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Response of *Bacillus subtilis* to Salt and Acid Stresses and the Optimal Effect on Fermentation of Locust Bean (*Parkia biglobosa*) Seeds into *Iru* (A Soup Condiment)

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Abstract

This study was aimed at evaluating the performance of *Bacillus subtilis* stressed in varying single-factor salt and acid concentrations as pre-treatments in the fermentation of African locust bean seeds (*Parkia biglobosa*) into *iru*, a commonly consumed soup condiment. Microorganisms were isolated from commercial *iru* and the result showed that there was a predominant bacteria growth, with a mild mold growth. The bacteria isolated were species of *Bacillus*, *Micrococcus* and *Pseudomonas*. Biochemical tests were carried out on the *Bacilus* species; and *Bacillus subtilis* was isolated and identified. The isolated *B. subtilis* was cultured under different acid (pH 3.5, 4.5 and 5.5) and salt (1%, 2.5% and 5% NaCl) concentrations. Notable growths were seen at all salt concentrations but no growth was observed at very high acid concentrations (pH 3.5 and 4.5). Isolates from varying cultural conditions were used as single starters to ferment the locust bean seeds into *iru* (at 37 ºC for 72 h), with subsequent evaluation of their performance in the fermentation process and final product. The results revealed that stressing the organism as a pretreatment step had a positive significant effect on the nutritional, antinutritional, physicochemical, and sensory properties of the fermented *iru*. This study therefore concludes that the introduction of appropriate starter culture such as *B. subtilis* with appropriate modifications could contribute to improved preservation, quality and safety of traditionally fermented foods. The overall acceptability of the samples fermented with the stressed *B. subtilis* compared favourably with the control samples.

Keywords: *Bacillus subtilis; salt and acid stresses; locust bean fermentation; iru quality; acceptability*.

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Introduction

Fermentative microorganisms are conventionally stressed for two main reasons, viz, to get them acclimatized to anticipated harsh and hostile conditions of subsequent fermentation environment and, to increase their productivity in real time or industrial processes. Coupled with the current prevailing need to convert most underutilized and neglected wild tropical bio-resources to more shelf-stable products to prevent fungal contamination and intoxication (Enujiugha, 2000; Enujiugha et al., 2023), the application of stressed environments in the culturing of fermentative organisms becomes even more appealing. *Bacillus subtilis* has been implicated as the main fermentative organism in legume- and oilseed-based fermentations that yield savoury condiments (Enujiugha, 2009; Chinma et al., 2023). Its performance has been found to be optimal in alkaline fermentations (Enujiugha et al., 2008), although it has also been touted as a probiotic organism with the behavior in food systems near that of lactic acid bacteria species (Enujiugha, 2020), and producing primary organic acids as fermentation by-products (Enujiugha, 2003). In a

previous study (Enujiugha and Badejo, 2002), *Bacillus subtilis* was isolated from traditionally-fermented and commercially-available condiment, *ugba*, and grown on plate count agar (PCA) under combinations of different salt concentrations, temperatures and pH prior to being used as single starter culture in a controlled fermentation of African oil bean (*Pentaclethra macrophylla*) seed slices. The results revealed that *Bacillus subtilis* grown under a combination of 5% w/w NaCl concentration, pH 6.8 and 37 ºC was found to give the best rated fermented product. However, to our knowledge, no known work has been done on optimizing the productivity of the fermenting organism, *Bacillus subtilis*, by growing it in a medium that is stressed with a single parameter such as salt or acid, as a pre-treatment step to controlled locust bean fermentation.

Legumes and oilseeds, apart from being consumed as cooked or roasted snacks (Talabi et al., 2023), are commonly fermented to increase their edibility, improve upon their nutritional values, yield final products with longer keeping quality and offer a route to effective removal or reduction of anti-nutritional and toxic factors (Enujiugha and Olagundoye, 2001; Chinma et al., 2023). All these qualities are obviously influenced mainly by the microorganisms involved in the diverse fermentations and their productivity levels (Enujiugha and Badejo, 2002). Legumes and oilseeds such as African locust bean seeds, African oil bean seeds, melon seeds, castor oil seeds, mesquite bean seed, and soybean seeds are fermented by *Bacillus* sp., especially *Bacillus subtilis*, into value-added products with increased flavour and taste (Enujiugha, 2009, 2020; Ndamitso et al., 2020). *Bacillus subtilis* produces greater quantities of protease, amylase and poly-γ-glutamic acid (which is responsible for mucilage production, that is very common in fermented vegetable proteins), while at the same time maintaining an alkaline fermentation environment (Oyedokun et al., 2016; 2020). The primary organic acids produced as secondary metabolic by-products during such fermentation, especially butyric acid (Enujiugha, 2003), are not enough to influence the pH towards creating acid environment.

Bacillus subtilis act on the food polymers to release various organic acids, alcohols, esters, aldehydes, and gases that confer characteristic aroma and flavour. Factors that influence the rate at which these products are formed include the pH, temperature, and moisture contents of the seeds. *Iru* is obtained from the traditional solid-state fermentation of African locust bean seeds (*Parkia biglobosa*) by *Bacillus* species, notably *Bacillus subtilis* (Enujiugha, 2009; Nwagu et al., 2020). A past study revealed that the seeds contain reasonable amounts of phenolics that possess high antioxidant capacity with remarkable free-radical scavenging activities (Enujiugha, 2010). The locust bean seeds also contain approximately 29.1% protein, 26.2% fat, 6.7% fibre and 2.5% ash contents, on a dry weight basis (Enujiugha and Ayodele-Oni, 2003). The present study evaluates the adaptation of enumerated *B. subtilis* to acid and salt stresses by monitoring its growth process under diverse salt and acid concentrations and its subsequent performance in a controlled fermentation of African locust bean seeds into *iru*. The concentrations of acid and salt solutions are programmed to be minimal such that, adaption, rather than mutation, was the desired goal, to enhance productivity without altering the nature of the fermentative microorganism. The study had four specific objectives, which included assessing the microbial density of fermenting locust beans using culturally-stressed *B. subtilis*, determining the chemical composition (proximate, minerals and antinutrients) of subsequent fermented product, evaluating the sensory acceptability of the fermented product from the different stressed organisms and determining the physicochemical properties (pH, TTA and Colour) of the fermented *iru*. This was with a view to contributing to the improvement of quality and safety of the fermented product.

