

Effect of Fermentation on the Proximate Composition of the Epicarp of Watermelon (*Citrullus lanatus*)

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Abstract

This research was carried out to study the effect of fermentation on the proximate composition, pH, titratable acidity and microbiological changes of the epicarp of watermelon with a view of harnessing it for consumption and possible industrial usage. The seeds were manually and aseptically separated from the fruit's pods, cleaned, washed with distilled water, air dried and the epicarp was removed with a sterile knife and grated. The sample was subjected to natural fermentation for 96 h. The following bacteria isolates were obtained from the fermentation; *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii*, *Staphylococcus epidermidis*, *Streptococcus lactis*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, and *Micrococcus luteus* of which *Lactobacillus* species were the most dominant throughout the period of fermentation. The pH of the sample decreased from 5.0 to 4.5 and the total titratable acidity increased from 1.0 to 2.6. The decrease in pH and increase in TTA during the fermentation could have been caused by the presence of lactic acid bacteria which are known to be aciduric. The result of the proximate composition indicated that the epicarp of the unfermented and fermented watermelon contained considerable amount of protein (7.69 ± 0.54 and 11.14 ± 0.41), carbohydrate (67.59 ± 0.69 and 61.54 ± 0.69), fat (7.04 ± 0.74 and 6.82 ± 0.37), fiber (6.73 ± 0.61 and 9.71 ± 0.44), ash (6.69 ± 0.32 and 6.70 ± 0.45) and moisture (7.92 ± 0.32 and 7.54 ± 0.37), respectively. These proximate values show that the sample is a potential food supplement.

Keywords: Watermelon fruit; Epicarp of fermented and unfermented watermelon; Natural fermentation; Proximate composition

Introduction

Citrullus lanatus (watermelon) is a fruit of about 93% water, hence the name "water" melon. The "melon" part came from the fact that the fruit is large and round and has a sweet, pulpy flesh. The scientific name of the watermelon is derived from both Greek and Latin roots. The *Citrullus* part comes from a Greek word "citrus" which is a reference to the fruit. The *lanatus* part is Latin, and has the meaning of being wooly, referring to the small hairs on the stems and leaves of the plant [1]. Every aspect of the fruit of watermelon has nutritional value, including the epicarp and the seeds. The most common way watermelon is eaten, is the consumption of the pink or yellow flesh, eaten raw, the way it was grown. However, other common ways by which it is eaten include watermelon rind pickles, deep fried watermelon, watermelon cake, and watermelon lemonade [2].

Watermelon has nutritional value, including the outer skin and the seeds. It is rich in Potassium, hence it is a great electrolyte and thus helps regulate the action of nerves and muscles in the body. It is also rich in beta-carotene which is converted in the body to vitamin A and this helps produce the pigments in the retina of the eye and prevents night blindness. The nutritional components of watermelon cannot be overemphasized as they are beneficial to health improvement [3]. According to Steinkraus [4], food fermentation has been said to serve five main purposes:

- Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates.
- Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations.
- Biological enrichment of food substrates with protein, essential amino acids, and vitamins.
- Elimination of anti-nutrients.

- A decrease in cooking time and fuel requirement.

However, this research is aiming at determining the effects of fermentation on the proximate composition of the epicarp of watermelon.

Materials and Methods

Source of sample

Watermelon (*Citrullus lanatus*) was purchased in Oja Oba, Akure metropolis in Ondo state, Nigeria. The epicarp was peeled off and blended; about 1000 g of the blended part of the epicarp of watermelon was homogenized in a sterilized transparent bucket containing 200 ml of sterile water.

Fermentation of sample

The fermentation was carried out at room temperature of $28 \pm 2^\circ\text{C}$ in a sterile transparent bucket and it was sealed with foil paper and chemical analysis (pH, temperature, TTA, and Proximate analysis) were carried out on daily basis. During the fermentation process, endogenous microfloras of the fruit (watermelon) were allowed for the fermentation. The blended epicarp of the watermelon was fermented between 0 to 96 h.

