BACTERIOLOGICAL QUALITY OF PROCESSED, PACKAGED AND STORED CONOPHOR *(Tetracarpidium conophorum)* NUTS

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Abstract: The present study dealt with the formulation and bacteriological quality assessment of two novel methods for processing of conophor *(Tetracarpidium conophorum)* nuts as food. Cooked-and-roasted (CR) and steeped-and-roasted (SR) conophor nuts stored for 28 days in air-tight transparent glass bottles at room temperature (30±2°C) were analyzed for total aerobic heterotrophic bacterial (TAHB) and total coliform counts. The CR sample recorded an insignificant population of TAHB up to day 7 followed by a viable count (cfu/g) of 3.0×10^3, 5.1×10^3 and 3.3×10^5 for day 14, 21 and 28, respectively. However, an insignificant population of TAHB was observed for the SR sample throughout the study period. There was no coliform growth on the nut samples irrespective of the processing method. Also, regardless of the processing method, the bacterial isolates successively encountered in the samples were identified as *Bacillus* sp., *Corynebactcrium* sp., *Lactobacillus* sp. *Staphylococcus aureus* and *Streptococcus* sp. In addition, the SR sample recorded the growth of *Micrococcus* sp. The most predominant bacteria in both the CR and SR samples were *Corynebacterium* sp. and *Lactobacillus* sp. There was persistent decline in moisture content from 6.04% at day 0 to 4.25 % at day 28 for CR sample, and 6.00% at day 0 to 4.26% at day 28 for SR sample. The bacteriological quality of conophor nuts which are processed, packaged and stored as reported herein does not appear to suggest a public health concern.

Key words: Bacteriological quality assessment, conophor nuts, processing, shelf-life

INTRODUCTION

Cashew nut, groundnut, African breadfruit seed and conophor nut (African walnut or *Tetracarpidium conophorum* nut) are amongst the most consumed indigenous edible nuts in Southern Nigeria. Cashew nut and groundnut are amongst the indigenous nuts which according to Little *et al.* (2010), their post-harvest processing, packaging and preservation have been commercialized, and modern technology and regulation adopted in major producing countries. However, in Nigeria, an earlier report by Irtwange and Oshodi (2009) that peasant processing, packaging and preservation are still adopted for indigenous edible nuts have not changed. This is particularly true for conophor nuts, which cooking without

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dehulling the shells is the sole traditional method of preparing it as food (Nwabunnia and Otogbor, in press).

In our earlier report (Nwabunnia and Otogbor, in press), it was established that *Salmonella, Shigella, Klebsiella, Staphylococcus aureus,* and a few other pathogens were associated with the shells of cooked conophor nuts. Hence, the age long practice of seasonally consuming cooked conophor nut as snack in Nigeria (Oke, 1995) by first breaking its shell with the teeth is of public health concern (Nwabunnia and Otogbor, in press).

Most of the studies on conophor nuts have been on the nutritional composition of the nuts (Enujiugha, 2003; Enujiugha and Ayodele-Oni, 2003; Ajaiyeoba and Fadare, 2006, Edem *et al.*, 2009) and there is little or no scientific report on the microbiological quality of processed, packaged and stored conophor nuts.

In view of the foregoing, the objectives of the present study were: i) to formulate novel methods for processing, packaging and storage of conophor nuts, and ii). to examine the bacteriological quality of the processed and packaged conophor nuts during short term storage.

MATERIALS AND METHODS

Sample collection

Fifty (50) raw conophor nuts used for this study were procured from the Relief market, Owerri, Imo State, Nigeria.

Processing of conophor nuts

Cooking and roasting

Twenty-five raw conophor nuts were washed and cooked for 45 min. After cooking, the water was decanted and the conophor nuts allowed to cool down, sun dried for 30 min before transferring into a cooking pot containing hot sand, and placed on a kerosene stove to roast for 90 min. Thereafter, the nuts were allowed to cool down to room temperature (30±2°C), the shells cleaned with a new handkerchief and removed before weighing. The weighed nuts (109.4 g) were transferred into a hot air oven at 110°C for 15 min in order to eliminate moisture and reduce microbial load before packaging.

