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Effect of Wrapping Materials on Physico-chemical and Microbiological Qualities of Fermented Melon Seed (*Citrullus colocynthis* L.) Used as Condiment

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Abstract The effect of six different wrapping materials on physico-chemical and microbiological qualities of fermented melon seed (*Citrullus colocynthis* L) was studied. Melon seeds were sorted, washed, boiled (48 hrs), dehulled and wrapped in blanched plantain leaf, then it was boiled again for 2 hours, drained, cooled and allowed to ferment naturally for 72 hours. The fermented melon seeds were then mashed, wrapped with six different wrapping materials include: fresh, blanched and dried of *thaumatococcus danielli* leaf, aluminum foil, black polyethylene and transparent polyethylene, placed in a warm environment for 72 hours (maturation period). The products which are known as *Ogiri* condiment were subjected to microbiological and physico-chemical evaluation. The results of the microbiological evaluation showed that the predominant bacteria involved were *Bacillus* spp, *Enterococcus* spp and *Corynebacterium* spp. While *Saccharomyces cerevisae* was isolated. The results of the physico-chemical evaluation showed increase in pH from 5.5 to 6.3, Titratable acidity (TTA) increased from 0.2% to 0.49% during fermentation and varied during maturation. Alumininum foil had the highest TTA of 0.43% while transparent polyethylene had the lowest TTA of 0.23%. During fermentation, the amino nitrogen increased from 2% to 5.5%. The amino nitrogen also increased appreciably during maturation. Black polyethylene had the highest amino nitrogen value (21.15%) while aluminum foil had the least value of amino nitrogen (15.65%) at 72 hours.

Keywords: *melon seed, Ogiri, fermentation, packaging, microorganisms*

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1. Introduction

The origin of food packaging can be said to be as old as civilization. It is an integral part of food processing and entails the use of some materials in the packaging of foods [5]. Packaging is an important descriptive area of food technology, as it concerns the preservation and protection of all types of food materials from microbial spoilage and oxidation. It also extends the shelf-life characteristics of products [16]. Packaging protects a food product from dirt and other contaminants prevent damage that could arise while handling, transporting and distributing the products. It protects food from light, moisture re-absorption, rodents etc and also facilitates distributions and marketing of the food product and raw materials [5]. Traditionally, all sorts of materials are used to package food. In Africa, corn sheet both dry and fresh are used for wrapping various types of foods. Plantain or banana leaves, other broad leaves, calabash gourds, cane and raffia leaves are all used as packaging materials [16,17]. All these leaves are used as packaging material because of the natural flavor they impacted on the food material [12,33]. The primary purpose of a manufacturer is to protect the food product, to keep it in good condition, and to preserve the flavor until it reaches the consumer [17]. The journey from the processor to the ultimate consumer may be a long one, but the product must still reach the consumers with its freshness, flavor, attractiveness and appearance unimpaired [17].

In Nigeria, traditional packaging materials such as *thaumatococcus Danielli*, banana leaves, cocoyam leaves and plantain leaves have gained ground for long [31]. The use of synthetic material such as tin can, aluminum foil, cellophane paper, glass, plastics etc. were invented with civilization to make packaging easier and presentable [28]. Leaves in general, only have limited protection against contamination of the food product packed in them, but have no resistance to impact injury and are easily tampered with [4]. The early man has practiced the preservation of food through various ways which includes the fermentation of food, drying of food over a fire or with the aid of sunlight and salting, and thereafter, packaging with various materials [31].

