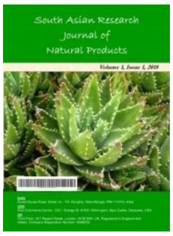

South Asian Research Journal of Natural Products

1(1): 16-22, 2018; Article no.SARJNP.39770

Microbiological, Proximate and Organoleptic Analyses of A Fermented Condiment Made from Seeds of *Citrullus lanatus* (Watermelon)

Fatimat O. Adebayo[1*], Mukhtar O. Adamu[1] and Rafiat B. Abdus Salaam[2]

[1]Department. of Microbiology, Nasarawa State University, Keffi, Nigeria.
[2]Departmentt of Food Technology, Lagos State Polytechnic, Ikorodu, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author FOA designed the study, conducted benchwork and statistical analysis. Author MOA wrote the protocol and the first draft of the manuscript. Author RBAS supervised the experimental analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SARJNP/2018/39770
Editor(s):
(1) M. V. N. L. Chaitanya, Lecturer, Department of Pharmacognsy and Phytopharmacy, Jagadguru Sri Shivarathreeswara University, India.
Reviewers:
(1) Fulden Sarac, Ege University, Turkey.
(2) Mariana-Atena Poiana, Banat's University of Agricultural Sciences and Veterinary Medicine, Romania.
Complete Peer review History: http://www.sciencedomain.org/review-history/23222

Original Research Article

Received 30th November 2017
Accepted 12th February 2018
Published 16th February 2018

ABSTRACT

Aim: This study was conducted to investigate the potential of the protein-rich oily seeds of *Citrullus lanatus* (watermelon) as raw material for fermented condiment production, and conduct the microbiological, proximate and organoleptic analyses of the condiment.
Study Design: This is a laboratory-controlled experimental design.
Place and Duration of Study: Dept. of Microbiology, Food and Industrial unit, Nasarawa State University, Keffi, Nigeria, between March and May 2017.
Methodology: A local condiment ('ogiri') was made from dried seeds of *Citrullus lanatus* (watermelon) by Nigerian traditional method of condiment-making. Microbial enumerations of the fermented product and its raw (unfermented) sample were conducted using standard techniques. The proximate composition of the samples was conducted. Also, sensory evaluations of soup formulations made with the condiment from watermelon seed and a similar product from 'egusi' melon seed were carried out to determine their consumer acceptability.
Results: The condiment obtained from traditional fermentation of *Citrullus lanatus* seeds was an

**Corresponding author: Email: fattysocioscope@yahoo.com;*

oily brownish paste that has a characteristic pungent smell. The enumeration of the fermentative organisms showed that total aerobic growth ranged from $3.2x10^{2}$ cfu/g at the starting time to 2.88 $x10^{8}$ cfu/g at the end of the fermentation period. There was no fungal growth at the beginning of the fermentation, till Day 1 that ranged from $8.0x10^{3}$ cfu/g to $6.0x10^{6}$ cfu/g on Day 5. The result of the proximate composition show that the moisture content of the dried fermented product was 3.5%, protein was 21.1%, fat was 36.9%, carbohydrate was 33.0% and ash was 5.5%. The result of sensory evaluation generally indicated that there was no significant difference ($p \geq 0.05$) in all the quality attributes of the soup samples analyzed.

Conclusion: It can be concluded that *Citrullus lanatus* seeds can yield a highly nutritious, microbiologically and organoleptically acceptable fermented condiment.

Keywords: Potential use; Citrullus lanatus; condiment-making; microbiological enumeration; organoleptic evaluation; proximate analysis.

1. INTRODUCTION

West African food cultures are rich in spontaneously fermented foods, the majority of which have been passed down from one generation to another. The fermentation of food in West Africa is said to account for 40% of the population's diet, a percentage that increases with decreasing income [1]. Africans usually ferment cereal-based foods like sorghum, millet and maize; roots such as cassava; fruits, vegetables, and less commonly meat and fish [2]. Also, fermented in different parts of West Africa (including Nigeria) are leguminous plants and oilseeds, to produce fermented condiments that are used as flavorant in soup. Such fermented condiments include 'ogiri' from castor bean (*Ricinus communis*), 'dawadawa' from African locust beans (*Parkia biglobosa*) and 'ugba' from African oil bean (*Pentaclethra macrophylla*).

