ABSTRACT

**Aim:** This study was conducted to assess fermented condiments that were made from watermelon seeds, using isolated organisms from traditional fermentation as starter-cultures.

**Study Design:** This is a laboratory-controlled experimental design.

**Place and Duration of Study:** Dept. of Microbiology, Nasarawa State University, Keffi, Nigeria, between March and May 2017.

**Methodology:** Traditionally fermented (chance-inoculated) ‘ogiri’ condiment was made from sun-dried watermelon seeds. Microbiological isolation and identification were carried out using standard techniques, to obtain the participating organisms. Afterwards, sterilized packages of another batch of boiled, mashed and banana-leaf packaged seeds were aseptically inoculated with the pure isolates obtained earlier as starter cultures for a subsequent laboratory-controlled 3-day-fermentation process at 37°C in an incubator. All the different fermented products (produced by both uncontrolled and controlled fermentation) were subjected to proximate analysis. Also, the organoleptic quality attributes of the products were determined by conducting sensory evaluation on the various samples.
Results: The traditional fermentation of the watermelon seeds yielded an oily brownish paste, which has a strong characteristic pungent aroma. The participating organisms obtained from traditional fermentation were Bacillus subtilis, Staphylococcus saprophyticus, Corynebacterium xerosis and Lactobacillus fermenti. The result of the proximate composition show that the moisture content of the samples ranges from 3.5% to 5.4%, protein content ranges from 13.4% to 21.1%, fat content ranges from 25.5% to 40.8%, carbohydrate content of the samples ranges from 29.4% to 49.5% and the ash (total minerals) content ranges from 4.5% to 6.5%. The result of sensory evaluation generally indicated that in terms of all organoleptic attributes assessed, the combined isolates fermented sample was most preferred by the panelists.

Conclusion: It can be concluded that a laboratory prepared watermelon seed ‘ogiri’ with starter cultures of Lactobacillus fermenti, Corynebacterium xerosis and/or Bacillus subtilis can yield an organoleptically acceptable and highly proteinous condiment.

Keywords: Condiment; starter-culture; fermented seed; watermelon; sensory evaluation; nutritional composition.

1. INTRODUCTION

All over the world, condiments have been an important part of various cultural dishes in different forms. Its consumption continues to increase due to population growth, increasing number of food-service outlets, growing consumer taste preferences and changing eating habits, amongst other reasons. According to an online statistics portal (https://www.statista.com/outlook/40070000/102/condiments/europe#), the year 2017 average per capita consumption of condiments and sauces in Europe, Asia, Australia, North-America stands at 9.7 kg, 2.8 kg, 13.8 kg and 30.1 kg respectively; and the market is expected to grow annually in the above-listed countries by 1.8%, 4.0%, 4.0% and 1.8% respectively.

Condiments are preparations that are added to food to impact a particular desired flavour or texture to the dish. The term was originally used to describe pickled or preserved foods but was later broadened to cover various spices and seasonings [1]. African condiments are usually introduced in fairly small quantities during cooking as they are generally pungent in flavour [2]. Examples of leguminous condiments are ‘dawadawa’ from African locust bean (Parkia biglobosa), ‘ogiri’ from the castor bean (Ricinus communis), ‘okpe’ from mesquite seed (Prosopis africana) and ‘ugba’ from African oil bean (Pentaclethra macrophylla). In West-African countries such as Nigeria, food condiments are usually prepared from oil-seeds by traditional methods of uncontrolled chance-inoculated solid state fermentation through the actions of various microorganisms especially Bacillus species and some members of the family Enterobacteriaceae [3]. These often result in varying degree of hydrolysis of the protein and carbohydrate components, thereby yielding end-products that differ greatly in quality.

Meanwhile, the choice of substrate for food condiment making often depends on the producing locality [4], but in Nigeria, the cost of various food condiments and their substrates has increased. This prompted the trial of other relatively cheaper and unpopular seeds for usage in the process. One of such potential substrates is the seeds of the watermelon fruit which are usually discarded after consuming the juicy flesh of the fruit. The watermelon seeds are known to be rich in protein, fat, vitamin B, minerals, as well as phytochemicals such as saponins, tannins, triterpenoids, glycosides and alkaloids [5-6]. This implies that they can contribute as cheap nutritious additives in soups for the densely populated developing nations like Nigeria, where the problem of protein deficiency is still a lingering issue till date.

