

MICROBIOLOGICAL AND SENSORY PROPERTIES OF *TEMPEH* AS INFLUENCED BY TYPE OF LEGUMES AND STORAGE TEMPERATURE

– Research paper –

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Abstract: Bambara groundnut and lima bean are lesser-known legumes with high protein content. These legumes were incorporated into *tempeh*; a soybean-based snack to advance their uses as food. This research studied effects of the legumes on microbiological, physicochemical and sensory properties of *tempeh* during storage at ambient and refrigeration temperatures. The results showed that the total viable count of bacteria and fungi count of *tempeh* mash ranged between 6.173 - 7.903 logcfu/g and 6.602- 6.799 log cfu/g respectively. The microbial load of the refrigerated fried *tempeh* snacks was lower than samples at room temperature. Blends of the three legumes (4.079 – 7.297 logcfu/g) had the highest total viable count while lima bean *tempeh* (3.903 -5.602 logcfu/g) had the lowest. *Bacillus cereus*, *Staphylococcus epidermidis*, *Bacillus coagulan*, *Bacillus subtilis*, *Bacillus alvei*, *Bacillus Licheniformis*, *Micrococcus cryophilus*, *Propionibacterium species*, *Rhizopus oryzae*, *Rhizopus stolonifer* and *Geotricum sp* were isolated from *tempeh* snacks during storage. The results of the sensory assessment showed that lima bean *tempeh* snack was the most preferred. This research therefore suggests the use of lima bean and blends of lima bean, Bambara groundnut and soybean in production of *tempeh* to advance the use of these underutilized legumes and increase food security.

Keywords: *tempeh*; lima beans; Bambara groundnut; *Rhizopus oryzae*; lima bean

INTRODUCTION

Legumes are staple crops consumed in different forms all over the world and serves as cheap source of protein especially for vegetarians and people living in areas where animal proteins are expensive (Malomo & Abiose, 2019). They are usually boiled, processed into curd, cake, milk or pudding or fermented to produce condiments. However, among the leguminous seeds cultivated, soybean, cowpea, pea and kidney beans are important in production of food and food ingredients while Bambara groundnut and lima bean and are among the lesser-known legumes (Adegbehingbe et al., 2014). Lima bean (*Phaseolus lunatus*), a lesser-known legume belonging to the family Fabbaceae is rich in protein, digestible carbohydrate, contains appreciable amount of dietary and soluble fibre which are important in the regulation of sugar level in the blood, reducing level of cholesterol, prevention of constipation, aiding digestion, prevention of stomach upset and inflammation of

intestinal pouches (Heuzé et al., 2015; Adegbehingbe and Daramola, 2019). Lima bean could be consumed boiled, milled into flour for baking or fermented to produce condiment such as *iru* (Farinde et al., 2018).

Bambara groundnut (*Vigna subterranean* L. Verdc) is also a lesser-known legume which originated from Africa. It could be eaten raw, boiled, roasted and processed into flour for baking and confectioneries. It is regarded as a balance food because it contains essential amino acids, richer in iron and its protein contains higher lysine and methionine than most legumes (Olaleye et al., 2013). Both vegetarian and non-vegetarian are seeking for a way of reducing animal protein by turning to plant protein due to health or ethical, religious reasons and economic status (Malomo and Abiose, 2020). Several meat analogues like tofu, kinema, soy *iru* and *tempeh* have been produced from soybean.

Tempeh, a cooked and mould fermented soyabean food is the native food of people living in east and central part of Java Island in Indonesia where it has

Received: 01.03.2023

Accepted in revised form: 20.04.2023

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been consumed for decades as cheap and affordable source of protein (Tahir et al., 2018). This mould fermented soybeans are knitted together into compact cake with soft texture by the white mycelium of *Rhizopus* species (Ahnan-Winarno et al., 2021; Wikandari et al., 2021). *Rhizopus* eliminates the unwanted beany flavour and also produces proteases that soften the bean thereby increasing its digestibility. Fresh *tempeh* is highly perishable with short shelf life between 1 to 3 days when stored at ambient temperature. Drying, freeze drying and frying increases its shelf life for a couple of months (Wan et al., 2018). Beans, maize, rice, lentils, and barley can also be used as raw materials for *tempeh* production (Erkan et al., 2019). *Tempeh* has gained more attention because it contains essential nutrients and desirable organoleptic characteristics. It is used in the production of green meat, vegetarian foods and also roasted or lyophilized into flour for production of confectionary. It serves as meat analogue and reduces malnutrition in poor regions especially in children and pregnant women (Scrimshaw and Wallerstein, 2012).

Fermentation of leguminous seeds such as soybean, lima bean and Bambara groundnut reduces the anti-nutrients, enhances digestion, reduces allergic reaction, stimulates the activities of antioxidants and could lead to the release of phenolic compounds. Health benefit such as reduction of chronic diseases is promoting the production of fermented leguminous seeds (Limon et al., 2015; Vital et al., 2018).

Previous researches focused on the microbiological and chemical properties of *tempeh* produced from soybean and other known legume (Omosebi and Otunola, 2013; Tahir et al., 2018; Vital et al., 2018) but there is inadequate data on the microbial and physicochemical characteristics of *tempeh* produced from lesser-known legume “lima bean and Bambara groundnut”.

This study was designed to determine the effect of lima bean and Bambara groundnut on the biochemical characteristics and sensory properties of *tempeh* snack produced from soybean, lima bean, Bambara groundnut and the blends of the three legumes to advance the use of lima bean and Bambara groundnut in the production of *tempeh*.

MATERIALS AND METHOD

Materials

Soybean, lima beans and Bambara groundnuts were purchased from Federal University of Technology Teaching and Research Farm, Akure, Ondo State. The starter culture “*Rhizopus oryzae*” was isolated from boiled rice. Agars (Lab M, USA) and chemical used were of analytical grade (Sigma, USA).

