Biochemical Changes of Soyiru (Fermented Soybean) During Storage

Adelodun L. Kolapo[1] *and Morenike O. Sanni*
Biology Department, Faculty of Science, The Polytechnic, Ibadan, Nigeria.
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Resumé

Kolapo, Adelodun L. & Sanni, Morenike O. *Les Changements Biochimiques du Soyiru (soja Fermenté) Pendant le Stockage.* Un usage augmenté du soja ferrmenté (*Glycine max.* L Merrill) comme soyiru similaire à iru (daddawa) des haricots du croquet (paricia filicoidea) améliorera la nutrition. Les sojas sont susceptibles aux changements péroxidatives et l'oxidation de l'acide gras. Les variétés TGX 1440-2E de la semence du soja étaient fermentées en utilisant un processus normal des trois jours. Puis le soyiru qui s'ensuit était stocké pour 14 jours des températures ambientes et réfrigératoires de 30 ± 1°C respectivement. L'évaluation proximative p^H, l'acidité titratable (TA) la valeur péroxidique (POV) le libre acide gras (FFA) et la capacité antioxidante de l'acide ascorbique (AEAC) étaient déterminés en trois exemplaires. La composition proximative du soyiru était 6.07% du gras cru 15.97% de la protéine crue (N x 6.25) 9.96% de l'hydrate de carbone avec 41.46 mg kg^{-1} du sucre réduit, une matière sèche de 35.6% correspondant à un contenu de l'humidité de 64. 4%, 1.96% du cendre cru et 1.64% de la fibre crue. A stockage d'ambient, le pH avait augumenté avec la réduction en TA indiquant fermentation alkaline lors de la réfrigération. Le pH a diminué avec l'augmentation en TA soulignant une fermentation acidique. Le contenu de l'hydrate carbone, le contenu réduit du sucre et FFA avaient diminué avec le stockage alors que le contenu de l'humidité et POV ont augmenté. AEAC a augmenté de 140 à 290 mg $100g^{-1}$ respectivenent avec le traitement mais diminué à 25 et 155mg $100g^{-1}$ respectivement avec la réfrigération. Pour les deux conditions de stockage, le contenu FFA a diminué alors que POV a augmenté. Un éssai de la mouche domestique a confirmé les signes imminentes de la détérioration aux quatriéme et sixiéme jours de l'ambient et du stockage réfrigéré respectivement. A partir de ces resultats, il parait que péroxidation est un principal étément biochimique dans la détérioration de soyiru. Le produit est périssable et donc il est recommandé qu'on l'utilise tout frais.

Mots clés: Antioxidant, péroxidation, pH, contenu proximatif, soyiru.

Abstract

An increased use of fermented soybean (*Glycine max* L. Merill) as soyiru similar to iru (daddawa) from Locust beans - *Parkia filicoidea* will enhance nutrition. Soybeans are susceptible to fatty acid oxidation and peroxidative changes. Seed of soybean variety TGX 1440-2E were fermented using a standard 3-day procedure and the ensuing soyiru was stored for 14 days under ambient and refrigeration temperatures of 30±1 °C and 6±1 °C respectively. Proximate evaluation, pH, titratable acidity (TA), peroxide value (POV), Free fatty acids

[1] *Corresponding author*

(FFA) and Ascorbic acid Antioxidant capacity (AEAC) were determined in triplicate. The proximate composition of soyiru was 6.07% crude fat, 15.97% crude protein (Nx6.25), 9.96% carbohydrate with 41.46 mg kg^{-1} reducing sugar, a dry matter of 35.6% corresponding to a moisture content of 64.4 %; 1.96 % crude ash and 1.64 % crude fibre. At ambient storage pH increased with reduction in TA, indicating alkaline fermentation while with refrigeration, pH decreased with increasing TA, underscoring acid fermentation. Carbohydrate content, reducing sugar content and FFA decreased with storage while moisture content and POV increased. AEAC increased from 140 to 290 mg100g^{-1} respectively with processing but decreased to 25 and 155mg 100g^{-1} respectively with ten days refrigeration and ambient storage. For both storage conditions, FFA content decreased while POV increased. A 'house-fly test confirmed signs of approaching spoilage on the fourth day and sixth day of ambient and refrigerated storage, respectively. From these results, it seems that peroxidation is a key biochemical factor in spoilage of soyiru and that the product is perishable and should be used fresh.

