

**Physicochemical Changes of *Citrullus Lanatus* (Watermelon) Seeds
during *Bacillus* Species mediated Fermentation and Sensory
Evaluation of Condiment Produced**

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Abstract

Watermelon (*Citrullus lanatus*), a drought tolerant and ubiquitous fruit, contains a lot of seeds that are generally discarded after consumption of the juicy portion. This study was designed to assess changes in proximate, amino acid, mineral and phytochemical compositions of watermelon seeds during a 72 hr fermentation period with mixed inocular of *Bacillus subtilis* and *Bacillus licheniformis* in the production of a local condiment similar to *ogiri* (a condiment produced from *Citrullus vulgaris* popularly consumed in Nigeria). The sensory quality was also evaluated for the resulting watermelon condiment. Proximate analysis revealed significant ($p < 0.05$) with an increase in crude protein (14.00-21.10 %), and ash (1.90-2.55%) at the end of fermentation. Some of the essential amino acids obtained include: threonine, valine, isoleucine, tryptophan and leucine, all of which increased with days of fermentation. Similar trend was observed in the mineral components: magnesium (41.02-45.06 mg/100 g), zinc (5.05-7.15 mg/100 g), phosphorus (14.52-17.00 mg/100 g), potassium (22.03-33.02 mg/100 g), calcium (13.45-16.03 mg/100 g), sodium (5.40-7.14 mg/100 g), and manganese (1.10-2.53 mg/100 g). Results showed that pH of the condiment ranged from 5.6 to 8.6 over 72 hrs of fermentation. Length of fermentation caused a steady decrease in hydrocyanic acid (1.57-0.03 mg/100 g), tannin (22.05-16.00 mg/100 g) and phytate (7.05-3.52 mg/100 g) but an increase in flavonoids (1.58-2.30 mg/100 g) and phenol (0.11-1.41 mg/100 g). Sensory evaluation showed

that *ogiri* produced from *C. vulgaris* was more generally accepted but that produced from *C. lanatus* was more preferred in taste with a score of 6.20. Increased days of fermentation with *B. subtilis* and *B. licheniformis* in consortium had varying effects on the nutritional and phytochemical compositions of watermelon condiment.

Keywords: Watermelon seed, *Bacillus* species, condiment, fermentation, physicochemical.

Introduction

Watermelon (*Citrullus lanatus*) is the fruit of an herbaceous creeping plant belonging to the family *Curcubitaceae* (Aguilera *et al.*, 2011). It has been described as one of the most widely cultivated crops with more than 1,200 varieties grown all over the world (Alfa-nla, 2014). Nigeria is one of the leading producers of watermelon in Africa, producing over 139, 000 tonnes in 2011 (Alfa-nla, 2014). Watermelon is highly nutritious with good contents of vitamin C as well as carotenoids, which are good antioxidant agents. In addition to the nutritional quality, as a fresh fruit, watermelon has an excellent thirst quenching property. The flesh of the fruit provides a sweet refreshing juice. As a result, only this part of the fruit is consumed. Obizoba *et al.* (2005) reported that only about 45-50 % of the total weight of watermelon is consumed; the rinds and seeds are usually discarded. This practice, aside constituting an economic loss, leads to increase in solid waste pollution and wastage of valuable biomass and nutrients (Acar *et al.*, 2012). Some recent researches have again reiterated the potential of some underutilised leguminous seeds, such as tamarind seeds (Olagunju *et al.*, 2018) and watermelon seeds (Adebayo, 2018; Adebayo *et al.*, 2018) for the production of protein-dense condiment.

Watermelon seeds are a good source of protein (16.33 – 17.75 %) (El-Adway and Jaha, 2001; Tabiri *et al.*, 2016), consisting of both essential and non-essential amino acids while watermelon rinds can be pickled and used as a condiment (Foster, 2015). Adebayo (2018) also showed that watermelon seeds are good potential for condiment production, and that *Bacillus subtilis* is one of the active microorganisms involved in the wild fermentation process.

