

Storage Stability of Butter Produced From Peanut, Crayfish and Ginger

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Abstract: Butters were produced from blends of peanut, crayfish and ginger to formulate samples A, (100% peanut), sample B (90 peanut:10% ginger), sample C (80% peanut:10% crayfish) and sample D (70% peanut.:20% crayfish:10% ginger). The 100% peanut butter served as the control. The effects of the substitution on storage for 12 weeks at ambient conditions were investigated. Pure peanut butter had higher initial bacterial and fungal counts of 2.7×10^3 Cfu/g and 2.6×10^2 Cfu/g respectively, which steadily decreased with storage time. With the addition of ginger, the bacterial and fungal counts were initially 1.5×10^3 Cfu/g and 1.4×10^2 Cfu/g respectively, at the end of 12 weeks of storage the values decreased to 0.8×10^3 Cfu/g and 0.4×10^2 Cfu/g respectively. Similar trends were observed when crayfish was added. The peroxide value, TBA value and acid value of the butter were 1.30 ± 0.20 Meq/kg, 0.11 ± 0.01 Mq/100g and 1.90 Mq/100g respectively. Addition of only ginger to the butter increased these values while addition of only crayfish lowered the indicators. There was significant ($p < 0.05$) increase in these parameters during storage. Oil separation was observed at the 8th week of storage, and this separation increased at week 12 of storage. Addition of 20% crayfish into the butter reduced this oil separation to 1.8mls and 2.0mls, while the addition ginger and crayfish reduced the oil separation of the butter to 2.0mls and 3.0mls. Conclusively, a mixture of ginger and crayfish into the butter significantly reduce its microbial activities, lowered its level of deterioration due to rancidity and reduced oil separation.

Keywords: Butter, Crayfish, Ginger, Peanut, Stability, Storage.

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I. Introduction

Shelf life is a major consideration in developing, producing, and marketing many food products. Consumers desire products that maintain a fresh appearance, odour, and flavour for as long as possible, and a longer shelf life reduces costs for the producer and, ultimately, for the consumer [1]. There has been an increasing consumer demand for foods free or with low synthetic preservatives, synthetic preservatives could be toxic to humans. Concomitantly, consumers have also demanded for wholesome and safe food with long shelf lives. Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices could also be exploited. The antimicrobial activity of spices is well documented and alarming interest continues to the present [2].

Groundnuts are liable to fungal contamination during handling, storage and transportation, exposing them to the risk of contamination with aflatoxin. Indeed, groundnuts can be contaminated with aflatoxin during pre- and post-harvest processing and the risk of contamination increases along the marketing chain due to poor handling practices. The main aflatoxin producing fungi in groundnuts are *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*, which mostly infect groundnuts as a complex [3]. Generally, peanut butter is produced by traditional methods characterized by unknown hygienic conditions and its storage for long durations is confronted by several storage issues such as oil separation, microbial activity and rancidity which require the incorporation of preservatives and emulsifier into the butter to combat these storage challenges [4].

Many investigators have demonstrated the antimicrobial activity of the constituents of some higher plants and quite a number of chemical compounds of plant origin shown to possess antimicrobial activities. In cases of food poisoning of microbial origin, the spices such as ginger and pepper are helpful because of their antimicrobial activity against the causative agents [5]. Ginger (*Zingiberofficinale*) is a perennial herb, with leafy stem up to 60 cm. The rhizome is horizontal, branched, fleshy, aromatic, white or yellowish to brown. The rhizome is rich in the secondary metabolites such as phenolic compounds (gingerol, paradol and shogaol), volatile compounds (zingiberene and bisabolene) and monoterpenoids (curcumene and citral) [6]. Previous studies have demonstrated that plant extracts and isolated compounds from *Z. officinale* possess strong antioxidant, antibacterial, antifungal, anticancer and anti-inflammatory effects [7]. In food industry, both pathogenic and food spoilage bacteria can attach and form a bio-film on food contact surfaces and food product.

The incorporation of spices like *Z. officinale* is widely considered to offer a decreasing effect on the activities of such microbes in food.

Crayfish are small fresh water crustaceans that can be found in many parts of the world. These distinctive invertebrates are rich protein sources and serve as good protein supplements in our diets [8]. They are proximately composed of 13.86% ash, 58.14% protein, 1.28% crude fibre, 89.92% dry matter and 2.87% gross energy [9]. Aside the high nutrient content of crayfish, it is also known for its oil binding and anti-oxidant activities. [10] In order to combat oil separation on the surface, inconsistent smoothness, spread ability, stickiness due to storage and rapid deterioration of peanut butter during storage, crayfish and ginger were substituted in different blends of peanut butter to assess the effects of ginger and crayfish addition on the microbiological, chemical and sensory attributes of fresh and stored butter.

II. Materials and Methods

2.1. Source of Raw Materials

Peanuts, ginger, crayfish, salt and spices (calabash nutmeg) were purchased from Modern market Makurdi, Benue State. All chemicals and reagents used were of analytical grade and purchased from credible scientific chemical suppliers.

2.2. Sample Preparation

The peanut seed (Red Boro specie), ginger and crayfish were subjected to pre-treatment and blended as shown in Table 1.