Keywords: Antioxidant, peroxidation, pH, proximate content, soyiru.

Introduction

Soybean (*Glycine max* L. Merill) is valued for its high protein content, edible oil and many nutrients (Popoola and Akueshi, 1986). There have been several attempts to introduce soybean as a protein supplement for Nigerians (Akinrele, 1967; Faryna, 1978; Sanni and Sobamiwa, 1994) . In addition, the use of soybean as an alternative to locust bean (*Parkia filicoidea* Welw) to produce iru - a condiment, has been shown to be an inventive means to increase soybean awareness (Odunfa, 1988). Odunfa (1988), further asserted that in northern Nigeria soybean fermentation has become an accepted family practice in some areas, and has even developed into rural cottage industry where it is dried and used as required. However the adoption of soyiru in southern Nigeria is suffering a setback because the fresh form, which the southern Nigerians prefer, deteriorates faster than the traditional locust bean iru (Obatolu *et al.,* 1998).

Food products naturally undergo various chemical and enzymatic reactions which determine their acceptability, storage life and safety (Tomassi, 1988); and the oxidation process is of particular relevance since unsaturated lipids are more susceptible to oxidation because of the presence of double bonds. Wilson and McDonald (1986) reported that peroxidative changes in polyunsaturated fatty acids have been associated with aging of soybean seeds. However, Bowler *et al.* (1992) stated that a protective mechanisms that could scavenge the peroxidatively produced free radicals and peroxides have evolved within the seed, keeping these deleterious compounds to a minimum. This protective mechanism is made possible through a series of enzymatic and non-enzymatic antioxidant defence system (Sies, 1985).

Published work on soyiru has centered on the science of processing and process optimization (Popoola and Akueshi, 1986; Suberu and Akinyanju, 1996; Obatolu *et al.*, 1998) with scanty information on spoilage studies. The present study investigates the biochemical aspect of spoilage of soyiru at two different storage temperatures and the level of acceptability with the days of storage.

Materials and methods

Preparation of Soyiru

The soybean samples of variety TGX1440-2E used for this work were obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria. About 2kg of sorted soybean seeds were roasted for 20 min under low heat, dehulled, winnowed and soaked in clean water overnight (12 hours). The seeds were rinsed and boiled on a stove for 3 hours. They were drained and transferred into a clean calabash lined and covered with clean, fresh banana leaves, and covered with another calabash. The set-up was put in a jute bag and left to ferment for three days, giving rise to a strong ammoniacal odour and sticky, fermented soybean seeds which is soyiru. After the completion of fermentation, the fermented soybean was packed into pre sterilized small plastic containers and stored for two weeks. Samples were obtained from the stored soyiru at 2 days interval for analyses.

Proximate composition analysis

The moisture, crude protein (Nx6.25), fat, ash and crude fibre of the Soyiru were determined according to the official methods of the Association of Official Analytical Chemists (AOAC) with carbohydrate content obtained by difference (AOAC, 1984). Analyses were in triplicate and on dry matter basis.

pH

The pH values of the soyiru were obtained according to the method described by Sanni (1989). Five gramme of soyiru was weighed into a beaker and 45ml of distilled water was added to make a one-tenth dilution. It was blended into slurry with stomacher (Seward Stomacher 400C Lab Blender) and filtered with glass wool. pH was measured using a standardised pH meter (Denver, pH215) with a glass electrode.

Titratable acidity

The method of Ikenebomeh *et al.* (1986) was employed in determining the titratable acidity of the soyiru samples. Distilled water was boiled to expel carbon dioxide, and cooled to room temperature to obtain decarbonated water. A one-tenth dilution of the soyiru was prepared and the slurry was filtered through Whatman No 1 filter paper and 10ml aliquot of the filterate was titrated with 0.1N NaOH. Phenolphthalein was used as the end point indicator. Ten millilitres of decarbonated water was also titrated. The water titre was subtracted from the sample titre.

Titratable acidity was calculated as mg lactic acid per g of sample (mg g^{-1}).

