Microbiological Quality of Dehydrated Yam (*Dioscorea rotundata*) Chips As Affected By Different Pre-Treatments During Storage

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ABSTRACT

The influence of pre-treatments and different drying temperatures on microbiological quality of yam (*Dioscorea rotundata*) chips was investigated. At the optimum conditions of 4 min blanching, 5% citric acid and 1% ascorbic acid samples were dried out using conventional oven dryer (SLN 75 POL-EKO-APPARATURA, Slaski Poland) under 80°C and 2.0 m/s air velocity. Microbiological study showed a decrease in all microbial count under the freezer storage condition. Stored products were found to be of good quality and safe for consumption since limits of counts of microbes fell within the standard allowed by ICMSF. However, signs of deterioration of the chips was evidenced with the presence of fungi and bacteria isolates including *A. niger*, *A. flavus*, *A. fumigatum*, *Penicillium*, yeast and *Bacillus aureus*. For all pre-treatments, citric acid pre-treated chips experienced the least microbial counts

Keywords: microorganisms, freezer storage, bacteria, fungi, contamination

INTRODUCTION

Microbiological contamination levels need to be brought down in order to extend the storage life of dried food products. Foods that have bacteria and fungi on them deteriorate over time, which shortens their shelf life. However, consumers of the product and industry actors are quite concerned about the danger of contamination in dried chips. Poor processing, storing, handling, packing, and shipping conditions might be the cause of the issue along the value chain (Solomon et al., 2002; Eni et al., 2010). The presence of bacteria, fungi, yeast, and mould on food products results in food poisoning, product contamination, infections, mouldy and discoloured products and loss of economic value on products (Pundir and Jain, 2011). Djeri et al., 2010 evaluation of the microbial contamination of dried yam chips revealed that moulds, coliforms, and mesophilic bacteria (*Bacillus* spp.) are all present in varying amounts on the yam chips (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus glaucus*). Some of the species of bacteria and mould found in these yam chips have been linked to food-borne illnesses. There is however, a knowledge gap on microorganisms that cause degradation of dehydrated yam chips during freezer storage. Therefore, the purpose of this study is to ascertain the microbiological quality of
dried Dioscorea rotundata (Pona) yam chips over a three-month period.

**MATERIALS AND METHODS**

**Study design**

The flow chart for evaluation of microbiological contaminations in stored yam chips is presented in Figure 1. Selected yam tubers were washed, sliced and then pre-treated at 4 min blanching, dipped separately in 5% citric acid or 1% ascorbic acid and oven dried at 80°C. Microbial examination was carried out at day 0, 30, 60, and 90 days of storage in the freezer.

**Sample collection and preparation**

*D. rotundata* (Pona) variety was obtained from the open market in Kumasi Central Market, Ashanti Region of Ghana. Yam tubers were washed using distilled water, peeled and sliced into sizes of 1 cm x 1 cm x 8 cm using a stainless steel kitchen knife. The middle portion of the yam tubers were used for the drying activity.

**Pre-treatment of yam slices**

**Blanching of yam slices**

Blanching was done by dipping 300 g of yam slices each in a 500 ml boiling water for 1, 2, 3, 4, and 5 min. Blanched yam slices were drained and blotted with tissue paper before oven drying. Unblanched yam slices served as control samples.

**Acid pre-treatments**

Yam slices (300 g) each was separately submerged in a 500 ml citric and ascorbic at 1% or 5% for 30 min. Following acid pre-treatment, yam samples were blotted with tissue paper before oven drying.

**Oven drying of pre-treated yam slices**

A typical oven (SLN 75 POL-EKO-APPARATURA, Slaski, Poland) was used for oven drying (Figure 3.5). Pre-treated yam samples were evenly spread on oven trays in a single layer and initial weight determined. Samples were dried independently at 50 °C, 60 °C, 70 °C, and 80 °C and air velocity of 2.0 m/s. Before loading samples, the drying system was allowed to rest for 30 min to establish stable drying conditions. The samples were taken out of the oven every hour, let them cool to room temperature, and then weighed them using a digital balance (PL2002, Mettler Toledo, Switzerland). Three replicates of each measurement were determined. Drying continued until constant weight was observed.

