World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Assessment of handling practices and microbial contamination of raw and cooked African walnut (*Tetracarpidium conophorum)* fruit snacks in Abuja Nigeria markets

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World Journal of Advanced Research and Reviews, 2024, 22(01), 888–897

Publication history: Received on 18 February 2024; revised on 11 April 2024; accepted on 13 April 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.22.1.0984

Abstract

Microbial contamination of walnut fruits can be of health risk concern. This study assessed the handling practices and the microbial contaminant of raw and cooked African walnuts (*Tetracarpidum conophorum* Mull. [Arg]), fruits in Abuja, Nigeria. An initial market survey involving the administration of 120 semi-structured questionnaires was conducted. Seventy-two raw walnuts and 72 cooked walnuts samples were collected from Gwagawlada, Bwari and Abuja municipal area councils and bulked separately into 24 composite samples and examined for the total bacterial count. Fifty-two (52.0%) of the traders, preserve the cooked walnuts by submerging them in cold water overnight. The raw ones were preserved by shade-drying monthly for 4-5 hrs and bagging jute sacks by (68.0%) traders. The bacterial colony count in the raw walnut sample ranged from 1.13 x 10^5- 2.30 x 10^6 and 1.53 x 10^5- 6.80 ×10^6 in the cooked samples. Three Gram-negative bacteria - *Pseudomonas aeruginosa*, *Escherichia coli,* and *Shigella sonnie,* and two Gram-positive bacteria; *Staphylococcus aureus* and *Salmonella* spp. were identified biochemically. *Staphylococcus aureus* and *E. coli* had the highest incidence of 42.0% and 25.2% in both the cooked and raw samples respectively. Thirty-three (67%) of the raw samples were in the satisfactory level while 83% of the cooked samples were in the satisfactory category when compared to the ICMSF food standard. Since some ready-to-eat cooked samples contained notable pathogenic microbes that are harmful to humans, this indicated a health risk concern.

Keywords: Bacteria; FCT-Abuja; Markets; Occurrence; Walnut fruits

1. Introduction

Nuts including walnuts are often exposed to microbial contamination during the processes of growth, harvesting, storage, and marketing. Contamination also occurs due to changing climatic conditions, and agricultural and storage practices (Brar and Danyluk, 2018; McDaniel and Jadeja, 2021). This is further assisted by adverse temperature and relative humidity, conducive to microbial growth (Qiu *et al*., 2022)

African walnut fruit (*Tetracarpidium conophorum* Mull. [Arg.]), in the family Euphorbiaceae also referred to as black walnut is a 3-6 m tall climbing vine. Walnut cultivations are majorly in West Africa, as well as the Southeastern and Southwest regions of Nigeria (Edeh *et al*., 2023). It is locally called "Bairi" by the Hausas in the Northern region of Nigeria, Asala in Yoruba, Ekporo in Efik and Ibibos, Ukpa in Ibo and Okwe in Edo and Gwandi (Chijioke et al. (2015). The nuts are a delicacy that is cooked and eaten as a snack. When broken, the nuts are pale and have black or brown shells. When it is split into two equal portions, there is typically a thin layer visible (Ayoola *et al*., 2011).

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African walnuts are rich in protein, carbohydrates, and fat but low in fiber and ash contents. Studies have also proved them to be very good sources of Vitamins A, B_1, B_2, B_6, E, copper, manganese, sodium, potassium, chloride, iron and the immune booster ascorbic acid. The green hulls which are the immature fruits provide a great supply of vitamin C. The nut is a good source of energy as it has about (16.90%) carbohydrates and calories of about 0.6 kJ. It is also a great supply of polyunsaturated fatty acids such as alpha-linolenic acid (ALA); a fantastic source of anti-inflammatory omega-3- essential fatty acids (Seyit *et al*., 2015). With regards to phytonutrients, walnuts contain antioxidants and anti-inflammatory compounds including more than a dozen phenolic acids, and a vast array of flavonoids. It also contains a high composition of vitamin E, most especially, gamma-tocopherol (Kanu *et al.,* 2015).

