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# Evaluation of changes in total amino acids and sugars during natural and controlled fermentation of lima bean into "daddawa"

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## Abstract
In Nigeria, a popular traditional condiment produced by fermentation of African locust bean is referred to as "Daddawa" in Hausa and "iru" in Yoruba. However, a less utilised legume, the Lima bean can also be used in the production of the condiment. Hence, this research work aims to evaluate the changes in amino acids and sugars during fermentation of lima beans into condiment and carry out sensory evaluation of the final condiment. Lima bean was fermented into "daddawa" by natural fermentation (NF) and also with the use of pure culture of *Bacillus subtilis* as starter (FBS). The amino acids, reducing sugars and total soluble sugars during the fermentation period were evaluated using standard methods. "Daddawa" samples produced (NF and FBS) were compared organoleptically with the commercial "daddawa" from locust beans. Total essential amino acids (TEAAs) content in NF and FBS were highest at 48 h of fermentation (33.49 and 35.77 g/100 respectively). Reducing sugar increased and attained peak at 24 h of fermentation in both NF and FBS after which it reduced till the end of fermentation period. There was no significant difference ($p > 0.05$) in total soluble sugar content of NF and FBS at 48 h and 72 h of fermentation. FBS was the most accepted in all the sensory attributes evaluated. In conclusion, lima bean could be fermented into "daddawa" by natural method and also by using pure starter culture. Total essential amino acids, reducing sugars and acceptability of the lima bean "daddawa" were enhanced by fermentation of lima bean with pure starter culture of *Bacillus subtilis* over the naturally fermented samples.

**Key words:** Amino acids, Controlled fermentation, Natural fermentation, Sugars

## Introduction
Condiments are substances added to soups, stews and dishes for flavour and taste enhancement. Some of them are culinary products produced by fermentation of legumes (Achi, 2005). A popular traditional condiment made from the African locust bean (*Parkia biglobosa*) is widely consumed in Nigeria. It is known as "daddawa" in Hausa and *iru* in Yoruba. "Daddawa" is rich in protein, fibre and minerals. It plays a vital role as an alternative to animal protein in the diet of poor families especially in the rural communities (Achi, 2005). Locust bean "daddawa" is becoming expensive because the parkia trees are going into extinction. Alternative beans such as soya beans have been used in the production of "daddawa" (Omafuvbe *et al*., 2000; 2002; 2004, Farinde *et al*., 2006) The desired state of fermentation of condiments into "daddawa" is indicated by the formation of mucilage and production of ammonia as a result of the breakdown of amino acids during the fermentation process (Omafuvbe *et al*., 2002). The characteristic ammoniacal odour and flavour of condiments enhance the taste of traditional soups and sauces especially the various soups used as accompaniment to the starchy root and tuber diets (Adelekan and Nwadiuto, 2012).

Lima bean (*Phaseolus lunatus*) is an underutilised legume in Nigeria. A lot of work has been carried out on the improvement of its utilisation (Farinde *et al.*, 2011; Adeniran *et al*., 2013; Farinde *et al.*, 2014; Baddi Jayalaxmi *et al*., 2016; Oraka, 2017; Farinde *et al*. 2017)**.** Lima beans could serve as alternative beans for production of "daddawa" as previously reported (Farinde *et al*., 2011; 2017). Lima bean "daddawa" has been reported to be rich in protein and low in fat (Farinde *et al*., 2011).

Protein and sugars are important constituents of legumes. Proteins can be broken down by hydrolysis into simple units called amino acids. Amino acids are biologically important organic compounds which are composed of an amino ($-NH_2$) group with basic properties and a carboxylic acid (-COOH) group with acidic properties attached to the same carbon along with a side-chain specific to each amino acid (Shakuntala and Shadaksharaswamy, 2008). Amino acids are joined together by peptide linkages in which the amino group of one amino acid is linked to the carboxyl group of another amino acid with elimination of a molecule of water to form short polymer chains called peptides or longer chains called either polypeptides or proteins (Rodnina *et al*., 2007). Sugars are the products of hydrolysis of carbohydrates, usually monosaccharides or disaccharides (Flitsch and Ulijn, 2003; Shakuntala and Shadaksharaswamy, 2008).They could be soluble sugars, reducing or non-reducing sugars.

