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Influence of Temperature and Enrichment on Storage Qualities of Soy-Enriched "Lafun", a Protein-Enriched Cassava Product

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Abstract

The influence of enrichment and temperature on the storage qualities of soy enriched fermented mashed lafun was investigated. Samples of the protein-enriched lafun were produced from fermented cassava obtained by soaking peeled cassava chunks in water, at ambient temperature (28 – 32^{0}C) for 2 – 5days. The enriched dried milled lafun flour was packaged in the high density polythene and stored under three different temperatures of 10^{0}C, 30^{0}C, and 40^{0}C. Samples were withdrawn at four weeks interval to measure the changes in their chemical compositions during storage. The moisture increased from 6.67 to 9.21% and 4.29 to 8.00% at 10^{0}C and 30^{0}C respectively, but followed another trend on the samples stored at 40^{0}C which decreases as the temperature increases. FFA values increased at 10^{0}C 0.50%-0.90% and 30^{0}C 0.56%-1.07%. TBA increased from 0.11-0.27, 0.12-0.67 and 0.24-0.45 mda/kg for control samples at 10^{0}C, 30^{0}C and 40^{0}C respectively, while sample supplemented with soy, increased from 0.25-0.37, 0.39-0.7 and 0.24-0.94mda/kg at 10^{0}C, 30^{0}C and 40^{0}C respectively. At higher temperature of 40^{0}C, browning increased sharply after about 16 weeks of storage from about 0.02nm to 0.017nm of change in absorbance per month (dA_{425}). Therefore, enrichment and high temperature reduces the shelf life of lafun, which could be better stored at temperature lower than 30^{0}C than at high temperature of 40^{0}C.

Keywords: *Soy Bean; "Lafun" Soy curd; Soy residue; Storage Stability; Protein Enrichment*

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1. Introduction

Cassava (*Manihot esculanta* Crantz), though originated from South America (Olanrewaju and Idowu, 2017) is now a common staple in many countries including Nigeria (Okpako *et al*., 2008; Okwulehie *et al*, 2021). Globally, its importance is rated sixth in terms of annual production (Fedrica, 2001) and Nigeria is considered the largest producer worldwide (Omolara, 2014). The processing vary from location to location but the commonly available forms in Nigeria and in order or popularity and acceptability are *gari, fufu and lafun* (Abiodun *et al*., 2017; Okafor *et al*., 2017). Besides this, cassava tubers can also be boiled and eaten (Oluba *et al*., 2018). Apparently, with up to 90% carbohydrate on dry weight basis, cassava is mainly a carbohydrate and an energy dense food. The tuber is estimated to be the source of energy for more than 800 million people (Oluwamukomi and Adeyemi, 2015).

Cassava is nutritionally deficient in many vital nutrients essential for growth and development, especially in children and young adults. This may be responsible for the observed stunted growth in people whose staples are mainly cassava and cassava products. Cassava is deficient in protein, mineral and vitamin. Previous study reported that the crude protein contents of most cassava foods ranged between 1 and 3% on dry weight basis (Anyaiwe *et al*., 2018). Obviously, this is too poor and the impact of such nutritionally deficient food may be significant for populations, especially children that largely depend on cassava products considering the fact they derive most of their daily nutritional needs from cassava and its products (Oluwamukomi and Adeyemi, 2015).

In areas where cassava is a staple food crop, people usually suffer from malnutrition because of the biochemical composition of the tubers and the fact that the supply of animal protein is inadequate in such areas (Lukuyu *et al*., 2014; John and Anger, 2010). Children happen to be the most vulnerable group in such areas suffering from both protein and calorie malnutrition. An easier or faster alternative route is to provide diets with greater amount of quality protein. This can be achieved through enrichment fortification programs (Anyaiwe *et al*., 2018). The use of plant protein supplement is a cheaper, more viable and most available option for this. Supplementary protein sources must therefore be provided if cassava is to maintain its role as a major source of calories (Bell and Labuza, 2000). Many attempts have been made to enrich cassava products with protein from vegetable sources (Some efforts have been made to improve the nutrient content of cassava products (Ogunlakin *et al*. 2015; Okwulehie *et al*., 2014: Oluwamukomi *et al*. 2005).