2.2.1. Preparation of Roasted Peanut Seed

Peanut seed was subjected to pre-treatment, the seed where cleaned and sorted for bad seeds, after which it was roasted at 116 degrees for 20 minutes, another cleaning and selection was done to remove the skin.

2.2.2 Preparation of Ginger Powder

Ginger roots were collected, cleaned, sorted and sliced to 2cm and dried in the oven at 60 degrees after which it was milled and sieved using 0.6mm sieved to get the ginger powder.

2.2.3. Preparation of Crayfish Powder

Crayfish was collected and it was winnowed and preheated in the oven for few seconds, milled and sieved using 0.6mm sieve to get the crayfish powder.

2.3 Formulation of Butter Preparation

The roasted peanut seed was milled with the ginger and crayfish powder for uniform homogenization using attrition mill, it was allowed to cooled and packed in hermetically sealed containers.

2.4. Determination of Microbial Load

Microbiological test was determined by [11], 0.1ml of the sample was aseptically pipette into the bottom of a sterile petri dish. 20ml of melted nutrient agar media was poured into each plate, flaming the neck of the flask between two plates poured. The plates were swirled to mix the inoculums and the medium, it was then allowed to solidify, the plates inverted and incubated at 37 degrees for 24-48hours. Potato dextrose agar, pH 5.6 was used for yeast and mould count serial dilutions and spread on the solidified PDA plates. These plates were incubated at 25 degrees for 4-5days.

2.5 Aflatoxin Determination

Analysis of aflatoxins was performed using high performance liquid chromatography according to the method described by [12] Samples of the butter were mixed with distilled water 1:1 and blended. A little of the sample 25g or 30g was placed in an Erlenmeyer flask containing NaCl (5g) and methanol/water (70:30).samples were placed in an orbital shaker for 30 minutes, and an aliquot(15ml) was transferred to a 50ml beaker. 30mls of ultra-pure water were added to the extract, and the homogenized mixture was filtered through a 1.5 micrometer filter. An aliquot 15 mls was then passed through the immune affinity column. After washing with ultra-pure water (10 mls) the column was eluted with methanol (1ml) and the diluents was collected in an amber vial. Solvent flow in the columns was kept at 2-3mls per minute.

The diluents were evaporated to dryness; trifluoroacetic acid and n-hexane (200 μ L) were added to it. The mixture was kept at 35°C for 10mins, then evaporated to near dryness and diluted in methanol/water/acetonitrile (500 μ L, 60:20:20 v/v/v). Final extracts were filtered through a 0.45 μ m PTFE membrane and 20 μ L were injected into a high performance liquid chromatography column. Reading of the HPLC were taken and recorded as the aflatoxin values of various peanut butter samples.

2.6 Assessment of the Peanut Butter oil

2.6.1 Peroxide value

This was carried out using a method as described by [13]. A 5.0 g of sample was weighed into a 250 ml Erlenmeyer flask and then added 30 ml acetic acid - chloroform (3:2) solution (under the hood). The flask was swirled until the sample was dissolved, then 0.5 ml saturated potassium iodine (KI) solution was added. The solution was allowed to stand with occasional swirling for one minute and then added 30 ml distilled water. It was later slowly titrated with 0.01 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) adding it with constant and vigorous shaking. Continued titrating until the colour changed to light yellow. A 0.5 ml of 1% soluble starch indicator was then added to the solution which gave a blue colour. Titration then continued and shook the flask vigorously near the endpoint which is a faint blue colour to liberate all of the iodine from the chloroform (CHCl_3) layer. Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) was added drop-wisely until the blue colour just disappeared. Peroxide value is recorded in ml-equivalent from peroxide in every 1000 g.

2.6.2 Acid value

This was carried out using a method as described by [13]. A 5.0 g of the sample was weighed and poured into a conical flask. About 50ml of hot neutral alcohol and a few drops of phenolphthalein were added to the sample inside the conical flask and shook vigorously, and then titrated with 0.5 N sodium hydroxide solution with constant shaking until the pink colour remains permanent. From the amount of 0.5N alkali used, the percentage of FFA was calculated in terms of oleic acid where 1ml of 0.5N NaOH = 0.141gm oleic acid.

2.6.3 TBA value

The food sample with 50ml water was macerated for 2 minutes and washed into a distillation flask with 47.5ml water, 2.5ml of 4M HCL acid was added to bring the pH to 1 followed by anti-foam preparation and a few glass beads, the flask was heated by means of electric mantle so that 50ml distillate is collected in 10 minutes from the time boiling commences, 5ml distillate was pipette into a glass stopper tube 5ml TBA reagent was added shook and heated in boiling water for 35minutes, a blank was prepared similarly using 5ml water reagent, then cooled in Water for 10 minutes and measured the absorbance D against the blank 538mm using 1cm cells.

2.7 Oil Separation Analysis

The samples were placed in 100ml graduated cylinders for the weekly oil separation determination. The graduated cylinders were stored undisturbed at ambient conditions for 12 weeks as described by [14].

