Effects of Fermentation on the Nutritional and Anti-Nutritional Components of Cooked/Boiled Water Melon (*Citrullus lanatus*) Seed

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Abstract: Watermelon is a commonly consumed fruit. However, only the fleshy pulp is usually consumed leaving the seed and rind to be discarded. The effects of processing (heat treatment and fermentation) on the proximate composition and anti-nutrient components of watermelon (*Citrullus lanatus* var. *lanatus*) seeds were investigated. Fermentation was carried out by first boiling seeds for 3h, wrapped in clean plantain (*Musa sapientum* var. *paradisiaca* Linn.) leaves and allowed to ferment for 96h. Proximate and phytochemical screenings were carried out on the raw and processed seeds. Microbial analyses were also carried out daily on the fermenting seeds. Results of proximate analysis revealed a significant difference ($p<0.05$) in contents of protein, crude fat, ash, moisture content and carbohydrate of the raw, cooked and fermented seeds while there was no significant difference in the crude fibre content of the raw and cooked seeds. Protein, crude fat and carbohydrate ranged from 8.55-13.14%, 4.64-9.76% and 49.78-60.29% respectively with the highest values (except for carbohydrate) in fermented seeds while the raw seeds had the least. The anti-nutrient investigation showed that oxalate was present in raw seeds but absent in the cooked and fermented seeds. Anti-nutrients present in the raw seeds showed variable reductions after processing. Nine bacterial genera identified as *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Lactobacillus*, *Micrococcus*, *Proteus*, *Pediococcus*, *Klebsiella* and *Serratia* with one fungal genera identified as *Aspergillus* were recovered from the fermenting seeds. The pH, titratable acidity and temperature of the seeds at the end of fermentation were 8.02, 0.20 and 28℃ respectively. The study showed that cooking and fermentation of watermelon seeds have direct relationships on its proximate and anti-nutrient compositions. The cooked and fermented watermelon seeds could be used in food and feeds formulation.

Keywords: valorisation, fermentation, feeds, fruit wastes, watermelon

INTRODUCTION

Watermelon, *Citrullus lanatus* var. *lanatus*, is a fruit commonly consumed in the tropics. It belongs to the family Cucurbitaceae and grows in almost all part of Africa and South East Asia (Koocheki *et al*., 2007). It has been shown to be good source of lycopene and other carotenoids (Tamburini *et al*., 2017) which have been shown to protect against certain forms of cancer (Jian *et al*., 2007; Sahin *et al*., 2017; 2018).

Many of the fruits eaten in the tropics are sometimes eaten without their seeds. Watermelon belongs to this group of fruits; with smooth, compressed seeds that are thickened at the margin and of black or yellow-white colour (Laghetti and Hammer, 2007). In countries such as Nigeria and some other parts of West Africa, when watermelon fruits are consumed, the seeds are seldom eaten with the pulp and are usually discarded. This is because they are considered unfit for human consumption. The seeds are mainly discarded into the environment and could become significant solid environmental waste in areas where the fruits are processed into juice or fruit salads. In some countries, however, the seeds are used as food, either in form of roasted snacks or as flour incorporated into flour used in the production of bread (El-Adway and Taha, 2011).

Fermentation is one of the oldest and most important traditional food processing and preservation techniques (Aworh, 2008). Food fermentation involves the use of microorganisms and enzymes for the production of foods with distinct quality attributes that are quite different from the original substrates. Cooking and fermentation have been shown to confer on foods qualities such as improved aroma, taste and flavour, and also reduction in toxic components such as hydrogen cyanide (Agbor-Egbe and LapeMbome, 2006). Studies on the proximate composition of watermelon rinds and seeds (Oseni and

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Okoye, 2013; Egbuonu, 2015; Omoboyowa However, there is a paucity of information on the effect of fermentation on the nutrient and anti-nutritional components of watermelon seeds. This warranted the present study which was set up to evaluate the potential food and feed uses of watermelon seeds and investigate the effects of fermentation on the raw and boiled seeds.

