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Microbiological, Essential Dietary Minerals and Amino Acids Composition of Malted and/or Fermented FARO 44 Rice Plus Soybean Based Complementary Foods

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Abstract

Objective: To produce complementary foods from malted and/or fermented FARO 44 rice cultivar combined with soybean and evaluate its microbiological, essential dietary minerals and amino acids composition. Targeting the nutritional and economic challenges faced by many people.

Methods: Paddy of FARO 44 rice cultivar and soybean (*Glycine max*) were obtained from good sources. Malting and fermentation were carried out on the rice and flours obtained from the rice were further formulated with a processed soybean flour. Microbiological counts, mineral and amino acids analysis were carried on the formulated malted and/or fermented rice plus soybean based complementary foods. Results obtained were statistically analyzed using IBM SPSS statistics version 22.

Results: Total plate count ranged from 2.3 × 10^3 to 3.3 × 10^3 CFU/g, yeast/mould count ranged from 3 × 10^2 to 4 × 10^2 CFU/g. Coliform and lactic acid bacteria count were observed to be absent (no growth) throughout all the formulations. Mineral composition of the formulated food products together with the raw materials is ranged from: 0.56 to 7.52 mg for iron, 0.34 to 4.51 mg for zinc, 5.60 to 62.87 mg for calcium, 6.60 to 53.11 mg for magnesium, 27.30 to 281.49 mg for potassium, 53.76 to 523.67 mg for phosphorus, 0.09 to 0.33 mg for copper and 0.30 to 3.09 for manganese. Amino acid composition of malted and/or fermented rice plus soybean based complementary foods ranged from: 7.25 to 7.49 mg for leucine, 6.53 to 6.64 mg for lysine, 3.55 to 3.76 mg for isoleucine, 7.98 to 8.09 mg for phenylalanine + tyrosine, 4.10 to 4.29 mg for valine, 0.99 to 1.10 mg for tryptophan, 2.71 to 2.90 mg for methionine + cysteine, 3.31 to 3.45 mg for threonine and 2.94 to 3.02 mg for histidine.

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While amino acid scores ranged from: 103.57 to 107.00% for leucine, 118.73 to 120.73% for lysine, 88.75 to 94.00% for isoleucine, 133.00 to 138.00% for phenylalanine and tyrosine, 82.00 to 85.80% for valine, 77.43 to 82.86% for methionine and cysteine, 82.75 to 86.25% for threonine and 113.08 to 116.15% for histidine.

Conclusion: All the formulated food products have no indication of being potentially hazardous to its consumers. Malting and fermentation caused slight reduction of mineral elements in rice, which were however restored by addition of soybean. All amino acids were within the recommended levels of FAO/WHO. However, isoleucine, methionine + cysteine, valine and threonine were slightly limiting.

Introduction

Complementary foods are foods often used to support breast milk. These includes breast milk substitutes ("follow-up formula") that are given gradually or fully to infants usually from the age of six months with the intention of complementing breast milk until the child adapts to solid foods [1]. In the northern parts of Nigeria, such preparations are called koko or kunu in Hausa. However, the names of these foods differs from ethnicities of Nigerian regions [2]. Such foods were also used not only for infant's feedings but to sustain severely ill persons who cannot take solids foods or help sick person regain strength or appetite. In some cases, Native doctors in North-eastern parts of Nigeria often advocate mixing of prepared powdered medicinal herbs with thin porridges (kunu) for better utilization. Such gruels are most often unhygienic and with low nutrients density [3].

Malting and fermentation have been used successfully for producing complementary foods. These processes are well established locally adaptable technologies for bulk reduction and hence increases nutrients density for a given gruel consistence and also for reduction of pH of gruels and hence reduction of food spoilage by pathogenic microorganisms [4, 5, 6]. Malting and fermentation aid in synthesizing enzymes, B-vitamins, essential amino acids, saccharification of starch, phytate degradation, production of bioactive component concentration etc. It has been used extensively for the production of medicine, cosmetics and food preservation [7, 6]. Besides these, it was reported that malting and fermentation help to reduce anti-nutrients and enhance digestion, absorption and utilization of nutrients in the body [8]. Products of malting alone is a 'cascade' of natural enzymes [9, 10]. In Nigeria, apart from the usage of malt to produce divers categories of foods with nutritional and health benefits, malted grains has been used to treat some diseases such as mumps in Children.

There are many cereals that can be malted or fermented to produce complementary foods. But rice is a common staple used by mothers for complementary feeding. In fact, rice has mild hypoallergenic properties, easily digestible, gluten free, low level of anti-nutrients, suitable organoleptic profiles, high lysine content compared to other cereal grains etc. [11, 12, 13, 14]. Global interest now is on how to boost rice production and utilization in other to combat economic and nutritional challenges (such as hunger and starvation) ravaging citizens across the entire world [15, 16]. Indian researchers have identified many rice varieties with specific nutritional and medicinal values. This rice are already into mass production [17]. Rice is rich in energy but low in protein quantity and quality as sole complementary food [17]. Complementation of low protein diets with readily available and cheap plant protein source e.g. soybean has been established by earlier workers [1, 18]. For this reason, soybean has become an important subject of interest for food complementation. This is because it was reported to contain high amount of proteins, essential minerals, dietary fibre, phyto-chemicals, and unsaturated fatty acids [19, 20].

Food fortification or complementation has been advocated by regulatory agencies for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population groups [21]. Combination of malted and/or fermented rice with soybean therefore would help in addressing many nutritional and economic problems within Nigeria and other countries of the world. These are the major target objectives of this research.

Materials and methods

Improved rice cultivar: FARO 44 (Federal Agricultural Research Oryza, the number 44th among the series of released varieties) was obtained from the National Cereals Research Institute, Badeggi, Bida, Niger State. While soybean (*Glycine max*) was procured in Gire Market, Gire Local Government, Adamawa State.

