Science World Journal Vol. 16(No 2) 2021
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

IMPROVING SHELF LIFE AND QUALITY OF DADDAWA MADE BY FERMENTATION OF SOYA BEANS AND SORREL SEEDS FROM MAIDUGURI, NORTH-EAST NIGERIA

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ABSTRACT
Daddawa is a Hausa name for a fermented product made from seeds of plants such as soya bean, locust bean, baobab and castor seeds. This product serves as flavour enhancers in soups in many homes in Nigeria. Sorrel seeds are less competitive sources of amino acids and proteins that is cultivated all year round in many parts of northern Nigeria. This study evaluated the biochemical properties of a fermented *daddawa* made from combination of soybeans (*Glycine max*) and sorrel (*Hibiscus sabdariffa*) seeds (3:1 and 1:3 ratios). Both combinations resulted in *daddawa* product with significantly higher ($p< 0.05$) soluble proteins, amino acids and titrable acidity relative to the unfermented sample. The product made from the 3:1 soybeans and sorrel seed combination ratio had the highest concentration of amino acid (29.0 ± 1.0 mg/g sample) at 60 h of fermentation while those made from the 1:3 ratio had the highest amino acid concentration (32.0 ± 1.0 mg/g sample) at 36 h. Reducing sugar concentrations were significantly lower ($p < 0.001$) in the fermented *daddawa* product compared to the unfermented sample for both combinations. Storage with vitamins C and E significantly decreased ($p < 0.001$) the levels of soluble proteins and amino acids in the product made from the 3:1 soybeans and sorrel seed combination. The vitamin containing stored *daddawa* product had significantly higher ($p < 0.05$) titrable acidity concentration. This study has shown the addition of vitamin C and E on *daddawa* made from a combination of soya bean and sorrel seeds to be inadequate in preserving nutrients during storage.

Keywords: *Daddawa*, fermentation, soya beans, sorrel.

INTRODUCTION

The production of *daddawa* represents one of the oldest traditional African technologies in food processing. However, the product has largely remained less acceptable in Nigerian markets, especially in urban centers compared to its counterparts; Maggi, Ajinomoto, Ajikama, etc which have the peptide monosodium glutamate as a constituent. Monosodium glutamate has been reported to be associated with some toxicities (Augustine *et al*., 2019; Chakraborty, 2019). Different types of the product *daddawa* are produced by the fermentation of a variety of plant seeds – locust beans, baobab seed, sorrel seed, soybeans, etc. However, the fermentation processes for production of the different types of the product may vary from place to place. It is of great importance to encourage the use of fermented legumes through appropriate studies aimed at improving their production and quality. *Daddawa* produced from locust beans has been found to be a highly nutritious flavour enhancer in foods (Abu, *et al*., 1995). It is a rich source of protein and lipids with low carbohydrates content. However, locust beans are a seasonal plant and its all-year round availability is highly limited. This has resulted into the shifting of emphasis from locust beans to soybeans which is a better source of protein and is cultivated annually taking only a few months to mature.

Fermentation is a process of metabolism of carbon source in which energy is generated by substrate level phosphorylation and in which organic molecules function as final electron acceptors (Okafor, 1987; McKinlay, 2020). It is a process of hydrolysis of complex plant constituents to simpler compound by enzymes produced by microorganism growing on these plants. In fermentation for *daddawa* production from the oil seed mentioned above various species of bacillus have been reported as important in the fermentation process notable among these being *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus licheniformis* (Ogbadu & Okague, 1988; Abu *et al.*, 1995).

