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Formulation and Nutritional Evaluation of a Nutraceutical with Antioxidant Activity Developed for the Management of Metabolic Syndrome

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Abstract

Background: Antioxidants are critical for maintaining optimum health and wellbeing and they protect the body against the development of diseases caused by oxidative stress. Nutraceuticals are foods or part of food that have medical benefit. The present study formulated and nutritionally evaluated an antioxidant rich nutraceutical.

Materials/Methods: Nutritional analysis of proximate composition, amino acids and fatty acids profile, antioxidants vitamins and minerals were determined using standard methods. Tomatoes, ginger, garlic, onion, watermelon seed, lemon juice and palm oil were used to formulate the nutraceutical.

Results: Proximate composition analysis revealed that the nutraceutical is rich in ash (3.00 ± 0.001 %), protein (22.09 ± 0.01 %) and Lipid (30.00 ± 0.06 %). The nutraceutical was also found to be low in moisture (4.00 ± 0.02 %) and Carbohydrate (38.78 ± 0.05 %) contents. Vitamins A, C and E concentrations obtained were 12.7 ± 3.7 mg/100g, 8.2 ± 0.04 mg/100g, and 5.5 ± 0.04 mg/100 g respectively. Concentration of the antioxidant minerals were Mn (590.2 ± 4.4 mg/100g), Cr (212.2 ± 16.6 mg/100g), Cu (53.1 ± 7.3 mg/100g) and Zn (5.3 ± 0.2 mg/100g). Both essential and non-essential amino acids were detected in the formulated nutraceutical with arginine and leucine having the highest concentration among the essential amino acids while glutamate and asparagine constitute the highest concentration among the non-essential amino acids. The fatty acids analysis revealed the presence of high amount of oleic and lauric acids.

Conclusion: The formulated nutraceutical can serve as a suitable source of amino acids, antioxidant vitamins and minerals hence might have potentials in the management of diseases related to oxidative stress.

Key words: Nutraceutical, Antioxidants, Oxidative stress, Amino acids.

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Introduction

Antioxidants are critical for maintaining optimum health and well being and they protect the body against the development of diseases caused by oxidative stress(1). Oxidative stress has been implicated in the progression of major health problems by inactivating the metabolic enzymes and damaging important cellular components, oxidizing the nucleic acids, leading to human ageing and diseases (2, 3) such as cardiovascular diseases (CVDs) (4, 5, 6), diabetes (7), obesity (8), neurological diseases, cancer and blood disorders among others (9). Oxidative stress occurs from the imbalance between reactive oxygen and nitrogen species (RONS) production and antioxidant defenses. Miyajima *et al.*, (10) indicated the role of oxidative stress in the pathogenesis of hypertension. Fermented papaya preparation, obtained from fermentation of *Carica papaya* Linn using yeast was considered a food supplement that exhibits antiinflammatory, antioxidant, and immunostimulatory functions that may positively affect the clinical damage induced by oxidative stress (11). Firuzi *et al.*, (12) conducted a study on the effect of intake of different vitamins and on the risk of CVDs, suggesting that higher intake of these vitamins significantly lowered the risk of these pathologic conditions. Given the reported inverse relationship between the dietary intake of antioxidant-rich food and the incidence of human diseases, there has been a global trend toward increasing the intake of natural antioxidants, especially in the geriatric population (13, 14).

Nutraceuticals can be defined as a food or part of a food that provides medical or health benefits, including the prevention and/or treatment of diseases (15). They are found in a number of products from food industry, herbal supplements, dietary supplements and pharmaceutical industries (16). Nutraceuticals are used in most therapeutic areas that include disorders related to sleep, digestion, cold and cough, prevention of cancer, blood pressure, pain killers, depression, obesity, hyperglycemia and hypoglycemia (17). Considering the role of oxidative stress in the pathogenesis of many diseases, development of antioxidants rich nutraceutical will provide a better treatment strategy with fewer side effects in such patients. The present study involved the formulation and nutritional analysis of a nutraceutical with antioxidant activity.