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Determination of the pH

At every 24 h, the pH of the fermenting must was determined using a HANNA pH meter 209. This was simply measured by immersing the pH electrode in 20 ml of the must sample. The readings were taken from the pH meter for 5 days. This was done by the method of Ofori and Hahn [5]. Before the pH measurements were taken, the pH meter was calibrated and the sample slurry was thoroughly stirred aseptically to homogenize the mixture and achieve uniformity.

Total titratable acidity (TTA)

The TTA analysis was done using AOAC [6] method. 10 ml of the sample was pipetted into a beaker and 3 drops of Phenolphthalein indicator was added. Titration was done using 0.1M NaOH to a faint pink colour for at least one minute compared against a white background. The titre volume was noted and used to calculate TTA which was expressed as percentage Lactic Acid. The TTA was determined and expressed as follows:

$$\% \text{ Lactic Acid} = A \times 0.009 \times 100/v$$

Where A=ml of 0.1 NaOH required for the titration,

Where V=ml of sample taken for the test.

The acidity was calculated as lactic acid using relationship:

$$\frac{\text{Volume of base used} \times \text{Normality of NaOH} \times 9}{\text{Volume of sample used (average titre)}}$$

Microbiological analysis

Daily changes in the microbial population (cfu/ml) of the total viable bacteria, lactic acid bacteria (LAB) and fungi were determined using Nutrient agar (NA), De Man Rogosa Sharpe agar (MRSa) and Potato Dextrose agar (PDA) respectively. Samples were enumerated by using appropriate serial dilution and spread plate method and pour plate method. At every 24 h, samples were aseptically withdrawn from the fermentor, serially diluted and 1ml each from dilution factor 10^{-3} , 10^{-4} and 10^{-5} was dispensed in triplicates on nutrient agar, de-man rogosa sharpe agar and potato dextrose agar. The fungal plates were incubated at 28°C for 2 to 5 days while the bacterial cultures were

incubated at temperatures ranging between 30 to 35°C for 1 to 2 days. The result of the respective incubated plates was checked, the colonies that developed on the plates were counted, recorded and expressed as Colony Forming Unit per mill (cfu/ml) and spore forming unit per mill respectively (sfu/ml). The isolated microbes were later sub cultured into their respective freshly prepared media in order to obtain pure strains, the sub culturing was done repeatedly to obtain pure isolate before they were later stored on slant bottles. Pure isolates were streaked on Nutrient agar while the fungi colonies were subcultured on Potato Dextrose agar. The isolates were stored in the refrigerator at 4°C for biochemical analysis. The isolates were characterized and identified with the criteria of Holt et al. [7]. Identification of the yeast isolates was confirmed using standard identification method based on the criteria of Kreger [8].

Proximate composition

Proximate composition, such as, ash, fiber, fat, protein, moisture were determined by Nout et al. [9] method. Carbohydrate content was determined by subtracting from 100 the sum of the percentage moisture, ash, protein, fat and fibre. The remainder value gives the carbohydrate content of the sample.

$$\% \text{Carbohydrate} = 100 - (\% \text{Moisture} + \% \text{Ash} + \% \text{Fat} + \% \text{Protein} + \% \text{Fibre})$$

Statistical analysis

Data obtained were subjected to simple statistical tools of analysis by using Mean, Standard Deviation and percentage in order to ascertain the significance of the variables obtained, as described by Stroud and Booth [10].

Results

Microscopy and biochemical characteristics of bacteria isolated from natural fermentation of the epicarp of watermelon

The different bacterial isolates obtained from the unfermented and fermented sample are shown in Table 1.

