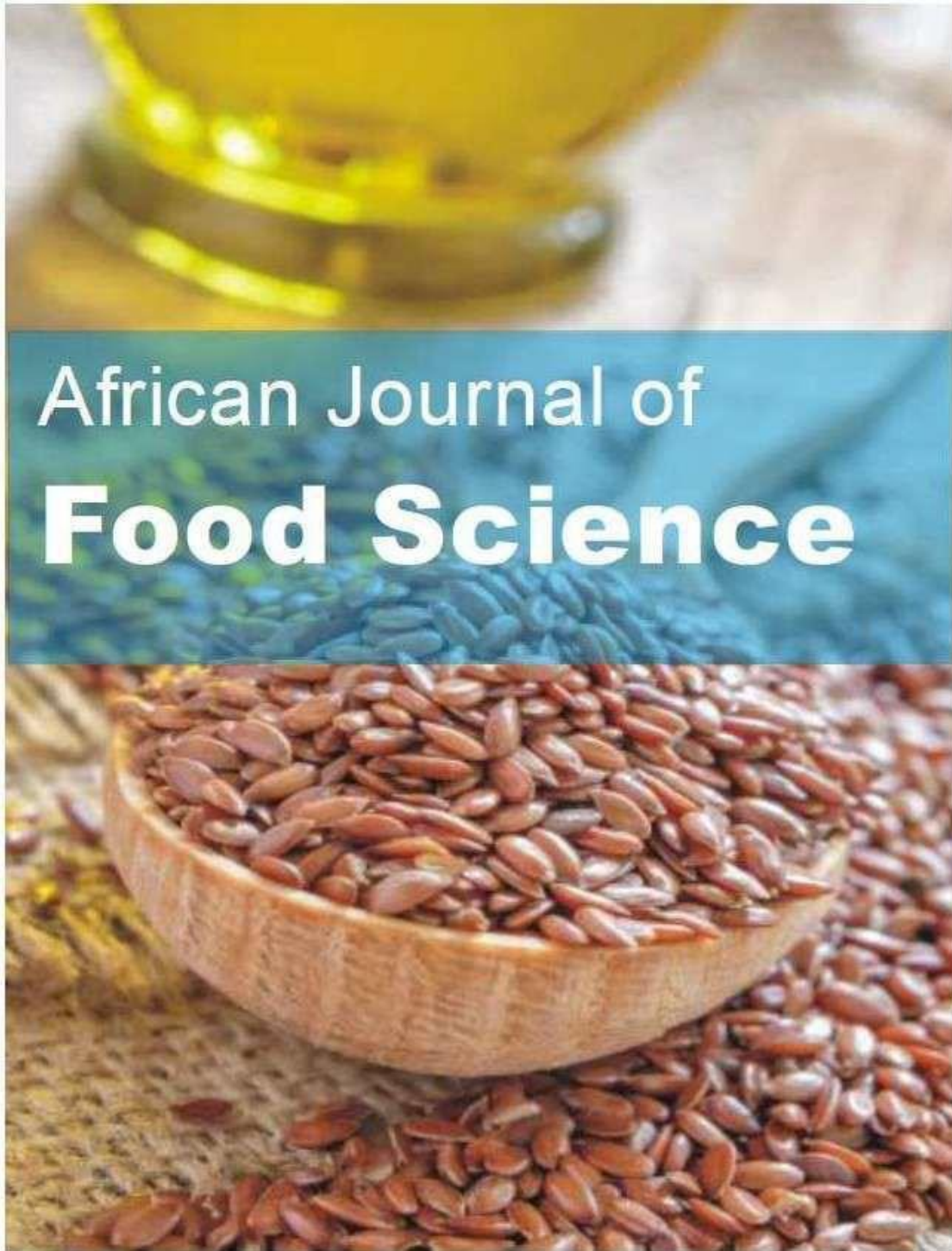


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Full Length Research Paper

A comparative study of the use of radiation, lemon juice, and vinegar for the preparation and preservation of African giant snails (*Achatina* and *Archachatina*)

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African giant snail is a popular alternative source of animal protein in Ghana and many other countries. The meat is high in lean protein and mineral elements. Ready-to-use meat obtained from snails can compete with animal proteins found on the market. After shelling, snails produced some slime that interfered with preparation and processing of the meat. This study aimed to provide consumers with ready-to-use fresh snails conveniently available on the market. The best treatment for eliminating slime from snails was determined using a 2×10×3 factorial design. Vinegar plus salt treatment was the most effective slime removal treatment which led to a significant weight loss. Irradiation at all doses most effectively reduced the microbial load of snails after slime removal. A 6×4×3 factorial design was used for the shelf-life study. Irradiation at 1.5 and 3 kGy extended the shelf life of fresh snails by 14 extra days with the lowest microbial load. Radiation did not affect the fat and mineral content, but the protein content increased. Panelists preferred irradiated snails even though they had different odours and aromas. This study concluded that irradiating fresh snails even at lower doses can extend the shelf-life of fresh snails under refrigeration temperature.

Key words: Contamination, irradiation, *Achatina achatina*, *A. marginata*.

INTRODUCTION

Apart from the conventional protein sources, snail meat is becoming an alternative source of protein for most people in Ghana. Already a delicacy, its consumption increased due to its lean meat nature, being very low in fat and rich in other essential nutrients. According to Cobbinah et al.

(2008), snail is a rich source of iron and calcium but low in fat and cholesterol than other protein sources such as poultry and pork.

Whether cultivated or wild, land snails are usually found in the soil and generally contact soil microorganisms. Soil

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and faecal coliforms are microorganisms of concern associated with snails. Some pathogens associated with fresh land snails included: *Salmonella*, *Staphylococcus*, *Shigella*, and *Arizona*. According to Okafor and Ogbo (2019), 86.7% of achatina obtained from different markets in Nigeria had coliform counts ranging from 10^6 to 10^8 CFU/g.

Despite the growing use of the meat, the studies had not been done on preserving the fresh snail in a ready-to-use state. Snails are still sold in their unshelled state. When shelled, snails contained some amount of slime or mucus naturally present on them. This is a secretory fluid produced from mucus glands located in the skin including the foot plate, mainly used to reduced friction and protected the foot during locomotion in live snails (Wiya et al., 2020).

Medicinally, snail mucus has the ability to facilitate wound healing and to prevent infections due to its many bioactive compounds (Adikwu and Enebeke, 2007).

In food industry however, the slime makes the meat less attractive during processing. According to Etengeneng et al. (2021), the flesh of snails contained some amount of slime and, when removed using the right method, rendered it more attractive and appetizing. Separating the meat from its shell during processing is also time-consuming and provided an opportunity for cross-contamination of the flesh during dressing.

