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Proximate analysis of different fermented *Citrullus vulgaris* products from different cultivars of melon and *Bacillus subtilis* strains in South West Nigeria

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Abstract

The strains of *Bacillus subtilis* obtained from different condiments such as Natto (USA), Kinema, Natto (Nigeria), 'Iru' (fermented *Parkia biglobosa*), Soy-iru, Cowpea 'iru' and commercial 'ogiri' were used to ferment three different cultivars of melon seeds which include *Citrullus lanatus* and *Cucumeropsis manii*. Proximate analyses of the products were also determined, while the fermented and unfermented seed products were compared. The moisture content ranges between 7.57 to 39.33%, dry matter (60.7 to 93.4%), ash (2.65 to 4.71%), soluble protein (24.4 to 35.9%), fatty acid (32.47 to 53.5%), crude fibre (1.89 to 3.75%), carbohydrate (7.60 to 25.17%), pH (6.10 to 7.7) and titratable acidity (0.10 to 0.24). The mineral contents and sensory evaluation of both fermented and unfermented melon seeds were done and the result revealed that *Citrullus vulgaris* seed is the best raw material for 'ogiri' production. This was supported by the proximate values of the seeds and fermented product.

Keywords: Proximate analysis, *Citrullus vulgaris*, Cultivars and *Bacillus subtilis* strains

1. INTRODUCTION

In Africa, the art of fermentation is wide spread. This include the processing of plant vegetable seeds into food condiment, which in Nigeria and many other countries of West and Central Africa have popular strong smelling fermented food artinary products that give pleasant aroma to soups sauces and other prepared dishes (Ogunshe *et al*., 2012). It is evident that fermented food condiments are good source of nutrient and could be used to produce complementary food supplements (Achi, 2005).

'Ogiri' is produced from fermentation of melon seed (*Citrullus vulgaris*) in the western part of Nigeria. The melon seed has high protein and low carbohydrate content. *Citrullus vulgaris* is a species of the genus *Citrullus* and family *cucurbitaceae* (Alfred, 1986). 'Ogiri' is mostly consumed in West Africa. The fact that these pastes are called the same name by different ethnic groups implies that they have a common origin. The art of making 'ogiri' in Sierra Leone was most probably introduced by the freed slaves, mostly of Yoruba origin who settled there (Odunfa and Oyewole, 1995). The different types of 'ogiri' are named after each ethnic group. 'Ogiri-Ijebu' is produced and essentially consumed among the 'Ijebus' of South Westhern Nigeria, 'Ogiri-Igbo' is consumed among Igbos of Easthern Nigeria, while 'Ogiri-Saro' is majorly consumed in Sierra Leone (Odunfa, 1985).

It is an established fact that different ogiris is produced from different towns because there is no standard starter culture for the product. In an attempt to industrialize the product there must be an established method of production with an attested strain of *Bacillus subtilis* and the best raw materials must also be identified. This research work tend to established more facts on the best attested strains and the raw materials that will give the best product of 'Ogiri' and this is done by evaluating the proximate analysis of different 'ogiri' fermented by different strains of *Bacillus subtilis* with different cultivars of melon seeds and subsequently subjecting it to sensory evaluation by a set of trained panelists.

2. Materials and Methods

Strains of *Bacillus subtilis* obtained from Natto from United States of America, Kinema local Natto, Iru (fermented *Parkia biglobosa*) 'Soy-iru, cowpea 'Iru' and commercial 'ogiri' from South west Nigeria were used as starter culture to ferment *Citrullus vulgaris* seeds. The best 'ogiri' fermenting strain of *Bacillus subtilis* from commercial 'ogiri' sample CRBS 23 was also used to ferment *Citrullus lanatus, Citrullus vulgaris* and *Cucumeropsis manii*. The proximate analyses of unfermented seeds were also evaluated. The following parameters were determined in both commercial and 'ogiri' samples produced by starter culture experiments. The methods AOAC (1990) were used except where otherwise stated. Determinations were in triplicates and the mean value calculated.