Condiment is a supplemental food ingredient, such as a sauce, added to foods in some cultures to impart or enhance flavour or to complement the

dish (Agboola and Oguntuase, 2006). Some locally produced condiments in Nigeria have been identified as low-cost meat substitutes, contributing to protein and calorie intake (Oboh, 2006) and helping to reduce the incidence of protein energy malnutrition among low-income families who cannot afford animal protein. Also, the controversy surrounding the safe consumption of monosodium glutamate-based seasonings has made some homes shift preference to the use of locally produced condiments (Oboh, 2006). Some locally produced condiments include *iru* or *dawadawa* (fermented locust bean), *okpehe* (fermented *Prosopis africana*) and *ogiri* (fermented melon seed). However, the production of these fermented condiments is still largely being done spontaneously (village art method) which leads to longer fermentation time, inconsistent condiment quality and unguaranteed microbiological safety. This has made it necessary for the use of starter cultures in the production of condiment.

Many genera of bacteria including *Bacillus*, *Micrococcus*, *Leuconostoc*, *Staphylococcus* and *Enterobacteria*, have been shown to be involved in fermentation during indigenous condiment production (Enujiugha, 2009; Adebayo *et al.*, 2018). Achi (2005) noted that the use of mixed cultures, through their physiological and metabolic properties, might be the best approach to achieving product of most desired nutritional and sensory qualities. Mono and mixed cultures of *Staphylococcus* and *Streptococcus* have been successfully used in the fermentation of soy *iru* (Agboola and Oguntuase, 2006). Balogun *et al.*, (2017) have also reported the production of *okpehe* using a mixed culture of *Bacillus subtilis* and *Bacillus licheniformis*. However, scanty information is available on the fermentation of watermelon seeds using starter cultures for condiment production, despite its established high protein content.

It was therefore necessary to determine the physicochemical changes and sensory properties of a fermented condiment produced from watermelon seeds using starter cultures of *Bacillus subtilis* and *Bacillus licheniformis*. The use of watermelon seed for the production of a locally fermented condiment will improve the economic importance of the seed, while also serving as a cheap source of plant protein for low-income populations in developing countries.

Materials and Methods

Source of materials

Dried watermelon seeds were obtained from Obasanjo Farms, Igbo-ora, and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin with the Voucher No: UILH/003/1056 Nigeria. Pure cultures of *Bacillus subtilis* and *Bacillus licheniformis* were obtained from the Microbiology Laboratory of Federal Institute of Industrial Research, Oshodi, Nigeria.

Production of fermented water melon condiment

The method of Balogun and Oyeyiola, (2011) was used with some modifications. The seeds were first softened by heating for 1 hr on a hot plate set at 60°C. The water used in heating the seeds was thereafter decanted, and the seeds were washed with water at ambient temperature (30±2 °C). The testae of the washed seeds were then removed using gloved hands. The cotyledons obtained were thoroughly washed with clean water, after which they were submerged in a clean pot filled with water, and then cooked at 60 °C for 30 mins. The cotyledons were drained over a sterile, dirt-free muslin cloth, and cooled to about 25-30 °C before being inoculated with the selected starter organisms.

The method described by Balogun *et al.*, (2014) was used for the inoculation of the starter organisms. Unfermented cotyledons in two separate portions (100 g each) were inoculated with mono cultures: one with *B. subtilis* and the other with *B. licheniformis*. Another 100 g was inoculated with 1 ml of a mixed starter culture prepared from 0.5 ml each from the mono cultures. Prior to inoculation, a suspension of each starter organism was made in nutrient broth and incubated at 37 °C for 24 hrs. The resulting inoculated mash was placed in banana leaves, which had been disinfected using a 75 % ethyl alcohol and rinsed with distilled water prior to their use. The mash was then wrapped in a 2-layered foil. The wrapped mash was kept at 30±2 °C for different time regimes (0, 24, 48, and 72 hrs).