This study aimed to provide consumers with ready-to-use fresh snails readily available to consumers. In doing so, the following will be determined: the best slime removal treatment and its effect on the microbial load of the meat, the shelf life and sensory characteristics of fresh snail meat, and the proximate analyses of irradiated meat snails.

MATERIALS AND METHODS

The study was conducted at the Radiation Technology Centre of Ghana Atomic Energy Commission. Live snail samples approximately of six months were bought from Nsawam farm in the Eastern region of Ghana and used for the study. A $2 \times 10 \times 3$ factorial design was used for this study. Thus, two species of snails were used for the study. The treatment included gamma irradiation (0, 2, 4, 6 kGy), lemon juice (400 and 600 ml), vinegar and salt solution (400 ml for 15 and 30 min) and blanching in water (for 10 and 15 s in three replicates). A $6 \times 4 \times 3$ factorial design was used for the shelf-life study (6 treatments- gamma irradiation at 0, 1.5, 3 kGy; 600 ml of lime juice and a solution of vinegar and salt for 30 min, blanching in water for 15 s; 4 sampling days and 3 replicates).

Removal of slime study

Sample preparation

This study used two snail species (*Achatina achatina* and *Archachatina marginata*). The snails were purged for two days and washed with distilled water to eliminate any soil matter and other debris that may be found on the shell. Shelling was done using a sterile metal pin, and 400g each of the shelled snail samples were

used for the study.

Determination of the effectiveness of the slime removal treatments by the change in weight

Four hundred grams (weight A) each of shelled snail specie were weighed separately into a plastic storage bowl, and different treatments were applied as described below:

Treatment A (Lemon juice): Two concentration of lemon juice were used. Fresh lemon juice of 400 and 600 ml volume were poured on separate snail samples. The snails were immersed in the lemon juice for 15 min, removed, and weighed to obtain weight B (Modification of known Ghanaian traditional method).

Treatment B (Vinegar and salt): Fifty (50) grams of salt were weighed and poured into each beaker containing 300 ml of malt extract vinegar and 100ml tepid water. Each solution was stirred to dissolve the salt. Immersion was done for 15 and 30 min. The snails were removed, and weighed to obtain weight B (A modification of Mezquita et al. (2007).

Treatment C (Blanching): Samples were immersed in boiling water for 10 and 15 s and removed. The weighing was done to obtain weight B.

Treatment D (irradiation): Four hundred grams of shelled snails were put into polyethylene zip-lock pouches and irradiated at 2, 4, and 6 kGy. The snails were weighed after irradiation to obtain weight B (Figure 1).

Chemical analysis

Sample preparation

Four hundred grams of shelled snails were cut into smaller sizes and oven-dried at 60°C overnight till the snails were dried (Babalola and Akinsoyinu, 2009). Dry samples were further broken with a mortar and pestle, and hammer milled to obtain the snail meat powder.

Determination of protein content

Nitrogen was determined using micro-Kjeldahl method. The percentages of nitrogen were converted to protein by multiplying by 6.25 (AOAC, 1990).

Elemental analysis

Atomic absorption spectroscopy

A quantity of 0.5 g of the powdered snail meat sample was weighed into a labelled 100 ml polytetrafluoroethylene (PTFE) Teflon bomb. A 6 ml aliquot of concentrated nitric acid (65%) and 1 ml of Hydrogen peroxide (30%) was added to the sample in a fume chamber. Samples were loaded in a microwave carousel, and the caps securely tightened with a wrench/ forgue. Microwave irradiation of the assembly was done for 18 min in a Milestone Microwave Labstation ETHOS 900 model (report code: 64, INSTR: MLS-200 MEGA) using the following microwave programme (Table 1). Five minutes were allowed for venting.

After digestion, the Teflon bombs were cooled in a water bath to reduce internal pressure and allow volatilized material to restabilise. The digestates were transferred into test tubes and assayed for the



Figure 1. Packaged snails for irradiation B. irradiated snails.
Source: Authors

Table 1. Microwave irradiation of samples.

Step	Time	Power	Pressure	Temp 1	Temp 2
1	00:01:00	250	100	400	500
2	00:01:00	0	100	400	500
3	00:05:00	250	100	400	500
4	00:05:00	400	100	400	500
5	00:05:00	650	100	400	500

Source (Milestone cookbook, update Jan 1, 1996).

presence of Fe, Zn, Mg, Ni, Cu, V, and Mn in Atomic absorption spectroscopy (AAS) on a (VARIAN AA240 FS) in an acetylene-air flame. Reference standards of the elements of interest, blanks, and duplicates of the samples were digested the same way as the actual samples. These served as internal positive controls. Reference standards were from FLUKA ANALYTICAL SIONRA-ALDRICH Chemie GmbH, CH-9471 Buchs product of Switzerland.

Working standards of Fe, Mg, Mn, Cu, Zn, and Ni were prepared by diluting concentrated stock solutions of 1000 mg/l in de-ionized water.

Determination of fat content

A nuclear magnetic resonance (NMR) instrument (Oxford Instruments, U.K.) was used for the analysis. The device was first auto-tuned using acetone. Powdered samples were then poured into a tarred glass tube up to the 4 cm mark. The sample mass was then recorded and transferred into the NMR instrument. The reading of the sample's fat content (%) was recorded.

Microbial analyses

Preparation of serial dilutions

Serial dilutions were done using the general rules for the preparation of initial suspension and decimal dilutions (ISO 6887-1-2017) to estimate the total viable counts of bacteria present in the snail meat. A 10g portion of the sample (snail) removed from the shell was aseptically transferred into 90 ml of sterile buffered peptone water under laminar flow hood to obtain a 1:10 dilution. The suspension was then agitated with a vortex for 5 min to ensure a homogenous mixture. Sevenfold serial dilution was made into McCartney bottles containing 9ml peptone water. 1ml of

the suspension was aseptically transferred into a 90 mm sterile and well labelled Petri dish (double trial for each dilution) beginning with the least dilution. Molten plate count agar and violet, red bile agar all at about 45°C were aseptically poured respectively according to labels; plates were swirled gently to mix and left for about 15 min to solidify. The solidified plates were incubated at 37 C for 18- 24 h in a microbiological incubator. After 24 h of incubation, cultures containing 30-300 colonies were selected and counted using a colony counter (Stuart Scientific).

Total viable count

The average count was calculated (arithmetic mean) with the duplicate plates at two consecutive dilutions. The total aerobic count (N) of CFU/g or ml of the test sample is calculated according to standard protocol (ISO7218:1996, ISO6887-1:2017).

$$\frac{c}{V(n_1 + 0.1n_2)} d$$

Where: C is the sum of colonies on all selected plates counted

V is the volume applied to each plate

n_1 is the number of plates counted at the first dilution

n_2 is the number of plates counted at the second dilution

d is the dilution from which the first count was obtained.