Preparation of raw materials

Malting of paddy (rice)

The rice cultivar (FARO 44) was first dry-cleaned using destoner (De-Stoner, Hunan Sunfied Machinary Co., Ltd, Model: TQS 320, Made in China) to remove contaminants (stones, dust, damaged paddy etc.). Paddy FARO 44 was divided into two equal portions. The first lot was germinated while the other was left un-germinated as described by Ariahu *et al*. [22] and Gernah *et al*. [23]. Malting of paddy was carried out with some slight modifications. Paddy was first washed twice with clean water. The cleaned paddy rice was steeped inside sufficient clean water to cover the surface of the grains completely. It was kept at 29 ± 2°C with good air circulation for 24 hours. The steeping process was interrupted after every 6 hours by draining. An "air-rest" period of one hour each for every interruption was provided until the grain reached about 42% moisture content [9, 24, 25, 26, 27].

The steeped paddy was then drained and wrapped in a wet jute bag to provide about 3 to 5 cm depths. The grains were germinated for 43 hours at 29 ± 1°C. The short periods of germinations were timed and was done to counter technical difficulties during dehulling of malted rice as experienced during pre-trials. After drying of germinated grains at 29 ± 2°C under constant air circulations for 48 hours, the germinated dried grains were polished by detaching the roots and rootlet [9, 24, 25, 26, 27].

Milling of rice into flour

The dried-polished paddy was then de-husked (Greep Rice Mill, Type: MBLN-115. Made in China.). After de-husking, all grains were finally dried again at 40°C in an air flow safety thermostat oven (air flow rate 140, Oven BS, Model OV-160, Gallen kamp, England) until constant weight were obtained [28]. Then all the rice (malted and un-malted rice) were milled using hammer miller into fine flour and let to pass through 0.8 mm fine sieve (Christy Hunt Agricultural Ltd, Foxhills Ind. Est Scunthorpe, Model DE DN15 8QW, South Humbers, England) as reported by Ojha and Micheal [29]; Mijinyawa *et al*. [30] and Sahay [31].

Fermenting of rice flours

Each of the rice flours obtained from germinated and un-germinated paddy were further divided into two equal parts. One lot from each of the germinated and un-germinated was fermented. From each batch selected for the fermentation, 120 g of flour was mixed with 80 ml of clean water and covered in plastic containers as reported by Ariahu *et al*. [22]. However, 60 (¾) ml of water was used for each of the 120 g of flour because the former was later giving out liquid not solid fermentation as intended.

Then after mixing the appropriate mixtures, it was allowed to undergo natural accelerated fermentation at 29 ± 2°C. Fermentation was then accelerated by adding 50% fermenting slurry to fresh concentrate for every 12 hour intervals until the pH of the mixtures get stabilized. pH was monitored for every 12 hour intervals using pH meter (Digital pH metre, Equip-Tronics, Model EQ-610, Mumbai) [22, 32, 33]

After fermentation, the final products were harvested and sprayed on drying trays. Fresh circulating air was allowed to pass through for 6 hours at 29 ± 2°C. After that, it was finally dried at 40°C in an air flow safety thermostat oven (air flow rate 140, Oven BS, Model OV-160, Gallen kamp, England) until constant weight were obtained [28].

Figure 1: Fermentation process and final packaged products in plastic containers.

Preparation of soybean flour

Soybean was sorted and washed with clean water. It was steeped for five hours in a clean water of three times its weight by volume until the seed coat become wet and soft (to reduce some soluble anti-nutrients and facilitate de-husking). After this process, soybean was further washed, drained and partially dried at 29 ± 2°C for 6 hours under fresh air circulation. After that, it was toasted at surface temperature of 180°C for 30 minutes and then de-husked by cracking the soybean kernel in a disc mill. Finally, it was winnowed and milled into fine flour in a hammer mill and let to pass through a 0.8 mm mesh size screen (Christy Hunt Agricultural Ltd, Foxhills Ind. Est Scunthorpe, Model DE DN15 8QW, South Humbers, England) as described by Badau *et al*. [1].

Formulation of complementary foods

An appropriate mixing ratio of rice to soybean flour using 16% protein as the constraint target recommended by the Protein and Advisory Group of the United Nation (PAG) [43] was obtained using material balance as described by Rao [35] and Eshun *et al*. [36].

Determination of microbiological count

Preparation of microbial apparatus and media

Glass wares were soaked in synthetic detergent and were allowed to stand for 30 minutes. These were then washed gently with sponge and rinsed thoroughly with distilled water. And then dried in an analytical hot air oven at 80°C. After drying and cooling, glass wares were assembled in canisters and sterilized in hot air oven at 160°C for 60 minutes. Glass wares were allowed to cool properly inside the oven before opening [37, 38].

Preparation of nutrient agar

This medium was prepared by weighing 28 g of nutrient agar powder and it was introduced into 1000 ml of distilled water (i.e., 7 g of agar into 250 ml distilled water) inside conical flask. The mouth of the conical flask was stoppered with sterile cotton wool and wrapped loosely with aluminium foil. Then the medium was heated slightly on heating mantle to get dissolved properly. It was then autoclaved (Prestige Medical Autoclave, Model No 21001, Prestige Medical, A Division of Meden, England) at temperature of 121°C for 15 minutes. It was then cold to 45°C for inoculation. The inoculated media were incubated (Gallenkamp Economy Incubator, Gallenkamp, Model 3A 4038, England) at 37°C for 24 hours [37, 38].

Preparation of macConkey agar

MacConky agar was prepared by weighing 48.5 g of macConkey agar powder into 1000 ml of distilled water (i.e., 12.13 g of agar into 250 ml distilled water) inside conical flask. The mouth of the conical flask was then stoppered with sterile cotton wool and wrapped loosely with aluminium foil. The medium was mixed thoroughly and heated slightly on a heating mantle to dissolve. This was autoclaved at temperature of 121°C for 15 minutes. The medium was cold to a temperature of 45°C inside water bath for inoculation. This was also done to prevent solidification of the medium [37, 38].

Preparation of potato dextrose agar

Potato dextrose agar (PDA) was also prepared by weighing 39 g of potato dextrose agar into 1000 ml distilled water (i.e., 9.75 g into 250 ml). It was autoclaved at a temperature of 121°C for 15 minutes and cold to 45°C and 1% gentamicin was introduced into it before inoculation [37, 38].

Preparation of de Man Rogosa and Sharpe agar (MRS agar)

This agar was prepared by suspending 66.73 g in 1000 ml of distilled water (i.e., 16.68 g into 250 ml of distilled water). It was dissolved completely and then sterilized by autoclaving at 121°C for 15 minutes before inoculation [37, 38].