Steeping and roasting

Twenty-five raw conophor nuts were washed and steeped in water for 3 h with their shells intact. After steeping, the water was decanted and the nuts sun dried for 30 min before transferring into a cooking pot containing hot sand, and placed on a kerosene stove to roast for 1h. Thereafter, the nuts were allowed to cool down to room temperature (30±2°C), cleaned with a new white handkerchief and weighed. The weighed nuts (207.7 g) were transferred into a hot air oven at 110°C for 15 min in order to eliminate moisture and reduce microbial load before packaging.

Packaging and storage of processed conophor nuts

Both the cooked-and-roasted (CR) and steeped-and-roasted (SR) conophor nuts were filled to brim in different air-tight sterile glass bottles, with their shells dehulled and intact, respectively. The set up was stored at room temperature for 28 days during which bacteriological examinations were undertaken.

Bacteriological Examination of processed, packaged and stored conophor nuts Enumeration and isolation of bacteria

Five (5) grams each of cooked-and-roasted nuts and steeped-and-roasted nuts were taken aseptically each week in duplicate from air-tight glass bottle during the 28-day storage period. Each 5 g sample was transferred into 45 ml of sterile peptone water (Oxoid). This was allowed to stand for 10 min, vigourously shaken and serially diluted 10-fold using the same diluent. Dilutions were plated out in duplicate on nutrient agar (Antec) and MacConkey agar (Oxoid) for total aerobic heterotrophic bacteria (TAHB) and total coliform counts, respectively, using the spread plate method of Miles and Misra (Collins *et al.*, 2004). Inoculated nutrient agar and MacConkey agar plates were incubated aerobically at room temperature (30±2°C) and 35°C, respectively for 18–24 h. Isolated microorganisms were purified by repeated streaking using the medium and incubation conditions of the original isolation, stored on agar slants at 4°C before being characterized.

Characterization and identification of bacterial isolates

Examination of the bacterial isolates for colony morphology, cultural appearance, cell micromorphology together with biochemical characterization tests for the identification of the isolates were all carried out according to the methods detailed by Collins *et al.* (2004). The tests undertaken include Gram staining, spore staining, motility, production of catalase, oxidase and urease; citrate utilization, oxidative/fermentative (O/F) utilization of glucose. Others include utilization of sugars; indole production; hydrolysis of casein, starch and tween 80; methyl red and Voges Proskaur test; gelatine liquefaction; coagulase production; haemolysis; gas production at 44°C; decarboxylation of amino acids; phenylalanine deaminase. Identification of bacteria followed the scheme of Holt (1982).

Determination of moisture content

Five (5) grams of steeped-and-roasted sample was ground and placed in a pre-weighed crucible and then dried to a constant weight at 80°C for 2 h in an oven. Moisture content was determined from loss in weight between the initial and final sample and expressed in percentage.

RESULTS

The viable counts of total aerobic heterotrophic bacteria in processed conophor nuts stored in air-tight glass bottle at room temperature is shown in Table 1. Insignificant growth was recorded for steeped-and-roasted (SR) samples throughout the 28-day study period. For the cooked-and-roasted (CR) samples, there was insignificant growth up to day 7 followed by a viable count (cfu/g) of $3.0 \times 10^3 - 3.3 \times 10^5$. There was no coliform growth on both CR and SR samples.