Determination of physicochemical parameters of fermented watermelon seeds condiment

The protein content of each watermelon condiment was determined and the sample with the highest crude protein content after 72 hrs of fermentation was selected for subsequent analyses. The pH, proximate and mineral compositions of the fermented watermelon condiments were determined using the method described by AOAC (2005). The carbohydrate content was determined by difference (i.e., 100 – moisture + crude protein + crude fibre + crude fat + ash). Amino acid profile was analysed using post-column Ninhydrin method described by Frank *et al.* (2000) while flavonoids and phenol contents were determined using AOAC (1990) method.

Some phytochemicals were also determined using standard methods. These include tannin (Jaffe, 2003), hydrocyanic acid (Drochioiu *et al.*, 2000) and phytate (Penuel *et al.*, 2013).

Sensory evaluation of fermented watermelon condiment

A local vegetable soup was prepared with the fermented watermelon seed condiment. This was then compared with vegetable soup prepared using *ogiri* (a fermented condiment produced from *Citrillus vulgaris*). Thirty (30) panelists, who were familiar with the peculiar sensory attributes of *ogiri*, were selected from the Department of Home Economics and Food Science, University of Ilorin. A duo-trio test was utilized. The panelists were given three samples, in which two were the same and one was different. The panelists were to identify the odd sample and compare it with the other two based on some stated sensory parameters (colour, taste, aroma, texture and general acceptability). A 9-point hedonic scale in which 9 represents ‘like extremely’ and 1 represents ‘dislike extremely’ was used.

Statistical analysis

Data generated from the various analyses above were subjected to Analyses of Variance using SPSS (Version 20.0; IBM SPSS) and the mean scores were separated using Multiple Range of Comparison Test.

Results and Discussions

Proximate compositions of *Bacillus subtilis* and *Bacillus licheniformis* fermented watermelon seeds

The protein content of watermelon seeds fermented with a mixed starter culture of *B. subtilis* and *B. licheniformis* was significantly ($p < 0.05$) higher (21.10 %) than those of other samples (those fermented singly and wildly) at the end of each fermentation period (Table 1). This shows a benefit of inoculation with either organism and an additional benefit when both organisms are present in the inoculum. Generally, the protein contents of all the condiments (both mono and mixed starter culture fermented watermelon seeds) increased with increased days of fermentation (Table 1). The increased protein content obtained in this study is similar to the findings of Gberikon *et al.*, (2010) who noted that the protein contents of *Parkia biglobosa*, *Glycin max*, and *Prosopis africana* seeds inoculated with a consortium of *B. subtilis* and *B. pumilus* were higher than those of the naturally fermented seeds. Balogun *et al.*, (2017) also reported an increase in the protein content of *Prosopis africana* seeds fermented with starter cultures.

There was a significant ($p < 0.05$) increase in the crude protein contents of the mixed-culture fermented watermelon seeds with increased days of fermentation (Table 2). This increase in protein could be as a result of significantly reduced carbohydrate contents with increased days of fermentation. It is plausible that the combined effects of *B. subtilis* and *B. Licheniformis* enhanced the fermentation process. Starter organisms are also known to secrete extracellular enzyme, which are protein in nature, during their metabolic activities (Oboh and Akindahunsi, 2003).

Table 1: Crude protein content (%) of watermelon seeds fermented with culture of either or both organisms (*B. subtilis* and *B. licheniformis*)

Bacterial isolate	0 hr	24 hrs	48 hrs	72 hrs
BS1M	14.00 ^a ± 0.20	15.06 ^b ± 0.02	16.50 ^b ± 0.00	18.43 ^c ± 0.01
BL1M	14.04 ^a ± 0.01	15.02 ^b ± 0.01	16.25 ^c ± 0.05	19.05 ^b ± 0.01
*B_sB_L	14.05 ^a ± 0.01	16.28 ^a ± 0.01	17.18 ^a ± 0.01	21.10 ^a ± 0.05
Control	14.05 ^a ± 0.00	15.00 ^b ± 0.02	15.05 ^d ± 0.05	16.05 ^d ± 0.01