**Freezer storage**

Using a Haier Thermocool (HRF-160EX), the dried yam chips were frozen. Following the sterilization of polyethylene films, the pre-treated, dried chips were packaged and stored at -18°C in the freezer. At days 0, 30, 60, and 90, the microbial loads in the samples were examined.

**Microbiological analysis of samples**

**Enumeration of total coliforms**

The total number of coliforms in the sample was calculated using the Most Probable Number (MPN) approach. By placing 10 g of the sample into 90 ml of sterile peptone water and pulsing for 15 s, serial dilutions 10^-1 - 10^-4 were prepared. One millilitre aliquot from each dilution were added to 5 ml of sterile MacConkey broth, and the mixture was incubated at 37°C for 18 - 24 hr to detect total coliforms. After 18 - 24 hr, tubes that had changed colour from purple to yellow were considered to be positive for total coliforms. For each pre-treated sample, microbial analysis was carried out in triplicates.

**Enumeration of total bacteria**

By using the pour plate method and growing them on Plate Count Agar (PCA), they were separated and counted. By dissolving 10 g of the sample into 90 ml of sterile peptone water and vortexing for 15 s, serial dilutions of 10^-1 - 10^-4 were prepared. PCA was already produced, and one milliliter aliquots from each dilution were added to petri dishes as an
inocula. The plates were then incubated for 24 hr at 37°C. Using a colony counter, all white spots or spread were counted after incubation and reported as total anaerobic bacterial counts. For each pre-treated samples, microbial analysis was carried out in triplicates.

**Isolation and enumeration of mould count (fungi)**

The samples were sorted and numbered after raising them on Potato Dextrose Agar (PDA) using the pour plate technique. Serial dilutions of $10^{-1}$ - $10^{-4}$ were created by dissolving 10 g of the specimen into 90 ml of sterile peptone water and pulsing for 15 s. 1 ml aliquots of each dilution were injected into petri plates with prepared PDA. After that, the plates were incubated for 24 hr at 25°C. After incubation, the colony counter was used to count and record each white spot or spread as mould. For each pre-treated samples, microbial analysis was carried out in triplicates.

**Isolation and enumeration of yeast count**

By using the pour plate method and growing them on Maltextract Yeast Extract Agar (MEYA), they were separated and counted. By dissolving 10 g of the sample into 90 ml of sterile peptone water and pulsing for 15 s, serial dilutions of $10^{-1}$ to $10^{-4}$ were prepared. MEYA that had already been produced was used to inoculate 1 ml aliquots from each of the dilutions into petri dishes. After that, the plates were incubated for 24 - 48 hr at 25°C. Using a colony counter, all white spots or spreads were tallied after incubation and identified as yeast. For each pre-treated samples, microbial analysis was carried out in triplicates.

**Identification of isolated fungi and bacteria from dried yam chips**

Based on culture and physical criteria provided by Samson et al. (2004), the isolated fungi were identified. A stereo microscope was used to look at the nature and shape of conidia. The Illustrated genera of imperfect fungi handbook served as a verification tool for all photos of fungi growths and spores (Barnett and Hunter, 1972). Based on colony morphology, microscopy, and biochemical analyses, distinctive bacterium isolates were found (Swaminathan and Feng et al., 1994).

**Statistical analysis**

Data from the studies were analysed using Origin-Pro 9.2 (Origin Lab Corporation, Northampton, MA, USA). Three replications were carried out and average values were used for calculation. Mean ± Standard deviations was used to express the data. ANOVA and Tukeys’ comparison tests were used to identify significant differences between the means at a significant level of 0.05%.

