Comparative amino acid and volatile flavor profile of dawadawa produced from the seeds of *P. biglobosa*, *G. max* and *H. sabdariffa*

Abstract

A comparative analysis of free amino acid and volatile organic compounds profile of dawadawa produced from the seeds *Parkia biglobosa*, *Glycine max* and *Hibiscus sabdariffa* was evaluated The free amino acid profile were analysed using amino acid analyser while the volatile organic compound profile were analysed using Gas –Chromatography-Mass Spectrometry (GC-MS). Difference was observed in the amino acid profile of the dawadawa with laboratory produced dawadawa recording an increased in the essential amino acid lysine, valine, methionine and leucine while tyrosine been the only non-essential amino acid that slight increased. Aspartic and glutamic acids seems to be the major amino acids in locally produced dawadawa with a value of 9.00 and 17.26 g/100 g protein. Fermentation increased the bioavailability of aspartic acid (9.00 to 9.31 g/100 g protein) while the glutamic acid decreased from 17.26 to 14.38 g/100 g protein after fermentation under laboratory conditions. The locally and laboratory produced dawadawa from *G. max*, the laboratory produced dawadawa showed increased in the six essential amino acid. The essential amino acid leucine and non-essential amino acids aspartic and glutamic acid are identified as the major amino acids in locally produced dawadawa from locust bean. The locally produced dawadawa from *H. sabdariffa* had the highest amino acid for lysine, valine glutamic acid and proline while threonine was the same in both local and laboratory produced. The locally and laboratory fermented seeds of *P. biglobosa* showed several volatile compounds in both dawadawa with locally produced dawadawa having 21 volatile organic compounds while dawadawa produced in the laboratory had 24 volatile organic compounds. The *G. max* produced dawadawa had 6 esters, 5 amides, 4 acids, 3 alcohols, 2 hydrocarbons and one heterocyclic compound. The volatile organic flavor compounds detected in dawadawa produced from *H. sabdariffa* seeds include 2 acids class flavor volatile, 1 alcohols, 2 aldehydes, 2 ketones, 2 amides, 4 carbonyl, 8 esters, 8 hydrocarbons and 1 phenol. The free amino acid and volatile profile varied between the laboratory and locally produced dawadawa from the three seeds.

Key words: Aspartic acid, dawadawa, locust bean, soya bean, roselle

Introduction

Several studies have evaluated the volatile constituents in some traditionally fermented condiments like *ogiri* from melon seeds (Ojinnaka and Ojimelukwe, 2013) and *daddawa* from locust bean and soybean (Ouoba *et al*. 2005), dawadawa botso from *Hisbiscus sabdariffa* (Ibrahim *et al*. 2011). The compounds identified were alcohols, aldehydes, ketones, esters, pyrazines, alkanoic acids, alkanes, alkenes and aldehydes being the predominant group (Onyenekwe *et al*., 2012). Volatile organic compounds profile have also been studied in some condiments in Benin Republic (Azokpota *et al*., 2010, 2008). Ouoba *et al*. (2005). The compounds responsible for the aroma of soumbala spontaneously produced with pure and mixed cultures of *B. subtilis* and *B. pumilus* and the volatiles were identified as alcohols, acids, aldehydes, ketones, esters, alkanes, alkenes, amines, pyrazines, pyridines, benzenes, phenols, sulphurs, furans and other compounds. The volatile organic compounds associated with the fermentation of baobab seeds for the production of maari has also been studied and 96 volatile organic compounds were identified in total and they include acids, alcohols, esters, and ketones (Parkouda *et al*. (2011).

Volatile organic compounds of soybeans fermented by *Bacillus* have been studied (Leejeerajumnean *et al*., 2001). The major volatile organic compounds in the soybeans includes 2-methylbutanoic acid, 3-hydroxybutane (acetoin), pyrazines, dimethyly disulphide and 2-pentylfuran. However, no aldehydes, aliphatic acids, esters and sulphur compounds were identified in natto samples though theses volatiles were abundant in the thua Nao samples (Leejeerajumnean *et al*., 2001). The organoleptic and physicochemical properties of ogiri was found to improve when 0.3% salt and 0.3% of lime was added (Ojimelukwe *et al*., 2011).