There is limited research evaluating the microbial activity of raw and cooked walnuts, despite the several studies that have been done on the nutritional benefits of walnuts. Therefore, the study's goals were to assess the local preservation methods for walnuts and to determine the bacterial load of both raw and cooked walnuts marketed in the FCT, Abuja, Nigeria.

2. Materials and Methods

2.1. The study region

Three Area Councils in the Federal Capital Territory, Abuja namely: Bwari Area Council, Abuja Municipal Area Council (AMAC), and Gwagwalada Area were involved in the study. The Territory lies on Latitude 9.0765˚ N and Longitude 7.3986˚ E with about 491 m elevation above sea level. The climate is warm with an annual average temperature of 32˚C and characterized by two distinct seasons, wet and dry season.

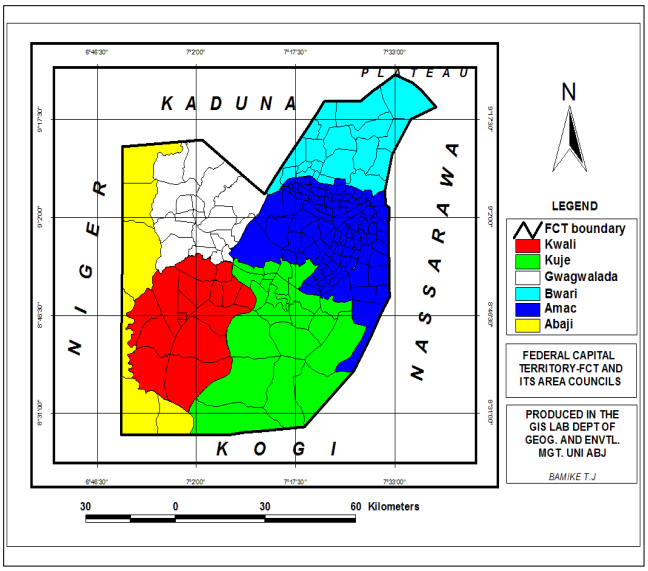

Figure 1 Map of the FCT, Abuja showing the Six Area Councils

2.2. Sampling Procedure and Sample Size

One hundred and twenty questionnaires were administered to walnut traders across the three Area Councils i.e. 40 per Area Council. Yamani's formula was applied in obtaining the sample size of the marketers i.e.:

$$n=N1+Ne\hat{}2$$

Where:

N= total population size
n =sample size
e= margin error (0.05)

2.3. Sample collection

One hundred and forty-four walnuts (72 raw and 72 cooked) were collected from retailers in three Area Councils in the FCT (Figure 2). Twelve raw and cooked walnut samples were randomly obtained from the selected markets in the Area Councils (AC). The two markets from each of the Area Councils were Dutse and Kubwa markets from Bwari AC, Zuba and Gwagwalada markets from Gwagwalada AC and Garki and Wuse markets from Abuja Municipal AC.

Figure 2 Unshelled walnut fruits

Figure 3 Shelled walnut

2.4. Enumeration of bacterial isolates

The Petri dishes, conical flask, beaker and universal bottles used were first washed, dried and sterilized in an autoclave at 121˚C for 15 min. and 15 psi. From each group of samples, 6 raw and cooked walnut fruits were randomly picked, cracked (Figure 3) and crushed using a pre-sterilized mortar and pestle, producing a total of twelve bulked samples for the analysis. Ten grams of the bulked ground sample was scooped into a sterile beaker containing ten milliliters of sterile H_2O in a ratio of 1:10 (walnut in gram: water) respectively. The combination was well mixed and became homogenized, this forms the stock solution.1 ml from each of the test tubes was collected using a sterile syringe to perform a 1:10 serial dilution of each sample. The media used were prepared by suspending 45 grams of commercial *Salmonella Shigella* Agar and Trypticase Soy Agar, Mannitol Salt Agar and Baird-Parker Agar, Tryptic soy agar (TSA) powder in 1000 milliliters of purified water; the mixture was heated to properly dissolve the medium. The solution was sterilized by autoclaving as earlier described and the pour plate technique was employed to isolate the bacteria. One ml from each inoculate of dilution factor 10^5 and 10^4 was aspirated into the centre of the labeled sterile plates; TSA was carefully poured into the Petri dishes. A smooth, sterilized spreader glass rod was utilized to evenly distribute the little volume of the bacteria suspension over the agar's surface in the plates. The dishes were tightly covered and sealed using masking tape and were inverted and kept warm at ambient temperature.