To produce the fermented products, the *Bacillus subtilis* from different condiments were used as starter culture for the fermentations. The method used by Sarkar and Tamang (1994) was adopted to prepare the inocula. To 24h old culture of each isolate on Plate count agar (PCA) slant was added to 5ml of distilled water

and shaken. The suspension was then used as inoculum for 200g of sterile melon seed (*Citrullus vulgaris*) in crystallizing dish. The bacterial cell count per ml of suspension used was estimated to be 3×10^6 (CFU). Inoculated samples were incubated at 37°C for 72h. Organoleptic/sensory evaluation was performed on the products by trained panellists.

2.1. Crude protein: Kjeldahl method used involved three stages. Five grams (5g) of the samples were weighed and placed in the digestion flask; copper and selenium (one tablet each) was used as the catalyst. Twenty five ml of sulphuric acid was added to each flask containing the samples and digested until a clear solution was obtained. After cooling, the digested samples were transferred into 250ml volumetric flask and made up with mark with distilled water.

The distillation apparatus (Markham semi-micro Kjeldahl apparatus) was connected up with delivery tube dipping below the boric acid in the receiving flask. Then 10ml of 2% boric acid was used for each sample distilled and five drops of screened methyl red was added as indicator. Then 10ml of the diluted digest was transferred into the digestion flask followed by addition of 15ml of 40% NaOH. After about 75ml has been distilled, the tip of the condenser was washed into the distillate. The distillate was titrated with 0.01N HCl and the percentage nitrogen calculated thus:

$$\text{Nitrogen (\%)} = \frac{\text{volume of acid (HCl) used } 0.1 \times 0.014 \times 50 \times 100}{\text{weight of the sample}}$$

Note : Crude protein 1ml of 0.01M HCl =Nitrogen % ×6.25

2.2. Determination of crude fibre content: Five gram of the sample were weighed into 1 litre conical flask (W1), followed by the addition of 200ml boiling 1.25% H_2SO_4. The solution was boiled gently for 30mins then filtered through a muslin cloth. Stretched over a Brichner funnel and rinsed with hot distilled water. The residue was scrapped back into the flask with a spatula and 200ml boiling 1.25% NaOH was added. The solution was allowed to boil gently for 30 minutes and cool thereafter. This was again washed thoroughly with hot distilled water and rinsed once with 10% Hcl and twice with industrial methylated spirit. The residue was rinsed finally three times with petroleum ether (40-60°C) and later drained, dried and scrapped into a crucible. The residue was dried overnight at 105°C in the oven cooled in a dessicators and then weighed (W_2). The residue was also ashed at 550°C for 90 minutes in a muffle furnace, then allowed to cool in a desiccators and weighed (W_3) percentage of crude fibre was estimated as bellow:

$$\text{\% Crude fibre} = \frac{W_2 - W_3 \times 100}{W_1}$$

W_1 = weight of sample
W_2 = weight of residue
W_3 = weight of residue after ashing

2.3. Determination of ash content: Five grams of each sample were added into a pre- weighed porcelain crucible and placed in a temperature controlled furnace pre-heated to 550°C, which was maintained for 2h. The crucible was transferred directly into desiccators, cooled and reweighed immediately. The ash content was then calculated thus:-

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dried sample}} \times \frac{100}{1}$$

2.4. Determination of Lipid: The Soxhlet extraction method was used (Pearson, 1990). Two grams (2g) of each dried sample was weighed into a pre-weighed filter paper and folded neatly. This was placed into the soxhlet extractor thimble containing petroleum ether (40 - 60°C). The extraction was done for six hours. The filter paper was taken out, air-dried and reweighed.

$$\text{Lipid (\%)} = \frac{\text{loss in weight of sample}}{\text{Original weight of sample}} \times \frac{100}{1}$$

2.4. Moisture content: moisture content was determined using the oven-drying method. A clean and dry dish was weighed and recorded (W_1). Five grams of the sample was weighed into the dish and re-weighed (W_2). The dish containing the sample was transferred into the oven, dried and maintained at 105°C for 4 hours after which the dish was transferred into the desiccators and allowed to cool before re-weighing. The process of heating, cooling and weighing was continued until a constant weight (W_3) was obtained. Moisture content was calculated as follows:

$$\text{Moisture content (\%)} = \frac{\text{loss in weight due to drying}}{\text{weight of sample take}} = \frac{W_2 - W_3}{W_3 - W_1} \times \frac{100}{1}$$

W_1 = weight of dish
W_2 = weight of dish + sample
W_3 = weight of dish + sample after drying

2.5. Determination of pH: One gram of each sample was grounded with mortal and pestle and transfer into a MacCartney bottle. Twenty millilitres of distilled water was added and mixed properly. The pH of suspensions using Pye Unican pH meter (model 290).

