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EFFECT OF STARTER CULTURE ON VOLATILE ORGANIC COMPOUNDS OF BAMBARA GROUNDNUT CONDIMENT

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ABSTRACT: *Bambara nut (Vigna subterranea) is one of the underutilized legumes in Africa that can be used to replace animal protein thereby combating malnutrition and shortage of food in Africa. In this study, Bambara nut condiments were produced by spontaneous and laboratory fermentation using starter cultures of Bacillus subtilis and Bacillus llicheniformis in combination. The bacteria associated with both fermentations were Streptococcus spp., Staphylococcus saprophyticus, Bacillus subtilis and Bacillus licheniformis. The mean heterotrophic bacterial counts were 3.1x106 cfug-1 and 3.8x106 cfug-1 for spontaneously and laboratory fermented condiments. Aroma and flavour volatile organic compounds were also determined using GC-MS analysis. The GC-MS analysis revealed 25 compounds that included 6 acids, 1 alcohol, 1 aldehyde, 6 aliphatics/ aromatics, 1 alkyl halide, 2 amines, 3 hydrocarbons, 2 ketones and 2 nitriles. These compounds play role in the flavour and aroma of Bambara nut condiment (daddawa). This study suggests that this condiment (daddawa) could be a good and cheap source of protein and a substitute to monosodium glutamate-based salts and can have health benefits due to the presence of probiotic bacteria.*

KEYWORDS: Bambara Nut, Daddawa, Starter Culture, Volatile Organic Compounds, Bacillus Spp.

INTRODUCTION

Bambara nut (*Vigna subterranean* L) is an indigenous African crop that is now grown across the continent from Senegal to Kenya and from the Sahara to South Africa (Atiku *et al.,* 2004*).* As the shortage of food continues to be a major problem in Africa, these legumes are being promoted more than before in order to help in combating malnutrition (Okafor *et al.,* 2003). The affordability of plant protein source relative to that of animal origin has led to the intensified development of legume processing as a means of enhancing the availability, palatability and diversity of leguminous source of dietary protein. Studies had revealed the detailed nutritional composition to be 16% crude protein, 9.7% moisture, 5.9% crude fat, 2.9% ash and 64.9% total carbohydrate (Aremu *et al.,* 2006). Studies have shown that although legumes are known for their high protein content, their utility is limited because of the low protein digestibility. A combination of sprouting and cooking resulted in an excellent digestibility coefficient (Ologhobo and Fetuga, 1986)

Different food products have been prepared through fermentation process which has led to the development of characteristic flavors, textures and changes in nutritive properties of the foods. Fermented Bambara nut is one of the popular food condiments in the regions of West Africa. This nutritious and delicious food spice is popularly called “ogiri” in Igbo, “iru” in

Yoruba, “bindo” in Bassa and “daddawa” in Hausa all in Nigeria. It is normally used to flavor soups and sauces and constitutes a source of proteins mainly for the diet of poor families (Odunfa, 1986). It is heavily consumed in Nigeria, Ghana, Sierra Leone and Togo as substitute for fish or meat.

MATERIALS AND METHODS

Raw Materials Procurement

Half (1/2) mudu of the seeds of *Vigna subterranea* were purchased from the central market of Sokoto State, Nigeria. The sample of Bambara nut was sent to the herbarium of Botany unit, department of Biological Sciences, Usmanu Danfodio University, Sokoto and was given the authentication number: ASN 0301

Preparation of Raw Material

Raw seeds were pre-processed before the real production steps. The pre-processing consisted of selection by manually sorting (Odunfa, 1981). The seeds were winnowed to eliminate stones and other impurities and were repeatedly washed with water (2 to 3 times). The water cleaning step was in fact a sorting by gravity in the sense that immature seeds and spoiled seeds as well as other light impurities floated while heavy impurities (stones, sand) were deposited as sediment.

Local Production of Daddawa

The production of Bambara nut condiment was done as described by Barimalaa *et al*., 1994 with modifications as follows:

Steeping

The step consisted of soaking the cleaned seeds in water at room temperature for two days to soften the seeds.

Cooking

After the initial cleaning process and soaking, the seeds were cooked for 1 hour in a pressure cooker. Seeds were considered well-cooked as they became soft and easily crushed with fingers.