MATERIALS AND METHODS

Sample Collection and Preparation

The watermelon used was bought from three different locations in Akungba-Akoko, Nigeria and authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko. The seeds were removed, sorted and washed to remove grit and dirt. They were then left to dry to a constant weight under room temperature. A total of 100g of the seeds were boiled for 3h in water. The water was drained and allowed to cool after which about 50g of the cooked watermelon seeds were wrapped in clean plantain (*Musa sapientum* var. *parasidiaca* Linn.) leaves. The seeds were then left in plastic containers at room temperature (28 to 30℃) for 96h to undergo spontaneous fermentation by autochthonous microorganisms in the seeds and plantain leaves. The fermented product was then allowed to dry to a constant weight under the room temperature and then ground into a smooth powder to check the nutrient and the anti-nutrient. In like manner, 50g of the remaining cooked seeds were allowed to dry to constant weight under room temperature and then ground into a smooth powder to check the nutrient and anti-nutrient. Furthermore, 50g of raw unfermented seeds were ground into a smooth powder to check the nutrient and anti-nutrient.

Sample Analysis

Proximate Analysis:

The proximate composition (crude moisture, crude fat, crude protein, crude ash, crude fibre, carbohydrates) of the raw, cooked and fermented seeds were analysed using the standard AOAC (1990) methodology. *et al*., 2015) are available. Carbohydrate content was determined by subtracting the crude moisture, crude fat, crude protein, crude ash and crude fibre from 100%. Calculation for percentage carbohydrate: % carbohydrate = (% moisture + % fat + % protein + % ash + % fibre) – 100%. The values recorded for the proximate composition were the mean of triplicate determinations.

Quantitative Phytochemical Screening

Samples were analysed for saponins, flavonoids, phytate, oxalate and tannins, according to the methods described by Trease and Evans (2002) and Tiwari *et al.* (2011).

Enumeration of Aerobic Bacteria and Fungi

A total of one gram of the dried uncooked seeds was mashed into paste with a sterile mortar and pestle for the determination of the microbial flora and succession by dissolving in 9ml of sterile distilled water. Six-fold serial dilution of the sample was carried out by pipetting 1ml of the stock sample into 9ml of sterile distilled water until the six-fold dilution was achieved. Isolations were made in duplicate on Petri dishes of Nutrient Agar and Potato Dextrose Agar containing 0.05mg chloramphenicol/ml using pour plating technique. The Petri dishes containing Nutrient Agar were incubated inverted at 37^{o}C for 24 h while the Petri dishes containing Potato Dextrose Agar were incubated at room temperature (25^{o}C) for 5 days. Cooked fermented and uncooked unfermented (control) seeds were used for the analysis.

Total bacterial count was estimated for the uncooked sample and for each day of the fermentation. This was done by counting the total number of colony present in the plates when cultured for 24h at 37^{o}C.

Isolation and Identification of Bacteria

Bacterial colonies observed on the plates were sub-cultured on Nutrient Agar until pure cultures were obtained. The bacterial isolates were identified based on their morphological characteristics and results

from various biochemical tests carried out on them.

Each of the test isolates were cultured on media and observed after 24h of incubation for their morphological appearances. All bacterial isolates were identified based on their morphological and biochemical characteristic according to Bergey's Manual of Determinative and Systematic Bacteriology (Holt *et al*., 1999).

Isolation and Identification of Fungi

Fungal colonies observed on the plates were sub-cultured on Potato Dextrose Agar until pure cultures were obtained. About 0.5ml of lactophenol-in-cotton blue solution was dropped on a grease-free glass slide and the mycelium of each fungal isolate was picked with two sterile inoculating loop and placed gently on the flooded glass slide. Cover slip was gently placed on the soaked fungal patch on the slide and viewed under the microscope with ×10 and ×40 objective lens for septate or aseptate hyphae, conidia and spore formation. Fungal isolates on plate were also observed for morphological features such as colony colour, background colour, conidia texture and shape.

Determination of pH and Temperature

The pH and temperature of the fermenting seeds were determined daily. To estimate the pH, 1.0g of the fermenting seeds was mashed with 10ml of distilled water and the pH of the homogenate was then determined with a pH meter. The temperature was determined by dipping thermometer in the fermenting mesh and the reading was taken by the movement of the mercury.