Analysis of free amino acid and volatile organic compounds profile of dawadawan botso produced from *H. sabdariffa* seeds have been previously studied. Fermentation was found to increase the quantity of all essential amino acids except of threonine and an increase in the total free amino acid was also observed following fermentation of the *H. sabdariffa* seeds. The profile of bitter, sweet and MSG-like free amino acids in the unfermented and fermented seeds were also different (Ibrahim *et al*., 2011). In the same study, 22 volatile compounds were identified from the fresh “dawadawan botso” and locally produced dried “dawadawan botso” and the compounds include alcohols, acids, aldehydes, esters, and alkanes. Certain volatiles were found to be dominant among them are Methyl (9Z) – 12- hydroxyl -9 – octadecenoate (40.66%) in fresh, Methyl (14E) – 14, 17- Octadecadienoate (33.97%) in dried and Cis -9- Hexedecenal (19.96%, 15.13%) in both samples (Ibrahim *et al*., 2011).

Similar approach has being exploited to analyse the free amino acid and volatile compounds of Chinese soy sauce (Yanfang and Wenyi, 2009). The study found that the bitter, sweet and MSG-like free amino acids values were significantly different in the soy sauces. The study also identified a total of 82 volatile organic compounds kinds that includes alcohols, aldehydes, acids, ketones, esters, alkynes, phenols, heterocyclic compounds and benzenes (Yanfang and Wenyi, 2009). To the best of my knowledge the present study is the only study that have exploited this approach to comparatively analysed three condiments commonly consumed in the Northern Nigeria. The aim of the present research is to study the amino acid and aroma volatiles organic compounds of locally and laboratory produced dawadawa from the seeds of *Parkia biglobosa*, *Glycine max* and *Hibiscus sabdariffa*.

Materials and Methods

Amino acid profile determination

The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer (Benitez, 1989).

Defatting Sample:

The sample was defatted using chloroform or methanol mixture of ratio 2:1. Three hundred (300) mg of the sample was put in extraction thimble and extracted for 15 hours in Soxhlet extraction apparatus (AOAC, 2006).

Nitrogen Determination:

A small amount (0.115 mg) of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing 4 drops of methyl red indicator added until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where :

a. = Titre value of the digested sample
b. = Titre value of blank sample
v. = Volume after dilution (100 ml)
W. = Weight of dried sample (mg)
C. = Aliquot of the sample used (10 ml)
14. = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. 7 ml of 6 NHCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystiene). The glass ampoule was then sealed with Bunsen burner flame and put in an oven present at 105 ^{O}C ± 5 ^{O}C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins.

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer

The amount loaded was 60 microliter of the filtrate in acetate buffer (hydrolysate) was loaded into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Method of calculating amino acid values

An integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids.

Extraction of volatile compounds

Extraction of volatile compounds was performed by direct solvent extraction method. Two gram of condiment was weighed into a bottle and saturated with 20 ml of chloroform. It was allowed to stand at room temperature for 24 hours, filtered using Whatman filter paper and the filtrate was collected in a sterile bottle, closed tightly before the GC-MS analysis.

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250 ^{O}C for 3 min by using an inlet of 0.75 mm i.d to minimize peak broadening. Chromatography separations were performed by using DB-WAX analytical column 30 m 0.25 mm, 0.25 mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/min.

The oven temperature was programmed at 60 ^{O}C for 5 min, followed by an increase (held for 5 min), and finally at 10 ^{O}C/min to 280 ^{O}C (held for 10 min). The temperature of the FID was set to 250 ^{O}C. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200 ^{O}C, ionization voltage of 70 eV and mass scan range of m/z 23- 450 at 2.76 scans/s.

