

Starter culture development for the production of 'Owoh'

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(Received: April 25, 2008; Accepted: June 12, 2008)

ABSTRACT

Species of *Bacillus* were earlier reported to be responsible for the fermentation of 'owoh', a fermented product of cottonseeds. Out of the three species (*Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*) screened *Bacillus subtilis* was discovered to produce the best quality 'owoh'. To develop a starter culture, *Bacillus subtilis* isolated from different condiments: 'iru', 'soy-iru', 'ogiri' and 'owoh' were used to ferment 'owoh' in the laboratory. The proximate analysis and sensory evaluation test were used to assess the quality of the final products. *Bacillus subtilis* from 'iru', used as a single starter culture, produced 'owoh' with highest amount of dry matter (71.5g/100g), ash, and soluble protein with 15.27 and 45.5g/100g of dry sample respectively. The naturally fermented 'owoh' produced the least protein content (30.87g/100g of dry matter). The pH of the fermented product ranged from 5.10 to 7.30 and *Bacillus subtilis* from 'iru' produced owoh with the least value, 5.10. The general acceptability of 'owoh' produced by *Bacillus subtilis* from 'iru' was highest (3.22) points while that of *Bacillus subtilis* from 'ogiri' has the least (3.10). However there were no significant differences in colour and appearance of the final products (at $P \geq 0.05$)

Key words: Starter culture, Owoh, *Bacillus* sp.

INTRODUCTION

'Owoh' is a traditionally fermented high protein food that is solely produced from cotton seeds (*Gossypium hirsutum*) Its fermentation, like other soup condiments is still a family art (Sanni and Ogbuna, 1991). Fermentation of 'owoh' is by chanced inoculation. This method exposes the seasoning agent to contamination by pathogens and results in lack of consistence in final product, hence reduces its acceptability. 'Owoh' serves as a low cost substitute for animal protein, since it ('owoh') is very rich in amino acids and essential fatty acids (Enjuigha and Agbede, 2000). This is responsible for the formation of ammonia that accounts for the characteristic pungent odour and high pH of 'owoh' (Cadwell, 1995)

Bacillus sp had been implicated in the fermentation of condiments especially those mainly consumed in West Africa (Campbell-Platt, 1980). In production of 'iru', fermented locust bean (*Parkia*

biglobosa), Odunfa (1985) and Oyeyiola (1988) identified *Bacillus* sp as most predominant organism. Obeta (1983) also reported the association of *Bacillus* sp with fermentation of 'ugba' a fermented product of Africa oil bean (*Pentaclethra microphyla* Benth) while Odunfa (1985) and Sanni *et al.*(1998) isolated *Bacillus* sp from 'ogiri', a fermented melon seeds (*Citrullus vulgaris* Schrad) and 'owoh', respectively.

Bacillus subtilis has been reported to be frequently encountered in the fermentation of oil-rich seeds (Campbell-Platt, 1980). This report made us investigated the advantage of single starter and the fermentative potentials of *Bacillus subtilis* isolated from other fermented oil-rich seeds.

In this work, interest was to develop a starter culture that produces 'owoh' with a better organoleptic property and without loss of acceptability among consumers.

MATERIALS AND METHODS

Source of cotton seeds

The cotton seeds used for this work were purchased from a market in Ado-Ekiti. They were collected in a new polythene bag and brought to the laboratory. The seeds were sorted to get rid of dirt and decomposing seeds. The seeds were boiled for one hour and dehulled. The cotyledons were washed and sorted again. The clean cotyledons were wrapped in aluminum foil and boiled for 2h. The water was drained off and allowed to cool before ground in a mortar with a pestle

Source and preparation of inocula

The bacteria used for this work was collected from stock culture of Microbiology Department, University of Ado-Ekiti. The bacteria were isolated from condiments and characterized accordingly. The test organisms were *Bacillus subtilis* (BSWH), *Bacillus subtilis* (BSGR), *Bacillus subtilis* (BSRU), *Bacillus subtilis* (BSSR), *Bacillus licheniformis* (BLWH) and *Bacillus pumilus* (BPWH) the viability of each of the organism was ensured by resuscitating it in buffered peptone (Oxoid) broth and incubated at 37°C for 18h. The starter cultures were standardized by the method of Bauer *et al*, (1966).

Twenty gram (20g) of mash was introduced into a 100 ml conical flask, properly corked and sterilized at 121°C for 15 min. After which two millilitre (2.00 ml) of standardized starter culture was introduced into the mash and mixed thoroughly with a surface sterilized spatula and incubated at 37°C for five days.

Determination of proximate composition of 'owoh'

The dry matter of the sample was determined by weighing 2g in a crucible; dry in an oven at 105°C. The sample was removed, cooled in desiccator and weighed intermittently until a constant weight was obtained. The weight was expressed in g/100g of freshly fermented 'owoh'.