Sugar Fermentation																									
S/N	GRAM STAINING	SHAPE	SPORE	INDOLE	CITRATE	STARCH	HYDROLYSIS	COAGULASE	TEST	CATALASE	MOTILITY TEST	ARRANGEMENT	OXIDASE	VOGES	PROSKAEUR	METHYL RED	UREASE	LACTOSE	SUCROSE	FRUCTOSE	GLUCOSE	MANNITOL	MALTOSE	DEXTROSE	PROBABLE MICROORGANISMS
1	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus plantarum</i>
2	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus acidophilus</i>
3	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus fermentum</i>
4	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus casei</i>
5	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus bulgaricus</i>
6	+	Rod	-	-	-	+	-	-	-	-	-	Singly	-	-	-	+	AG	AG	AG	AG	A	A	A	A	<i>Lactobacillus delbrueckii</i>
7	+	Cocci	-	-	-	+	-	-	-	-	-	Cluster	-	-	-	+	AG	AG	A	AG	A	A	A	A	<i>Staphylococcus epidermidis</i>
8	+	Cocci	-	-	-	+	+	-	+	-	+	Chain	-	-	+	-	AG	AG	AG	AG	A	A	A	A	<i>Streptococcus lactis</i>
9	+	Cocci	-	-	+	-	+	+	-	-	-	Cluster	-	-	-	-	AG	A	AG	AG	A	A	A	A	<i>Staphylococcus aureus</i>
10	+	Rod	+	+	-	-	+	+	+	+	+	Singly	-	-	+	+	-	A	A	AG	AG	-	-	A	<i>Bacillus cereus</i>
11	+	Cocci	+	-	-	-	-	+	-	-	-	Singly	-	-	+	+	A	A	-	-	A	A	-	-	<i>Micrococcus luteus</i>
12	+	Rod	+	+	+	+	-	+	-	-	-	Singly	-	+	-	+	-	AG	A	AG	AG	-	-	-	<i>Bacillus subtilis</i>

KEYS: A=Acid production; + = Positive reaction; AG=Acid and Gas production; - = Negative reaction

Table 1: Microscopy and biochemical test for the bacterial isolates in the epicarp of unfermented watermelon and fermented watermelon.

Changes in total titratable acidity

The changes in the total titratable acidity of the samples are shown in Figure 1. At 96 h of fermentation, the values of titratable acidity in g/100 lactic acid for the fermented sample increased compared to the unfermented sample.

Changes in pH

The changes in the pH of the samples are shown in Figure 2. At 96 h of fermentation, the values of pH for the fermented sample were lower compared to the unfermented sample.

Changes in temperature

The changes in the temperature of the samples are shown in Figure 3. At 96 h of fermentation the values of temperature in °C for the fermented sample was greater compared to the unfermented sample.

Changes in moisture content

The moisture content of the unfermented epicarp of watermelon was 7.50% but there was a reduction in the moisture content of the fermented epicarp of watermelon to 6.91% (Figure 4).

Changes in carbohydrate content

Carbohydrate content of the unfermented epicarp of watermelon as shown in Figure 4 recorded greater values than that of the fermented epicarp of watermelon.

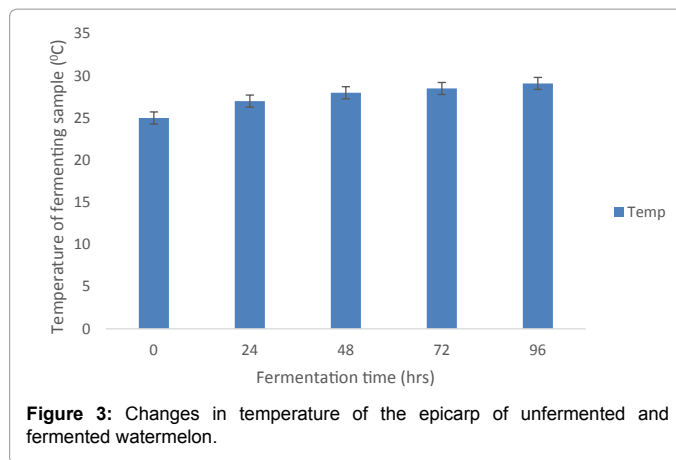


Figure 3: Changes in temperature of the epicarp of unfermented and fermented watermelon.