Plating out

Serial dilution

One gram of the food material was weight weighed and aseptically mixed thoroughly with 9 ml of sterile distilled water in a sterile bijou bottle. Then 1 ml out of this mixture was taken using 1 ml sterile pipette and was transferred into another 9 ml of sterile distilled water in a sterile bijou bottle. It was mixed thoroughly and a desired serial dilution of 10^2 was made [37, 38].

Determination of total plate count

The total plate count was determined by taken 1 ml of the diluents (i.e., 1 ml suspension of the mixture) using sterile 1 ml pipette. This was introduced into the sterile labelled microbial plate. Then about 18 ml out of the prepared nutrient agar (medium) was poured into the sterile labelled microbial plate containing the 1 ml of the inoculums. It was swirled gently, mixed thoroughly and was allowed to solidify. The inoculated plate was then incubated at 37°C for 24 hours. Colonies was counted using colony counter [37, 38]. The result obtained was expressed using: Eqn. 1

$$\text{Total plate count (CF U/g} = \frac{\text{Number of colonies}}{\text{Volume transferred to plate} \times \text{Dilution blank factor}} \times 100$$

Eqn 1.

Determination of coliform count

Coliform count was determined using pour plate method. One millilitre (1 ml) of the diluents (i.e., 1 ml suspension of the mixture) was taken out with sterile 1 ml pipette. The diluent was introduced into the sterile labelled microbial plate. Then about 18 ml out of the prepared macConkey agar was poured into the sterile labelled microbial plate and was swirled gently to mix. The inoculated plates containing the culture medium were allowed to solidify. This was incubated at 37°C for 24 hours. Colonies were then counted using colony counter [37, 38].

$$\text{Coliform count} \left(\frac{\text{CFU}}{\text{g}}\right) = \frac{\text{Number of colonies}}{\text{Volume transferred to plate} \times \text{Dilution blank factor}} \times 100$$

Eqn 2.

Determination of coliform count

Coliform count was determined using pour plate method. One millilitre (1 ml) of the diluents (i.e., 1 ml suspension of the mixture) was taken out with sterile 1 ml pipette. The diluent was introduced into the sterile labelled microbial plate. Then about 18 ml out of the prepared macConkey agar was poured into the sterile labelled microbial plate and was swirled gently to mix. The inoculated plates containing the culture medium were allowed to solidify. This was incubated at 37°C for 24 hours. Colonies were then counted using colony counter [37, 38].

$$\text{LAB count} \left(\frac{\text{CFU}}{\text{g}}\right) = \frac{\text{Number of colonies}}{\text{Volume transferred to plate} \times \text{Dilution blank factor}} \times 100$$

Eqn.3.

Where:

LAB = Lactic Acid Bacteria

Determination of yeast-moulds count

Yeasts-moulds count was also determined using pour plate method. This was done by weighing 1 ml of the diluents (i.e., 1 ml suspension of the mixture) using sterile 1 ml pipette. These 1 ml suspensions were introduced into the sterile labelled microbial plates. Then about 18 ml out of the prepared potato dextrose agar (PDA) were also poured into each sterile labelled microbial plate and were swirled gently to mix. The inoculated plates containing the culture media were allowed to solidify. It was then incubated at room temperature for 5 days and observed daily. Colonies were counted using colony counter [39].

$$\text{Yeas t/m ould count (CF U/g} = \frac{\text{Number of colonies}}{\text{Volume transferred to plate} \times \text{Dilution blank factor}} \times 100$$

Eqn 4.

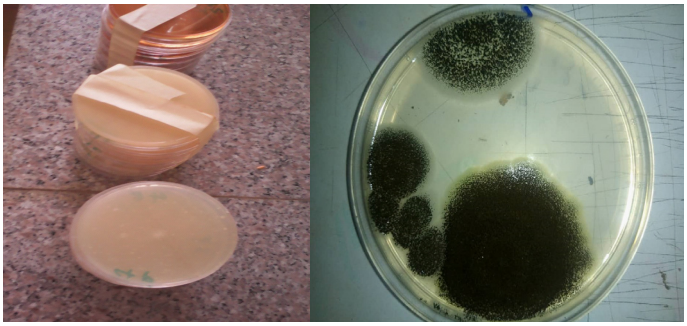

Figure 2: Microbial colonies.

Mineral analysis

The equipment and instruments used for this study were all calibrated and thoroughly checked before the experiments. All glass wares (Borosilicate volumetric flasks and measuring cylinders: 25, 50, 100, 1000 ml and micro/macro pipettes 1-10 ml, 100-1000 ml) and digestion flasks were washed with detergents and tap water. Then also with 10% concentrated nitric acid (HNO_3) in order to remove any heavy metal contaminants on their surfaces and was thoroughly rinsed with distilled water. Also, the digestion tubes were soaked in a solution containing 1% (w/v) potassium dichromate and 98% (v/v) solution of sulphuric acid (H_2SO_4). These were finally dried in oven and kept in dust free environment for use. Prior to use, glass wares were rinsed again with distilled water [28].

Then 200 mg of the food materials was weighed (Analytical balance: 250 g capacity, resolution 0.0001 g, OHAUS, PA214 Pioneer, USA) in to microwave tube. Then 6 ml of 65% nitric acid (HNO_3) and 2 ml of hydrogen peroxide ($H_2O_{2)}$ were added and allowed to stand for 5 minutes. The plastic container (microwave tube) was then covered and placed in to microwave digester (Master 40 serial No: 40G106M) and was digested at 75°C for 10 minutes. It was then ramped at 10°C per minute and to 95°C and it held for 30 minutes. After digestion, it was cooled to room temperature in the microwave [40].

Preparation of 1000 mg/liter stock AAS standard solution

The determination of a given metal concentration in the experimental solution was based on its respective calibration curve. In plotting the calibration curves for lead, cadmium, zinc and other metals, a stock solution of each metal ion of (1000 ppm) supplied by manufacturers company was used. From which a standard working solution of 100 ppm was prepared [28].