2.6. Determination of Total Titratable Acidity (TTA): The method of AOAC (1990) was used to 20ml of sample extract was added to two drops of 1% phenolphthalein indicator. This was titrated against 0.1N NaOH solution until a permanent pink colour was observed. The acidity was calculated as:

$$\text{TTA (\%)} = \frac{M_b V_b}{V_S} \times \frac{M_{eq}\, C_3H_6O_3}{1} \times \frac{100}{1}$$

M_b = Molarity of NaOH used
V_b = Volume of NaOH used
V_s = Volume of sample used
$M_{eq}\, C_3H_6O_3$ = Millilitre equivalent of lactic acid = 90g
The titratable acidity was expressed as a percentage of lactic acid.

2.7. Mineral content: The mineral content for each sample was analysed. The ash (0.25g) prepared and described above was dissolved in 10ml of 1N Hcl and made up to the mark in 100ml standard flask with distilled water. The mineral contents of the sample was analysed with the aid of atomic absorption spectrophotometer (AAS), Buck scientific inc. Model 200A/210 Connecticut (1993). The metals qualified were Na, Ca, Fe, Mg, Cu, Co, Zn and Mn. Standard for each metal was prepared using their respective salts. The instrument was switched on and lamp for each metal was fixed. All metals analysed used hollow cathode lamps and air acetylene flame. The standard of each metal as well as each sample was aspirated into the flame and the absorbance of the standards and the samples read. The respective concentrations in milligram per litre (mg/l) were calculated for each sample.

2.8. Determination of carbohydrate content: carbohydrate was determined by the difference 100 – (Ash % + crude fibre % + crude fat % + Crude protein % + moisture %).

2.9. Determination of soluble protein: The method of Lowry *et al*., (1951) was used. Four reagents A, B, C, and E were prepared. Reagent A was prepared by dissolving 0.4g for Na_2CO_3 in 0.1N sodium hydroxide to give 2.0% sodium carbonate. Reagent B contains 0.5g of $CuSO_4$ in 100ml 0f 1% sodium or potassium tartarate solution. Reagent C was prepared by adding 1ml of reagent B to 50ml of reagent A while reagent E was a 1:2 dilution of concentrated folin-ciocalteaus phenol with distilled water.

Two gram (2g) of each sample was ground into a pulp in mortal. Twenty millilitres (20ml) of 50% of ethanol was added and mixed. The slurry was filtered into a McCartney bottle through a Whatman No1 filter paper. A 0.3ml of each extract was pipette into 3 test tubes, mixed and allowed to stand for 10 minutes. Then 0.3ml of reagent E was added, mixed properly and allowed to stand for 30 minutes. The optical density (O.D) was determined at 500nm using a visible Bruker DPX – 400MHZ) spectrophotometer. The instrument was standardized with a blank of 03ml of 50% ethanol and reagents. The quantity of soluble protein (mg/ml) was read from a standard curve of bovin serum albumin (BSA). All statistical analyses were done using SPSS 0.05.

3.0 Results and Discussion

The microbial load of the raw materials showed their ability to support the growth of the fermenting organisms. They all supported the growth of *Bacillus subtilis* from the commercial 'ogiri' (CRBS 23) but the highest growth was observed in *Citrullus vulgaris*. Which equally served as the control because it was the most commonly used cultivar of melon in the South-West Nigeria.

The proximate composition of both unfermented and the fermented melon seeds (Table 1) showed that 'ogiri' production is a moist solid state fermentation, the moisture content observed in the study was in agreement with the findings of Omafuvbe *et al*. (2003).

