Food Research 4 (1) : 152 - 160 (February 2020)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of natural fermentation on nutritional composition and anti-nutrients in soy-wara (a Nigerian fried soy-cheese)

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Article history:
Received: 10 May 2019
Received in revised form: 17 July 2019
Accepted: 19 July 2019
Available Online: 5 August 2019

Keywords:
Soy-wara,
Soybeans,
Fermentation,
Nutritional and anti-nutrients

DOI:
https://doi.org/10.26656/fr.2017.4(1).187

Abstract

This study was carried to evaluate the effect of natural fermentation on nutritional composition and anti-nutrients in soy-wara. A total of 100 samples consisting of five treatments of 20 samples per each treatment were studied. Yellow soybeans were soaked and fermented for 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively at 27±2°C with 0 hr as control. Fermented soybeans were used for soy-wara production and soy-wara samples were evaluated for nutritional (amino acid, vitamin and mineral profile) and anti-nutritional (phytate, tannin and trypsin inhibitor activity) qualities. Results of amino acids analysis showed that tryptophan, isoleucine, methionine, phenylalanine, leucine and lysine contents in the fermented soy-wara ranged from 3.49 to 6.75, 21.94 to 24.41, 20.60 to 23.98, 22.11 to 27.15, 33.16 to 36.51 and 24.16 to 26.27 mg/100 g respectively. The values of thiamine, riboflavin and niacin contents in the fermented soy-wara ranged from 1.60 to 1.87, 1.44 to 1.71 and 2.21 to 2.78 mg/100 g respectively. The mineral elements calcium, iron, potassium, sodium, phosphorus and magnesium contents in the fermented soy-wara ranged from 17.65 to 19.08, 6.94 to 8.41, 29.76 to 30.69, 8.31 to 9.42, 27.52 to 28.55 and 21.02 to 24.83 mg/100 g respectively. Soaking and fermentation reduced the tannin content from 115.64 to 43.26 mg/100 g; phytate content from 153.81 to 47.16 mg/100 g, trypsin inhibitor from 96.56 to 1.10 mg/100 g and protease inhibitor from 98.11 to 1.2 mg/100 g respectively. In conclusion, natural fermentation of the legume reduced anti-nutritional factors and improved the nutrient composition of the product.

1. Introduction

Soybeans (*Glycine max* MERILL), are good sources of plant proteins, complementing grain proteins, in many Asian and African countries (Yang *et al*., 2011). Together, protein and oil content account for 56% of dry soybeans weight (36% protein and 20% fat). The remainder consists of 30% carbohydrates, 9% water and 5% ash. Soybeans comprise approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ (Corke *et al.,* 2004, Tripathi and Misra, 2005).

Most soy protein is a relatively heat-stable storage protein. This heat stability enables soy food products requiring high-temperature cooking, such as tofu, soy milk and textured vegetable protein (soy flour) to be made (Wang *et al*., 2011). The Protein Digestibility Corrected Amino Acid Score (PDCAAS) of soybeans have been found to be nutritional equivalent of meat, eggs, and casein and good for human growth and health. Soybean protein isolate has a biological value of 74, whole soybeans 96, soybean milk 91, and eggs 97 (Corke *et al.,* 2004).

Soy protein is essentially identical to the protein of other legume seeds and pulses (Derbyshire *et al*., 1976; Hefnawy and Ramadan, 2011). Moreover, soybeans can produce at least twice as much protein per acre than any other major vegetable or grain crop besides hemp, five to 10 times more protein per acre than land set aside for

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eISSN: 2550-2166

grazing animals to make milk, and up to 15 times more protein per acre than land set aside for meat production (FAO/WHO,1989; Corke *et al.,* 2004).