Consequently, this study was conducted to assess fermented condiments that were made from watermelon seeds, using isolated organisms from traditional fermentation as starter-cultures. The outcome of this work shall serve to guide cottage industry in the use of watermelon seeds for condiment making, and selection of its starter culture for controlled, uniform fermentation processes.

2. METHODOLOGY

2.1 Sample Preparation

Traditionally fermented (chance-inoculated) ‘ogiri’ condiment was made from watermelon seeds that were sun-dried at a daytime average
temperature of 38°C (Abuja, Nigeria) for 8 hours. The seeds were then shelled and washed. 600 g of the seeds were boiled in excess water (5 litres) in a pressure cooker for 4hrs, then allowed to boil to dryness, aseptically mashed and wrapped in flamed banana leaves to ferment at ambient room temperature (30°C±2°C) for 5 days, as adapted from documented traditional method [3]. The fermented condiment was made in seven (7) replicates to facilitate daily microbiological isolation of participating microorganisms during the 5-day traditional fermentation period.

2.2 Microbiological Isolation and Identification

These were carried out using standard techniques described in Collins and Lyne’s Microbiological Methods [7]. These involve preliminary pour-plate culturing of the organisms on Nutrient agar, observing the cultural characteristics of isolates on agar plates, conducting gram staining and spore staining reactions, and observing microscopic cell morphology from heat fixed smears. Pure cultures of the different colony isolates were obtained by streaking on sterile nutrient agar plates. Thereafter, pure isolates were subcultured and preserved on agar slants at refrigeration temperature (4°C) for further biochemical tests that were adopted to identify the isolates.

One banana-leaf-wrapped replicate of fermenting samples was taken aseptically at 24-hour interval over the 5-day period for the microbiological isolation and identification, and to obtain the pure culture isolates for subsequent inoculation as starter cultures.

2.3 Laboratory-Controlled Fermentation

Fermented condiments were produced from sterile watermelon seeds by inoculating the pure isolates obtained from the previous traditionally fermented condiments (as described above). Following the steps outlined for traditional fermentation above, banana leaf-packaged boiled and mashed watermelon seeds were packed in the autoclave to sterilize at 15 psi, 121°C for 15 minutes. Meanwhile, the starter cultures for inoculation were previously prepared by aseptically transferring a loopful of each isolate obtained from the traditionally fermented condiment into sterile 10ml Nutrient broth in different bottles and incubated at 37°C for 24 hrs. Afterwards, the cell suspensions obtained were shaken together and 1ml each was taken for inoculating each of the sterilized samples (six replicates/wraps in all), using a sterile pipette for each of the isolate-cultured broths. A separate sterile sample was also inoculated with 1ml of combined-isolates cultured broth. All inoculated samples were subsequently subjected to 3-day-fermentation process at 37°C in an incubator.

At the completion of the fermentation stages, all condiment products obtained were oven dried at 60°C for 24hrs for proximate analysis.

2.4 Proximate Analysis

All the different products (by both uncontrolled and controlled fermentation) were subjected to proximate analysis in triplicates. Moisture content, fat, protein and ash (total minerals) were done using the AOAC methodology [8]. The percentage nitrogen was determined by the macro-Kjeldahl method and converted to protein content by multiplying with factor 6.25. Carbohydrate content was determined by difference method, whereby the values of all the other parameters are added together and then subtracted from 100%.

2.5 Sensory Evaluation

The organoleptic quality attributes of the products were determined by conducting a sensory evaluation on the various samples. The tests were conducted in a well-lighted and ventilated spacious laboratory during the mid-morning period. Twenty-five (25) panelists were selected to score each of the samples in terms of colour, aroma, texture and overall acceptability. The 9-point Hedonic scale questionnaire was used to score the samples, from 1 (disliked extremely) to 9 (liked extremely). The order of presentation of the samples was randomized for each judge to avoid bias. The responses of the panelists were then statistically analyzed by comparing means using the IBM SPSS Statistic 20 package.