Determination of Total Titratable Acidity (TTA)

This was determined by titrating 0.2M NaOH against a known amount of the water that contains a known mashed amount of the fermenting seeds for each day. Phenolphthalein indicator was used. About 10ml of sodium hydroxide was pipetted into a conical flask, a drop of phenolphthalein indicator was added to give a deep pink colour. The steeped water was placed in 50ml capacity of burette and its tap was opened to the conical flask containing the base and the indicator until the end point of light pink was reached.

TTA= Volume of NaOH used (ml)×0.009×100/Volume of sample used.

STATISTICAL ANALYSIS

Data obtained were statistically analysed using GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California USA. Multiple comparisons of mean ± SEM were carried out by correlation and two- way ANOVA. A probability level of less than 5% was considered significant.

RESULTS

Microbial Counts and Succession

A wide range of bacterial genera was recovered from the fermenting seeds, with a total of nine genera identified as *Bacillus* sp, *Enterobacter* sp, *Staphylococcus* sp, *Pediococcus* sp, *Lactobacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Klebsiella* sp and *Proteus* sp. *Aspergillus*sp was the only fungus isolated. Table 1: the identification of fungal isolates based on their microscopy, cultural and morphological characteristics.

The succession of the bacterial isolates during the fermentation of the cooked seeds is presented in Fig. 1. It shows *Staphylococcus* sp being the only organism isolated at 0h; *Staphylococcus* sp. persisted till after 48h and disappeared during 72h. *Bacillus* sp appeared after 0h and persisted till 96h. Four isolates, *Lactobacillus* sp., *Micrococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. appeared after 24h. *Pseudomonas* sp. *Micrococcus* sp. and *Lactobacillus* sp. persisted till the end of the fermentation, while *Proteus* disappeared after 72h. One isolate, *Pediococcus* sp. was observed at 48h, but disappeared after 72h. *Enterobacter* sp. and *Klebsiella* sp. were encountered as from the 72h until the end of the fermentation, with the population of *Enterobacter* at 96h stable at 1.0 X 10^5cfu/ml.

Table 1: Identification of Fungal Isolates

Code name	Background Colour	Colony colour	Texture and Shape of Conidia	Probable Fungi
FUa	Black to dark brown	Grey	Finely wrinkled and ellipsoidal	*Aspergillus niger*
FUa1	Dark brown/gray tones	Pale to Yellow	Spiny and globular	*Aspergillus* sp.
FUb	Cream	Grey white	Spiny/finely wrinkled and globular	*Aspergillus* sp.
FUc	Black/Brown	Cream	Spiny/finely wrinkled and globular	*Aspergillus* sp.
F0	Black/Brown	Cream	Spiny/finely wrinkled and globular	*Aspergillus* sp.
F24a	Black to dark brown	Grey	Finely wrinkled and ellipsoidal	*Aspergillus* sp.
F24b	Dark brown/grey tones	Pale to Yellow	Spiny and globular	*Aspergillus* sp.
F48a	Black/Brown	Cream	Spiny/finely wrinkled and globular	*Aspergillus* sp.
F48b	Dark brown/black	Tones of grey to brown centre	Delicately spiny/smooth and globular	*Aspergillus* sp.
F48c	Dark brown/grey tones	Pale to Yellow	Spiny and globular	*Aspergillus* sp.
F48d	Black	Colourless	Wrinkled and globular	*Aspergillus* sp.
F96a	Black	Colourless	Wrinkled and globular	*Aspergillus* sp.
F96b	Dark brown/black	Tones of grey to brown centre	Delicately spiny/smooth and globular	*Aspergillus* sp.