On some Nigerian fermented condiments, hydrolytic decomposition of the fermenting substrate has contributed to the moisture content of the samples. With the used of starter cultures and controlled fermentation conditions, the quality of the condiment can be uniform (Beaumont, 2002). Selection of appropriate strains of *B. Subtilis* group as starter cultures for the production of 'ogiri' in the study contributed a database for the optimization of traditional fermentation of condiments in Nigeria. In Table 3 the result of sensory evaluation favoured the fermented *Citrullus vulgaris* which had the highest score from the panellists.

This can be supported by the observation that it was the richest in minerals. When the melon seeds were analysed after fermentation. The proximate composition of 'ogiri' produced by using strains of *Bacillus subtilis* showed that samples from commercial 'ogiri' had the highest score in the Table 5 since in Table 4 the product is among those having the highest reducing sugars and soluble protein.

Fermented *Citrullus vulgaris* has the lowest fatty acids content and also the strains (CRBS 23) when used in fermented product. The most abundant mineral was calcium. This study confirms that both fermented and unfermented melon seeds are good sources of potassium and calcium. The differences in the mineral contents of plant product might be due to the soil composition and the rate of uptake of minerals by each plant (Nelson, 1980). The presence of these important minerals (i.e. iron, magnesium, phosphorus, potassium and calcium) in sufficient quantities indicates that both fermented and unfermented melon seeds flour are potential choices for future food and feed supplements.

Therefore, the best raw material is *Citrullus vulgaris* and the best strain of *Bacillus substilis* is CRBS23 from commercial sample of ogiri (fermented *Citrullus vulgaris*).

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Table 1: Proximate composition of both unfermented and fermented melon seeds

Parameters	**UCM**	**FCM**	**UCL**	**FCL**	**UCV**	**FCV**
Moisture (%)	7.57^{d}	35.20^{b}	34.70^{b}	39.33^{a}	6.60^{e}	33.40^{c}
Dry matter (%)	92.00^{a}	65.40^{b}	65.30^{b}	60.70^{c}	93.40^{a}	66.60^{b}
Ash (%)	3.05^{b}	2.91^{b}	2.83^{b}	2.97^{b}	2.65^{b}	4.71^{a}
Soluble protein (%)	35.90^{a}	32.00^{b}	29.00^{c}	31.50^{b}	32.00^{b}	24.40^{d}
Fatty acid (%)	45.00^{b}	32.47^{d}	52.10^{a}	39.00^{c}	53.50^{a}	43.17b
Crude fibre (%)	3.30^{b}	1.89^{c}	3.43^{b}	1.97^{c}	3.81^{a}	3.75^{a}
Carbohydrate (%)	12.40^{c}	24.50^{ab}	13.30^{c}	25.17^{a}	7.60^{d}	23.60^{b}
pH	6.10^{f}	7.60^{b}	6.40^{c}	7.7^{a}	6.20^{e}	6.77^{c}
Titratable acidity	0.21^{c}	0.21^{c}	0.13^{d}	2.40^{a}	0.10^{e}	1.60^{b}

Key:

UCM = unfermented *Cucumeropsis manii* FCM = fermented *Cucumeropsis manii*
UCL = unfermented *Citrullus lanatus* FCL = fermented *Citrullus lanatus*
UCV = unfermented *Citrullus vulgaris* FCV = fermented *Citrullus vulgaris*
Starter culture used: *Bacillus subtillis* CRBS23

Table 2: Mineral contents (Mg/100g) of both unfermented and fermented melon seeds (mg/100g)

Minerals	UCM	FCM	UCL	FCL	UCV	FCV
Calcium	84.96^{ab}	76.99^{ab}	37.65^{b}	77.93^{ab}	77.03^{ab}	98.68^{a}
Copper	6.54^{d}	11.39^{c}	13.67^{bc}	0.12^{cd}	16.32^{b}	21.69^{a}
Iron	23.90^{b}	12.07^{e}	24.44^{b}	14.50^{d}	18.08^{c}	28.53^{a}
Magnesium	79.90^{d}	13.05^{f}	113.96^{b}	58.72^{e}	85.19^{c}	124.72^{a}
Manganese	1.84^{b}	75.85^{a}	1.09^{c}	1.16^{c}	1.10^{c}	1.60^{b}
Phosphorus	179.02^{b}	139.17^{e}	1531.37^{c}	1075.00^{d}	1624.07^{bc}	1834.39^{a}
Sodium	550.37^{bc}	429.56^{c}	630.82^{b}	270.15^{d}	497.67^{bc}	793.15a
Zinc	1.91^{b}	1.13^{c}	1.18^{c}	1.18^{c}	1.85^{b}	2.26^{a}