The principal soluble carbohydrates of mature soybeans are the disaccharide sucrose (2.5–8.2%), the trisaccharide raffinose (0.1–1.0%) composed of one sucrose molecule connected to one molecule of galactose, and the tetrasaccharide stachyose (1.4 to 4.1%) composed of one sucrose connected to two molecules of galactose (Danielsson, 1949; Derbyshire *et al*., 1976; Wang *et al*., 2011). While the oligosaccharides raffinose and stachyose protect the viability of the soybean seed from desiccation (see above section on physical characteristics) they are not digestible sugars, so contribute to flatulence and abdominal discomfort in humans and other monogastric animals, comparable to the disaccharide trehalose. Undigested oligosaccharides are broken down in the intestine by native microbes, producing gases such as carbon dioxide, hydrogen, and methane.

Soluble carbohydrates are found in soy whey and are broken down during fermentation and in sprouted soybeans. On the other hand, there may be some beneficial effects to ingesting oligosaccharides such as raffinose and stachyose, namely, encouraging indigenous bifidobacteria in the colon against putrefactive bacteria (Corke *et al.,* 2004).

Apart from the fact that soybeans are sources of essential nutrients, soybean products, especially fermented soybean products, have functional components which include peptides, isoflavonoids and other components (Yang *et al*., 2011). Previous research studies have suggested that soybeans and soyfoods could lower the risks of several cancers including breast, prostate, and colon cancers and cardiovascular diseases (Peterson and Barnes, 1993; Anderson *et al*., 1998; Messina, 1999; Butler *et al*., 2007) and improves bone health (Yang *et al*., 2011).

USDA – (2016) forecasted world production of soybeans to be 324 million tonnes in 2016, a 5% increase from the 2014 world total (FAOSTAT, 2015; USDA, 2016). The world largest producers of soybeans are the United States, Brazil and Argentina which produced over 80% of world soybean production (USDA. 2012; FAOSTAT, 2015; USDA, 2016).

Soy-wara (Tofu) is one of the most important food products made from soybean protein (Kohyma *et al*., 1995). It is becoming an important traditional food in Nigeria because of its good nutrition and digestibility (Tsai *et al*., 1981; Adeyeye *et al*., 2017). The non-availability of cow milk coupled with high availability and the cheapness of soybeans in Nigeria as well as other benefits of soybeans to human health have recently increased interest in eating soya-wara, an analogue of warankasi from cow milk well known in Nigeria (Adeyeye, 2017).

Soy-wara (Tofu) is a salt- or acid-coagulated water-based gel, with soy lipids and proteins trapped in its gel networks (Kohyma *et al*., 1995; Adeyeye, 2017). This could be used as an inexpensive milk and meat substitutes. Soy-wara is cholesterol-free, a rich source of proteins, minerals and omega-6 polyunsaturated fatty acids (PUFA) (Adeyeye, 2017). Therefore, soy-wara can form an alternative protein source, which is higher than soybean (on dry body weight) (Akinola *et al*., 2014; Adeyeye, 2017).

Soy-wara is prepared by soaking and grinding of soybeans in water, filtering, boiling and coagulation of soymilk, molding and pressing. The quality and texture of soy-wara is affected by cultivar of soybean (Shen *et al*., 1991; Sun and Breene 1991) processing methods (Beddows and Wong, 1987) and type of coagulant used (Tsai *et al*., 1981; deMan *et al*., 1986; Lim *et al*., 1990). The taste of soy-wara is significantly affected by its final texture (Kohyama and Nishinari 1993; Jackson *et al*., 2002, Adesokan, *et a l.,* 2009).

Fermentation is one of the major processing of cereals and pulses. Fermented soybean paste is indigenous to the cuisines of East and Southeast Asia (Yang *et al*., 2011). Steinkraus (2004) pointed out that the traditional fermentation of foods by microorganisms serves several functions such as enrichment of food substrate biologically with proteins, essential amino acids, essential fatty acids and vitamins; this improves the digestibility and acceptability of foods, detoxification of toxic substances in foods, preservation of substantial amounts of food through production of anti-bacterial compounds such as lactic acid and acetic acid, a decrease in time and fuel requirement during cooking and enrichment of the diet through the development of flavours, aroma, taste, palatability and texture of such foods.

