4].

The *Syzygium aromaticum* flower buds oil has anti-inflammatory properties (due to the presence of flavonoids) and antibacterial properties which make it useful as mouth washes, dental creams, throat sprays and tooth paste. Mixture of eugenol (compound in clove) and zinc oxide is use for short time dental cavities filling [8].

Paste of *Syzygium aromaticum* flower powder and honey is used to cure skin conditions. Paste of *Syzygium aromaticum* flower buds and water aids healing process of bites and cuts.

It is also used in the treatment of many digestive disorders such as diarrhea, flatulence, nausea and vomiting, dyspepsia. The flower buds (*Syzygium aromaticum)* has been reported to be good for diabetic patients because it controls the glucose level in the blood [9], posses aphrodisiac properties, improves human memory and also used as mosquito repellent [10].

Therefore, the objective of this work is to determine the proximate, amino acids and fatty acids composition of the *Syzygium aromaticum* flower buds in order to ascertain its usefulness in healthcare system and for the production of medicaments, food supplements and animal feeds.

Materials and Methods

Sample Collection

Syzygium aromaticum flower buds were purchased at Ojajagun herbs market situated opposite the Soun of Ogbomoso,s palace, Ogbomoso North Local Government Area, Oyo State, Nigeria.

Sample Identification

The sample was authenticated by Prof. A.T.J. Ogunkunle a taxonomist in the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Sample Preparation

The samples were thoroughly checked to remove dirt, stones and other visible particles that were present and five hundred grammes (500 g) of the flower buds were weighed into a beaker, thereafter, ground to coarse powder using mechanical grinder. The powdered sample was air dried by spreading on a white cardboard in the laboratory for fourteen days (14) days, sieved and transferred into air tight plastic sample container with a lid and kept for analysis.

Determination of Proximate Compositions

Part of the sample was taken separately, weighed and used for the determination of moisture content by drying to constant

weight in air, ash content by ignition at 550^{0}C in a muffle furnace, oil content by soxhlet extraction with hexane (an organic solvent), crude protein by the Kjeldahl method, and crude fibre by the acid and alkaline digestion methods. The carbohydrate content was estimated by subtracting the sum of moisture, protein, fat, crude fibre and ash percentages from one hundred [11]. The calorific value was calculated by multiplying the mean values of the crude protein, fat and carbohydrate by Atwater factors of 4, 9 and 4 respectively, taking the sum of the products and expressing the result in kilocalorie [12].

Determination of Amino acids

The free amino acids concentrations were determined with ninhydrin reagent using phenylalanine as standard and reading the developed color at 570 nm and extrapolating the values from a standard curve of phenylalanine. Ninhydrin in acetone (0.1%) was diluted with distilled water in the ratio 1:4. Exactly, 20µL each of the diluted extracts was added to 4ml portions of the diluted ninhydrin. The resulting solutions were heated to boiling for 5 min, cooled and the absorbance read in a spectrophotometer at 570nm using distilled water as blank [13].

The amino acids constituents of the samples were also determined with the aid of Technicon Sequential Multi sample Amino acid Analyzer (TSM) [14].

Determination of Fatty acids

The powdered sample of *Syzygium aromaticum* flower buds which had previously been weighed (200g) was poured into an amber coloured bottle of 2.5L and 1L of n-hexane solvent was added into the bottle with the powdered sample. It was left for 72 h with intermittent shaking. The mixture, after the 72 h was filtered using filter in glass funnel and the filtrate was concentrated in a water bath.

Gas chromatography

The oil obtained was subjected to GC analyses using GC 2010 gas chromatograph. Column oven temperature was 60^{0}C injection temperature of 250^{0}C· split injection mode at 100, 2k Pa: Column flow of 1.61 ml/min and total flow of 6.2 ml/min: 1.0 split ratio: oven temperature programming is 60^{0}C for 5 min and at the rate of 5^{0}C/min till 140^{0}C, 15^{0}C/min till 280^{0}C.

Gas chromatography-Mass spectrometry

The GC-MS analyses were performed on GC-MS QP2010 Plus ion, source temperature 200^{0}C; interface temperature 250^{0}C; solvent cut time 2.5 min; with relative detector gain mode and threshold 3000; scan MS ACQ mode; detector FTD; mass range of m/z 40-400.

Identification of Components

The components were identified based on their retention indices which were determined with a reference to a homologous series of n-alkane, along with comparison of their mass spectral fragmentation patterns in computer matching against the inbuilt data and commercials such as library as well as in-house Baser Library of essential oil constituents built up by genuine compounds and components of known oils [15,16].