2.8 Sensory Properties of Peanut Butter Incorporated with Crayfish and Ginger

The peanut butter samples were kept in the sensory laboratory for sensory evaluation, A 9-point Hedonic scale (where 1 = dislike extremely, 9 = like extremely) was used to rate the sensory attributes of appearance, taste, aroma, texture and overall acceptability of the products. Each panelist was presented with four different samples. A sachet of drinkable water and carrot were also given for mouth rinsing and taste removal respectively in-between evaluation of samples [15]. The procedure was carried out weekly for the period of storage.

2.9 Statistical Analysis

Statistical package for social sciences (SPSS) version 16 statistical software package was used for the statistical analysis of all the datas except for the microbial load. Similarities and differences amongst data were subjected to analysis of variance (ANOVA).Duncan multiple range test (DMRT) was used for mean separation and significance was accepted at 5% probability level.

III. Result and Discussion

3.1. Aflatoxin content of peanut Butter incorporated with Crayfish and Ginger

Result of the aflatoxin content of peanut butter incorporated with ginger and crayfish is presented in Table 2. Production of aflatoxins occurs when the crops are exposed to high temperatures, drought, high moisture, oxygen concentration and infestation by insects and occur both at pre-harvest and post-harvest period [16]. The result of this study showed that, aflatoxin was detected only in samples A (100% peanut butter) and sample B (90% peanut and 10% ginger). This is in agreement with [17] who reported that foods composed purely of plants parts are more vulnerable to fungi attacks. In the zero week of storage, sample A and B had the same aflatoxin content of 0.01 ± 0.01 ppb which showed that the peanut butter had low aflatoxin content that was safe for human consumption. At the end of 12 weeks of storage, there was significant increase in the aflatoxin content of sample A and B. the rise indicates a slake in storage process of the peanut butter samples [18]. There was no trace of aflotoxin in samples C and D. Although the aflatoxin content of samples A and B increased considerably during storage it remained within the NAFDAC stipulated standard of 15ppb and poses no health threat to consumers of the product especially adults who have a high resistance ability to aflatoxin infections.

3.2 Microbiological Load of Peanut Butter Produced with Crayfish and Ginger

The result of the microbiological load of the peanut butter incorporated with crayfish and ginger is presented in Table 3. The bacterial count of the peanut butter decreased when ginger was incorporated into the

peanut butter but there was no change in the bacteria count when crayfish was incorporated into the peanut butter. In both samples, the bacteria load decreased with storage time except in sample D where the initial bacteria count decreased for the first four weeks of storage, then, increased in the 8 and 12 weeks of storage. Fungal counts decreased on the incorporation of ginger, crayfish and a mixture of ginger and crayfish into the butter. Fungal count decreased continually as the storage time increased. The result showed that microbiologically, peanut butter is a pretty safe food. [19] reported that peanut butter is microbiologically stable and attributed such microbial safety to the production processes of blanching, roasting and grinding of the peanut at high temperature during which a lot of microbial loads must have been eliminated. [20], also attributed the microbial safety and stability of peanut butter to their low moisture and high oil content.

Pure peanut butter (sample A) contained a bacteria load of 2.7×10^3 Cfug and the bacterial load decreased with increasing storage time. During the 4th, 8th and 12th weeks of storage, the bacterial load decreased to 2.1×10^3 , 1.6×10^3 and 1.4×10^3 respectively. This result agreed with the findings of [21] that the microbial load of some foods decreased with storage time. The authors attributed such decrease in the bacteria content of foods to the depletion of nutrients used by the bacteria and the building up of an anaerobic environment in the package due to the accumulation of carbon dioxide gas (CO₂) produced from the respiratory activities of the bacterial.

In sample B, the microbial analysis revealed that, the incorporation of 10% ginger into the butter reduced the amount of bacteria contained in the butter from 2.7×10^3 to 1.5×10^3 . [22] reported that, ginger exhibits a very high anti-microbial activity on foods. The authors explained that ginger contains 5-acetoxy-gingerol, 3, 5-diacetoxy-gingerdiol and galanolactone which inhibit microorganisms and their activities in foods.

The addition of 20% crayfish (sample C) to the peanut butter created a significant difference on the initial bacteria load of the butter. This result compares favourably with the result of [19] who incorporated crayfish into *ogi* and came out with the conclusions that crayfish imposed no increasing or decreasing effect on the bacteria content of the *ogi*. Like in all other samples, the bacterial load of sample C decreased with increase in storage time, agreeing with the explanations of [21]. A lower bacterial load was observed in sample D where both ginger and crayfish were incorporated into the butter. This decrease might be due to the anti-bacterial activity of ginger as discussed earlier like in samples A, B, and C above, the microbial load of peanut butter decreased from 1.3×10^3 to 1.0×10^3 in the first four (4) weeks of storage, then, to 1.8×10^2 in the 8th week and 1.6×10^2 Cfug in the 12th week.

A low fungal count was recorded in the peanut butter. Pure peanut butter (sample A) had a fungal count of 2.6×10^2 Cfug which was slightly higher than the load recorded by [10]. The fungal count of the peanut butter reduced to 2.2×10^2 Cfug, 1.7×10^2 Cfug and 1.2×10^2 Cfug when it was stored for 4, 8 and 12 weeks, respectively. The incorporation of 10% ginger in the butter resulted to a decrease in fungal count from 2.6×10^2 Cfug to 1.4×10^2 cfug due to the anti-microbial activities in the ginger [22]. As the storage time increased to 4, 8 and 12 weeks, the fungi count of the ginger incorporated peanut butter reduced to 1.0×10^2 Cfug, 0.6×10^2 Cfug and 0.4×10^2 Cfug respectively. Sample D with 10% ginger and 20% crayfish incorporation had the least fungal count at a storage time of 12 weeks.