Materials and Methods

Materials

African locust bean seeds and commercially fermented *iru* used for the study were procured from *Oja-Oba* market in Akure, Nigeria. Nutrient agar (ISO-6579, ISO-10273), hydrochloric acid (HCl) and table salt (NaCl) used in the study were purchased from Pascal Scientific laboratory store in Akure, Nigeria. All reagents and chemicals used were of analytical grade

Isolation of Microorganisms

Fermented samples were taken aseptically from the purchased commercially fermented *iru* and pulverised. One gram (1 g) of the sample was mixed with 9 ml of 1% sterile peptone water as diluent in a McCartney bottle and the content was shaken properly to give the 10^{-1} dilution level. From this, dilution levels of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made and cultured on Nutrient Agar at 37 ºC for 24 hours using pour plate technique. Subsequent sub-culturing and streaking on plates and slants was done to obtain pure cultures of the isolated organisms (Enujiugha et al., 2008).

Characterization and Identification of Isolates

Representative colonies of isolates were streaked on the NA medium and incubated for 24 hours at 37 ºC. The cultural characteristics of the isolates were observed and noted. The motility of the isolates was examined by the hanging drop technique. Gram staining and cell morphology were examined from heat smears. The isolates were identified using appropriate morphological and biochemical tests outlined by Enujiugha et al. (2008). The pure culture of the desired organism (*Bacillus subtilis*) was sub-cultured on NA slant at 4 ºC.

Culturing the Organism under different Single-factor Stressed Conditions

The isolated *Bacillus subtilis* was cultured in varying salt and acid concentrations following the method of Enujiugha and Badejo (2002) with slight modifications, to examine which of the concentrations will favour its growth and the subsequent fermentation of African locust bean seeds.

Salt Stress (Application of Sodium Chloride)

The manufacturer of nutrient agar (NA) prescribes the suspension of 2.8 g in 100 ml of distilled water. About 1.12 g of NA was weighed into a conical flask and 40 ml of distilled water was added to it. It was placed in an autoclave and left to dissolve for 5 minutes. The same was repeated for 3 other conical flaks and they were labelled ASE, BSE, CSE and CTN. Then, 1% w/w of NaCl (0.007 g) was added into ASE, 2.5%

w/w of NaCl (0.0175 g) was added into BSE. 5% w/w of NaCl (0.035 g) was added into CSE, and CTN served as control, without any salt added into it.
The agar were collectively sterilized in the autoclave at 121 ºC for 15 minutes and allowed to cool down before pouring in duplicate petri dishes, respectively. The plates were allowed to solidify and labelled accordingly before inoculation with the pure culture of *Bacillus subtilis*. They were all incubated at 37 ºC for 24 hours.

Acid Stress (Hydrogen Ion Concentration)
Exactly 1.12 g of NA was weighed into a conical flask and 40 ml of distilled water was added to it. It was placed in an autoclave and left to dissolve for 5 minutes. The same was repeated for 3 other conical flaks and they were labelled BAE, CAE, DAE and CTN. Flask BAE was adjusted into a pH of 3.5 using 0.1 M HCl and the pH meter. Flask CAE was adjusted into a pH of 4.5 using 0.1 M HCl and the pH meter. Flask DAE was adjusted into a pH of 5.5 using 0.1 M HCl and the pH meter while CTN served as the control without any pH alteration. The agar were collectively sterilized in the autoclave at 121 ºC for 15 minutes and allowed to cool down before pouring in duplicate petri dishes, respectively. The plates were allowed to solidify and labelled accordingly before inoculation with the pure culture of *Bacillus subtilis*. They were all incubated at 37 ºC for 24 hours.
After incubation, the plates were examined to see which of the conditions favoured the growth of the *Bacillus subtilis*. The organisms that survived any of the conditions were used to inoculate the locust bean seed in a controlled fermentation.

Laboratory Fermentation of *Iru*
The fermentation was carried out as shown on Figure 1. Parboiling was done using a pressure cooker for 2 h, and after dehulling, the cotyledons were cooked for 1 h at 100 ºC. The cooked cotyledons were soaked in water for 12 h under ambient conditions (28 ± 2 ºC), washed in 3 changes of clean water, drained and autoclaved at 10 psi for 15 min. Then the seeds were inoculated with the sub-cultured *Bacillus subtilis* under aseptic conditions at 3% w/w (bacteria: seed ratio). Fermentation was carried out at 37 ºC for 72 h to yield *iru* from the different variants. A portion of the prepared seed cotyledons was fermented spontaneously using the traditional method (Enujiugha, 2009; Oyedokun et al., 2016), without inoculation of *B. subtilis* and served as negative control (CTR); while a portion was fermented with organism that was not subjected to either salt or acid stress and served as positive control (CTN). All the fermented samples were dried at 50 ºC for 6-8 h in a cabinet drier, milled into powder (~250 µm) using hammer mill and kept at 4 ºC prior to further analyses.

Determination of Microbial Density
After the controlled laboratory fermentation, two (2) loopfulls (approx. 1 g) of the *Bacillus subtilis* were taken from each sample and homogenized in 10 ml peptone water. From the 10^{-1} dilution, other decimal dilutions (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were prepared. The total microbial density was determined by the pour plate method, using Nutrient agar (ISO-6579 and ISO-10273) as the medium. Plates were incubated at 37 ºC for 24 h (Enujiugha and Akanbi, 2008), and examined for growth. Discrete colonies were counted, multiplied against dilution level, and expressed as CFU/g (colony-forming units per gram).