The production, storage and marketing of lafun is still mainly carried out by local farmers, processors and foodstuff traders, while only a few highly mechanised processing plants market their products in consumer packaged forms (Alphonse *et al*., 2018). lafun is still being packaged, transported and stored in woven sacks with attendant fluctuations in climatic conditions and sometimes it is being sold in the market in bowls with exposed surfaces thus increasing its susceptibility to environmental contaminations (Ogiehor and Ikenebomeh, 2006). The producers of lafun go about the storage and packaging of this product in a non-scientific way (Oyelade *et al*., 2001) using hessian bags and transparent plastic polyethylene sheets. Polyethylene is widely used as a packaging material because of its good mechanical properties and low cost however these qualities have been overshadowed by its high non-biodegradable nature and waste disposal problems (Sailaja and Chanda, 2001). The products may look alright from outside, while its quality may be musty and completely bad when it is touched. This is an indication that faulty packaging can conveniently undo all that a food processor has attempted to accomplish by the most meticulous method of manufacturing practice (Fedrica, 2001). The hygroscopic nature of dried lafun products is a major constraint to its keeping quality. The use of polythene by the local producers of lafun for its packaging is due to the fact that the material is cheap, readily available and durable. The material also has ease of bulk packing and transportation of products with little or no attention paid to the quality of products stored. The polythene is not moisture proof or airtight on dried lafun which is hygroscopic in nature makes the use of polythene grossly inadequate. Lafun stored in polythene in a humid atmosphere can absorb sufficient moisture making them vulnerable to microbial growth (Adejumo and Raji, 2012).

With the continued interest in the enrichment of lafun with local protein sources from soy bean, it is necessary to study the effects of the conditions of storage on the quality and storage stability of soy enriched lafun during storage (Oluwamukomi, 2008). The objectives of this study are therefore to produce soy enriched lafun, subject it to storage stability study and determine the effects of enrichment and the temperature of storage on the quality parameters of critical importance to deterioration during storage of the enriched lafun.

2. Materials and Methods

2.1 *Sources of raw materials*

Cassava roots (*Manihot, esculenta crantz*) were obtained from the Teaching and Research Farm of the Federal University of Technology, Akure, Ondo State, Nigeria. Soybean (*Glycine max (TGX)*) was purchased from Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 *Soy curd and residue extraction*

Soy bean seed (150 g) were sorted, cleaned, soaked (12 h) in 2 L of tap water containing 0.5 g $NaHC0_3$ in a cooking pot and boiled for 25 min. The boiled and dehulled soybean seeds were then wet milled in a hammer mill. Water was added in ratio 1:8 and a muslin cloth was used to extract the milk (pH 6.40) and the residue was kept separate. Thereafter, the pH of the extracted milk was adjusted to 4.6 by adding 1 M citric acid. The soy milk was allowed to stand and the clear whey at the upper part was decanted while the lower part (curd) was collected after six hours. The curd and residue was oven dried (at 60 ^0C for 24 h), milled, packaged in high density polyethylene HDPE and stored in the refrigerator until needed for further use. Figure 1 shows the production chart for the curd and residue.

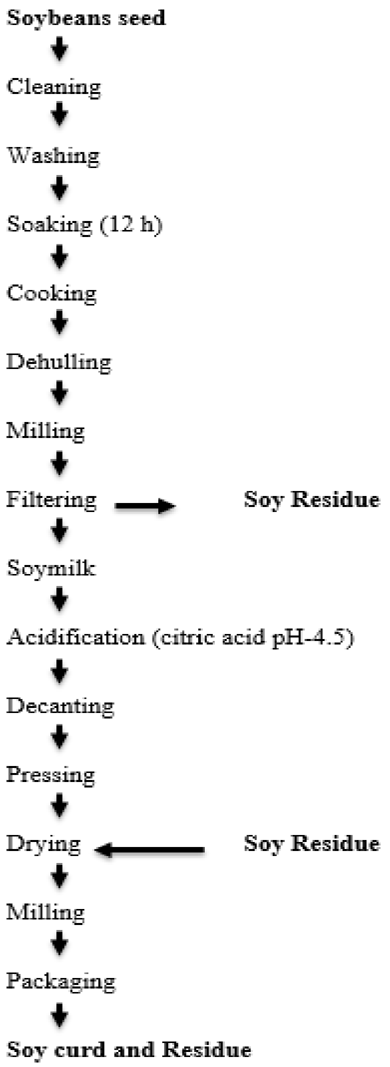

Figure 1: Production of Soy Curd and Soy Residue
Sources: Anyaiwe and Osuji (2010)

2.2 Lafun production and enrichment

Freshly harvested cassava roots were peeled with knife, washed and cut into chunks, fermented for 4 days (pH 3.67), washed, sifted, milled into pulp and divided into two portions (Figure 2). One portion was used as control (CL) while the other portion was enriched with either dry soy curd or residue using Pearson scale with, 10% enrichment level and also taking into consideration the water content of the mash at 100%. Sample supplemented with curd was named *Lafun* enriched with curd" (LEC) and the other sample *Lafun* enriched with residue" (LER) A commercial *Lafun* sample (CS) was obtained from FIIRO Oshodi, Lagos for comparison. Figure 2 shows the production chart for the enriched lafun samples.