Table 2 shows the succession of bacteria in processed conophor nuts stored in air-tight glass bottles at room temperature (30±2°C). Bacteria identified as *Staphylococcus aureus*, *Streptococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Lactobacillus* sp. and *Corynebacterium* sp. were isolated from processed conophor nuts. *Corynebacterium* sp. appeared in the CR nuts at day 7 and

persisted until the end of the storage period. *Bacillus* sp. was isolated only on day 7 of the storage period for both CR and SR nuts. *Micrococcus* sp. was implicated only on day 7 in the SR nut sample. *Corynebacterium* sp. and Lactobacillus sp. were the most predominant bacteria associated with processed, packaged and stored conophor nuts irrespective of the processing method.

There was persistent decline in moisture content from 6.04% at 0 day to 4.25 % at day 28 for CR sample, and 6.00% at 0 day to 4.26% at day 28 for SR sample. The CR and SR samples of conophor nuts were crispy throughout the study period.

Table 1. Viable count of total aerobic heterotrophic bacteria in processed conophor nuts stored in air-tight glass bottles at room temperature (30±2°C)

	Viable counts (cfu/g)	
Storage period (day)	Cooked-and-roasted	Steeped-and-roasted
0	3×10^1	1×10^1
7	7×10^1	1×10^1
14	3.0×10^3	3×10^1
21	5.1×10^3	9×10^1
28	3.3×10^5	7×10^1

Table 2. Succession of bacteria and changes in moisture contents of processed conophor nuts stored in air-tight glass bottles at room temperature (30±2°C)

	Identity of isolates		
Storage period (day)	Cooked-and roasted	Steeped-and-roasted	Moisture content (%)*
0	*Staphylococcus aureus, Lactobacillus* sp.	*Staphylococcus aureus, Corynebacterium* sp.	6. 04 (6.00)
7	*Bacillus* sp., *Corynebacterium* sp., *Lactobacillus* sp.	*Bacillus* sp., *Micrococcus* sp.	ND (ND)
14	*Corynebacterium* sp., *Staphylococcus aureus, Streptococcus* sp.	*Corynebacterium* sp., *Streptococcus* sp. *Lactobacillus* sp.	4.97 (4.95)
21	*Corynebacterium* sp., *Lactobacillus* sp.	*Staphylococcus aureus*	4.46 (4.43)
28	*Corynebacterium* sp.,	*Corynebacterium* sp., *Lactobacillus* sp.	4.25 (4.26)

** Values outside and inside parenthesis are for cooked-and-roasted and steeped-and-roasted samples, respectively; ND, not determined*

DISCUSSION

The very low or insignificant population of total aerobic heterotrophic bacteria in steeped-and-roasted (SR) conophor nuts throughout the 28-day storage period may have been due to the protection given to the cotyledons by the intact shells. On the other hand, the dehauling of the shells of the cooked-and-roasted (CR) conophor nuts may have exposed CR samples to comparatively higher bacterial growth than SR samples (Table 1).

The relatively low bacterial growth on the processed, packaged and stored conophor nuts and more so, the complete absence of coliforms in the nut samples are pointers to good aseptic and hygienic measures at different levels of this study. Mirrored with the guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale (PHLS-ACFDP, 2000), the highest aerobic colony count of 3.3×10^5 cfu/g at day 28 for CR was the only conophor nut sample with unsatisfactory microbiological quality condition during the study period. This is so far as these said guidelines provide that aerobic colony count of > or = 10^5 cfu/g is unsatisfactory for category 2 foods which include ready-to-eat nuts. The absence of coliforms and in particular, *Escherichia coli* in conophor nut samples is a positive deviation from earlier reports on edible nuts and seeds by Adebesin *et al.* (2001), Vural and Erkan (2008), Willis *et al.* (2009) and Little *et al.* (2009; 2010). These independent researchers implicated *E. coli* and *Salmonella* spp. in varieties of edible nuts and dried seeds. Also, Alwakeel and Nasser (2011) recorded total and faecal coliform counts in pine seeds and salted pistachio nuts sold in Riyadh, Saudi Arabia. Accordingly, since *E. coli* is an indicator of faecal contamination, its absence will make the conophor nut samples so handled as in this study, to be more acceptable to the public. Interestingly, *E. coli* was implicated in the shells of ready-to-eat conophor nuts on retail sale in Owerri, South-Eastern, Nigeria as detailed in our earlier report (Nwabunnia and Otogbor, in press). This suggests that the processed and packaged conophor nuts as detailed in this study are safer for consumption than the traditionally prepared ready-to-eat conophor nuts available to Nigerian consumers.