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly (p<0.05) different

Keys: BS1M: *Bacillus subtilis*; BL1M: *Bacillus licheniformis*; B_sB_L: *Bacillus subtilis* and *Bacillus licheniformis*

* Sample selected for further analyses

Table 2: Proximate composition (%) of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time (hr)	Moisture	Carbohydrate	Protein	Ash	Fiber	Fat
0	6.05 ^c ±0.01	52.9 ^a ±0.02	14.05 ^d ±0.01	1.90 ^b ±0.01	7.05 ^a ±0.03	18.05 ^d ±0.13
24	6.07 ^c ±0.00	47.58 ^b ±0.10	16.28 ^c ±0.01	2.00 ^b ±0.00	6.05 ^b ±0.00	22.02 ^c ±0.20
48	7.02 ^b ±0.0	45.25 ^c ±0.00	17.18 ^b ±0.03	2.04 ^b ±0.02	5.01 ^c ±0.01	23.50 ^b ±0.00
72	7.75 ^a ±0.05	38.35 ^d ±0.02	21.10 ^a ±0.15	2.55 ^a ±0.01	5.05 ^c ±0.01	25.20 ^a ±0.02

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly (p<0.05) different

The crude fat content of watermelon condiment produced from a mixed starter culture of *B. subtilis* and *B. licheniformis* significantly ($p < 0.05$) increased from 18.05 % at 0 hr to 25.20 % after 72 hrs (Table 2). This is likely due to the significant reduction of carbohydrate and crude fibre contents. The high amount of crude fat obtained is in line with the finding of Murkovic *et al.*, (2002) that watermelons are a good source of fat. It has also been reported that the fat content of locust beans increased when fermented (Achi, 2005). There was a significant reduction in the carbohydrate content of the watermelon seeds fermented with a mixed culture as increased days of fermentation. This could be associated with the utilization of carbohydrate by fermenting organisms. Crude fibre ranged from 5.05 to 7.05 % while ash content was between 1.90 and 2.55 %. Oboh (2006) earlier reported 5.9-11.2 % and 1.9-4.7 % as the crude fibre and ash contents, respectively, for some fermented underutilized leguminous seeds. Crude fiber is of great importance in human metabolism; it helps to form complexes with triglycerides, and tends to reduce low density lipoprotein (LDL) thereby reducing the risks of heart attack (Bello *et al.*, 2008). Ash content on the other hand is a measure of the amount of minerals present in food.

Amino acid profile of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Seventeen amino acids were found present in varying proportions in the seeds (Table 3). These include: all the essential amino acids (threonine, valine, isoleucine, tryptophan, lysine, histidine, methionine, leucine and phenylalanine), most of which steadily increased with increased days of fermentation. A similar trend was reported by Balogun and Oyeyiola (2012). The rapid increase in total amino acids is consistent with the increase in protein content. Low molecular weight peptides and amino acids produced through enzymatic breakdown of protein exhibit a desirable flavouring property (Achi, 2005). It was actually found that serine decreased steadily with increase in fermentation days, suggesting its depletion by microbial activity during fermentation.

Table 3: Amino acids profile (g/100 g) of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Amino acids	0 hr	24 hr	48 hr	72 hr
Lysine	0.52	1.05	1.55	1.79
Histidine	0.39	0.56	0.64	0.79
Asparagine	0.73	0.88	1.46	1.58
Aspartic acid	0.85	0.92	1.04	1.07
Threonine	1.04	1.45	3.88	2.64
Serine	4.03	3.07	3.03	2.07
Glutamine	0.03	0.07	0.53	0.87
Proline	2.85	2.82	2.90	2.98
Glycine	2.30	2.42	1.65	2.45
Alanine	1.51	1.64	2.01	2.75
Valine	2.54	2.73	2.02	3.53
Cysteine	0.06	0.10	0.19	1.10
Methionine	1.25	1.29	2.20	2.43
Isoleucine	2.00	2.50	2.43	2.44
Leucine	1.65	2.75	1.05	2.02
Tryptophan	2.11	1.32	2.21	2.45
Phenylalanine	0.25	1.44	1.77	1.97