Preparation of starter culture

Rhizopus oryzae was isolated from rice by boiling, cooling, and leaving for 3 days at room temperature. The isolate obtained were streaked on solid potato dextrose agar (PDA) to obtain pure culture. Slide cultures were prepared and *Rhizopus oryzae* was identified using the schemes described by Harrigan (1998). The isolate was transferred into an agar slant and incubated at 28 °C for 5 days. Afterwards the spores were washed with sterile distilled water, dispensed into McCartney bottles and kept in a refrigerator at 4 °C. Spore count of the *Rhizopus oryzae* suspension was obtained by pour plate method on PDA plates.

Production of *tempeh*

Tempeh was produced using modified method of Bavia et al. (2012). Each legume was sorted, soaked in water for 12 h, drained, dehulled and boiled for 45 min. The cotyledon (1 kg) was thereafter drained, cooled by spreading in tray, transferred into

rectangular mould, inoculated with *R. oryzae* spore suspension (10 ml) containing 1.0×10^6 cfu/ml and incubated at 28 °C for 48 h. Thereafter, the cotyledons were covered with white mycelia knitting it together to form a soft compact cake known as *tempeh*. The *tempeh* was steamed in rice cooker at 100 °C for 10 mins and pulverised.

Production of blends of soybean, lima bean and Bambara groundnut *tempeh*

The mash (200 g) obtained from each legume was mixed with 300 g of wheat flour, 10 g of salt and dried pepper, 75g of margarine and 3 cubes of seasoning and fried at 160 °C for 3 min in a delonghi deep fryer until it was golden brown (Lesly, 2005). The snacks obtained were cooled at room temperature, packaged in low density polyethylene bags (Ziplock bag) and kept at room temperature (28 ±1 °C) and refrigeration temperature (4 °C) for 4 weeks.

Microbiological analyses of the fresh *tempeh* and *tempeh* snacks

Each sample (5 g) was homogenized in 45 ml of peptone water in stomacher bag and diluted appropriately. Appropriate dilution (1 ml) was dispensed into sterile plate and molten agar (20 ml) was poured. Plates were swirled clockwise and anticlockwise to ensure even distribution of growth.

Total viable count (TVC) was determined using Nutrient agar, Eosin methylene blue for Coliform count and potato dextrose agar (PDA) for fungi count (Harrigan, 1998). Plates were incubated at inverted position for total viable count and Coliform count at 35 °C for 24 h and yeast and mould count at 25 °C for 72 to 120 days respectively. The colonies formed on each Petri dish were counted with the aid of Gallenkamp colony counter. Distinct colonies were streaked on solidified molten agar to obtain pure isolate (Harrigan, 1998).

Bacteria were identified based on appearance on plate, morphology, reaction to Gram's staining and selected biochemical tests according to Harrigan (1998). Each mould isolate was streaked on a solidified agar on a sterile glass slide placed on a U-tube in sterile petri dish and incubated at 28 °C for 48 h. Fungi morphology such as: colour of spores, presence of conidia or sporangium, hyphae; septate or non-septate and special structures were observed under the microscope by placing the glass slide on the stage.

Determination of pH of the *tempeh* mash

The pH was determined by homogenizing 10g of *tempeh* in 100 ml of distilled water. The suspension was thoroughly mixed and the pH was determined by dipping the probe of the pH meter into the sample after standardization with buffer 4 and buffer 7 (Farinde et al., 2011).

Determination of total titratable acidity (TTA) of the *tempeh*

Percentage lactic acid in samples were determined by titrating 10 ml of the homogenized sample

against 0.1N sodium hydroxide using 0.5 ml of phenolphthalein indicator (0.25% in 100% alcohol) (AOAC, 2000) (Equation 1).

$$\text{Lactic acid (\%)} = \frac{\text{ml NaOH} \times 0.009}{\text{weight of sample}} \quad (1)$$

Determination of the texture of *tempeh* snacks

Tempeh samples were subjected to compression test using Universal testing machine (Instron 3369, Germany). Each *tempeh* snack was cut to a dimension of anvil height 14 mm and diameter 20 mm. Compression was done to determine the response of samples to mechanical loading of loading rate 2mm/min. The each sample was placed between a fixed plate and a plate moving vertically downwards. Force was exerted on the samples until breaking point was attained. A graph of Compressive stress against compressive strain generated a young modulus for the samples (Modified method of Panea et al., 2018).

Sensory evaluation of *tempeh* snacks

A panel consisting of twenty trained panelist who are familiar with *tempeh* snack, evaluated the coded *tempeh* products for taste, texture flavour, appearance, aroma, and overall acceptability using Seven Point Hedonic Scale: where 7 represent like extremely and 1 represent dislike extremely (Osegbhun, 2006).

Statistical analysis

Data obtained from the experiment were analyzed using SPSS (IBM SPSS Statistic 20, Inc., USA). Means obtained were separated using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Microbial count of *tempeh* mash

Table 1 show the microbial counts of the freshly prepared *tempeh* from lima beans, soybeans, bambara are microbiologically safe for consumption. BLT had the highest TVC (7.903 log cfu/g) while BGT had lowest count (6.173 log cfu/g). BLT had significantly higher count ($p < 0.05$) than BGT and LBT. There was no significant difference ($p > 0.05$) in the TVC of LBT and BGT.

The fungi count of all *tempeh* mash samples were not significantly different ($p > 0.05$). Sample BLT also had the highest fungi count (6.799 log cfu/g) while SBT had the lowest (6.602 log cfu/g). The population of mould was similar to the quantity used for inoculation. *Rhizopus* species is intentionally added to soybean as starter culture for *tempeh* fermentation due to its ability to improve its flavour, texture and digestibility (Wan et al., 2018).