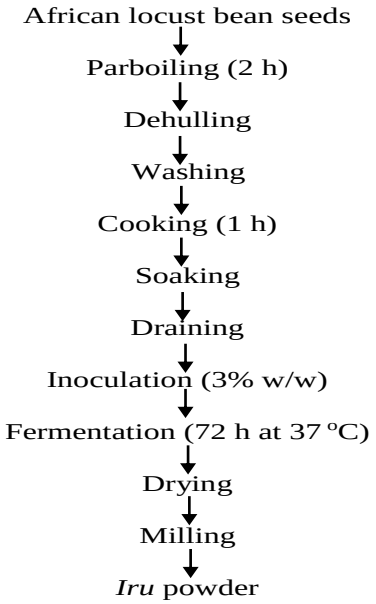

Figure 1: Flow chart of fermentation process of *iru*.

Determination of Proximate Composition
The proximate composition (moisture content, crude fiber, crude fat, total ash, and crude protein contents) of the flour blends were determined as described by AOAC (2012). Crude protein was calculated by multiplying the percent Kjeldahl total nitrogen by the factor 6.25. Total carbohydrate was determined as difference between 100 and the total sum of fat, moisture, ash, crude fibre and crude protein contents. All determinations were carried out in triplicates.

Determination of mineral concentrations in the samples
Minerals were determined by dry-ashing 1 g of the sample at 550 °C in a muffle furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Flame photometer (Sherwood Flame Photometer 410, Sherwood Scientific Ltd. Cambridge, UK) was used to determine sodium and potassium contents of the sample, while Ca, Fe, Mg, Zn, Cu and Mn were determined using atomic absorption spectrophotometer (AAS) (Perkin - Elmer Model 403, Norwalk, CT, USA). Phosphorus was determined by using phospho-vanado molybdate (yellow) method (AOAC, 2012).

Determination of physicochemical parameters
Determination of pH
The pH of the samples was determined using a standard pH meter (Expandable Ion Analyzer EA 920). The pH meter was calibrated using buffer solutions of pH 4 and 7. About 10 mL of sample homogenate was measured into a beaker and the pH probe was dipped into the beaker to measure the pH of the sample AOAC (2012). The pH values were then recorded

Determination of total titratable acidity (TTA)
One gram (1 g) of sample was weighed into a conical flask and 10 ml of distilled water was added as described by AOAC (2012). About 200 ml of 0.1 M NaOH was poured into a

burette and was titrated against the sample in the flask using three drops of phenolphthalein as indicator. It was titrated until a pink coloration was observed and the corresponding burette reading was taken using the following formula.

$$\textbf{Titratable acidity}\ (\%) = \frac{(\text{Titre} - \text{blank})\ \text{x M x ml of 0.1 NaOH x ml equiv. of acetic acid}}{\text{Weight of sample}}$$

ml equivalent of acetic acid (meq) = 0.0640

Colour measurement

Colour was measured using a spectrophotometer CM-700d (Konica Minolta Sensing, Osaka, Japan) which was calibrated against a white plate (Makanjuola and Enujiugha, 2018). The colour difference of the samples were read in L* (brightness – 0 : black, 100 : white), a* (+ : red, - : green), b* (+ : yellow, - : blue) values (Yangilar, 2016). While the chroma (c*) and hue (h*) values were calculated from *a and *b values (Hirata, 2020).

Determination of Anti-nutrients

The modified method of Reddy *et al.* (1982) was used for phytic acid and phytate-phosphorus determinations. Phytic acid was extracted from each 3 g *iru* sample with 3% trichloroacetic acid by shaking at room temperature followed by high- speed centrifugation (30,000 x g for 5 min). The phytic acid in the supernatant was precipitated as ferric phytate, and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron:phosphorous molecular ratio according to Enujiugha and Olagundoye (2001).

Tannin contents were determined by the modified vanillin-HCl method (Price *et al.*, 1978), with some minor adaptations. A 2 g sample was extracted with 50 ml 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 x g, 5 ml of vanillin–HCl (2% vanillin, 1% HCl) reagent was added to 1 ml aliquots, and the colour developed after 20 min at room temperature (28±2 ^{o}C) was read at 500 nm. Correction for interference from natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction, but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank.

Determination of oxalate was by the AOAC (2012) method. Exactly 1 g of finely ground sample was dissolved in 75 ml of 1.5 N H_2SO_4. The solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and filtered using Whatman no. 1 filter paper. A 25 ml sample of the filtrate (extract) was collected and titrated hot (80 – 90 ^{o}C) against 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30 seconds.

Sensory evaluation

The best starters were determined through test panel assessment of the fermented products. A 20-member sensory panel was constituted, based on familiarity with *iru* flavour (aroma and taste), colour and texture, to assess the products on the characteristic sensory parameters of appearance, aroma, and texture. The samples for evaluation included the fermented products using the single pure isolates of *B. subtilis* and the controls. A seven-point Hedonic scale was adopted, with seven equaling 'like very much' and one equaling 'dislike very much'.

Statistical analysis

All results obtained by measurements were subjected to one-way analysis of variance (ANOVA) in the SPSS software ver. 22.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). The significance of each experimental value was analyzed by Duncan's multiple-range test ($p < 0.05$), which was also utilized in the separation of the mean values.