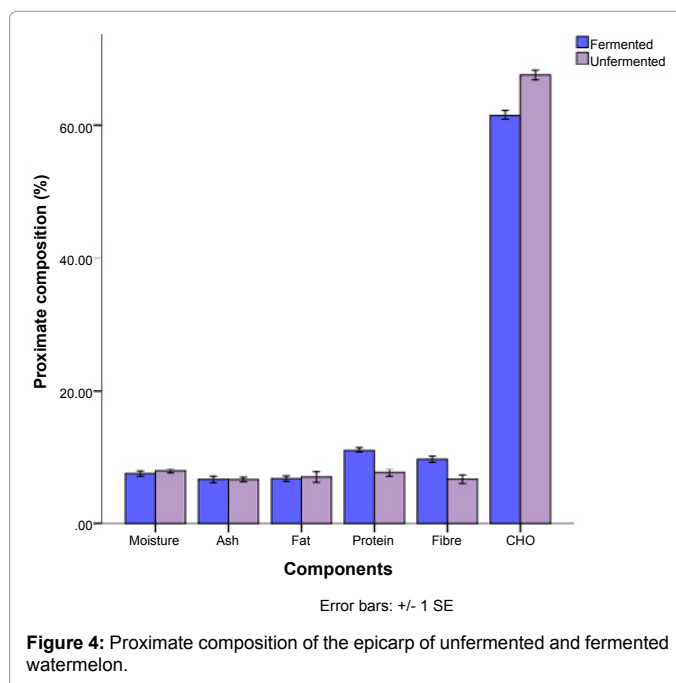


Figure 4: Proximate composition of the epicarp of unfermented and fermented watermelon.

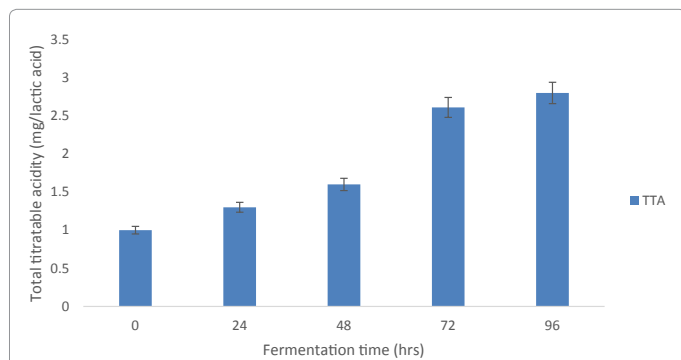


Figure 1: Changes in the total titratable acidity of the epicarp of unfermented and fermented watermelon.

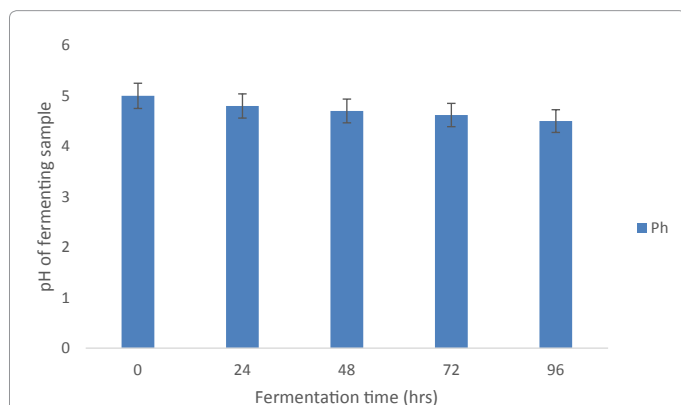


Figure 2: Changes in pH of the epicarp of unfermented and fermented watermelon.

Changes in protein content

Fermentation increased the protein content of the fermented watermelon to 10.50%, while the protein content of the unfermented sample was reduced to 7.00% (Figure 4).

Changes in crude fibre content

Changes in the crude fibre content of the fermented epicarp of watermelon as shown in Figure 4, recorded greater values of crude fibre 9.26%, while that of the unfermented epicarp of watermelon was reduced to 6.00% .

Changes in ash content

The ash content of the unfermented epicarp of watermelon was 6.32% but there was a little reduction in the ash content of the fermented epicarp of watermelon to 6.30% (Figure 4).

Changes in fat content

The fat content for the fermented watermelon was reduced to 6.19% while the unfermented sample was 6.25% (Figure 4).