Total coliform count

Sample preparations, serial dilutions, and determination of the CFU/g were made as described for the total aerobic bacterial count. The molten Violet Red Bile Agar was cooled to 50°C and poured into a petri dish. The mixture was swirled gently and allowed to cool. Incubation was done at 37°C for 18-24 h.

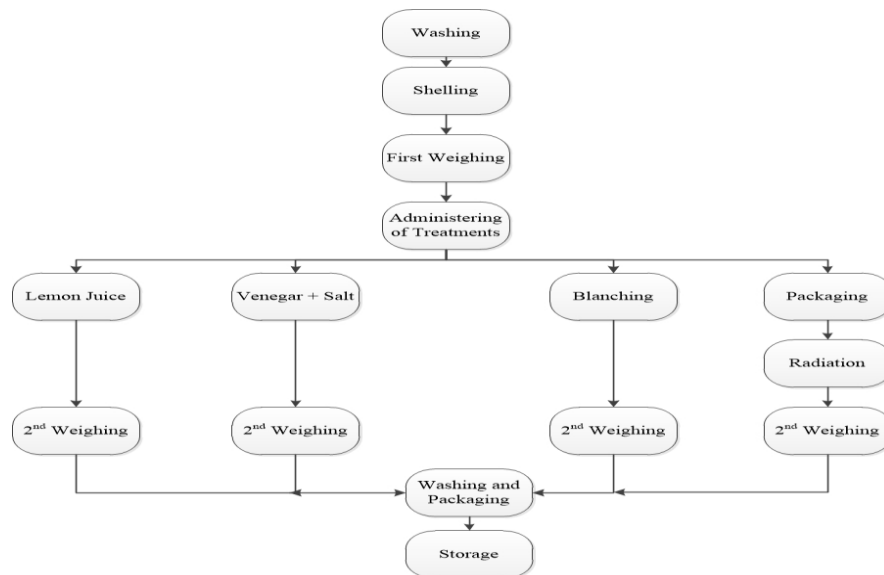


Figure 2. Flow chart for snail processing.
Source: Authors

Detection and enumeration of *Staphylococcus*

Serial dilution was made as described above. Plating was done on molten Baird-Parker medium enriched with egg -yolk emulsion, and incubation was done at 37°C for 24 - 48 h. Incubated plates were observed for blackish colonies with mucoid surroundings and counted according to standard methods.

Detection and enumeration of *Salmonella*

Twenty five grams of each snail sample was aseptically transferred into 225 ml of buffered peptone water homogenized with a vortex mixer and incubated at 37°C for 18 to 24 h. 0.1 ml and 1ml of the incubated stock culture were transferred into 10ml of sterile Rappaport-Vassiliadis soya (RVS) peptone broth and Muller-Kauffman Tetrathionate-novobiocin broth (MKTn), respectively. These were then incubated at 37°C for 20 - 24 h proceeded by a subculture onto xylose lysine deoxy-chocolate agar (XLD agar). Inoculated plates were incubated at 37°C for 20 - 24 h and examined for typical blackish colonies. Further biochemical identification was performed using API 20E (BioMerieux).

Detection and enumeration of *E. coli*

Sub-cultures were made from typical pinkish colonies from VRBA agar with a sterile inoculation loop. The cultures were streaked on eosin methylene blue agar plate and incubated at $44 \pm 1^\circ\text{C}$ for 18 to 24 h. Further, plates were observed for a shiny metallic sheen (typical of fecal coliforms on EMB agar); Sub-culturing was done to obtain a pure culture. The effect of the slime removal treatment on the microbial load of snails was determined. Immediately after treatment, the Total Viable Count (TVC), coliform count, *Salmonella* and *Staphylococcus* count were determined as described above.

Shelf-life study

Achatina achatina was used for this study. Four hundred grams

each of shelled snails were administered 6 different treatments and observed during a 21-day storage period at a refrigeration temperature of 4°C. Samples were taken at (0, 7, 14 and 21 days) for microbial and sensory analyses during the storage period. All analysis was done in triplicates. Figure 2 shows the flow chart of snail processing.

Sample preparation

A. achatina approximately 6 to 8 months, from a snail farm (Assin Fosu) with the same growth condition was used for the shelf-life study. Shelling and slime removal was done as described above. Irradiation was, however, done at 1.5 and 3 kGy. All samples were stored under a refrigeration temperature of 4°C. Microbial analyses and sensory evaluation, were done within 21 days (0, 7, 14, and 21 days).

Microbiological analyses

Enumeration of total coliforms and total viable counts was done during storage as described above.

Sensory evaluation

Sample preparation

The sensory properties of snails were analysed by 20 trained panelist from the Department of Food Science and Radiation Processing; Ghana Atomic Energy Commission. Samples were cut into equal sizes, boiled in 200ml of water for 15 min and randomly selected for sensory evaluation. Panelists were asked to evaluate cooked snails for colour, taste, flavour, aroma, and tenderness, and fresh snails for colour, firmness, odour, and sliminess. Structured questionnaires aimed at evaluating the effect of each treatment on snails were used and a 9-point hedonic test was used to express their degree of preference for the models. Data from the sensory analysis were pooled and analyzed for the study.

Table 2. Total means weight loss of two species of snail after treatment.

Treatment		<i>A. achatina</i>	<i>A. marginata</i>
Doses of gamma irradiation	0 kGy	-23.0±12.7 ^a	-22.0±9.48 ^b
	2 kGy	-113.8±12.7 ^{cd}	-101.2±9.48 ^d
	6 kGy	-114.3±12.7 ^{cd}	-94.9±9.48 ^d
	4 kGy	-124.5±12.7 ^{cd}	-102.2±9.48 ^d
Vinegar and salt solution	15min	-126.6±12.7 ^{cd}	-101.8±9.48 ^d
	30min	-144.7±12.7 ^d	-95.3±9.48 ^d
Blanching in water	15 sec	-39.3±12.7 ^{ab}	+16.0±9.48 ^a
	10 sec	-48.2±12.7 ^{ab}	+17.5±9.48 ^a
Lemon juice	600ml	-70.7±12.7 ^{bc}	-76.5±9.48 ^{cd}
	400ml	-89.2±12.7 ^{bc}	-57.2±9.48 ^c

Values are means ± standard deviation. Different superscripts within the same columns are significantly different ($p \leq 0.05$).