Results and Discussion

Enumeration, characterization and isolation of the fermentative microorganism

The results of morphological and biochemical characterization of the isolates from traditionally fermented commercial *iru* samples are shown in Table 1 and Table 2, respectively. The major organisms isolated from the commercial *iru* samples were *Bacillus subtilis, Bacillus licheniformis, Pseudomonas fluorescens* and *Micrococcus* species. Reports have it that microorganisms involved in *iru* fermentation include species of *Escherichia, Proteus, Pediococcus, Micrococcus, Staphylococcus, Streptococcus, Alcaligenes, Pseudomonas, Corynebacterium and Enterococcus* (Odunfa, 1981; Antai and Ibrahim, 1986; Ogbadu and Okagbue, 1998; Ogueke and Aririatu, 2004). *Bacillus* species are notably identified in their numerous numbers during fermentation of *iru* (Obeta, 1983; Sanni, 1993; Isu and Ofuya, 2000; Sanni *et al.*, 2002), with *B. subtilis* appearing to be the most predominant (Enujiugha, 2009). The isolated *Bacillus* species are nonpathogenic, saprophytic mesophiles commonly associated with soil, air and water. According to Isu and Njoku (1997), *Bacillus* species constitute over 95 per cent of the total microbial population density in African oil bean seed fermentation into ugba, a soup condiment. The isolated *Bacillus* species have been variously identified as playing major parts in the fermentation of other unconventional legumes for the production of condiments (Enujiugha, 2009; Enujiugha and Badejo, 2002; Chinma et al., 2023).

Table 1: Colony characteristics of the isolates

Isolates	Colony Morphology on Agar	Cell Characteristics	Probable Identification
A	Greenish-white, raised, entire, smooth	Gram+ve, short rods, non-sporing	*Pseudomonas spp*
B	White, flat, rough, large and spreading	Gram+ve, long rods, central spores	*Bacillus spp*
C	Yellowish, raised, entire, smooth	Gram-ve, cocci rods	*Micrococcus spp*

The result shown in Table 3 is in agreement with the findings of Enujiugha and Badejo (2002) in their study of the effect of changes in the culture conditions of *B. subtilis* for African oil bean fermentation. Growth characteristics or level in CTN was same because the condition of the media is same, hence only

one was used. Samples ASE, BSE, CSE and DAE were used for the fermentation because the organisms survived the stressed cultural environments. *B. subtilis* did not survive acid stresses at pH 3.5 and 4.5. According to Enujiugha and Badejo (2002), the optimum medium condition of *B. subtilis* was reached at 37 ^{o}C and pH 5.5. There was a very good growth at all salt concentrations and this agrees with previous observations that showed that *B. subtilis* grows well at 2-7% NaCl concentrations.

Table 2: Biochemical and morphological characteristics of *B. subtilis* in *iru*

Characteristics	***B. subtilis***
Colony morphology	White-cream,Large and spreading
Cell morphology	Rods,central spores
Gram's reaction	+ve
Catalase test	+ve
Motility test	+ve

Table 3: *B. Subtilis* under salt and acid stress

Conditions	Salt conc.	Salt conc.	Salt conc.	Salt conc.	pH	pH	pH	pH
	0%	1%	2.5%	5%	3.5	4.5	5.5	N^{+}
Level of growth	++	++	++	++	-	-	++	++
Label	CTN	ASE	BSE	CSE	BAE	CAE	DAE	CTR

Where N^{+} is the normal pH of the agar; - = no growth; + = good growth; ++ = very good growth

ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

Table 4 shows the changes in pH during the period of locust bean fermentation using *B. subtilis*. There were general increases in pH of the fermenting seeds after 48 h. This trend was sustained after 72 h of fermentation using *Bacillus subtilis* as starter culture. Sample CTR had a pH of 6.66; others fermented with *B. subtilis* had pH ranging between 7.27 and 7.54, with CSE recording the highest. This reveals a strongly alkaline fermentation. As reported by previous researchers, most bacteria are favoured by reactions near neutrality while a few are favoured by an alkaline reaction. High pH inhibits many microorganisms and determines the types of organisms that grow in the fermenting seed (Enujiugha *et al.*, 2008). In the present study, the increase in pH during fermentation could have been contributed by the strong proteolysis of the African locust bean seed proteins into some basic products like ammonia. In the fermentation of soybeans by *Bacillus subtilis*, Owens *et al*., (1997) observed a rise in pH value from an initial value of 6.5-7.0 to 7.5-8.5 and often the development of a strong ammoniacal odour, because of the hydrolysis of the seed proteins and the metabolism of the resultant amino acids. There was no significant difference between samples ASE and BSE.

Table 4: pH and TTA (g/100g lactic acid) of the samples after 72 h fermentation

Samples	**pH**	**TTA (g/100g)**
ASE	7.27 ± 0.01^{d}	2.35 ± 0.49^{a}
BSE	7.26 ± 0.01^{d}	2.50 ± 0.71^{a}
CSE	7.54 ± 0.01^{a}	1.63 ± 0.18^{ab}
DAE	7.39 ± 0.01^{c}	2.05 ± 0.00^{ab}
CTN	7.47 ± 0.03^{b}	2.08 ± 0.11^{ab}
CTR	6.66 ± 0.03^{e}	1.18 ± 0.04^{b}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≤0.05.

ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

The total titratable acidity (TTA) was highest in the sample fermented with 2.5% salt (BSE) with a value of 2.50 g/100g. The TTA of the naturally fermented seed (CTR) was lowest with 1.18 g/100g. There was no significant difference in all samples fermented with *B. subtilis* including the unstressed control CTN. The resulting increase in the pH and reduction in TTA of samples obtained by natural fermentation and *Bacillus subtilis* may have been the effect of accumulation of ammonia. A similar increase was also reported by Barber and Achinewhu (1992) during the fermentation of *Citrullus vulgaris* to 'ogiri'. A decrease in TTA and increase in pH was also reported by Omafuvbe *et al.*, (1999) during the fermentation of *Prosopisa africana*. Table 5 shows that the colour of all samples was significantly different from each other at P≤ 0.05.