Bacillus sp., *Staphylococcus aureus*, *Streptococcus* sp. and *Micrococcus* sp. which were amongst the six bacterial species implicated in this study have earlier been reported by Douglas *et al.* (1970) to be associated with processed almond, an edible nut. Report by Adebesin *et al.* (2001) showed *Staphylococcus aureus*, *Micrococcus* sp. and *Bacillus cereus* to be associated with roasted groundnuts hawked in Bauchi, a Nigerian city. Also, a related research on microbiological quality of some types of edible nuts by Vural and Erkan (2008) implicated *Staphylococcus-Micrococcus* sp. and *Bacillus cereus*. More recently, Ibeabuchi (2012) and Oladapo *et al.* (2014) independently implicated *Micrococcus lutae*, *Bacillus brevis*, *Bacillus subtilis and Staphylococcus epidemics* amongst three species of fungi in roasted, packaged and stored cashew nuts.

The isolation of *Micrococcus* sp. and coagulase positive *Staphylococcus aureus* from the processed, packaged and stored conophor nuts is of public health concern since the organisms according to Frazier and Westhoff (2000)

are known to cause food poisoning. This is more so when the ready-to-eat conophor nuts do not require further heat treatment since subjecting of food containing *Staphylococcus* toxin to cooking will destroy such toxin due to high heat treatment. The presence of *Staphylococcus aureus* is an indication of improper handling (Odibo and Umeh 1989) as this organism is a normal flora of the skin.

Also, the high protein content of conophor nut as reported by Enujiugha (2003) may have encouraged the growth of *Lactobacillus* sp., one of the most predominant microbes encountered in this study, as the organism is known to be associated with proteinous foods. The spore forming ability of *Bacillus* sp. may have occasioned its survival in the processed, packaged and stored conophor nut samples. Also, its presence poses a risk to public health since some species of the genus such as *B. cereus* are known to cause food poisoning (Douglas *et al.* 1970).

Physical observation of the processed, packaged and stored conophor nut samples showed that they remained crispy throughout the 28-day storage period. This is a pointer to the fact that the packaging containers were truly air-tight and the storage temperature appropriate. This is encouraging in that crispiness is one of the characteristics which most consumers of roasted edible nuts expect of such products.

Conclusion

From the points of view of microbial safety and moisture content, steeping-and-roasting proved to be a better method for the processing of conophor nuts than cooking-and-roasting. Also, the storage containers duly served the purpose of shielding the processed nuts from the elements of the storage environment thus ensuring crispiness all through the study period. Starting with shelf-life extension studies, these findings will give impetus to further laboratory and pilot scale investigations, which will culminate to industrial processing, packaging and preservation of conophor nuts. In this lies the hope of over-coming the existing seasonal barrier thus ensuring the consumption of wholesome and safe conophor nuts all-round the year.

REFERENCES

Adebesin A. A., Saromi O. T., Amusa N. A. & Fagade S. O. (2001). Microbial quality of some groundnut products hawked in Bauchi, a Nigerian city. *J. Food Technol. Afr.* 6: 53–55.

Ajaiyeoba E. O. & Fadare D. A. (2006). Antimicrobial potential of extracts and fractions of the African walnut (*Tetracarpidium conophorum*). *Afr. J. Biotechnol.* 5: 2322–2325.

Alwakeel, S. S. and Nasser, L. A. (2011). Microbial contamination and mycotoxin from nuts in Riyadh, Saudi Arabia. *Amer. J. Food Technol.* 6(8): 613–630.