Source: Authors

Data analysis

Data obtained were analyzed using Microsoft Excel and StatsGraphix Centurion XVI. Means were separated using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Effect of various treatments on slime removal

The effects of the various treatments on the slime removal are shown in Table 2. There were significant differences ($p \leq 0.05$) among each treatment. Vinegar + salt had the highest weight loss of -144.7 and -126 for 30 and 15 min dipping time, respectively. Blanching had the lowest weight loss of -48.2 at 10 s and -39.3 at 15 s for the *A. achatina* and a weight gain of 17.5 at 10 s and 16.0 at 15 s for *A. marginata*.

After shelling, snails produced some amount of slime. Burton (2003) compared this slime to blood released when the foot is irritated. It consisted of 95.2% of moisture (Danladi and Haruna, 2020), and the remaining part of mucus without water consisted of a mixture of proteoglycans, glycosaminoglycans, glycoprotein enzymes, hyaluronic acid, copper peptides, antimicrobial peptides, and metal ions (Griestorfer et al., 2017). It is therefore expected that the removal of the slime will be led to some weight loss in the meat.

Vinegar + salt treatments were most effective, achieving the highest mean weight loss. Followed with irradiation at 4, 6, and 2 kGy, then lemon juice and the least was blanching. Irradiation is known to break-down the viscosity properties of polysaccharides such as starch because the β - Glucan components of starch responsible for its gelling properties decreases in viscosity upon irradiation due to the radiolysis of the glycosidic bonds (Eui-Hong et al., 2007). Therefore, it was anticipated that

irradiation might break-down the slime associated with the meat.

The efficacy of these treatments measured as weight loss was less significant in the *A. marginata* and gave in weight gain in the blanched treatments. This may be due to the difference between the components and the slime structure from the *Archachatina* species. The slime of *A. marginata* had a thicker consistency than the *A. achatina*. This increased its tendency to absorb water resulting in weight gain.

Effects of slime removal treatment on the microbial load of snails

The TVC and coliform count of snails before and after slime removal are shown in Table 3. Irradiation at 2, 4, and 6 kGy led to 4, 5, and 5 \log_{10} cycle reductions of TVC; a 6 \log_{10} cycle reduction each in coliform counts. Both levels of blanching were least effective for reducing the TVC and the coliform counts, in which gave a one \log_{10} reduction in both TVC and coliform counts. All doses of irradiation and vinegar + salt treatments led to a 5 \log_{10} cycle reduction in *Salmonella* counts. Blanching for 10 s, however, gave a one \log_{10} reduction of *Salmonella*. Again, vinegar + salt treatment and irradiation at 6 kGy reduced *Staphylococcus* counts by a 6 \log_{10} reduction each. Blanching for 10 s, however, led to a 2 \log_{10} cycle reduction.

Effect of slime removal treatments on the microbiological quality of snails

All treatments had significant effect on the TV and coliform counts of the snail meat. Irradiation at 2, 4, and 6 kGy was most effective for reducing the TVC on the snail

Table 3. Effect of removal of slime treatments on the microbial load of snails.

Treatment	Microbial count log ₁₀ CFU/g			
	TVC	Coliform	Salmonella	S. aureus
0 kGy	7.61±0.07 ^g	6.38±0.53 ^d	5.60 ± 0.07 ^d	6.06 ± 0.51 ^e
2 kGy	3.44± 0.11 ^b	<1	<1	2.68 ± 0.07 ^c
4 kGy	2.18 ± 0.01 ^a	<1	<1	2.11 ± 0.07 ^{bc}
6 kGy	2.06 ± 0.02 ^a	<1	<1	<1
Lemon juice(400ml)	4.48± 0.15 ^c	3.83± 0.34 ^a	3.38 ± 0.42 ^b	1.13 ± 1.24 ^a
Lemon juice(600ml)	4.64± 0.13 ^c	4.05± 0.11 ^{ab}	2.14 ± 0.11 ^a	1.42 ± 1.69 ^{ab}
Vinegar+salt (15m)	4.82±0.10 ^d	4.60±0.42 ^c	<1	<1
Vinegar+salt (30m)	4.55± 0.18 ^c	4.39±0.34 ^{bc}	<1	<1
Blanching (15sec)	6.32±0.45 ^e	6.16±0.84 ^d	2.28 ± 0.35 ^a	4.67 ± 0.07 ^d
Blanching (10sec)	6.71±0.21 ^f	6.24±0.49 ^d	4.15 ± 0.69 ^c	4.23 ± 0.11 ^d

Values are mean count ± standard error.* Values with the different superscripts within the same columns are significantly different (P≤0.05).
Source: Authors

meat samples, and the least was blanching.

The immediate effect of ionizing radiation on the bacterial load of snails confirmed the effective use of ionizing radiation alone in reducing microbial count on food substances. This was observed with a sharp decline in log CFU of the total aerobic and fungal count, and a complete decontamination *Triphala* exposed to gamma-radiation at 5 kGy (Kumari et al., 2009). According to Brewer (2009), a 90% reduction of most vegetative cells can be accomplished with 1-1.5 kGy; hence a higher dose of 2, 4, and 6 kGy was expected to cause higher reduction both in the vegetative parts and some of the sensitive spores. Moini *et al.* (2009) reported that irradiation at 1, 3, and 5 kGy significantly reduced the total viable count of rainbow trout fillets. Radiation of the snail meat, at 2, 4, and 6 kGy, caused complete elimination of coliforms achieving a 6 log₁₀ cycle reduction. The high count of *Salmonella* was eliminated from the sample after irradiation. *Salmonella* is radio-resistant; hence, a treatment designed to eliminate *Salmonella* will ultimately destroy all Gram-negative pathogens (Belle and Tofana, 2010).

Commercially, food substances have been treated with preservatives such as salt and vinegar to reduce the microbial load, prolong the shelf life, and impact the sensory characteristics. According to Eyabi et al. (2001), several studies had been carried out on the microbial effects of treatment with salt and other preservatives such as vinegar, BHT, BHA, citric acid, potassium sorbate etc. of food. Some of these treatments have been used together to have a synergistic effect on the samples. In this study, vinegar + salt treatments not only eliminated the slime associated with the snail but reduced the TVC by a 3-log₁₀ cycle and the coliform count by a 2 log₁₀ cycle. The combination of vinegar and salt had a better effect of eliminating all *Salmonella* and *Staphylococcus* from the samples. Similarly, in a study to

evaluate the impact of various concentrations of vinegar on *Staphylococcus* and other microbial loads of fish, commercial vinegar reduced *Staphylococcus* and other bacterial population due to its acetic acid content (Mohamed et al., 2011). According to Bibek (2005), acetic acid, salts, and vinegar (which contained 5 to 40% acetic acid and many other compounds) are used in different foods for inhibiting growth and reducing the viability of Gram-positive and Gram-negative bacteria, yeasts, and molds.

