



Amino Acid Profile and Vitamin C Content of Selected Condiments Used as Thickeners in Soup Preparation

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Authors' contributions

This work was carried out in collaboration among all authors. Author LHZ designed the study and wrote the protocol, performed the amino acid and vitamin c content analysis, data analysis and wrote the draft of the manuscript. Author MPC managed the analyses of the study. Authors MPC, MY and OOD made the necessary corrections in the write up. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2021/v20i930339

Editor(s):

(1) Dr. Nelson Pérez Guerra, University of Vigo, Spain.

Reviewers:

(1) Sudheer Kumar Dokuparthi, JNTUH, India.

(2) Brij Bhushan, Banaras Hindu University, India.

Complete Peer review History: <https://www.sdiarticle4.com/review-history/70147>

Original Research Article

Received 01 May 2021

Accepted 03 July 2021

Published 30 July 2021

ABSTRACT

Aim: This study aimed to analyze the amino acid profile and determine the vitamin C content of the condiments named Detarium microcarpum (DM), Cissus populnea (CP), Grewia mollis (GW) and Parkia biglobosa (PB).

Study Design: The condiments obtained from Gwagwa market, Federal Capital Territory (FCT), Abuja were used for this study to analyze and determine the amino acid profile and vitamin C content.

Place and Duration of the Study: The study was conducted in Abuja, Nigeria at the Department of Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development [NIPRD], from February 2020 to September 2020.

Methodology: The amino acid profile of the condiments was analyzed using methods described

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by (Maria et al., 2004). The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer while the Ascorbic acid content of the condiments was determined by titration method.

Result: The result indicated that non- essential amino acids are higher in concentration in all the samples (PB - 33.77, DM - 27.51, GM - 18.21 and CO - 25.86) compared to the essential amino acids (PB -27.16, DM - 19.46, GM - 16.47 and CO - 22.38) and semi-essential amino acid (PB - 22.22, DM - 16.59, GM - 9.64, CO - 14.01). Among the essential amino acids, leucine is the predominant acid, while glutamic, aspartic acids were found to be the major non-essential amino acids in the samples. High concentrations of aspartic acid, glutamic acid valine, alanine and leucine predominate in all the samples analyzed. The sample Parkia biglobosa has the highest concentration of vitamin C with 0.3 mg/L followed by Detarium Microcarpum 0.18 mg/L, Grewia Mollis 0.12 mg/L and Cissus populnea 0.11 mg/L.

Conclusion: Results indicate that these condiments contained amino acids in appropriate quantities that can serve as supplementary potential sources of essential amino acids to man and appreciable amount of vitamin C.

Keywords: Condiments; amino acid; vitamin C; titrimetric method.

1. INTRODUCTION

Food condiments play important role in the diet of many Africans. They are used to enhance the flavour of many dishes including soups and sauces and they are also known to be good sources of protein and vitamin [1]. With high content of protein, leguminous condiments serve as a tasty complement to sauces and soups and can substitute for fish or meat. In addition to the benefits of leguminous condiments mentioned, they are also employed in dietary strategies to control obesity due to their high fibre, low carbohydrate and fat contents rather than physical exercise [2,3]. They are healthy diets which boosts the brain power, protect the heart and prevents cancer [4]. Food condiments are helpful in food garnishing and as appetizers.

Nutritional status of the general population in Nigeria especially children, pregnant women and lactating mothers have been a major source of concern. Several reports indicate protein deficiency as the commonest form of malnutrition in the developing countries, particularly in regions where diets are mainly based on roots and tubers crops. In most part of Nigeria, starch based foods are the main staple food which supply both energy and protein. Protein is essential for growth and development of living organisms and it constitute 80 – 90% of all organic substances in animal body. Protein quality is measured by the type of amino acids present. There are twenty different types of amino acids, eight of which are essential because they are not manufactured by the animal's body. This include Lysine, Leucine, Isoleucine, Methionine, Phenylalanine,

Threonine, Tryptophan and Valine [5]. The amino acid histidine is essential for the growth and development of children but it is only synthesized by adults. Other non-essential amino acids that are required to maintain health, can be synthesized by the body if supplied with necessary nitrogen. These non-essential amino acids include alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyproline, proline, serine and tyrosine [6]. Dietary protein with all the essential amino acids in the proportion required by the body is said to be a high quality protein. If the protein is low in one or more of the essential amino acids, the protein is of low quality. The amino acid that is in short supply is called limiting amino acid [7]. Generally, many plant proteins are low in one of the essential amino acids. A combination of plant proteins, such as grains, with pulse or seeds leads to a high quality protein which is just as good as protein from animal foods. Most condiments used as food soup thickeners contains vegetable proteins rich in glutamine and asparagine. These can be hydrolyzed enzymatically or chemically to Glutamic and aspartic acid. Glutamic acid is considered as a flavour agent [8].