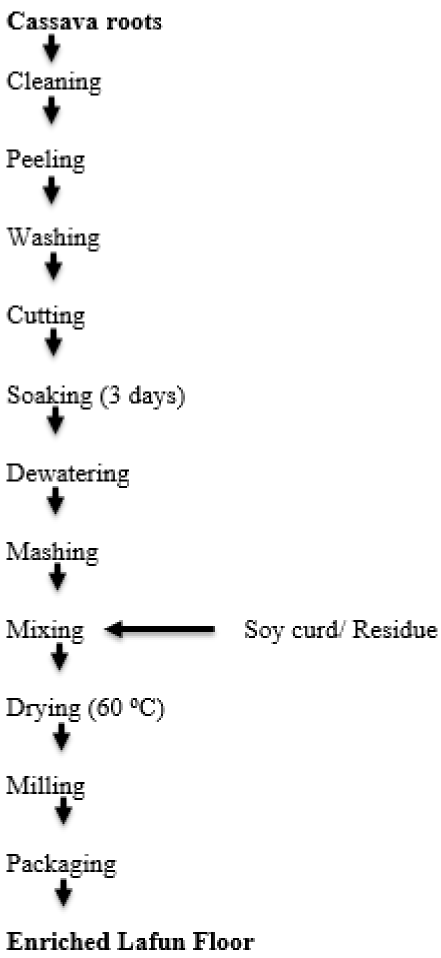

Figure 2: Production of Enriched Lafun Flour
Source: Njoku *et al.* (2013).

2.3 Accelerated Storage Stability Studies

To the enriched "lafun" samples was added Butylated Hydroxyl Toluene (BHT) as an antioxidant at 200ppm level based on 10% fat content. 200gms of each sample was packaged into high density polyethylene (HDPE) film of size 15 x 20cm (50µm [2.15 mils] thickness; water vapour transmission rate: 1.33x 10-3kgmil/m2/PA). The packaged samples were stored under three (3) different temperatures, viz: 10 ± 2^{0}C, 30 ± 2^{0}C, and 40 ± 2^{0}C. A Thermo hydrograph was placed in the storage room to record the temperature and relative humidity of the room atmosphere. Un-enriched "lafun", without soy supplement, served as control. A control sample was kept in a glass bottle flushed with nitrogen and kept at 30 ± 1^{0}C. Samples were removed at monthly intervals and subjected to physicochemical and sensory analyses.

2.4. Analyses

The samples were analysed at monthly intervals to determine their quality factors critical to deterioration of oily food materials during storage such as moisture content, thiobarbituric acid number, non-enzymic browning and free fatty acid. Moisture content was determined by the oven dry method (AOAC, 2005) by drying triplicate samples in a hot-air circulating oven (Galenkhamp) at 105oC for 5 hours The analysis of thiobarbituric value was carried out according to the method described by AOAC (2000). About 5 g of the 'lafun' sample were placed in a beaker before adding 50 ml of a 20% trichloroacetic acid and 1.6% of m-phosphoric acid solution for about 30 minutes, before filtering the slurry. The residue were diluted with 5 ml of freshly prepared 0.02 M (1.44g in 500 ml of distilled water) 4, 6-dihydroxypyrimidine-2-thiol and mixed. Tubes were stored in the dark for 15 hours to develop the colour, before the colour was measure by a spectrophotometer at a wavelength of 538 nm**.** The non-enzymic browning of lafun samples was measured according to the method described by Oluwamukomi and Adeyemi (2015). This was done by monitoring the melanoidin pigment production using the colorimetric method. The extract used was prepared by suspending a 5g sample in 50 ml 60% ethanol (v/v) and allowing it to stand for 12hrs. The extract was filtered and its absorbance (A) was measured at 420nm wavelength using ethanol as a blank. The rate of browning was expressed as change in absorbance per month (δA_{420}/ month). The free fatty acid was analysed by first of all extracting the oil used for the test, from the sample with petroleum ether (40-60°C). 5g of the oil was dissolved in 50 ml neutral alcohol and allowed to boil. This was quickly titrated with aqueous 0.1 M sodium hydroxide against phenolphthalein indicator shaking constantly until a pink colour persisted for 15 seconds (Oluwamukomi and Adeyemi, 2015).