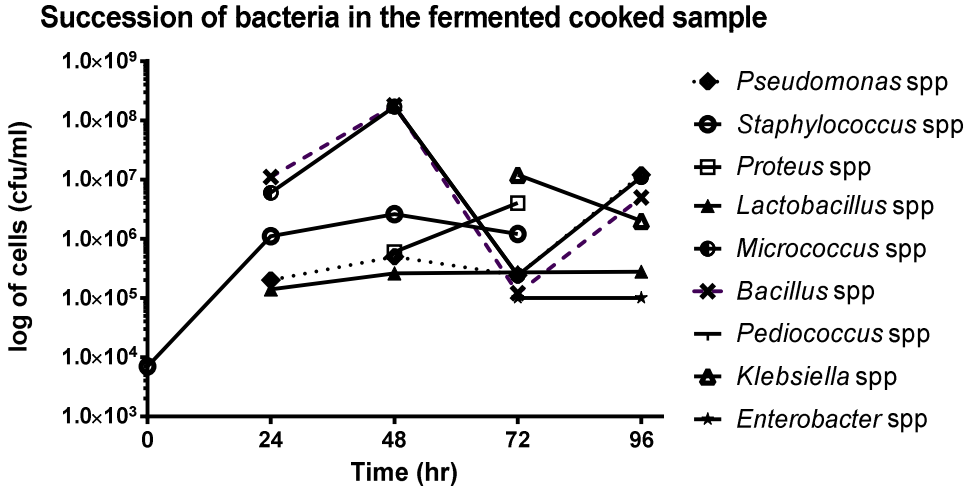

Fig.1: Succession of Bacteria in Fermenting cooked Watermelon Seeds.

Figure 2 shows the effects of cooking and fermentation on the anti-nutrient composition of watermelon seeds. The anti-nutrient components tested were phytate, tannin, flavonoids, saponin and oxalate. All the anti-nutrient composition tested for were present in the raw seeds in varying quantities. Quantities of phytate, tannin, flavonoids, saponin and oxalate were 1.43, 0.46, 2.11, 2.06 and 0.06mg per 100g of the raw seeds. Cooking and fermentation were found to reduce the contents of all the anti-nutrients. At the end of the fermentation of the cooked seeds, oxalate was completely eliminated.

Table 2 shows the proximate composition of raw, fermented and cooked water melon seeds. Results showed that moisture contents in the raw water melon seeds were lower than their corresponding cooked and fermented products. It was higher in the cooked water melon seeds. The crude fat was higher in the fermented samples than in the raw and cooked samples. It was least in the raw sample. The values of crude fibre were higher in the cooked sample than in the raw and fermented sample. It was least in the fermented sample. The protein content was higher in the fermented sample than in the raw and cooked sample, while it was least in the raw sample. The values of carbohydrate were higher in raw sample than in the fermented and cooked sample. It was least in the fermented sample.

Figure 3 shows the pH of the fermenting water melon seed. The figure shows that there is a general increase in the pH during the fermentation. The pH ranged between 7.62 and 8.02. Figure 4 shows the temperature of the fermenting water melon seeds. The figure shows a slight fluctuation in the temperature during the fermentation, with the temperature of the fermenting seeds gradually increasing and then reaching a peak of 32^{o}C at 72h. The temperature decreased to 29.5^{o}C at 96h. Figure 4 shows the titratable acidity of the fermenting water melon. The figure shows a decrease in the titratable acidity till 96h of the fermentation