Sieving

The cooked seeds were sieved to drain the cooking water and allowed to cool.

Dehulling

The cooked seeds were dehulled with fingers after cooling.

Pounding

After dehulling step, the seeds were pounded in a pestle and mortar to form a paste.

Fermentation

Fermentation took place by placing the formed paste into an airtight container and kept for three days to ferment spontaneously.

Laboratory Production of Daddawa

As described by Fadahunsi and Olubunmi, 2009, 200g of Bambara nut seeds were weighed using an electric weighing balance. They were then washed and steeped 500ml of distilled water for 18 hours and then put on hotplate containing 400ml of distilled water and boiled for about 90 minutes until they became soft. They were then sieved and allowed to cool for 15 minutes, dehulled and mashed in a sterile pestle and mortar to form a pulp. The pulp was then sterilized in an autoclave at 121^{o}C for 15 minutes after which it was aseptically inoculated with starter cultures, wrapped in a polythene bag and placed into an airtight container and incubated at 37^{o}C for 72 hours.

Processing of 'Daddawa'

At the end of the fermentation, the ammonia-like flavor condiments were dried in open sun and were repeatedly turned to form balls and enable a good drying for 2 to 3 days and packaged in polythene bags.

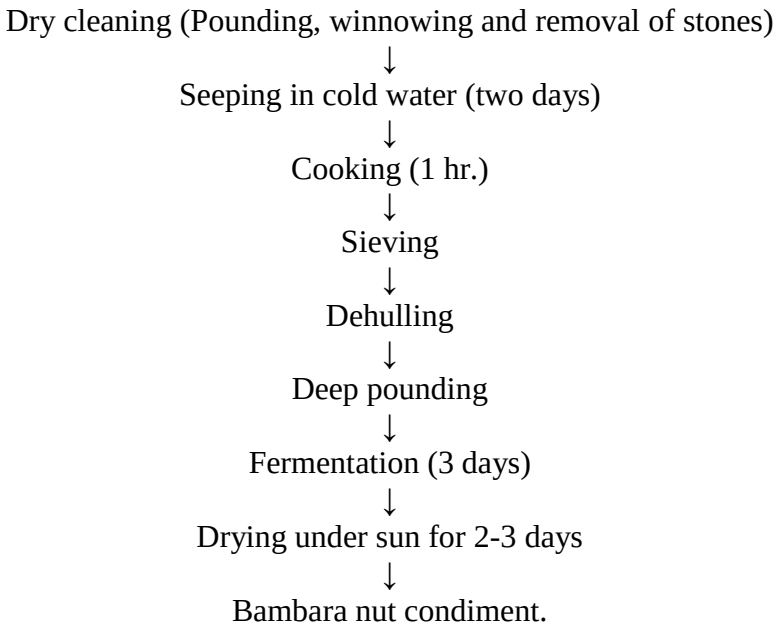

Figure 1: Summary of Bambara Nut Condiment Production

Bacteriological Analyses

Serial Dilution

Ten (1 g) gram of each of the samples was weighed and dissolved in 9 ml. of distilled water and was serially diluted to 10^2, $10^3$and 10^5. 0.1ml. from each test tube was transferred using a fresh syringe onto nutrient agar plate, spread using a sterile bent glass rod and incubated at 370C for 24 hours.

Processing, Maintenance and Identification of Isolates

The isolates that emerged after 24 hours of incubation were continually subcultured until pure cultures were obtained. The pure cultures were then being subcultured on nutrient agar slants, incubated for 24 hours and refrigerated. The isolates were maintained on the slant until required. The isolates were identified following a series of biochemical tests as described by Cheesebrough (2003) and Oyeleke and Manga (2008).

Extraction of Volatile Organic Compounds

Volatile compounds were extracted using general purpose solvent Parliment (1997) as described by Ibrahim *et al.* (2011). Extraction of volatile compounds was done by direct solvent extraction method. Five grams of each sample were weighed into bottles and saturated with 20 ml of diethyl ether. They were allowed to stand at room temperature for 24 hours, filtered using Whatman No. 1 filter Paper and the filtrate was collected in sterile bottles, closed tightly for GC-MS analysis.