3. Results and Discussion

3.1 Effect of Temperature and enrichment on Moisture Content

The moisture content increased slightly with storage time in 10°C and 30°C but decreased significantly at 40°C (Fig.3). All the sample (CL, LER, LEC and CS) stored in refrigerator at 10^{0}C increase in this order 6.67%-8.65%, 4.61%-6.18%, 5.95%-7.68% and 6.67%-9.21 respectively, while the same samples CL, LER, LEC and CS stored under ambient temperature (30^{0}C) also increase from about 6.39%-9.89%, 4.29%-8.00%, 5.76%-8.73% and 6.48%-7.54% respectively, within the six months interval of storage. This trend was not followed in the same samples CL, LER, LEC, and CS stored at 40^{0}C (incubator). The samples decrease with the days of storage from LEC 6.43%-5.41%, LER 5.84%-4.18%, CL 6.48-5.41 and CS 6.54-5.39. These changes in moisture content with changes in storage temperature might have been due to the hygroscopic properties of soy enriched lafun granules and the relative humidity of the environment. It was also observed that enrichment reduces the level of moisture absorption of the samples under the three storage conditions (10^{0}C, 30^{0}C and 40^{0}C). From figure 3, it was observed that at 40°C, there was a significant decrease in the moisture content (P ≤ 0.05). The increase in moisture content at 10^{0}C and 30ºC may be due to gradual equilibration with the high ambient relative humidity in the refrigerator and the ambient temperature (atmosphere), while the decrease in moisture content at 40ºC may be due to gradual equilibration with the low ambient relative humidity of the hot air in the incubator leading to evaporation of water from the lafun granules. In a similar storage study, Gopika *et al*. (2014) observed that the moisture of sunflower kernel was reduced from 5% for samples stored at 4oC and 60-80% R.H to 2.1% at 21^{0}C and 40% R.H and to 1.8% at 38^{0}C and 25% RH. This is also similar to findings of Alphonse *et al.* (2018) who found out that the moisture of ghewar, an Indian traditional sweet decreased in moisture content from 9.0 to 7.6% in LDPE bag.

Ajum *et al*. (2013) observed that a food blend from corn sugar and fat picked up additional moisture of 2.1% in HDPE at the end of 4½ month storage at 27ºC. Alphonse *et al*. (2018) also observed that white "gari" packed in polythene bag increased from a moisture content of 18.6 to 22.3% in two months.