Table 5: Colour Analysis of fermented Iru

	Colour				
Sample	**L***	**a***	**b***	**c***	**h***
ASE	30.75 ± 0.01^{e}	13.65 ± 0.00^{d}	21.20 ± 0.01^{e}	23.88 ± 2.30^{c}	57.21 ± 0.01^{d}
BSE	28.95 ± 0.05^{f}	14.16 ± 0.01^{c}	20.83 ± 0.03^{f}	25.19 ± 0.02^{bc}	55.79 ± 0.02^{f}
CSE	39.35 ± 0.33^{b}	11.35 ± 0.10^{f}	22.02 ± 0.12^{d}	24.77 ± 0.16^{c}	62.73 ± 0.09^{a}
DAE	33.44 ± 0.07^{c}	14.54 ± 0.02^{b}	23.03 ± 0.04^{a}	27.23 ± 0.04^{a}	57.74 ± 0.02^{c}
CTN	33.15 ± 0.04^{d}	14.76 ± 0.01^{a}	22.48 ± 0.01^{c}	26.89 ± 0.01^{ab}	56.71 ± 0.01^{e}
CTR	52.04 ± 0.01^{a}	11.91 ± 0.02^{e}	22.62 ± 0.03^{b}	25.57 ± 0.02^{abc}	62.22 ± 0.06^{b}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≤0.05.

ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

The bacterial load increased during fermentation. The increase in bacterial load may have resulted from the ability of the bacteria to make use of the available nutrients in the locust bean seeds in the production of more cells. Adelekan and Nwadiuto (2012) reported a similar increase in microbial density during the fermentation of *Parkia biglobosa* seeds to

produce *iru*. The highest bacterial load was found in samples naturally fermented, this may have been the result of some of the first colonizing bacteria producing secondary metabolites for the growth of others (Atere *et al.*, 2019). In this study, sample DAE had the highest microbial density with $2.4x10^7$ cfu/g while sample ASE had the lowest with $1.0x10^6$ cfu/g (Table 6). The increase in the counts of the fermentative organisms during the fermentation implies that the seed cotyledons have the required substrates for their growth and multiplication. Some other workers have observed increases in microbial density during fermentation of plant protein sources (Odunfa, 1981; Obeta, 1983; Njoku *et al.*, 1990; Enujiugha et al., 2008).

Table 6: Microbial density of fermented *iru*

Samples	Total viable counts cfu/g
ASE	$1.0x10^6$ cfu/g
BSE	$1.5x10^6$ cfu/g
CSE	$1.2x10^7$ cfu/g
DAE	$2.4x10^7$ cfu/g
CTN	$1.2x10^6$ cfu/g

ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control).

Table 7 shows the proximate chemical composition of the fermented *iru* samples. The increase in protein content of the fermented sample compared to the unfermented had earlier been reported (Aremu *et al.*, 2015). Omafuvbe *et al.* (2004) also reported a slight increase in the protein composition of fermented *Parkia biglobosa* seeds. Makonjuola and Ajayi (2012) also observed a similar increase in protein content during fermentation of *Parkia biglobosa* seeds. The increase in the protein content was attributed to the proteolytic activities of the bacteria involved in the fermentation (Ojewunmi *et al.*, 2016). *Bacillus subtilis* fermented samples had the highest protein contents; and CSE with protein content of about 56% was similar to what Omodara and Aderibigbe (2014) reported when starter culture of *Bacillus subtilis* was used during the fermentation of *Parkia biglobosa* seeds. The samples fermented with the organism had lower carbohydrate content (BSE and CSE recorded lowest at 24.16% and 25.57 % respectively). The reduction in the carbohydrate content may have been the effect of increase in population of the bacteria which resulted in the increase in metabolism thereby making use of the available sugar for energy and biomass production. Oluwaniyi and Bazambo (2016) reported an increase in the crude fat and this increase was attributed to the loss of some other constituents of the seeds due to heat treatment during the fermentation process thereby leading to an overall increase in the fat content of the fermented seeds. All samples had an ash content <3% with CSE recording highest at 2.85%. The reduction in the ash content after fermentation was attributed to the loss of some minerals during the fermentation processes (Oluwaniyi and Bazambo, 2016).

Table 7: Proximate composition (g/100g wet wt.) of the fermented *iru* samples

Sample	Moisture content	Ash	Fat	Crude fibre	Protein	Carbohydrate
ASE	10.53 ± 0.11^a	1.98 ± 0.05^b	25.94 ± 0.01^a	4.16 ± 0.09^e	43.75 ± 0.01^f	13.64 ± 0.01^a
BSE	10.01 ± 0.06^c	1.89 ± 0.02^c	24.16 ± 1.80^c	6.11 ± 0.01^d	54.60 ± 1.20^c	$3.22\pm0.11_f$
CSE	9.81 ± 0.31^d	2.85 ± 0.01^a	25.57 ± 0.01^b	2.42 ± 0.10^f	56.00 ± 0.90^a	3.34 ± 0.01^e
DAE	10.11 ± 0.06^b	1.85 ± 0.03^d	20.82 ± 0.08^d	8.15 ± 0.01^a	51.80 ± 0.05^e	7.26 ± 0.13^d
CTN	9.52 ± 0.01^e	1.63 ± 0.01^e	20.80 ± 0.01^e	7.61 ± 0.01^b	$52.92\pm0.01_d$	7.53 ± 0.02^c
CTR	8.07 ± 0.11^f	1.30 ± 0.07^f	17.65 ± 0.01^f	6.44 ± 0.03^c	55.20 ± 0.24^b	11.34 ± 0.07^b

**Values along the column are the means of triplicate determinations and those with the same superscripts are not significantly different, at $P\leq0.05$.
ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

The mineral composition of the fermented locust bean product is presented in Table 8. Na/K ratios were all less than 1 and the Ca/mg ratios were more than 1, which are desirable for healthy and sustainable diets. The accumulation of some elements like calcium, potassium sodium and iron during fermentation of *Parkia biglobosa* seeds was earlier reported by Oluwaniyi and Bazambo (2016). Minerals are trace elements hence, they are present in small amounts and *iru* is particularly very rich in phosphorus. The decrease in both Phytate and oxalate (Table 9) may be due to the activities of the enzymes produced by the fermenting bacteria (Enujiugha and Olagundoye, 2001). A similar trend on the reduction of phytate was also reported by Omodara and Aderibigbe (2014).