Identification and quantification of volatile compounds

The identification of chromatographic peak was carried out by comparing their mass spectra with those of the bibliography data of known compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value being 1,000). According to the method of Wanakhachornkrai and Lertsiri (2003) approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results are presented as the peak area normalized (%).

Results

The amino acid profile of locally and laboratory produced dawadawa from *P. biglobosa* was analyzed (**Table 1**). Difference was observed in the amino acid profile of the dawadawa with laboratory produced dawadawa recording an increased in the essential amino acid lysine, valine, methionine and leucine while tyrosine been the only non-essential amino acid that slight increased. Aspartic and glutamic acids seems to be the major amino acids in locally produced dawadawa with a value of 9.00 and 17.26 g/100 g protein. Fermentation increased the bioavailability of aspartic acid (9.00 to 9.31 g/100 g protein) while the glutamic acid decreased from 17.26 to 14.38 g/100 g protein after fermentation under laboratory conditions.

With respect to the locally and laboratory produced dawadawa from *G. max*, the laboratory produced dawadawa showed increased in the six essential amino acid namely histidine, methionine, isoleucine, leucine, tryptophan and phenylalanine while all except two (glutamic acid and proline) non-essential amino acid also increased in the laboratory produced dawadawa (**Table 2**). The essential amino acid leucine and non-essential amino acids aspartic and glutamic acid are identified as the major amino acids in locally produced dawadawa with the first two increasing after fermentation while glutamic acid decreased after fermentation under laboratory conditions for dawadawa production.

The result obtained for the amino acid profile of locally and laboratory produced dawadawa botso from *H. sabdariffa* was same as those obtained with *G. max* where the laboratory recorded in increased in almost all amino acid (**Table 3**). The locally produced recorded the highest amino acid for lysine, valine glutamic acid and proline while threonine was the same in both local and laboratory produced dawadawa from *H. sabdariffa*. Leucine, arginine, aspartic and glutamic acids are the major amino acids in locally produced dawadawa with the first three showing increase after fermentation under laboratory conditions for dawadawa production while glutamic acids was higher in locally produced dawadawa.

The analysis of the volatile organic compound profile of locally and laboratory fermented seeds of *P. biglobosa* showed several volatile compounds were identified in both dawadawa with local dawadawa having 21 volatile organic compounds while dawadawa produced in the laboratory had 24 volatile organic compounds (**Table 4**). Of the diverse volatiles identified, certain volatile organic compounds namely decanoic acid, undecanoic acid methyl ester, dodecanoic acid methyl ester, heptadecanoic acid methyl ester, hexadecanamide and nonadecanamide were common to both dawadawa. The major volatile organic compound in the locally produced dawadawa was undecanoic acid with 33.27% while Z-11-Tetradecenoic acid (30.64%) was the most abundant compound in the laboratory produced dawadawa.

When the seeds of *G. max* were fermented either locally or under laboratory conditions, two volatile organic compounds namely hexadecanoic acid (25.62 and 31%) and 9-Octadecenoic acid (z) (45.16 and 45.66%). were found as the most abundant and common volatile compounds in the local and laboratory produced dawadawa from *G. max* seeds (**Table 5**). A total of fourteen and eleven volatile organic compounds were identified in the local and laboratory produced dawadawa from *G. max* seeds. Some volatile organic compounds were unique to the locally and laboratory produced dawadawa.

Analysis of the volatile organic compounds of local and laboratory condition produced dawadawa botso allowed the identification of nineteen volatile organics in the locally produced dawadawa botso from *H. sabdariffa* seeds while the dawadawa botso produced from *H. sabdariffa* seeds after fermentation under laboratory conditions allowed the identification of ten volatile organic compounds (**Table 6**). Iridecanoic acid, methyl ester, tetradecanoic acid and hexadecanoic acid, 15-methyl-, methyl ester were common to both local and laboratory fermented *H. sabdariffa* seeds dawadawa. The major volatile organic compounds in the locally produced dawadawa botso are 9-Octadecanoic acid (z) (35.97%), tetradecanoic acid (31.00%) and octadecanoic acid (15.31%).