On a general note, the bacterial and fungal counts of peanut butter were low and this could be as a result of many factors. Several thermal processing unit operations were involved in the production of the peanut butter which must have affected the survival of the bacteria and fungi [19]. The product was also found to have contained low moisture with an appreciable percent of the water in bounded form resulting in low water activity in the product, thus affecting microbial activities in the product as explained by [22]. The peanut butter was also incorporated with ginger, a rhizome containing high anti-microbial substances [10] and it was stored in air tight containers that were hermetically sealed to promote anaerobic respiration that affects the survival of most microbes.

3.3. Storage Stability of peanut Butter oil

The various storage stability indices of the peanut butters such as peroxide value, thiobabutriuric acid value and acid value are presented in Table 4. The peroxide value of the peanut butter increased insignificantly when ginger was incorporated but decreased significantly when crayfish and a mixture of ginger and crayfish were incorporated into the butter. In both samples, the peroxide value of the butter increased with storage time. The TBA value decreased insignificantly as ginger, crayfish and a mixture of both ginger and crayfish were incorporated into the peanut butter and values for all samples decreased with storage time. The acid value was significantly increased when ginger was incorporated into the butter but the incorporation of crayfish and a mixture of ginger and crayfish created no significant difference in the butter's acid value. The acid value in all samples increased continuously with storage time.

Peroxide value

The peroxide value of the butter is the amount of peroxide contained in the butter and is expressed a mili-equivalent of peroxides per kg of the sample (Meq/kg). It indicates the butters level of primary oxidation

and its likeness to oxidative rancidity [23]. The peroxide value is also used to predict the shelf life of some products like butter. A lower peroxide value indicates good quality butter and a good preservation status while large peroxide values indicate poor quality butter [24].

The peroxide value of pure peanut butter (sample A) was found to be 1.30 ± 0.20 Meq/kg which is in agreement with the findings of [24] that the peroxide value of peanut butter ranges from 1.30 Meq/kg to 1.34 Meq/kg depending on the production process, the production sites and atmospheric conditions. This result also proves that peanut butter is rancidity free and suitable for consumption within its first week of production since its peroxide value falls between the maximum limits of 2.0 Meq/kg set by NAFDAC for all edible oils and butter. Storing the peanut butter for a period of 4 weeks, 8 weeks and 12 weeks showed significant increases ($p < 0.05$) in the peroxide values of the oil to 1.80 ± 0.10 Meq/kg, 2.53 ± 0.23 Meq/kg and 3.27 ± 0.40 Meq/kg, respectively. This shows that, the butter became rancid with storage time which is an indication of the butters depletion in quality. [15] pointed out that, the peroxide value of butters and oils increased with storage time due to the products interaction with the atmospheric oxygen, exposure to high temperature and/or excess sunlight. In respect to the maximum peroxide value standard set by NAFDAC (2.0 Meq/kg), pure peanut butter stored for a period of 0-4 weeks is safe for consumption while that stored for 8-12 weeks is too rancid and unsuitable for human consumption.

The incorporation of 10% ginger into the peanut butter showed an increase but insignificant difference in the peroxide value of the butter. Peanut butter incorporated with 10% ginger and stored for zero weeks had a peroxide value of 1.50 ± 0.17 Meq/kg which is insignificantly higher than that of pure peanut butter (sample A) stored for zero week. These findings compared favorably with the results of [25] that the incorporation of rhizomes into oil rich meals possesses no significant effects on its peroxide value. Storage of the ginger incorporated peanut butter for a further duration of 4, 8 and 12 weeks significantly increased its peroxide values to 2.20 ± 0.17 Meq/kg, 2.93 ± 0.23 Meq/kg and 3.30 ± 0.17 Meq/kg, respectively. This result showed that, the peroxide value of the butter increased greatly as a result of storage time. It also showed that, ginger incorporated peanut butter stored for 4, 8 and 12 weeks were very rancid and not suitable for human consumption as stipulated by NAFDAC. [25] attributed the increased rancidity of butter with storage time to the level of oil separation. He explained that the oil contained in the butter increasingly separated to the top layer of the packaging material with storage time and shows more affinity to trap oxygen molecules which greatly increase its peroxide value.

In sample C where the peanut butter was incorporated with 20% crayfish, the peroxide value of the butter was reduced to a significantly different value of 0.83 ± 0.23 Meq/kg. This decrease in peroxide value of the butter is best explained by attributing it to the conclusions of [8] that crayfish when incorporated in foods binds its constituents together and leaves little or no room for their chemical separation, thus making no oil available for interaction with agents of rancidity. Storage of the crayfish incorporated butter for an increased period of 4 and 8 weeks showed an increased level of peroxide value of the butter to slightly significant levels. These changes are known to be due to the high temperature (40 degrees) in the storage room. However, such significant changes could not affect the overall quality of the butter since it still remained within the limit of 2.0 Meq/kg recommended by the regulatory agency. Storing the butter to an increased period of 12 weeks significantly affected both the peroxide value of the oil and its quality. This is because the peroxide value was increased to a significant level of 2.10 ± 0.0 Meq/kg which is above the approved regulatory value of 2.0 Meq/kg for the product.