Standard working solution

A standard working solution of 100 ppm (i.e., 10 ml into 100 ml calibrated flask and diluted with the distilled water up to the 100 ml mark) was prepared as working solution from the 1000 ppm already supplied by the manufacturer. A simple dilution formula ($C_1V_1 = C_2V_2$) was used to calculate the volume of the stock solution to be diluted to the new desired concentration [41]. Then 1 ml of concentrated HNO_3 was added to each working standard and finally diluted to the desired volume with distilled water [28].

Preparation of calibration curve

Calibration curves were prepared to determine the concentration of the metals in the sample solution. The instrument was calibrated using series of working standards. The working standard solutions of each metal were prepared from standard solutions of their respective metals and their absorbance were taken using the AAS. Then calibration curve for each metal ion to be analyzed was prepared by plotting the absorbance as a function of metal ion standard concentration [28].

Determination of mineral contents by Atomic Absorption Spectrophotometer (AAS)

Concentration of the metal ions present in the sample solution was determined by reading their absorbance using AAS (Buck scientific, equipped with hollow cathode and air-acetylene lamps, Model 210VGP, USA) and was compared with their respective standard calibration curve. Three replicate determinations were carried out on each sample. The metals were determined by absorption/concentration mode and the instrument readout was recorded for each solution manually. Blanks were used simultaneously in each batch of the analysis to authenticate the analytical quality. The same analytical procedure was employed for the determination of elements in digested blank solutions and for the spiked samples [28, 40].

Determination of amino acid profiles

Principle: The amino acid profile of malted and/or fermented rice plus soybean based complementary food products were determined using method described by Benitez [42]. These foods were dried to a constant weight, defatted, hydrolysed, evaporated in a rotary evaporator before it was loaded into the Applied Biosystems Phenyl Thiohydantoin (PTH) Amino Acid Analyser (Applied Biosystems PTH Amino Acid Analyser, Model 120A PTH Applied Biosystems Inc. 850 Lincoln Centre Dr. Foster City CA. 94404. USA. Serial no.704520 Patents: U.S.A 4347131)

Defatting

Soxhlet apparatus was used to de-fat the food materials. Chloroform and methanol (solvents) were mixed in a ratio of 2:1. Then 4 g of each of these rice foods was placed in extraction thimble inside Soxhlet extraction apparatus and were defatted for 15 hours [43].

Nitrogen determination

Nitrogen content of the defatted food was determined as follows: The defatted food was weighed (200 mg portion) and wrapped in Whatman filter paper (No.1) and placed in Kjeldahl digestion flask. Then 10 ml of concentrated sulphuric acid was added; also 0.5 g catalyst mixture 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. Then four pieces of anti-bumping granules was added. The flasks were fixed into the Kjeldhal digestion apparatus and food material was digested for 3 hours (until clear light green solutions were obtained). The digested food material was cooled and diluted with distilled water up to the 100 ml mark of the standard volumetric flask. An aliquot of 10 ml of the diluted solution was then taken for distillation. Also 10 ml of 45% sodium hydroxide was taken and it was introduced into the Marham 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 titrated with standard 0.01 N hydrochloric acid to a grey-coloured solution [43].

$$\text{Percentage nitrogen} = \frac{(A-B) \times 0.01 \times 14 \times V}{W \times C} \times 100 \quad \text{Eqn. 5}$$

Where:

A: Titre value of the digested food material; B: Titre value of the blank; C: Aliquot of the food materials used (10 ml); V: Volume after dilution (100 ml); W: Weight of the food taken (mg); 14: Nitrogen constant in mg.

Acid hydrolysis of the food materials

Defatted malted and/or fermented rice plus soybean based complementary foods were then hydrolyzed. A known weight of 0.7800 g of the defatted food was weighed into glass ampoule and 7 ml of hydrogen chloride (6 N HCl) was added. Oxygen was expelled by passing nitrogen into each of the ampoules (this was done to avoid possible oxidation of some amino acids during hydrolysis (e.g., methionine and cysteine). The glass ampoules were sealed with Bunsen burner flame and were place in an oven at 105°C ± 5°C for 22 hours. The ampoules were allowed to cool before opening at the tip and its contents were filtered. Tryptophan usually gets destroyed by 6 N HCl during hydrolysis [42, 43].

The filtrates were evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residues were dissolved with 5 ml acetate buffer solution (pH 2.0) and stored in plastic specimen bottles in the freezer for subsequent use [42].

Then 60 microliter of the hydrolysate was loaded into the Applied Biosystems PTH (Phenyl thiohydantoin) Amino Acid Analyzer. It was dispensed into the cartridge of the analyzer. The analyzer then separated and analyzed free acidic, neutral and basic amino acids of the hydrolysates [42].

Method of calculating amino acid values

An integrator attached to the analyzer calculated the peak area proportional to the concentration of each of the amino acids. The norcleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$\text{Norcleucine Equivalent} = \frac{\text{Area of norcleucine peak}}{\text{Area of each amino acid}} \quad \text{Eqn. 6}$$

A constant S was calculated for each amino acid in the standard mixture,

Where:

$$S_{std} = NE_{std} \times \text{Molecular weight} \times \mu MAA_{std}) \text{ Eqn. 7}$$

Finally, the amount of each amino acid present in the food was calculated in g/16 g N or in g/100 g protein using the following formula:

$$\text{Concentration (g per 100 g protein)} = \frac{NH \times W@NH}{2 \times S_{std} \times C} \text{ Eqn. 8}$$

Where:

$$C = \frac{\text{Dilution} \times 16}{\text{Sample Wt(g)} \times N\% \times 10 \times \text{Vol.loaded}}$$

$$\div NH \times W(\text{Nleu}) \text{ Eqn. 9}$$

Where:

NH: Net height; W: Width @ half height; Nleu: Norcleucine.

Determination of tryptophan

Tryptophan is a difficult amino acid to be determined in proteins and peptides because chemically it decomposes during acid hydrolysis. It should be noted that tryptophan gets destroyed by adding 6 N HCL during hydrolysis. Antioxidants such as thioglycolic acid or dodecanethiol have been used to 6 N hydrochloric acid (HCl) to preserve tryptophan. Alkaline hydrolysis has also been studied and was shown to produce higher tryptophan recovery than acid hydrolysis. The addition of phenol has also been reported. Alkaline hydrolysis was improved by using sodium hydroxide (NaOH) instead of barium hydroxide to prevent problems with both precipitation and adsorption of tryptophan [44].