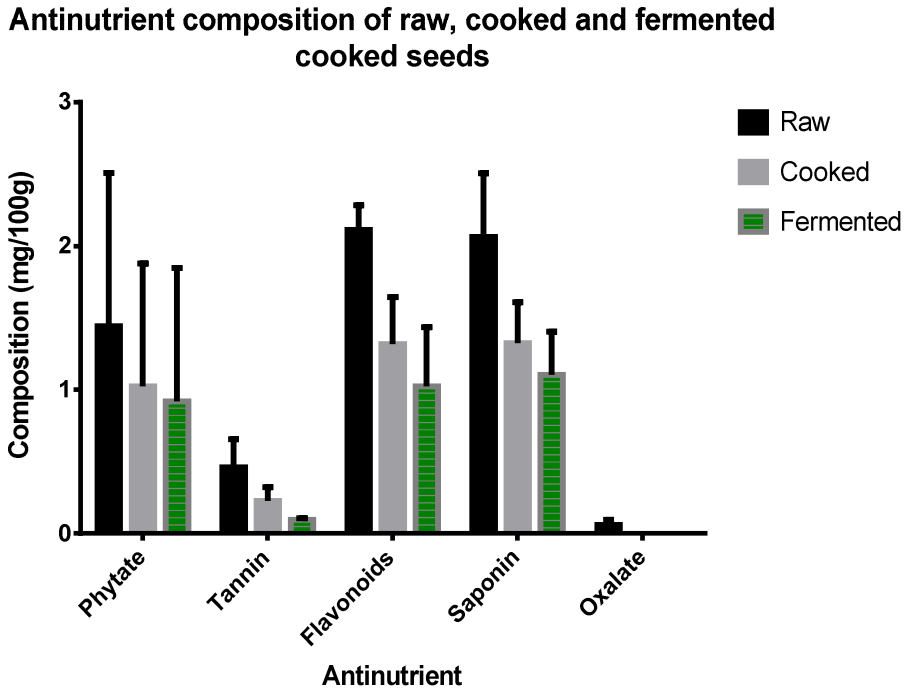

Figure 2. Anti-nutrient composition of the raw, cooked and fermented cooked seeds (Data represented in Mean ± SEM)

TABLE 2: Proximate Composition of Ground Raw, Fermented and Cooked Watermelon Seed

Parameters	Raw	Cooked	Fermented cooked
Moisture content	10.4100± 1.0[a]	12.0467± 2.092[c]	11.2533± 0.31[b]
Crude fat	4.6433± 0.866[a]	5.6733±1.278[c]	9.7600± 0.46[b]
Crude fibre	9.2300± 1.00[a]	9.6400±0.861[a]	8.9333± 1.53[b]
Protein	8.5500± 1.00[a]	9.7600±1.322[c]	13.140± 1.72[b]
Ash	6.8833± 0.866[a]	8.0800±0.975[c]	7.1433± 0.857[b]
Carbohydrate	60.2900± 0.500[a]	54.8000±1.228[c]	49.7800±1.462[b]

Results are means of 3 determinations ± S.E.M, values along the same row with the same superscript are not significantly different (P> 0.05), and are significantly different if the superscripts are different.