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 C for 3min by using an inlet of 0.75mm i.d to minimize peak broadening. Chromatographic separations were performed by using DB-WAX analytical column 30 m 0.25 mm, 0.25mm (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 60oC for 5min, followed by an increase (held for 5min), and finally at 100C/min to 280oC (held for 10min). The temperature of the FID was set to 250oC. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200oC, 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 chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). Approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna).

RESULTS AND DISCUSSION

In this study, four different bacterial isolates from genera of *Bacillus, Staphylococcus and Streptococcus* were identified based on colonial, morphological and biochemical characterization. The species were *Bacillus subtilis, Bacillus licheniformis, Staphylococcus saprophyticus* and *Streptococcus* spp. respectively as shown in Table 1 and 2. Both *S. saprophyticus* and *Streptococcus* spp. are naturally present in the fermentation of Bambara nut as reported by Barimalaa *et al.,* 1994; Suberu and Akinyanju, 1996. The genus of *Bacillus* is made up of Gram-positive, aerobic or facultative anaerobic spore-forming, rod bacteria and includes mesophiles and extremophiles.

Table 1: Result of Mean Heterotrophic Bacterial Count after Production of Daddawa

Sample	Bacterial count (cfug-1)
Uninoculated	3.1 x 106
Inoculated	3.8 x 106

Table 2: Morphological and biochemical characteristics of bacteria isolated

MO	SP	CA	MR	VP	IT	CO	CI	UR	HM	SC	GL	LC	SR	HS	GA	Probable organism
Sample: Inoculated																
+rods	+	+	-	+	-	-	+	-	Γ	+	+	+	+	+	-	B. licheniformis
+rods	+	+	+	+	-	-	+	-	Γ	+	+	-	+	+	-	B. subtilis
Sample: Uninocuated																
+rods	+	+	-	+	-	-	+	-	Γ	+	+	+	+	+	-	B. licheniformis
+rods	+	+	+	+	-	-	+	-	γ	+	+	-	+	+	-	B. subtilis
+cocci(Ch)	-	-	-	-	-	-	+	+	α	+	+	+	+	-	-	*Streptococcus* spp
+cocci(Ch)	-	-	-	-	-	-	+	+	γ	+	+	+	+	+	-	S. saprophyticus

Key: + = positive, - =Negative, MO = Morphology, SP = Spore stain, CA = Catalase, MR = Methyl- red, VP = VogesProskauer, IT = Indole test, CO = Coagulase, CI = Citrate, UR = Urease, HM = Hemolysis, SC = Starch hydrolysis, GL = Glucose, LC = lactose, SR = Sucrose, HS =H ydrogensulphide, GA = Gas.

They are metabolically organotrophs, depending on organic compounds as source of carbon and energy. Due to their highly resistant endospores they are able to colonize wide varieties of environments. They get contact with food through production containers, handlers, air and dust (Jay *et al.,* 2005). The use of starter cultures in the production of fermented foods is very crucial as they play significant roles in the process such as reducing of production period, enhancing their nutritional contents, organoleptic properties of the product and increasing the shelf life of the final product. *Bacillus* spp. that were found in the inoculated sample and not in the uninoculated fermented sample testified that they were exogenous fermentation spp., thus were inoculated before fermenting thesample as starter cultures. Most authors agree that *B. subtilis* is predominant in the fermentation of legumes (Ouoba *et al.,* 2003). However, the use of *B. subtilis* in combination with *B. licheniformis* is very essential for laboratory fermentation (Omafuvbe *et al.,* 2003).

Several studies have been done to characterize the aroma profiles of fermented and non-fermented foods (Komthong *et al.,* 2007). Volatile organic compounds are considered essential in the fermentation industries as they affect the quality of the product and enhance consumer acceptance (Liu *et al.,* 2005; Guillaume, *et al.,* 2009).