3. RESULTS AND DISCUSSION

The traditional fermentation of the watermelon seeds yielded an oily brownish paste, which has a strong characteristic pungent aroma. The brownish colour of the product deferred greatly from the original creamy colour of the raw seeds used. Also, the smell of the condiment obtained was completely different from that of the starting
seeds. It is indicated in a research work [9] that the flavour 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. A similar fermented seed product ‘ogiri-saro’ is brown, soft and sticky condiment with an ammoniacal odor that is usually used as a flavoring ingredient in soups [10]; ‘ogiri-okpei’ is characteristically dark-brown in appearance and is said to play a major role as a nutritive protein substitute, as well as containing some phytochemicals (such as phytate, flavonoid, alkaloid and phenol) that are the reasons behind its health functions to humans [11].

From the results of microbial isolation and identification, the participating organisms obtained from traditional fermentation were Bacillus subtilis, Staphylococcus saprophyticus, Corynebacterium xerosis and Lactobacillus fermenti. The isolation of Bacillus subtilis, Staphylococcus spp., Corynebacterium xerosis and Lactobacillus fermenti from the fermenting mash agreed with some other research work, as these bacteria are among common microbial genera that have been isolated from different fermented protein condiment by various researchers [12-15]. Amongst these, Bacillus spp. are said to be the major group of fermenting organisms as a result of their presence and population throughout the period of fermentation, and because Bacillus cells are known to exhibit very high protease activity compared with the other bacteria isolates [14]. Corynebacterium and Lactobacillus are two common genera that are regularly isolated from different solid-state fermentation of African staples, such as fermented cassava grits (‘gari’), cassava flour (‘lafun’), maize gruel (pap), and so on.

Although, the Staphylococcus spp. that were isolated from this study may be regarded as contaminants, they have been regularly isolated from different fermenting mash by various researchers [4,16-17]. Also, in some food processing applications, the addition of some species of Staphylococcus (especially S. carnosus) to a lactic culture in the production of some European sausages is a common practice in Europe; the non-lactic bacteria help to reduce nitrates to nitrites and produces catalase that benefits the lactic culture [18]. Thus, it may be suggested that the Staphylococcus spp. isolated in this present work are normal microflora of the fermenting seeds.

The results of the proximate composition of the different samples are presented in Table 1. The moisture content of the samples ranges from 3.5% (traditionally fermented) to 5.4% (Bacillus-fermented). These low moisture contents for dried sample allows for better keeping quality of the products, as compared with fresh (high moisture) sample that readily develop mould growth after five days of production at room temperature, if it is not salted or refrigerated. The protein content ranges from 13.4% (raw sample) to 21.1% (traditionally fermented). The increase in the protein content of fermented products is expected of the fermentative organisms (especially Bacillus spp.) which are known to exhibit proteolytic activity and high level of free amino acid. This implies that one of the desirable effects of the fermentation process is the increment in the protein content. This relatively high content of the protein makes the samples suitable for use as cheap sources of protein for vulnerable people, including PEM patients.

The fat content ranges from 25.5% (raw sample) to 40.8% (Lactobacillus- and Staphylococcus-fermented). Some short-chain fats 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. On the other hand, the carbohydrate content of the samples ranges from 29.4% (Lactobacillus-fermented) to 49.5% (raw sample). The main substrate in the sample that serves as food (energy) for the fermenting organisms is the carbohydrates. Thus, it is an expected outcome that the carbohydrate content decreased in all the products of fermentation, as compared to its value in the raw sample. Similarly, the ash (total minerals) content ranges from 4.5% (Staphylococcus-fermented) to 6.5% (raw sample). The decrease in the total minerals (as measured by the ash content) could be attributable to the oily nature of the fermented products that made it slightly difficult for uniformity in surface area for complete ashing process.

Meanwhile, the values obtained from literature indicated that the macronutrient composition of shelled watermelon seeds is 28% protein, 47% fat and 15% carbohydrate (www.nutritiondata.com). A similar work on fermented melon seeds reported the following values: 3.38% ash, 11.59% crude fibre, 35.28% fat, 23.71% protein and 7.27% moisture [3]; whereas another research work reported the following values for the proximate composition of
3-day fermented African locust beans ('iru'): 3.0% ash, 15.6% crude fibre, 36.3% fat, 19.9% protein and 25.2% carbohydrate [19].