The incorporation of both ginger and crayfish into the butter (sample D) resulted in increased the peroxide value of the oil. Storage for 4, 8 and 12 weeks increased its level of rancidity but maintained its peroxide value within the regulatory point of 2.0 Meq/kg. The peroxide values at 4th, 8th and 12th weeks of storage were 0.97 ± 0.23 Meq/kg, 1.70 ± 0.00 Meq/kg and 2.0 ± 0.17 Meq/kg, respectively. Thus, the shelf life of peanut butter incorporated with ginger and crayfish can be predicted to be 12 weeks from the date of production depending on storage conditions. [27] also agreed that the peroxide value of oils and their overall level of rancidity increased with storage time.

Thiobarbituric acid (TBA) value

The thiobarbituric acid (TBA) value is used to investigate secondary oxidation of the butter. High TBA values indicate the butter's high level secondary oxidation and possible formation of aldehydes, TBA values are usually small because secondary oxidation of oils is highly undesirable and its occurrence in a well processed and packaged oil or butter is a rare phenomenon. The regulatory standard for TBA has been set at 0.35 mg/100g. The TBA value of pure peanut butter (sample A) was 0.11 ± 0.01 mg/100g. This TBA value of peanut butter is lower than that of sesame seed butter (0.15 mg/100g) reported by [28]. Storing sample A for 4, 8 and 12 weeks increased the TBA value of the peanut butter to significant levels of 0.12 ± 0.10 mg/100g, 0.21 ± 0.01 mg/100g and 0.28 mg/100g, respectively. [29] had earlier reported that the secondary oxidation of vegetable oils and butter increases with storage time.

The incorporation of 10% ginger into the peanut butter showed an insignificant decrease in the TBA value of the butter from 0.11 ± 0.01 mg/100g to 0.08 ± 0.01 mg/100g. This result proved the antioxidant activity of ginger reported by [30]. Storing the butter (sample B) for a period of 4, 8 and 12 weeks resulted in a significant increase in the TBA value of the butter. The significant increase could be due to the low concentration of ginger (10%) incorporated into the butter. [30] also explained that, as storage time increases, the rhizome gradually drops in concentration reducing its antioxidant and microbial effect and as a result secondary oxidation sets in, resulting in increases in the TBA value of the oil or butter.

The incorporation of 20% crayfish into the butter (sample C) decreased the TBA value of the butter to a lesser level of 0.04 ± 0.01 mg/100g, this could be due to the ability of the chitosans contained in the chitin of crayfish to bind oil contained in the butter, making them saturated and less reactive to agents of secondary oxidation. Famous Chinese food scientist [10] explained that, antioxidants prevent oxidation of foods by binding their available fats and oils which could have readily caused rancidity. Storage of the crayfish incorporated butter for a period of 4, 8 and 12 weeks resulted in a significant increase in the TBA value of the butter from 0.04 ± 0.01 mg/100g to 0.11 ± 0.01 mg/100g, 0.18 ± 0.01 mg/100g and 0.26 ± 0.02 mg/100g, respectively. This increase in TBA could be due to the limited content of crayfish to act as an antioxidant for the aforementioned time. The result is in total agreement with the claims of [31] that for crayfish to exhibit a steady and long lasting antioxidant activity in foods with lots of oil, it must be incorporated in a good amount of 20% and above. On a general note, the storage of peanut butter (incorporated with ginger, crayfish or not) for a time frame of 4, 8 and 12 weeks led to increase in TBA values but all the values were below the regulatory standard value of 0.35mg/100g.

Acid value

The acid value analysis is a good measure of the oils degree of hydrolytic rancidity. Hydrolytic rancidity occurs as a result of the interaction of the oil or butter with water and enzyme, High acid values indicate high level of liberated free fatty acids in the oil or butter while low levels show a lower level of liberated free fatty acids and a better quality product. The result showed that, pure peanut butter (sample A) contained an acid value of 1.90 ± 0.10 mg/100g. This is within the range (1.79-2.52) by [32] but lower compared to the acid value of cone oil as reported by [33]. This low acid value of the peanut butter could be as a result of the low moisture content of peanut butter. Storing the peanut butter for 4, 8 and 12 weeks produced a corresponding significant increase in the acid value of the butter to 2.17 ± 0.06 mg/100g, 2.43 ± 0.06 mg/100g and 2.77 ± 0.12 mg/100g respectively. This result agrees with that of [34] who reported that the acid value of most oils increase with storage time when they analysed the acid value of sunflower oil stored for an interval of 2-24 weeks. The incorporation of 10% ginger led to a significant increase in the acid value of the butter from 1.90 ± 0.10 mg/100g to 2.17 ± 0.12 mg/100g while subsequent storage of the butter for a period of 4, 8 and 12 weeks produced further significant increases in the acid values of the butter to 3.00 ± 0.17 mg/100g, 3.20 ± 0.17 mg/100g and 3.67 ± 0.00 mg/100g, respectively. The increases in the acid values of the butter show that the glyceride in the oil has been decomposed by actions such as light and heat during the storage time as earlier reported by [35].