Vitamin C is a component of food needed by all animals especially humans to prevent scurvy, a disease of the gums, bones and blood vessels and to increase the body's resistance to infection. It acts as an antioxidant, a nutrient that chemically binds and neutralizes the tissue damaging effects of substances in the environment known as the free radicals [9]. L-Ascorbic acid is the main biologically active form of vitamin C. It is a water- soluble vitamin whose

dietary sources include fruits and vegetables. Humans are unable to synthesize vitamin C endogenously, and as such, it is consumed as essential dietary component. Vitamin C deficiency is known to cause scurvy in humans [10], scurvy can be prevented with as little as 10 mg vitamin C per day, an amount easily obtained through consumption of fresh fruits and vegetables.

The underutilization of these condiments used as thickeners in soup preparation and medicinal purposes prompted the need for this study in order to analyze the amino acid composition and vitamin C content of *Detarium microcarpum*, *Cissus populnea*, *Grewia mollis* and *Parkia biglobosa* collected from Gwagwa market, in the Federal Capital Territory, Abuja to ascertain their nutrients contribution to man.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The dried leaves of *Detarium microcarpum*, *Cissus populnea*, stem bark of *Grewia mollis* and seeds of *Parkia biglobosa* were purchased from Gwagwa market. The samples were identified and authenticated by a Taxonomist at the Herbarium unit, National Institute for Pharmaceutical Research and Development, Idu-Abuja. A voucher specimen was deposited at the herbarium of the institute with voucher specimen numbers NIPRD/H/7241, NIPRD/H/7242, NIPRD/H/7243, NIPRD/H/7244 for *Detarium microcarpum*, *Cissus populnea*, *Grewia mollis* and *Parkia biglobosa* respectively. The cleaned samples were pounded into powder form using mortar and pestle. The powdered samples were stored in an air tight container, properly labelled and kept at room temperature for subsequent analysis.

2.2 Amino Acid Analysis

The Amino acid profile of the samples was determined using methods described by [11]. The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

2.3 Defatting Sample

The samples were defatted using chloroform/methanol mixture of ratio 2:1. About 500 mg each of the sample was inserted into the

extraction thimble and extracted for 15 hours in soxhlet extraction apparatus [12].

2.4 Nitrogen Determination

Each of the sample (115 mg) was weighed, wrapped in whatman filter and was inserted into the Kjedahl digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) 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 Kjedahl digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 m1) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey colored end point. The percentage Nitrogen content was determined using equation 7 below.

$$\text{Percentage Nitrogen} = (a - b) \times 0.01 \times 14 \times V \times 100 / W \times C \quad (7)$$

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 (5ml)

14. = Nitrogen constant in mg

2.5 Hydrolysis of the Sample

The defatted sample was weighed into glass ampoule. 7ml of 6 NHCL was added and oxygen was expelled by passing Nitrogen into the ampoule (this is 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 $105^\circ\text{C} \pm 5^\circ\text{C}$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was

filtered to remove the humins. 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.

2.6 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.

2.7 Method of Calculating Amino Acid Values

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

2.8 Vitamin C Content Determination

Ascorbic acid content of the condiments was determined by titration method described by [13].

2.9 Preparation of Extracts

About 0.250 g vitamin C (ascorbic acid) was dissolved in 100cm³ distilled water. And it was diluted to 250cm³ with distilled water in a volumetric flask which was used as standard. For the samples, 10g of the powdered samples were macerated in 50cm³ distilled water for each for 24hours and were filtered, the filtrate was collected for the analysis.

2.9.1 Titration

2.9.1.1 For vitamin c standard solution

About 25.00 cm³ of vitamin C standard solution was measured into a 250 cm³ conical flask, 10 drops of 1% starch solution was added to it. The burette was rinsed with a small volume of the iodine solution and then filled. The initial volume was recorded. The solution was titrated until the endpoint was reached. That is when the first sign of color was observed by swirling the solution. The final volume of iodine solution was recorded. The volume that was required is the starting volume minus the final volume. The procedure was repeated twice.

2.9.1.2 For the samples solution

2 ml aliquot of the sample solution was measured into a 250cm³ conical flask and 15cm³ of distilled water was added to it and 10 drops of 1% starch indicator solution was also added. The sample solution was titrated with iodine solution. Until the end point was identified. The titration was repeated with further aliquots of the samples to obtain concordant results.

2.9.1.3 Calculation

The average volume of iodine solution used was calculated from the concordant titres and the amount of the titrant iodine solution required for the standard was determined. The concentration in mgL⁻¹ of ascorbic acid in the solution and the concentration in mgL⁻¹ of samples were also calculated.