Soya bean (Glycine max) is a leguminous crop that grows in tropical, sub-tropical and temperate climates. It is one of the most cherished and valuable oil seed legumes widely cultivated and utilized in many parts of the world. The legume seeds are a good source of food for infants, adults and animals providing proteins, minerals, essential fats and vitamins. They are widely used in making various types of foods such as soya bean cakes (local popular condiment *daddawa*), biscuits, non-diary milk and plant-based cheeses (*awara*) by fermentation. Soya beans oil is used in the manufacture of paints, soap, insecticides and disinfectants. It is also used in making hay and silage or fodder crops for animal nutrition. The ability of soya bean seeds to convert atmospheric nitrogen into organic soil nitrogen is utilized to improve soil fertility, hence serving as a green manure. Soya bean meal is a major raw material in the animal feed production. Soya bean meal production in Nigerian in the year 2020 was estimated at 368,000 metric tonnes. Between 2010 and 2020, the soybean meal crop in the country increased, registering the highest growth in 2011, when the production grew by about 25 percent compared to the previous years (Anonymous, 2020). There are many varieties of soya bean with varying colors and compositions. Most common varieties are yellow, green, brown, black and mixed colored varieties being the richest in protein and the lowest in oil (Willis, 2021). The seeds contain about 20% oil on a dry matter basis and this is 85% unsaturated and cholesterol-free. Soybean also has an average protein content of 40% and is more protein rich than any of the common vegetable or animal food sources found in Nigeria (Omoigui *et al*., 2020). Soya bean contains antinutritional factors; trypsin inhibitor and haemagglutinin which inhibit the digestion of proteins in the intestine and agglutinate red blood cells at low

temperatures, respectively. These antinutrients can be inactivated by processing methods such as soaking and heat treatment.

The sorrel (*Hibiscus sabdariffa*) is commonly referred to as *yakuwa* in Hausa language. It has been cultivated in Asia for over 300 years and now in many tropical areas of the world. The plant is adapted to a wide range of soil conditions and often grows on relatively infertile soil which is supplied with organic and essential nutrition. The seeds of the sorrel plant have been used in various localities for making condiment, particularly in some parts of northern Nigerian where it is used in making fermented sorrel *daddawa*. The sorrel seeds contain crude proteins, fibres and mineral elements (Dashak & Nwanegbo, 2002; Ismail *et al*., 2008) and some antinutrients such as tannins, cyanide and phytates (Nnam & Onyeke, 2020).

Free radicals; reactive oxygen and reactive nitrogen species are generated by our body by various endogenous systems, exposure to different physiochemical conditions or pathological states (Lobo *et al*., 2010). A balance between free radicals and antioxidants is necessary for proper physiological function. Antioxidants are substances that help prevent the oxidation of organic materials by radicals generated from metabolic processes and the environment. Many plant parts; fruits, seeds, barks, leaves etc, are documented to possess antioxidant potentials. During the course of radical chain reaction, breakage can occur with the introduction of an oxygen function into the molecule which then acts as a photosensitive material, absorbing light or ultraviolet radiation and generating further free radicals to initiate further oxidation. This process leads to discolouration and most importantly to breakdown of the substrate molecules. In unsaturated fatty acids, the free radicals can add to centers of unsaturation (double bonds) to generate new free radicals and join two substrate molecules together. Antioxidants such as the phenolic antioxidant and the aromatic amine (butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), methylene bisphenol and diphenylamines) have been widely used in industries to serve as antioxidants in foods. However, BHA and BHT are feared to be potent carcinogens (Lobo *et al*., 2010). Natural antioxidants such as L-tocopherols (vitamin E), ascorbic acid (vitamin C), carotenoids (vitamin A), quinines and uric acid have also been recognized to have antioxidant properties that can be exploited in food processing.

Soybeans are popular ingredients in preparation of *daddawa* with the addition of wheat as an adjunct (Nkeiruka *et al*., 2020). Apart from soybeans, sorrel seed have also found use in making "*daddawa*" though to a lesser extend in some parts of Nigeria (Ibrahim *et al*., 2012). Literature on the utilization of sorrel seeds in *daddawa* production has not gained popularity despite the availability, cheapness and nutritional quality of the seeds. It would therefore be important to study the suitability of sorrel seeds as flavouring agent and as an adjunct to soya beans in *daddawa* production. Indeed, some types of *daddawa* e.g., *daddawan batso*, are produced from combination of various seeds including locust beans, soybeans, baobab seeds and sorrel seeds in varying proportions (Ibrahim, 2014). Determination of the right proportion of each seed in making the best product will be highly useful in improving the quality of *daddawa*. The present study aims to evaluate the effect of the addition of vitamins C and E on biochemical properties and the shelf life of *daddawa* produced from a combination of soya beans and sorrel seeds.