Egusi Melon (*Citrullus colocynthis L.*) belongs to the species of the genus *Citrullus* of *Cucurbitaceae* family, which usually consists of a large number of varieties that

are generally known as melons. Egusi (*Citrullus colocynthis L.*) is among the 300 species of melon found in tropical Africa and it is cultivated for its seeds, which have been reported to be rich in oil and protein. The regions of its cultivation are Middle East, West African (Nigeria, Ghana, Togo, Benin) and other African countries for the food in the seeds and as a crop inter-planted with maize, cassava and yam. As reported by Giwa *et al.*, 2010, in Nigeria only, ''egusi'' is cultivated over an area of 361,000 ha with a production figure of 347,000 tonnes (as seeds) in 2002. It is used both as condiment and thickener in Nigerian local soup, and the industrial scale production of the oil is yet to be utilized despite the huge potential.

'Ogiri' generally refers to as an oily paste made from oil seeds in West Africa. They are also used as soup condiments with strong smell. It is a product of fermentation of melon seeds (*Citrullus vulgaris*) [2] consumed by the Ijebu and Ondo tribes in the forest zone of South Western Nigeria. *Ogiri egusi* is a food flavouring condiment prepared by traditional methods of uncontrolled solid state fermentation of melon seeds involving the use of chance fermentation. In ogiri preparation, melon seeds are boiled until they are very soft and mashed. The mashed melon seeds are then wrapped tightly in banana leaves and left to ferment for five to seven days. Thereafter, the fermented mashed melon is placed in earthen well pot and covered with jute bags which provide low oxygen tension [23,26]. The fermenting mashed melon is still wrapped in leaves, placed on a wire mesh, smoked over charcoal heat at a distance for about two hour and pulverished before it can be used in cooking [1,23].

In traditional fermentation processes, natural microorganisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhancing flavor and other desirable qualities associated with digestibility and edibility. The fermentation techniques are often characterized by the use of simple, non-sterile equipment, chance or natural inoculum, unregulated conditions, sensory fluctuations, poor digestibility, and unattractive packaging of the processed product [20]. Ogiri is a fermented food condiment of flavoring agent, whose character and organoleptic properties depends on microbial activities [21]. The production process is still a traditional family art and the fermentation is by chance inoculation [37]. It is consumed mainly in Southern Nigeria especially by the Igbos [36].

Nigeria is endowed with a wide range of indigenously fermented foods and condiment [1], which are traditionally packaged with leaves [22]. The traditional food condiments are often stigmatized partly due to poor packaging practices and are such regarded as food for the poor. However, inadequate packaging of this product for improved patronage and effective commercialization has been identified as a major problem as it is still being packaged with leaves and sometimes covered with cement papers which are unhygienic and unsafe for consumption, therefore, this research is aimed at determining the effects of both treated and untreated (fresh, blanched and dried) *thaumatococcus Danielli* leaves and modern (aluminum foil, polyethylene transparent and black) wrapping materials on microbial and physico-chemical qualities of fermented melon seeds as condiment.

2. Materials and Method

2.1. Sample Collection

Melon seed (*Citrullus colocynthns. L*) used for this work were purchased from, Ekeonunwa market in Owerri, the capital city of Imo State, Nigeria. Aluminum foil, (Raynolds, USA) and polyethylene were purchased from Maris Super Market, Ikenegbu in Owerri Municipal L.G.A Imo State Nigeria. The *thaumatococcus Danielli* leaves used were also sourced from Eziobodo village, Owerri in Imo State, Nigeria. All the equipment used were available at food science and Technology Department, Federal University of Technology Owerri, in Imo State of Nigeria and all the chemicals used are of analytical grade.

2.2. Methods

2.2.1. Sample Preparation

2.2.2. Traditional Production of Fermented Melon Seed (Ogiri)

Undehulled melon seeds were properly washed, boiled for 3 hours, cooled and dehulled. The dehulled seeds were wrapped tightly in layers of blanched plantain leaves and pierced with fork. The wrapped cotyledons were thereafter boiled for 2 hours, removed from water and placed on a wire mesh to drain for 1 hour. The wrapped cotyledon was then left to ferment at the prevailing ambient temperature (28^oC) for 72 hours. At the end of the fermentation period, the seeds were pounded into paste. The paste was subsequently distributed into the various wrapping material which comprised blanched, unblanched and dried leaves of *thaumatococcus danielli*, and aluminum foil, transparent polyethylene and black polyethylene bags and were left to ferment for 72 hours over a fire place for maturation. Figure 1 shows the flow diagram of ogiri egusi condiment production.