Results are expressed as means of two determinations (n=2)

Mineral composition of fermented watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Major minerals found in the watermelon condiment are magnesium (41.02-45.06 mg/100 g), potassium (22.03-33.02 mg/100 g), phosphorus (14.52-17.00 mg/100 g), and calcium (13.45-16.03 mg/100 g) (Table 4). This is similar to the finding of Tabiri *et al.* (2016) who reported that watermelon seeds had appreciable contents of the aforementioned minerals. Oboh (2006) also reported that condiments produced from underutilised legumes in Nigeria such as soybeans (*Glycine max*), locust beans (*Parkia filicoidea* L.), pigeon pea (*Cajanus cajan*) and watermelon seed (*Citrullus lunatus*) were generally high in potassium, calcium, magnesium and sodium. The mineral contents analysed increased with increased days of fermentation. This might be linked with reduction of some antinutrients such as phytate (Table 5) which are known to bind with some minerals thereby making them biounavailable. Minerals are important for vital physiological functions in the body. Calcium is essential for bone formation and strength (Samir-El *et al.*, 2012) while potassium is required for proper sodium-potassium balance in the body.

Table 4: Mineral composition (mg/100 g) of fermented watermelon seed using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time (hr)	Magnesium m	Zinc	Phosphorus	Potassium	Calcium	Sodium	Manganese
0	41.02 ^d ±0.02	5.05 ^d ±0.05	14.52 ^c ±0.02	22.03 ^c ±0.01	13.45 ^c ±0.05	5.40 ^c ±0.02	1.10 ^d ±0.01
24	42.05 ^c ±0.05	5.52 ^c ±0.02	15.64 ^b ±0.02	25.04 ^b ±0.02	15.00 ^b ±0.00	6.01 ^b ±0.01	1.57 ^c ±0.03
48	44.00 ^c ±0.09	6.20 ^b ±0.01	16.75 ^b ±0.05	25.01 ^b ±0.01	16.01 ^a ±0.01	7.00 ^a ±0.00	2.01 ^c ±0.01
72	45.06 ^a ±0.02	7.15 ^a ±0.00	17.00 ^a ±0.02	33.02 ^a ±0.02	16.03 ^a ±0.01	7.14 ^a ±0.12	2.53 ^a ±0.03

Data presented as means ± S.D

Mean values with different superscripts within the same column are significantly ($p < 0.05$) different

pH of fermented watermelon seeds using a mixed starter culture of *Bacillus subtilis* and *Bacillus licheniformis*

Significant increase was noticed in the pH (5.8 – 8.6) of the watermelon seeds fermented with a mixed starter culture of *B. subtilis* and *B. licheniformis* with increase in duration of fermentation from 0 to 72 hrs (Fig. 1). The initially acidic pH became basic after 72 hrs. This may be as a result of ammonia, a by-product of protein, produced during fermentation (Balogun and Oyeyiola, 2012). Ammonia production is accompanied by a characteristic off-flavour. Fermented condiments from vegetable seeds have a fairly alkaline pH (Ogunshe *et al.*, 2007).