MATERIALS AND METHODS

All chemicals and reagents used in this study were of analytical grade.

Source of Seeds

Soya beans (*Glycine max*) and the sorrel seeds (*Hibiscus sabdariffa*) used in the production of *daddawa* were purchased from the Monday Market, Maiduguri Metropolis, Borno State.

Microbial Starter Cultures

The microbial starter cultures *Lactobacillus spp* used in the fermentation of seeds for the production of *daddawa* were provided by the Department of Veterinary Microbiology and Parasitology, University of Maiduguri.

Processing of Raw Materials

Dirt and stones were removed from the purchased soya beans and sorrel seeds. *Daddawa* was prepared by the fermentation of a mixture of soybeans and sorrel seeds in different proportions. Raw soya beans (1 kg) were soaked in sufficient water to cover seeds for 3 hours after which seeds were dehusked to remove pericarp. One kilogram (1 kg) of cleaned sorrel seeds and the dehusked soya beans were then soaked in water separately for 6 hours after which both were washed, drained and then boiled for 2 hours to soften the seeds. The boiled seeds were then taken into two 2-Litre Buckner flasks in the ratios of 3:1 and 1:3 soya bean/sorrel seeds. The first contains 600 g of soya beans and 200 g of sorrel seeds (3:1) while the second contains 200 g of soya beans and 600 g of sorrel seeds (1:3). The flasks were covered with aluminium foil paper and sterilized in an autoclave for 15 minutes at 121^0C. After cooling both flasks were inoculated with *Lactocillus* starter cultures. One end of a rubber tube was fixed to a hole at the side of the Buckner flask and the other end inserted into a container containing a solution of sodium metabisulphite (1%). Samples for analysis were taken under aseptic conditions at specified time intervals. Control samples were taken before the commencement of fermentation. Fermented samples were divided at the end of fermentation into two groups of 200 g each. Two grams (2 g) of vitamin C was added to one of the samples and to the other 2 g of vitamin E was added. The two samples were then stored for 10 weeks after which they were analyzed for soluble proteins, amino acids, reducing sugars and titrable acidity.

Preparation of Samples for Analysis

Ten grams (10 g) of the fermented *daddawa* was homogenized and centrifuged at 1000 x g to remove the dregs. The clear supernatant was separated and used for the determination of reducing sugars, titrable acidity and total amino acids. For the total extractable proteins, 5 mL of calcium chloride solution was added to 10 mL of the supernatant and then centrifuged for 10 minutes. The clear supernatant was used for the determination of extractable proteins.

Determination of Total Titrable Acidity (AOAC 2010)

The total titrable acidity of the fermented mash was determined at various stages of fermentation by titrating samples with standardized solution of a base. Twenty-five milliliters (25 mL) of the homogenized fermented mash were pipetted out and placed in a conical flask. The sample solution was then titrated with 0.2 N NaOH and using phenolphthalein as an indicator to determine the

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end point. Thus, total titrable acidity was calculated using tartaric acid as an index.

Determination of Reducing Sugar by Slight Modification of Method of Asatoor and King (1954)

The estimation of reducing sugar is based on their ability to reduce alkaline (Cu^{2+}). This method uses isotonic copper sulphate – sodium tungstate to precipitate the protein. The reducing sugars diffuse out of the cells while the proteins are retained and get precipitated. Sodium tungstate is added to the samples as protein precipitated copper tungstate formed in the solution will precipitate the proteins. The precipitated proteins and cells are removed by centrifugation. The supernatant is then heated with alkaline copper sulphate forming cupric ions which are reduced to cuprous oxide in the mixture. The intensity of the blue colour formed was measured by a spectrophotometer. The intensity of the colour is proportional to the amount of Cu_2O that has reacted. To 3.8 mL sodium sulphate-copper sulphate solution in a clean tube, 0.1 mL of sample was added. This was properly mixed and kept for 5 minutes at room temperature. One hundred microlitres (0.1 mL) of 10% sodium tungstate was then added to the mixture and allowed to stand for 5 minutes. This was centrifuged at 1000 x g for 10 minutes. One mililitre (1 mL) of the supernatant was pipetted out in a clean test tube. To this was added 1 mL of alkaline tartrate solution and mixed well. The test tube was plugged in cotton wool and put in boiling water bath for 10 minutes. It was then cooled and 3 mL of phosphomolybdic acid was added and mixed properly. Three mililitres (3 mL) of distilled water was added and left to stand for 5 minutes. The optical density readings were taken at 680 nm against a blank. The standard curve was prepared as follows:

Test tube number	1	2	3	4	5	6	7
Standard solution (mL)	0.2	0.4	0.5	0.6	0.8	0.9	1.0
Distilled water (mL)	0.8	0.6	0.5	0.4	0.2	0.1	-

Determination of Total Amino Acids by Modification of Method of Moore and Stein (1948)

This method is based on the formation of blue purple colouration by reaction of ninhydrin with compounds containing free amino groups. On heating, two molecules of ninhydrin react with an L-amino acid to produce an intensely coloured product. The purple colouration is given by all L-amino acids with the exception of proline and hydroxyproline which gives a yellow colour. Two mililitres (2 mL) of ninhydrin was added to 0.2 mL of the working standard or test solution. The test tubes are transferred to a boiling water-bath for 10 minutes. The test tubes were cooled and 2 mL of the diluent solvent was added to each of the tubes and allowed to stand for 10 -15 minutes. The optical density readings were taken at 570 nm against a blank. The standard curve was prepared as follows.

Test tube number	1	2	3	4
Standard solution (mL)	0.2	0.4	0.6	0.8
Distilled water (mL)	0.8	0.6	0.4	0.2

Determination of Total Soluble Protein by Modification of Method of Layne (1957)

This method is based on the formation of blue purple colouration when substances containing two or more - CONH groups are treated with alkaline copper reagent. The CONH groups can be connected either directly or through a single carbon atom or nitrogen atom. Tripeptide and other higher peptides containing two or more CONH groups connected through a single carbon atom can give positive test. The intensity of the colour was spectrophotometrically determined. One mililitre (1 mL) of sample was put in a clean test tube and 1 mL of distilled water was added and mixed well, 5 mL of Biuret working reagent was then added to the test tube and allowed to stand for 10 minutes at room temperature. The optical density readings were taken at 540 nm against a blank. The standard curve was prepared as follows:

Test tube number	1	2	3	4
Standard solution (mL)	0.4	1.8	1.0	1.6
Distilled water (mL)	1.6	1.2	1.0	0.4

RESULTS

The profile of water-soluble proteins during the course of fermentation of soya bean and sorrel seed *daddawa* is shown in fig. I. The fermentation of *daddawa* made from the 3:1 ratio (soya bean: sorrel seed) showed a sharp decrease in protein from the zero hour to the 12 h. The protein value then rose again to a peak (163 ± 1.7 mg/g of fermenting mash) at the 36 h and 48 h which gradually decreased until the end of the fermentation time (72 h). In the case of the product made from the 1:3 ratio soya bean and sorrel seed, the fermentation proceeded with a gradual increase to a value of 93 ± 4.0 mg/g sample of the fermenting at the 24 h before a sharp decrease to a 70± 4.6 mg/g
sample at 36 h. The protein level however rose again to the highest peak (127 ± 3.5 mg/g) at the 60 h before decreasing to a value of (97.0 ± 1.0 mg/g) at the end of the fermentation time (72 h).

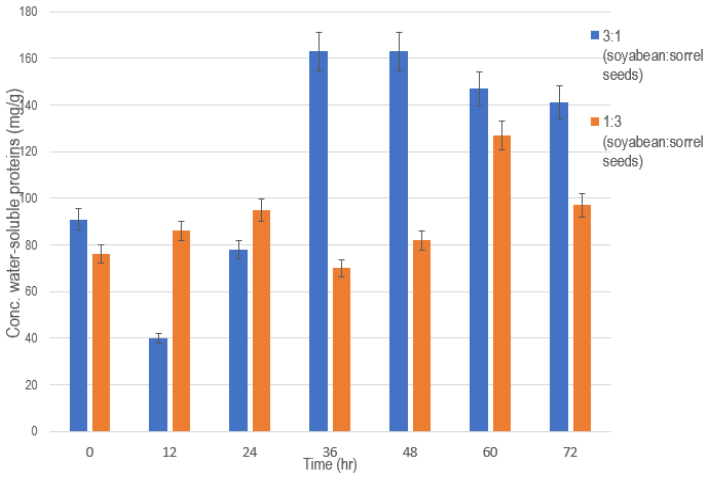

Fig. I: Concentration of water-soluble Proteins (mg/g sample) at various stages of fermentation
Values are means of triplicate determinations ± standard deviation.