Food material was dried to a constant weight and defatted. The food material containing tryptophan was hydrolysed with 4.2 M sodium hydroxide. This was evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyser [44].

Defatting and nitrogen determination

A known weight of the dried sample was weighed into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using Soxhlet extraction apparatus as described by AOAC [43]. The extraction lasted for 15 hours. Nitrogen content determination of the food materials were also carried out as described by AOAC [43].

Alkaline hydrolysis of the food materials

A known weight of the defatted food was weighed into glass ampoule. And then 10 ml of 4.2 M of NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and placed in an oven at 105°C ± 5°C for 4 hours. The ampoule was allowed to cool before it was opened at the tip and the content was filtered to remove humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was then dissolved with 5 ml of borate buffer (pH 9.0) and store in plastic specimen bottles in a freezer for use [44].

Loading of the hydrolysate into the applied bio systems PTH amino acid analyser

Sixty (60) microliter was loaded and dispended into the cartridge of the Analyser.

Method of calculating amino acid values

An integrator attached to the Analyser calculated the peak area proportional to the concentration of each of the amino acid.

Amino acid scores

Amino acid score of the food materials were determined as described by Onabanjo *et al*. [45] and Hayes *et al*. [46].

$$\text{Amino acid scores} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in 1 g of reference protein}} \times 100 \text{ Eqn. 10}$$

Statistical analysis

Data generated were subjected to analysis of variance (ANONA) using IBM SPSS statistics version 22 and mean values were separated by Duncan's Multiple Range Test (DMRT) at 5% significant level [47].

Results & discussions

Mineral contents of FARO 44 and soybean

Table 1 showed effects of malting and fermentation on essential mineral content of FARO 44 cultivar. These results showed significant difference ($p < 0.05$) and is ranged from: 0.56 to 7.52 mg for iron, 0.34 to 4.51 mg for zinc, 5.60 to 62.87 mg for calcium, 6.60 to 53.11 mg for magnesium, 27.30 to 281.49 mg for potassium, 53.76 to 523.67 mg for phosphorus, 0.09 to 0.33 mg for copper and 0.30 to 3.09 for manganese. Malting and fermentation generally affected mineral elements of the raw materials throughout as observed. Significant effect was observed during malting. For all the mineral elements, Non - Malted - Non Fermented FARO 44 rice recorded the highest mineral values followed by Non Malted-Fermented FARO 44 Rice, Malted - Fermented FARO 44 Rice and Malted - Non Fermented FARO 44 rice, respectively.

Processing conditions has been reported to have either negative or positive effect on food components. Location has also been reported to influence the levels of mineral elements in plants foods. Hence the available minerals for humans' and animals' consumption [48]. Humans and animals may be at advantage then where the plants vicinity grown for consumption is on fertile soil rich in mineral elements. Study by Chaudhari *et al*. [17] showed higher levels of iron (3 mg) and zinc (2 mg) in brown rice compared to this study. But is deficient to be used as complementary foods. Upadhyay and Karn [49] reported nutritional composition and health benefits of rice with comparable levels of mineral elements to this study. However, calcium, magnesium and zinc were observed to be higher.

Table 1: Effects of malting and fermentation on essential mineral content of FARO 44 cultivar.

Dietary minerals (mg/100 g)	FARO 44 and Soybean Products					
	$NMNFR_{44}$	$MNFR_{44}$	$NMFR_{44}$	MFR_{44}	S	LSD
Iron	$1.05^{b} \pm 0.11$	$0.56^{c} \pm 0.07$	$0.87^{bc} \pm 0.04$	$0.85^{bc} \pm 0.05$	$7.52^{a} \pm 0.68$	0.8
Zinc	$0.71^{b} \pm 0.10$	$0.34^{c} \pm 0.04$	$0.52^{bc} \pm 0.03$	$0.51^{bc} \pm 0.03$	$4.51^{a} \pm 0.41$	0.5
Calcium	$11.53^{b} \pm 1.22$	$5.60^{d} \pm 0.72$	$8.73^{c} \pm 0.42$	$8.47^{c} \pm 0.46$	$62.87^{a} \pm 3.95$	5.1
Magnesium	$12.61^{b} \pm 0.46$	$6.60^{c} \pm 0.22$	$9.61^{c} \pm 0.46$	$9.31^{c} \pm 0.51$	$53.11^{a} \pm 4.49$	5.5
Potassium	$52.17^{b} \pm 1.89$	$27.30^{d} \pm 0.91$	$39.74^{c} \pm 1.89$	$38.52^{cd} \pm 2.10$	$281.49^{a} \pm 17.18$	21.1
Phosphorus	$102.74^{b} \pm 3.73$	$53.76^{d} \pm 1.79$	$106.87^{b} \pm 5.28$	$75.86^{c} \pm 4.14$	$523.67^{a} \pm 3.79$	10.5
Copper	$0.17^{b} \pm 0.01$	$0.09^{d} \pm 0.00$	$0.13^{c} \pm 0.01$	$0.13^{c} \pm 0.01$	$0.33^{a} \pm 0.01$	0.0
Manganese	$0.57^{b} \pm 0.02$	$0.30^{d} \pm 0.01$	$0.44^{c} \pm 0.02$	$0.42^{cd} \pm 0.02$	$3.09^{a} \pm 0.19$	0.7

Each result is mean ± SD of triplicate determinations. Values with common superscripts along each raw are not significantly (P > 0.05) different. LSD: least significant difference.

NMNFR44: Non-Malted - Non Fermented FARO 44 Rice; MNFR44: Malted - Non Fermented FARO 44; NMFR44: Non Malted – Fermented FARO 44 Rice; MFR44: Malted – Fermented FARO 44 Rice; S: Soybean.