**RESULTS AND DISCUSSION**

**Effect of pre-treatment and freezer storage on the growth of total plate count, total coliform, yeast and mould counts**

The impact of pre-treatment and freezer storage on the development of various microorganisms is presented in Table 6.1. It can be observed from the table that there was a general decrease in all microbial counts over the storage period. At day 0, total plate count for blanched pre-treated samples was observed to be higher than counts in citric and ascorbic pre-treated samples. The total plate count was $2.02 \times 10^5$ cfu/g for blanched samples, $1.80 \times 10^5$ cfu/g and $4.36 \times 10^4$ cfu/g for citric and ascorbic acid samples respectively. Over the storage period total plate counts decreased from $4.36 - 2.02 \times 10^4$ cfu/g in ascobic acid samples, $1.80 - 1.12 \times 10^5$ cfu/g in blanched samples and $1.80 - 1.16 \times 10^5$ cfu/g in citric acid pre-treated samples. No total coliform count was recorded for all samples at day 0 and over the storage period. Yeast count of $1.15 \times 10^3$ cfu/g was established for ascorbic acid samples, $5 \times 10^2$ cfu/g.
Microbiological Quality of Dehydrated...

cfu/g for blanched samples and no count for citric acid pre-treated samples at day 0. At the end of storage, yeast formation in ascorbic acid samples decreased from 1 - 1.78 x 10^3 cfu/g whereas blanched samples also experienced a decreased from 5 - 2.17 x 10^2 cfu/g. Mould count of 7.14 x 10^3 cfu/g was recorded for ascorbic acid samples whereas a count of 9.5 x 10^1 cfu/g and 4.12 x 10^2 cfu/g was also established for blanched and citric acid samples respectively at day 0. Over the storage duration, ascorbic acid pre-treated samples experienced a decrease in counts from 7.14 - 2.17 x 10^3 to 1.78 x 10^3 cfu/g whereas blanched samples decreased in counts from 9.50 - 4.4 x 10^1 to 4.4 x 10^1 cfu/g. Mould count of 7.14 x 10^3 cfu/g was recorded for ascorbic acid samples whereas a count of 9.5 x 10^1 cfu/g and 4.12 x 10^2 cfu/g was also established for blanched and citric acid samples respectively at day 0. Over the storage duration, ascorbic acid pre-treated samples experienced a decrease in counts from 7.14 - 2.17 x 10^3, blanched samples decreased in counts from 9.50 - 4.4 x 10^1 cfu/g whereas the citric acid samples decreased in counts from 4.12 x 10^2 - 2.40 x 10^1 cfu/g.

**Total plate count**

The metric indicates the overall number of bacteria identified in experimented samples. Stored samples obtained plate counts between 2.04 x 10^4 - 2.02 x 10^5 cfu/g over the storage period. The International Commission for Microbiological Specification for Foods states that ready–to-eat products with plate counts of ≤ 10^3 are acceptable, between 10^3-10^5 are tolerable whereas counts ≥ 10^6 are unacceptable. Because stored samples had counts lower than the limit of 1 x 10^6 cfu/g it made their consumption permissible. However, the decrease in the bacterial counts from day 0 to the end of the storage period is a clear indication that the lower storage temperature (-18°C) hindered the multiplication of food pathogens. Freezing leads to reducing temperature and creating an uncomfortable atmosphere for growth and survival of microorganisms (Gould, 2000). The bacteria count present on samples before being frozen could have resulted from cross contamination during cooling of samples after drying and also improper handling at the time of packing the samples into the polyethylene films (Babajide et al., 2000). It could also be that because the organisms are thermophiles they were able to thrive in the relatively high temperature of 80°C. In a study by Asiedu-Larbi, 2010 on storage of Dioscorea alata chips, bacteria counts were found to have decreased over the storage period of three (3) months from 9.9 - 2.2 x 10^3 cfu/g and 4.2 - 1.7 x 10^3 cfu/g for TDa 98/01176 and TDa 291 samples respectively. Spoilage of most foods is largely due to the presence of bacteria as a result of bad hygienic practices (Elmahmood, 2007).