Discussion

The essential amino acids show a significant increase in the laboratory fermentation of *Parkia biglobosa* and glycine max as compared to unfermented seeds. Similarly there was also increased in non-essential amino acids notably aspartic and glutamic acid which were found to be more abundant. The observed increase in the amino acids content after fermentation may be due to the proteolytic activities of the bacteria during fermentation.

Similar observations were made during the production of the condiment using roselle (Ayodele and Musa, 2008) and other leguminous seeds (Ikenebome *et al*, 1986).

The essential amino acids were found to decrease after local and laboratory fermentation of *H. Sabdariffa*. This was probably due to the removal of the hulls that contain higher level of sugar and amino acids. The cooking stage of the seeds that have been reported as the most important step in the preparation of raw materials for fermentation may have also resulted in partial loss of soluble solids in the cooking water (Wang *et al*, 1979). Similar result was also obtained from non-essential amino acids.

The volatile compounds identified in the condiment produced from *P. biglobosa* seeds had 6 acidic compounds namely butanoic acid, 2-methyl, 4-Methyloctanoic acid, undecanoic acid, Z-11-tetradecenoic acid pentadecanoic acid and 9-Hexadecanoic acid (Z). Three alcohols were identify as 2-Hydroxymethyl-2-methylcyclo-pentanol, 2,5-Anhydro-1-0-octylhexitol(Z), (Z)6, (Z)9-pentadecadien-1-ol and 12 esters namely octanoic acid, methyl ester, phthalic acid, di-(1-hexen-5-yl) ester, nonanoic acid, methyl ester, decanoic acid, methyl ester, dodecanoic acid, methyl ester, tridecanoic acid, methyl ester, 2-propenoic acid, 2-(dimethylamino) ethyl ester, heptadecanoic acid, methyl ester, hexadecanoic acid, 2,3-dihydroxypropyl ester, 13,16-octadecadienoic acid, methyl ester, octadecanoic acid, 2,3-dihydroxypropyl ester, dichloroacetic acid, 4-pentadecyl ester. Only one aldehyde and ketone was identified namely 9,12-Octadecadienal and 2-Dimethylaminomethyl-4-methoxycyclohexanone. Two fatty acids were identified namely propyl decanoate and Methyl 12-methyltetradecanoate. Squalene 2, 3-Dimethylbutane, 3,4-Dimethylheptane, 2,7-Dimethylnonane, 1,2-Hexadecane, and 1-Eicosene were the six (6) alkanes detected in the *P. biglobosa* produced dawadawa. Five (5) amides were also identified in the condiment and they include hexadecanamide, octadecanamide, octanamide, nonadecanamide and eicosadecanamide. The volatile organic compounds play very important role in the overall flavor and taste profile of the condiments.

The *G. max* produced dawadawa had 6 esters, 5 amides, 4 acids, 3 alcohols, 2 hydrocarbons and one heterocyclic compound. The esters include pentadecanoic acid, methyl ester; 7,10-Hexadecanoic acid, methyl ester 9,12-Hexadecadienoic acid, methyl ester, 11-Octadecenoic acid, methyl ester, 16-Octadecenoic acid, methyl ester, Tetradecanoic acid, 12-methyl-,

methyl ester. The acid compounds are tetradecanoic acid, pentadecanoic acid, hexadecanoic acid and 9-Octadecenoic acid (z). The amide includes hexanamide, 8-Methyl-6- nonenamide, 9-Octadecenamide, (z), (9E)-n-Butyl-9-octadecenamide, and butanamide, 3-methyl while the alcohols are 2,2-Dimethyl-3-hexanol, 12-Methyl-E,E-2,13-octadecadien-1-ol Z,Z-3,13-octadecadien-i-ol. Cyanocyclobutane and 2,6-Dimethyl-1,5-heptadiene are the two hydrocarbons detected while 5H-1-Pyrindine was the only heterocyclic compound detected in the *G. max* produced dawadawa.