Microbiological count of malted and/or fermented rice plus soybean based complementary foods

Table 2 indicated results of the microbiological count of malted and/or fermented rice plus soybean based complementary foods. These results vary slightly with total plate count ranged from 2.3×10^3 to 3.3×10^3 CFU/g, yeast/mould count ranged from 3×10^2 to 4×10^2 CFU/g, while coliform and total lactic acid bacteria count showed no indication of colonies as observed (found nil). It was observed that malting and fermentation has increased total plate and yeast/mould count. Malted - Fermented FARO 44 Rice + Soybean recorded the highest total plate count (bacteria enumeration), followed by Non Malted - Fermented FARO 44 Rice + Soybean, Malted - Non Fermented FARO 44 + Soybean and Non-Malted - Non Fermented FARO 44 Rice + Soybean. All the malted and/or fermented rice plus soybean based complementary foods recorded same number of yeast/mould counts, except Non Malted and Non Fermented FARO 44 rice plus soybean. Coliform and lactic acid bacteria count were observed to be absent (no growth) throughout in all the malted and/or fermented rice plus soybean based complementary foods.

Infants and young children are often being susceptible to food borne diseases. Numerous studies in developing countries have shown that weaning foods prepared under unhygienic conditions are usually get contaminated [50]. Microbial study of complementary foods is important to ascertain safety for its consumers. In this study, the absence of coliforms throughout the study is an indication that the foods itself pose no health implications (not potentially hazardous to its consumers). Levels of $\geq 10^4$ CFU are considered as potentially hazardous and may result in food borne illness if consumed [51]. Also the absence of lactic acid bacteria is an indication that some important microbial peptides (often associated with them) which are of health benefits may not be present in this formulations [52].

Similar study carried out by Gernah *et al*. [23] recorded total aerobic count from 1.2×10^4 to 2.2×10^4 CFU/g for unmalted and malted food respectively, which corresponds to this study. But the fermented had much higher count as compared to this study. Yeast/mould count also were observed to be higher compared to this study. In another study by Asma *et al*. [53] on sorghum, legumes and oil seeds complementary foods recorded also higher value of total bacteria count (7.2×10^2 to 7.6×10^4 CFU/g) as compared to this study.

Table 2: Microbiological count of malted and/or fermented rice plus soybean based complementary foods.

Microbiological count (CFU/g)	FARO 44 Rice + Soybean Product			
	$NMNFR_{44}S$	$MNFR_{44}S$	$NMFR_{44}S$	$MFR_{44}S$
Total plate count	2.3×10^3	2.8×10^3	3.7×10^3	3.8×10^3
Yeast/mould count	3×10^2	4×10^2	4×10^2	4×10^2
Coliform count	Nil	Nil	Nil	Nil
Total lactic acid bacteria count	Nil	Nil	Nil	Nil

Each value is a mean of triplicate determinations.

$NMNFR_{44}S$: Non-Malted - Non Fermented FARO 44 Rice + Soybean; $MNFR_{44}S$: Malted - Non Fermented FARO 44 + Soybean; $NMFR_{44}S$: Non Malted – Fermented FARO 44 Rice + Soybean; $MFR_{44}S$: Malted – Fermented FARO 44 Rice + Soybean.

Dietary mineral composition of malted and/or fermented rice plus soybean based complementary foods

Table 3 indicated essential dietary mineral composition of malted and/or fermented rice plus soybean based complementary foods. The dietary mineral content of malted and/or fermented rice plus based complementary foods vary significantly ($p < 0.05$) and are as follows: iron ranged from 2.26 to 2.62 mg, zinc ranged from 1.35 to 1.63 mg, calcium ranged from 20.34 to 24.43 mg, magnesium ranged from 17.95 to 22.42 mg, potassium ranged from 86.06 to 107.72 mg, phosphorus 168.32 to 204.69 mg, copper ranged from 0.12 to 0.21 mg and manganese ranged from 0.98 to 1.19 mg. It was observed that upon addition of soybean, the mineral composition of the malted and/or fermented rice flours were improved significantly. Similarly, Non Malted -Non Fermented FARO 44 rice plus soybean had the highest levels of mineral elements, followed by Non Malted- Fermented FARO 44 rice plus soybean, Malted-Fermented FARO 44 rice plus soybean and Malted-Non Fermented FARO 44 rice plus soybean, respectively.

The importance of mineral nutrients in foods, their major functions, deficiency symptoms and factors affecting the concentration and availability have been reviewed extensively by Soetan *et al* [48]. Micronutrient deficiencies are major public

health problems especially in developing countries, with high number of infants and pregnant women are often predispose to high risk [48]. In fact, without mineral elements in our diet, life could have been impossible for humans and animals. For these reasons, regulatory agencies have to set limits of micronutrients in complementary foods to safeguards the lives of the consumers and enable them obtain or enjoy the value of the commodity being purchased. The recommended levels of dietary minerals recommended by the World Food Programme for any complementary food containing essential dietary mineral elements are: Zinc 4.2 to 8.4 mg, copper 0.22 to 0.44 mg, iron 11.6 to 23 mg, manganese 0.6 to 1.2 mg, calcium 260 to 800 mg, magnesium 54 to 108 mg, phosphorus 180 to 550 mg and potassium 700 to 773 mg [34]. However, among all the mineral elements of malted and/or fermented rice plus soybean based complementary foods, only cupper, manganese and phosphorus were found slightly corresponded to the standard recommendations. Development of weaning food by Asma *et al*. [53] from sorghum supplemented with legumes and oil seeds showed higher level of iron (5.3 to 9.1 mg) and calcium (150 to 220 mg) as compared to this study but are still bellow quality standard. Pobee *et al.* [54] produced complementary foods from rice and six other Ghanaian foods but it was observed to be still low in iron and calcium. However, this finding corresponds slightly with this study. High level of zinc was also observed. Formulation and evaluation of complementary foods from flour blends of sprouted paddy rice, sprouted African yam bean and pawpaw fruits investigated by Obasi *et al*. [55] reviles much high levels of iron and low levels of calcium and magnesium compared to recommended standard range. Similar study by Chinelo *et al*. [18] investigated very low levels of zinc, iron and calcium in complementary foods from blends of roasted rice and soybean flours. Nutritive value of three potential complementary foods based on cereals and legumes studied by Marian [56] found slightly lower levels of mineral elements as compared to this study except for magnesium and manganese as compared to the quality standards. Traditional enrichment of the flour of rice for unearth flours and soya for the confection of weaning flour is limited in iron and zinc [57]. Mugalavai *et al*. [5] produces rice composites flours using five different raw materials but the study reviles very low in mineral elements compared to mineral content of the malted and/or fermented rice plus based complementary foods. From this study, it ca be envisage that most complementary foods produced from plants sources would require appropriate mineral fortification.