Table 1. Microbial analyses of fresh *tempeh* mash (log cfu/g)

Samples	Coliform count	Total viable count	Fungi count
BLT	Nil	7.903±0.848 ^a	6.799±0.430 ^a
SBT	Nil	7.580±0.636 ^{ab}	6.602±0.438 ^a
LBT	Nil	6.230±0.184 ^b	6.748±0.156 ^a
BGT	Nil	6.176±0.422 ^b	6.740±0.707 ^a

All mean values with different alphabets at the superscript in the same row are significantly different ($P < 0.05$). LBT – Lima bean *Tempeh*; SBT – Soybean *Tempeh*; BLT – Blend of the legumes *Tempeh*; BGT – Bambara groundnut *Tempeh*

Changes in the TVC of the *tempeh* snacks during storage

Total viable count was between the range of 4.000 and 4.431 log cfu/g at the beginning of storage (Table 2). There was no significant difference ($p > 0.05$) in the total viable count of all *tempeh* snack samples at the beginning of storage. Counts were generally higher in samples stored at room temperature than at refrigerated temperature. TVC generally increased in *tempeh* samples store at ambient temperatures from 4.000 log cfu/g at week 0 to 7.297 log cfu/g at week 4. The highest count was recorded in BLTS from week 1 to week 4 (5.845 – 7.297 log cfu/g) followed by SBTS (5.778 – 7.196 log cfu/g) while LBTS had the lowest count from week 2 to week 4 (4.763 – 6.041 log cfu/g).

At refrigeration storage, LBTS had the lowest count at week 1 (3.903 log cfu/g) and 4 (5.845 log cfu/g) while BLTS had the highest count (5.477 to 6.813 log cfu/g) from week 1 to week 4. It was observed that addition of soybean had significant effect ($p < 0.05$) on the TVC of the BLTS during storage. Lower count at refrigeration temperature is an indication that low temperature storage can slow down the growth of microorganisms and reduce spoilage of food (Todar, 2012).

Changes in the fungi counts of *tempeh* snacks

The fungi count of *tempeh* snacks are shown in Table 3. There was a significant difference ($p < 0.05$) in the fungi count of all *tempeh* snacks at the beginning of storage. Sample LBTS (4.000 log cfu/g) had the lowest count while BGTS (4.431 log cfu/g) had the highest fungi count. The fungi count increased progressively to week 4 and was generally lower in *tempeh* snacks kept at refrigeration (3.301 – 6.556 log cfu/g) than ambient temperature (4.000 – 7.093 log cfu/g). Fungi count was generally higher in SBTS (4.176 – 6.929 log cfu/g) than LBTS (4.342 – 4.568 log cfu/g) and BGTS (4.114 – 4.940 log cfu/g) throughout the period of storage. High count recorded in SBT could be due to the high oil content of soybean which facilitates the activities of lipolytic activities of the fungi. More et al. (2019) reported that addition of soybean oil enhanced fungi activities in Czapek agar medium. The influence of soybean also had direct effect on the increase in fungi count on BLTS (4.431 – 7.093 log cfu/g). Higher count at ambient temperatures could be attributed to the exponential growth of the fungi due to availability of nutrient and favourable temperature. Low temperatures have been reported to reduce the metabolic activity of microorganisms thereby slowing down their growth and reproduction (Adepoju et al., 2016).

Table 2. Total Viable count (log cfu/g) of the *Tempeh* snacks stored for 4 weeks

Samples	Storage period (Weeks)				
	0	1	2	3	4
LBTS(Am)	4.000 ±0.127 ^a	4.431 ±0.003 ^c	4.763 ±0.006 ^e	5.954 ±0.249 ^d	6.041 ± 0.058 ^e
SBTS(Am)	4.204 ±0.007 ^a	5.778 ± 0.003 ^a	6.653 ± 0.130 ^b	7.114 ± 0.010 ^a	7.196 ±0.014 ^a
BGTS(Am)	4.431 ±0.001 ^a	4.079± 0.007 ^{cd}	4.580 ± 0.040 ^f	4.398 ±0.010 ^f	6.875 ± 0.005 ^b
BLTS(Am)	4.079 ±0.010 ^a	5.845 ± 0.016 ^a	6.707 ±0.010 ^a	7.233± 0.047 ^a	7.297 ± 0.008 ^a
LBTS (Ref)	4.000 ±0.127 ^a	3.903±0.100 ^d	4.398 ± 0.006 ^f	5.602 ± 0.003 ^d	5.845 ± 0.007 ^g
SBTS (Ref)	4.204 ±0.001 ^a	5.477 ± 0.080 ^b	5.778 ±0.006 ^d	6.041 ± 0.014 ^c	6.301 ± 0.013 ^d
BGTS (Ref)	4.431 ±0.007 ^a	4.000 ±0.004 ^d	3.778 ± 0.011 ^h	4.146 ±0.003 ^e	5.903 ± 0.011 ^f
BLTS (Ref)	4.079 ±0.010 ^a	5.477 ±0.008 ^b	6.322 ± 0.003 ^c	6.633 ± 0.006 ^b	6.813 ± 0.009 ^c

All values with different alphabets at the superscript in the same row are significantly different at ($P < 0.05$); LBTS – Lima bean *Tempeh* snacks; SBTS – Soybean *Tempeh* snacks; BLTS – Blend of the legumes *Tempeh* snacks; BGTS – Bambara groundnut *Tempeh* snacks; Am – ambient; Ref – Refrigeration