The pH of the sample was determined by taking 2g of fermented sample into a clean mortar and homogenized to a pulp using a pestle. ten millilitre (10 ml) of distilled water was added to

make fine slurry. The pH meter (ELE model) standardized using buffer at the pH of 4.00 and 9.00 was dipped into the samples to determine their pH values.

The standard method of AOAC (1990) was used to determine the ash and the lipid content of the sample. Dried sample was weighed into a platinum dish and ashed by heating at 500°C in a muffle furnace until residue turned whitish gray. The ash content per unit weight was calculated and expressed as percentage (%). The lipid content of the samples was extracted quantitatively with petroleum ether (b.p. 40-60°C) in a Soxhlet apparatus.

The indicator method of AOAC (1984) was adopted for the determination of titratable acidity. Twenty grams of sample was ground into pulp using a mortar and pestle. Then 100 ml of distilled water was added to make fine slurry. The slurry was filtered using Whatman No 1 filter paper. To 10 ml of filtrate in 100 ml conical flask was added 5 drops of 1% phenolphthalein indicator. Sample was titrated against 0.1N NaOH (in burette) until a pink colour developed. The result was expressed as normality (N) of the predominant citric acid.

The soluble protein was determined according to Lowry *et al*, (1951) while the crude fibre was determined according to AOAC (1984). Dried samples were extracted with petroleum ether; air dried and boiled in H₂SO₄ for 30 min. after cooling, insoluble matter (in water) was washed free of acid then boiled in alkali (NaOH solution). Residue was washed in 95% ethanol and then ether, before drying in oven at 100°C.

Sensory evaluation of owoh

A randomly selected nine-man panel conducted sensory evaluations. The panel consists of students and staff members of the University of Ado-Ekiti. A scorecard was six-point hedonic scale (with excellent, very good, good, poor, and very poor representing 6, 5, 4, 3, 2 and 1 respectively) was supplied to each of the panelists. Attributes scored include appearance, colour texture, odour, and general acceptability. The identity of each of the samples was concealed from the panelists as suggested by Moskowitz (1999)

The results were subjected to statistical analysis using analyses of variance (ANOVA). The level of significance was determined at 0.05 level of probability.

RESULTS

The mean value of triplicate determination of proximate composition analyses of 'owoh' fermented by different *Bacillus* species was presented in Table 1. The protein content of the samples varied from 14.03 in sample BSPLO to 33.79g/100g in sample BLO. 'Owoh' produced by single starter culture of *Bacillus* species had higher amount of crude fibre than the sample with the combination of the starter cultures. The amount of free nitrogen extract (NFE) was lower in pure starter cultures.

The dry matter of 'owoh' fermented by *Bacillus subtilis* isolated from different condiment ranged from 61.65 to 71.53g/100g. Sample BSRU had the highest value (71.52g/100g). Amount of

soluble protein, ash, and crude fibre was highest in BSRU with values 45.15, 1.52 and 6.90g/100g respectively. Naturally fermented 'owoh' had the highest amount of NFE (28.68g/100g). The titratable acidity of the product ranged from 0.052 to 0.086. The least value (0.052) was recorded in sample BSRU.

The acceptability of 'owoh' produced by different strains of *Bacillus subtilis* varies. Sample SRBS had the highest value in general acceptability with sample GRBS.

DISCUSSION

'Owoh' is produced from an oil seed, it is very rich in amino acids and essential fatty acids (Enjuigha and Agbede, 2000). 'Owoh' is nutritionally better than raw cotton seeds (Enjuigha *et al.*, 2002). *Bacillus subtilis* was found to be the best for the fermentation of 'owoh'. This report is in agreement with the work of Oyeyiola and Odunfa (1985)

Table 1: Proximate composition of 'owoh' fermented by different *Bacillus* species (g/100g dry matter)

Sample	Dry matter	Lipid	Ash	Soluble protein	Crude fibre	Nitrogen free extract	pH	Acidity (% citric acid)
BSO	62.14	44.28	1.45	20.62	4.46	29.29	5.30	0.07
BSO	62.18	44.28	1.45	20.61	4.46	29.29	5.30	0.07
BPO	70.38	48.83	1.14	19.54	4.73	30.71	6.70	0.08
BLO	65.05	44.94	1.59	33.79	4.88	16.64	7.30	0.65
BSPLO	67.76	48.69	2.01	14.03	4.00	31.55	6.20	0.07
BSPO	65.44	48.70	1.79	16.95	4.45	29.05	7.40	0.06
BSLO	71.15	37.22	1.34	19.92	3.55	38.28	6.31	0.05
BPLO	69.45	35.05	1.14	21.28	4.01	39.90	7.92	0.07
Control	69.48	43.09	1.03	20.16	5.03	31.79	6.50	0.09