Peroxidative changes

Free fatty acid (FFA) and peroxide value (POV) were used as indexes of peroxidation in the soyiru stored at both ambient and refrigeration temperatures. The FFA content (% oleic acid) was estimated using an alkali titration method. The peroxide value (meq kg^{-1} oil) was measured by titration with 0.1M sodium thiosulphate solution using starch as an indicator (Pearson, 1985).

Antioxidant capacity

The extract that was used for antioxidant capacity measurement was prepared according to the method reported by Sanchez *et al.* (2003).Ten grammes of the soyiru sample was homogenized using a stomacher with 10ml of ice cold extraction solution (methanol/formic acid, 98:2v/v) for one minute. The homogenate was filtered through muslin cloth, then centrifuged using Hermle Refrigerated centrifuge (Z323K) at 11,340xg for 3 minutes and was passed through a 0.45m type HA filter (Millipore S. A. Molshiem, France). The filterate thus obtained was subsequently used as soyiru extract.

The antioxidant capacity of the stored soyiru samples was estimated using a modified thiocyanate method as reported by Zia-ur-Rehman *et al.* (2003). First, 200l of soyiru extract was added to a solution of linoleic acid (0.13ml) in 99.0% ethanol (10ml) and 0.2mM Phosphate buffer (pH 7.0,10ml) and the volume was made up to 25ml with distilled water. The solution was then incubated in an airtight conical flask at 40C. To 0.1ml of this solution were added 9.7ml of 75% ethanol and 0.1ml of 30% ammonium thiocyanate. Precisely 3 minutes after the addition of 0.1ml of 20mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the resultant red colour was measured at 500*nm* with a Griffin Model 40 colorimeter (Griffin and George Leicestershire, UK). Distilled water was used as control.

Aqueous standard solutions of 50mM Analar grade L-ascorbic acid was prepared. Diluted standards were used within 1h of preparation. The results were expressed as ascorbic acid equivalent antioxidant capacity-AEAC (Gil *et al.*, 2000).

Sensory evaluation of stored soyiru

Consumer acceptability of the stored Soyiru was assessed by a panel of 10 known users of Soyiru. Parameters such as flavour, texture, colour and taste were evaluated on a 5 point hedonic scale with 5=Excellent and 1=Poor.

The data was subjected to Duncan Multiple range test to separate the means using SPSS (version 7.5) statistical package.

Results

It was evident that the same pattern of change occurred for crude fat, crude protein and crude fibre (Fig. 1a-d), which generally decreased both at refrigerated and ambient storage temperatures. However, at the end of storage, the value of fat content of the soyiru stored at ambient temperature was slightly greater than that of refrigerated storage. The moisture content of the stored soyiru increased marginally from 64.40% to 69.03% and 68.11% at ambient and refrigeration temperature respectively corresponding to a marginal decrease in dry matter (Fig. 2b). The carbohydrate level of the stored soyiru also reduced marginally at both storage conditions while the change in the reducing sugar level at ambient condition was more than that observed at refrigeration storage (Fig. 2b). The initial pH of soyiru was 7.87. At ambient storage, the fermented soybean had a very low pH by day 4 and that of refrigeration storage by day 8. However a further pH increase at ambient storage resulted into alkaline pH while that of refrigeration storage did not increase beyond the starting pH (Fig. 3b). In general, there was a decrease in FFA content at both storage temperatures, while an increase in POV was observed at both storage conditions (Table 1).

There was no significant differences between the two storage conditions with regard to the FFA and POV values, while a significant difference existed for AEAC values of the two storage conditions. The AEAC value for unprocessed soybean was 1400.00 mg100g^{-1}. Results from the present