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Materials and Methods

Chemicals/Reagents: All Chemicals and reagent used for this study were of analytical grade. Metaphosphoric acid, thiourea, 2,4-Dinitrophenylhydrazine (DNPH), potassium hydroxide and xylene were purchased from Sigma-Aldrich, UK. Per chloric acid, α - dipyridyl, nitric acid (HNO₃) and sodium 3 hydroxide were purchased from Fisher Scientific, USA.

Ingredients for Nutraceutical Preparation

Fresh onion, tomato, garlic, ginger, lemon, water melon and palm oil were purchased from Ramin Kura market Sokoto, Sokoto State, Nigeria. The samples were identified by Mal. A. A. Umar, a taxonomist in the Herbarium, Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, voucher specimen was dropped at the Herbarium. The samples were taken to the laboratory immediately for formulation of the nutraceutical.

Formulation of the Nutraceutical

Tomatoes, Ginger, Onion, Garlic, Water melon seed, Lemon juice and Palm oil were used in the preparation in ratio of 2:2:2:2:1:0.5:0.5 respectively. Water melon seeds were collected by dissecting water melon with a knife, the seeds were then pounded using mortar and pestle, ten grams of the pounded seed material and twenty grams each of sliced and pounded fresh tomatoes, ginger, Onion and garlic were placed in a beaker. The content were emptied into a blender, one hundred millilitre (ml) of distilled deionized water was added and blended for two minutes. The content was then transferred into a conical flask. Lemon juice and palm oil (five grams each) were measured and added in to the conical flask respectively. The content was mixed thoroughly; after which the conical flask was covered with aluminium foil. The sample was then stored in a refrigerator at four degrees Celsius (4°C) for further analysis.

Proximate Composition of the Nutraceutical

For the determination of moisture, ash, crude lipid and crude fibre, method of the Association of Official Analytical Chemist (AOAC) (18) was used. For crude protein, AOAC (18) was also used. Available carbohydrates were estimated by subtracting the total percentages of crude protein, crude lipid, crude fibre and ash content from 100 % moisture free sample (19).

Analysis of Antioxidant Vitamins and Minerals

Vitamin C was determined by the method of Rutkowski and Grzegorzczak (20). To 0.5 ml of sample, 2 ml of freshly prepared 6 % metaphosphoric acid was added, mixed thoroughly and centrifuged for 10 minutes (10 mins) at three thousand five hundred revolutions per minute (3500 rpm). Similarly, blank and standard were prepared but instead of sample, 0.5 ml of distilled water and standard were added respectively. To 1.2 ml of supernatant, 0.4 ml of Dithiocarbamate (DTC) reagent (5 ml of 5% thiourea solution + 5 ml of 0.6 % copper sulphate

+ 10 ml 2,4-Dinitrophenylhydrazine (DNPH) reagent) was added, mixed and incubated in a water bath at 37°C for 3 hours. The test tubes were transferred into ice bath for 10 mins. Two millilitres (2 ml) of 12 M sulphuric acid was added and mixed. The absorbance was read at five hundred and twenty nanometre (520 nm) after zeroing the instrument with blank. Concentration of vitamin C was calculated by dividing the absorbance of sample by the absorbance of standard, then multiplying the result by concentration of standard.

Vitamin A was estimated by the method of Rutkowski *et al.*, (21). One milliliter (1 ml) each of the sample and potassium hydroxide (KOH) solution were placed into a centrifugal test tube, then it was shaken vigorously for 1 min. The tube was heated in a water bath (60°C, 20 mins), then cooled. One millilitre (1 ml) of xylene was added and the test tube was shaken vigorously again for one min. The mixture was centrifuged for ten min at 4000 rpm, the supernatant was transferred to a glass test tube and the absorbance of the solution was read at 335 nm against xylene (A₁). The xylene extract 1 was irradiated to UV light for 30 minutes, until a steady absorbance (A₂) was obtained at 335 nm. The absorbance of retinol was obtained by subtracting the second absorbance (A₂) from the 2 initial absorbance (A₁). The concentration C_x of vitamin A in the sample was calculated by multiplying 22.23 to the difference of A₂ from A₁. Where: 22.23 is the multiplier on basis of the absorption coefficient of 1 % solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette of about thickness one centimetre.