The results of this study agreed with a research work in which the authors concluded that fermentation had effect on the composition of the raw locust bean and castor oil seeds, as the protein increased from 32.4% to 39.5% in raw to fermented locust beans ('iru') and 14.07% to 18.1% in raw to fermented castor seeds ('ogiri') [20]. They also indicated that the values of ether extract and moisture increased during fermentation, while ash, crude fibre and carbohydrates decreased in both seeds. Also, in a different study, it was reported that during the fermentation of ‘egusi’, the total of unsaturated fatty acids increased with hydrolysis of protein into amino acids and peptides [21]. Ammonia was said to be 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 ammonical odor and flavor.

More so, it is important to note that the seed oils are not extracted before converting the seeds into condiments, thus the lipophylic components of the seeds transfer the organoleptic properties of the seed into the diets [2]. Table 2 shows the result of sensory evaluation conducted on the various samples. Generally, in terms of all the organoleptic attributes, the combined isolates fermented sample was most preferred by the panelists. The average rank score for all parameters of each sample was highest for the combined isolates fermented ‘ogiri’. This was followed by preference for Corynebacterium -fermented, and Staphylococcus-fermented, then Bacillus- and Lactobacillus-fermented samples for overall acceptability ranking as shown below:

<table>
<thead>
<tr>
<th>Rank</th>
<th>Sample Description</th>
<th>Mean Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.82</td>
<td>Combined &gt; Corynebact. &gt; Staphylo. &gt; Bacillus &gt; Lactobacillus</td>
<td></td>
</tr>
</tbody>
</table>

Specifically, in terms of the appearance (color) of the products, the judges’ preference was for the sample that was fermented by combined isolate (with a mean score of 7.90), closely followed by Bacillus-fermented (6.40) and then Lactobacillus-fermented samples. For aroma, the panelists mostly preferred the combined-isolate fermented sample (mean score of 8.10), followed by Corynebacterium-fermented (6.30), Staphylococcus-fermented (6.00) and then Bacillus-fermented (5.80) samples. Regarding texture attribute, the combined-isolate fermented sample (mean score of 8.10) was most preferred, followed by Corynebacterium-fermented (6.40), Staphylococcus-fermented (6.30) and then Bacillus-fermented (5.80) samples. Similarly, for overall acceptability of the products, the judges mostly preferred the combined-isolate fermented sample (mean score of 8.50), followed by Corynebacterium-fermented (6.70), Staphylococcus-fermented (6.60) and then Bacillus-fermented (6.00) samples.

Table 1. Nutritional evaluation of raw and fermented products from isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>samples</th>
<th>Moisture content (%)</th>
<th>Protein (%)</th>
<th>Carbohydrate (%)</th>
<th>Fat (%)</th>
<th>Minerals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raw sample</td>
<td>5.1±0.05 b</td>
<td>13.4±0.02 a</td>
<td>49.5±0.01 a</td>
<td>25.5±0.01 b</td>
<td>6.5±0.00 a</td>
</tr>
<tr>
<td>2</td>
<td>Traditionally Fermented</td>
<td>3.5±0.03 b</td>
<td>21.1±0.03 g</td>
<td>33.0±0.09 g</td>
<td>36.9±0.00 a</td>
<td>5.5±0.00 c</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus- fermented</td>
<td>5.4±0.10 c</td>
<td>18.2±0.02 a</td>
<td>43.9±0.09 g</td>
<td>27.5±0.01 b</td>
<td>5.0±0.01 b</td>
</tr>
<tr>
<td>4</td>
<td>Corynebacterium- fermented</td>
<td>5.1±0.10 c</td>
<td>18.5±0.00 d</td>
<td>34.7±0.05 d</td>
<td>35.7±0.01 c</td>
<td>6.0±0.00 d</td>
</tr>
<tr>
<td>5</td>
<td>Lactobacillus- fermented</td>
<td>5.1±0.17 c</td>
<td>18.7±0.01 e</td>
<td>29.4±0.00 a</td>
<td>40.8±0.01 f</td>
<td>6.0±0.01 d</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus- fermented</td>
<td>5.1±0.05 b</td>
<td>17.6±0.00 b</td>
<td>32.0±0.17 b</td>
<td>40.8±0.01 f</td>
<td>4.5±0.00 a</td>
</tr>
<tr>
<td>7</td>
<td>Combined isolates</td>
<td>4.5±0.23 c</td>
<td>19.2±0.01 f</td>
<td>34.8±0.02 d</td>
<td>36.0±0.00 a</td>
<td>5.5±0.00 c</td>
</tr>
</tbody>
</table>