Fermentation is an important food processing unit operation in which food materials are acted on by microorganisms during which biochemical changes occur resulting in improved texture, taste and flavour of the food material. Farinde *et al*. (2017) previously reported the microbial and biochemical changes during the natural and controlled fermentation of lima beans. This study therefore evaluated the changes that occur in the protein and sugar contents of lima bean during its natural and controlled fermentation into "daddawa".

## Materials and Methods

### Source of materials

Local brown variety of mature dry lima bean seeds (*Phaseolus lunatus*) were purchased from a local market at Ita-Ogbolu, via Akure, Ondo State Nigeria. Processing materials such as cooking utensils and fermenting containers were obtained from the Food Processing Laboratory of the Product Development Programme, Institute of Agricultural Research and Training, Moor Plantation, Ibadan, Nigeria. Pure starter culture (*Bacillus subtilis*) was isolated from naturally fermenting lima beans.

### Natural fermentation of lima beans into "daddawa"

Natural fermentation of lima beans into "daddawa" was carried out according to the method used by Farinde *et al*. (2014). Lima bean seeds (1kg) were roasted in a frying pan, dehulled and cooked in boiling water for 45 minutes on a gas cooker. The cooked beans were drained and divided into four portions. One portion was left unfermented while the remaining three portions were poured while still warm into three clean calabashes lined with clean banana leaves, covered with more clean banana leaves and then with the calabash lids. The beans were fermented in an incubator at 35 ± 2°C for 72 hours. Fermented samples were taken out in duplicates for each fermentation stage at 24 hour intervals.

**Controlled fermentation of lima bean seeds into "daddawa"**

Controlled fermentation of lima bean seeds was carried out using the method described by Omafuvbe *et al*. (2002) with slight modification. Roasted and dehulled lima bean seeds (1kg) were washed with water and cooked for 30 minutes on a gas cooker. Cooked beans (50g) were dispensed into 250 mls conical flasks plugged with cotton wool. Contents of the flasks were autoclaved at 121°C for 15 minutes at 15 psi pressure to obtain sterile dehulled cooked beans. The sterile cooked beans were cooled to ambient temperature before inoculation. Suspension of 18 hour old pure culture of *B. subtilis* isolated from naturally fermenting lima bean seeds and maintained on tryptone soya agar slant in refrigerator was used as starter to inoculate lima bean seeds. Suspension (500 µL of culture) was used to inoculate 50g of sterile lima bean seeds in the conical flasks (this gave approximately 4.0 log CFU/g wet weight of the lima bean seeds). A control was set up with one flask containing 50g sterile lima bean seeds without inoculum. The flasks and their contents were incubated at 35°C for 72 hours after which samples were taken out in duplicates at 24 hour interval for analysis.

**Determination of total amino acids (Amino acid profile)**

Quantification of total amino acids of the fermenting lima bean samples was determined using the method described by Ijarotimi and Olapade (2009). Samples were freeze-dried and then hydrolysed for 24 hours at 110°C with 6 M HCL. After hydrolysis, the samples were freeze-stored in sodium citrate buffer at pH 2.2. When ready for analysis, 50 µl of the hydrolysate was directly injected into the amino acid analyser (S433 amino acid SYKAM, Eresing Germany). Cystein and methionine were determined after performing acid oxidation prior to hydrolysis in 6 M HCL and measured as cysteic acid and methionine sulphone respectively.

**Determination of reducing sugar**

Reducing sugar was determined using dinitrosalicylic reagent method described by Falegan (2012). Ethanolic extract was diluted tenfold. To 1ml of the aliquot was added 2mls of 3, 5, dinitrosaliccylic acid (DNSA). The mixture was heated in a boiling water bath for 5 minutes, cooled in cold water and diluted with 7mls of distilled water. The absorbance of the resultant solution was read at 540 nm in a spectrophotometer (Spectrumlab 752x). The amount of reducing sugar was estimated from a standard curve of varying concentration of glucose.