In sample C where 20% crayfish was incorporated, a lower acid value of 1.37 ± 0.15 mg/100g was recorded. This implies that crayfish exerted a retarding effect on the hydrolysis of triglycerols contained in the butter into free fatty acids [36]. Storage of the butter for periods of 4, 8 and 12 weeks, led to significant ($p < 0.05$) increase in acid value to 1.73 ± 0.11 mg/100g, 1.93 ± 0.11 mg/100g and 2.70 ± 0.10 mg/100g, respectively. This means that as the butter aged, the crayfish lost its retarding impact and hydrolysis of the triglycerols set in. When 10% ginger and 20% crayfish were incorporated into the butter, the hydrolysis and autolysis of triglycerols to free fatty acids was reduced to its minimal level and a significant decrease in the acid value of the butter was observed (1.20 ± 0.10 mg/100g) producing a peanut butter of better quality. This result is in a state of total agreement in [37] that no matter how good the storage conditions of an oil product may be, a slight increase in its acid value will always be observed.

3.4 Oil Separation Profile of Peanut Butter Incorporated with Crayfish and Ginger

Oil separation did not occur in any of the samples at the first weeks of storage (Table 5). However, in the fourth week, samples B and D showed oil separation at varying degrees with that of sample B standing at 8% and sample D at 0.5%. In the 8th and 12th weeks of storage, oil separation occurred in all the samples, with that of samples A and B being higher while samples C and D were relatively lower.

In the 8th and 12th weeks of storage, the oil content of the pure peanut butter (sample A) separated to worrisome levels of 15mls and 20mls respectively. This result of an increased oil separation level with storage time in peanut butter is in agreement with the report of [28, 36, 39] that the rate of oil separation in peanut butter during storage is the greatest factor militating against its storage, worldwide distribution and marketing. In the same manner, the storage of sample B (butter incorporated with ginger) for the storage period of 8 and 12 weeks

showed a significant level of oil separation of 10 and 15mls respectively. The presences of oil in sample B at the 4th weeks indicate that ginger contributed to the oil content of the peanut butter. This shows that ginger has little or no effect in reducing the level of oil separation of foods when it is incorporated into the food as earlier highlighted by [21].

Storage of sample C (peanut butter incorporated with crayfish) for a period of 8 and 12 weeks showed a very low level of oil separation of 1.8mls and 2.0mls. Sample D where both ginger and crayfish were incorporated into the butter had slightly higher oil separation level of 2.0mls and 3.0mls respectively when compared with sample C incorporated with 20% crayfish. These results indicate the ability of crayfish to bind oils when incorporated into oil rich foods. Similar results were also reported by [10, 31, 38]. [10] explained that, the oil binding ability of crayfish is as a result of its high content of chitosans. He explained further that crayfish have chitin which act as an exoskeleton that gives crayfish its hard shape and chitin is chiefly composed of chitosans that are capable of binding lipids. [10] explained that, chitosans binds oils because it possess a positive charge that chemically react with and binds the negatively charged oil molecules. [31] added that, when the positive charges of chitosans and the negative charges of oil molecules are strongly bonded and a thick film is formed which leaves little or no chances for oil separation.

3.5 Sensory Evaluation

Result of the sensory properties of peanut butter incorporated with crayfish and ginger during storage is presented in Table 6. Sample A had an appearance score of 7.05 which increased insignificantly to 8.00 in sample B, 7.65 in sample C and 7.80 in sample D. These appearance levels decreased significantly with storage time. The taste of the butter increased significantly when ginger (sample B), crayfish (sample C) and mixture of ginger and crayfish (sample D). In all the samples, the taste of the butter decreased significantly with storage time. The aroma of the butter was insignificantly improved as the ginger, crayfish and a mixture of both was incorporated into the peanut butter, sample B where ginger was incorporated had a highest aroma improvement. Sample D was the most accepted as incorporation of ginger and crayfish induced the overall acceptability of the butter.

Sample A, with 100% peanut butter had the best appearance during the first week of storage. The quality of the products in terms of its appearance decreased significantly with storage time (4-12weeks). This decrease could be due to oil separation in which the 100% peanut butter recorded the highest value. The taste of the pure peanut butter was most preferred at the first week of storage. The taste of the butter like its appearance depreciated as the product was stored for a period of 4, 8 and 12 weeks. The decrease in the taste of the butter from the first week to the 4th week of storage was not significant whereas the mean scores on the 8th and 12th weeks were significant. Sample A also had a better aroma during its first week of storage and its aroma was significantly affected during storage for a period of 4, 8 and 12 weeks. This depreciation in the taste and aroma of the butter might be due to an increased rancidity level of the butter where the peroxide and acid value of the butter increased and hence, its level of rancidity increased with storage time. [15] pointed out that, activities of rancidity in foods produces volatile products which does not only affects its aroma but also its taste. The overall acceptability of sample A was higher in the first week of storage and reduced to an insignificant level during the 4th week of storage. Storage of the product for an increased period of 8 and 12 weeks saw a significant drop in its level of acceptability.