The volatile compound identified in dawadawa was more the number previously reported by Ibrahim *et al*. (2011). The difference could be attributed to either the solvent used for extraction of the volatile compound or the column used for the gas chromatography. The compounds identified in this study include 12 esters namely Decanoic acid methyl ester, Decanoic acid ethyl ester, Phthalic acid, d:- (-1-hexen-5-yl) ester, Dodecanoic acid, methyl ester, Iridecanoic acid, methyl ester, 9,12-Pentadecadienoic acid, methyl ester, 6-Pentadecenoic acid, methyl ester, Hexadecanoic acid, 15-methyl-, methyl ester, 10-Octadecenoic acid methyl ester, Tricosanoic acid, methyl ester, Tetracosanoic acid, methyl ester, Heptacosanoic acid, methyl ester. The acids volatile organic compounds were 7, namely Hexanoic acid, Nonanoic acid, Tetradecanoic acid, 12-Pentadecenoic acid, 9-Heptadecenoic acid (z), 9-Octadecanoic acid (z), Octadecanoic acid while only 2 amides namely octanamide and nonanamide. Four alcohol volatile compound class detected and they include 2, 5-Dimethyl-3,4-hexanediol, 2-Methyl-Z,Z-3,13-octadecadienol Nonanol acetate and Z,Z-10-Hexadecadien-1-ol acetate while only one hydrocarbon namely 4,5-Dimethylnonane was detected. The volatile organic compound class identified in this study are similar to those previously reported for same or similar condiment (Onyenekwe *et al*., 2012; Azokpota *et al*., 2010, 2008; Ouoba *et al*. (2005; Ibrahim *et al*., 2011; Parkouda *et al*., 2011).

The volatile organic flavor compounds detected in dawadawa produced from *H. sabdariffa* seeds include 2 acids class flavor volatile, 1 alcohols, 2 aldehydes, 2 ketones, 2 amides, 4 carbonyl, 8 esters, 8 hydrocarbons and 1 phenol. The acids are 9, 12-Octadecadienoic acid (Z,Z)- and Erucic acid, alcohol, 6,17-Octadecadien-1-ol and the aldehyde are Cis-9-Hexadecenal and 8-Hexadecenal, 14-methyl-, (Z)- while 4-Hydroxy-3-pentyl-cyclohexanone and Cyclopentadecanone, 2-methyl- and Phenol, 2,4-bis(1,1-dimethylethyl)- as the only phenol. The hydrocarbons are 2, 6, 11-trimethyldodecane, Hexadecane, Heptadecane, Dodecane, 2,6,10-trimethyl, 2-methyltetracosane, Eicosane, Bicyclo[10.1.0]tridec-1-ene and 6-Ethyl-3-trimethylsilyloxydecane while 9-Octadecenamide, (Z)- and Octadecanamide are the two amides. Methyl 10-trans,12-cis-octadecadienoate, Methyl 2-octylcyclopropene-1-octanoate, Ethyl 9-Pentadecenoate and Ethyl 9-hexadecenoate are the carbonyl volatile flavor compounds found in the dawadawa from the crushed defatted and undefatted fermented *H. sabdariffa* seeds. The esters include Pentadecanoic acid, ethyl ester, Hexadecanoic acid, ethyl ester**,** Heptadecanoic acid, 10-methyl-, methyl ester**,** Ethyl Oleate**,** Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester**,** Heptadecanoic acid, ethyl ester**,** 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester**,** and Octadecanoic acid, 2,3-dihydroxypropyl ester. The importance of these esters that contribute to food aroma is an undisputed fact as esters with low carbon atoms are known to be highly volatile at ambient temperatures with the perception thresholds been 10 times lower than their alcohol precursors (Izco and Torre, 2000; Nogueira *et al*., 2005).