Fig. II shows the amino acid concentration profile during the fermentation of soya bean and sorrel seed *daddawa*. Fermentation of the 3:1 ratio (soya bean and sorrel seed) *daddawa* showed an initial increase in amino acid from a mean value of 18.5 ± 0.50 mg/g at the zero hour to a value of 24 ± 1.5 mg/g at 12 h. This initial increase was followed by a sharp decrease to 20 ± 1.5 mg/g sample at the 36 h before rising again to the highest peak of 29.0

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ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
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± 1.0 mg/g sample at 60 h. Similarly, a sharp increase from a value of 21.5 ± 1.0 mg/g sample at zero hour to a value of 25.5 ± mg/g sample at the 12 h was observed for the 1:3 ratio of soya bean and sorrel seed combination. This initial increase was also followed by a decrease in amino acid concentration at 24 h but which subsequently rose again to the highest peak of 32.0 ± 1.0 mg/g sample at the 36 h. The amino acid values then gradually decreased till the end of the fermentation at 72 hours.

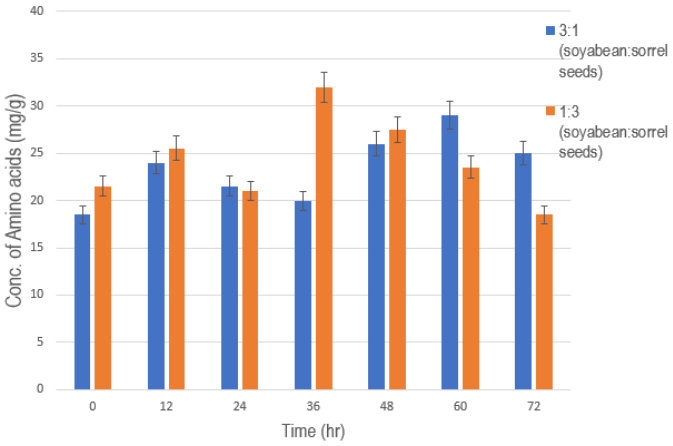

Fig. II: Concentration of Amino Acids (mg/g) at various stages of fermentation.
Values are means of triplicate determinations ± standard deviation.

Fig. III shows the reducing sugars of the *daddawa* during the course of fermentation of soya bean and sorrel seed combination. Fermentation started with a gradual decrease in reducing sugar from a value (166.7 ± 11.55 mg/g) at the zero hour to 100 ± 10 mg/g sample at the 36 h. The value of the reducing sugar however increased slightly (126.7 ± 23.09 mg/g) before decreasing again till the end of the fermentation. In the fermentation of the 1:3 soya bean and sorrel seed combination a gradual increase from a value of 120 ± 23.09 mg/g sample at zero hour to a value of 133.3 ± 23.09 mg/g sample at the 24 hours was followed by a sharp decrease to a value of 53.33 ± 11.55 mg/g at 36 hours. However, the value rose again to 133.3 +mg/g sample at the 48 h before falling again at the end of the fermentation time.

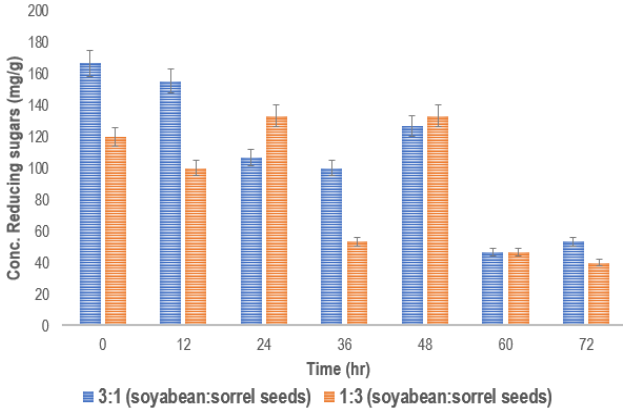

Fig. III: Concentration of reducing sugars (mg/g) at various stages of fermentation.
Values are means of triplicate determinations ± standard deviation.