Determination of Microbiological Properties of the Samples

Sample preparation and enumeration of microbial isolates: Microbial isolation and identification was by standard microbiological techniques [25]. Ten grams of each sample for microbiological evaluation was aseptically homogenized in 90 ml of 0.1% sterile peptone water, shaken thoroughly, and appropriate dilutions (up to 10^{-9}) were prepared for microbiological studies. Aliquot 0.1 mL of appropriate dilutions was spread inoculated in duplicate onto Nutrient agar (Oxoid, Hampshire, U.K.), and Potato Dextrose Agar (PDA) (PDA, Oxoid). The inocula were spread with sterile spreader to ensure even distributions before incubating the plates. Nutrient Agar was incubated at 37± 2°C for 24 – 48h for the growth of heterotrophic bacteria, while PDA plates were incubated at 25 ± 2°C for 3 days [3]. Colonies were enumerated at the end of incubation period using digital colony counter (Gallenkamp, England). The isolates were characterized on the bases of colonial morphology, microscopic and biochemical characteristics [7,8] to include: indole production, methyl red, Voges-Proskauer, citrate utilization, motility, spore stain, urease production, catalase, oxidase, coagulase, starch hydrolysis, gelatin liquefaction, fermentation of glucose, lactose, sucrose, maltose, mannitol, xylose, raffinose, arabinose, temperature and salt tolerance tests.

Further identification of bacteria isolates was by Biomerieux® sa API kit and with reference to standard identification manuals [8,15,19].

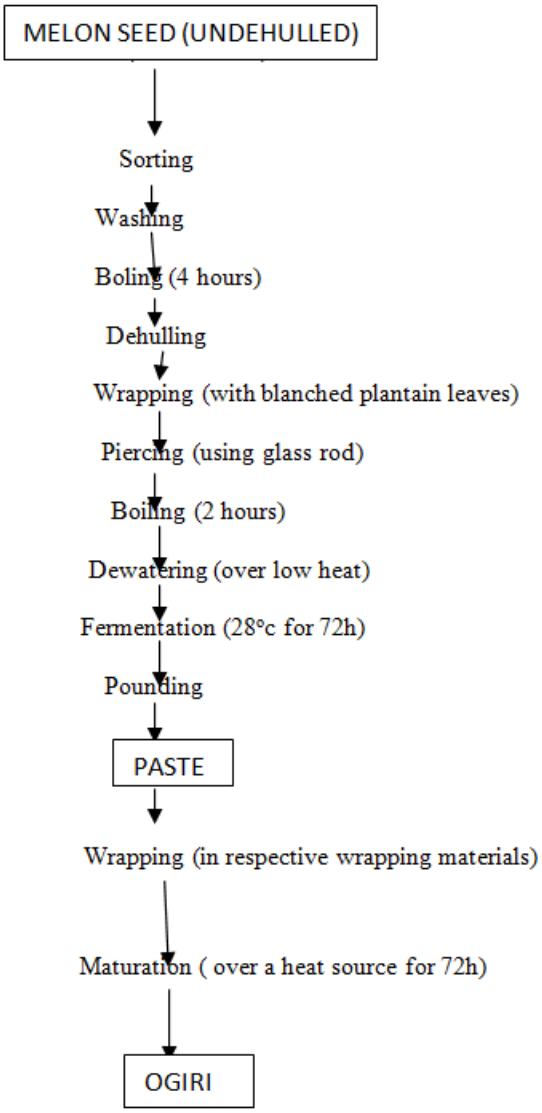

Figure 1. The Flow diagram of Production of Ogiri

Physico-chemical Analyses

Physico-chemical analysis was carried out as described by Ogueke *et al*., [24], Onwuka, [29] and Han *et al*., [11].