Vitamin E was estimated by the method of Rutkowski *et al.*, (22). To 1.5 ml of sample, 1.5 ml of ethanol and 2.0 ml of xylene were added and centrifuged (2500 rpm, 10 mins). Similarly, standard and blank test tubes were prepared by adding standard vitamin E and distilled water respectively instead of the sample. One millilitre of xylene layer was pipetted into another set of identically labeled test tubes and 1 ml of α -dipyridyl reagent was added to the test tubes, contents were mixed and the absorbance of sample and standard was read against blank at 460 nm. After 3 minutes, 0.33 ml of ferric chloride was added to the test tubes and another absorbance was taken at 520 nm. Concentration of vitamin E was calculated by subtracting the absorbance of sample at 460 nm from the absorbance of sample at 520 nm, multiplying the result by concentration of standard, then dividing the result by absorbance of standard at 520 nm.

Atomic absorption spectrophotometry (AAS) (23) was used for the determination of Copper, Zinc, Chromium and Manganese. The sample was digested before mineral quantification by adding 5 mls of concentrated nitric acid (HNO₃) to 2 mls of the sample in a beaker; the solution was heated on a hot plate until a clear solution was formed. Per chloric acid (1 ml) was added and the content was further heated to a clear solution. Distilled deionised H₂O was added to 2 make 10 mls and the solution was then filtered. The volume was then made up to 50 mls with distilled deionised HO using a clean volumetric 2 flask (24).

Fatty Acid Analysis: The fatty acids in the sample were determined by the method described by Lokonge *et al.*, (25). The sample (50 mg) was dissolved in 1 dm³

dry toluene in a test tube to which 2 cm³ 0.5 M sodium hydroxide in methanol was added, and the solution was maintained at 50°C for 10 minutes. The required esters were extracted into 5 cm³ hexane twice using a Pasteur pipette to separate the layers. The extract was dried over anhydrous sodium sulphate and filtered; and the solvent was later evaporated at low temperature. The fatty acid methyl esters generated were used for GC-MS analysis.

Amino acid Profiles: The Amino acid profile of the sample was determined as described by Benitez, (26). The sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Applied Bio systems PTH Amino Acid Analyser (Technicon Sequential Multiple Amino Acid Analyser, Dublin, UK).

Statistical Analysis: All data are presented as mean± SEM. SPSS version 20 was used for the analysis of data.

RESULTS

The result obtained in this study revealed the proximate composition, antioxidant vitamins and minerals, fatty acids and amino acids content in the formulated nutraceutical. The result of the proximate composition of the nutraceutical is presented in Table 1. The result revealed that the nutraceutical is rich in proteins, lipids and carbohydrate, while the moisture and crude fibre content were low. The antioxidant vitamins content is shown in Table 2. The amount of vitamin A (12.70±3.7 mg/100g) present in the nutraceutical is highest compared to vitamins C and E though their levels are appreciable. The result of the antioxidant minerals composition of the nutraceutical is as presented in Table 3. Of all the minerals analysed (Mn, Cr, Cu and Zn), Mn is the most abundant (590.10±4.4 mg/100g) while Zn and Cu were low. The fatty acids detected in the nutraceutical are presented in Table 4 and include Oleic, Lauric, Stearic acids. However, no essential fatty acids (Linoleic and Linolenic acids) were detected in the nutraceutical. Amino acid profiles of the nutraceutical indicated the presence of essential and non essential amino acids (Table 5).

Table 1: Proximate Composition of the Formulated Nutraceutical

COMPOSITION	% COMPOSITION
Moisture	4.00±0.02
Ash	3.00±0.001
Lipid	30.00±0.06
Protein	22.09±0.01
Fibre	2.12±0.07
Carbohydrates	38.78±0.05

Results are expressed as mean ± standard error of mean.

Discussion

The proximate composition of the formulated nutraceutical in the present study revealed the presence of moisture, ash, carbohydrate, protein, crude fibre, and lipids. The moisture content of the nutraceutical was

Table 2: Antioxidant Vitamins Content of the Formulated Nutraceutical

Antioxidant vitamins	Concentration (mg/100g)
Vitamin A	12.70±3.7
Vitamin C	8.20±0.04
Vitamin E	5.40±0.04

Results are expressed as mean ± standard error of mean.