Table 3. Fungi count (log cfu/g) of the *Tempeh* snacks stored for 4 weeks

Samples	Storage period (Weeks)				
	0	1	2	3	4
LBTS(Am)	4.000 ±0.127 ^c	4.362 ±0.003 ^f	4.342 ±0.005 ^d	4.462±0.113 ^f	4.568 ± 0.113 ^f
SBTS(Am)	4.176 ±0.007 ^b	6.462± 0.003 ^b	6.851 ±0.004 ^a	6.785± 0.006 ^c	6.929 ± 0.013 ^b
BGTS(Am)	4.431 ±0.014 ^a	4.505 ± 0.007 ^e	4.785 ±0.005 ^c	4.903± 0.004 ^e	4.940 ± 0.057 ^e
BLTS(Am)	4.114 ±0.010 ^b	6.653 ± 0.011 ^a	6.845± 0.009 ^a	6.940± 0.056 ^b	7.093 ±0.004 ^a
LBTS (Ref)	4.000 ±0.127 ^c	3.301± 0.007 ^h	3.845± 0.007 ^f	3.954± 0.006 ^g	4.176 ±0.008 ^h
SBTS (Ref)	4.176 ±0.007 ^b	5.700±0.055 ^d	6.398± 0.007 ^a	6.279± 0.003 ^d	6.415 ±0.007 ^d
BGTS (Ref)	4.431 ±0.014 ^a	4.279±0.003 ^g	4.255 ±0.006 ^e	4.462± 0.003 ^f	4.505 ±0.070 ^g
BLTS (Ref)	4.114 ±0.010 ^b	6.146±0.006 ^c	6.415± 0.007 ^b	7.358±0.0113 ^a	6.556 ±0.007 ^c

All values with different alphabets at the superscript in the same row are significantly different at ($P < 0.05$); LBTS – Lima bean *Tempeh* snacks; SBTS – Soybean *Tempeh* snacks; BLTS – Blend of the legumes *Tempeh* snacks; BGTS – Bambara groundnut *Tempeh* snacks; Am – ambient; Ref – Refrigeration

Bacteria isolated from tempeh

Morphological and biochemical characteristics of the isolated microorganisms are shown in Tables 4a and 4b. Bacteria isolated from BGT were *Bacillus cereus*, *Staphylococcus epidermidis*, *Bacillus coagulans* and *Micrococcus cryophilus*. The microorganisms isolated from BLT were *Propionibacterium species*, *Bacillus alvei* and *B. Licheniformis*. *Bacillus licheniformis*, *B. Subtilis* and *B. Coagulans* were isolated from SBT and *Propionibacterium species*, *Bacillus alvei*, and *B. Licheniformis* were isolated from LBT (Table 6). The predominant microorganisms isolated belong to genus *Bacillus*. Frequent occurrence of *Bacillus* species could be due ability to metabolise carbohydrate, protein and fat which are the major constituents of legume. *Bacillus licheniformis* has been reported to be important during fermentation of some oil seeds because of its high lipolytic activity (Olajuyigbe and Ajele, 2008). Presence of *Bacillus* and *Micrococcus* species have been reported during fermentation of legume-based food by many researchers (Farinde et al., 2014; Malomo

et al., 2018; Adegbehingbe and Daramola, 2019). Previous researches also reported the importance of *Bacillus* in fermentation of lima bean and soybean by impacting desirable flavour and improving the rheological properties of the fermented products (Omafuvbe, 2007; Farinde et al., 2014).

Fungi isolated from the tempeh snacks

Rhizopus oryzae, *Rhizopus stolonifer* and *Geotricum species* were identified in all tempeh snacks during storage (Tables 5 and 6). *Rhizopus oryzae* has black spores with long sporangiophore and non septate hyphae. *Rhizopus stolonifer* has the same characteristics with *Rhizopus Oryzae* but has a longer rhizoid. *Geotricum candidum* colony was cream in colour on plate and microscopic examination show fragmenting undifferentiated hyphae. *Rhizopus species* accounted for 85 % of mould isolated in tempeh snack probably because it was used as the starter culture, thereby colonizing other moulds. This result is in agreement with Omosebi and Otunola (2013).

Table 4a. Bacteria isolated from tempeh

	A	Isolates B	C	D	E
Colony morphology					
Colour	Light cream	Yellow	cream	Cream	cream
Shape	Irregular	Rough	Round	Jagged	Rhizoid
Cell morphology					
Shape	Rods	Long rods	rods	Long rods	Long rods
Spores	Central	Ab	center	center	center
Gram's staining	+	+	+	+	+
Biochemical test					
Catalase test	+	+	+	+	+
Lecithinase production	+	-	-	-	-
Starch Hydrolysis	+	-	+	+	+
Growth at 10 °C	-	-	-	-	-
Growth at 50 °C	+	-	+	+	+
Growth at 55 °C	-	-	-	+	+
Growth at 60 °C	-	-	-	-	-
Indole test	+	-	+	-	-
Oxidase test	+	-	+	+	+
Voges – Proskauer	+	+	+	-	-
Methyl Red Test	-	-	-	-	+
Spore Staining	+	-	+	+	+
Acid from Glucose	+	+	+	+	+
Acid from Maltose	-	-	-	+	+
Acid from Galactose	-	-	-	+	+
Acid from Sucrose	+	-	-	+	+
Acid from Fructose	-	-	-	+	+
Acid from Raffinose	-	-	-	+	+
Coagulase Test	ND	ND	ND	ND	ND
Acid from Mannitol	ND	-	-	+	-
Probable microorganism	<i>Bacillus cereus</i>	<i>Propionibact erium sp</i>	<i>Bacillus alvei</i>	<i>Bacillus licheniformis</i>	<i>Bacillus licheniformis</i>