In addition to fruity floral character ester impart on food, esters are known to diminish or mask the sharpness of unpleasant free amino acid-derived notes. These esters are known to be formed by esterification between the short-chain acids and the alcohols. The carbonyl compounds evolution may be due to the fact that ketones and aldehydes are intermediate unstable compounds being easily reduced to alcohols (Estrella *et al*., 2004). Aldehydes such as 3-methylthio-propanal are known to impart a powerful meaty and soy sauce-like odour and flavour at high dilution (Chung, 1999). The phenols might have been produced during the amylolytic phase of the fermentation. Phenol and 4-ethylphenol are thought to have been generated from lignin glycoside degradation during the fermentation (Kobayashi and Sugawara, 1999). Other phenols are thought to be the thermal degradation products of lignin-related phenolic carboxylic acids (Chung, 1999). Pyridine could be the products of the Maillard reaction and might be contributing to the floral note of the condiments (Chung, 1999). Alkyl pyrazines are known to have nutty aroma and could be have been generated naturally during the aging process by the condensation of amino ketones formed through the Maillard reaction and Strecker degradation (Sarkar *et al*., 2002).

Table 1: Amino acid profile of locally and laboratory produced 'dawadawa botso' by fermenting seeds of *P. biglobosa*

Amino acids	Locally produced	Laboratory produced	Difference
Essential amino acids	g/100g protein		
Lysine	4.29	4.64	-0.35
Histidine	2.20	2.17	0.03
Threonine	3.38	2.99	0.39
Valine	3.94	3.97	-0.03
Methionine	1.01	1.20	-0.19
Isoleucine	3.40	3.34	0.06
Leucine	7.29	7.91	-0.62
Tryptophan	0.89	0.86	0.03
Phenylalanine	4.61	4.52	0.09
Non-essential amino acids			
Arginine	5.59	6.28	0.69
Aspartic acid	9.00	9.31	0.31
Serine	4.18	3.78	0.4
Glutamic acid	17.26	14.38	2.88
Proline	4.47	3.96	0.51
Glycine	4.20	4.08	0.12
Alanine	4.47	4.28	0.19
Cystine	1.45	1.33	0.12
Tyrosine	3.27	3.61	-0.34

Table 2: Amino acid profile of locally and laboratory produced 'dawadawa' by fermenting seeds of *Glycine max*

Amino acids	Locally produced	Laboratory produced	Difference
Essential amino acids	g/100g protein		
Lysine	4.21	5.14	0.93
Histidine	2.11	2.23	-0.12
Threonine	3.27	3.27	0.00
Valine	3.39	4.79	1.4
Methionine	0.91	1.33	-0.42
Isoleucine	3.34	3.50	-0.16
Leucine	7.00	8.35	-1.35
Tryptophan	0.84	0.89	-0.05
Phenylalanine	4.43	4.96	-0.53
Non-essential amino acids			
Arginine	5.33	6.71	-1.38

Aspartic acid	8.00	8.96	-0.96
Serine	3.94	4.16	-0.22
Glutamic acid	16.96	15.21	1.75
Proline	4.26	4.16	0.1
Glycine	3.99	4.27	-0.28
Alanine	4.25	4.47	-0.22
Cystine	1.21	1.57	-0.36
Tyrosine	2.92	3.44	-0.52

Table 3: Amino acid profile of locally and laboratory produced 'dawadawa' by fermenting seeds of *H. sabdariffa.*

Amino acids	**Locally produced**	**Laboratory produced**	**Difference**
Essential amino acids	g/100g protein		
Lysine	4.21	5.14	0.93
Histidine	2.11	2.23	-0.12
Threonine	3.27	3.27	0.00
Valine	3.39	4.79	1.4
Methionine	0.91	1.33	-0.42
Isoleucine	3.34	3.50	-0.16
Leucine	7.00	8.35	-1.35
Tryptophan	0.84	0.89	-0.05
Phenylalanine	4.43	4.96	-0.53
Non-essential amino acids			
Arginine	5.33	6.71	-1.38
Aspartic acid	8.00	8.96	-0.96
Serine	3.94	4.16	-0.22
Glutamic acid	16.96	15.21	1.75
Proline	4.26	4.16	0.1
Glycine	3.99	4.27	-0.28
Alanine	4.25	4.47	-0.22
Cystine	1.21	1.57	-0.36
Tyrosine	2.92	3.44	-0.52