When the butter was incorporated with 10% ginger (sample B), the product had an improved appearance level which was higher than that of sample A. The products level of appearance decreased significantly with storage time of 4, 8 and 12 weeks but it is worth mentioning that the products appearance at a storage time of 12 weeks looked as good as that of sample A stored for 8 weeks. The incorporation of ginger also provided the product with an improved taste that decreased insignificantly when stored for 4, 8 and 12 weeks. The aroma of the peanut butter incorporated with ginger was greatly improved and the improved aroma decreased insignificantly when stored 1 to 4 weeks but decreased at significant levels when stored for 8 and 12 weeks. This insignificant decrease in the taste and aroma of the butter is as a result of the antioxidant and antimicrobial activity of ginger which prevented the activities of fungi whose growth is capable of aflatoxins formation.

In sample C, butter was incorporated with 20% crayfish and its good appearance decreased insignificantly between its 1st and 4th weeks of storage. Storage for a further period of 8 and 12 weeks saw a tremendous significant decrease in its appearance score. The taste of the butter also followed the same trend as its appearance. The zero and fourth week of storage recorded no significant decreases in its taste but further storage for 8 and 12 weeks significantly reduced its taste. The aroma of the butter also decreased with storage time and was significantly different at storage times of 0, 4, 8 and 12 weeks. This significant difference in the aroma of the butter is as a result of the formation of foul smelling volatile compounds. This could be because of the non-antimicrobial activity of crayfish, where the microbial load of sample C was not affected by the incorporation of crayfish.

Sample D where 10% ginger and 20% crayfish were incorporated into the butter, an appearance score of 7.80 was recorded which decreased insignificantly to 7.30 at a storage time of 4 weeks. The decrease in the appearance of the butter however, became significant as the storage time increased to 8 and 12 weeks. A taste score of 6.95 was also obtained which decreased insignificantly when the butter was stored for duration of 4 and 8 weeks but was significantly affected when stored for 12 weeks. The aroma score was also great at 7.60 due to the ginger's flavour. This aroma decreased constantly at significant levels as the butter was stored for 4, 8 and 12 weeks. The product recorded the highest level acceptability due to its better appearance, taste, and flavour in relation to the other peanut butter products.

IV. Conclusion

The results from this study have shown that, the storage stability of peanut butter was significantly improved when the butter was incorporated with crayfish. The challenges usually encountered during the storage of peanut butter such as oil separation, microbial activities, and its general deterioration due to oxidative and/or hydrolytic rancidity were reduced to minimal level when ginger and crayfish were incorporated to the butter. This also improved the shelf life of the butter to a period that ensures its stability during safe storage, distribution and marketing thereby, ensuring peanut butter's availability all year round. A mixture of both ginger and crayfish offered more desirable effects to the product than when only ginger or crayfish were individually incorporated into the butter. As such, the incorporation of a mixture of ginger and crayfish into the butter is preferable and should be the widely practiced blend

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Table 1: Blend Formulation

Sample	A	B	C	D
Peanut	100	90	80	70
Ginger	-	10	-	10
Crayfish	-	-	20	20

Table 2: Effect of Storage on the Aflatoxins Level of the Butter Blends

	A	B	C	D	
Aflatoxins (ppb)	Week 0	0.01±0.01 ^a	0.01±0.01 ^a	ND	ND
	Week 4	0.02±0.00 ^{ab}	0.01±0.00 ^{ab}	ND	ND
	Week 8	0.02±0.01 ^b	0.03±0.01 ^b	ND	ND
	Week 12	0.04±0.01 ^c	0.05±0.01 ^c	ND	ND

Values are means of duplicate determination + Standard Deviation

Mean with different superscript column wise are significantly difference (p<0.05).

Key: A= Control (Peanut) 100%, B= 90% Peanut, 10% Ginger, C= 80% Peanut, 20% Crayfish.

D= 70% Peanut, 10% Ginger and 20% Crayfish, ND= Not Detected

Table 3: Effect of Storage on the Microbial Load Samples

A	B	C	D (Cfu/g)		
Total Bacteria count	Week 0	2.7 x10 ³	1.5 x10 ³	2.6 x 10 ³	1.3 x10 ³
	Week 4	2.1 x10 ³	1.2 x10 ³	1.6 x 10 ³	1.0 x10 ³
	Week 8	1.6 x 10 ³	1.0 x10 ³	1.2 x 10 ³	1.8 x10 ²
	week 12	1.4 x 10 ³	0.8x10 ³	1.0 x 10 ³	1.6 x10 ²
Total Fungal count	Week 0	2.6 x10 ²	1.4x 10 ²	1.8x10 ²	1.2 x10 ²
	Week 4	2.2 x10 ²	1.0x 10 ²	1.5x10 ²	1.0 x10 ²
	Week 8	1.7 x10 ²	0.6 x10 ²	1.1x10 ²	0.4 x10 ²

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week 12 1.2×10^2 0.4×10^2 0.8×10^2 0.2×10^2

Key: A= Control (Peanut) 100%, B= 90% Peanut, 10% Ginger, C= 80% Peanut, 20% Crayfish, D= 70% Peanut, 10% Ginger and 20% Crayfish.