Table 3: Essential dietary mineral composition of malted and/or fermented rice plus soybean based complementary foods.

Dietary minerals (mg/100 g)	FARO 44 Rice + Soybean Product				LSD
	$NMNFR_{44}S$	$MNFR_{44}S$	$NMFR_{44}S$	$MFR_{44}S$	
Iron	$2.62^{a} \pm 0.08$	$2.26^{b} \pm 0.23$	$2.42^{ab} \pm 0.17$	$2.31^{ab} \pm 0.16$	1.4
Zinc	$1.63^{a} \pm 0.13$	$1.35^{b} \pm 0.13$	$1.44^{ab} \pm 0.09$	$1.39^{b} \pm 0.10$	0.9
Calcium	$24.03^{a} \pm 1.40$	$20.34^{b} \pm 1.32$	$21.27^{b} \pm 0.91$	$20.42^{b} \pm 0.80$	3.0
Magnesium	$22.42^{a} \pm 0.94$	$17.95^{b} \pm 0.93$	$19.68^{b} \pm 1.36$	$18.94^{b} \pm 1.31$	3.0
Potassium	$107.72^{a} \pm 4.34$	$86.06^{b} \pm 9.43$	$95.73^{b} \pm 3.61$	$97.58^{b} \pm 11.30$	20.6
Phosphorus	$204.69^{a} \pm 2.54$	$168.32^{c} \pm 0.69$	$203.37^{a} \pm 3.59$	$174.29^{bc} \pm 3.84$	7.7
Copper	$0.21^{a} \pm 0.01$	$0.12^{c} \pm 0.04$	$0.18^{b} \pm 0.01$	$0..17^{b} \pm 0.01$	0.0
Manganese	$1.19^{a} \pm 0.05$	$0.98^{c} \pm 0.05$	$1.05^{bc} \pm 0.04$	$1.01^{bc} \pm 0.05$	0.4

Each result is mean ± SD of triplicate determinations. Values with common superscripts along each raw are not significantly (P > 0.05) different. LSD: least significant difference.

$NMNFR_{44}S$: Non-Malted - Non Fermented FARO 44 Rice + Soybean, $MNFR_{44}S$: Malted - Non Fermented FARO 44 + Soybean; $NMFR_{44}S$: Non Malted – Fermented FARO 44 Rice + Soybean; $MFR_{44}S$: Malted – Fermented FARO 44 Rice + Soybean.

Essential amino acid composition of malted and/or fermented rice plus soybean based complementary foods

Table 4 showed essential amino acid composition of malted and/or fermented rice plus soybean based complementary foods. Amino acid composition of malted and/or fermented rice plus soybean based complementary foods did no vary much and are ranged from: 7.25 to 7.49 mg for leucine, 6.53 to 6.64 mg for lysine, 3.55 to 3.76 mg for isoleucine, 7.98 to 8.09 mg for phenylalanine + tyrosine, 4.10 to 4.29 mg for valine, 0.99 to 1.10 mg for tryptophan, 2.71 to 2.90 mg for methionine + cysteine, 3.31 to 3.45 mg for threonine and 2.94 to 3.02 mg for histidine. Fermentation increased essential amino acids while malting did not have much effects. Threonine was increased much significantly by either malting or fermentation. Malting alone did not affect histidine, methionine + cysteine and lysine. Fermentation of the malted rice caused reduction of lysine as observed.

Amino acids are basic unit of protein which is needed for healthy growth, development and body maintenances. Amino acids play major role in regulating multiple processes related to gene expression, including modulation of the function of the proteins that mediate messenger RNA (mRNA) translation. If amino acids are deficient, then protein synthesis does not occur, protein deficiency diseases then set on and death may occur [58]. In fact, without essential amino acids in our diets, there could have been no human or animal lives.

In this study, it was observed that amino acids composition of rice greatly improved upon addition of soybean as compared with results of amino acids of milled rice from literatures reported by Houston [59]. Most of the essential amino acids of the formulated foods in this study met the standard requirement of FAO/WHO for complementary foods except isoleucine, methionine + cysteine, threonine and valine which were observed to be slightly lower. Asma *et al*. [53] studied development of weaning food from sorghum supplemented with legumes and oil seeds showed much lower level of essential amino acids compared to this study except for isoleucine. In another study by Onabanjo *et al*. [45] on complementary foods from cassava and soybean recorded low level of amino acids compared to this study. Again study by Ijarotimi and Keshinro [60] on formulation and nutritional quality of infant formula produced from germinated pop-

corn, Bambara groundnut and African locust bean flour showed lower amount of amino acids. Nutritive value of three potential complementary foods based on cereals and legumes studied by Marian [56] found slightly lower levels of amino acids compared to this study. About seven essential amino acids are lower than the recommended range.

Table 4: Essential amino acid composition of malted and/or fermented rice plus soybean based complementary foods.

Essential amino acid (mg/100 g)	FARO 44 Rice + Soybean Product					
	$NMNFR_{44}S$	$MNFR_{44}S$	$NMFR_{44}S$	$MFR_{44}S$	FAO/WHO 1973	LSD
Leucine	$7.30^{a} \pm 0.74$	$7.35^{a} \pm 0.49$	$7.25^{a} \pm 0.07$	$7.49^{a} \pm 0.02$	7.00	1.3
Lysine	$6.63^{a} \pm 0.23$	$6.63^{a} \pm 0.15$	$6.64^{a} \pm 0.01$	$6.53^{a} \pm 0.01$	5.50	0.4
Isoleucine	$3.76^{a} \pm 0.51$	$3.72^{ab} \pm 0.30$	$3.55^{ab} \pm 0.01$	$3.74^{a} \pm 0.01$	4.00	0.9
Phenylalanine + Tyrosine	$8.21^{a} \pm 0.43$	$7.98^{b} \pm 0.24$	$8.09^{a} \pm 0.03$	$8.28^{a} \pm 0.04$	6.00	1.8
Valine	$4.25^{a} \pm 0.35$	$4.27^{a} \pm 0.25$	$4.29^{a} \pm 0.02$	$4.10^{a} \pm 0.01$	5.00	0.6
Tryptophan	$1.10^{ab} \pm 0.23$	$1.08^{ab} \pm 0.19$	$0.99^{abc} \pm 0.02$	$1.00^{abc} \pm 0.00$		0.4
Methionine + Cysteine	$2.71^{a} \pm 0.15$	$2.71^{a} \pm 0.06$	$2.90^{a} \pm 0.35$	$2.74^{a} \pm 0.01$	3.50	0.5
Threonine	$3.31^{a} \pm 0.43$	$3.34^{a} \pm 0.21$	$3.41^{a} \pm 0.02$	$3.45^{a} \pm 0.01$	4.00	0.7
Histidine	$2.94^{a} \pm 0.18$	$2.94^{a} \pm 0.08$	$2.96^{a} \pm 0.07$	$3.02^{a} \pm 0.03$	2.60	0.2