Table 4: Volatile organic compound profile of local and laboratory produced dawadawa by fermenting the seeds of *P. biglobosa*

		Abundance	(%)
RT^{-1} (Min)	Volatile organic compound*	Local	Lab
3.09	Butanoic acid, 2-methyl	-	0.76
4.41	2-Hydroxymethyl-2-methylcyclo-pentanol	0.14	-
5.18	2,3-Dimethylbutane	-	0.17
9.00	3,4-Dimethylheptane	-	0.04
10.65	Octanoic acid, methyl ester	-	0.20
11.25	Propyl decanoate	0.89	-
11.45	2,7-Dimethylnonane	-	0.08
11.76	Phthalic acid, di-(1-hexen-5-yl) ester	-	0.47
12.94	Nonanoic acid, methyl ester	-	0.23
13.71	2,5-Anhydro-1-0-octylhexitol	1.15	-
14.53	4-Methyloctanoic acid	-	2.18
15.60	Decanoic acid, methyl ester	0.12	2.02
15.69	Methyl 12-methyltetradecanoate	0.31	-
17.13	Undecanoic acid	33.27	14.98
18.94	Dodecanoic acid, methyl ester	1.90	6.14
19.29	Tridecanoic acid, methyl ester	-	1.28
20.27	Z-11-Tetradecenoic acid	-	30.64
20.48	Pentadecanoic acid	-	11.97
20.72	Hexadecanamide	8.04	11.87
20.78	(Z)6, (Z)9-Pentadecadien-1-ol	9.03	-
21.12	9-Hexadecanoic acid (Z)	13.66	-
22.70	9-Heptadecenamide (Z)	-	7.65
22.90	9,12-Octadecadienal	4.93	-
22.92	Octadecanamide	-	4.49
23.11	Octanamide	4.23	-
23.45	2-Propenoic acid, 2-(dimethylamino)ethyl ester	1.87	-
23.52	1,2-Hexadecane	-	0.17
23.65	2-Dimethylaminomethyl-4-methoxycyclohexanone	2.89	-
23.90	Heptadecanoic acid, methyl ester	2.06	0.26
24.21	Hexadecanoic acid, 2,3-dihydroxypropyl ester	3.23	-
24.77	Nonadecanamide	1.41	0.88
24.90	Heptanamide, 4-ehtyl-5-methyl	2.03	-
25.63	13,16-Octadecadienoic acid, methyl ester	-	0.77
25.83	9,12-Octadecadienal	2.69	-
25.98	Octadecanoic acid, 2,3-dihydroxypropyl ester	2.84	-
26.34	Dichloroacetic acid, 4-pentadecyl ester	-	0.58
26.46	1-Eicosene	1.08	-
26.52	Squalene	-	0.14
26.63	Eicosadecanamide	-	2.04

*The unknown compounds were removed.

Table 5: Volatile organic compound profile of local and laboratory produced dawadawa by fermenting the seeds of *G. max*

RT^{-1} (Min)	**Volatile organic compound***	Abundance **Local**	(%) **Lab**
4.38	Butanamide, 3-methyl	-	0.40
5.18	Cyanocyclobutane	0.24	-
8.47	5H-1-Pyrindine	1.35	-
13.68	Tetradecanoic acid	-	0.61
14.52	2,2-Dimethyl-3-hexanol	0.56	-
14.63	Pentadecanoic acid	0.76	0.33
15.64	Pentadecanoic acid, methyl ester	1.53	0.21
16.94	Hexadecanoic acid	25.62	31.00
18.84	7,10-Hexadecanoic acid, methyl ester	1.27	-
18.87	9;12-Hexadecadienoic acid, methyl ester	-	0.96
18.90	11-Octadecenoic acid, methyl ester	1.98	-
18.93	16-Octadecenoic acid, methyl ester	-	0.75
19.27	Tetradecanoic acid, 12-methyl-, methyl ester	0.32	-
20.98	9-Octadecenoic acid (z)	45.16	45.66
20.50	Hexanamide	5.22	-
22.61	8-Methyl-6- nonenamide	3.33	-
21.12	9-Octadecenamide, (z)	-	7.49
23.50	12-Methyl-E,E-2,13-octadecadien-1-0l	1.35	-
24.52	(9E)-n-Butyl-9-octadecenamide	-	3.09
25.71	Z,Z-3,13-octadecadien-i-ol	-	3.69
26.50	2,6-Dimethyl-1,5-heptadiene	2.43	-