**Total coliform growth**

Coliforms on food products are a clear indicator of faecal contamination (Tahir and Oyawole, 1993). There was no colony count for total coliform in Table 1. The lack of coliforms may be the consequence of spores dying off during the 80°C drying process. Once more, during the storage period, the microorganisms' rapid multiplication was inhibited by the low temperature regime of -18°C. Uriah and Izuagbe (1990) suggest that the absence of coliforms during specific periods of the storage period may have been caused by the low temperature in the storage region.

**Yeast and mould**

The fungal counts recorded on the samples were within the standard limit of 10^5 cfu/g according to ICMSF (1996). This can be attributed to the drying of the yam chips to optimum moisture levels below < 20%. Again, the polyethylene films used for packaging prevented air movement around samples and thus hindered fungal activity. Foods with minimal moisture content keep well for longer and are less likely to get contaminated by bacteria that ruin food (Uriah and Izuagbe, 1990). Food products with low moisture content inhibit fungal growth and activity (Aryee et al., 2006). Mould and yeast contamination on samples before being frozen is a signal that although heating samples to a temperature of 70-80°C can destroy the microorganisms some of
these microbes might make resistant spores for their continuous survival during heat treatment (Adams and Moss, 2000). High fungal counts in food products is however a sign of spoiling agents that cause changes in food products’ appearance, colour, texture, and flavour (Gacheru et al., 2016). The least microbial count recorded on citric pre-treated chips could be because the acidic solution decreased the pH of the samples, making it difficult for the survival of microorganisms in the acidic environment.

Effect of pre-treatment and freezer storage on identified isolates from dried yam chips after 90 days’ storage

Five fungus and one strain of bacteria were discovered in the dried yam chips after 90 days of storage. Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium, and yeast species were among the fungal isolates. Mostly Bacillus aureus was isolated from the bacterium. The decline in yam chips is connected to comparable isolates discovered in 2010 by Ojokoh and Gabriel.

It has been established that the presence of A. flavus, A. niger, A. fumigatus, Penicillium, and yeast in food products poses a risk to both human and animal health since these organisms create mycotoxins, which, depending on the amount taken, can either cause illness or death (Nida’M and Ahmad, 2010). Similar findings of isolation of Aspergillus spp., including A. fumigatus, A. flavus, and Penicillium from dried yam chips was reported by Djeri et al. (2010). According to Ofor et al. (2009) and Eni et al. (2010), inadequate sanitation during after-harvest handling, shipping, preservation, processing, and drying may be to blame for the occurrence of fungal loads on the food items. Due to their dependence on hot, dry surroundings with little aw, which were present during the final stages of drying, Aspergillus spp are commonly discovered on food products. The samples may have acquired significantly greater fungal load shortly after drying and before being stored, according to the results of the substantial amount of fungus loads on the blanched samples. White filamentous, white irregular, and white circular colony forms all displayed positive (+) gram results for the isolated Bacillus cereus for all pre-treatments throughout the bacterium identification process. Because some strains of Bacillus cereus are known to be hazardous and associated with food poisoning, there is cause for worry (Elise et al., 2019; Amor et al., 2018). According to a study by GFIRA, 2020 (diarrhoeal type), the bacteria produces two distinct diseases, one of which is marked by nausea (emetic disorders) and the other of which is characterized by diarrhoea. The incorrect storage conditions in the storage regions may have contributed to the bacterium's presence in the dried yam chips after storage. Poor handling of food, the use of unclean utensils, and the conditions of storage were determined to be the main contributors to the existence of B. cereus and S. aureus affecting the nutritional value of fermented cassava in a similar research by Olopa et al. (2014).