Table 3: Antioxidant Minerals Content of the Formulated Nutraceutical

Antioxidant minerals	Concentration (mg/100g)
Manganese	590.10±4.4
Zinc	5.20±0.2
Copper	5.30±7.3
Chromium	212.20±16.6

Results are expressed as mean ± standard error of mean.

Table 4: Fatty acids Content of the Formulated Nutraceutical

Fatty acid	Observation
Lauric acid	+++
Oleic acid	+++
Stearic acid	++
Linoleic acid	-
Linolenic acid	-

KEY: + Abundant, ++; More abundant, +++; Most abundant, -; Not detected.

Table 5: Amino acids Content of the Formulated Nutraceutical

Amino acid	Concentration (mg/100g)
Leucine	1365.16
Lysine	689.20
Isoleucine	709.09
Phenylalanine	1252.50
Valine	793.03
Methionine	293.80
Arginine	1614.78
Histidine	627.36
Threonine	881.39
Proline	852.67
Tyrosine	682.58
Cysteine	293.80
Alanine	804.08
Glutamic acid	2909.00
Glycine	976.38
Serine	821.75
Aspartic acid	1875.44

Results are expressed as mean ± standard error of mean.

found to be low which suggests relatively long shelf life of the nutraceutical since high moisture content of food materials has been reported to be associated with increased growth of microorganisms (27) and consequently spoilage of food materials. Ash is an indication of the micronutrient content of food materials (28). The high amount of ash in the nutraceutical indicates high amount of minerals and this may be one of the reasons for it to have pharmacological effect.

The considerable amount of protein that was detected in the formulated nutraceutical suggest that it can reduce the risk of cardiovascular diseases since protein lowers plasma triglycerides (29), and reduces LDL, VLDL

and cholesterol levels (30), The significant amount of protein contents of the nutraceutical could have supplementary effect for daily protein requirement. The low carbohydrate content of the nutraceutical will make it suitable for metabolic syndrome patients as carbohydrate restriction is part of recommended strategy in metabolic syndrome management because high carbohydrate intake increases the requirement for insulin secretion in order to maintain glucose homeostasis (31).

The dietary fibre content of the newly developed nutraceutical was not high but can be improved because dietary fibre is one of the factors that influence the post prandial glucose and insulin tolerance (32). Cross sectional studies have shown that lack of fibre may be a causative factor of type 2 diabetes and have also shown an inverse relationship with insulin level (33, 34). Chandalia *et al.* (35) reported that high fibre supplements could improve glucose tolerance. In addition, the nutritional implications of fibre are to prevent diverticulosis and aid absorption of trace elements in the gut as well as elimination of undigested food material through the bowel (36, 37) and to prevent certain form of cancer (38) so necessitating the need to improve the dietary fibre content of the formulated nutraceutical. The formulated nutraceutical was found to be rich in lipids and since lipids from vegetable sources have been reported to lower the risk of type 2 diabetes mellitus (T2DM) (39) and cardiovascular diseases (40), for energy source, transport of fat soluble vitamins and also for insulation and protection of internal tissues, it can have advantageous effect on various diseases.

The concentration of vitamin A in the nutraceutical is appreciable. It has been reported that onion, garlic and ginger are good sources of vitamin A (41) and could be the reason for high amount of vitamin A in the nutraceutical. The value obtained is higher than that obtained in white ginger (8.4 ± 0.0 mg/100 ml) and garlic (13.4 ± 0.0 mg/100 ml) (42). Vitamin A supplementation has been reported to lower systolic blood pressure in salt induced hypertensive rats, it also lowers LDL oxidation and improves endothelial function in metabolic syndrome (43). Being an antioxidant, vitamin A can play significant roles in the management of CVDs and obesity through scavenging of free radicals.

The amount of Vitamin C in the formulated nutraceutical is appreciable; the presence of which may be due to the presence of tomato, ginger, lemon, watermelon seed and garlic. However, this is lower than the vitamin C level in garlic and onion (18.0 mg/100 ml) (44), and lemon (53.0 mg/100 ml) reported by Bilbis *et al.*, (43). However the value is higher than the one obtained from fresh watermelon seed (45). Vitamin C supplementation in T2DM improved blood glucose level (46) which is a component of metabolic syndrome. Vitamin C is known for its antioxidative activity thus can protect the body against the development of diseases caused by oxidative stress such as cancer, Parkinson's disease, Alzheimer's disease, myocardial infarction and Sickle Cell Disease.