Results and Discussion

The moisture content was low and this is an indication of stability, quality, shelf life and high yield [17]. The ash and fat contents were also low while the crude protein and crude fibre were a bit higher (18.38% and 12.24%). The carbohydrate content and total energy value were higher than other parameters indicating that the *Syzygium aromaticum* flower bud could be energy given (Table 1).

Syzygium aromaticum flower buds are good sources of essential amino acids such as; leucine, phenylalanine, tryptophan, isoleucine, valine,lysine, threonine and methionine (Table 2). The non essential amino acids present in the buds according to their concentration were; arginine, glutamic acid, aspartic acid, alanine, glycine, serine, cystine and tyrosine (Table 3).

Leucine was the most abundant essential amino acid present in the *S. aromaticum*. It aids the healing of muscle tissue, skin and bones and being recommended for those recovering from surgery, lower blood sugar level and aids in increasing growth hormone. Deficiency in the essential amino acids may hinder healing recovery process [18].

Phenylalanine was the next essential amino acid present in abundance. It is used for the production of epinephrine in the brain. It promotes alertness and vitality, elevates mood, decreases pain, aids memory and learning, used to treat arthritis, depression, menstrual cramps, migraine obesity, Parkinson's diseases and schizophrenia. Valine is used for muscle metabolism and coordination, tissue repair and as energy source by muscle tissue, helpful in treating liver and gall bladder diseases [19].

Tryptophan is a natural relaxant, helps alleviate insomnia by inducing normal sleep, reduce anxiety and depression and stabilizes mood, helps in the treatment of migraine, headache, helps the immune system function properly, aids in weight control by reducing appetite, enhances the release of growth hormones, helps control hyperactivity in children. Isoleucine

Table 1. *Proximate Compositions of the Syzygium aromaticum flower buds*

Parameter	% Composition
Moisture content	16.21± 0.01
Crude fibre	12.24 ± 0.02
Ash	02.21 ± 0.03
Protein	18.38 ± 0.008
Fat	01.41 ± 0.01
Carbohydrate	49.55 ± 0.05
Energy value(kcal/g)	284.31± 0.005

Values are means (±SD) of triplicate determinations

Table 2. *Essential Amino acids Composition of Syzygium aromaticum flower buds as a percentage of total protein*

Amino Acid	Composition mg/100g
Tryptophan	6.17 ± 0.02
Leucine	9.23 ± 0.01
Isoleucine	5.23 ± 0.01
Lysine	3.77 ± 0.01
Methionine	0.45 ± 0.01
Phenylalanine	7.66 ± 0.02
Threonine	2.22 ± 0.05
Valine	5.05 ± 0.01

Values are means (±SD) of triplicate determinations

Table 3. *Non Essential Amino acids Composition of Syzygium aromaticum flower buds as a percentage of total protein*

Amino Acid	Composition (mg/100g)
Glutamic acid	8.13 ± 0.01
Cystine	1.04 ± 0.05
Alanine	5.22 ± 0.01
Aspartic acid	7.58 ± 0.02
Tyrosine	0.15± 0.01
Glycine	4.24 ± 0.02
Serine	2.12 ± 0.03
Arginine	71.66± 0.01

Values are means (±SD) of triplicate determinations

aids in the healing, repair of muscle tissue, skin, and bone and needed for hemoglobin formation, stabilizing, regulating blood sugar and energy [20].

Lysine aids calcium absorption and maintain a proper nitrogen balance in adults. Also aids in the production of antibodies which have the ability to fight cold sores and herpes outbreaks. It also helps to form collagen, which makes up cartilage and connective tissue. Threonine helps maintain proper protein balance in the body, it is important for the formation of collagen, elastin and tooth enamel, aids liver and lipotropic function when combined with aspartic acid and methionine, it prevents the buildup of fat in the liver. Methionine is a powerful antioxidant and a good source of sulphur which prevents disorders of the hair, skin and nails. It helps to detoxify harmful agents such as lead and other heavy metals. Beneficial for women who take oral contraceptives because it promotes the excretion of estrogen, reduces the level of histamine in the body which can cause the brain to relay wrong messages [20].

Evaluation of non essential amino acids composition result shows that arginine was the most abundant in the flower bud followed by glutamic acid. Arginine helps the body to build protein, regulation of blood pressure, boost immune system, helps the kidney to work more efficiently [4].

Glutamic acid is important in the metabolism of sugars and fats. It aids in the transportation of potassium into the spinal fluid, it acts as fuel for the brain, it helps to correct personality disorders and is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers [19]. Aspartic acid increases stamina and is good for chronic fatigue and depression.