Each result is mean ± SD of triplicate determinations. Values with common superscripts along each raw are not significantly (P > 0.05) different. LSD: least significant difference.

$NMNFR_{44}S$: Non-Malted - Non Fermented FARO 44 Rice + Soybean; $MNFR_{44}S$: Malted - Non Fermented FARO 44 + Soybean; $NMFR_{44}S$: Non Malted – Fermented FARO 44 Rice + Soybean; $MFR_{44}S$: Malted – Fermented FARO 44 Rice + Soybean.

Amino acid scores of malted and/or fermented rice plus soybean based complementary foods

Table 5 indicated amino acid scores of malted and/or fermented rice plus soybean based complementary foods. Amino acid score of malted and/or fermented rice plus soybean based complementary foods ranged from: 103.57 to 107.00% for leucine, 118.73 to 120.73% for lysine, 88.75 to 94.00% for isoleucine, 133.00 to 138.00% for phenylalanine and tyrosine, 82.00 to 85.80% for valine, 77.43 to 82.86% for methionine and cysteine, 82.75 to 86.25% for threonine and 113.08 to 116.15% for histidine. It was observed that isoleucine, methionine + cysteine, valine and threonine were slightly limiting in the food materials throughout. However, the percentage limiting was observed to be minimal.

A lower score for any of the essential amino acids designates the limiting quality of the amino acid and it gives an indication of the percentage protein quality relative to the reference amino acid as described by Asma *et al*. [53]. In a similar study conducted by Asma *et al*. [53] found leucine as the most limiting amino acid in most of the blends (at 58 to 75%). But mostly lysine, threonine, valine and tryptophan are also limiting amino acids in the developed complementary foods. Nutritive value of three potential complementary foods based on cereals and legumes studied by Marian [56] found mostly limiting amino acids except for tryptophan, phenylalanine and tyrosine. However, amino acids may be limiting in a certain diet but may fulfil its function in the body due to its nutritional bioavailability [61, 62, 63, 64].

Table 5: Amino acid scores of malted and/or fermented rice plus soybean based complementary foods.

Essential amino acid (%)	FARO 44 Rice + Soybean Product				
	$NMNFR_{44}S$	$MNFR_{44}S$	$NMFR_{44}S$	$MFR_{44}S$	LSD
Leucine	$104.29^{a} \pm 10.57$	$105.00^{a} \pm 7.00$	$103.57^{a} \pm 1.00$	$107.00^{a} \pm 0.29$	18.4
Lysine	$120.55^{a} \pm 4.18$	$120.55^{a} \pm 2.73$	$120.73^{a} \pm 0.18$	$118.73^{a} \pm 0.18$	8.7
Isoleucine	$94.00^{ab} \pm 12.75$	$93.00^{ab} \pm 7.50$	$88.75^{ab} \pm 0.25$	$93.50^{a} \pm 0.25$	21.4
Phenylalanine + Tyrosine	$136.83^{a} \pm 7.17$	$133.00^{b} \pm 4.00$	$134.83^{a} \pm 0.50$	$138.00^{a} \pm 0.67$	29.6
Valine	$85.00^{a} \pm 7.00$	$85.40^{a} \pm 5.00$	$85.80^{a} \pm 0.40$	$82.00^{a} \pm 0.20$	12.5
Methionine + Cysteine	$77.43^{a} \pm 4.29$	$77.43^{a} \pm 1.71$	$82.86^{a} \pm 10.00$	$78.29^{a} \pm 0.29$	12.8
Threonine	$82.75^{a} \pm 10.75$	$83.50^{a} \pm 5.25$	$85.25^{a} \pm 0.5$	$86.25^{a} \pm 0.25$	17.3
Histidine	$113.08^{a} \pm 6.92$	$113.08^{a} \pm 3.08$	$113.85^{a} \pm 2.69$	$116.15^{a} \pm 1.15$	12.0

Each result is mean ± SD of triplicate determinations. Values with common superscripts along each raw are not significantly (P > 0.05) different. LSD: least significant difference.

$NMNFR_{44}S$: Non-Malted - Non Fermented FARO 44 Rice + Soybean; $MNFR_{44}S$: Malted - Non Fermented FARO 44 + Soybean; $NMFR_{44}S$: Non Malted – Fermented FARO 44 Rice + Soybean; $MFR_{44}S$: Malted – Fermented FARO 44 Rice + Soybean.

Conclusion

Microbiological counts of the complementary foods post no potential health hazards. The mineral contents of the raw materials are comparable to many data information on food composition. Malting and fermentation also caused reduction of mineral elements in the processed rice flours. However, addition of soybean to processed rice significantly improve it. But mostly lower than the standard recommended range for complementary foods except cupper, manganese and phosphorus. All amino acids were within the recommended levels of FAO/WHO. However, isoleucine, valine, threonine and Sulphur containing amino acids (methionine and cysteine) were the limiting amino acids found in all blends. But the percentage limiting is minimal.

Recommendations

Mineral fortification of rice and soybean combination is required to enhance its nutritional value. Other plant products produce as complementary foods should be fortified appropriately to meet standard requirements. Advanced technology, appropriate for dehulling of malted rice is required to enhance further research studies and also to meet the demand for dehusk malted rice by the local market traders in Nigeria and possibly in other countries for other food applications.

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