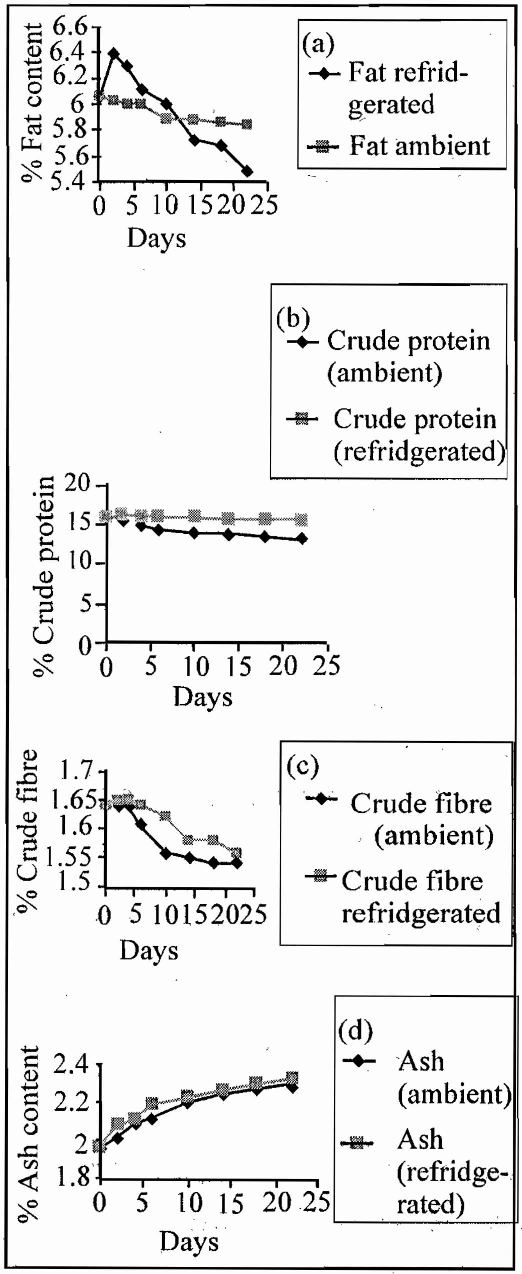

Figure 1. Changes in (a) crude fat, (b) crude protein, (c) crude fibre, (d) ash of stored Soyiru.

Table 1. Effect of storage conditions on free fatty acids[a] (FFA) content (% Oleic acid), peroxide value[a] (POV; meqkg^{-1} oil) and antioxidant capacity[b] (ascorbic acid equivalent antioxidant capacity, AEAC) of stored Soyiru.

Storage time (Days)	*FFA*	*Refrigerated (6±1C)* *POV*	*AEAC*
0	0.065(0.003)	2.00(0.000)	290.00(9.30)
2	0.065(0.004)	7.000(0.020)	113.33(4.86)
4	0.052(0.005)	11.400(0.800)	90.16(3.27)
6	0.043(0.003)	14.600(0.400)	80.00(1.73)
8	0.030(0.003)	17.066(0.305)	90.16(3.27)
10	0.022(0.006)	19.000(0.200)	25.00(0.00)
12	0.019(0.005)	19.200(0.200)	165.00(1.73)
14	0.017(0.003)	19.400(0.200)	91.67(5.27)
Storage time (Days)	*FFA*	*Ambient (30±1C)* *POV*	*AEAC*
0	0.065(0.003)	2.000(0.000)	290.00(9.30)
2	0.074(0.003)	6.333(0.305)	458.33(4.86)
4	0.059(0.004)	10.000(0.200)	341.67(3.01)
6	0.056(0.006)	13.466(0.470)	280.00(3.00)
8	0.044(0.003)	16.400(0.400)	240.00(1.73)
10	0.059(0.002)	18.533(0.330)	155.00(3.00)
12	0.044(0.005)	18.733(0.300)	101.67(1.84)
14	0.047(0.004)	19.400(0.200)	60.00(0.00)

a. Values are means.
Standard deviation (n=3) in parentheses.
b. Values are means in mg 100g^{-1}.
Standard deviation (n=3) in parentheses.

study revealed that the antioxidant capacity of soyiru increased with processing (290±9.30 mg 100g^{-1}).

The organoleptic ratings of the stored Soyiru is shown in Table 2. At the onset of spoilage, there was a gradual loss of a characteristic strong ammoniacal odour, colour and taste. A repulsive off-flavour development, complete loss of taste and colour were evidences of overt spoilage. To this end, Soyiru samples stored at ambient temperature manifested signs of approaching spoilage on the fourth day of storage while the symptoms of overt spoilage characterized the product by the sixth day of storage. On the other hand, signs of approaching spoilage characterized the refrigerated Soyiru on the sixth day of storage while the

Table 2. Organoleptic characteristics of soyiru stored at ambient and refrigerated temperatures.