2.5. Identification of bacteria isolates from raw and cooked walnut samples

The bacteria growth observed on the agar was sub-cultured on selective media at 35˚C ± 2 for 24 - 48 hours for pure cultures. The total number of bacterial colonies on the plates was determined by counting the visible colonies that were formed on the incubated plates, The formula for computing the colony forming unit (CFU) per ml is given below:

$$CFU/g = \frac{\text{number of bacteria colony} \times \text{reciprocal of the dilution factor}}{plating\ volume\ (\ 1ml\ )}$$

2.6. Biochemical tests on isolated bacteria

Isolated bacteria were put through the following biochemical tests: Gram's reaction, Motility, Lactose, glucose, and catalase test respectively as carried out by Balogun *et al,* 2012.

2.7. Statistical analysis

The collected data were analyzed using the mean, frequency and percentages. Incidence of bacterial colonies was expressed in means ± standard errors (SE) using SPSS package version 20.

3. Results

3.1. Procurement and handling of the walnuts by the FCT marketers

Owing to the fact that walnuts are not grown in the territory, the respondents indicated that all the nuts were procured from middlemen who bought them from farmers in the Southern and Western parts of the country. From the findings, there were two walnut seasons in Nigeria which makes retailers store the walnuts for business continuity when the nuts are out of season.

Figure 4 also shows that 52.0% of the traders locally preserved the cooked walnuts by traditionally submerging them in cold water overnight to lower the storage temperature and preserve the nuts; while only 31.0% use refrigerators, a modern and more effective method of food preservation. The majority (77%) of the marketers in Figure 5 preserve the raw walnuts by airing the nuts under shade for about 4 - 5 hours and bagging them in sacks. This revealed that temperature is essential to the preservation of food products as it either fastens or lowers the microbial build-up process. Zhang (2016) reported a significant decrease in the moisture, fat, protein, saccharine, amino acids, ascorbic acid, phenol and flavonoid compound contents, and antioxidant activities of walnut male inflorescences due to storage temperature.