**Determination of total soluble sugar**

Total soluble sugar was determined using the anthrone reagent method described by Omafuvbe *et al*. (2000). Ethanolic extract was diluted tenfold. To 1ml of the aliquot was added 5mls of anthrone reagent. The reaction mixture was mixed thoroughly, heated in boiling water bath for 10 minutes and allowed to cool to room temperature. The absorbance of the mixture was read in a spectrophotometer (Spectrumlab 752x) set at 620nm. The amount of soluble sugars in the samples was estimated from a standard curve of varying concentrations of glucose.

**Sensory evaluation of the lima bean "daddawa" samples**

Naturally fermented (NF) and *Bacillus subtilis*-fermented "daddawa" (FBS) and the commercial locust bean "daddawa" (ZAP) were used to prepare okro soup and coded accordingly. The coded "daddawa" okro soup samples were presented to twenty semi-trained panel of judges (Postgraduate students and members of staff of the Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife) who are familiar with the traditional locust bean "daddawa". The panelists were provided with water for mouth rinsing after each round of tasting. The panelists were asked to score the samples for colour, texture, stickiness, flavour, taste and overall acceptability. The attributes were scored using a 7 point Hedonic scale where 7 = like extremely, 4 = neither like nor dislike and 1 = dislike extremely (Montgomery, 2004)

**Statistical Analysis**

Data obtained were subjected to descriptive and inferential statistics (ANOVA) using SPSS (Version 17). Means of samples were separated using the Duncan Multiple range Test. Significance was accepted at 5% level.

**Results and Discussion**

**Changes in amino acid profile during fermentation of lima bean to produce "daddawa"**

Lysine content increased from 6.25g/100g protein at 0 hour to 7.48g/100g protein at 48 hours of fermentation in naturally fermented lima beans after which it dropped to 7.20g/100g protein at 72 hours of fermentation (Table 1). Lysine content of *Bacillus subtilis*-fermented lima beans on the other hand increased from 6.25g/100g protein at 0 hour to 7.40g/100g protein at 48 hours and further increased to 7.71g/100g protein at 72 hours of fermentation. *Bacillus subtilis* was used as starter for the controlled fermentation of the lima bean seeds because it was found to be predominant and majorly involved in fermentation of lima beans to produce "daddawa" as previously reported by Farinde *et al*. (2014).

Methionine content also increased from 1.88g/100g protein at 0 hour to 2.35g/100g protein at 48 hours of fermentation in naturally fermented lima beans after which it dropped to 1.95g/100g protein at 72 hours of fermentation (Table 1). Methionine content of *Bacillus subtilis*-fermented lima beans similarly increased from 1.88g/100g protein at 0 hour to 2.25g/100g protein at 48 hours after which it dropped to 2.08g/100g protein at 72 hours of fermentation. Methionine content of all the lima bean samples fell within the recommended daily allowance of 2g/100g methionine as reported by FAO/WHO (2013). All the other essential amino acids, valine, threonine, isoleucine, leucine and phenylalanine had their content fluctuating with fermentation time. However, isoleucine, leucine and lysine contents of 2.8g/100g, 6.25g/100g and 5.8g/100g protein respectively in the fermented lima bean samples were still within the recommended daily allowance recommended by FAO/WHO (2007). There was no significant difference ($p > 0.05$) in the methionine and phenylalanine contents of lima beans fermented naturally and lima beans fermented with pure starter culture of *B. subtilis* at 48 hours of fermentation. There was also no significant difference ($p > 0.05$) in the valine content of lima beans fermented naturally and lima beans fermented with pure starter culture of *B. subtilis* at 72 hours of fermentation. Lima beans fermented with *B. subtilis* at 48 hours was significantly higher ($p < 0.05$) in histidine compared with all the other lima bean samples. Drop in lysine and the other amino acid contents after 48 hours was probably as a result of the decrease in the microbial activities at the later stages of fermentation process. Farinde *et al*. (2017) reported that during fermentation of lima beans into *daddawa*, the fermenting bacteria (*Bacillus* species) had their optimal growth at 48 hours of fermentation and that the more their count, the more the protease enzymes being produced and the more their activities to break down protein into smaller molecules of amino acids; an indication that their growth were reduced after 48 hours and subsequently their activities which could be due to factors such as reduced nutrients and water activity.