3. RESULTS AND DISCUSSION

The results for the amino acid profile and vitamin C content analysis of *Detarium microcarpum*, *Cissus populnea*, *Grewia mollis* and *Parkia biglobosa* are presented in Table 1 and 2.

3.1 Amino Acid Analysis

Table 1 shows the amino acid profile of *Grewia mollis*, *Cissus populnea*, *Detarium microcarpum* and *Parkia biglobosa*. In this study, fourteen amino acids were detected as a result of the conversion of glutamine and asparagine to glutamic and aspartic acids respectively and complete destruction of tryptophan during acid hydrolysis. The result obtained showed that among the essential amino acids, leucine was highest in *Parkia biglobosa* 7.76g/100g protein than in *Detarium microcarpum* 6.10 g/100g protein, *Cissus populnea* 6.10g/100g protein and *Grewia mollis* 4.20 g/100g protein. Leucine is responsible for regulating the blood sugar concentrations, growth and repairs of muscles/tissues, hormone production, wound healing and energy production. Its deficiency causes dizziness, headaches, fatigue, depression, confusion, irritability and hypoglycemia in infants [14]. Lysine insures the adequate absorption of calcium, help the formation of collagen, in addition it aids the production of antibodies, hormones and enzymes. Lysine concentrations in these condiments were 5.37g/100g protein in *Cissus populnea*, 5.14 g/100g protein in *Parkia biglobosa*, 4.03g/100g protein in *Grewia mollis* and 3.02g/100g protein in *Detarium*

microcarpum. Lysine deficiency may result in tiredness inability to concentrate, irritability, bloodshot eyes, retarded growth, hair loss, anemia and reproductive problems [15].

From the result obtained, histidine had the highest concentration occurring in *Parkia biglobosa* 3.39 g/100g protein, *Cissus populnea* 3.20 g/100g protein, while *Detarium microcarpum* content was 2.20g/100g protein and 2.14g/100g protein in *Grewia mollis*. Histidine is essential especially in children, it is used for growth, tissue repairs and histamine development [16], this amino acid was found to be higher than 1.9g/100g protein set as reference standard [17]. Valine promotes mental vigor, muscle coordination and calm emotions [18]. Its concentration was highest in *Parkia biglobosa*, while *Detarium microcarpum* had 3.86g/100g protein and 2.92g/100g protein in *Cissus populnea* and 2.54 g/100g protein in *Grewia mollis* respectively. It functions as a supplier of sulphur, which prevents disorders of hair, skin and nails. It prevents arterial fat buildup, regulate ammonia formation and creates ammonia free urine which reduces bladder irritations; its deficiency results in similar symptom like phenylalanine [19].

Tryptophan is an amino acid needed for growth in infants and for the production and maintenance of the body's proteins, muscles, enzymes and neurotransmitters [20]. Tryptophan concentration in the condiments were 1.66g/100g protein in *Parkia biglobosa*, 1.26g/100g *Detarium microcarpum*, 1.05g/100g *Cissus populnea* and 0.71g/100g protein in *Grewia mollis* respectively. Isoleucine is essential for the production and formation of hemoglobin and the production of red blood cells. It is primarily present in muscle tissue and regulates energy levels [21]. Older adults may be more prone to isoleucine deficiency than younger people. This deficiency may cause muscle wasting and shaking [22]. Its concentration was highest in *Parkia biglobosa* 4.65g/100g protein than *Cissus populnea* 3.20g/100g protein while *Detarium microcarpum* has 2.20g/100g protein and 2.14g/100g protein in *Grewia mollis*.

Semi-essential amino acids are those that can be synthesized by the body's metabolic pathways but possibly not in sufficient quantity especially in children. Arginine participates in a number of metabolic pathways, it also influences blood pressure, muscle building, muscle regeneration and erection in males [23].

Table 1. Amino acid profile of the samples (g/100g protein)

Amino acid	PB	DM	GM	CO
Histidine ^a	3.39	2.20	2.14	3.20
Isoleucine ^a	4.65	3.02	2.85	3.73
Leucine ^a	7.76	6.10	4.20	6.10
Lysine ^a	5.14	3.02	4.03	5.37
Tryptophan ^a	1.66	1.26	0.71	1.05
Valine ^a	4.56	3.86	2.54	2.92
Arginine ^b	11.36	9.20	3.18	4.64
Glycine ^b	5.16	3.14	2.99	4.70
Cysteine ^b	1.57	1.15	1.33	1.57
Tyrosine ^b	4.13	3.10	2.14	3.10
Aspartic acid ^c	9.30	7.63	7.63	9.64
Alanine ^c	5.50	4.17	1.90	2.24
Glutamic acid ^c	14.38	12.20	6.28	8.98
Serine ^c	4.59	3.51	2.40	4.00
TEAA	27.16	19.46	16.47	22.37
SEAA	22.22	16.59	9.64	14.01
NEAA	33.77	27.51	18.21	24.86