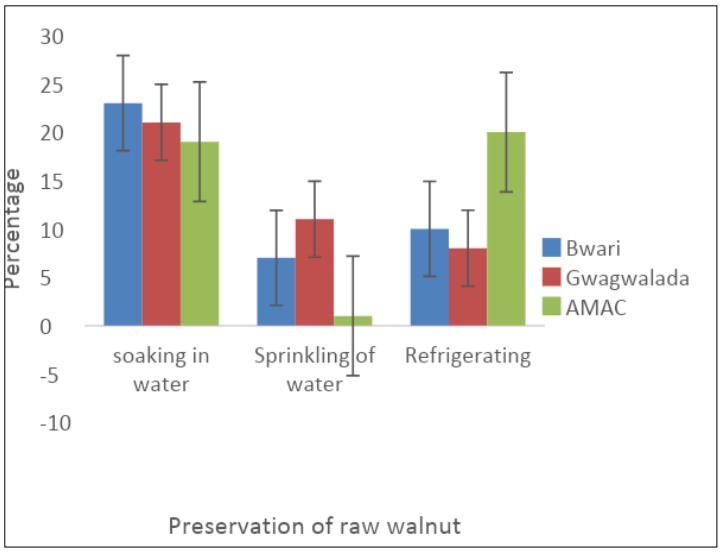

Figure 4 Preservation of cooked walnuts by the FCT retailers

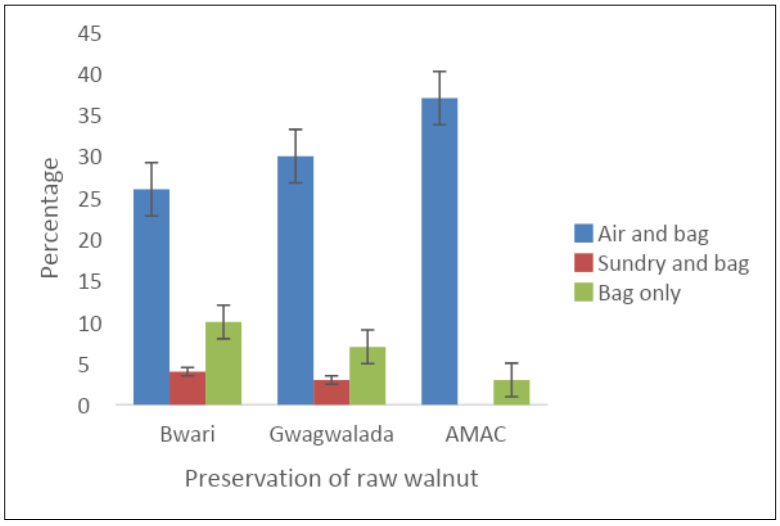

Figure 5 Preservation of raw African walnuts by the FCT retailers

Figure 6 shows that 68% of the respondents use sacks and jute for storage of walnuts while only 11% of the traders use polythene for storage of the nuts

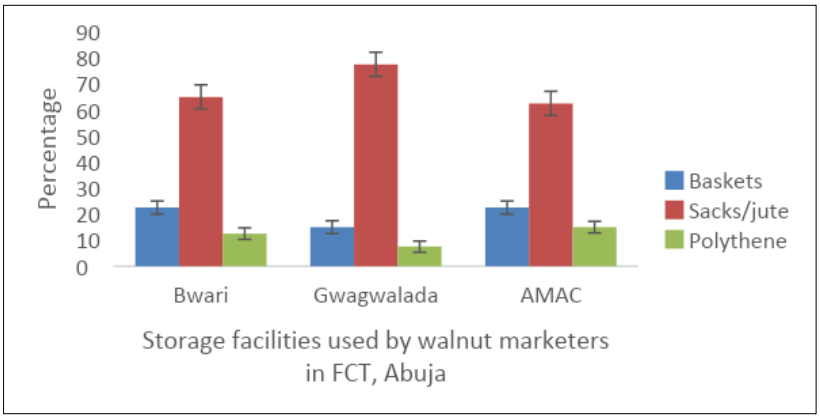

Figure 6 Storage facilities used by walnut retailers in FCT, Abuja

3.2. Bacterial colony count on walnuts obtained from three Area Councils in the FCT Abuja

Table 1 shows that raw walnut samples from Kubwa-Bwari had the highest total bacterial colony count at 3 DAI (2.30×10^6) while Giri-Gwagwalada samples had the lowest total bacterial colony count at 3 DAI (1.13×10^6). For cooked samples, a sample from Garki AMAC had the highest bacterial colony count at 3 DAI (6.80×10^6) while the lowest bacterial colony count at 3 DAI was seen in a sample from Giri in Gwagwalada (1.53×10^6).

Table 1 Bacterial colony count on walnuts obtained from three Area Councils in the FCT Abuja

S/N	Area Council	Sample state	Market location	Total bacterial colony count 3DAI Cfu/ml(10^6) (10^5)
1	Bwari	Uncooked	Dutse	1.36×10^6
2	Bwari	Uncooked	Kubwa	2.30×10^6
3	Gwagwalada	Uncooked	Zuba	1.51×10^5
4	Gwagwalada	Uncooked	Giri	1.13×10^5
5	AMAC	Uncooked	Wuse	1.52×10^5
6	AMAC	Uncooked	Garki	1.84×10^5
7	Bwari	Cooked	Dutse	3.62×10^5
8	Bwari	Cooked	Kubwa	3.86×10^5
9	Gwagwalada	Cooked	Zuba	2.32×10^5
10	Gwagwalada	Cooked	Giri	1.53×10^5
11	AMAC	Cooked	Wuse	3.28×10^5
12	AMAC	Cooked	Garki	6.80×10^6