The total essential amino acids (TEAAs) content in naturally fermented lima beans increased from 31.90g/100g at 0 hour to 33.49g/100g at 48 hours of fermentation after which it dropped to 31.83g/100g at 72 hours of fermentation (Table 1). The same trend was observed for lima beans fermented with *Bacillus subtilis* in which the TEAAs increased from 31.90g/100g at 0 hour to 35.77g/100g at 48 hours after which it dropped to 34.18g/100g at 72 hours of fermentation. Amino acids are the building blocks of proteins. They are important taste and flavour enhancers in fermented vegetable proteins (Kpikpi *et al.*, 2009). Amino acid content of fermented foods can change with fermentation time because of liberation of proteolytic enzymes which set the hydrolysis of proteins in motion. The increase observed

**Table 1: Changes in Essential Amino Acid (EAAs) profile during fermentation of Lima bean to produce "*Daddawa*"**

| Essential amino acids (EAAs) | Sample / amino acid concentration (g/100 g protein) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0h | NF 24h | FBS 24h | NF 48h | FBS 48h | NF 72h | FBS72h |
| Valine | 5.02± 0.03 [b] | 5.70 ± 0.05[a] | 4.51 ± 0.03[c] | 4.07 ± 0.09[d] | 4.96 ± 0.02[b] | 4.34 ± 0.01[c] | 4.37 ± 0.04[c] |
| Threonine | 2.45 ± 0.08[b] | 2.22 ± 0.05[c] | 1.84 ± 0.01[de] | 1.70 ± 0.00[e] | 2.91 ± 0.05[a] | 1.91 ± 0.03[d] | 2.13 ± 0.04[c] |
| Isoleucine | 2.41 ± 0.00[bc] | 2.67 ± 0.06[a] | 2.77 ± 0.01[a] | 2.64 ± 0.06[a] | 2.51 ± 0.01[b] | 2.75 ± 0.01[a] | 2.32 ± 0.02[c] |
| Leucine | 6.75 ± 0.02[c] | 6.32 ± 0.06[d] | 7.59 ± 0.09[a] | 7.32 ± 0.05[b] | 8.33 ± 0.10[a] | 6.99 ± 0.13[c] | 8.33 ± 0.03[a] |
| Lysine | 6.25 ± 0.02[e] | 7.03 ± 0.12[d] | 7.82 ± 0.03[a] | 7.48 ± 0.19[ab] | 7.40 ± 0.05[bc] | 7.20 ± 0.12[c] | 7.70 ± 0.13[b] |
| Methionine | 1.88 ± 0.01[cd] | 1.77 ± 0.01[d] | 2.25 ± 0.05[a] | 2.35 ± 0.01[a] | 2.25 ± 0.07[a] | 1.95 ± 0.14[c] | 2.08 ± 0.04[b] |
| Phenylalanine | 5.43 ± 0.01[a] | 5.32 ± 0.04[b] | 4.99 ± 0.09[b] | 5.59 ± 0.05[bc] | 4.94 0.08[bc] | 4.71 ± 0.21[c] | 5.05 ± 0.14[b] |
| Histidine | 1.67 ± 0.00[e] | 1.73 ± 0.03[e] | 2.16 ± 0.03[c] | 2.30 ± 0.02[b] | 2.44 ± 0.04[a] | 1.96 ± 0.01[d] | 2.16 ± 0.01[c] |
| TEAAs | 31.90 ± 0.07[d] | 32.80 ± 0.01[cd] | 33.96 ± 0.01[c] | 33.49 ± 0.03[c] | 35.77 ± 0.02[a] | 31.83 ± 0.06[d] | 34.18 ± 0.02[b] |

Means followed by different superscript in the same row are significantly different at $p < 0.05$
NF = Naturally fermented, FBS = Fermented with *Bacillus subtilis*, EAAs = Essential Amino acids, TEAAs = Total Essential Amino Acids

in total essential amino acids up till 48 hours was probably as a result of enzymatic breakdown of proteins by the fermenting microorganisms (proteolysis). Similar trend was observed by Song *et al*. (2008) during soya bean meal fermentation.