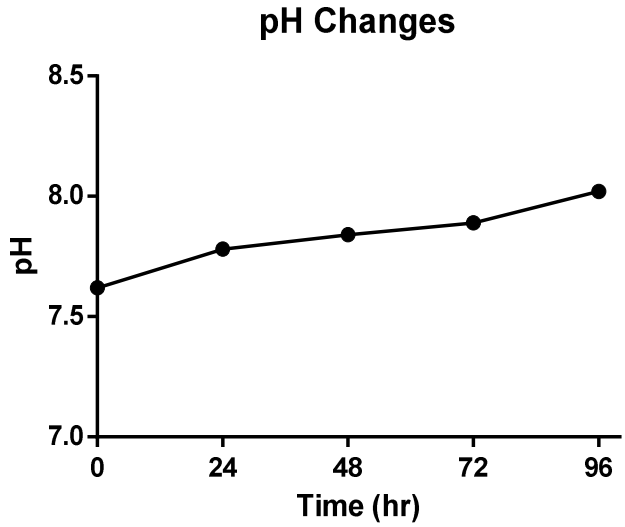

Figure 3: Mean Daily pH of the Fermenting Seeds

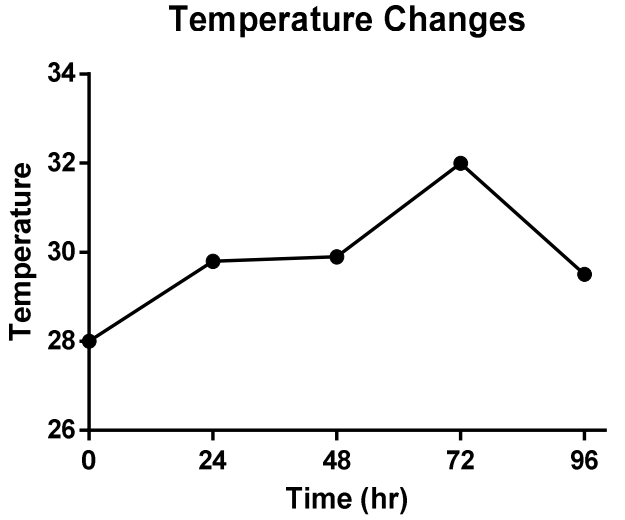

Figure 4: Mean Daily Temperature of the Fermenting Seeds

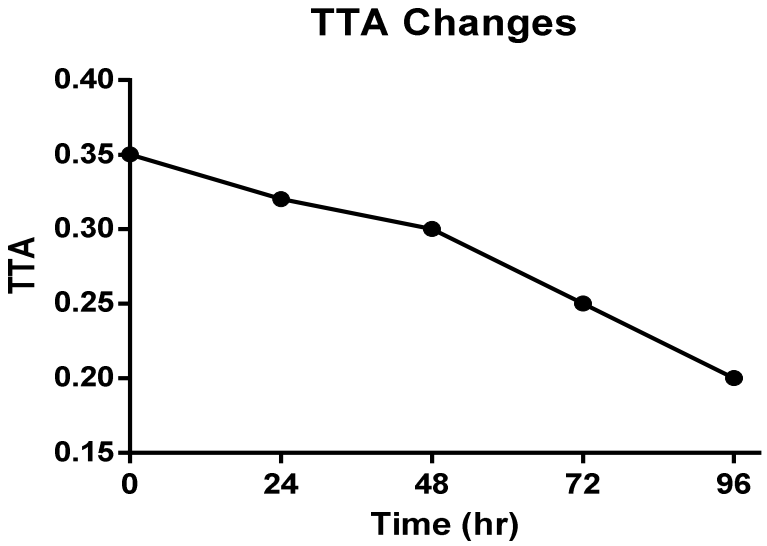

Figure 3: Mean Daily Total Titratable Acidity of the Fermenting Seeds

DISCUSSION

This study was aimed at identifying the effects of fermentation on the nutrient and antinutrient components of boiled watermelon seeds. Bacteria such as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., among others were isolated from the cooked fermented seeds, while Aspergillus sp. was the only fungus isolated. It was observed that, overall, fermentation reduced all the antinutrients tested (Fig. 2), while it was able to improve some nutritional qualities when compared to raw seeds (Table 2). Anti-nutrients are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as foods and feeds (Soetan and Oyewole, 2009). They could become toxic at high concentration and exert their toxicity by the formation of insoluble complexes with important cellular nutrients. This leads to a marked reduction in the bioavailability of these nutrients and impairs their adsorption (Francis *et al*., 2001; Udensi *et al*., 2007). However, some of these anti-nutrients are usually heat labile and volatile and can be reduced by food processing methods like cooking and frying (Akwaowo *et al*., 2000; Egbuonu *et al*., 2014).

The reduction in tannin contents of the seeds after cooking agrees with the studies of Alagbaoso *et al.* (2015) who found a significant reduction in the tannin content of *Canavaliaplagiosperma piper* seeds after cooking them for 50 minutes. The reduction in the content of tannin after cooking could be due to the fact that they are water soluble and heat labile, which makes them break down during cooking of the seeds (Kumar *et al*., 1979; Udensi *et al.*, 2007). The reduction of tannin and other anti-nutrients after fermentation observed in this current study agrees with the studies of Adegbehingbe *et al*. (2014) who found significant reductions in the anti-nutrients of ground-cooked Lima bean (*Phaseolus lunatus*) seeds which the authors fermented using *Bacillus subtilis* and *B. pumilus.*

A significant reduction of saponin and flavonoids observed in this study is in tandem with the reports of Effiong and Umoren (2011) when they examined the effects of several processing methods on the chemical compositions of horse eye beans after cooking them. Although cooking and fermentation reduced the flavonoid contents of watermelon seeds in this study, at low concentrations flavonoids have been shown to be anti-inflammatory, anti-allergic, antioxidant and anti-microbial (Adeniyi *et al.,* 2012; Adeolu and Enesi, 2013).