+ = positive, - = negative, ND = Not determined

Table 4b. Bacteria isolated from *tempeh*

	F	Isolates G	H	I
Cultural characteristics				
Colour	Cream	White	cream	transparent
Shape	cocci	Glossy spherical	spreading	Spherical
Cell morphology				
Shape	Short rods	Cocci in cluster	rods	cocci
Spores	center	Ab	centralized	ab
Gram's staining	+	-	+	+
Catalase test	+	+	+	+
Biochemical tests				
Lecithinase production	-	ND	-	ND
Starch Hydrolysis	+	+	-	-
Growth at 10 °C	-	-	+	-
Growth at 50 °C	-	-	+	-
Growth at 55 °C	-	-	+	-
Growth at 60 °C	-	-	+	-
Indole test	-	-	+	+
Oxidase test	+	+	-	+
Voges – Proskauer	+	+	+	+
Methyl Red Test	+	-	+	+
Spore Staining	+	ND	+	ND
Acid from Glucose	+	-	+	+
Acid from Maltose	+	-	-	-
Acid from Galactose	+	+	-	-
Acid from Sucrose	+	-	+	+
Acid from Fructose	+	-	-	+
Acid from Raffinose	-	+	-	-
Coagulase Test	ND	ND	ND	-
Acid from Mannitol	-	-	-	-
Probable microorganism	<i>Bacillus subtilis</i>	<i>Staphylococcus epidermidis</i>	<i>Micrococcus cyophilus</i>	<i>Micrococcus coagulans</i>

+ = positive, - = negative, ND = Not determined

Table 5. Morphological characteristics of the fungi isolate from *tempeh*

Colony	Morphological Characteristics	Identity
A	Black sporangium, long sponrangiophore, non –septate hyphae with large diameter. Whitish mycelia like cotton wool.	<i>Rhizopus oryzae</i>
B	Long Sporangiphore with black sporangium at the head rhizoid at the base of the sporangiphore, non – septate.	<i>Rhizopus stolonifer</i>
C	Fragmenting off white, flattened, chains of hyaline, fragmenting of undifferentiated hyphae, white to cream colony.	<i>Geotrichum candidum</i>

Table 6. Occurrence of microorganisms in *tempeh* snacks during storage

	SBT	BGT	LBT	BLT
<i>Bacillus subtilis</i>	+	-	-	-
<i>Bacillus cereus</i>	-	+	-	-
<i>Staphylococcus epidermidis</i>	-	+	-	-
<i>Bacillus coagulans</i>	+	+	-	-
<i>Micrococcus cryophilus</i>	-	+	-	-
<i>Propionibacterium species</i> ,	-	-	+	+
<i>Bacillus alvei</i>	-	-	+	+
<i>B. Licheniformis</i>	+	-	-	+
<i>Rhizopus oryzae</i>	+	+	+	+
<i>Rhizopus stolonifer</i>	+	+	+	+
<i>Geotricum candidum</i> .	+	+	+	+

LBT – Lima bean *Tempeh*; SBT – Soybean *Tempeh*; BLT – Blend of the legumes *Tempeh*; BGT – Bambara groundnut *Tempeh*; + - Positive; I- : Negative

Rhizopus species is important in production of *tempeh* because it produces enzymes amylases, lipases and proteases that breaks down carbohydrate, protein and lipid respectively thereby improving its appearance, chemical properties and texture. It also imparts desirable flavour on *tempeh*, increase the pH and soften the legumes (Pedizzi et al., 2017; Wikandari et al., 2021).

pH and titratable acidity (TTA) of the *tempeh* mash

Table 7 show the pH and TTA of the freshly prepared *tempeh* from lima beans, bambara groundnut, soybean and their blend. The pH ranged from 4.73 to 6.89 with LBT having the lowest (4.73) while SBT (6.89) had the highest. The pH of soybean *tempeh* obtained from this study was in the same range with value obtained by Omosebi and Otunola (2013). There was significant difference ($p < 0.05$) in the TTA of the *tempeh* snack samples. SBT had the lowest TTA of 0.081% which could be due to high composition of amino acid. Deamination of amino acid to ammonia by proteolytic enzymes could be responsible for low acid production (Wikandari et al., 2021).

Changes in pH of the *tempeh* snacks during storage

The pH of sample BLTS increased from 5.48 in the first week to 6.41 at the fourth week at ambient temperature (Figure 1). The pH of soybean *tempeh* was within the range reported by Reyes-Bastidas *et al.* (2010). The pH of LBTS, BGTS and BLTS increased progressively and were within the range of 4.73 to 6.45 at ambient temperature. The increase could be due to the proteolytic activity of microorganisms (Todar, 2012). LBTS had the lowest pH from the beginning of storage (4.73) to the fourth week of storage (5.83). This pH range is in agreement with values obtained for fermented lima bean (Adegbehingbe and Daramola, 2019).

The pH also increased at refrigerated temperature (Figure 2). LBTS had the lowest pH range (5.01 to 5.77) while SBTS had the highest (5.66 to 6.52). The pH of BGTS increased from 5.43 to 6.28 at week four and BLTS increased from 5.48 to 6.27. The pH of *tempeh* snack samples was generally lower at refrigeration temperature than ambient temperature probably due to inhibition microorganisms or lower rate of metabolic activities of microorganisms which also prevented rapid deterioration of the snacks. Farinde et al. (2014) also reported increase in pH during fermentation of lima bean.