The health benefits of soy-wara (tofu) include its ability to help lower cholesterol levels, prevent anemia and manage weight, among others (Yang *et al*., 2011). It is a by-product of soybeans that essentially helps in maintaining cardiovascular health. It also boasts a wealth of different health benefits, including a lower risk of cancer, anemia, osteoporosis, and kidney diseases (Yang *et al*., 2011). Soya protein from which soy-wara is derived is believed to help lower levels of bad cholesterol (LDL). Soy-wara contains phytoestrogens

called isoflavones – a group of chemicals found in plant foods. They have a similar structure to the female hormone oestrogen and therefore mimic the action of oestrogen produced by the body. They naturally bind to oestrogen receptor sites in human cells including breast cells – potentially reducing the risk of breast cancer (Yang *et al*., 2011).

Soybeans have been used in the production of soy-wara in many parts of Nigeria and African continent, or tofu in many Asian countries. However, much works have not been done on "fermented soy-wara" or "fermented tofu", its effects on the product and the nutritional implications have not been well studied. The main objective of this study, therefore, is to evaluate the effect of natural fermentation on nutritional composition and anti-nutrients in soy-wara.

2. Materials and methods

2.1 Sample collection

Healthy seeds of soybean (*Glycine max*) used for this study was purchased from Bodija market in Ibadan, Oyo State.

2.2 Preparation of soymilk

Soy-milk was prepared by the method reported by Obadina *et al*. (2013). Soybean seeds were sorted manually to remove stones, damaged and immature seeds. The soybean seeds were soaked in tap water at room temperature (27±2°C) in a beaker for 18, 48, 72 and 96 hrs respectively. The water was drained from the soybean and beans thereafter were blanched at 98°C in boiling distilled water for 2 mins and dehulled manually to remove their testa. They were placed in a blender and boiled water at 87-90°C was added before blending for 3 min. The boiled water inactivates the enzyme, lipoxygenase during blending (Wilkens *et al.*, 1967 as reported by Obadina *et al*., 2013). The slurry obtained was filtered through two layers 50 µm mesh cheesecloth. The resulting soymilk was boiled at 85 - 90°C for 5 mins (Adeyeye *et al*., 2018).

2.3 Preparation of soy-wara

Soy-wara was prepared following the method of Hou *et al*. (1977). Soymilk (200 mL) was heated to 95°C for 5 mins and then cooled to 80°C with constant stirring at room temperature. Seasonings, pepper, onion and salt were added. Then, lemon juice was added to soymilk as a coagulant and stirred for 5, 10, 15 and 20 mins at different batches. Soy-milk was allowed to coagulate for 15 mins, without disturbing. The coagulated soy-milk was transferred into cheesecloth lined on porous plastic mould. The curd was pressed with 1,000 g initially for 15 mins and then reduced to 500 g weight for the next 15 mins. At the end of pressing, the cloth was removed and the soy-wara was cut into shapes and deep-fried in vegetable oil (Adeyeye *et al*., 2018).

2.4 Determination of nutritional composition of soy-wara

2.4.1 Determination of amino acid

The amino acid compositions of the samples were determined by the method of (AOAC, 2000). The amino acid profile of samples taken from the soy-wara was determined in four phases: the amino acid extraction phase, the chromatographic phase, the quantitative determination of amino-acid profile phase and the colorimetric analysis of amino-acid profile phase.

2.4.2 Colorimetric analysis of amino-acid

The extracts obtained above from the samples were used for amino-acid profiles analysis using the method of Rosen (1957) as reported by Opere *et al*. (2012). To 1 mL of the diluted extracts of each amino-acid was added 0.5 mL cyanide-acetate buffer (pH 5.4) and 0.5 mL 3% (w/v) Ninhydrin in methyl cellosolve. The mixture was heated in a boiling water-bath at 100^{o}C for 15 mins. Immediately after the mixture was removed from the water-bath, 5.0 mL isopropyl alcohol-water mixture (ratio 1:1) was added as diluents and mixed by shaking vigorously, then cooled to room temperature (25^{o}C). The amino-acid profile was estimated by determining the optical density at 570 nm wavelength using UV/visible spectrophotometer. The blank was similarly treated as sample above and used as the control to set the absorbance to zero (distilled water). The amount of amino-acid content was each calculated from the standard curve of known concentration of leucine (10 mg/mL).