In traditionally fermented condiments, microbes are used to prepare and/or preserve the products, add to their nutritive value, flavor and other edibility qualities. These processes are characterized by their limited need for energy input, allowing microbial fermentations to proceed without external heat sources [3]. The processing is a natural un-inoculated solid-state fermentation of boiled and de-hulled cotyledons of the various oilseeds used as substrates. Different genera of bacteria are reported to be involved in the individual indigenous fermentations via natural inoculations [4]. These include *Bacillus*, *Micrococcus*, *Leuconostoc*, *Staphylococcus* and *Enterobacteriaceae*.

Meanwhile, an under-utilized protein-rich oily seed with high potential for condiment production is the usually discarded seeds of watermelon (*Citrullus lanatus*) fruit, which is a sweet, juicy, fleshy fruit that belongs to the family of *Cucurbitaceae*. All parts of the watermelon fruit are believed to be valuable. The rind and juice are used medicinally [5,6], the flesh is consumed for its nutrition and refreshing properties, the seeds have been used in the cosmetic industry [7], and suggested as a potential coagulant for water treatment [8]. Apart from these, the seed of the watermelon fruit can be used in other areas such as food application. For example, in a number of traditional African cuisines, the seeds (rich in edible oils and protein) and flesh are used in cooking; roasted seeds are eaten throughout Asia and the Middle East, and fresh dried seeds are ground into flour and baked as bread in some parts of India [9]. Although there is a challenge of manual shelling of the seeds that consumes a considerable amount of man-hour, this problem could be overcome by the use of shelling machine such as that designed by [10].

Hence, this study was conducted to investigate the potential of the protein-rich oily seeds of *Citrullus lanatus* as raw material for 'ogiri' condiment production, and to conduct the microbiological, proximate and organoleptic analyses of the condiment.

.

2. METHODOLOGY

The watermelon fruits and banana leaves used were obtained from Karu market in Abuja, Nigeria. The banana leaves were obtained from a backyard garden at Abuja. For condiment production, the watermelon fruits were sliced to remove the seeds, which were then sun-dried. The dried watermelon seeds (*Citrullus lanatus*) were shelled and washed and boiled for 12 hrs, allowed to cool to about 30ºC, mashed and wrapped in banana leaves to ferment at ambient room temperature for 5 days. The dried watermelon seeds (*Citrullus lanatus*) were processed into condiment, as adapted from the traditional method that was documented by [11], which is summarized in the flowchart below:

Watermelon seeds
↓
Shelling
↓
Washing and boiling (12 hours)
↓
Mashing of boiled seed
↓
Packaging in banana leaves
↓
Fermentation at 28º± 2ºC, 5days
↓
'Ogiri'

2.1 Microbiological enumeration of the Sample

The microbiological enumerations of the raw and fermented samples conducted were total viable counts, total coliform counts and total fungal counts. Enumerations of the samples were carried out as described in Collins and Lyne's Microbiological Methods [12]. The total viable count was made on Nutrient Agar (NA), coliform count was made on MacConkey agar (MA), while fungal count was made on Sabouraud Dextrose Agar (SDA), to which chloramphenicol antibiotics was added to suppress bacterial growth.

2.2 Proximate Analysis

For proximate analysis, raw (unfermented) and fermented samples were subjected to determination of the proximate composition of protein, carbohydrate, fat, ash and moisture content by AOAC [13] standard procedures.

2.3 Sensory Evaluation

For sensory evaluation, the fermented watermelon seed 'ogiri' and a similarly fermented protein condiment 'ogiri-egusi', which was made from 'egusi' melon (*Citrullus vulgaris*) seeds were added into soups as follows:

- Soup 1 = 'ogiri-egusi' condiment
- Soup 2 = Watermelon seed 'ogiri' and 'ogiri-egusi' (1:1)
- Soup 3 = Watermelon seed 'ogiri' condiment

To determine the preferences and overall acceptability of the watermelon seed 'ogiri', twenty-five (25) untrained panellists that were familiar with fermented condiments were used as judges. The order of presentation of the samples was randomized such that half of the judges tasted Soup 1 before Soup 3, while the other half tasted Soup 3 before Soup 1. The judges were asked to score their preferences on a 9-point hedonic scale, ranging from 1 (Disliked extremely) to 9 (Liked extremely). The responses of the panellists were then statistically analyzed by comparing means using the IBM SPSS Statistic 20 package.