Discussion

It has been observed from the results obtained from this research that the epicarp of fermented watermelon contained lactic acid bacteria which aided the process of the fermentation. This is also related to the findings of Zhou et al. [11], who stated that lactic acid bacteria are present in fermented samples. The decrease in pH during the fermentation of the sample could also have contributed to the viable growth of lactic acid bacteria which are known to be aciduric. The decrease in pH of this sample is related to the result obtained by Alavi et al. [12], who stated that the increase in the viable cell counts of watermelon juice corresponds to the decrease in pH and this enhance acidity during fermentation. It was also observed that fermentation led to the reduction of the pH and increase in titratable acidity of the fermented sample. The observed increase in titratable acidity and decrease in pH could be due to the dominance of the environment by lactic acid bacteria which degrades carbohydrates resulting in acidification. These observations were in agreement with earlier studies by Ojokoh and Babatunde [13], who stated that the increase in titratable acidity of millet-soya bean blends and decrease in pH of millet – soya bean blends was as a result of the presence of lactic acid bacteria that degrades the carbohydrates. The temperature of the samples ranges between 25°C to 29°C, the ranges in this temperature could serve as the reason for the presence of the mesophilic bacteria that were obtained in the sample. The results of this research show that fermentation has great effect on the proximate composition of the epicarp of watermelon. The result of the proximate analysis revealed that the moisture content of the fermented sample (7.54 ± 0.37) was lower than the unfermented sample (7.92 ± 0.32). The moisture content of these samples is lower compared with the range (91.22 ± 0.65 and 87.06 ± 0.29) reported for fermented and unfermented rind of watermelon by Erukainure et al. [14]. The relatively low moisture content of the samples is an indication that the epicarp of watermelon will have high shelf life especially when properly packaged against external conditions. The result of the proximate analysis also revealed that the ash content of the fermented sample (6.70 ± 0.45) was higher than the unfermented sample (6.69 ± 0.32). The increase in the ash content of the fermented sample could be as a result of partial consumption of minerals by fermenting microorganisms in the process of metabolism. This observation is related to the research of Ojokoh and Babatunde [13], who stated that the increase in ash content of millet-soybean blends is caused by incomplete utilization of minerals by fermenting organisms during their metabolism. The result of the proximate analysis also revealed that the fat content of the fermented sample (6.82 ± 0.37) was lower than the unfermented sample (7.04 ± 0.74). The observed decrease of fat content in the fermented sample could be as a result of the breakdown of fatty acid and glycerol by lipolytic microorganisms present in the sample during fermentation, and the breakdown of the fatty acid and glycerol resulted in the increase of aroma, taste, odour and texture of fermented sample [15]. The fact that reduction in the lipid content of millet - soybean blends increased the shelf life of food sample as stated by Ojokoh and Babatunde [13], could also connote that the reduction in the fat content of the fermented epicarp of watermelon will increase the shelf life of the sample. The protein content of the fermented sample (11.14 ± 0.41) was significantly higher than the unfermented sample (7.69 ± 0.54). According to Oboh and Akindahunsi [16], the high protein content in the fermented sample could be attributed to the ability of *Saccharomyces cerevisiae* that was present in the course of the fermentation to secrete some extracellular enzymes (protein) into the fermented sample during their metabolic activities on the sample. The high protein content of fermented food samples has a good implication in a society with high protein deficiency and will no doubt complement protein from cereals and other plant foods

in the diet of Nigerians [17]. The fibre content of the fermented sample (9.71 ± 0.44) was significantly higher than the unfermented sample (6.73 ± 0.61). According to Eromosele and Eromosele [18], high fibre food expands the inside walls of the colon, easing the passage of waste, thus making it an effective anti-constipation. They also stated that high fibre food lowers cholesterol level in the blood, reduce the risk of various cancers, bowel diseases and improve general health and well-being. The percentage of the carbohydrate content of the unfermented sample was significantly higher (67.59 ± 0.69) than the fermented sample (61.54 ± 0.69). Some of the seeds of plants most especially pear and watermelon seeds can be considered as a potential carbohydrate source when compared to the content of conventional source like cereals that contains 72-90% carbohydrate and these can be good supplements to scarce cereal grains as sources of energy in feed formulation [19].

Conclusion

Fermentation of foods has many advantages such as improvement of nutritional value and protection against pathogens. At the end of this research, it was observed that the sample contains some beneficial microbes which are probiotics and that the epicarp is rich in nutritional components and hence it is good for consumption. It can be concluded that the nutritional benefit of the epicarp of watermelon can be enhanced when it is subjected to fermentation.

Recommendation

Fermented food may be recommended for human consumption for both adults and children, since its nutritional value is high when compared with raw unfermented food products. Further work should be carried out on the functional properties of the epicarp of watermelon.

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