The decrease observed in the TEAAs after 48 hours of fermentation might be due to depletion of food supply, oxygen and water activity and increase in respiration of the microorganisms (Okpokwasli and Nweke, 2005). During fermentation, biochemical changes take place and high amounts of amino acids are released. However, as fermentation progresses, nutrients become depleted and growth of the fermenting microorganism are reduced and subsequently the amount of amino acids produced become reduced (Okpokwasli and Nweke, 2005). The decrease in the concentration of most amino acids after 48 hours also suggests metabolism of amino acids by the bacteria responsible for fermentation. This process is important for the development of the aroma and characteristic flavour of the product (Odibo *et al*., 2008; Dakwa *et al*., 2005). The total essential amino acids (TEAAs) in naturally fermented lima bean samples and lima bean samples fermented with *B. subtilis* compared well with the recommended total essential amino acids' daily requirement of 33g/100g (FAO/WHO 2013). The results obtained for total essential amino acids are similar to the report of Ijarotimi and Keshinro (2012) during fermentation of locust beans.

Glycine, a non-essential amino acid increased with fermentation time in lima bean samples fermented with *B. subtilis*. It increased from 3.51g/100g at 0 hour to 4.35g/100g at 48 hours and further increased to 4.46g/100g at 72 hours of fermentation (Table 2). There was no significant difference ($p > 0.05$) in the glycine content of naturally fermented lima beans and lima beans fermented with pure starter culture of *B. subtilis* at 48 hours of fermentation. Glycine is found in appreciable amounts in structural proteins and it is important for the synthesis of nucleic acids, bile acids and non-essential amino acids in the body (Kitamura *et al*., 2011). Glycine has been reported to impart sweet flavour to foods (Norziah and Ching, 2000). Serine content increased from 4.11g/100g protein at 0 hour to 5.45g/100g protein and 5.74g/100g protein at 48 hours and 72 hours respectively in naturally fermented lima beans. Similarly, it increased from 4.11g/100g protein at 0 hour to 4.82g/100g protein and 5.52g/100g protein at 48 hours and 72 hours respectively in lima beans fermented with *B. subtilis*. Tyrosine also increased with fermentation time in both naturally fermented lima beans and lima beans fermented with pure starter culture of *Bacillus subtlis*.

Glutamate content was highest in lima beans fermented with *B. subtilis* at 72 hours of fermentation. Glutamate has been reported as the most abundant amino acid in fermented and unfermented legumes (Ijarotimi and Keshinro, 2012). Glutamate, an amine of glutamic acid has also been reported as one of the compounds contributing to the characteristic flavour of fermented seeds. It was reported as the most important flavour-enhancing amino acid (David, 2004). It is also used in the production of seasoning agents such as monosodium glutamate (Piyarat, 2008).

The total non-essential amino acids (TNEAAs) increased from 47.98g/100g at 0 hour to 55.51g/100g at 48 hours of