Figures with different letters along a column are significantly different (p<0.05)

Table 2. Mean scores of sensory evaluation of the starter culture-fermented samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Color</th>
<th>Aroma</th>
<th>Texture</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus-fermented</td>
<td>6.40±0.58 a</td>
<td>5.80±0.89 a</td>
<td>5.80±0.53 a</td>
<td>6.00±0.56 a</td>
</tr>
<tr>
<td>Corynebacterium-fermented</td>
<td>5.30±0.62 a</td>
<td>6.30±0.26 a</td>
<td>6.40±0.31 a</td>
<td>6.70±0.47 a</td>
</tr>
<tr>
<td>Lactobacillus-fermented</td>
<td>5.90±0.77 a</td>
<td>5.80±0.29 a</td>
<td>5.40±0.65 a</td>
<td>6.20±0.25 a</td>
</tr>
<tr>
<td>Staphylococcus-fermented</td>
<td>5.60±0.56 a</td>
<td>6.00±0.56 a</td>
<td>6.30±0.58 a</td>
<td>6.60±0.37 a</td>
</tr>
<tr>
<td>Combined isolates</td>
<td>7.90±0.54 b</td>
<td>8.10±0.54 b</td>
<td>8.10±0.10 b</td>
<td>8.50±0.28 b</td>
</tr>
<tr>
<td>Traditionally fermented</td>
<td>8.13±0.99 b</td>
<td>8.38±0.74 b</td>
<td>8.50±0.76 b</td>
<td>8.50±0.76 b</td>
</tr>
</tbody>
</table>

Figures with the same letters along a column are not significantly different (p≥0.05)
Consequently, from the result of the organoleptic evaluation, it is evident that the judges preferred samples that were fermented by the single isolate of Corynebacterium to those of the other individual isolates, after the judges’ preference for the combined isolate fermented samples. This result is in contrast with a research work that studied laboratory fermentation of African oil bean (‘ugba’), locust bean (‘iru’), mesquite seed (‘okpei’) and castor bean (‘ogiri’), using isolated microorganisms from the traditionally fermented ones [22]. Therein, the author stated that all the judges preferred the samples that were fermented with Bacillus spp. to those fermented with the other starter organisms, which included Staphylococcus spp., Lactobacillus fermenti and Micrococcus spp.; as it gave the desired color, texture and aroma to the respective fermented products (‘ogiri’, ‘iru’, ‘ugba’ and ‘okpei’).

From the foregoing result and discussion, it becomes evident that there is a great window of opportunities for condiment makers in Africa (and the world at large), as various novel sources of the substrate are being discovered and analyzed for use in food processing. Also, under-utilized edible plants and seeds are constantly been researched upon for their value-addition. These often help to reduce the pressure of demand on the commonly used substrates and raw materials for food processing, thereby impacting positively on the cost of production and making the eventual products more easily affordable to the consumers.

4. CONCLUSION AND RECOMMENDATION

The results obtained from the study have shown the prevalence of bacteria throughout the period of fermentation in an increasing population. Bacillus spp. were isolated throughout the fermentation period, thereby proving to be one of the major fermentative organisms.

The result of the sensory evaluation indicated that the combined-isolates fermented sample was most preferred by the panelists, followed by Corynebacterium-fermented sample, Staphylococcus-fermented and then Bacillus-fermented samples for overall acceptability score. Comparison of the nutritional composition of the fermented samples shows that in terms of protein content, Lactobacillus-fermented, Corynebacterium-fermented and Bacillus-fermented samples ranked closely to the combined isolate protein content which has the highest value amongst laboratory inoculated samples.

Hence, to obtain a fermented condiment from watermelon seeds that can serve as a good additive in the diet of protein deficient people (including children), a laboratory prepared ‘ogiri’ with Lactobacillus fermenti, Corynebacterium xerosis and/or Bacillus subtilis starter cultures can yield an organoleptically acceptable and highly proteinous condiment.

It is however recommended that in order to fully ascertain their suitability for human consumption, Toxicology study should be conducted on the fermented products obtained from novel seeds or food sources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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