Collins C. H., Lyne P. M., Grange J. M. & Falkinnam, J. O. (2004). *Collins and Lyne's microbiological methods*, 8th Edition. Hodder Arnold Publication, New York, 464 pp.

Douglas K. A., Miller, M. J. & Eldridge, L. C. (1970). Almond harvesting, processing and microbial flora. *Appl. Environ. Microbiol.* 20: 208–214.

Edem C. A., Dosunmu M. I. & Bassey F. I. (2009). Determination of proximate composition, ascorbic acid and heavy metals content of Africa walnut (*Tetracarpidium conophorum*). *Pak. J. Nutr.* 8 (3): 225–226.

Enujiugha V. N. (2003). Chemical and functional characteristics of conophor nut. *Pak. J. Nutr.* 2(6): 335–338.

Enujiugha V. N. & Ayodele-Oni O. (2003). Evaluation of nutrient and antinutrients in lesser known under-utilized oil seeds. *Intl. J. Food Sci. Technol.* 38: 525–528.

Frazier W. C. & Westhoff, D. C. (2000). *Food microbiology*, 4th edition. Tata McGraw-Hill Publication Inc, New Delhi, pp. 17–34.

Hall H. E. (1971). The significance of *Escherichia coli* associated with nut meats. *Food Technol.* 25: 230–323.

Holt, J. G. (Ed.) (1982). *The shorter Bergey's manual for determinative bacteriology*, 8th edition. The Williams and Wilkins Inc., Baltimore, Maryland, U.S.A, pp. 45–50.

Ibeabuchi J. C. (2012). Microbial and physicochemical changes in processed cashew nuts stored in different packaging materials. *Intl J. Agric. Rural Dev.* 15(2): 1119–1128.

Irtwange, S. V. and Oshodi, A. O. (2009). Shelf-life of roasted cashew nuts as affected by relative humidity, thickness of polythene packaging material and duration of storage. *Res. J. Appl. Sci. Engr. Biotechnol.* 1 (3): 149–153.

Little, C. L., Jemmott,W., Surman-Lee, S., Hucklesby, L. and de Pinna, E. (2009). Assessment of the microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on *Salmonella*. J. *Food Protect.* 72 (4): 853–855.

Little, C. L., Rawal, N., de-Pinna, E. and McLauchlin, J. (2010). Survey of *Salmonella* contamination of edible nut kernels on retail sale in the U. K. *Food Microbiol.* 27 (1): 171–174.

Nwabunnia, E. and Otogbor, C. C. (2015). Microflora of the shells of ready-to-eat conophor (*Tetracarpidium conophorum*) nuts on retail sale in Owerri. *Nig. J. Microbiol* 29 (1): in press.

Odibo, F. J. C. and Umeh, A. I. (1989). Microbiology of the fermentation of *Telfaria* seeds for *Ogiri* production. *MIRCEN Journal* 5: 217–222.

Oke, O. L. (1995). *Leaf protein research in Nigeria*, 2nd edition. University of Ibadan Press, Ibadan, Nigeria, pp. 89–94.

Oladapo, A. S., Olufunmilola, A. A., Akintoyese, O. A. and Adepeju, A. B. (2014). Effect of packaging materials on moisture and microbiological quality of roasted cashew nuts (*Anacadium occidentale* L). *Res. J. Eng. Appl. Sci.* 3(2): 98–103.

PHLS-ACFDP (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Commun. Dis. Pub. Hlth* 3(3): 163–167.

Vural, A. and Erkan, M. E. (2008). The research quality in some edible nut kinds. *J. Food Technol.* 6(1): 25–28.

Willis, C., Little, C. L., Sagoo, S., de Pinna, E. and Threlfall, J. (2009). Assessment of the microbiological safety of edible dried seeds from retail premises in the United Kingdom with focus on *Salmonella* spp. *Food Microbiol.* 26 (8): 847–852.