Table 10 shows the results of organoleptic tests on the samples. It was observed by the panelists that the samples became darker after fermentation, which is similar to the report of Babalola and Giwa (2012) that there was a change in the colour of soybeans during fermentation. Komolafe (2002) attributed this change to the degradation of protein, fat and nucleic acid. The ammonia odour was highest in samples fermented naturally; this may have supported the claim of Aderibigbe *et al.* (2011) who reported that starter culture delays the onset of the ammonia odour. There was no significant difference in the overall acceptability of the spontaneously fermented sample and sample fermented with *Bacillus subtilis*. This actually supports the earlier researchers who claim that *Bacillus subtilis* is the main fermenter of *iru* (Aderigbe *et al.*, 2011, Odunfa, 1981; Odunfa and Adewuyi, 1985).

Table 8: Mineral composition of fermented *iru*

Sample	Mineral							
	P (mg/100g)	K (mg/100g)	Na (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Fe (ppm)	Mn (ppm)	Zn (ppm)
ASE	13.99±0.07^{e}	0.45±0.01^{e}	0.43±0.01^{d}	1.62±0.03^{d}	0.79±0.01^{e}	1.64±0.03^{e}	0.48±0.01^{f}	2.86±0.07^{c}
BSE	17.62±0.02^{d}	0.50±0.01^{d}	0.44±0.01^{cd}	1.25±0.01^{e}	0.61±0.01^{f}	1.86±0.02^{d}	0.68±0.03^{b}	3.17±0.02^{b}
CSE	13.24±0.03^{f}	0.53±0.01^{d}	0.46±0.01^{c}	2.15±0.07^{b}	0.98±0.03^{c}	2.09±0.02^{c}	0.52±0.01^{e}	2.56±0.10^{f}
DAE	29.54±0.06^{a}	1.02±0.03^{b}	0.89±0.01^{a}	1.76±0.03^{c}	0.89±0.01^{d}	1.87±0.01^{d}	0.61±0.02^{c}	3.52±0.04^{a}
CTN	19.72±0.03^{c}	0.75±0.01^{c}	0.64±0.01^{b}	2.18±0.03^{b}	1.05±0.01^{b}	2.15±0.01^{b}	0.93±0.01^{a}	2.85±0.09^{d}
CTR	23.44±0.05^{b}	1.29±0.01^{a}	0.92±0,02^{a}	2.36±0.08^{a}	1.16±0.05^{a}	2.51±0.10^{a}	0.59±0.02^{d}	2.60±0.10^{e}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≤0.05.
ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

Table 9: Anti-nutrient Composition of fermented Iru

	Antinutrient		
Sample	Oxalate (mg/g)	Phytate (mg/g)	Tannin (mg/g)
ASE	9.99±0.13^{b}	26.78±0.58^{b}	2.05±0.00^{b}
BSE	11.21±0.06^{a}	42.44±1.75^{a}	2.22±0.01^{a}
CSE	3.11±0.06^{e}	10.30±0.58^{d}	1.66±0.03^{e}
DAE	3.74±0.06^{d}	14.83±1.17^{c}	1.85±0.05^{d}
CTN	6.80±0.06^{c}	27.19±0.00^{b}	1.93±0.00^{c}
CTR	2.93±0.06^{e}	7.83±0.58^{e}	1.08±0.04^{f}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≤0.05.
ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

Table 10: Mean scores for the sensory evaluation of fermented Iru

Sample	Sensory Evaluation			
	Appearance	Aroma	Texture	Overall acceptability
ASE	5.65±1.31^{a}	5.35±0.99^{abc}	6.05±0.76^{a}	5.80±1.01^{a}
BSE	6.05±1.28^{a}	5.80±1.11^{a}	5.60±1.14^{ab}	5.80±1.36^{a}
CSE	5.35±0.86^{a}	4.75±1.21^{c}	4.90±1.17^{bc}	5.30±0.98^{a}
DAE	5.95±0.83^{a}	5.50±1.10^{ab}	5.75±1.02^{a}	5.80±1.01^{a}
CTN	5.30±1.08^{a}	5.40±0.94^{abc}	5.75±1.16^{a}	5.50±0.83^{a}
CTR	5.55±1.28^{a}	4.95±1.05^{bc}	4.70±1.56^{c}	5.15±1.42^{a}

Values in the same column with the same following letters in superscript are not significantly different (P≤0.05)
ASE = Sample fermented with *B subtilis* grown at 1% salt concentration; BSE = Sample fermented with *B subtilis* grown at 2.5% salt concentration; CSE = Sample fermented with *B subtilis* grown at 5% salt concentration; DAE = Sample fermented with *B subtilis* grown at pH 5.5; CTN = Sample fermented with *B subtilis* grown at 0% salt and normal pH (positive control); CTR = Sample fermented spontaneously (negative control).

Conclusion

In the present study, the performance of *B. subtilis* stressed under varying salt and acid concentrations in Iru fermentation is very much acceptable. There was a low level of anti-nutritional factors except for phytate in all samples of fermented iru. The protein content was high in all samples, although highest in CSE (yet with a moderate ammonia odour) and least in ASE. The overall acceptability of the samples fermented with the stressed *B. subtilis* faired favourably when compared with the spontaneously fermented (CTR) and unstressed *Bacillus subtilis* fermented product (CTN). However, samples ASE, BSE, CTN and DAE appeared darker than the spontaneously fermented *iru*. Nevertheless, they were very much accepted for consumption because the nutritional and organoleptic characteristics were generally improved. Indeed, the stressed environmental and cultural conditions greatly impacted the colour of the fermented iru.