Lemon juice contained citric acid which has some bactericidal effect. This was confirmed with a 3-log₁₀ cycle reduction in the TVC of snails treated with lemon juice. Both levels of lemon juice did not eliminate all microorganisms of coliform, *Salmonella*, and *Staphylococcus* but resulted in significant decreases in their counts. Similarly, Bingol et al. (2011) observed that treatment of lemon juice for different exposure times caused reduction ranging between 0.1 and 1.7 log₁₀ CFU/g for *Salmonella*. Some bactericidal effect of lemon juice on *Salmonella* inoculated in stuffed mussels was observed by Kisla (2007), where 0.08 to 0.25 log₁₀ CFU/g reductions in mussels treated with lemon juice and 0.22 to 0.78 log₁₀ CFU/g reductions treated with lemon juice dressing were observed. Similar observations were made of carrot samples treated with lemon juice and vinegar, for different exposure times, which caused significant reductions ranging between 0.79 to 3.95 and 1.57 to 3.58 log₁₀ CFU/g, respectively. Lemon juice and other organic acids are usually pre-treatments in many food processing cycles. The initial reduction achieved, therefore, was desirable in most situations.

Pipek et al. (2004) observed that citric acid reduced mesophilic and psychrotrophic microorganisms on pork and beef. When compared to several other mild preservation procedures, lemon juice is inexpensive and uncomplicated as a method of extending shelf-life.

Table 4. Pooled means of microbial counts of snails with different treatment during storage.

Days	Total aerobic plate count	Total Coliform count	Total <i>Salmonella</i> Count	Total <i>Staphylococcus</i> Count
	Log₁₀ CFU/g			
0	5.51 ±0.02 ^a	5.03±0.03 ^a	4.79±0.03 ^c	5.0.8±0.03 ^c
7	6.16 ±0.02 ^b	6.22±0.03 ^c	5.04±0.03 ^d	5.16±0.03 ^c
14	6.82 ±0.02 ^d	5.08±0.03 ^a	4.07±0.03 ^a	3.54±0.03 ^a
21	6.30 ±0.02 ^c	5.17±0.03 ^b	4.58±0.03 ^b	4.04±0.03 ^b
Treatment				
0 kGy	7.97 ±0.03 ^f	8.01±0.03 ^f	7.85±0.04 ^f	7.47±0.04 ^f
1.5 kGy	5.26 ±0.03 ^b	3.13±0.03 ^b	1.96±0.04 ^b	2.19±0.04 ^b
3 kGy	3.91 ±0.03 ^a	1.36±0.03 ^a	1.11±0.04 ^a	1.02±0.04 ^a
Vinegar + salt	6.75 ±0.03 ^d	6.74±0.03 ^d	5.16±0.04 ^d	4.89±0.04 ^d
Lemon juice	6.04 ±0.03 ^c	5.73±0.03 ^c	4.97±0.04 ^c	5.97±0.04 ^e
Blanching	7.28 ±0.03 ^f	7.29±0.03 ^e	6.66±0.04 ^e	4.60±0.04 ^c

*Values are mean count ± standard error. *Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

High-temperature treatments such as blanching have been used in several processing steps as an initial treatment to reduce the microbial load and maintain color or stop enzyme action (Shaheen et al., 2012). Both treatment levels of blanching used in this study led to 1 log₁₀ cycle reduction in the TVC and coliform counts; however, significant decreases were observed in *Salmonella* and *Staphylococcus* counts. These observations were expected since blanching was known to reduce the number of contaminating microorganisms on the surface of foods and assist in subsequent preservation operations (Fellows, 2000). These results indicated that snail meat's initial high microbial load can be significantly reduced by most of these treatments.

Effect of storage on microbial quality of snails

At refrigeration temperatures, most microbial actions are reduced but not completely halted; however, treatments administered before storage influenced the rate at which microorganisms proliferated on the samples. Microbial counts increased in all samples as storage progressed; however, the irradiated samples recorded the lowest counts while blanched samples had the highest count (Table 4).

The lethal effect of ionizing radiation on microorganisms in radiation processed fresh foods usually results from the radiolysis of water and the production of hydrogen peroxide and other free radicals during radiation. This free radical, especially hydrogen peroxide, caused damages to the cells and DNA of microorganism, resulting in mutations and cell death (Lewis et al., 2002). This study showed that gamma radiation was effective in

reducing the TVC on snail samples. A 1.5 kGy led to a 2 log₁₀ cycle reduction in TVC, while a higher dose of 3 kGy led to a 4 log₁₀ cycle reduction (Table 4).

Salmonella population on the snail samples reduced with radiation dose, but there was an increase and some reductions during storage. Radiation at 3 and 1.5 kGy led to a 6 log₁₀ cycle reduction in the *Salmonella* count and a 5 to 6 log₁₀ cycle reduction in *Staphylococcus* count (Table 4). These reductions observed are due to the combined effect of radiation and refrigeration temperatures on the microorganisms. Ionizing radiation alone can be destroyed most microorganisms in food; however, the radio-resistant ones are further eliminated by refrigeration. Similarly, irradiating raw oysters at 1.5 kGy was enough to make them safe from pathogenic bacteria such as *E.coli*, *Shigella*, and *Vibronaceae* (Gelli et al., 2001).

The citric acid content (pH) of lemons acts as a growth inhibitor of many microorganisms. Lemon juice treatment on snail samples led to 1, 3, 3, and 2 log₁₀ cycle reductions in TVC, coliform, *Salmonella*, and *Staphylococcus* counts, respectively (Table 4). Similarly, lemon juice treatment for different exposure times caused a reduction range of 0.1 to 1.5 log₁₀ CFU/g for *Salmonella enteritidis* and 0.1 to 2.1 log₁₀ CFU/g for *E. coli* (Bingol et al., 2011). More than 5 log₁₀ reduction of stationary phase cells of 5 strains of *E. coli* 0157:H7 was achieved in both lemon and lime juice treatment (Enache et al., 2009).

Salts and organic acids are known to cause reductions in microbial loads in foods. Organic acids and extend the shelf life of refrigerated meat, poultry, and fish products by inhibiting the growth of spoilage and pathogenic bacteria (Sallam, 2007). From this study, a combination treatment of vinegar and salts for immersion time of 30

Table 5. Pooled mean scores of multiple comparison of cooked snails with different treatments during storage.