Key: As in Table 1 above

Table 3: Sensory scores* for the Bacillus CRBS23- fermented products of *Cucumeropsis manii*, *Citrullus lanatus* and *Citrullus vulgaris*

Attributes	FCM	FCL	FCV
Flavour (50)	20	30	38
Body and texture (45)	20	28	27
Colour (5)	5	2	3
Total Score (100	42	60	68

*Mean score from five (5) panellists

Table 4: Proximate composition of 'ogiri' produced by using strains of *Bacillus subtilis* group obtained from different condiments isolates

Parameters	LN	KE	AN	IR	SI	CP	CO
pH	6.51^{b}	7.01^{a}	6.43^{b}	6.48^{b}	7.05^{a}	6.54^{b}	6.53^{b}
Moisture (%)	41.83^{b}	41.65^{c}	41.48^{d}	41.88^{b}	41.20^{e}	40.38^{f}	42.91^{a}
Crude protein (%)	20.34^{a}	14.81^{d}	13.16^{f}	14.92^{d}	14.07^{e}	15.68^{b}	15.36^{c}
Ash (%)	2.21^{b}	2.72^{a}	1.92^{c}	2.18^{b}	2.57a	1.57d	2.01^{c}
Soluble protein	6.34^{f}	5.86^{g}	6.65^{e}	8.61^{d}	12.46^{b}	9.75^{c}	13.21^{a}
Reducing sugars (mg/100g)	4.64^{ab}	4.44^{b}	5.00^{ab}	4.82^{ab}	4.43^{b}	5.41^{a}	5.31^{a}
Calcium (mg/100g)	140.85^{a}	77.85^{c}	61.03^{d}	49.79^{e}	13.42^{g}	82.90^{b}	35.46^{f}
Potassium (mg/100g)	17.57^{b}	10.37^{f}	13.25^{e}	14.55^{d}	16.75^{c}	22.18^{a}	17.45^{b}
Phosphorus (mg/100)	32.31^{a}	69.04^{de}	72.48^{bc}	70.75^{cd}	69.26^{de}	67.03^{e}	73.64^{b}
Crude fibre (%)	8.27^{e}	6.63^{g}	8.18^{f}	8.90^{c}	10.01^{a}	8.54^{d}	9.54^{b}
Titratable acidity (%)	3.41^{b}	2.39^{f}	2.58^{e}	3.14^{c}	2.93^{d}	2.26^{g}	3.66^{a}
Fats (%)	23.27^{bc}	23.80^{ab}	23.85^{a}	23.67^{ab}	23.90^{a}	24.16^{a}	23.04^{c}
Carbohydrate (%)	4.17^{f}	10.39^{b}	11.41^{a}	8.99^{d}	9.65^{c}	9.66^{c}	8.15^{e}

Key:
LN = Natto (USA) KE = Kinema AN = Local Natto (NIG)
IR = 'Iru' SI = 'Soy-iru' CP = Cowpea 'Iru'

CO = Commercial 'ogiri'

Table 5: Sensory scores of 'ogiri' produced using strains of *Bacillus subtilis* group from condiments as starter cultures

Attributes	LN	KE	AN	IR	SI	CP	CO
Flavour (50)	18.13^{d}	18.23^{d}	7.80^{e}	25.10^{b}	20.27^{c}	26.06^{b}	39.90^{a}
Body and texture (45)	10.13^{f}	20.50^{d}	18.03^{e}	27.73^{b}	28.27^{b}	22.00^{c}	44.50^{a}
Colour (5)	2.10^{c}	1.93^{c}	2.17^{c}	3.07^{c}	4.20^{a}	20.7^{c}	4.10^{a}
Total Score (100)	30.40^{d}	40.70^{c}	28.00^{e}	55.90^{b}	52.80^{ab}	50.13^{bc}	88.50^{a}

Key: As in Table 4 above