Amino Nitrogen Determination

The amino nitrogen of the mashed seeds for each fermentation process was measured at 24 h interval, for fermentation and maturation periods. Two grams of the mashed sample were placed in a conical flask, 0.5 ml of phenolphthalein (0.5%) and 0.4 ml of neutral saturated potassium oxalate were added. The mixture was allowed to stand for few minutes and this was neutralized with NaOH (0.1N) to a standard pink colour. Two (2.0 ml) of 40% formaldehyde solution was added and allowed to stand for few minutes (until mixture was colourless), This was titrated with NaOH (0.1N) to pink colour. The percentage amino nitrogen was calculated using the titre value (V) as shown below;

$$\%\ \text{amino nitrogen} = \frac{NNaOH \times VNaOH \times 100}{Mass\ of\ sample}$$

Where N = Normality; V = Titre value.

Titratable Acidity Determination

The titratable acidity of the inoculated seeds (expressed as percentage lactic acid) for each fermentation process was measured on inoculation and at 24 hours interval, for fermentation maturation periods Five grams of mashed seeds were dissolved in 10.0ml distilled water and 2-5 drops of phenolphthalein was added. The solution was carefully neutralized with 0.1N sodium hydroxide (NaOH) and titrated with aqueous 0.1N sodium hydroxide (NaOH), constantly swirled until a pink colour persisted for 15 seconds. The titre value was recorded and the titratable acidity was calculated as % lactic acid thus;

$$\%\ \text{TTA}\ (\%\ \text{Lactic Acid}) = \frac{Titre\ value \times 0.09 \times N \times 100}{Wt}$$

Where N = Normality of NaOH; 0.09 = Equivalent weight of predominant acid, wt = Weight of sample used.

Temperature

Temperature was measured with a thermometer

pH Value

The pH value of the samples was determined using a standard pH meter (P3935100, PHYWE, GERMANY)

DATA ANALYSIS

The results obtained from the data were subjected to Analysis of variance (ANOVA) according to Onuh and Igwemma [27] and SAS [35]. Significant means at $p \leq 0.05$ were separated using Fisher's least significant difference (LSD) test [27].

3. Results and Discussion

3.1. Microbial Assessments

3.1.1. Total Viable Count for Fermentation and Maturation Periods

The result of the total viable count on fermented melon seed is shown in Table 1. The total viable count increased exponentially from zero time to72 hrs both for bacteria and fungi. This indicates that the organisms were at their exponential phase of growth [6] and that the fermenting melon seed was a suitable substrate for the microorganism to grow. At zero time, the melon substrate had 8.0 x10^9cfu /g of total viable bacterial count and increased to 2.32 x10^{10}cfu /g while yeast and fungi count was 5.3 x10^7cfu /g which increased to uncontrollable number.

Table 1. Total viable count of fermented melon seeds (*Citrullus colocynthns* L.) during fermentation at 28°C

Fermentation period (hrs)	Total viable bacteria count	Yeast and mold count
	cfu g^{-1}	
Zero time	8×10^9	5.3×10^7
24	7.5×10^9	2.05×10^8
48	1.95×10^{10}	2.89×10^8
72	2.32×10^{10}	TNC

Note; TNC –TOO NUMEROUS TO COUNT.

The result of the total viable bacterial count on fermented melon seeds wrapped in various wrapping materials during maturation at 24 hrs and 72 hrs is shown in Table 2. An increase was observed in the total viable count bacteria, yeast and fungi. The sample wrapped in Aluminum foil had the least bacteria count of 1.28 $x10^{12}$cfu /g after 72 hrs while fresh leaf was the highest in bacteria count ($2.37x10^{12}$cfu/g). Sample wrapped in dried leaf had the least yeast and fungi count of 9.5 $x10^{9}$cfu /g at 72 hrs, followed by aluminum foil, while fresh leaf had the highest ($2.12x10^{10}$cfu/g). The least fungi count observed in dried leaf may be due to drying which reduced the moisture level of the leaf making it unavailable to the substrate for microbial activity [31]. On the other hand, the least bacteria count observed in aluminum foil could be attributed to the non-porous nature of aluminum foil which prevented the entrance of external growth factors such as moisture, air for microbial growth and oxygen [32].