In this study, a total number of 25 volatile organic compounds from both inoculated and uninoculated samples were detected by GC-MS analysis, presented in Table 3**.** They were then classified into different classes (Cortacero-Ramirez *et al.*, 2003; Liu *et al.*, 2005) including 6 acids, 1 alcohol,1 aldehyde, 6 aliphatics/ aromatics, 1 alkyl halide, 2 amines, 3 hydrocarbons, 3 ketones and 2 nitriles as shown in Table 3**.**

Table 3: Volatile Organic Compounds from Fermented Bambara Nut Condiment

RT -1(min.)	Volatile Organic Compound (by functional group)	Area normalized (%)		Molecular Weight
		Ino.	Unino.	
Acids				
4.060	Propionic acid	*	0.69	144
14.256	n-decanoic acid	4.1	*	172
14.275	1-pentadecanecarboxylic acid	*	36.37	256
16.022	Oleic acid	*	22.53	282
16.270	Stearic acid	*	7.71	284
16.358	Acetic acid	1.07	*	296
Alcohols				
17.074	1-trydecyn-4-ol	*	1.28	196
Aldehydes				
5.564	1-(4-bromobutyl)indole-3-carboxaldehyde	0.59	*	281
Aliphatics/ Aromatics				
9.879	Diethyl phthalate	11.95	37.49	222
13.330	4-ethylformalinidine	2.60	*	149
13.916	Aminoguanidine	0.80	*	74
14.033	2,6-diamino-8-azapurine	11.95	*	151
14.034	2,2-dimethyldecarhydronaphthalene	*	8.25	166
Alkyl halides				
20.788	1-decyliodide	*	2.13	226
Amines				
4.577	1-butanamine	0.76	0.30	118
Hydrocarbons				
3.462	3,4-dimethyloctana	6.32	24.09	142
Ketones				
4.092	Mentha-6,8-diene-2-one	0.98	*	207
15.706	3,4-methylenedioxybenzylidineacetone	*	2.08	190
15.707	7-methoxy-2-benzofuranylmethylethanone	1.73	*	190
Nitries				
7.911	6-heptenenitrile	2.93	*	163
16.054	Propanedinitrile	4.55	*	140

*Key: RT-1 = Retention time, Ino. = Inoculated sample, Unino. = Uninoculated sample, * = Absent*

Volatile organic compound has been shown to be partly responsible for the aroma of several fermented foods (Ogueke *et al.,* 2010). These volatile organic compounds are probably important in the flavour of daddawa as they have been shown to be in a variety of *Bacillus* spp. fermented foods. A great variety of organic acids present in fermented Bambara daddawa possesses significant roles as they affect theorganoleptic properties (e.g. taste, aroma and color), stability, nutrition, acceptability and quality of the product. This is in proximity to the findings of Aka *et al.,* 2008 and Guillaume *et al.,* 2009. The aliphatic/ aromatic compounds have been reported to provide odor attributes to fermented foods (Meigui *et al.,* 2017). Many alcohols have been described to possess undesirable odor which partially contributes to raw and beany flavour of soya bean (Wilkens and Lin, 1970). Hydrocarbons are assumed to be formed from fatty acid lipoxygenase catalysis (Belitz *et al.,* 2009). This may explain their abundance as volatile compounds in fermented seeds, since they are rich in oil (Irvine, 1961). Nakamura *et al.,* 1989 suggested that hydrocarbons do not play a significant role as flavour compounds in roasted sesame since they possess relatively weak aroma. Alcohols formation, in general is limited as seen in low consumption of reducing sugar (Lertsiri *et al.,* 2001). Aldehydes are important aroma-active compounds in foods because of their low threshold aromas, as well as rancid odours and flavour (Blanco *et al.,* 2016). Relatively, small amounts of aldehydes, ketones, and amines were identified in this study, they are considered to play important role in aroma of daddawa because of their low detection/ aroma thresholds as in roasted chicory (Baek and Cadwallader, 1998).

CONCLUSION

The use of a mixture of microorganisms with proximate physiological and biochemical properties as starter cultures in the fermentation of Bambara nut seems to be the best way of producing daddawa with nutritional and sensory properties desired. Understanding the phenomena of succession of the different populations in this natural process of is required. Flavour and taste are important quality characteristics of traditional foods. Fermented foods are particularly appreciated for their pleasant flavour and taste. Several groups of volatile organic compounds were identified after fermentation of Bambara nut which included acids, alcohols, aldehydes, aliphatics/ aromatics, alkyl halides, amines, ketones, hydrocarbons and nitriles that influence the flavour and aroma of Bambara nut daddawa. Bambara nut condiment should be utilized more because it does not only serve as a substitute to the monosodium glutamate-based flavour agents but also has health benefits as it contains probiotic bacteria.

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