Days	Taste	Flavour	Aroma	Tenderness	Colour
0	6.06±0.05 ^c	6.12±0.05 ^a	5.79±0.05 ^a	6.08±0.51 ^b	6.12±0.04 ^a
7	5.99±0.05 ^c	6.48±0.05 ^a	6.00±0.05 ^b	6.58±0.51 ^c	6.46±0.04 ^c
14	4.53±0.05 ^b	6.12±0.05 ^b	6.04±0.05 ^{bc}	6.06±0.51 ^b	6.26±0.04 ^b
21	4.36±0.05 ^a	6.28±0.05 ^c	6.15±0.05 ^c	5.75±0.51 ^a	6.28±0.04 ^b
Treatment					
0 kGy	2.98±0.07 ^a	6.0±0.07 ^{ab}	5.9±0.06 ^{ab}	5.69±0.06 ^a	5.85±0.06 ^a
1.5 kGy	6.29±0.07 ^c	6.78±0.07 ^d	6.16±0.06 ^d	6.78±0.06 ^c	6.87±0.06 ^d
3 kGy	6.29±0.07 ^c	6.74±0.07 ^d	6.15±0.06 ^d	6.62±0.06 ^c	6.82±0.06 ^d
Lemon juice	6.19±0.07 ^c	6.08±0.07 ^{bc}	5.91±0.06 ^{ab}	6.04±0.06 ^b	6.34±0.06 ^c
Vinegar+salt	6.04±0.07 ^b	6.07±0.07 ^{bc}	6.02±0.06 ^{cd}	6.11±0.06 ^b	6.16±0.06 ^b
Blanching	6.02±0.07 ^b	6.25±0.07 ^c	6.04±0.06 ^{cd}	6.08±0.06 ^b	6.12±0.06 ^b

Values are mean count ± standard error. *Different uppercase superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

min led to a 1, 2, 2, and 3 log₁₀ cycle reductions in the TV, coliform, *Salmonella*, and *Staphylococcus* counts; however, microbial counts increased during storage (Table 4). The microbial load in pork was reduced during storage by 0.5 to 2 log₁₀ CFU/g after treatment with organic acids and salts mixture under refrigeration (Ratanatriwong et al., 2009).

Even though blanching did not lead to a significant decrease in the TVC of snail samples, coliform, *Salmonella*, and *Staphylococcus* counts have reduced by 1, 1, and 3 log₁₀ cycles, respectively. The main objective of blanching is to inactivate enzymes and surface microorganisms associated with food spoilage (Fellows, 2017). Therefore, it is important to note that microorganisms may be increased during storage after blanching has been done as a pre-treatment.

Sensory evaluation of selected attributes for cooked snail after different treatments

Table 5 show the scores for the attributes measured of cooked snails. Significant differences ($P \leq 0.000$) were recorded in the values for colour, flavour, tenderness, and taste; and significant differences ($P \leq 0.05$) in the aroma. Irradiation at 1.5 and 3 kGy had no significant effect ($P \geq 0.05$) in all attributes measured, but significant differences ($P \leq 0.05$) were observed in samples treated with lemon juice and vinegar + salt. Hedonic test for cooked snails had significant differences ($P \leq 0.05$) in all attributes measured. Pooled mean values for irradiated samples of cooked snails had no significant difference; however, there were significant differences ($P \leq 0.05$) between the irradiated samples and other treatments (Table 7).

Sensory evaluation of raw snail after different treatments

The scores of the attributes measured for raw snails with different treatments are shown in Tables 6 and 8. Values showed significant differences for firmness and sliminess ($P \leq 0.05$) and highly significant differences ($P \leq 0.000$) in the colour and odour for raw snails with different treatments during storage. Samples irradiated at 1.5 and 3 kGy showed no significant effect in the color and sliminess of raw snails but showed a significant difference in both samples' firmness.

Lemon juice, vinegar + salt, and blanched samples had substantial differences in all attributes measured significant differences ($P \leq 0.05$) were recorded in all the attributes in all attributes (Table 8) measured during storage, except aroma which showed a highly significant difference ($P \leq 0.000$). Pooled means of irradiated samples showed no significant difference ($P \geq 0.05$) in all attributes measured, but significant differences ($P \geq 0.05$) existed between irradiated samples and other treated samples. There were significant differences ($P \geq 0.05$) in all attributes measured for samples treated with lemon juice, vinegar + salt, and blanching.

Effect of radiation on elemental, protein, and fat content of *A. achatina*

Snail meat is a rich source of trace elements for human development. Generally, the effect of irradiation on these elements had no specific trend. Iron and manganese concentrations increased with increased radiation dose; however, copper, zinc, magnesium, and nickel

Table 6. Pooled means scores of multiple comparison of raw snails with different treatments during storage.

Days	Colour	Firmness	Odour	Sliminess
0	6.10±0.04 ^a	6.18±0.04 ^b	5.78±0.05 ^a	5.78±0.35 ^a
7	6.10±0.04 ^a	5.81±0.04 ^a	6.52±0.05 ^b	5.97±0.35 ^b
14	6.26±0.04 ^b	6.06±0.04 ^b	6.67±0.05 ^c	5.85±0.35 ^a
21	6.31±0.04 ^b	5.73±0.04 ^a	6.67±0.05 ^c	5.84±0.35 ^a
Treatment				
0 kGy	5.71±0.05 ^a	5.57±0.06 ^a	6.33±0.06 ^a	5.79±0.04 ^b
1.5 kGy	6.83±0.05 ^c	6.68±0.06 ^d	6.39±0.06 ^{ab}	6.0±0.04 ^d
3 kGy	6.85±0.05 ^c	6.47±0.06 ^c	6.42±0.06 ^{ab}	5.99±0.04 ^d
Lemon juice	6.01±0.05 ^b	5.85±0.06 ^b	6.46±0.06 ^{ab}	5.86±0.04 ^{bc}
Vinegar + salt	6.13±0.05 ^b	5.7±0.06 ^{ab}	6.52±0.06 ^b	5.85±0.04 ^{bc}
Blanching	6.06±0.05 ^b	5.8±0.06 ^b	6.44±0.06 ^{ab}	5.95±0.04 ^{cd}

*Values are mean count ± standard error *Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

Table 7. Pooled mean scores of hedonic scale of cooked samples with different treatments during storage.