Saponin has been reported to show medicinal as well as exhibiting physiological activities. A high saponin content had earlier been reported for *Garcinia kola* and *Aframomum melegueta* respectively which were observed to possess inhibiting roles on microorganisms, that explained their utilization in traditional medicine as a cough suppressant, an anti-tumor agent and an aphrodisiac (Hour *et al*., 1980). Therefore, the presence of a certain level of saponin in the cooked and fermented seed of water melon can be of usefulness and importance in preventing some diseases in man and farm animals. The increase in protein in the cooked water melon seed contradicts report by Idoko *et al.* (2014) in the effect of heat treatment on nutrient and anti-nutrient components of melon (*Citrullus colocynthis*) husks in which there was a significant reduction in the protein contents of boiled melon husk. This contradiction could be attributed to the differences in material analysed from the watermelon as the husks, and not the seeds, were boiled in their study.

The rise in temperature observed till 72h suggests that the fermentation of the cooked seeds is exothermic and the changes in temperature can be attributed to the metabolic activities of the microorganisms present in the fermenting mash. The heat generated likely provided ideal temperature conditions for the optimal activity of the proteolytic enzymes (Odunfa, 1985).

The appearance of *Staphylococcus* sp. after 24h of fermentation is in keeping with the observation of Odibo and Umeh (1989) or ogiri from *Telfaria* seeds, Odibo *et al*., 1992) orogiri-okpei from *Prosopis* seeds and Jideani and Okeke (1991) for ogiri from African Oil bean, soya bean and castor oil seeds. According to these reports *Staphylococcus* sp. were isolated after 24h and persisted till the end of fermentation except in the case of castor oil seeds in which the organism appeared after 24h and disappeared after 48h. However, the appearance of *Staphylococcus* sp after 24h of fermentation is in contrast with the reports of Popoola and Akueshi (1984) in the fermentation of *Cucumeropsis* seeds that *Staphylococcus* sp. were present only within 24h of fermentation of 'daddawa', a nutritionally related condiment produced from soya bean fermentation.

The increase in amount of soluble protein with fluctuations in population of *Bacillus* sp, suggests non-utilization by the organism while the decrease in the amount of carbohydrate suggests utilization by the organism. The steady increase in the microbial load during fermentation of the water melon seeds could have been influenced by the accumulation of compounds such as organic acids and other metabolites that support their growth. Yong and Wood (1976) also observed fluctuations in microbial load during the fermentation of soy sauce. The fluctuations in pH during fermentation of watermelon seed could have contributed to the fluctuations in the appearance and disappearance of *Lactobacillus* sp. which had been reported to be aciduric (Aderiye and Ojo,1987). The increase in the moisture contents of the fermented products agree closely with the report of Omafuvbe *et al*. (2004). This may be as a result of the decomposition of the fermenting bacteria on the products. The appearance of *Serratia* sp. at 72h agrees with the report of Odibo *et al*. (2012) in the fermentation of *Cucumeropsis*, an uncommon substrate for ogiri production. The plantain leaves used to wrap the fermenting seeds are presumably a major source of the bacteria. Previous report by Odibo and Umeh, (1989) revealed that seven out of the nine isolated bacteria were similar microbial flora that were isolated during the fermentation of *Telfaria* seeds including two unidentified fungi isolates which were recovered from the plantain leaves used in wrapping the *Telfaria* seeds. With the consortia of bacterial genera found in the fermentation mash, their contributions to the fermentation of the cooked seeds are unclear. It is suggested that some of the bacteria are accidental members of the flora and serve no important purpose.

However, the major microorganisms responsible for the fermentation will be further pursued in subsequent studies in order to develop efficient starter cultures for the fermentation of the seeds and to evaluate the relationship between the mixed populations of bacteria found in the fermentation mash.

CONCLUSION AND RECOMMENDATION

From the results obtained in this study, the fermented seeds proved to be good source of valuable nutrients such as proteins, carbohydrates, fats, and fibre. This could be exploited in managing malnutrition by incorporation in various foods and feed formulations. The qualitative screening of the differently processed seeds revealed important phytochemicals of pharmaceutical and medicinal importance. Since the various anti-nutrient parameters monitored in the seeds of watermelon were observed to be lower in the cooked and fermented sample in comparison to the raw seed, it could be inferred therefore that the cooked and fermented seed will be better suited for adsorption and bio-availability of important nutrients. Considering this, the use of the cooked and fermented seeds in foods and feeds is tentatively recommended as safe, pending the results of *in vitro* activities of the said seeds on appropriate laboratory animals.

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