2.4.3 Determination of vitamin B_1 (thiamin)

Vitamin B_1 was analysed in samples using the AOAC (2005) method. Accurately weighed 1.5 g of test sample was introduced into a 200 mL volumetric flask; 100 mL of 0.1N HCL solution was added and the mixture heated in a water bath at 100^{o}C for 30 mins. After cooling, the content of the flask was made up to mark with 0.1M HCL solution and mixed thoroughly. The solution was filtered using Whatman No. 1 filter paper. The first 20 mL of the filtrate was discarded. The remaining filtrate (100 mL) was transferred into centrifuge tube containing 0.5 g frankonite powder (a flocculant which precipitates the particles faster during centrifugation) stirred for 10 mins using RAM 2718 stirrer, then centrifuged at 5000 rpm for 5 mins to separate layers. The supernatant liquid was discarded while 5 mL of absolute alcohol and 5 mL of the

potassium ferric-cyanide solution in sodium hydroxide solution were added after it was previously frozen at 0°C. A pinkish colouration of mixture was observed after 10 mins of mixing, and then 10 mL of toluene solution was added, stirred for 10 mins and centrifuged for 10 mins at 5000 rpm. A very clear pink colour was transferred to the toluene layer. Thiamine standard (0.5 mg) was prepared and 10 mL of the thiamine standard solution was treated the same as sample above. The standard and sample solution was read at 530 nm wavelength using the SP 30UV spectrophotometer (Pye Unican). The amount of thiamine present in each sample was calculated as thus:

$$\text{Thiamine (mg/100g)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of Standard}} \times \frac{\text{Weight of Standard (mg)}}{\text{Weight of sample (g)}} \times 100$$

2.4.4 Determination of vitamin B_2 (riboflavin)

Vitamin B_2 was analyzed in samples using the AOAC (2005) method. Accurately weighed 1.5 g of sample was introduced into 200 mL volumetric flask; 100 mL of acetic acid: water mixture (50:50) was added and heated in a boiling water bath at 100°C for 30 mins. The mixture in the flask was cooled to 20°C, then made up to the mark with acetic acid-water solution. The mixture was stirred for 10 mins using the stirrer and then filtered in the dark. The first 20 mL of the filtrate was discarded, 0.5 mg of riboflavin standard solution was prepared, and 10 mL of the standard solution was transferred into 200 mL volumetric flask and treated similarly as sample above. The fluorescence of the standard and sample solutions was read using spectrophotometer at 460 nm wavelength. The amount of riboflavin in each sample was calculated as follows

$$\text{Riboflavin (mg/100g)} = \frac{\text{sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Weight of Std (mg)}}{\text{Weight of sample}} \times 100$$

2.4.5 Determination of vitamin B_3 (niacin)

Vitamin B_3 was analyzed in samples using the AOAC (2000) method. Sample (1.5 g) was accurately weighed into 200 mL volumetric flask. Hydrochloric acid solution (5N; 5 mL) was added, and 5.0 mL of dichloromethane and 90 mL of deionized water were added to the mixture, stirred and heated in a boiling water bath at 100°C for 30 mins. It was then cooled and the flask content made up to the mark with distilled water, filtered using Whatman No. 1 filter paper discarding the first 20 mL of the filtrate. The niacin standard solution of 0.5 mg was prepared, and 10ml of the stock solution was taken and treated the same as sample above. The absorbance of the standard and sample solutions were taken at 410 nm wavelength using spectrophotometer and calculation followed thus:

$$\text{Niacin (mg/100 g)} = \frac{\text{Sample reading}}{\text{standard reading}} \times \frac{\text{standard weight (mg)}}{\text{sample weight}} \times 100$$

2.5 Determination of mineral elements of soy-wara

The mineral contents of the soy-wara samples were determined by the procedure of AOAC (2000). Magnesium, calcium, iron and zinc were determined using the Atomic Absorption Spectrometer (Thermo Scientific S Series Model GE 712354) after digestion with a perchloric-nitric acid mixture (AOAC, 2000). Prior to digestion, 0.50 g of soymilk samples were weighed into a 125 mL Erlenmeyer flask with the addition of perchloric acid (4 mL), concentrated HNO_3 (25.00 mL) and concentrated sulphuric acid (2.00 mL) under a fume hood. The contents were mixed and heated gently in a digester (Buchi Digestion unit K-424) at low to medium heat on a hot plate under perchloric acid fume hood and heating was continued until dense white fume appeared. Heating was continued strongly for half a minute and then allowed to cool, followed by the addition of distilled water (50.00 mL). The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was read on the Atomic absorption spectrometer.