Day	Aroma	Colour	Flavour	Taste	Tenderness
0	6.34±0.12 ^b	6.59±0.11 ^b	6.37±0.11 ^c	6.69±0.10 ^c	6.41±0.11 ^c
7	5.10±0.12 ^a	5.70±0.11 ^a	5.90±0.11 ^b	5.86±0.10 ^b	5.41±0.11 ^a
14	5.37±0.12 ^a	6.33±0.11 ^{ab}	5.47±0.11 ^a	4.73±0.10 ^a	5.97±0.11 ^b
21	5.28±0.12 ^a	6.21±0.11 ^b	5.50±0.11 ^a	4.66±0.10 ^a	6.04±0.11 ^b
Treatment					
0 kGy	3.7±0.16 ^a	6.04±0.15 ^a	4.68±0.15 ^a	2.77±0.13 ^a	5.53±0.04 ^a
1.5 kGy	6.74±0.16 ^d	6.3±0.15 ^{ab}	6.89±0.15 ^c	6.99±0.13 ^c	6.45±0.04 ^c
3 kGy	6.78±0.16 ^d	6.09±0.15 ^{ab}	6.84±0.15 ^c	7.01±0.13 ^c	6.24±0.04 ^{bc}
Lemon juice	5.92±0.16 ^c	6.31±0.15 ^{ab}	5.98±0.15 ^b	6.16±0.13 ^b	5.94±0.04 ^{ab}
Vinegar + salt	5.65±0.16 ^c	6.5±0.15 ^b	6.21±0.15 ^b	6.33±0.13 ^b	6.08±0.04 ^{bc}
Blanching	5.67±0.16 ^c	6.11±0.15 ^{ab}	5.85±0.15 ^b	6.22±0.13 ^b	5.91±0.04 ^{ab}

Different uppercase superscripts within the same columns are significantly different (P≤0.05).

Source: Authors

concentrations increased or decreased with no particular direction (Table 9). The mean value of iron concentration in the control sample was 7.04. This value fell in the range of 6.79 and 11.09 mg/kg were obtained for *Nucellalapillus* and *Archachatina marginata ovum* (Eneji et al., 2008). The mean values of 0.847, 11.16, and 9.80 mg/kg concentrations were recorded for manganese, copper, and zinc. Although, magnesium values were highest at 13.55 for the control, this value was lower than 28.00 mentioned with Ogbuagu (2011) for *Achatina* samples in Nigeria. The concentration of copper in control was lower than the recommended limit of 10 mg/kg.

Micro-nutrients such as Fe and Mg are essential trace

elements due to the major role played in the metabolic processes of humans and the total well-being of humans. Magnesium functions as a co-factor in enzymatic activities, while the iron is a major component of hemoglobin in human blood. A zinc deficiency is marked by retarded growth, loss of taste, and hypogonadism, leading to decreased fertility (Adebayo-Tayo et al., 2011).

Copper is necessary for the synthesis of haemoglobin, and its deficiency can result in anaemia in children. In snails, trace element content varies due the feeding habits, location (where the snail is found or picked from) and as well as where it feeds from (Ozogul et al., 2005).

The fat content of snails was rather very low. Samples irradiated at 2 and 6 kGy recorded no significant

Table 8. Pooled mean scores of hedonic scale of raw samples with different treatments during storage.

Days	Colour	Firmness	Odour	Sliminess
0	6.52±0.12 ^b	6.60± ^a	6.06±0.13 ^b	5.84±0.12
7	6.17±0.12 ^a	6.16± ^a	5.06±0.13 ^a	5.84±0.12
14	6.36±0.12 ^b	6.23± ^a	5.16±0.13 ^a	5.97±0.12
21	6.19±0.12 ^b	5.98± ^a	5.14±0.13 ^a	5.91±0.12
Treatment				
0 kGy	6.23±0.16B ^{cd}	5.7±0.17 ^a	2.86±0.17 ^a	5.28±0.16 ^b
1.5 kGy	6.84±0.16 ^e	6.84±0.17 ^b	7.01±0.17 ^e	6.73±0.16 ^d
3.0 kGy	6.67±0.16 ^e	6.65±0.17 ^b	6.78±0.17 ^e	6.69±0.16 ^d
Lemon juice	5.73±0.16 ^a	5.94±0.17 ^a	5.89±0.17 ^{cd}	6.09±0.16 ^c
Vinegar + salt	6.04±0.16A ^b	5.99±0.17 ^a	5.13±0.17 ^c	5.47±0.16 ^b
Blanching	6.14±0.16A ^{bc}	6.63±0.17 ^b	5.95±0.17 ^d	6.31±0.16 ^{cd}

Different uppercase superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

Table 9. Fat, protein, and mineral analysis of *Achatina achatina* before and after irradiation.

Sample	Mineral (elemental) composition (mg/kg)						Proximate composition (g/ 100g)	
	Fe	Mn	Cu	Zn	Mg	Ni	Fat	Protein
0 kGy	7.04	0.847	11.16	9.8	13.55	0.26	0.733±0.004 ^a	16.85±1.21 ^a
2 kGy	18.2	1.48	6.44	6.88	7.73	0.12	0.723±0.004 ^a	30.93±1.21 ^c
4 kGy	22.84	1.36	6.4	6.84	20.44	1.2	0.753±0.004 ^b	22.89±1.21 ^b
6 kGy	20.6	1.32	7.68	7.68	27.75	4.8	0.736±0.004 ^a	26.61±1.21 ^b

*Values are mean count ± standard error *Different superscripts within the same columns are significantly different ($P \leq 0.05$).

Source: Authors

differences in the fat content at 2 and 6 kGy (Table 9). When rainbow trout was irradiated at 1, 3 and 5 kGy, there was no significant change in the fatty acid composition (Oraei et al., 2011) an indication that lower doses and other factors may not have any significant difference in the fat content of food.

The increase in protein content of the snail samples did not follow any trend (Table 9). This is similar literature (Nour et al., 2009), where protein contents in pea nuts increased significantly from 25.84 to 29.75%.

Conclusions

This study shows that irradiation, organic acids, and other substances like salts (which are readily available) can eliminate slime from snails and help in the production of ready-to-use fresh snails. Irradiation effectively reduced

the microbial load of snails and extended the shelf life by 14 days and these samples were accepted by panelist. Gamma irradiation had no significant effect on the fat content of the snails; however, the protein content of the snail increased after irradiation. The findings of this study give a promise for producing packaged ready-to-use snail meat of safe microbiological quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Microbial safety and biodiversity of bacterial communities in traditional Egyptian cheese types

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In this study, 19 cheese samples from six common Egyptian cheese types were evaluated for microbiological safety and bacterial diversity using 16S rRNA gene amplicon sequencing analysis. Results showed that the total viable count exceeded $5.2 \log \text{CFU g}^{-1}$ in 13 cheese samples. Further, the count of Enterobacteriaceae varied in the range 2.0 to $6.7 \log \text{CFU g}^{-1}$ in 17 samples. PCR-based detection of pathogenic strains confirmed the absence of *Listeria* spp. and *Salmonella* spp. Furthermore, 16S rRNA gene amplicon sequencing analysis revealed the richness and diversity of the bacterial communities inhabiting the Egyptian cheese samples. Besides unfavorable bacteria, such as Enterobacteriaceae, 10 genera were present in 74% of the examined samples, predominantly including lactic acid bacteria and salt-tolerant bacteria. These insightful findings provide a deeper understanding of the typical bacterial profiles of different Egyptian cheese types and can be applied to standardize cheese production and improve cheese quality and safety.