Table 2. Total viable count of fermented melon seeds (*Citrullus colocynthns* L.) during maturation

Packaging materials	Total viable bacteria count		Yeast and mold count	
	cfu g^{-1}			
	24 hrs	**72 hrs**	**24 hrs**	**72 hrs**
Unblanched leaf	1.84×10^{12}	2.37×10^{12}	1.74×10^{10}	2.12×10^{10}
Blanched leaf	1.41×10^{12}	2.07×10^{12}	1.85×10^{10}	1.95×10^{10}
Dried leaf	8.3×10^{11}	1.62×10^{12}	1.32×10^{10}	9.5×10^{9}
Aluminum foils	9.3×10^{11}	1.28×10^{12}	1.47×10^{10}	1.22×10^{10}
Polyethylene (Transparent)	1.76×10^{12}	1.86×10^{12}	TNC	1.62×10^{10}
Polyethylene (Black)	1.22×10^{12}	2.15×10^{12}	2.05×10^{10}	1.42×10^{10}

Note; TNC – TOO NUMEROUS TO COUNT.

3.1.2. Cultural, Morphological and Microscopic Characteristics of Bacteria and Fungi Detected

The result of the cultural and morphological characteristics of bacteria detected during fermentation is shown in Table 3. From the result, three types of colonies were observed on the nutrient agar plate. The length of the colonies observed ranged from 1-5 mm. Azu [6] reported that the usefulness of using size as a distinguishing feature is however limited by the fact that the sizes change with change in prevailing condition. Colonies grown in conditions of over –crowding tend to be considerably smaller than those of the same organism growing on plates containing only a few colonies. So, it could be assumed that the length of the organism in the agar plate was small due to the overcrowdiness in the agar plate. The organisms on the agar plate showed regular to irregular shape, they were yellow creamy in colour with entire or serrated margin. The bacteria have low convex, flat and unbonate elevation with surface appearances which were moist and shiny or dull and dry. The characteristics showed a continuous and consistent succession of microorganisms with similarities.

Table 3. CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF BACTERIA ORGANISMS

Sample code	Colony code	Size (mm)	Shape	Colour	Elevation	Margin	Appearance
0 day	Aa	2	Regular	Yellow	Low convex	Entire	Moist and Shiny
	Ab	1	Regular	Creamy	Low convex	Entire	Moist and Shiny
	Ac	4-5	Irregular	Creamy	Flat	Serrated	Dull and dry
24 hours	Aa	1-2	Irregular	Creamy	Flat	Serrated	Dull and dry
	Ab	2-4	Regular	Creamy	Umbonate	Entire	Moist and Shiny
	Ac	1	Regular	Creamy	Low convex	Entire	Moist and Shiy
48 hours	Aa	2-3	Irregular	Creamy	Flat	Serrated	Dull and dry
	Ab	1-2	Regular	Creamy	Low convex	Entire	moist and shiny
	Ac	3-1	Regular	Creamy	Umbonate	Entire	Moist and Shiny

The result in Table 4 showed the cultural and microscopic characteristics of fungi isolated during fermentation of the melon seeds. No growth was observed on the potato dextrose agar plate on the zero time. Furthermore, after 24 hrs of fermentation, *sacharomyces cerevisae*) was identified on the potato dextrose agar plate, which is an organisms known to ferment carbohydrates, breaking them down to sugar in cereal based product mainly. Therefore, it is present due to the carbohydrate content of the melon seed. The presence of fungi during fermentation was due to contamination [31] possibly from the processing environment.