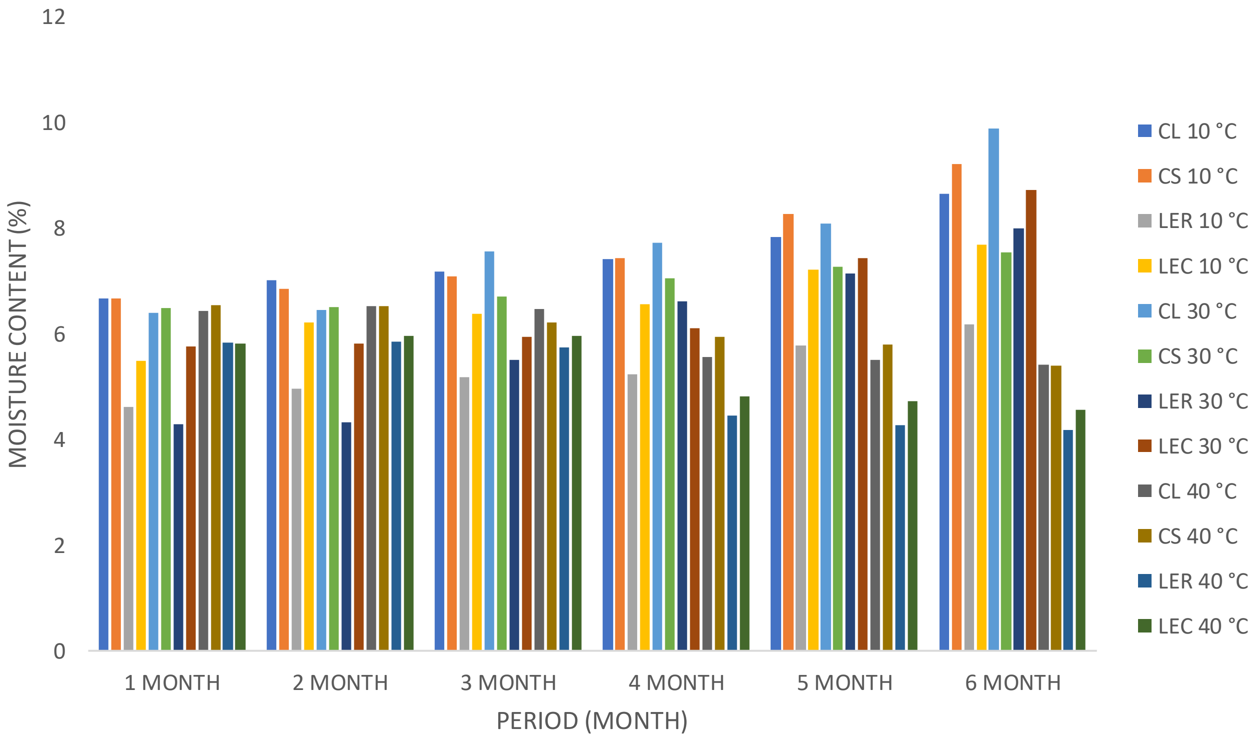

Figure 3: Effect of temperature and enrichment on the moisture content of soy-enriched and control "lafun" stored at 10^{0}C, 30^{0}C and 40^{0}C.

3.2. Effect of temperature and enrichment on non-enzymatic browning

In figure 4 there were little or no significant difference in the browning reaction at 10^{0}C and 30^{0}C during the six months of storage rather, the changes noticed was as a result of enrichment that slightly increased the level of browning in the samples stored at 10^{0}C for LER (0.02-0.03 nm) LEC (0.02-0.03 nm) at 30^{0}C is given as LER (0.02-0.06 nm) and LEC (0.02-0.06 nm). This observation was quite different from the trend noticed in the same lafun samples stored under 40^{0}C which had a significantly increased non- enzymic browning for the enriched samples LER (0.03-0.17 nm) and LEC (0.04-0.2 nm). At 40^{0}C browning increased sharply after about three months of storage in all the samples but remarkably higher in the enriched samples. Enrichment and temperature increased the browning reaction which could be due to maillard reaction at higher temperature (40^{0}C) leading to non-enzymic browning**.** The browning reaction did not start from the beginning of the storage which agreed with earlier observation by Jian *et al*. (2018) for skim milk, and Ashleigh *et al*. (2018) for grape fruit juice. Non-enzymic browning increased with increased in temperature (30-40^{0}C) enrichment and storage time. Germah *et al*. (2011) observed this behaviour or trend in a little way for soy-fortified fermented maize meal stored at 25^{0}C and 35^{0}C for 130 days; but at 60^{0}C sample did not store for more than 10 days or 60 days at 45^{0}C.

There were no significant difference in the value of non-enzymic browning at lower temperature for all the samples (enriched and control), but at 40ºC the enriched samples became more brownish in colour.