2.6 Atomic Absorption Spectrophotometer (AAS) analysis of digested samples

A standard curve was obtained for each of the minerals using the serially diluted concentration. After obtaining the standard curve at a particular wavelength, the digested samples of soy-wara in the containers were sucked into the AAS for analysis. For the different minerals analysed, their lamps and individual wavelengths were potassium (K lamp; 766nm), phosphorus (P lamp; 213nm), calcium (Ca lamp; 317nm), sodium (Na lamp; 589nm), magnesium (Mg lamp; 279nm), and iron (Fe lamp; 259nm).

2.7 Determination of anti-nutritional factors

2.7.1 Determination of tannin

Each sample of one gram was weighed into a beaker. Each was soaked with solvent mixture (80 mL of acetone and 20 mL of glacial acetic acid) for 5 hrs to extract tannin. The samples were filtered through a double layer filter paper to obtain the filtrates which were stored for further use. A standard solution of tannic acid was prepared ranging from 10 ppm to 30 ppm. The absorbances of the standard solution, as well as that of the filtrates, were read at 500 nm on a Spectronic 20, England spectrophotometer (AOAC, 1990).

2.7.2 Determination of phytates

Two grams of each sample was weighed into a 250 mL conical flask. A total of 100 mL of 2% hydrochloric acid was used to soak each sample in a conical flask for

3 hrs. This was filtered through a double layer of hardened filter paper Whatman No. 3. 50 mL of each filtrate was placed in 250 mL beaker and 107 mL of distilled water was added in each case. 10 mL of 0.3% ammonium thiocyanate solution was added into each solution as an indicator. This was titrated with standard iron (III) chloride solution, which contained 0.00l95 g iron per ml. The endpoint is slightly brownish yellow, which persisted for 5 m. The percentage phytates were calculated using the formula:

$$\% \text{ Phytates} = \frac{X \times 1.19 \times 100}{0.00195}$$

Where X = Titre value (AOAC, 1990).

2.7.3 Determination of trypsin inhibitors

Trypsin inhibitors were determined following the method of AOAC (1990). Two batches each of the samples (0.2 g each) were weighed into a screw-capped centrifuge tube. A total of 10 mL of 0.1M phosphate buffer was added and shaken vigorously. The contents were left at 25°C for 1 hr on a UDY 60 shaker, England. The suspension obtained was centrifuged at 5000 rpm for 5 mins and filtered through Whatman No. 42 filter paper. The volume of each was adjusted to 2 mL with phosphate buffer. The test tubes were placed in a water bath, maintained at 37°C. A total of 6 mL of 5% Trichloroacetic Acid (TCA) solution was added to one of the tubes to serve as a blank. A total of 2 mL of casein solution was added to all the tubes, which was previously kept at 37°C. These were incubated for 20 mins. The reaction was stopped after 20 mins by adding 6 mL of TCA solution to the experimental tubes and shaken. The reaction was left for 1 hr at room temperature after which it was filtered through Whatman No. 42 filter paper. The absorbance of filtrate from sample and trypsin standard solutions was read at 380 nm on a Spectronic 20, England spectrophotometer. The trypsin inhibitor in mg/g sample was calculated using the formula:

$$\text{Trypsin (mg/g)} = \frac{\text{A STD} - \text{A sample} \times \text{Dilution factor}}{19 \times \text{sample weight (g)}} \times 1000$$