Microscopic and biochemical characteristics of bacteria isolated.

The result of the biochemical test conducted on the bacteria colonies during fermentation period is shown in Table 5. The most predominant organisms identified from the biochemical test were *Enterococcus faecalis*, *Corynebacterium* species and *Bacillus* species, Osho *et al* [30], reported that quite a number of *Bacillus* species have been isolated from various fermented food condiments, although yeast and other bacteria were also seen, only part of them can be considered to play a substantial role in fermentation process. Iwuoha and Eke [14] also reported that the combination of *Bacillus* species and Alkaligence species in Ogiri-egusi was capable of producing the quality characteristics of a good Ogiri which was confirmed from the result of biochemical test in conformity with the above report, It could be deduced that Bacillus species were the main fermenting organisms in melon seed (*citrullus colocynthns L*). However, from the above result, it could be detected that *Bacillus* spp were not the only microorganism that effected the fermentation of melon seed especially with the dependence on natural innoculium. There is evidence that a combination of *Bacillus* species, *micrococcus leteus*, *enterococcus*

feacalis, and *corynebacterium* species which were seen in the study effected natural fermentation of the melon seed.

Table 4. Microscopic characteristics of yeast isolates

Hours	Colony Code	Colonial characteristics	Microscopic appearance	Identity of isolates
0	Ax	None	No definite appearance	*No growth*
	Ay	Tiny Creamy circular colonies	Gram- positive large spherical Budding cells	*Saccharomyces cerevisiae*
24	Az	Creamy circular mucoid Butyrous colonies	Gram –positive large ellipsoidal and spherical budding cells.	Saccharomyces cerevisiae *var. ellipsoideus*
	Ay	Tiny creamy circular colonies	Gram- positive large spherical Budding cells.	*Saccharomyces cerevisiae*
48	Az	Creamy circular mucoid Butyrous colonies	Gram positive large ellipsoidal spherical budding cells.	*Saccharomyces cerevisiae var.ellipsoideus*
	Ay	Tiny creamy circular colonies	Gram positive large spherical Budding cells	*Saccharomyces cerevisiae*

Table 5. Microscopic and biochemical characteristics of bacteria isolates

Hour	Type of colony	Microscopic characteristics	Catalase	oxidase	Coagulase	Indole	Citrate	Motility	Sugar fermentation					Most probable Organism Identified
									Glue	Sucr	Lact	Malt	Mann	
Zero time	1	Gram +ve spherical chain	+	-	-	-	+	-	+	+	+	+	+	*Enterococcus faecalis*
	2	Gram +ve rod short chain	+	-	-	-	+	+	+	+	+	+	+	*Bacillus* spp
24	1	Gram +ve spherical chain	+	-	-	-	+	-	+	+	+	+	+	*Enterococcus faecalis*
	2	Gram +ve rod Short chain	+	-	-	-	+	+	+	+	+	+	+	*Bacillus* spp
48	1	Gram +ve rod Pleamorphic	+	-	-	-	-	-	-	-	-	-	-	*Corynebacterium* spp
	2	Gram +ve rod Short chain	+	-	-	-	+	+	+	+	+	+	+	*Bacillus* spp

Where +ve =positive, Gluc= Glucose, Sucr =Sucrose, Lact =Lactose, Malt= Maltose, Mann= Manitol.