*The unknown compounds were removed for the purpose of this presentation

Table 6: Volatile organic compound profile of local and laboratory produced dawadawa by fermenting the seeds of *H. sabdariffa*

RT^{-1} (Min)	Volatile organic compound	Abundance Local	(%) Lab
4.15	Hexanoic acid	0.58	-
7.83	Nonanoic acid	0.32	-
10.65	Decanoic acid methyl ester	-	0.80
11.45	Decanoic acid ethyl ester	0.21	-
11.76	Phthalic acid, d:- (-1-hexen-5-yl) ester	0.52	-
12.94	Dodecanoic acid, mrthyl ester	-	0.61
13.70	2,5-Dimethyl-3,4-hexanediol	0.52	-
15.65	Iridecanoic acid, methyl ester	1.87	6.28
17.46	Tetradecanoic acid	31.00	16.92
18.92	9,12-Pentadecadienoic acid, methyl ester	3.88	-
18.95	6-Pentadecenoic acid, methyl ester	-	5.91
18.99	12-Pentadecenoic acid	3.03	-
19.30	Hexadecanoic acid, 15-methyl-, methyl ester	0.41	1.15

20.30	9-Heptadecenoic acid (z)	-	45.48
20.54	9-Octadecanoic acid (z)	35.97	
20.68	Octadecanoic acid	15.31	-
21.67	Nonanol acetate	1.96	-
22.65	Octanamide	-	1.64
22.70	Nonanamide	0.68	-
23.52	Z,Z-10-Hexadecadien-1-ol acetate	-	0.53
23.56	2-Methyl-Z,Z-3,13-octadecadienol	0.35	-
23.70	10-Octadecenoic acid methyl ester	0.09	-
23.92	Tricosanoic acid, methyl ester	1.20	-
25.62	Tetracosanoic acid, methyl ester	1.64	-
26.19	4,5-Dimethylnonane	-	1.45
27.61	Heptacosanoic acid, methyl ester	0.07	-

*The unknown compounds were removed for the purpose of this presentation

Conclusion

Fermentation increased the bioavailability of aspartic acid (9.00 to 9.31 g/100 g protein) while the glutamic acid decreased from 17.26 to 14.38 g/100 g protein after fermentation under laboratory conditions. The locally and laboratory produced dawadawa from *G. max*, the laboratory produced dawadawa showed increased in the six essential amino acid. The essential amino acid leucine and non-essential amino acids aspartic and glutamic acid are identified as the major amino acids in locally produced dawadawa from locust bean. The locally produced dawadawa from *H. sabdariffa* had the highest amino acid for lysine, valine glutamic acid and proline while threonine was the same in both local and laboratory produced. The locally and laboratory fermented seeds of *P. biglobosa* showed several volatile compounds in both dawadawa with locally produced dawadawa having 21 volatile organic compounds while dawadawa produced in the laboratory had 24 volatile organic compounds. The *G. max* produced dawadawa had 6 esters, 5 amides, 4 acids, 3 alcohols, 2 hydrocarbons and one heterocyclic compound. The volatile organic flavor compounds detected in dawadawa produced from *H. sabdariffa* seeds include 2 acids class flavor volatile, 1 alcohols, 2 aldehydes, 2 ketones, 2 amides, 4 carbonyl, 8 esters, 8 hydrocarbons and 1 phenol. The free amino acid and volatile profile varied between the laboratory and locally produced dawadawa from the three seeds.

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