Fig. IV shows the titrable acidity profile for the fermentation of the two combinations of soya bean and sorrel seed. The concentration of titrable acidity for the 3:1 soya bean and sorrel seed combination ratio increased gradually from a value of 1.52 ± 0.05mg acid/g of fermenting mash at the zero hour to a value of 2.68 ± 0.12 mg acid/g at the end of the fermentation time. Similarly, the 1:3 soya bean and sorrel seed combination showed a gradual increase from a value of 1.8 ± 0.18 mg acid/g at the zero hour to a value of 3.04 ± 0.07mg acid/g at 72 h. Fermentation increases titratable acidity with increases in fermentation time.

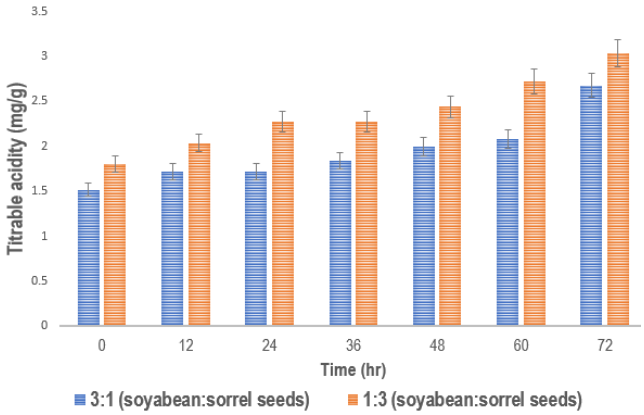

Fig. IV: Titrable Acidity (mg/g) at various stages of fermentation.
Values are means of triplicate determinations ± standard deviation

The effect of the addition of vitamins C and E on the levels of some biochemical parameters after 10 weeks of storage of the two types of *daddawa* produced is presented in Table 1. *Daddawa* produced from a soya bean and sorrel seed combination of 3:1 and the addition of vitamins C and E appeared to cause significant ($P < 0.001$) decreases in the levels of soluble proteins and amino acids when compared with the vitamin free control. The vitamin E containing-*daddawa* had a significantly ($P < 0.001$) lower soluble protein concentration but significantly ($P < 0.001$) higher amino acid concentrations when compared with the stored *daddawa* product containing vitamin C. Reducing sugars was higher though not significant ($P > 0.05$) in the stored products containing vitamins C and E. Titrable acidity was insignificantly ($P > 0.05$) higher in vitamin E containing stored *daddawa* compared with the vitamin free control, but significantly ($P < 0.001$) lower when compared with the vitamin C containing samples.

The *daddawa* product made from 1:3 soya bean and sorrel seed combination ratio showed significantly ($P < 0.001$) higher protein but lower amino acid values when stored with the two vitamins. The product containing vitamin C had significantly ($P < 0.001$) higher protein and lower amino acid when compared to the "*daddawa*" containing vitamin E. There was a significant decrease in the reducing sugar of the products stored with the two vitamins when compared with the vitamin free control. This decrease was significant ($P < 0.001$) in the product containing vitamin E but insignificant in the finished product stored with vitamin C. Titrable acidity was insignificantly higher ($P > 0.05$) in the vitamin E containing product but significantly higher ($P < 0.001$) in the vitamin C containing *daddawa* product.