Physico-chemical Assessment

The result of the chemical parameters that were monitored during fermentation of melon seed (*citrullus colocynthis* L.) is shown in Table 6. Temperature was fairly constant (33°C) at 24 hours to 48 hrs, which reduced to 29°C at 72 hrs of fermentation. The initial temperature (33°C) was as a result of the drop in the cooking temperature of the melon seeds which finally reduced to the prevailing ambient temperature of 29°C as at the time of fermentation. The pH of the fermented seed was observed to increase during fermentation from 5.5 to 6.3; similar report was made on *Citrullus vulgaris* L. series [31]. Also Odunfa [22] reported the pH of Ogiri-egusi increase from 6.5 to 8.1 and fermentation temperature from 29°C – 30°C. The rise in pH during production of alkaline fermented food is due to the ability of the dominant microorganisms *Bacillus* species to hydrolyze protein into amino acid and ammonia. Sanni *el al* [34] reported that the higher pH values of fermented legumes compared to other materials under similar condition were as a result of their higher protein content. Therefore, the increase in the pH of fermented melon (*citrulls colocynthis L.*) could be attributed to its high protein content, which was hydrolyzed to amino acid and ammonia. There was an increase in the amino nitrogen produced during fermentation of melon seed (Table 6) as the period of fermentation increased. Amino nitrogen can be used as an indicator for autolysis and microbial degradation of food protein [13] as well as a good index of yeast growth [18]. It is also used as a good indicator for increase in pH value.

Table 6. Physico-chemical properties of melon seeds during fermentation

Parameters	24 hours	48 hours	72 hours
Temperature (^{o}C)	33	33	29
p^{H}	5.5	6.0	6.3
Amino Nitrogen(%)	2 %	4%	5.5%
Total Titratable Acidity(%)	0.2%	0.45%	0.49%

Table 6 reveals an increase in total titratable acidity (TTA) from 0.21% to 0.49%. Titratable acidity is calculated as lactic acid produced by bacteria cells or enzyme from the raw material [38]. The result of the total titratable acidity obtained showed the percentage of lactic acid that was produced during fermentation of melon seeds. In alkaline fermented foods, lactic acid production would not be much beneficial due to the desired alkaline nature of the product expected at the end.

Table 7. Physico- chemical properties of melon seeds during maturation

	p^{H}		TTA		Amino Nitrogen	
Packaging Material	24 hours	72 hours	24 hours	72 hours	24 hours	72 hours
Unblanched leaf	6.8	7	0.66%	0.36%	13.65%	19.50%
Blanched leaf	6.5	7.5	0.64 %	0.25%	11.25%	18.15%
Dried leaf	6.4	7	0.54%	0.32%	9.35%	17.65%
Aluminum foil	6.8	7.5	0.66%	0.43%	10.50%	15.65%
Polyethylene (Transparent)	6.8	7.8	0.66%	0.23%	16.58%	20.00%
Polyethylene (Black)	6.5	7.7	0.57%	0.36%	17.15%	21.15%

Physico-chemical assessment of melon seeds during maturation

The result of the physico-chemical parameters that were examined during maturation period of fermented melon seed paste was wrapped into various wrapping materials (aluminum foil, leaves, transparent and black polyethylene) are shown in Table 7. The value of pH of the fermented melon paste increased from 6.4 to 7.8 as the maturation periods increased from 24 to 72 hrs. The total titratable acidity at this stage varied among the packaging materials.

The total titratable acidity (TTA) result showed that the samples packaged in transparent polyethylene, blanched leaf and dried leaf had low TTA of 0.23%, 0.25% and 0.32% respectively at 72 hrs. While the aluminum foil has the highest TTA (0.43%).

The amino nitrogen increased appreciably during the maturation. Black polyethylene had the highest amino nitrogen value (21.15%) while aluminum foil had the least value of amino nitrogen (15.65%) at 72 hours. According to Ogueke *et al*., [24], increased amino nitrogen is due to the breakdown of proteins to amino acids by proteases. The putrid aroma which is a characteristic of fermented melon seed (Ogiri) is due to the ammonium ion produced from the amino nitrogen content of the melon.

4. Conclusion

Modern packaging materials maintained and improved the chemical composition of fermented melon seed such as pH and amino nitrogen, which are greatly responsible for the characteristic flavor of the condiment. Therefore, packaging this condiment with modern packaging materials like aluminum foil and polyethylene will enhance the chemical quality, aesthetic value and marketability in the world.

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