3.3. Bacteria incidence of occurrence in raw and cooked samples sold in FCT, Abuja

Table 2 shows the bacteria Incidence of raw and cooked walnut samples sold in FCT, Abuja. Bwari samples had an incidence of *Staphylococcus aureus, Escherichia coli, Salmonella* and *Pseudomonas aeruginosa.* The Gwagwalada samples had an incidence of *S. sonnei, P. aeruginosa Salmonella* spp, and *E. coli.* Sample from AMAC had *S. aureus, P. aeruginosa* and *S sonnei.* In the cooked walnut, *P. aeruginosa, E. coli, Salmonella* spp were observed in the Bwari samples, while *E. coli, S. sonnei, S. aureus* were found in the Gwagwalada samples and AMAC sample had the incidence of only *Salmonella* spp, and *E. coli.*

Table 2 Bacteria Incidence in raw and cooked walnut samples sold in FCT, Abuja

Area council	Location	Bacteria incidence of raw sample	Bacteria incidence of cooked sample
Bwari	Dutse	*E. coli, S. aureus*	*P. aeruginosa, E. coli*
Bwari	Kubwa	*P. aeruginosa, S. aureus*	*Salmonella* spp, *E. coli*
Gwagwalada	Zuba	*S. sonnei, P. aeruginosa,*	*S. aureus*
Gwagwalada	Giri	*Salmonella* spp, *E. coli*	*E. coli, S. sonnei, S. aureus*
AMAC	Wuse	*P. aeruginosa, S. aureus,*	*Salmonella* spp, *E. coli*
AMAC	Garki	*P. aeruginosa, S sonnei*	*Salmonella* spp, *E. coli*

Fig 1 Percentage of bacteria Incidence in raw and cooked walnut samples sold in FCT, Abuja

Figure 7 shows that *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* had 25% incidence, while *Salmonella* spp had only 8% in the raw sample. In the cooked samples, there was evidence that *E. coli* had the highest incidence of 42% while *Pseudomonas aeruginosa* and *Shigella sonnei* had the lowest incidence of 8%.

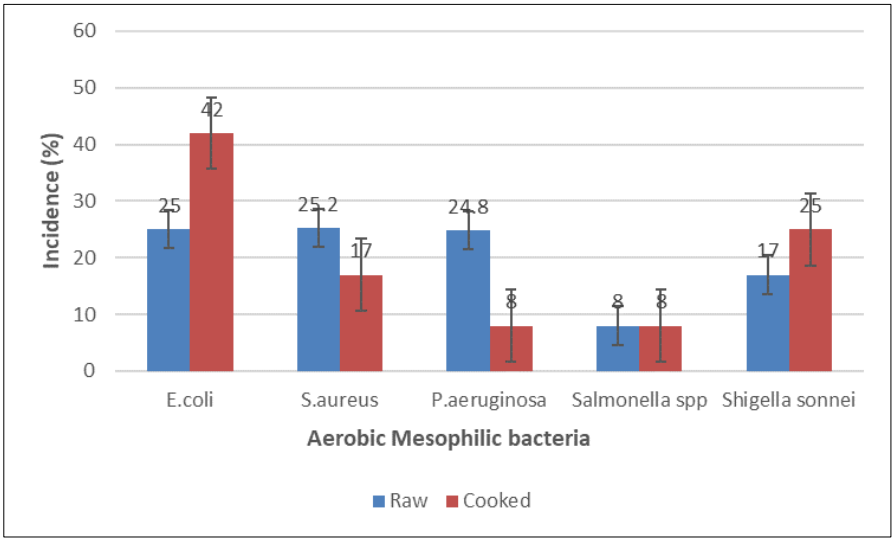

Figure 7 Incidence of Isolated Aerobic Mesophilic bacteria

3.4. Morphological and Biochemical Characteristics of Suspected Bacteria in

3.4.1. Raw and Cooked samples

Tables 3 and 4 show that five species of bacteria were found in both the raw and the cooked samples. *E. coli; P. aeruginosa*; S*. sonnei; Salmonella* spp and *Staphylococcus aureus.* The isolated bacteria consisted of two Gram-positive and 3 Gram-negative bacteria (GRM R^{DX}). Four of them were rod-shaped (*Escherichia coli, Pseudomonas aeruginosa*, *Shigella sonnei and Salmonella* spp) and one was coccus shaped (*Staphylococcus aureus*). While all the tested samples were positive for catalyze (CAT) in both the raw and cooked samples, only some tested positive for Motility, Lactose and Glucose tests