2.7.4 Determination of protease inhibitors

Protease inhibitors were determined following the method of AOAC (1990). Egg albumin 2% solution and 0.1% solution of Bromelain, both in pH 7 phosphate buffer, were prepared. A total of 5 mL of the egg albumin substrate and 1 mL of the Bromelain enzyme was incubated at 55°C for 10 mins. Then, 5 mL 10% TCA was added to stop the reaction. The precipitate was filtered off with Whatman No. 1 filter paper and the absorbance of the filtrate was measured at 280 nm on the Atomic Absorption Spectrophotometer (AAS) labelled (Ai). The entire procedure was repeated but incubating with the enzyme and substrate mixture, i.e. 1 ml of the extract of the material for protease inhibitor determination labelled (As). The absorbance of the filtrate was measured at 280 nm. This was denoted Ai.

$$\% \text{ Protease Inhibitor} = \frac{\text{As} - \text{Ai}}{\text{As}} \times 100$$

Where As = Absorbance of sample and Ai = Absorbance of blank/initial (Cuatrecasas and Anfisen, 1991)

2.8 Statistical analysis

Mean values of triplicate determinations were reported with their standard deviations. Data obtained were subjected to analysis of variance (ANOVA) at $\alpha = 0.05$ level of significance with the use of the Statistical Package for Social Sciences (SPSS) version 16.0. Significant means ($P<0.05$) were separated using Duncan multiple range test.

3. Results

Table 1 shows the nutritional composition of soybean samples before and after fermentation for soy-wara. The essential amino acids, tryptophan, isoleucine, methionine, phenylalanine, leucine and lysine increased as fermentation period increased. The values of tryptophan, isoleucine, methionine, phenylalanine, leucine and lysine contents in the fermented soy-wara were 3.49 to 6.75, 21.94 to 24.41, 20.60 to 23.98, 22.11 to 27.15, 33.16 to 36.51 and 24.16 to 26.27% respectively. This could be due to the biodegradation of some chemical components of the soybean during soaking and fermentation processes. This agreed with the reports of other researchers (Obadina *et al*., 2013; Akanbi and Usoh, 2016).

The effect of fermentation on the vitamin profile of

Table 1. Amino acid profile of the different fermented soy-wara samples

	Time (h)	Tryptophan (mg/100 g)	Isoleucine (mg/100 g)	Methionine (mg/100 g)	Phenylalanine (mg/100 g)	Leucine (mg/100 g)	Lysine (mg/100 g)
Soybeans (Raw)	0	2.46±0.01^{a}	20.16±0.00^{b}	19.94±0.00^{b}	21.36±0.10^{c}	32.40±0.05^{b}	23.70±0.25^{a}
Soaking phase	24	3.49±0.00^{b}	21.94±0.00^{b}	20.60±0.01^{c}	22.11±0.02^{b}	33.16±0.01^{a}	24.16±0.01^{a}
	48	3.86±0.00^{c}	22.60±0.01^{b}	21.80±0.01^{a}	24.11±0.01^{c}	34.99±0.01^{b}	25.14±0.00^{a}
	72	4.90±0.00^{c}	24.20±0.01^{b}	23.60±0.01^{a}	25.21±0.02^{c}	34.09±0.01^{b}	25.37±0.01^{a}
	96	6.75±0.00^{c}	24.41±0.00^{b}	23.98±0.00^{a}	27.15±0.01^{c}	36.51±0.01^{b}	26.27±0.01^{a}

Values are mean ± standard deviation of triplicate determinations. Different alphabet superscripts in the same row are statistically different ($p \leq 0.05$).

fermented soy-wara is presented in Table 2. Soy-wara is rich in vitamins especially the vitamin B-complex such as thiamine, riboflavin and niacin. The values of thiamine, riboflavin and niacin contents in the fermented soy-wara were from 1.60 to 1.87, 1.44 to 1.71 and 2.21 to 2.78% respectively. Soaking and fermentation increased the vitamin contents of all the vitamins studied (Table 2).