Table 4: Effect of Storage on Oil Quality Indices

Storage Time	Peroxide Value	TBA	Acid Value		
(Weeks)	(Meq/lkg)	Mg/100g	Mq/100g		
A	Week 0	1.30 ± 0.20^d	0.11 ± 0.01^d	1.90 ± 0.10^d	
	Week 4	1.80 ± 0.10^c	0.12 ± 0.10^c	2.17 ± 0.06^c	
	Week 8	2.53 ± 0.23^b	0.21 ± 0.01^b	2.43 ± 0.06^b	
	Week 12	3.27 ± 0.40^a	0.28 ± 0.01^a	2.77 ± 0.12^a	
	LSD	0.48	0.01	0.16	
B.	Week 0	1.50 ± 0.17^d	0.08 ± 0.01^d	2.17 ± 0.12^c	
	Week 4	2.20 ± 0.17^c	0.18 ± 0.02^c	3.00 ± 0.17^{bc}	
	Week 8	2.93 ± 0.23^b	0.23 ± 0.01^b	3.20 ± 0.17^b	
	Week 12	3.30 ± 0.17^a	0.32 ± 0.01^a	3.67 ± 0.00^a	
	LSD	0.36	0.02	0.25	
C.	Week 0	0.83 ± 0.23^b	0.04 ± 0.01^d	1.37 ± 0.15^d	
	Week 4	1.10 ± 0.00^b	0.11 ± 0.01^c	1.73 ± 0.11^c	
	Week 8	1.97 ± 0.23^a	0.18 ± 0.01^b	1.93 ± 0.11	Week 12
	2.10 ± 0.00^a	0.26 ± 0.02^a	2.70 ± 0.10^a		
	LSD	0.31	0.02	0.19	
D.	Week 0	0.60 ± 0.17^d	0.03 ± 0.00^d	1.20 ± 0.10^d	
	Week 4	0.97 ± 0.23^c	0.07 ± 0.01^c	1.53 ± 0.11^c	
	Week 8	1.70 ± 0.00^b	0.21 ± 0.01^b	1.90 ± 0.00^b	Week 12
	2.00 ± 0.17^a	0.25 ± 0.02^a	2.40 ± 0.10^a		
	LSD	0.32	0.02	0.14	

Values are means of duplicate determination + Standard Deviation

Mean with different superscript column wise are significantly difference (p<0.05).

Key: A= Control (Peanut) 100%, B= 90% Peanut, 10% Ginger, C= 80% Peanut, 20% Crayfish D= 70% Peanut, 10% Ginger and 20% Crayfish, LSD= Least Significant Difference

Table 5: Oil Separation from the Peanut Butter during Storage

	Samples			
	A	B	C	D (mls)
Week 0	-	-	-	-
Week 4	-	8	-	0.5
Week 8	15	10	1.8	2.0
Week 12	20	15	2.0	3.0

Key: A= Control (Peanut) 100%, B= 90% Peanut, 10% Ginger, C= 80% Peanut, 20% Crayfish D= 70% Peanut, 10% Ginger and 20% Crayfish

Table 6: Effect of Storage on the Sensory Attributes of the Produced Butters

Storage Time (Weeks)	Appearance	Taste	Aroma	Overall acceptability	
A	Week 0	7.05 ^a	6.85 ^a	6.90 ^a	7.00 ^a
	Week 4	6.35 ^{ab}	6.70 ^a	6.45 ^{ab}	6.35 ^a
	Week 8	5.90 ^b	5.85 ^b	5.75 ^{bc}	5.30 ^b
	Week 12	5.05 ^c	4.05 ^c	5.20 ^c	4.00 ^c
	LSD	0.83	0.71	1.10	0.97
B	Week 0	8.00 ^a	6.75 ^a	7.80 ^a	6.45 ^a
	Week 4	7.20 ^b	6.50 ^a	7.10 ^a	6.10 ^a
	Week 8	6.50 ^c	6.45 ^a	6.10 ^b	5.30 ^{ab}
	Week 12	5.90 ^c	5.80 ^a	5.05 ^c	5.15 ^b
	LSD	1.03	1.10	1.05	1.20
C	Week 0	7.65 ^a	6.55 ^a	6.95 ^a	6.50 ^a
	Week 4	7.10 ^a	6.35 ^a	5.80 ^b	5.80 ^a
	Week 8	5.90 ^b	5.05 ^b	5.25 ^{bc}	5.55 ^a
	Week 12	5.05 ^c	3.75 ^c	4.45 ^c	4.90 ^b
	LSD	1.11	1.10	1.11	1.10
D	Week 0	7.80 ^a	6.95 ^a	7.60 ^a	7.15 ^a
	Week 4	7.30 ^a	6.10 ^a	6.05 ^b	7.05 ^a
	Week 8	6.50 ^b	5.85 ^a	5.90 ^b	5.10 ^b
	Week 12	5.25 ^c	3.75 ^b	5.35 ^b	4.85 ^b
	LSD	0.64	1.03	0.91	0.70

Key: A= Control (Peanut) 100%, B= 90% Peanut, 10% Ginger, C= 80% Peanut, 20% Crayfish D= 70% Peanut, 10% Ginger and 20% Crayfish, LSD = Least Significant Difference.

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