Table 3 Morphological and biochemical characteristics of suspected bacteria in raw samples

Location	GRM R^{DX}	BS	CAT	MO	LAC	GLU	Suspected Bacteria
Dutse	+ve	Rd	+ve	+	+ve	+ve	*Escherichia coli*
Dutse	+ve	Coc	+ve	-ve	+ve	+ve	*S. aureus*
Kubwa	+ve	Coc	+ve	-ve	+ve	+ve	*S. aureus*
Kubwa	-ve	Rd	+ve	-ve	-ve	-ve	*P. aeruginosa*
Zuba	-ve	Rd	+ve	-ve	-ve	+ve	*Shigella sonnei*
Zuba	-ve	Rd	+ve	-ve	-ve	-ve	*Pseudomonas aeruginosa*
Giri	+ve	Rd	+ve	+ve	+ve	+ve	*Salmonella* spp
Giri	-ve	Rd	+ve	-ve	+ve	+ve	*Escherichia coli*
Wuse	-ve	Rd	+ve	-ve	+ve	+ve	*Esherichia coli*
Wuse	+ve	Coc	+ve	-ve	+ve	+ve	*S. aureus*
Garki	-ve	Rd	+ve	- ve	- ve	- ve	*P. aeruginosa*
Garki	-ve	Rd	+ve	- ve	- ve	+ve	*Shigella sonnei*

Note: Rd=rod; Coc- Cocci; +ve - Positive; -ve – Negative; BS- Bacteria shape; CAT- Catalase test; Motility; LAC- Lactose test; GLU- Glucose test

Table 4 Morphological and Biochemical Characteristics of Suspected Bacteria in Cooked samples

Location	GRM R^{DX}	BS	CAT	MO	LAC	GLU	Suspected Bacteria
Dutse	-ve	Rd	+ve	+ve	-ve	-ve	*P. aeruginosa*
Dutse	-ve	Rd	+ve	+ve	+ve	+	*E. coli*
Kubwa	+ve	Rd	+ve	-ve	+ve	+ve	*Salmonella* spp
Kubwa	-ve	Rd	+ve	+ve	+ve	+ve	*E. coli*
Zuba	+ve	Coc	+ve	-ve	+ve	+ve	*S. aureus*
Zuba	+ve	Coc	+ve	-ve	+ve	+ve	*S. aureus*
Giri	-ve	Rd	+ve	+ve	+ve	+ve	*E. sonnei*
Giri	-ve	Rd	+ve	-ve	-ve	-ve	*Salmonella* spp
Wuse	+ve	Rd	+ve	-ve	+ve	+ve	*E. coli*
Wuse	-ve	Rd	+ve	+ve	+ve	+ve	*Salmonella* spp
Garki	+ve	Rd	+ve	-ve	+ve	+ve	*E. coli*
Garki	-ve	Rd	+ve	+ve	+ve	+ve	*Salmonella* spp

Note: Rd=Rod; Coc- Cocci; +ve - Positive; -ve- Negative; Note: Rd=rod; Coc- Cocci; +ve - Positive; -ve – Negative; BS- Bacterial shape; CAT- Catalase test; Motility; LAC- Lactose test; GLU- Glucose test

4. Discussion

Walnut marketers revealed that all the walnuts sold within the FCT were purchased from other Southern States, for the reason that the climatic condition of the Territory does not support the cultivation of walnuts. According to reports, walnuts are commonly grown in the South-eastern and South-western States of Nigeria, with the production regions spanning from Uyo, Akamkpa, Akpabuyo, Lagos, Akure, Kogi, Ajaawa, Ogbomosho, Ibadan, Ife, Ekiti and Ijeshaland (Balogun *et al.,* 2021; Akin *et al.,* 2018). Most of the marketers preserved their walnut based on indigenous knowledge

by Asogwa, (2017). Rellinger (2013) observed and reported that walnuts can be better preserved in the refrigerator for a longer duration of up to three months and kept safe for up to one year by freezing the cooked walnuts.