Values are expressed as g/100g dry weight of sample

^aTEAA= Total essential amino acids

^bSEAA= Semi- essential amino acids

^cNEAA= Non- essential amino acids

Key: GM = *Grewia mollis*

CP = *Cissus populnea*

DM = *Detarium microcarpum*

PB = *Parkia biglobosa*

Table 2. The vitamin C content of the samples (mg/100ml)

Detarium microcarpum	0.18
Cissus populnea	0.11
Grewia mollis	0.12
Parkia biglobosa	0.30

The result obtained showed that among the semi-essential amino acids, arginine was highest in *Parkia biglobosa* 11.36g/100g protein than in *Detarium microcarpum* 9.20 g/100g protein, *Cissus populnea* 4.64g/100g protein and *Grewia mollis* 3.18 g/100g protein. Cysteine concentration in the samples was 1.57g/100g protein in *Parkia biglobosa*, 1.57g/100g protein in *Cissus populnea*, 1.33g/100g protein in *Grewia mollis* and 1.15g/100g protein in *Detarium microcarpum*. This shows that cysteine values are relatively low in all the samples. Tyrosine is particularly important in the production of epinephrine. It helps the body produce enzymes, thyroid hormones and skin pigment melanin [24]. Its concentration was 4.13g/100g protein in *Parkia biglobosa*, 3.10g/100g, protein in *Detarium microcarpum* and *Cissus populnea*, while 2.14g/100g protein in *Grewia mollis*. Tyrosine supplements can cause insomnia, restlessness, palpitations, headache and heartburn [25]. Glycine is an amino acid that our body uses to create proteins, our bodies naturally produces glycine from other amino acids, which it needs for the growth and maintenance of tissue and for making important substances such as hormones and enzymes [26]. It is also found in protein rich foods [27]. The concentration of glycine in the samples were 5.16g/100g protein *Parkia biglobosa*, 4.70g/100g protein in *Cissus populnea*, 3.14g/100g protein in *Detarium microcarpum* and 2.99g/100g protein in *Grewia mollis*.

Among the non-essential amino acids, *Parkia biglobosa* had 14.38, 9.30, 4.59 and 5.50 (g/100g protein) of glutamic acids, aspartic acid, serine and alanine respectively. On the other hand, *Detarium microcarpum* had 12.20, 7.63, 3.51 and 4.17 (g/100g protein) as concentrations for glutamic acids, aspartic acid, serine and alanine respectively. While glutamic acids in *Grewia mollis* was 6.28g/100g protein, aspartic acid 7.63g/100g protein, serine 2.40g/100g protein and alanine 1.90g/100g. The result obtained for *Cissus populnea* indicated that aspartic acid 9.64g/100g protein was higher than glutamic acids 8.98g/100g protein, serine 4.00g/100g protein and alanine 2.24g/100g

protein. The result indicates that *Parkia biglobosa* is a good source of essential amino acids. This agrees with Ram [28] who reported that, *Parkia biglobosa* contained all the essential amino acid in appreciable quantities. Similarly, the amino acid composition of *Detarium microcarpum*, *Grewia mollis* and *Cissus populnea* confirmed that, the plant is a good source of amino acids [29].

To evaluate the nutritional quality of the samples, the percentages of the essential amino acids in the samples were compared with those of reference standard amino acid profile by WHO/FAO/UNU (1985) for adults (0.9 - 1.9g/100g protein) and for preschool (1.9 - 6.6g/100g protein). The result presented in Table 1 shows that all the essential amino acids exceeded the reference value for adults and preschool children. High concentrations aspartic acid, glutamic acid valine, alanine and leucine predominate in all the samples analyzed. Specific metabolic processes in which these amino acids participate may be related to the therapeutic properties of plants as per their use in traditional medicine and therefore may facilitate the understanding their beneficial properties.

3.2 Vitamin C

The result obtained for the concentration of vitamin C contents of the condiments reveals low content of vitamin C. The sample *Parkia biglobosa* has the highest concentration of vitamin C with 0.3mg/L followed by *Detarium Microcarpum* 0.18mg/L, *Grewia Mollis* 0.12mg/L and *Cissus populnea* 0.11mg/L. The result obtained for this study is compared with those reported by [30]. Ascorbic acid, an essential vitamin for human health is required for many physiological functions. It can be suggested that incorporation of small amount of spices in once daily food items may be beneficial to human health.

4. CONCLUSION

This study has shown that these condiments contained amino acids in appropriate quantities

that can serve as supplementary potential sources of essential amino acids to man and appreciable amount of vitamin C.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

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