3. RESULTS AND DISCUSSION

The virtual image showing freshly made 'ogiri' condiment produced from watermelon seeds is presented in Fig. 1.

Fig. 1. Freshly made 'ogiri' condiment

The condiment obtained from traditional fermentation of *Citrullus lanatus* seeds was an oily brownish paste that has a characteristic pungent aroma that is known to impact the desired flavor in soups. The brownish color of the product deferred greatly from the original creamy color of the raw seeds used. Also, the smell of the product was completely different from that of the starting seeds. Its peculiar flavor has similar function with commercial seasonings and can serve as a cheaper substitute in homes since it can easily be produced by uncontrolled chance fermentation.

As indicated in [14], the flavor of a food is created by aromatic substances that are biosynthesized during normal metabolic processes in plants and animal, possibly further modified by processing of the food. Specifically, it is stated that reactions leading to flavor development may include pyrolysis of amino acids and peptides, carbohydrate degradation, interaction of sugars with amino acids and peptides, breakdown of ribonucleotides and lipids [15]. Evidently, most non-volatiles are odorless and extremely hydrophilic and include compounds such as table salt, citric acid and sugar [16]. These are known to impact significantly on the taste of substances and thus regarded as flavorants. A number of other non-volatiles such as amino acids, peptides, fats, carbohydrates and organic acids also provide and enhance tastes in food [16]. Although generally odorless, these also generate characteristic volatiles. Their chemical interaction such as during hydrolytic cleavages usually leads to the formation of specific aromas [17].

3.1 Microbial Enumeration of Traditionally Fermented 'Ogiri'

Table 1 shows the enumeration of the microbial counts of the organisms over a period of 5-day fermentation. It can be seen that total aerobic growth (presumably bacteria) was in an increasing population that ranged from 3.2×10^2 cfu/g at the starting time to 2.88×10^8 cfu/g at the end of the fermentation period. This is an expected outcome, as the seeds provide the required conditions for rapid growth and multiplication of the bacteria population, although the substrate is equally capable of supporting the growth of many pathogenic and spoilage microorganisms that may be present. However, the fermentative microorganisms are usually known to produce inhibitory compounds at a rate that ensure the safety and stability of the fermented product. Fermentations of food are often monitored using simple indicators of microbial growth such as the total aerobic count (aerobic plate count, APC), which is based on the assumption that each living microbial cell or cell clump will form a single visible colony when incubated aerobically for a period of time on a given medium and other specific growth conditions. But, as indicated by [18], the application of microbiological criteria to fermented foods is dictated by the nature of the product and its intended use.

There was no fungal growth at the beginning of the fermentation, till from Day 1 that ranges from 8.0×10^3 cfu/g to 6.0×10^6 cfu/g on Day 5. The presence fungi could have been introduced from the leaves used in wrapping the samples during the traditional fermentation process. Conversely, the fungi may have developed from the micro-flora of the watermelon seeds, considering the initial decrease in their population from 8.0×10^3 cfu/g at Day 1 to 1.0×10^3 cfu/g at Day 2 and 3, and subsequent increase to 5.0×10^6 cfu/g and 6.0×10^6 cfu/g at Day 4 and 5, respectively. Specifically, mould growth was observed only from the Day 5 period of fermentation. The mould growth could most probably be a spoilage agent, in which case, it should be noted that after 3-4 days of fermentation, the aromatic product should be adequately preserved to inhibit the growth of mould and other spoilage agents.

Usually, for foods, the acceptable microbiological limit for fungal growth is 1.0×10^3 cfu/ml [19], while for the total aerobic count (indicator of aerobic bacterial contamination) is 5.0×10^5 cfu/g or ml. However, the guidelines for microbiological limits do not apply to certain fermented foods as these foods fall into the 'Not Applicable' category. Acceptability of such foods is based on appearance, smell, texture, and the levels or absence of indicator organisms or pathogens, as stated in the Working Document on Microbial Contaminant Limits for Microbial Pest Control Products by the European Commission Health and Consumer Protection Directorate-General (available at: https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_ppp_app-proc_guide_phys-chem-ana_microbial-contaminant-limits.pdf).