References

Adelekan AO. Nwadiuto E. (2012). Bacterial Succession Studies during Fermentation of African Locust Bean (*Parkia biglobosa*) to Iru Using Molecular Methods. *British Biotechnology Journal*, **2**(1):49-59.

Aderibigbe EY, Visessanguan W, Sumpavapol P. Kongtong K. (2011). Sourcing starter cultures for *Parkia biglobosa* fermentation I: Phylogenic grouping of *Bacillus* species from commercial 'iru' samples**.** *International Journal for Biotechnology and Molecular Biology Research*, **2**(7):121-127.

Antai SP, Ibrahim MH. (1986). Microorganisms associated with African locust bean (*Parkia filicoidea*) fermentation for dawadawa production. *Journal of Applied Bacteriology*, **61**:145-148

AOAC. (2012). Association of Official Analytical Chemist. Official Methods of Analysis of the Analytical Chemist International, 18th ed. Gathersburg, MD USA.

Aremu MO, Awala EY, Opaluwa OD, Odoh R, Bamidele TO. (2015). Effect of Processing on Nutritional composition of African Locust Bean (*Parkia biglobosa*) and Mesquite Beans (*Prosopis Africana*) seeds. *Communication in Applied Sciences*, **3**:22-41.

Atere A.V., Oyetayo V.O. and Akinyosoye F.A. (2019). Effects of starter culture on the Proximate, Antioxidant, Antinutritional and Mineral composition of fermented *Parkia biglobosa* seeds to produce *Iru*. *International Journal of Food Science and Nutrition*, **4**(4), 61-65.

Babalola R.O. and Giwa E.O. (2012). Effect of fermentation on nutritional and anti-nutritional properties of fermenting Soy beans and the antagonistic effect of the fermenting organism on selected pathogens. *International Research Journal of Microbiology*, **3**(10):333-338.

Barber, L. I. and Achinewhu, S. C. (1992). Microbiology of *Ogiri* production from melon seeds (*Citrullus vulgaris*). *Nigerian Food Journal*, **10**, 129-135.

Chinma, C.E., Ezeocha, V.C., Adedeji, O.E., Inyang, C.U., Enujiugha, V.N., Adebo, O.A (2023). African legumes, pulses and oil seeds-based fermented products. In: *Indigenous Fermented Foods for the Tropics*, Adebo, O.A., Chinma, C.E., Obadina, O., Panda, S., Soares, A., & Gan, R.Y. (Eds.). Elsevier, Netherlands. pp. 73-84.

Enujiugha, V.N. (2000). Development of a new food paste from seeds of *Pentaclethra* species. *Applied Tropical Agriculture*, **5**(2), 89-94.

Enujiugha, V.N. (2003). Nutrient changes during the fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Pakistan Journal of Nutrition*, **2**(5), 320-323.

Enujiugha V. N. (2009) Major fermentative organisms in some Nigerian soup condiments. *Pakistan Journal of Nutrition*, **8**(3), 270-283.

Enujiugha, V.N. (2010). The antioxidant and free radical-scavenging capacity of phenolics from African locust bean seeds (*Parkia biglobosa)*. *Advances in Food Sciences*, **32**(2), 88-93.

Enujiugha V. N. (2020). Biotechnology for healthy nutrition and productive lifestyle. Inaugural lecture series 120. Federal University of Technology, Akure, Nigeria, 91p.

Enujiugha V. N., Adeyemo M. B. and Adisa A. M. (2023) Nutritional and safety implications of consuming melon seeds and impacts on international trade: A review. *Food and Humanity*, 1, 241–249. https://doi.org/10.1016/j.foohum.2023.05.020

Enujiugha, V.N., and Akanbi, C.T. (2008). Quality evaluation of canned fermented oil bean seed slices during ambient storage. *African Journal of Food Science*, **2**, 54-59.

Enujiugha, V.N., Akanbi, C.T., and Adeniran, H.A. (2008). Evaluation of starters for the fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Nutrition and Food Science*, **38**(5), 451-457.

Enujiugha, V.N., and Ayodele-Oni, O. (2003). Evaluation of nutrients and some anti-nutrients in lesser-known underutilized oilseeds. *International Journal of Food Science and Technology*, **38**(5), 525-528.

Enujiugha, V.N., and Badejo, A.A. (2002). Cultural alterations for the improvement of *Bacillus subtilis* in the fermentation of African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Applied Tropical Agriculture*, **7**, 6-11.

Enujiugha, V.N., and Olagundoye, T.V. (2001). Comparative nutritional characteristics of raw, fermented and roasted African oil bean (*Pentaclethra macrophylla* Benth) seeds. *La Rivista Italiana delle Sostanze Grasse*, **78**(4), 247-250

Hirata, S., Hayashi, S. and Ohta, M. (2020). Color measurements according to three sections of wood. *Journal of Wood Science*, **66**(36), 1-13.

Isu, N.R. and Njoku, H.O. (1997). An evaluation of the micro flora associated with fermented oil bean (*Pentaclethra macrophylla* Benth) seeds during *Ugba* production. *Plant Foods for Human Nutrition*, **51**: 145-157.

Isu N.R. and Ofuya C.O. (2000). Improvement of the traditional processing and fermentation of African oil bean (*Pentaclethra macrophylla* Bentham) seeds into a food snack ugba. *International Journal of Food Microbiology*, **59**:235-239. DOI: 10.1016/S0168-1605(00)00318-4

Komolafe I.O. (2002). Cereal crops and their foods in the tropics. *African Journal of Agriculture*, **62**:105-116.