Storage	*Refrigerated(6±1C)*				*Ambient (30±1C)*			
time (Days)	*Flavour*	*Texture*	*Colour*	*Taste*	*Flavour*	*Texture*	*Colour*	*Taste*
0	3.40^{b}	3.60^{a}	3.50^{b}	3.80^{a}	3.40^{d}	3.60^{d}	3.50^{a}	3.80^{a}
2	3.90^{ab}	3.50^{a}	4.40^{a}	4.10^{a}	3.00^{d}	2.60^{d}	3.10^{a}	3.00^{b}
4	4.30^{a}	3.20^{a}	3.20^{bc}	3.60^{a}	2.220^{b}	2.10^{b}	2.00^{b}	2.30^{c}
6	2.40^{c}	2.40^{bc}	2.30^{cd}	1.90^{b}	1.40^{c}	1.40^{c}	1.50^{bc}	1.50^{d}
8	2.40^{c}	2.90^{ab}	2.30^{cd}	1.20^{c}	1.20^{d}	1.40^{c}	1.30^{c}	1.30^{d}
10	1.90^{c}	2.40^{bc}	2.00^{d}	1.20^{c}	1.20^{d}	1.20^{c}	1.10^{c}	1.30^{d}
12	1.70^{c}	2.10^{c}	2.00^{d}	1.00^{c}	1.00^{c}	1.10^{c}	1.10^{c}	1.10^{d}

Within each column values with different superscripts differ ($p < 0.05$).
Hedonic scale: 5= Excellent; 1= Poor.

symptoms of overt spoilage were evident by the tenth day of storage. A particular circumstantial evidence, called `House-fly test' was used to confirm the rating reported in Table 2. When all the samples were opened, it was noticed that house-flies alighted on the overtly spoiled samples much more readily than the sound samples or those in which spoilage was just setting in.