Alanine aids in the metabolism of glucose, a simple carbohydrate that the body uses for energy; guards against the buildup of toxic substances that are released into muscle cells when muscle protein is broken down quickly to meet energy needs, such as what happens with aerobic exercise; strengthens the immune system by producing antibodies. Amino acids like aspartic acid, glycine and glutamic acid are known to play a key role in the process of wound healing [21]. Glycine retards muscle degeneration, improves glycogen storage thus freeing up glucose for energy needs, promotes a healthy prostrate, central nervous system and immune system, useful for repairing damaged tissue and promotes healing [22].

Serine is needed for the proper metabolism of fats and fatty acids, the growth of muscle and the maintenance of a healthy immune system. It is important in RNA and DNA function and cell formation aids in the production of immune globulins and antibodies. Cysteine functions as a powerful antioxidant in detoxifying harmful toxins. It protects the body from radiation damage, protects the liver and brain from damage due to alcohol, drugs and toxic compounds found in cigarette smoke. It is used in the treatment of rheumatoid arthritis and hardening of the arteries. Promotes the recovery from severe burns and surgery, promotes the burning of fat and the building of muscle. Slow down aging process [19].

Table 4. *Saturated Fatty acids Composition of Syzygium aromaticum flower buds*

Fatty Acid	Double bond	Systematic name	Chemical structure	Composition (%)
Capric acid	10:00	Decanoic acid	CH3(CH2)8 COOH	0.03±0.01
Lauric acid	12:00	n-dodecanoic acid	CH3(CH2)10COOH	0.24± 0.02
Myristic acid	14:00	n-tetradecanoic acid	CH3(CH2)12COOH	0.10± 0.01
Palmitic acid	16:00	n-hexadecanoic acid	CH3(CH2)14COOH	5.44±0.20
Stearic acid	18:00	n-octadecanoic acid	CH3(CH2)16COOH	3.12±0.020

Values are means (±SD) of triplicate determinations

Table 5. *Unsaturated Fatty acids Composition of Syzygium aromaticum buds*

Fatty Acid	Double bond	Systematic name	Chemical structure	Composition (%)
Oleic acid	18:01	Cis-9-Octadecenoic	$CH_3(CH_2)7CH=CH(CH_2)7COOH$	20.44±0.5
Linoleic acid	18:02	Cis-9-,cis-12-octadecadienoic acid	$CH_3(CH_2)4CH=CHCH_2CH=CH(CH_2)7COOH$	15.33±0.08
Linolenic acid	18:03	Cis-9-,cis-12-,cis-15-octadecatrienoic acid	$CH_3CH_2CH=CHCH_2CH=CHCH_3CH=CH(CH_2)7COOH$	0.17±0.01

Values are means (±SD) of triplicate determinations

Tyrosine is a precursor of adrenaline, nor epinephrine and dopamine, which regulate mood and stimulates metabolism and nervous system, acts as a mood elevator, suppresses the appetite and helps reduce body fat, aids in the production of melanin (the pigment responsible for hair and skin color) and in the function of the adrenal, thyroid and pituitary glands, has been used to help chronic fatigue, narcolepsy, anxiety, depression, low sex drive, allergies and headaches [23].

The saturated fatty acids present were palmitic (5.44%), stearic (3.12%), lauric (0.24%), myristic (0.10%) and capric (0.03%) acids (Table 4). The unsaturated fatty acids present were oleic (20.44%), linoleic (15.33%) and linolenic (0.17%) acids (Table 5). The unsaturated fats, which are liquid at room temperature, are considered beneficial fats because they can improve blood cholesterol levels, ease inflammation, stabilize heart rhythms, and play a number of other beneficial roles. Fatty acids have many important functions in the body, including energy storage. If glucose (a type of sugar) isn't available for energy, the body uses fatty acids to fuel the cells as alternative. Diet rich in unsaturated fatty acids promote weight loss and body composition in women with obesity [24].

High unsaturated fatty acids diets can also lower blood pressure, reduce blood cholesterol and other heart disease risk factors, particularly if they replaced saturated fats in the diet. May also help to reduce the risk of certain cancer such as prostate cancer in elderly men and breast cancer in women and may also reduce inflammation which could lead to chronic diseases [26].

Conclusion

This study shows that the *Syzygium aromaticum* flower buds could serve as a nutrient, energy source and medicine. However, more research could be embarked upon to establish bioactive compounds present in the flower buds that could be used in the production of healthcare medicaments, food supplements, formulation of drugs and feeds for animals.

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