Table I: Effects of vitamins C and E on the storage of soya bean/sorrel seed *daddawa*

Parameters	3:1 SOYA BEANS/SORREL			1:3 SOYA BEANS/SORREL		
	+ Vit. C	+ Vit. E	Control	+ Vit. C	+ Vit. E	Control
Protein	183 ± 3.2[a]	92 ± 1.7[b]	104 ± 40[c]	84 ± 3.46[a]	98.6 ± 2.31[b]	110.6 ± 2.31[c]
Amino Acids	32 ± 0.87[a]	25.3 ± 2.9[a]	21.0 ± 0.87[c]	28.7 ± 0.58[a]	22.3 ± 0.57[b]	18.8 ± 1.76
Reducing Sugars	100 ± 20.0	106 ± 23.00	126.7 ± 11.55	106.7 ± 30.55	66.67 ± 11.55[b]	86.66 ± 23.09[ba]
Titrable Acidity	2.04 ± 0.07[a]	2.88 ± 0.08[a]	7.8 ± 0.32[b]	2.8 ± 0.32[b]	2.96 ± 0.12[a]	9.32 ± 0.87[c]

Values are presented as mean ± S.D. of triplicate determinations. Values with different superscripts along a row are significantly ($P < 0.05$) different.

DISCUSSION

Locust bean (*Parkia filicoidea*), soya bean (*glycine max*) and baobab seeds (*Adansonia digitata*) are tropical seed plants used as protein supplements to improve the nutritive quality of feeds and food products. One of the important uses to which the seed of these crops are put, particularly in northern Nigerian, is in the production of *daddawa*, a fermented product of oil seeds which serves as flavour enhancers in soups. Production of *daddawa* is by a process of fermentation in which enzyme induced chemical alteration of these oil seed impact meat-like taste and flavour to the product. The enzymes involved may be indigenous to the seeds or produced by the fermentation microorganism.

Raw sorrel seeds are bitter in taste and contain anti-nutritive factors such as tannins and phytic acid (Aliyu *et al*., 2020). Anti-nutrients reduce the bioavailability of nutrients, however processing by fermentation, boiling, soaking, sprouting or germination can inactivate these factors (Aliyu *et al*., 2020). The fermentation profile of the soya bean and sorrel seed (3:1) combination showed an initial sharp decrease in soluble protein at the 24 h. This decrease may be due to the breakdown of proteins by proteolytic enzymes secreted into the medium by the fermenting organism (Nkhata *et al*., 2018). Fermenting microorganism also uses amino acid which could lower the protein content and quality of some fermented food (Osman, 2011). The gradual rise in the soluble protein after this initial decline to a peak value between the 36 h and 48 h may represent a gradual increase in both the levels of enzymes secreted by the organism as well as increased levels of peptide fragments to which the biuret reagent also responds.

The combination of 1:3 soya bean and sorrel seed showed a gradual increase in soluble protein until the 24 hour before declining slightly at 36 h. This may be due to delayed commencement of the breakdown of the proteins of the fermenting seed and the synthesis/secretion of the enzymes for the breakdown of the proteins and other substrates. The drop in the level of soluble protein at the 36 h may represent active proteolysis of the seed proteins while the peak increase in protein level at the 60 h may be due to increase in the level of enzymes for the breakdown and conversions of the various substrates in the medium. Processing of seed oils like soya beans and sorrel seeds improved soluble proteins as similarly reported by Ayo-Qmogie & Osanbikan, (2019).

The amino acid profiles in both combinations used in this study showed an initial increase at the 12 h before dropping again to about the zero-hour values. The increase may be due to initial hydrolysis of proteins to peptides/polypeptides and amino acids. The return of the aminoacyl values to near zero hour values may be due to the utilization of the amino acids by the fermenting organism in synthesizing the necessary enzymes it requires to grow in the fermentation medium. In the 3:1 combination ratio the initial decrease in amino acid levels persisted until the 36 h before it rose again to the highest peak at the 60 h. Findings from this study may suggest that the organism used amino acids for a longer period perhaps to synthesize the necessary enzymes which corresponded with the high protein values between the 36 h and 60 h of fermentation (Fig. I). In the case of the ratio 1:3 soya bean and sorrel seed the initial decline in amino acid level to about the zero-hour lasted for a shorter period and increased to the highest peak at the 36 h, this may suggest that there was faster breakdown of protein in the medium by the proteolytic enzymes produced by the organisms. In both combinations, the highest amino acid levels were obtained between the 36 h and 60 h of fermentation with the amino acid levels being significantly higher in the 1:3 combination relative to the 3:1 ratio. Long fermentation times of up to 96 h have been reported to increase amino acid concentrations of *Ricinus communis* seeds *daddawa* (Ojinnaka & Ojimelukwe, 2012). The flavour enhancing property of a condiment or food seasoning agent is dependent on the amino acid level. Fermentation of oil seeds release free amino acids such as glutamic and aspartic acids that contribute to texture and flavour enhancement (Ojinnaka & Ojimelukwe, 2012).