Table 3 shows that soy-wara is rich in minerals such as calcium, iron, potassium, sodium, phosphorus and magnesium. The values of calcium, iron, potassium, sodium, phosphorus and magnesium contents in the fermented soy-wara were 17.65 to 19.08, 6.94 to 8.41, 29.76 to 30.69, 8.31 to 9.42, 27.52 to 28.55 and 21.02 to 24.83% respectively. The calcium, iron, potassium, sodium, phosphorus and magnesium contents in soy-wara increased with the increase in natural fermentation period from 24 hrs to 96 hrs (Table 3).

Table 2. Vitamin profile of the different fermented soy-wara samples

	Time (h)	Thiamine (mg/100 g)	Riboflavin (mg/100 g)	Niacin (mg/100 g)
Soybeans (Raw)	0	1.54±0.10^{c}	1.25±0.05^{b}	1.24±0.25^{a}
Soaking phase	24	1.60±0.00 ^{b}	1.44±0.00^{b}	2.21±0.01^{c}
	48	1.65±0.00^{c}	1.51±0.01^{b}	2.47±0.01^{a}
	72	1.72±0.00^{c}	1.63±0.01^{b}	2.62±0.01^{a}
	96	1.87±0.00^{c}	1.71±0.00^{b}	2.78±0.00^{a}

Values are mean ± standard deviation of triplicate determinations. Different alphabet superscripts in the same row are statistically different ($p \leq 0.05$).

Table 4 shows the reduction of anti-nutrients in soybean during pre-treatment and fermentation. It was observed that soaking and fermentation reduced the different anti-nutritional factors in soybeans. The raw soybeans contain 125.16 mg/100 g tannin; 174.37 mg/100 g phytate, 120.62 mg/100 g trypsin inhibitor and 120.11mg/100 g protease inhibitor respectively. Soaking and fermentation reduced the tannin content from 115.64 to 43.26 mg/100 g; phytate content from 153.81 to 47.16 mg/100 g, trypsin inhibitor from 96.56 to 1.10 mg/100 g and protease inhibitor from 98.11 to 1.2 mg/100 g respectively. This result agreed with reports of Adeyemo and Onilude (2013) who observed a reduction in the levels of anti-nutrients after fermentation.

4. Discussion

It was observed that the nutritional composition of the soy-wara improved significantly after soybean was fermented because the anti-nutritional factors which may affect nutrient availability had reduced. Soaking and fermentation increased the essential amino acid contents of all the essential amino acids studied (Table 1). The essential amino acids, tryptophan, isoleucine, methionine, phenylalanine, leucine and lysine increased as fermentation period increased. This could be due to the biodegradation of some chemical components of the soybean during soaking and fermentation processes. This agreed with the reports of several researchers (Omojasola 2000; Osundahunsi *et al*., 2007; Obadina *et al*., 2013; Akanbi and Usoh, 2016). The improvement in the amino acid profile of fermented soy-wara could encourage the use of soybeans for other culinary products and the utilization of the crop in Nigeria and other African countries. Soy-wara is one of the major and common household products that are widely consumed in many parts of Nigeria and African

Table 3. Mineral profile of the different fermented soy-wara samples

	Time (h)	Ca++ (mg/100 g)	Fe++ (mg/100 g)	K+ (mg/100 g)	Na+ (mg/100 g)	PO_3++ (mg/100 g)	Mg++ (mg/100 g)
Soybeans (Raw)	0	17.45±0.05^{a}	6.72±0.02^{a}	29.25±0.02^{a}	7.79±0.01^{a}	27.29±0.05^{a}	20.05±0.01^{a}
Soaking phase	24	17.65±0.00^{b}	6.94±0.00^{b}	29.76±0.01^{c}	8.31±0.02^{b}	27.52±0.01^{a}	21.02±0.01^{a}
	48	18.03±0.00^{c}	7.60±0.01^{b}	30.08±0.01^{a}	8.81±0.01^{c}	27.99±0.01^{b}	22.51±0.00^{a}
	72	18.69±0.00^{c}	8.20±0.01^{b}	30.26±0.01^{a}	9.02±0.02^{c}	28.11±0.01^{b}	23.64±0.01^{a}
	96	19.08±0.00^{c}	8.41±0.00^{b}	30.69±0.00^{a}	9.42±0.01^{c}	28.55±0.01^{b}	24.83±0.01^{a}