From this study, 33% of raw samples were contaminated beyond the acceptable limits of ICMSF. This contamination was suspected to be from sites of walnut collection as stated by the study conducted by Zhang *et al*. (2017) that bacterial abundance on walnut surface is proportional to its abundance in the community where they were gathered.

Seventeen (17%) of the cooked sample were contaminated beyond the acceptable limit of the ICMSF standard, this might be because of a rise in the moisture content of the walnut due to cooking which gave way to microbial activity over and since the antimicrobial properties embedded in the raw walnut are already denatured during cooking process this makes deterioration process to set in at a very fast as shown by Hintz *et al.* (2015) Pardeepinder and Michelle.

Pathogenic microbial counts in some of the raw and cooked walnuts sold to consumers exceeded the acceptable limit of $\leq 10^3$ and also outside the tolerable level (10^4–10^5) for total viable counts in ready-to-eat foods as specified by the ICMSF (Izah *et al*., 2022; Kigigha *et al*., 2018

The identified bacteria contaminants on the raw samples show that *E. coli*, *S. aureus* and *P. aeruginosa* were identified in Bwari samples. *S. sonnei, Salmonella* spp, *P. aeruginosa,* and *E. coli* were identified in Gwagwalada samples. While *E. coli, S. aureus, S. sonnei* and *P. aeruginosa* were isolated from AMAC raw samples. In the cooked samples *P. aeruginosa, E. coli, Salmonella* spp and *E. coli* were found in Bwari. *S. aureus, S. sonnei* and *E. coli* were isolated from Gwagwalada samples while AMAC samples had only. These identified microbial contaminants are similar to those found in fruits, nuts and other ready-to-eat foods reported by Sharma and Mazumdar (2014); Wei *et al*. (2019); and Micheal e*t al*. (2022). Samuel *et al*., 2020) carried out a study on food-borne diseases in Nigeria and found that the majority of the bacteria contaminants are enteric and could occur in the human intestine. From the study on the distribution of bacteria on in-shell walnut surfaces in China by Zhang *et al*. (2017), it was indicated that the bacterial community's composition and abundance on walnut surfaces differed depending on the location where they were gathered, and that sample storage duration also affected the bacterial community's abundance. Pardeepinder and Michelle (2018) also confirmed that increased moisture content is a factor that could lead to bacteria proliferation. Low water activity of nuts and grains prevents the growth of most food-borne pathogen on their surfaces. Some of the retailers sold the walnuts on the tray leaving the reserves inside the bag for hours thereby leading to increased temperature, humidity and moisture. (2018)

Escherichia coli has a greater incidence among the bacteria contaminants, the reasons could be because of the use of contaminated water in the washing process of the walnuts as some studies have proven *Escherichia coli* are water-borne organisms (Charles *et al.,* 2015).

5. Conclusion

Bacteria contaminations were noticed in some of the raw and cooked walnut samples sold in the FCT markets, although the raw walnut samples had a higher percentage of contamination as compared to the cooked samples.

Five pathogenic organisms (*E. coli, Salmonella* spp, *P. aeruginosa S. sonnei* and *S. aureus*) were isolated from the raw and cooked walnut samples marketed in the FCT. The isolated microbe's mean counts were above the tolerable limits (10^4–10^5) for ready-to-eat foods.

Walnut is a common fruit snack consumed by the populace believed to be medicinal and nutritious, necessary precautions should be taken during storage, handling, as well as packaging to reduce microbial contaminations that constitute human health hazards.

Compliance with ethical standards

Acknowledgments

We acknowledge the contribution of Joy A. Edeh who designed the experiment and executed the survey research, Toba S. Anjorin, who supervised the project and proofread the manuscript, Shatu W. Asala, who contributed to the literature review, Stella C. Onyeiwu who gave some useful documents and technical advice and Glory Ephraim Akpan who participated in the laboratory experiment.

Disclosure of conflict of interest

There is no conflict of interest to be disclosed.

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