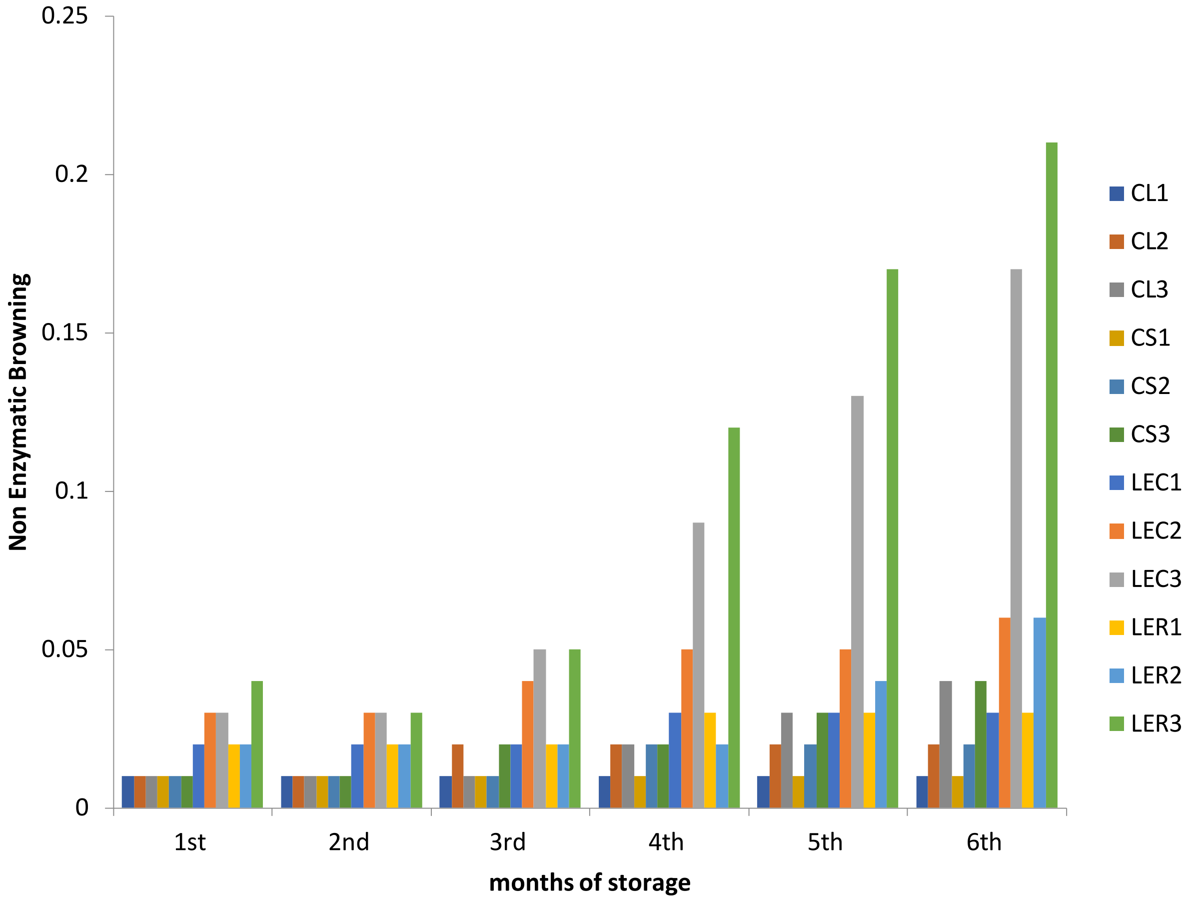

Figure 4: Effect of temperature and enrichment on the non enzymic browning of soy-enriched and control "lafun" stored at 10^0C, 30^0C and 40^0C.

3.3 Effect of temperature and enrichment on free fatty acid

Figure 5 showed the FFA value which is a measure of the extent to which the triglyceride in the oil has been decomposed by lipase action increased slightly with period of storage. Significant difference (P<0.05) was observed in FFA value of lafun enriched and stored under 10^0C and ranged from LER 0.50-0.77%, LEC 0.56-086%, 30°C LER 0.59-0.90%, to 0.56-1.07% respectively. This value obtained could be as a result of the increased in lipid content present in the soy enriched samples. This is similar to the report of Goyal *et al*. (2012) that soy bean products has the potential of increasing its lipid content leading to increase in FFA values at 10^0C and 30^0C which could be due to permeability of the packaging material and the humidity of the environment. The slight deterioration that occurred in the lafun samples stored at 40^0C, would not have been due to hydrolytic rancidity but oxidative rancidity and lipid oxidation as a result of the moisture content being less than 10% and the humidity being lower than 70% (Oluwamukomi and Adeyemi, 2015). This is similar to the findings of Savali *et al*. (2017) who observed that the FFA content of whole wheat flour increased with time which might probably due to the higher activity of lipase and lipoxidase enzyme present in the germs or aleurone layers of the wheat couple with high moisture content. Period of storage also affected free fatty acid content of lafun enriched with soy supplement in Figure 3.3. The free fatty acids value for enriched and control samples stored at 10^0C and 30^0C increased with storage period but the values remained in acceptable level in terms of aroma appearance, texture and colour. This also agreed with the findings of Botsoglou *et al*. (2012) who reported that the FFA content increased from 1.90-2.36% during refrigerated storage.