**Table 2: Changes in Non-Essential Amino Acids (NEAAs) profile during fermentation of Lima bean to produce "Daddawa"**

| | Sample/ amino acid concentration (g/100 g protein) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Non Essential amino acids (NEAAs)** | **0h** | **NF 24h** | **FBS 24h** | **NF 48h** | **FBS 48h** | **NF 72h** | **FBS72h** |
| Glycine | 3.35 ± $0.03^{b}$ | 3.42 ± $0.02^{b}$ | 4.35 ± $0.11^{a}$ | 4.43 ± $0.18^{a}$ | 4.37 ± $0.02^{a}$ | 4.13 ± $0.12^{a}$ | 4.46 ± 0.21 |
| Alanine | 4.26 ± $0.07^{d}$ | 4.20 ± $0.08^{d}$ | 4.77 ± $0.05^{a}$ | 4.66 ± $0.02^{b}$ | 4.50 ± $0.09^{c}$ | 4.36 ± $0.11^{c}$ | 4.71 ± 0.07 |
| Serine | 4.11 ± $0.08^{d}$ | 4.75 ± $0.05^{c}$ | 5.73 ± $0.10^{ab}$ | 5.45 ± $0.05^{b}$ | 4.82 ±$0.09^{c}$ | 5.74 ± $0.02^{a}$ | 5.52 ± $0.12^{ab}$ |
| Proline | 2.12 ± $0.01^{b}$ | 2.69 ± $0.07^{a}$ | 2.11 ± $0.06^{b}$ | 1.86 ± $0.03^{c}$ | 1.79 ± $0.06^{c}$ | 2.17 ± $0.04^{b}$ | 1.98 ± $0.11^{bc}$ |
| Aspartate | 6.75 ± $0.10^{de}$ | 6.59 ± $0.06^{e}$ | 7.99 ± $0.05^{a}$ | 7.29 ± $0.04^{b}$ | 7.46 ± $0.11^{b}$ | 6.94 ± $0.04^{d}$ | 7.02 ± $0.09^{c}$ |
| Glutamate | 13.65 ± $0.04^{d}$ | 14.62 ± $0.11^{c}$ | 15.75 ± $0.06^{b}$ | 16.52 ± $0.16^{a}$ | 15.48 ± $0.10^{b}$ | 14.47 ± $0.01^{c}$ | 16.62 ± $0.12^{a}$ |
| Arginine | 8.85 ± $0.04^{d}$ | 9.09 ± $0.05^{bc}$ | 8.56 ± $0.05^{e}$ | 9.43 ± $0.04^{b}$ | 9.38 ± $0.01^{c}$ | 8.41 ± $0.21^{f}$ | 9.73 ± $0.21^{a}$ |
| Tyrosine | 2.64 ± $0.02^{e}$ | 3.24 ± $0.01^{d}$ | 3.38 ± $0.01^{cd}$ | 3.63 ± $0.12^{b}$ | 3.45 ± $0.01^{c}$ | 3.95 ± $0.05^{a}$ | 3.62 ± $0.05^{b}$ |
| Cysteine | 2.23 ± $0.01^{d}$ | 3.54 ± $0.05^{a}$ | 2.21 ± $0.03^{d}$ | 2.21 ± $0.02^{d}$ | 2.41 ± $0.02^{c}$ | 2.99 ± $0.04^{b}$ | 2.44 ± $0.04^{c}$ |
| TNEAAs | 47.98 ± $0.01^{f}$ | 52.17 ± $0.05^{e}$ | 54.89 ± $0.05^{d}$ | 55.51 ± $0.10^{b}$ | 53.64 ± $0.06^{c}$ | 53.19 ± $0.01^{c}$ | 56.14 ± $0.01^{a}$ |

Means followed by different superscript in the same row are significantly different at $p < 0.05$
NF = Naturally fermented, FBS = Fermented with *Bacillus subtilis,* NEAAs = Non-Essential Amino acids, TNEAAs = Total Non-Essential Amino Acids

fermentation after which it dropped to 53.19g/100g at 72 hours of fermentation in naturally fermented lima beans. However the total non-essential amino acids increased throughout the fermentation process in lima beans fermented with pure starter culture of *B. subtilis*. Uaboi-Egbemi *et al.* (2009) similarly reported an increase in amino acid content during "daddawa" production from African locust beans.