Table 1. Enumeration of microbial counts (cfu/g)

Days	Total aerobic count	Total fungi count
0	3.20×10^2	0
1	3.16×10^3	8.0×10^3
2	6.70×10^7	1.0×10^3
3	1.11×10^8	1.0×10^3
4	2.48×10^8	5.0×10^6
5	2.88×10^8	6.0×10^6

3.2 Proximate Evaluation of the Different Samples

The result of the proximate composition of the fermented 'ogiri' sample is as shown in Table 2. The moisture content of the samples is very low, thereby allowing for better keeping quality of the product. The protein content of fermented 'ogiri' implies that one of the desirable effects of the fermentation process is the increment in protein content. Different researchers have also reported

Table 2. Proximate composition of the fermented 'Ogiri' sample

Parameters (%)	Raw sample	Fermented 'ogiri'
Protein	13.4	21.1
Carbohydrate	49.5	33.0
Fat	25.5	36.9
Ash	6.5	5.5
Moisture	5.1	3.5

Table 3. Mean scores of the different soup formulations

Attributes	Soup 1	Soup 2	Soup 3
Appearance	$8.38 \pm 0.74^{a*}$	8.25 ± 0.71^{a}	8.38 ± 0.92^{a}
Taste	8.50 ± 0.76^{b}	8.13 ± 0.99^{b}	8.50 ± 0.76^{b}
Aroma	8.38 ± 0.74^{c}	8.25 ± 0.89^{c}	8.50 ± 0.76^{c}
Acceptability	8.25 ± 0.71^{d}	8.25 ± 0.89^{d}	8.13 ± 0.84^{d}

*Figures with the same letters along a row are not significantly different ($p \geq 0.05$)

increase in protein content of various fermented condiments. One of such is [20] that reported increased protein value resulting from an increase in activity of proteinase as well as increase in amino acids during fermentation and a decrease in thiamine and niacin levels during the fermentation of castor oil beans and African melon seeds. This relatively high content of the protein content makes the product of this study suitable for use as cheap sources of protein for those who cannot easily afford proteins of animal sources.

The fat content was relatively high. Some short-chain fatty acids are known to enhance the flavor of food. This high proportion of fat in the samples may have some contribution to the desired aroma that the condiment impact in soup. More so, it is important to note that the seed oils are not extracted before converting the seeds into condiments, thus the lipophilic components of the seeds transfer the organoleptic properties of the seed into the diets [21]. Also, a study in reported that during the fermentation of egusi, the total of unsaturated fatty acids increased with hydrolysis of protein into amino acids and peptides [22], while ammonia is released due to the proteolytic activity taking place during fermentation which, therefore, raises the pH of the final products and giving the food a strong ammoniacal odor and flavor.

As the main substrate in the fermenting mash that serves as food (energy) for the fermentative organisms is the carbohydrate, it is an expected outcome that the carbohydrate content decreased in the product of fermentation of this study ('ogiri'), as compared to its value in the raw sample. A research by [23] studied the effect of fermentation on the nutrient content of locust beans and reported that protein and fat increased when fermented whereas the quantity of carbohydrates decreased.

3.3 Sensory Evaluation

Above Table 3 shows the result of the sensory evaluation. There was no significant difference ($p \geq 0.05$) in all the quality attributes of the soup samples analyzed. The mean scores of the different soup formulations revolved around 8.33 for appearance, 8.37 for taste, 8.37 for aroma and 8.21 for overall acceptability. The judges could not detect any perceptible differences between the soup made with 'ogiri-egusi' sample and that made with watermelon seed 'ogiri' sample, and thus, they readily accepted the use of watermelon seeds 'ogiri' as much as they accepted the soup made with 'ogiri-egusi'.

Hence, the result confirms the acceptability of 'ogiri' made from *Citrullus lanatus* (watermelon) seeds and thereby suggests possible commercialization.

4. CONCLUSION AND RECOMMENDATION

As the result of this study confirms the acceptability of 'ogiri' made from *Citrullus lanatus* (watermelon) seeds, it can be concluded that watermelon seeds can be used to produce 'ogiri' condiment of acceptable microbiological, organoleptic and nutritional standard, and thereby suggests possible commercial utilization of the seeds for cottage industry.