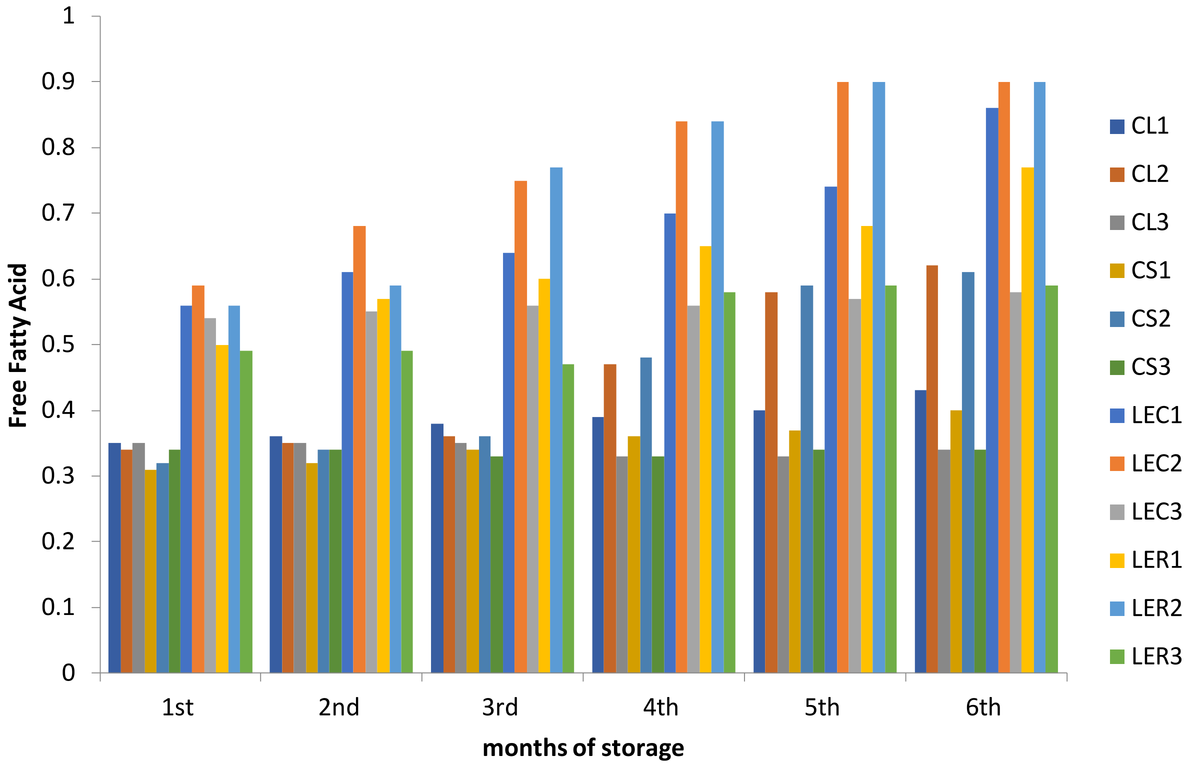

Figure 5. Effect of temperature and enrichment on the free fatty acid content of soy-enriched and control "lafun" stored at 10^0C, 30^0C and 40^0C.

Key: CL1-control lafun store at10^0C, CL2 - 30^0C, CL3 - 40^0C, s
CS1-commercial control stored at10^0C, CS2 - 30^0C, CS3 - 40^0C,
LEC1-lafun enriched with soy curd stored at10^0C, LEC2 - 30^0C, LEC3 - 40^0C,
LER1-lafun enriched with residue 10^0C, LER2 - 30^0C, LER3 - 40^0C

(16^0C) and also agreed with Gupta (2006) who reported that high FFA values are due to triacylglycerol hydrolysis that takes place upon released of water from the fried food.

3.4 Effect of temperature and enrichment on the thiobarbituric acid number

TBA is a measure of incipient oxidation of three or more double bonds in a fatty system, with the formation of secondary lipid oxidation products Meenakshisundaram *et al.* (2016) like carbonyls which are responsible for the sensory impact of lipid oxidation (Park and Drake, 2017). The higher the temperature the higher the thiobarbituric acid number measured as malonaldehyde/kg in a food sample (Amadi and Adebola, 2008). Figure 3.4 observed that TBA value of samples stored in the refrigerator (10^0C) increased slightly from 0.11-0.19, 0.20-0.27 and 0.25-0.37 for CL, LER and LEC respectively while the samples stored at 30^0C have their value as CL 0.11-0.22, 0.39-0.63 LER and 0.51-0.74 LEC, with the same lafun samples stored at 40^0C ranged CL 0.24-0.45, 0.24-0.87 LER and 0.46-0.94 LEC respectively. This showed that TBA increased with increase in temperature ($p \leq 0.05$). This corroborates with the findings of Kumar and Anasdaswamy (1981) that 'Balahar' a maize based product increased in TBA with increase in temperature. Ho *et al*. (2011) also reported that thiobarbituric acid increased steadily in milk powder stored at accelerated temperature of 45^0C for 60 days. Enrichment also had a significant effect on the thiobarbituric acid number of lafun samples. The increased in TBA value could have been as a result of the soy supplement used in the enrichment of lafun sample coupled with the accelerated high temperature which might have led to faster production of malonaldehyde in the lafun samples under storage. The thiobarbituric acid value was still within the acceptable limit which was less than 1 mg malonaldehyde kg-1 and indicates good quality lafun products (Goyal *et al*. 2012).