Table 7. pH and Total titratable acidity of fresh *Tempeh*

Samples	pH	TTA (%)
SBT	6.89± 0.10 ^a	0.081± 0.02 ^d
LBT	4.73± 0.19 ^c	0.117± 0.03 ^c
BGT	5.43± 1.10 ^b	0.153± 0.031 ^b
BLT	5.48± 0.94 ^b	0.162± 0.83 ^a

All values with different alphabets at the superscript in the same row are significantly different ($P < 0.05$). LBT: Lima bean *Tempeh*; SBT: Soybean *Tempeh*; BLT: Blend of the legumes *Tempeh*; BGT: Bambara groundnut *Tempeh*

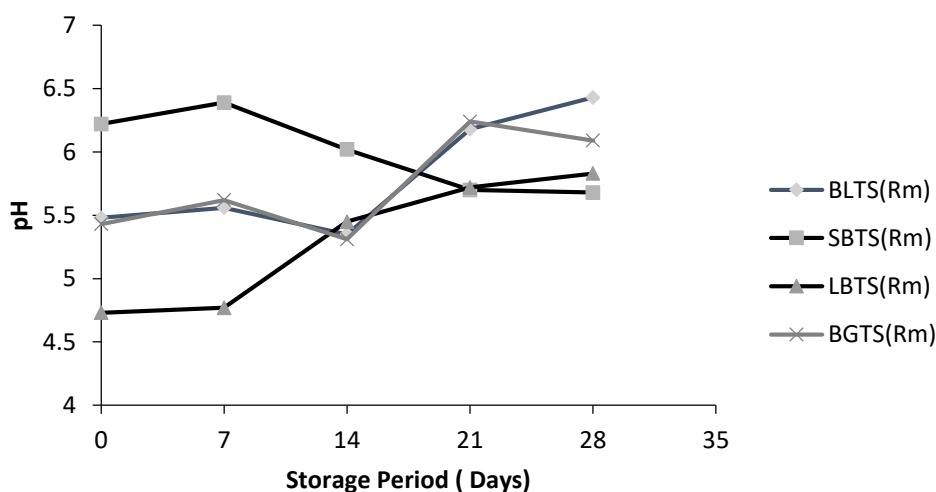


Figure 1. Changes in pH of the *tempeh* snacks at ambient temeperature for 4 weeks

LBTS – Lima bean *Tempeh* snacks; SBTS – Soybean *Tempeh* snacks; BLTS – Blend of the legumes *Tempeh* snacks; BGTS – Bambara groundnut *Tempeh* snacks; Rm – ambient

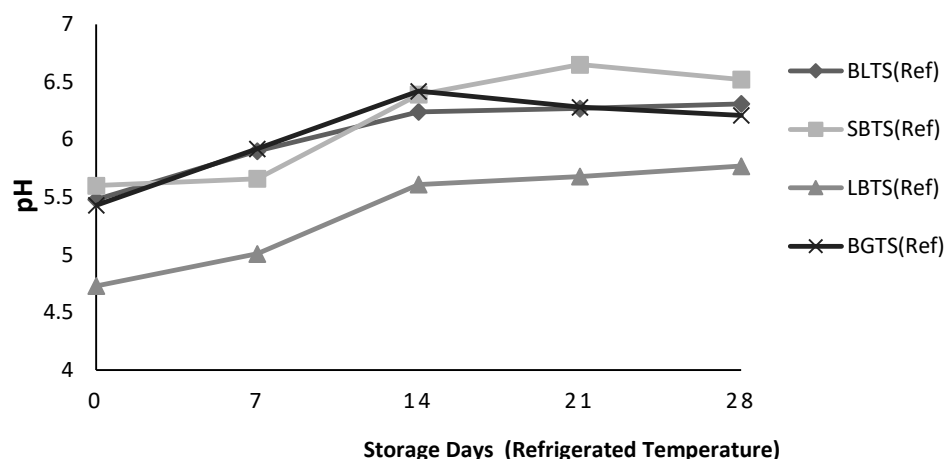


Figure 2 Changes in the pH of *tempeh* snacks at refrigerated temperature for 4 weeks
 LBTS – Lima bean *Tempeh* snacks; SBTS – Soybean *Tempeh* snacks; BLTS – Blend of the legumes *Tempeh* snacks; BGTS – Bambara groundnut *Tempeh* snacks

Changes in the titratable acidity of the *tempeh* snacks

The titratable acidity of *tempeh* snacks generally decreased at both refrigeration and ambient temperatures (Figures 3 and 4). TTA was between the range 0.040% to 0.162 % during the period of ambient storage. BLTS had the highest TTA (0.162 %) while SBT had the lowest (0.081 %) at the beginning of storage. TTA was generally higher in *tempeh* snacks stored at room temperature than in samples stored at refrigeration temperature. Reduction in the titratable acidity can be attributed

to the activities of microorganisms which breaks down the protein compounds in the *tempeh* snacks and increase the pH with production of pungent ammonia. Ammonia is produced from protein oxidation and deamination of amino acids by proteolytic enzymes produced by the fermenting microorganisms (Visessanguan et al., 2005; Wikandari et al., 2021). Omafuvbe et al. (2007) and Adegbehingbe et al. (2014) reported decrease in TTA and increase in pH during fermentation of soybean.