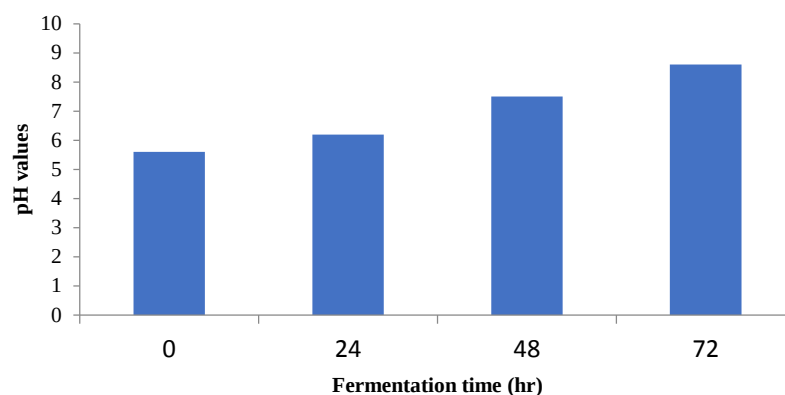


Figure 1: Changes in pH during fermentation of watermelon seeds using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Phytochemicals present in watermelon seeds fermented using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Fermentation brought about a significant reduction in the tannin, hydrocyanic acid and phytate contents of the watermelon seeds (Table 5). This is desirable as these food components, when present in high quantity, can interfere with the bioavailability of essential nutrients. For example, phytate forms insoluble complexes with zinc, iron, copper, and calcium (Lee and Kader, 2000) thereby limiting the absorption of these minerals. Tannin binds with protein forming a protein-limiting complex. Cyanohydric acids, also known as prussic acid, is extremely toxic due to its ability to link with metals such as Fe^{2+} , Mn^{2+} , and Cu^{2+} , which are functional groups of many enzymes (Alfred, 2010). This consequently

inhibits physiological processes like reduction of oxygen in the cytochrome respiratory chain as well as the activity of some important metabolic enzymes in the body (Doymaz, 2007). There was however an increase in the flavonoid (1.58-2.30 mg/100 g) and phenol (0.11-1.41mg/100g) contents. Egbebi *et al.* (2016) reported that these phytochemicals are in low amounts in fermented locust bean (*Parkia biglobosa*) and fermented melon (*Citrullus vulgaris*).

Table 5: Phytochemical composition (mg/100 g) of watermelon seeds fermented using mixed cultures of *Bacillus subtilis* and *Bacillus licheniformis*

Time	Hydrocyanic acid	Tannin	Flavonoids	Phytate	Phenol
0 hr	1.57	22.05	1.58	7.05	0.11
24 hr	1.02	20.94	1.80	5.78	0.58
48 hr	0.82	17.01	2.08	4.45	1.11
72 h	0.03	16.00	2.30	3.52	1.41

Sensory evaluation of fermented watermelon seeds

Though the soup prepared with *ogiri* was more generally accepted, the one prepared with the fermented watermelon seeds had higher mean score in taste and also compared closely in other sensory attributes (Table 6). In terms of taste, the mean score (6.20) obtained for fermented watermelon seed is similar to that reported for *ogiri-iru* blend (Ibeabuchi *et al.*, 2013). These results showed that, with regards to taste, the condiment from fermented watermelon seeds is a promising substitute for *ogiri*.

Table 6: Sensory scores of watermelon seed condiment

Samples	Colour	Taste	Aroma	Texture	General acceptability
A	4.05 ^b ±0.03	6.20 ^a ±0.01	5.00 ^b ±0.50	5.03 ^b ±0.51	5.32 ^b ±0.20
A1	5.05 ^a ±0.03	5.03 ^b ±0.00	6.55 ^a ±0.30	6.04 ^a ±0.41	7.30 ^a ±0.20

Data presented as means ± S.D

Means with different subscript letters in the same column are significantly different ($p < 0.05$)

Keys:

A: Watermelon seed condiment

A1: *Ogiri*

Conclusion

This study has revealed the suitability of starter cultures of *Bacillus subtilis* and *Bacillus licheniformis* as inocula for fermentation of watermelon seeds in the production of condiment. The combined use of *Bacillus subtilis* and *Bacillus licheniformis*, as well as increased days of fermentation resulted in an increase in protein and ash contents, and thus nutritional value. The condiment was rated quite close to *ogiri* by the panelists, and was, in fact, more preferred in taste. These findings indicate that through fermentation, the proportionate contents of protein and other nutrients can be increased at the expense of carbohydrate.