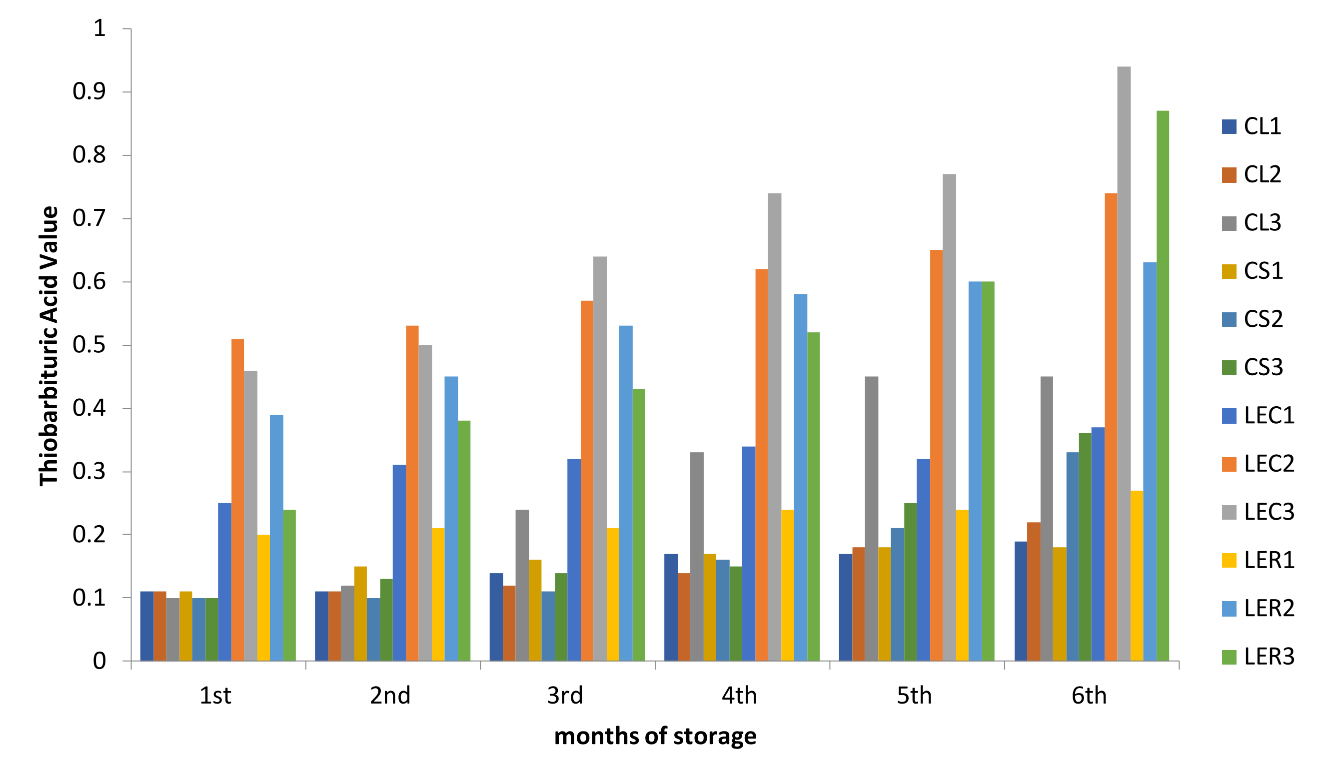

Figure 6: Effect of temperature and enrichment on the thiobarbituric acid number of soy-enriched and control "lafun" stored at 10^{0}C, 30^{0}C and 40^{0}C.

Key: CL1-control lafun store at10^{0}C, CL2 - 30^{0}C, CL3 - 40^{0}C, s
CS1-commercial control stored at10^{0}C, CS2 - 30^{0}C, CS3 - 40^{0}C,
LEC1-lafun enriched with soy curd stored at10^{0}C, LEC2 - 30^{0}C, LEC3 - 40^{0}C,
LER1-lafun enriched with residue 10^{0}C, LER2 - 30^{0}C, LER3 - 40^{0}C

Conclusion

In storage stability, the free fatty acid (FFA) was affected mostly by moisture content which increased its value as the days of storage increased. Lafun samples stored under 10°C and 30^{0}C absorbed more moisture with the days of storage which could be due to permeability of the packaging material and the humidity of the environment. Furthermore, enrichment and temperature had a significant effect on the thiobarbituric acid number (TBA) of lafun samples which was observed to have increased faster in the samples enriched with soy curd and residue than the control samples. The same factor also influenced the non-enzymic browning reaction of lafun samples which must have been due to the maillard reaction at higher temperature (40^{0}C) leading to non-enzymic browning

Competing interests

The authors report no conflicts of interest.

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