Generally, both naturally fermented lima beans and lima beans fermented with pure culture of *Bacillus subtilis* contain appreciable amounts of amino acids particularly at 48 hours of fermentation probably because food supply, oxygen and water activity and protease enzyme needed by the microorganisms for production of the amino acids were still optimal at that stage of fermentation. The result compared well with the amino acid composition of germinated and fermented African locust beans (Ijarotimi and Keshinro 2012).

### Changes in reducing sugar during natural and controlled fermentation of lima bean into "daddawa"

Reducing sugars increased and attained peak at 24 hours of fermentation in both naturally fermented (NF) and pure starter culture-fermented samples (FBS) (Table 3). Reducing sugars increased from 1.24

and 1.23mg glucose $g^{-1}$ at 0 hour to 2.87 and 3.37mg glucose $g^{-1}$at 24 hours in NF and FBS respectively after which the reducing sugar content reduced till the end of the fermentation process. The increase in reducing sugar level in the first 24 hours of fermentation is probably due to microbial activities which might have improved amylase production at the initial stage of fermentation. Amylolytic microorganisms begin to liberate amylases within the first 24 hours if the conditions are favourable. Decrease in reducing sugar content after 24 hours till the end of fermentation was also a reflection of microbial activities. This could probably be because most of the reducing sugars have been used up by the fermenting microorganisms at the later stages of fermentation. Similar trend of increase in reducing sugars at early stages and subsequent decrease at later stages of fermentation was reported by Omafuvbe (2006) when soya beans was fermented with *B. subtilis* with or without addition of salt. The trend of reduction in reducing sugar level observed in this study also agrees with the report of Omafuvbe *et al.* (2002). Reducing sugar level was significantly lower ($p< 0.05$) at 72 hours in lima bean fermented naturally (1.38mg glucose $g^{-1}$) compared to the pure starter culture-fermented samples.

**Table 3: Changes in Reducing Sugar during Fermentation of Lima Bean into "Daddawa"**

| Fermentation time (h) | Reducing sugar (mg glucose $g^{-1}$ dry wt.) | |
|---|---|---|
| | NF | FBS |
| 0 | $1.24 \pm 0.02^{a}$ | $1.23 \pm 0.03^{a}$ |
| 24 | $2.87 \pm 0.02^{b}$ | $3.37 \pm 0.02^{a}$ |
| 48 | $1.57 \pm 0.03^{b}$ | $2.28 \pm 0.01^{a}$ |
| 72 | $1.38 \pm 0.06^{b}$ | $2.25 \pm 0.03^{a}$ |

Values are mean of three replicate ± standard errror
Means in the same row followed by different superscript are significant different at $p < 0.05$
NF = Lima bean fermented naturally, FBS = Lima bean fermented with *B. subtilis*

**Table 4: Changes in Total Soluble Sugar during fermentation of Lima Bean to Produce "Daddawa" (mg glucose $g^{-1}$)**

| Fermentation time (h) | Total soluble sugar (mg glucose $g^{-1}$ dry wt.) | |
|---|---|---|
| | NF | FBS |
| 0 | $44.50 \pm 0.50^{a}$ | $47.00 \pm 0.10^{a}$ |
| 24 | $36.00 \pm 1.00^{a}$ | $25.00 \pm 0.50^{b}$ |
| 48 | $23.75 \pm 2.75^{a}$ | $20.00 \pm 2.0^{b}$ |
| 72 | $13.50 \pm 1.50^{a}$ | $15.75 \pm 1.75^{a}$ |

Values are mean of three replicate ± standard errror
Means in the same row followed by different superscript are significant different at $p < 0.05$
NF = Lima bean fermented naturally, FBS = Lima bean fermented with *B. subtilis*

**Changes in Total soluble sugar content during natural and controlled fermentation of lima bean into "daddawa"**

Presented in Table 4 are changes in total soluble sugar content during natural and controlled fermentation of lima beans to produce "daddawa". Highest total sugar was recorded at 0 hour in both naturally fermented and pure starter culture-fermented samples. However, the total soluble sugars decreased with fermentation time in all the samples. It decreased from 44.50 and 47.25mg glucose $g^{-1}$ at 0 hour to 13.50 and 17.75mg glucose $g^{-1}$ at 72 hours of fermentation in NF and FBS respectively. Omafuvbe *et al.* (2004); Omafuvbe (2006) and Yabaya (2006) reported that decrease in total soluble sugars with fermentation time in African locust beans and melon seeds, soya beans and lima beans respectively was probably due to utilisation of the soluble sugars by the fermenting microorganisms for metabolic activities.