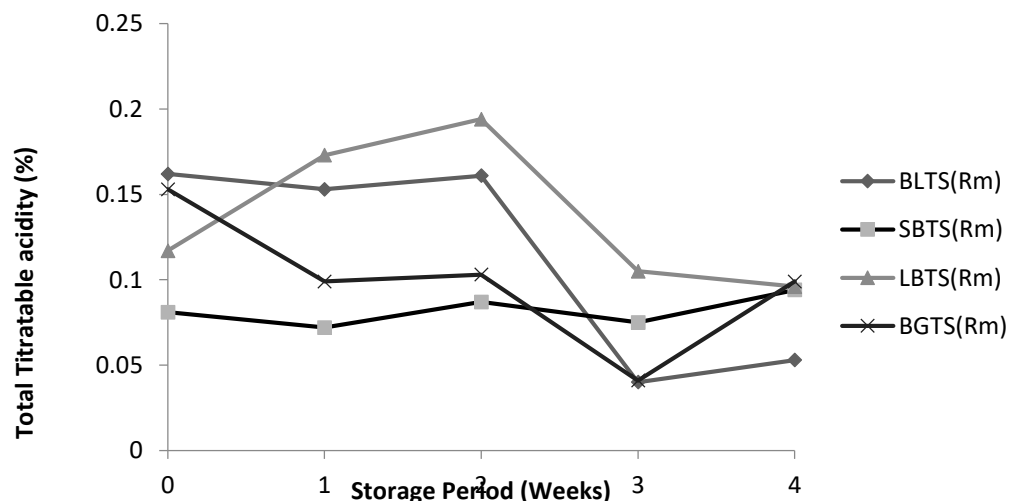


Figure 3 Changes in the total titratable acidity of the *tempeh* snacks at ambient temperature for 4 weeks.
 LBTS – Lima bean *Tempeh* snacks, SBTS – Soybean *Tempeh* snacks, BLTS – Blend of the legumes *Tempeh* snacks, BGTS – Bambara groundnut *Tempeh* snacks, Rm – room temperature

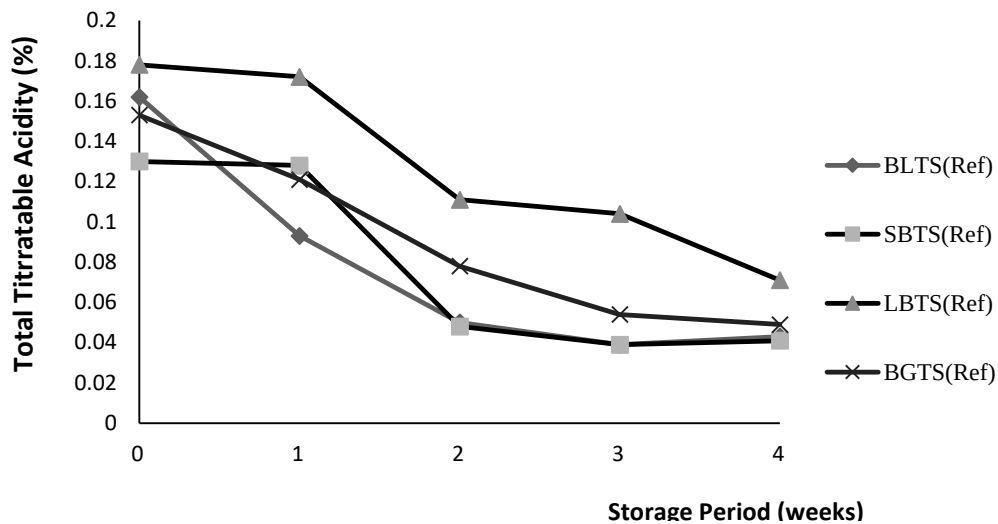


Figure 4. Changes in the Total Titratable Acidity of the *Tempeh* snacks at Refrigerated Temperature for 4 weeks
LBTS – Lima bean *Tempeh* snacks, SBTS – Soybean *Tempeh* snacks, BLTS – Blend of the legumes *Tempeh* snacks, BGTS – Bambara groundnut *Tempeh* snacks, Ref – Refrigeration temperatures

Physical appearance of the samples during storage

There were no visible changes in the flavour, aroma and appearance of *tempeh* snacks stored at ambient and refrigeration temperature at week 1. They all retained the qualities of freshly prepared snacks. At week two, the refrigerated samples retained the crisp, brown colour while samples stored at room temperature became soft and slimy. Observation at week three revealed noticeable crumbling of snacks at ambient temperature and growth of mould on the sample especially BLTS. At week four, the refrigerated sample was moist and slimy but it was still hard while the *tempeh* snacks stored at ambient temperature were totally covered with spores. The softness of the *tempeh* samples at week four could be as a result of continuous activity of *Rhizopus*. *Rhizopus* is intentionally added to *tempeh* to improve both its rheological and organoleptic properties (Tahir et al., 2018).

Results of the compression test on the *tempeh* snacks

Compressive test measure properties such as elastic and compressive fracture of brittle or low – ductility materials by pushing and squeezing the materials together (Chua et al., 2017). The response of the *tempeh* snacks to mechanical loading at 2mm/min deformation rate are shown in Figures 5 to 8. Sample LBTS had the highest compression stress before break point at 0.17 Mpa followed by BLTS with 0.134 MPa while the least was sample BGTS at 0.0133 MPa. The maximum load required to break the snacks is 57.4N with the sample LBTS withstanding it making it the hardest of the four snacks and the minimum load was 4.75N for sample BGTS making it the softest. This show that meat analogue can be produced from soybean, lima bean and Bambara groundnut. Meat analogue and textured vegetables has been produced from mushroom, chicken pea, soybean, soy concentrate and cowpea (Tahir et al., 2018; Vital et al., 2018).

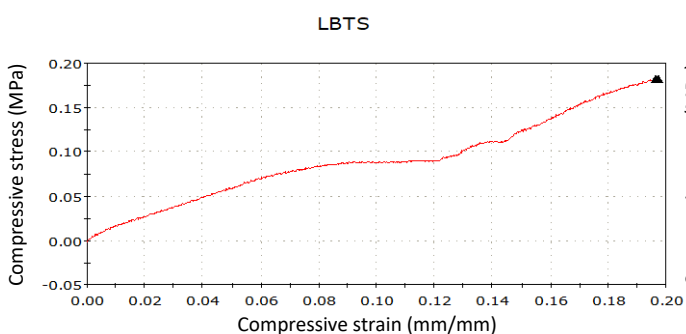


Figure 5 Compression test on lima beans *tempeh* snacks (LBTS)