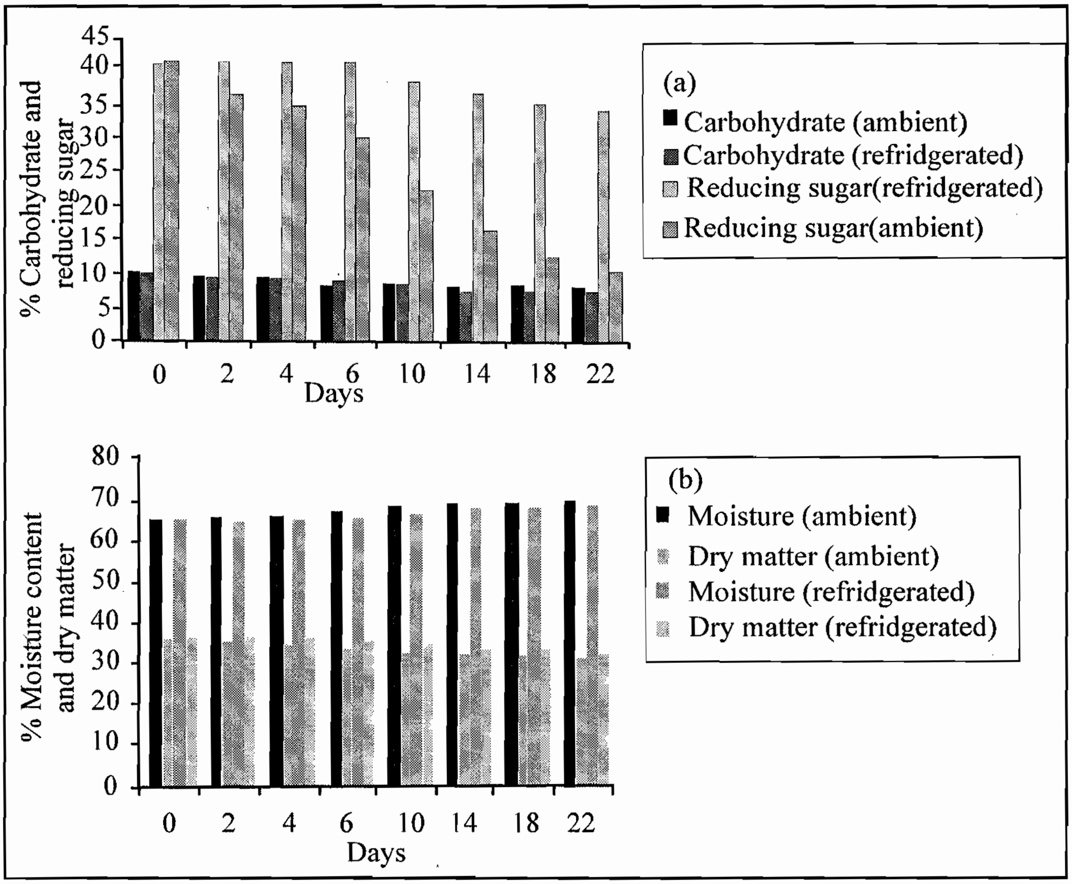

Figure 2. Changes in (a) carbohydrate and reducing sugar, (b) moisture and dry matter of stored Soyiru.

- Proximate analysis was determined in % based on Dry Matter.
- Reducing sugar are in mg kg^{-1}.

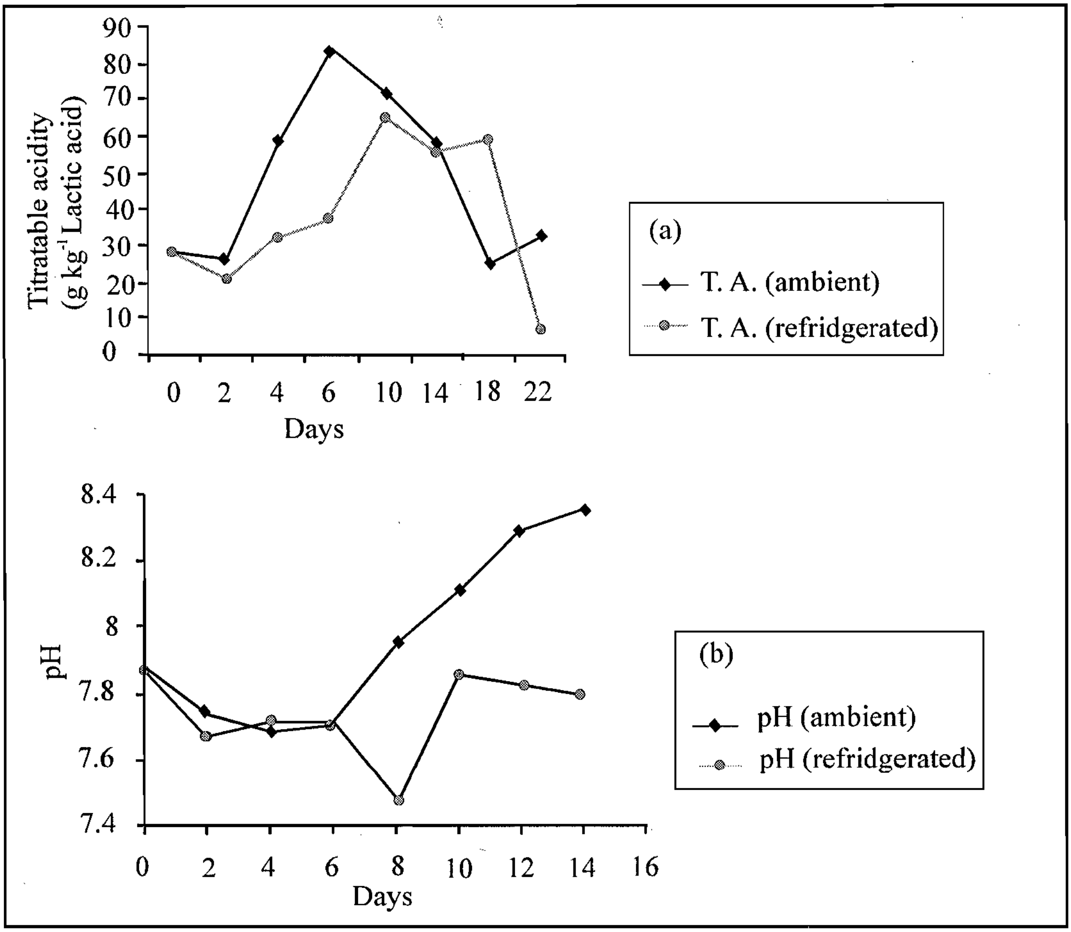

Figure 3. Changes in (a) Titratable acidity and (b) pH of stored Soyiru.