BSO = 'Owoh' fermented by *Bacillus subtilis*

BPO = 'Owoh' fermented by *Bacillus pumilus*

BLO = 'Owoh' fermented by *Bacillus licheniformis*

BSPLO = 'Owoh' fermented by *Bacillus subtilis*, *Bacillus pumilus* and *Bacillus licheniformis*

BSPO = 'Owoh' fermented by *Bacillus subtilis* and *Bacillus pumilus*

BSLO = 'Owoh' fermented by *Bacillus subtilis* and *Bacillus licheniformis*

BPLO = 'Owoh' fermented by *Bacillus pumilus* and *Bacillus licheniformis*

Control = Naturally fermented 'owoh'

Bacillus subtilis had been reported to produce antibiotics that act against other bacteria (Campbell-Platt, 1980). The combination if the starter cultures produced low quality of 'owoh'. This may be as a result of competition for the available nutrients or antagonistic interaction between the organisms that consequently reduced their efficiency (Woodburn, 1992).

The pH of fermented 'owoh' skates between 5.10 and 7.30. This agrees with the findings of Whitaker (1978) who reported that the pH of

fermenting oil seeds tends to alkalinity as against the starchy substrates that increase acidity with fermentation period. Gilbert (1973) reported that the genus *Bacillus* utilizes different carbon sources. It was also noted that the amount of nitrogen free extracts (carbohydrates) of the fermented cotton seed compare to the value reported for the raw seeds by Faulkner (1974).

Sample BSRU produced 'owoh' with highest amount of protein. Aderibigbe and Odunfa (1988) reported that *Bacillus subtilis* produces

Table 2: Proximate composition of 'owoh' produced by *Bacillus subtilis* strains isolated from different condiments (g/100g dry matter)

Sample	Dry matter	Lipid	Ash	Soluble protein	Crude fibre	Nitrogen free extract	pH	Acidity (% citric acid)
BSRU	71.53	32.75	1.52	45.15	6.90	13.62	5.10	0.05
BSSR	62.54	33.50	1.90	45.64	6.18	15.84	6.40	0.07
BSWH	64.00	34.27	2.95	38.22	6.65	19.31	7.00	0.06
BSGR	61.65	36.90	1.47	39.69	5.61	16.22	6.21	0.06
Control	71.48	42.40	1.13	20.07	5.06	28.68	7.30	0.09

BSRU = 'Owoh' fermented by *Bacillus subtilis* isolated from 'iru'
 BSSR = 'Owoh' fermented by *Bacillus subtilis* isolated from 'soy-iru'
 BSWH = 'Owoh' fermented by *Bacillus subtilis* isolated from 'owoh'
 BSGR = 'Owoh' fermented by *Bacillus subtilis* isolated from 'ogiri'
 Control = Naturally fermented 'owoh'

Table 3: Sensory evaluation of 'owoh' fermented by *Bacillus subtilis* from different soup condiment

Samples	Texture	Odour	Colour	Appearance	General acceptability
BSRU	3.25	3.65	2.75	2.50	3.03
BSSR	2.88	3.60	3.25	3.13	2.32
BSWH	3.38	3.50	2.63	3.13	3.16
BSGR	3.00	2.63	2.89	3.88	3.10
Control	3.35	3.63	2.38	2.00	3.12

BSRU = 'Owoh' fermented by *Bacillus subtilis* isolated from 'iru'
 BSSR = 'Owoh' fermented by *Bacillus subtilis* isolated from 'soy-iru'
 BSWH = 'Owoh' fermented by *Bacillus subtilis* isolated from 'owoh'
 BSGR = 'Owoh' fermented by *Bacillus subtilis* isolated from 'ogiri'
 Control = Naturally fermented 'owoh'

protease that degrades the protein in the *Parkia biglobosa* during the production of 'iru'. This process resulted in steady increase in the amino acids content of the final product.

High measure of dietary fibre (5.61 to 6.90g/100g) of fermented 'owoh' is in close agreement with 6.50% reported by Sanni *et al.*, (1998) the high crude fibre has been reported to aid digestibility and prevents constipation and appendicitis Ibitoye, (2005).

The result of the sensory evaluation test of 'owoh' fermented by *Bacillus subtilis* strains is shown in Table 3. Based on texture, the control

sample was rated best with the score of 3.50 while in term of odour BSRU, BSSR, and control were rated same with 3.63 points the general acceptability presents sample BSSR to be the best. According to Sarkar and Tamang (1994) this product judgment was the best way of rating and selecting the highest quality amongst the total products. There was no significant difference in the attributes examined (at $p \geq 0.05$)

It may be concluded that single starter culture of *Bacillus subtilis* produced a better 'owoh' than other species. The combination of the organisms does not produce a nutritionally better 'owoh'.

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