Makanjuola OM and Ajayi A. (2012). Effect of natural fermentation on the nutritive value and mineral composition of African locust beans. *Pakistan Journal of Nutrition*, **11**(1):11-13.

Makanjuola, S.A., and Enujiugha, V.N. (2018). Modelling and prediction of selected antioxidant properties of ethanolic ginger extract. *Journal of Food Measurement and Characterization*, **12**(2), 1413-1419.

Ndamitso, M.M., Musah, M., Mathew, J.T. and Bissala, V.T. (2020). Comparative Nutritional Analysis of Daddawa Made from Fermented Parkia biglobosa and Glycine max Seeds. *Comminication in Physical Science*, **5**, 263-269

Njoku, H.O., Ogbulie, J.N. and Nnubia, C. (1990). Microbiological study of the traditional processing of African oil bean (*Pentaclethra macrophylla*) for ugba production. *Food Microbiology*, **7**, 13-26.

Nwagu, T.N., Ugwuodo, C.J., Onwosi, C.O., Inyima, O., Uchendu, O.C. and Akpuru, C. (2020). Evaluation of the probiotic attributes of Bacillus strains isolated from traditional fermented African locust bean seeds (*Parkia biglobosa*),"daddawa". *Annals of Microbiology*, **70**(1), 1-15.

Obeta J.A.N. (1983). A note on the microorganisms associated with the fermentation of seeds of African oil bean (*Pentaclethra macrophylla*). *Journal of Applied Bacteriology*, **54**:433-435

Odunfa S.A. (1981). Microbiology associated with fermentation of African locust bean during iru preparation. *International Journal of Plant Foods*, **25**:245-250.

Odunfa SA. and Adewuyi EY. (1985). Optimization of process conditions for the fermentation of African locust bean (*Parkia biglobosa*). I. Effect of time, temperature and humidity. *Journal of Chemical Technology and Food Microbiology*, **9**:6-10.

Ogbadu C.O. and Okagbue R.N. (1988). Bacterial fermentation of soybean for *dawadawa* production.

Journal of Applied Bacteriology, **65**:353-356. DOI: 10.1111/j.1365-2672.1988.tb01902

Ogueke C.C. and Aririatu L.E. (2004). Microbial and organoleptic changes associated with *ugba* stored at ambient temperature. *Nigerian Food Journal*, **22**:133-140.

Ojewumi E.M., Omoleye J.A. and Ajayi A.A. (2016). The effect of different starter culture on the protein content in fermented African Locust Bean (*Parkia biglobosa*) seeds. *International Journal of Engineering Research and Technology*, **5**(4):249-255.

Oluwaniyi O.O., Bazambo I.O. (2016). Nutritional and Amino acid analysis of Raw, Partially Fermented and Completely fermented Locust Bean (*Parkia biglobosa*) seeds. *African Journal of Food Agriculture, Nutrition and Development*, **16**(2):10866-10883.

Omafuvbe, B.O., Falade, O.S., Osuntogun, B.A. and Adewusi, S.R.A. (2004).Chemical and Biochemical Changes in African Locust Beans (*Parkia biglobosa)* and Melon (*Citrullus vulgaris*) Seed sduring Fermentation to Condiments. *Pakistan Journal of Nutrition*. **3**(3): 140-145.

Omodara TR. Aderibigbe EY. (2014). Effects of Starter culture and different Componets of 'Kuuru' on the Nutritional Quality of Fermented *Parkia biglobosa*. *International Journal of Applied Microbiology and Biotechnology Research*, **2**:72-78.

Owens, J.D., Allagheny, N., Kipping, G. and Ames, J.M. (1997). Formation of volatile compounds during *Bacillus subtilis* fermentation of soya beans, *Journal of the Science of Food and Agriculture*, **74**, 132-40.

Oyedokun, J., Badejo, A.A., and Enujiugha, V.N. (2016). Biochemical changes associated with poly- γ –glutamic acid synthesis during spontaneous and *Bacillus subtilis* fermentation of *Parkia biglobosa* seed into iru. *Advances in Food Sciences*, **38**(3), 117-124.

Oyedokun, J., Badejo, A. A., Oluwayomi, S. F., and Enujiugha, V. N. (2020). Synthesis of poly-γ-glutamic acid during fermentation of African locust bean (*Parkia biglobosa*). *Applied Tropical Agriculture*, **25**(2), 74-78.

Price M. L., Scoyoc S. V. and Butler L. G. (1978). A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum grain. *Journal of Agricultural and Food Chemistry*, **26**, 1214-1218.

Reddy, N. R., Sathe, S. K. and Salunkhe, D. K. (1982). Phytates in legumes and cereals. *Advances in Food Research*, **28**, 1-9.

Sanni AI. (1993). Biochemical changes during production of okpehe—A Nigerian fermented food condiment. *Chemical Microbiological Technology Lebensmittel*, **15**:97-100

Sanni AI, Onilude AA, Fadahunsi IF, Ogubanwo ST and Afolabi RO. (2002). Selection of starter cultures for the production of *ugba*, a fermented soup condiment. *European Food Research and Technology*, **215**:176-180. DOI: 10.1007/ s00217-002-0520-3

Talabi, J. Y., Oguntoyinbo, O. O., and Enujiugha, V. N. (2023). Serum Lipid Profile and Organ Histology of Albino Rats Fed on Conophor (*Tetracarpidium conophorum*) Nut Oil-Based Diets. *IPS Journal of Nutrition and Food Science*, **2**(1), 9–12. https://doi.org/10.54117/ijnfs.v2i1.18.

Yangılar, F. (2016). Production and evaluation of mineral and nutrient contents, chemical composition, and sensory properties of ice creams fortified with laboratory-prepared peach fibre. *Food and Nutrition*, **60**, 31882.