References

- Acar, R., Özcan, M.M., Kanbur, G. and Dursun, N. (2012). Some physicochemical properties of edible and forage watermelon seeds. *Iran. Journal of Chemistry and Chemical Engineering*, 31(4): 41-47.
- Achi, O.K. (2005). Traditional fermented protein condiments in Nigeria. *African Journal Biotechnology*, 4: 1612-1621.
- Adebayo, F.O. (2018). Microbiological Profile of ‘Ogiri’ Condiment Made from Seeds of Watermelon (*Citrullus lanatus*). *Asian Food Science Journal*, 1(1): 1-9.
- Adebayo, F.O., Mukhtar O.A. and Abdussalaam, R.B., (2018). Microbiological, Proximate and Organoleptic Analyses of a Fermented Condiment Made from Seeds of *Citrullus lanatus* (Watermelon). *South Asian Research Journal of Natural Products*, 1 (1): 1-7
- Agboola, Y.A. and Oguntuase O.S. (2006). Effect of *Bacillus sphaericus* on proximate composition soybean iru (*Glycine max*) for the production of soy-iru. *Pakistan Journal of Nutrition* 5(6): 606-607

Aguilera, Y., Benítez, V., Mollá, E., Esteban, R.M. and Martín-Cabrejas, M.A. (2011). Influence of dehydration process in *castellano* chickpea: changes in bioactive carbohydrates and functional properties. *Plant Food, Human Nutrition* 22(5): 43-49

Alfa nla, M.B.A. (2014). Economic analysis of watermelon production in selected local government areas of Kano State. Masters Thesis, Department of Agricultural Economics and Rural Sociology, Uploaded at Open Access Institutional Repository, Ahmadu Bello University, Nigeria, pp 12 and 22 <http://hdl.handle.net/123456789/6660>

Alfred O.U. (2010). Cyanogenic Glycosides and the fate of cyanide in soil. *Australian Journal of Crop Science*, 4(4):223-237

AOAC (1990). Official Method of analysis. Association of official Analytical Chemists, Washington DC, pp. 205-228A

AOAC (2005). Official Methods of Analysis (I8th Edition): Association of Official Analytical Chemist, Washington D.C.

Balogun, M.A. and Oyeyiola, G.P. (2011). Microbiological and Chemical Changes During the Production of *Okpehe* from *Prosopis africana* Seeds. *Journal of Asian Scientific Research*,1 (8): 390 – 398.

Balogun, M.A. and Oyeyiola, G.P. (2012). Changes in the Nutrient Composition of *Okpehe* during fermentation. *Pakistan Journal of Nutrition*, 11(3): 270-275

Balogun, M. A., Oyeyiola, G. P. and Kolawole, F. L. (2014). Physicochemical and sensory characteristics of fermented seeds of *Prosopis africana*. *Ethopian Journal of Environmental Studies and Management*, 7 (4): 411-422

Balogun, M.A., Oyeyiola, G.P. and Kolawole, F.L. (2017). Comparative study of physicochemical analysis of *Prosopis africana* seeds fermented with different starter cultures. *Croatian Journal of Food Science and Technology*, 9(1): 25-30

Bello, M.O., Falade, O.S., Adewusi, S.R.A., and Olawore, N.O. (2008). Studies on the chemical compositions and anti-nutrients of some lesser known Nigeria fruits. *African Journal of Biotechnology*,7 (21): 3972-3979