There was no significant difference ($p > 0.05$) in the total soluble sugar content at 0 hour in both naturally fermented and starter culture-fermented lima bean samples. There was also no significant difference ($p > 0.05$) in total soluble sugar content of NF and FBS at 48 hours and 72 hours of fermentation. Lima beans fermented naturally was significantly higher ($p < 0.05$) in total sugar content at 24 hours compared to the pure starter culture-fermented lima bean samples. This might be due to the fact that the microbial population in the natural fermentation is a mixed one and may contain more amylolytic bacteria which may produce more soluble sugars at the early stages of fermentation than the *Bacillus subtilis*-fermented sample that contains single pure culture.

**Sensory evaluation of lima bean "daddawa" produced by natural and pure starter culture fermentation**

The result of the sensory evaluation of lima bean "daddawa" (produced from natural and controlled fermentation) used as condiment in okro soup is presented in Table 5. There was no significant difference ($p > 0.05$) in the flavour of lima bean fermented with *B. subtilis* (FBS) and the commercial "iru" (ZAP) when added to okro soup. There was also no significant difference ($p > 0.05$) in the texture of naturally fermented lima bean (NF) and the commercial iru (ZAP). Lima bean fermented naturally (NF) was least accepted in all the attributes evaluated compared with the other "daddawa" samples when added to okro soup. Nevertheless, it was accepted. Lima beans fermented with *B. subtilis* (FBS) was the most accepted in terms of overall acceptability. It is also worthy of note that FBS recorded the highest scores in all the attributes evaluated compared to the other "daddawa" samples.

**Conclusion**

Total essential amino acids (TEAAs) increased with fermentation time, reached peak at 48 hours and then decreased till the end of the fermentation period in all the samples. Total non-essential amino acids (TNEAAs) increased throughout the fermentation period in pure culture-fermented lima bean samples. Reducing sugars increased at the early stages and decreased at the later stages of fermentation in both naturally fermented and pure starter culture-fermented lima beans. Total soluble

**Table 5: Sensory Evaluation of Lima Bean "Daddawa" Produced from Natural and Controlled Fermentation as Condiment in Okro Soup**

| Sample | Colour | Flavour | Texture | Stickiness | Taste | Overall acceptability |
|---|---|---|---|---|---|---|
| FBS | 5.85± 0.24 [a] | 5.15± 0.19[a] | 5.25± 0.23[a] | 5.50± 0.25[a] | 5.35± 0.27[a] | 5.55± 0.27[a] |
| NF | 4.20± 0.26[c] | 4.20± 0.32[b] | 4.05± 0.24[b] | 3.30± 0.53[c] | 4.05± 0.31[c] | 4.35± 0.27[c] |
| ZAP | 5.00± 0.30[b] | 5.05± 0.31[a] | 4.80± 0.23[b] | 5.10± 0.23[b] | 5.10± 0.30[b] | 5.35± 0.30[b] |

Values are mean scores ± standard error where n = 20
Means followed by different superscript within the same column are significantly different ($p < 0.05$).
FBS = Fermented with *B. subtilis,* NF = Naturally fermented, ZAP = Commercial *iru*

sugars decreased with fermentation time in both naturally fermented and pure starter culture-fermented lima beans. *Bacillus subtilis*-fermented "daddawa" was the most accepted in all the sensory attributes evaluated. Lima beans could be fermented into "daddawa" by natural method and also by using pure starter culture. Total essential amino acids, reducing sugar content and acceptability of the lima bean "daddawa" were enhanced by fermentation of lima beans with pure starter culture of *Bacillus subtilis* over the naturally fermented samples.

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