CONCLUSIONS

The present findings indicated the microbiological quality of stored dried yam chips in a freezer over a period of 90 days. Storage under freezing condition of -18°C resulted in the decrease of all microbial counts during the storage period. The stored chips were generally found to be wholesome for consumption since counts of total bacteria, yeast and mould were within the tolerable and acceptable limits as prescribed by the ICMSF. However, contamination or careless handling during sample cooling after drying and sample packing into polyethylene films before freezer storage could be the cause of microorganisms on samples prior to
storage. The presence of fungal isolates such as Aspergillus species, yeast and Penicillium and bacteria isolate primarily Bacillus cereus was a major concern of spoilage of the product since the microorganisms can break down the nutrient source of the food products to meet their energy requirements. The samples that were pre-treated with citric acid had the lowest microbial counts across all pre-treatments, exhibiting zero total coliform and yeast counts, minimal mould counts, and fewer fungal isolates.

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pp.1-22.
**Table 1.** Effect of pre-treatment and freezer storage on the growth of total plate count, total coliform, yeast and mould (cfu/g)

<table>
<thead>
<tr>
<th>Days</th>
<th>Pre-treatment</th>
<th>Total Plate Count (cfu/g)</th>
<th>Total coliform (cfu/g)</th>
<th>Yeast count (cfu/g)</th>
<th>Mould count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Ascorbic acid</td>
<td>4.36 x 10^4</td>
<td>-</td>
<td>1.15 x 10^3</td>
<td>7.14 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Blanching</td>
<td>2.02 x 10^5</td>
<td>-</td>
<td>5x10^2</td>
<td>9.50 x 10^4</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1.80 x 10^5</td>
<td>-</td>
<td>-</td>
<td>4.12 x 10^2</td>
</tr>
<tr>
<td>30</td>
<td>Ascorbic acid</td>
<td>3.65 x 10^4</td>
<td>-</td>
<td>1.12 x 10^3</td>
<td>4.90 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Blanching</td>
<td>1.85 x 10^5</td>
<td>-</td>
<td>3.12 x 10^2</td>
<td>8.71 x 10^1</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1.63 x 10^5</td>
<td>-</td>
<td>-</td>
<td>3.90 x 10^2</td>
</tr>
<tr>
<td>60</td>
<td>Ascorbic acid</td>
<td>3.26 x 10^4</td>
<td>-</td>
<td>1.09 x 10^3</td>
<td>3.82 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Blanching</td>
<td>1.50 x 10^5</td>
<td>-</td>
<td>2.76 x 10^2</td>
<td>6.62 x10^1</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1.30 x 10^5</td>
<td>-</td>
<td>-</td>
<td>1.22 x 10^2</td>
</tr>
<tr>
<td>90</td>
<td>Ascorbic acid</td>
<td>2.04 x 10^4</td>
<td>-</td>
<td>1.78 x 10^2</td>
<td>2.17 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Blanching</td>
<td>1.12 x 10^5</td>
<td>-</td>
<td>2.17 x 10^2</td>
<td>4.4 x 10^3</td>
</tr>
<tr>
<td></td>
<td>Citric acid</td>
<td>1.16 x 10^5</td>
<td>-</td>
<td>-</td>
<td>2.40 x 10^1</td>
</tr>
</tbody>
</table>

**Table 2.** Fungal and bacteria isolates from dried yam chips

<table>
<thead>
<tr>
<th>Storage medium</th>
<th>Pre-treatment</th>
<th>Fungal isolates</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refrigerator</td>
<td>Ascorbic</td>
<td><em>A. fumigatum, penicillium, Yeast</em></td>
<td><em>Bacillus aureus</em></td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td><em>A. niger, A. flavus, A. fumigatum, penicillium, yeast</em></td>
<td><em>Bacillus aureus</em></td>
</tr>
<tr>
<td>Citric</td>
<td></td>
<td><em>A. niger, A. fumigatum</em></td>
<td><em>Bacillus aureus</em></td>
</tr>
</tbody>
</table>
**Figure 1.** Flow chart of microbial analysis on stored yam chips

**Figure 2.** Identification of isolated fungi: A: Subculture from Potato Dextrose Agar (1% ascorbic acid samples); B: Subculture from Potato Dextrose Agar (5% citric acid samples); C: Subculture from Potato Dextrose Agar (4 min blanched samples)