- Doymaz, I., (2007). The Kinetics of forced convective air drying of pumpkin slices, *Journal of Food Engineering* 76 (1): 562-567
- Drochioiu, G., Mangalagiu, I. and Tataru, V. (2000). Specific spectrophotometric determination of hydrocyanic acid in the environment. *Analyst*, 125: 939-941
- Egbebi, A.O., Seidu, K.T. and Muhammad, A.A. (2016). Nutritional and Microbiological Analyses of Fermented Locust Beans (*Parkia biglobosa*) and Fermented Melon (*Citrullus vulgaris*). *Savant Journal of Agricultural Research*, 2 (1): 1-6
- El-Adway, T. A. and Jaha, K. M. (2001). Characteristics and composition of water melon, pumpkin and paprika seed oils and flours. *Journal Food Chemistry*, 49(3): 1253-1259
- Enujiugha V.N. (2009). Major fermentative organisms in Some Nigerian Soup Condiments. *Pakistan Journal of Nutrition*, 8: 279-283.
- Foster, K. (2015). 5 Reasons to Reconsider Trashing Your Watermelon Rind, Tips from the Kitchn. Kitchn, <https://www.thekitchn.com/5-reasons-to-reconsider-tossing-your-watermelon-rind-tips-from-the-kitchn-219949>
- Frank, D.M., Felicity, J.S, Rodney, G.K. and Reed J.H. (2000). Amino Acid Analysis, Using Postcolumn Ninhydrin Detection, in a Biotechnology Laboratory. *Methods in Molecular Biology*, 159: 9-30
- Gberikon, G. M., Ameh, J. B., Ado, S. A. and Umoh, V. J. (2010). Comparative studies of the nutritional qualities of three fermented African legumes seeds using *Bacillus subtilis* and *Bacillus pumilus* as starters. *Croatian Journal of Food Science and Technology* 4: 60-64.
- Ibeabuchi, J.C., Ojukwu, M. and Olawunmi, I. (2013). Comparative study of proximate and sensory qualities of *iru* powder, ogiri powder, and *iru*-ogiri powder blend. *Indian Journal* 9(7): 288-293
- Jaffe, C.S. (2003). *Analytical Chemistry of Food*. Blackie Academic and Professional, New York, pp.1: 200.
- Lee, S.K. and Kader, A.A. (2000). Pre-harvest and Post-harvest factors influencing vitamin C content of horticultural crops. *Post-harvest Biology and Technology*, 256-271.

Murkovic, M., Mulleder, U. and Neunteufl, H. (2002). Carotenoid content on different varieties of pumpkins: *Journal of Food Composition and Analysis*, 15(6): 633-38.

Obizoba, A.S., Vellon, L., Colomer, R. and Lupu, R. (2005). Oleic acid: the main monounsaturated fatty acid of olive oil suppresses. *Annals of Oncology*, 16(3): 359-371

Oboh, G. and Akindahunsi, A.A. (2003). Biochemical changes in cassava products (flour and gari) subjected to *Saccharomyces cerevisiae* solid media fermentation. *Food Chemistry*. 82, 4: 599-602.

Oboh, G. (2006). Nutrient and antinutrient composition of condiments produced from some fermented underutilized legumes, *Journal of Food Biochemistry*, 30 (5): 579-588

Ogunshe, A.A.O., Omotoso, M.O., Ayansina, A.D.V. (2007). Microbial studies and biochemical characteristics of controlled fermented *afiyo*- a Nigerian fermented food condiment from *Prosopis africana* (Guill and Perr.). *Pakistan Journal of Nutrition*, 6 (6): 620-627.

Olagunju, O.F., Ezekiel, O.O., Ogunshe, A.O., Oyeyinka, S.A. Ijabadeniy, O.A. (2018). Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment, *LWT*, 90: 455-459

Penuel, B.L., Khan, E.M. and Maitera, M.O. (2013). Properties of proximate composition and elemental analysis of *Citrullus vulgaris* seed. *Life Science*, 2(2): 39-46.

Samir-El, S.F., Rabab, H.S. and Abd El-Ghany, M.E., (2012). Chemical and nutritional evaluation of different seed flours as novel sources of protein. *World Journal of Dairy and Food Science* 7(1): 59-65

Tabiri, B., Agbenorhevi, J.K., Wireko-Manu, F.D. and Ompouma, E.I. (2016). Watermelon seeds as Food: nutrient composition, phytochemicals and anti-oxidant activity. *International Journal of Nutrition and Food Science*, 5 (2): 139-144.