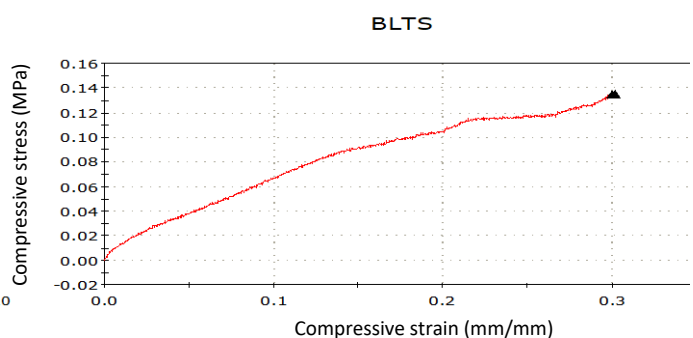


Figure 6 Compression test on blend of the legumes *tempeh* snacks (BLTS)

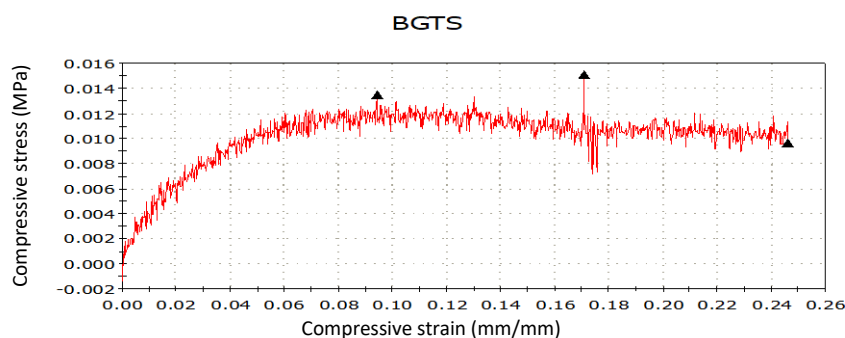


Figure 7 Compression test on bambara groundnut *tempeh* snacks (BGTS)

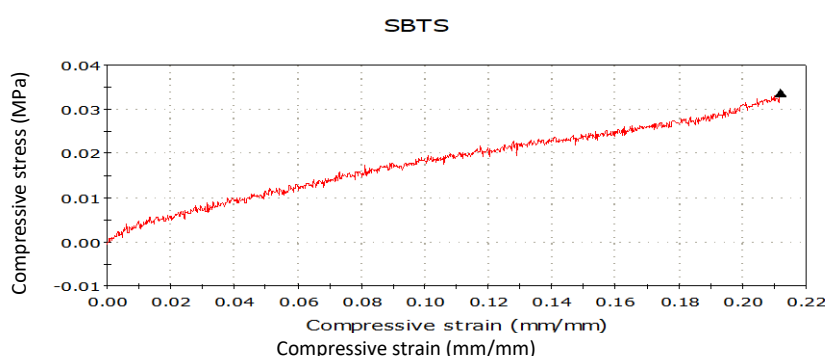


Figure 8. Compression test on soybean *tempeh* snacks (SBTS)

Sensory evaluation of the fresh *tempeh* snacks

BGTS had the lowest score for appearance and overall acceptability probably because it sporulated and appeared blackish at 24 h of fermentation unlike other legumes. Sample LBTS had the highest score in terms of appearance, texture, aroma and overall acceptability (Table 7). The score for texture corroborates the result of the compressive force of LBTS as presented in Figure 5. LBTS produced from lima bean scores for appearance, texture, aroma and overall acceptability was significantly higher ($p < 0.05$) than SBTS which is produced from the traditional *tempeh* legume.”

Lima beans *tempeh* snack (LBTS) was the most preferred followed by soybean *tempeh* snack (SBTS). Therefore, it can be concluded that *tempeh* from other legumes such as lima bean and blends of lima bean, Bambara groundnut and soybean can be used for *tempeh* production as this can increase utilization of lima bean and Bambara groundnut in *tempeh* production. Use of 100 % Bambara groundnut is not appropriate because it had negative effect on the organoleptic properties of *tempeh* but its addition to soybean and lima bean *tempeh* increased its organoleptic properties.

Table 7 Sensory evaluation of the the fresh *tempeh*

Samples	Appearance	Aroma	Texture	Overall Acceptability
BGT	3.40 $\pm$ 0.34 ^c	4.60 $\pm$ 1.25 ^b	4.45 $\pm$ 0.79 ^b	3.70 $\pm$ 0.34 ^d
LBT	6.10 $\pm$ 0.68 ^a	5.55 $\pm$ 0.86 ^a	5.70 $\pm$ 0.81 ^a	6.05 $\pm$ 0.68 ^a
SBT	5.10 $\pm$ 1.23 ^b	4.05 $\pm$ 0.74 ^b	4.79 $\pm$ 0.70 ^{ab}	5.26 $\pm$ 1.23 ^b
BLT	4.19 $\pm$ 0.76 ^c	4.81 $\pm$ 0.98 ^{ab}	4.29 $\pm$ 0.41 ^c	4.46 $\pm$ 0.76 ^c

All mean values with different alphabets at the superscript in the same row are significantly different at ($P < 0.05$).

CONCLUSIONS

Lima beans and Bambara groundnut can be incorporated into the production of *tempeh* due to similarities in the microbiological and physicochemical characteristics of the blends. This will advance the use of these underutilized legumes and encourage scientist to explore their use in gastronomy, improve their value chain and increase

food security in areas where lima bean and Bambara groundnut is being produced. The absence of coliform in the snacks also indicate that *tempeh* samples produced from these legumes are safe for consumption. Results of organoleptic assessment revealed that *tempeh* produced from lima bean was the most preferred.

ACKNOWLEDGEMENTS

I would like to thank the technical staff of the Department of Food Science and Technology, Obafemi Awolowo University, Ile – Ife, Nigeria for their support during this work.

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