Table 4. Anti-nutritional factors in the different fermented soy-wara samples

	Time (h)	Tannin (mg/100 g)	Phytate (mg/100 g)	Trypsin Inhibitor (mg/100 g)	Protease Inhibitor (mg/100 g)
Soybeans (Raw)	0	125.16±0.35^{a}	174.37±0.57 ^{a}	120.62± 0.12^{a}	120.11± 0.01^{a}
Soaking phase	24	115.64±0.21^{a}	153.81±0.49 ^{a}	96.56 ± 0.01^{a}	98.11 ± 0.01 ^{b}
	48	69.32±0.92^{a}	83.65±0.35^{a}	63.84 ± 0.01^{a}	68.43 ± 0.01 ^{c}
	72	65.19±0.49^{a}	68.39±0.07^{a}	19.32 ± 0.01^{a}	23.21 ± 0.01 ^{c}
	96	43.26±0.53^{a}	47.16±0.35^{b}	1.10±0.02^{a}	1.20 ±0.03^{c}

Values are mean ± standard deviation of triplicate determinations. Different alphabet superscripts in the same row are statistically different ($p \leq 0.05$).

eISSN: 2550-2166

continent.

Soaking and fermentation increased the vitamin contents of all the vitamins studied (Table 2). The increase in values of vitamin B-complex such as thiamine, riboflavin and niacin in the "fermented soy-wara" could be as a result of biological and enzymatic activities during soaking and fermentation processes. This result agreed with the reports of several researchers (Obadina *et al*., 2013; Adeyemo and Onilude, 2013).

The calcium, iron, potassium, sodium, phosphorus and magnesium contents in soy-wara increased with the increase in natural fermentation period from 24 hrs to 96 hrs (Table 4). Gabriel *et al*. (2011) reported the increase in some mineral contents of Jack beans as affected by the use of Mould starter cultures for fermentation. Obadina *et al*. (2013) also reported similar results for fermented soymilk. However, the increase in the mineral contents of fermented soy-wara was an indication that these minerals were released from chelated complex compound through the activities of microorganisms responsible for the fermentation (Gabriel, 2002). Soaking and fermentation increased the mineral contents of all the minerals studied (Table 3). This agreed with the works of Gabriel (2002) and Gabriel *et al*. (2011).

Legumes contain some natural toxicants which include tannins, phytic acid, protease and trypsin inhibitors, saponins, metal chelates, cyanogens, isoflavonoids, phytoalexins, flatus factors, etc. (Pariza and Johnson, 2001). Some of these substances reduce the nutritional value of the food by interfering with mineral bioavailability and digestibility of proteins and carbohydrates (Salunkhe *et al*., 1990; Haard, 1999; MacDonald *et al*., 2012). Since legumes are usually consumed along with cereals in form of additives, or as a protein source, proper processing of these food substances should, therefore, be encouraged to eliminate these anti-nutrients before they are consumed (Ogbulie, 1991; Reddy and Pierson, 1994; Riaz, 2006; Orhevba, 2011).

Soybeans contain appreciable amounts of anti-nutritional factors like tannin, phytates, trypsin and protease inhibitor. It was therefore pre-treated by soaking and fermentation to reduce the amount of these anti-nutritional factors in soybeans. It was observed that soaking and fermentation reduced the different anti-nutritional factors in soybeans. These findings could improve soybeans utilization; improve community nutrition, as well as soybeans production in developing countries as more nutrients, will be obtained from the same quantity of soybeans.

5. Conclusion

Natural fermentation of the legume reduced anti-nutritional factors and improved the nutrient composition of the product. This resulted in an increase in vitamin and mineral contents and reduction in anti-nutrients in "fermented soy-wara" at the end of fermentation. This product could contain more nutrients since the amounts of anti-nutrients have been reduced. "Fermented soy-wara" could be used to improve nutrients availability and reduce malnutrition as well as help in soybean utilization in developing countries.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgement

The authors appreciate the Technical staffs of Multidisciplinary Central Research Laboratory of University of Ibadan for their technical support.

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eISSN: 2550-2166