However, it is recommended that further work is done to produce a potable sheller that would be easily accessible and affordable to common man. Also, it is recommended that other usually discarded seeds of edible fruits could be examined for possible use in production of condiment and other food applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Koss LH. Fermentation creates sustainable growth in the West African food sector. 2017.
   Available:http://www.science.ku.dk/english/press/news/2017/fermentation-creates-sustainable-growth-in-the-west-african-food-sector/)
   (Accessed 26 January 2018.)
2. Mokoena P. What ancient African fermentation techniques reveal about probiotics; 2016.
   Available:https://theconversation.com/what-ancient-african-fermentation-techniques-reveal-about-probiotics-61221]
   (Accessed 26 January 2018.)
3. Achi OK. The potential for upgrading traditional fermented foods through biotechnology. African Journal of Biotechnology. 2005;4(5):375-380.
4. Enujiugha VN. Major fermentative organisms in Some Nigerian Soup Condiments. Pakistan Journal of Nutrition. 2009;8:279-283.
5. Pons L. Exploring important medicinal uses for watermelon rinds; 2003.
   Available:http://www.ars.usda.gov/is/pr/2003/030221.htm
   (Accessed 31 October 2017.)
6. Tarazona-Díaz MP, Alacid F, Carrasco M, Martínez I, Aguayo E. Watermelon juice: Potential functional drink for sore muscle relief in athletes. Journal of Agriculture and Food Chem; 2013.
7. Vincentz F. Watermelon Seed Oil. Homepage of Oil Health Benefits; 2015.
   Available:http://oilhealthbenefits.com/watermelon-seed-oil/
   (Accessed 31 October 2017.)
8. Muhammad IM, Abdulsalam S, Abdulkarim A, Bello AA. Watermelon seed as a potential coagulant for water treatment. Global Journal of Researches in Engineering: Chemical Engineering. 2015;15(1).
9. Maynard D, Maynard DN. Cucumbers, melons, and watermelons. In: Kiple KF, Ornelas KC, editors. Cambridge World History of Food. UK: Cambridge University Press; 2000.
10. Olusegun HD, Adekunle AS. The Design, construction and testing of melon sheller. Global Journal of Engineering & Technology. 2008;1(4).
11. Akinyele BJ, Oloruntoba OS. Comparative Studies on *Citrullus vulgaris, Citrullus colocynthis* and *Cucumeropsis mannii* for Ogiri Production. British Microbiology Research Journal. 2013;3(1):1-18.
12. Collins CH, Lyne PM, Grange JM, Falkinham III JO. Collins and Lyne's microbiological methods. 8th ed. London: Arnold Hodder Headline Group Publishers; 2004.
13. Association of Official Analytical Chemists, AOAC. Official Method of Analysis. 18th ed. Washington DC: AOAC International; 2005.
14. Olabode OO, Arueya GL. Analysis and identification of the volatile compounds in melon-bitter leaf soup. African Journal of Food Science. 2016;10(11):302-312.
15. Shahidi F. Flavor of muscle foods - An overview. In: Flavor of Meat, Meat Products, and Sea-foods. 2nd ed. London: Blackie Academic and Professional; 1998.
16. Choudhury B. Volatile and non-volatile components of beef marrow bone stocks. PhD. Thesis. NewJersey: The State University of New Jersey; 2000.
17. Chen J, Ho CT. The flavour of poultry meat. In: Shahidi F, editor. Flavor of Meat, Meat Products and Seafoods. London: Blackwell Publishers; 1998.
18. Bell C, Greenwood M, Hooker J, Kyriakides A, Mills RG. Development and use of microbiological criteria for foods. Food Science and Technology Today. 1997;11(3).
19. Gulf Standard Organization, GSO (2014). Microbiological Criteria for Foodstuffs. GCC Standardization Organization.
   Available:https://www.gso.org.sa/gso-website/gso-website/activities/standards
   (Accessed 11 January 2018.)
20. Odunfa SA. Dawadawa. In: Reddy NR, Pierson MD, Salunkhe DK, editors. Legume-based fermented foods. Florida: CRC Press Inc.; 1986.

21. Dosumu OO, Oluwaniyi OO, Awolola GV, Oyedeji OO. Nutritional composition and antimicrobial properties of three nigerian condiments. Nigerian Food Journal. 2012;30(1):43–52.
22. Ibeabuchi JC, Olawuni IA, Iheagwara MC, Ojukwu M, Ofoedu CE. Microbiological evaluation of 'Iru' and 'Ogiri-Isi' used as Food Condiments. IOSR J of Envi Sc Tox Food Tech. 2014;8(8):45-50.
23. Eka OU. Effect of fermentation on the nutrient status locust beans. Food Chemistry. 1980;5:305-308.

© 2018 Adebayo et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/23222