Discussion

The involvement of microbiological factors in food spoilage has been well established (Frazier and Westhoff, 1978), and this may be responsible for the observed decrease in the proximate parameters. The observed increase in crude ash content at both storage conditions may be consequent upon the decrease in other proximate parameters.

The observed increase in moisture content corroborates the submission of Frazier and Westhoff (1978) who stated that it is possible for microorganisms growing in food to change the level of available moisture by release of metabolic water or by changing the substrate so as to free water. This phenomenon may perhaps explain the reason for greater increase of moisture content observed for ambient storage, which is normally characterized with greater microbial activity.

A large reduction of reducing sugar level obtained at ambient storage might be likely responsible for the consistently higher titratable acidity obtained for ambient storage. This result is in agreement with the earlier submission that simple sugars in food products can be fermented by microorganisms to produce acids (Frazier and Westhoff, 1978). The observed pH changes is in perfect agreement with that reported by Obatolu *et al.* (1998). The high pH of the freshly fermented soyiru on day 0 was due to the ongoing fermentation, and the production of ammonia (Odunfa, 1988). The rise in pH during storage is likely due to microbial activity, possibly proteolysis. The simultaneous increases in pH and titratable acidity in this stored food sample was unusual but not unique, as similar results have been obtained from studies on burong dalang-a fermented product made from fish and moist rice (Orillo and Pederson, 1968), tempeh fermentation (Wagenknecht *et al.*, 1961), and locust bean fermentation (Ikenebomeh *et al.*, 1986). In these previous studies, it was suggested that liberated ammonia or other basic end products of protein decomposition during fermentation were the cause of this phenomenon. The same type of decomposition may have taken place in stored soyiru. The decrease in FFA content with storage contrasts the result obtained from studies on stored sunflower oil (Zia-ur-Rehman *et al.*, 2003). The observed decrease in FFA content in the present work may be consequent upon the corresponding decrease of crude fat content with storage as the initial step in fat deterioration is the formation of free fatty acids (Sattar and Demen, 1973). The peroxide value which is a measure of the extent of rancidity was increasing with storage period at both storage conditions. These results compare favourably with that obtained from soymilk spoilage (Iwe, 1991). The rate of peroxide production in the earlier day of storage is greater in refrigeration storage and this may be consequent upon a corresponding lower Ascorbic acid equivalent antioxidant capcity (AEAC) of soyiru stored at refrigeration temperature. This result is not particularly strange as storage at low temperature above freezing point can result in chilling injury in susceptible plant species (Shewfelt and del Rosario, 2000); and increased lipid peroxidation has been observed in the development of chilling injury in cucumber (Hariyadi and Parkin, 1993), Zucchini squash (Wang and Sass, 1994) and soybean seeds upon imbibition (Punturalo *et al.*, 1991).

Nicholi *et al.* (1997) presented a concept, which emphasizes that losses of a known antioxidant could be counterbalanced by the development of others during processing. The present results may lend support to that concept as there was an increase in the antioxidant capacity of soyiru subsequent to processing. On a general note, the AEAC of the stored soyiru at both storage temperatures decreased up to the tenth day of storage. However, a more critical look at the values show that ambient storage values were consistently multiples of the corresponding refrigeration storage equivalent within the first ten days of storage. Beyond the tenth day there was an irregular pattern of change for refrigerated samples. It has been stated earlier that peroxidation, both in foods and living organisms, is counteracted by a series of enzymatic and non-enzymatic antioxidant defence system. A lower AEAC value observed at refrigeration storage up to the tenth day of storage suggests that antioxidant enzymes whose optimum temperatures is non-psychrophilic may be responsible for the conferment of the antioxidant activity to soyiru. However, this should be a subject of further investigation. From the present organoleptic data, it seems that Soyiru is not acceptable beyond 2 days (ambient storage) and 3 days (refrigerated storage). The biochemical deterioration in the stored product may have been responsible for the decreased acceptability. The use of preservatives to extend the shelf life of this product should be evaluated.

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