The soya bean: sorrel seeds in the 1:3 combination ratio may be a better combination for the production of *daddawa* as analyzed here.

Reducing sugars are produced as a result of breakdown of carbohydrates by enzymes so that energy can be released. The fermentation profiles of reducing sugar during the production of *daddawa* using the two-combination ratio showed that reducing sugar levels both decrease drastically at 36 h, this may be due to the conversion or breakdown of the sugars/carbohydrates to provide energy for the growth of the organisms such as *Lactobacillus spp* used in this study. Sugars are further metabolized to organic acids such as lactic acids which contribute to flavour and texture of fermented foods. This conversion may be responsible for the sharp and prolonged decline in reducing sugar. The reducing sugar also increased significantly ($P < 0.05$) at 48 h after the initial decline at the 36 h.

Titrable acidity is a fermentation parameter which measures the total acidity in the fermented food. Increased titrable acidity is a function of increased storage ability of any fermented food. The titrable acidity showed a gradual and continuous increase with fermentation time. The titrable was significantly higher ($P < 0.05$) at 72 h of fermentation relative to the zero-hour values for both combination ratios of soya bean and sorrel seed. The combination of 1:3 had a higher though insignificantly ($P > 0.05$) titrable acidity compared to the 3:1 combination ratio. The increase in the titrable acidity with fermentation time has been similarly reported (Kolapo *et al*., 2007; Wang *et al*., 2019). The conversion of carbohydrates into organic acids as well as the breakdown of lipids and proteins to fatty acids and amino acids may have contributed to increased free acid concentrations. These authors have similarly reported changes in functional properties during storage of soya beans *daddawa.*

Vitamins are essential micronutrients required for many

biochemical processes in the body. They act as antioxidant molecules that maintain balances between free radical generation and neutralization. Vitamins C and E are found to be rich in *Hibiscus sabdariffa* (Onyeke, 2001) and *Glycine max* respectively (Banaszkiewicz, 2011). Addition of vitamins C and E in *daddawa* from this study were observed to have some effect on the protein, amino acids, reducing sugar and titrable acidity during the 10 weeks storage period. Proteins and amino acids were significantly ($P < 0.001$) decreased in the stored product made from the combination ratio of 3:1 soya bean and sorrel seed. In the 1:3 combination ratio, the stored product containing the two vitamins had significantly higher protein concentrations but significantly lower amino acids when compared to the vitamin free control. This apparently suggests that the two vitamins do not seem to adequately preserve the nutrients during storage, reducing sugar was also adversely affected in the product made from the combination of 1:3 where it was observed that significant reductions in the levels of reducing sugar occurred during storage even in the presence of this vitamins. However, the reducing sugar levels in the fermented product made from the combination ratio of 3:1 were higher though insignificantly ($P > 0.05$) in the stored products containing the two vitamins with the one containing vitamin C exhibiting the highest level. This appears to suggest that the vitamins to some extend have preserved the reducing sugar during the storage. In both combinations of stored product containing the vitamins resulted in increase in titrable acidity which was significant ($P < 0.001$) in the product containing vitamins appear to indicate that microbial metabolic conversions leading to the production of organic acids may be taking place.

Conclusion

In this study decrease in protein and reducing sugar with increases in amino acids and titrable acidity were observed at the later stages of fermentation. The 1:3 (soya bean: sorrel seed) combination has the highest concentrations of amino acids and may likely give a better condiment. Fermentation should be best stopped between the 48 h and 60 h to obtain a desirable nutritive value. For the products stored with the vitamins, there was a decrease in protein and amino acid in the 3:1 (soya bean: sorrel seed) combination with increases in titrable acidity and reducing sugars in both combinations. It may be suggested that the vitamins do not seem to adequately preserve the nutrients during storage. Other antioxidants and natural preservatives be sourced through research to come up with better ways of improving the shelf-life of *daddawa* from a combination of soya bean and sorrel seeds.

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