The amount of vitamin E in the nutraceutical is also appreciable and is likely attributable to the presence

of ginger and onion. The value is higher than (3.6 ± 0.0 mg/100 ml) and (1.6 ± 0.00 mg/100 ml) those obtained from white ginger and onion respectively (47). This may likely be due to the different variety of constituents used in the preparation of the nutraceutical. The significant level of vitamin E in the nutraceutical could play a vital role in the antioxidant defence system. It has been reported that increased dietary level of vitamin E results in higher tissue α -tocopherol concentration and greater stability toward lipid peroxidation (48).

The antioxidant vitamins A, C and E play a significant role as antihypertensive agents (47), help in the management of diabetes mellitus (49) as well as other complications that are components of metabolic syndrome probably due to their roles in scavenging free radicals. The antioxidant minerals (Mn, Cr, Cu and Zn) were also detected, the result indicates that the minerals increase in the order $Mn > Cr > Cu > Zn$. Antioxidant minerals important roles in maintaining immune function and neutralizing the reactive oxygen intermediates produced by activated macrophages and neutrophils and act as cofactors for antioxidant enzymes (50). Mn has been reported to play a significant role in insulin synthesis and secretion (51). Type II diabetic subjects also respond well to oral doses of Mn (49). Mn is a cofactor of many enzymes including mitochondrial superoxide dismutase (52), it activates enzymes that play important roles in the metabolism of carbohydrates, amino acids and cholesterol. Cr has been known to be a component of glucose tolerance factor required for optimal glucose utilization by cells (53), increase insulin binding to cells and triggers insulin receptor kinase leading to increase insulin sensitivity (49). The concentration of Copper and Zinc are lower than those of Mn and Cr in the nutraceutical. Cu and Zn are components of superoxide dismutase which are responsible for mopping up free radicals (54) hence prevent oxidative stress which is associated with metabolic syndrome. Apart from having antioxidative properties, they also play other vital roles such as regulation of cellular growth and participate as co-factors of enzymes responsible for carbohydrate, proteins and nucleic acid metabolism in the system (55). Low level of the minerals has also been reported to correlate with the activity of antioxidant enzymes (56). Thus, the appreciable amount of the antioxidant minerals in the nutraceutical could help in the management of metabolic syndrome by increasing the activity of antioxidant enzymes.

Although many fatty acids were detected in the formulated nutraceutical, no essential fatty acid was detected. Free fatty acids have roles in host defence against potential opportunistic or pathogenic microorganisms (57). Indeed, fatty acids are normally identified as active ingredients in ethnic and herbal medicines (58), free fatty acids also serve as a source of energy and play pivotal roles in the body (59).

Amino acid profile revealed that all the essential and non-essential amino acids were detected in the formulated nutraceutical. The essential amino acids most importantly, have to be provided in the diet due to inability of synthesis by humans (60). Presence of appreciable amount of essential amino acids in the

nutraceutical could be a good source of nutrient for patients having diseases related to oxidative stress. Arginine and Leucine have the highest concentration among the essential amino acids. It has been reported that Arginine plays important roles in weight loss (61), antihypertensive (62), management of obesity and type 2 diabetes (63). Leucine helps in lowering LDL and cholesterol (64). These properties of essential amino acids could help in the management of metabolic syndrome.

Among the non-essential amino acids, glutamic acid (2909.25 mg/100g) and aspartic acid (1875.44 mg/100g) were found to have the highest concentration. Glutamic acid has anticancer effect (65) while aspartic acid has immune function, increase resistance to fatigue and energy production (66). The considerable amount of amino acids (essential and nonessential) of the formulated nutraceutical can serve as good source of amino acids for protein synthesis and other physiological roles (67).

Conclusion: The presence of appreciable amount of antioxidant vitamins and minerals, essential and non-essential amino acids as well as macronutrients in the formulated nutraceutical could serve as a good source of these nutrients which can play significant roles in health maintenance and contribute to reducing the risk of CVDS and other degenerative diseases associated with free radical damage.

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