Key words: Egyptian cheese, microbiota, food safety, fermentation product, Amplicon sequencing.

INTRODUCTION

Based on cheese processing-related archeological evidence, it is believed that Egypt, as the heart of the Middle East, was the origin and first provider of cheese. This has not only been illustrated by old Egyptian drawings (El-Gendy, 1983), but has also been proven by a recent discovery of the oldest cheese material (Greco et al., 2018). In addition to its historical significance, Egyptian cheese is also of great importance from economic, social, nutritional, and food safety perspectives. Presently, Egypt is ranked as one of the top two cheese-producing and consuming countries in Africa and the Middle East. Further, in Egypt, cheese, owing to

its pleasant taste as well as its high protein, fat, and mineral contents, is a main part of meals, including lunch in schools (PM Food & Dairy Consulting, 2016).

Traditional cheeses, including soft and hard types, are primarily obtained from cow and buffalo milk. Specifically, hard-type Romy cheese, also known as Ras cheese, is produced in the shape of wheels following processing steps similar to those employed in the production of Gouda cheese, except for a few differences, especially in the subsequent dry salting step (for 4 to 5 weeks) following cheese brining, which gives Ras cheese its distinct sharp and salty flavor (Ismail et al., 2014). Further,

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soft traditional Egyptian cheeses include Karish, Mish, Domiati, Tallaga and Istanbuli cheeses. Specifically, Karish cheese is a fresh, healthy, and defatted cheese that is processed, usually using traditional methods, without any ripening step. Furthermore, it is used for the production of a yellowish-brown fermented cheese known as Mish cheese via pickling in microaerophilic conditions, with the addition of salt, milk, nutritive substances, oriental spices, and some plants, using old Mish cheese as the starter. This is usually followed by storage in warm conditions for a period of approximately one year to ensure ripening. Thus, Mish cheese with a sharp, salty, and pungent taste is obtained (Ismail et al., 2014).

Domiati cheese, the most popular white brined cheese in Egypt and other Arab countries that shows close resemblance with Feta cheese in Greece, is known for its unique processing technique that involves “milk salting,” during which 5 to 14% salt is directly added to the milk at the initial production step. The cheese obtained may be eaten fresh or as is often the case, the salted whey is removed, and the cheese is further stored for 4 to 8 months for ripening. Additionally, Tallaga Cheese with a creamy, spreadable soft texture and a minimal salty taste is obtained by the same method if low salt is added. Finally, to obtain Istanbuli cheese, which is characterized by a crumbly texture and Jalapeno chili studding that gives it a remarkable spicy flavor, the salt starter is added to pasteurized milk before culturing (Ismail et al., 2014).

Generally, the safety of cheese, which was previously considered as a potentially hazardous food (PHF) that facilitates microbial growth, especially when good hygiene and safety practices are not followed during its preparation, is considered to be time/temperature control (TCS) dependent. Outbreaks related to the consumption of dairy products were reported all over the world, so it is necessary to verify the safety of cheese in order to achieve the safer diet (Costard et al., 2017; Makino et al., 2005; Lance et al., 2005; MacDonald et al., 2005). It has been suggested that traditional Egyptian cheeses do not comply with both Egyptian and international cheese safety standards (Sayed et al., 2011). Various pathogenic bacteria, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria* spp., which are possible sources of human infection and food poisoning, have been isolated from traditional Egyptian cheeses (El-Eyiby, 2017; Hassan and Goma, 2016). In particular, *Salmonella* spp. and *L. monocytogenes* are relatively resistant to osmotic pressure and acid stress, and can survive and proliferate during child storage (Melo et al., 2015; Leyer and Johnson, 1992). Cheese is a ready-to-eat food that is eaten without heating, so if these harmful microorganisms are contaminated in cheese, it may cause serious food poisoning.

Even though current industrial development standards for the commercial production of cheeses require milk pasteurization, most traditional Egyptian cheeses are made without pasteurization (Ismail et al., 2014) since it is

well-known that these traditional practices contribute significantly to the original sensory characteristics of cheese, in addition to the associated health benefits (Montel et al., 2014). Cheeses made with raw milk are increasingly preferred by consumers due to their richer and more varied flavors than cheeses made with pasteurized milk (Yoon et al., 2016; Casalta et al., 2009). The development of cheese flavor is largely due to the naturally occurring microbial community, primarily in raw milk (Montel et al., 2014). However, such practices may be a threat to public health. It has also been reported that traditional cheeses are characterized by microbial community richness and diversity. Microorganisms such as lactic acid bacteria involved in cheese fermentation also contribute to suppressing the growth of pathogenic bacteria by producing bacteriocins and organic acids during cheese production (Montel et al., 2014; Yoon et al., 2016). Therefore, understanding the microbial flora in cheese is essential for producing safer cheese. However, analyzing these bacterial communities using culture-dependent methods is still challenging.

In this regard, the importance of using culture-independent methods, particularly 16S rRNA gene amplicon sequencing, which is based on the use of next-generation sequencing (NGS) technology, has been highlighted in several food science-related studies. Specifically, 16S rRNA gene amplicon sequencing analysis has been used for the characterization of the microbial communities in Danish raw milk cheeses (Masoud et al., 2011), the Polish cheese, Oscypek (Alegria et al., 2012), water buffalo mozzarella (Ercolini et al., 2012), artisanal Irish cheeses (Quigley et al., 2012), Latin-style cheeses (Lusk et al., 2012), and Iranian Liqvan cheese (Ramezani et al., 2017). However, as far as we know, this method has not been previously applied to characterize traditional Egyptian cheeses.

Therefore, the objective of this study was to assess the microbiological safety and biodiversity of the bacterial communities in six types of traditional Egyptian cheeses (Karish, Mish, Domiati, Tallaga, Istanbuli, and Romy cheeses) using both culture-dependent and culture-independent methods, particularly, NGS technology.

MATERIALS AND METHODS

Cheese samples

A total of 19 different commercial cheese samples corresponding to six traditional Egyptian cheese types were obtained from the Cairo and Kalyobia governorates in Egypt. Thereafter, the samples were categorized under two major groups: Soft cheese samples (Karish, Domiati, Tallaga, Mish, and Istanbuli cheeses, samples C1-C3, C4-C6, C7-C10, C11-C13 and C14-C16, respectively) and hard cheese samples (Romy cheese, samples C17-C19). After transportation to the laboratory, the cheese samples were stored at 4°C until analysis. Detailed information regarding the cheese types as well as the markets from which they were obtained are presented in Table 1.

Table 1. Details of traditional Egyptian cheese samples used in this study.

Cheese type	Name of cheese	Sample code	Source	Location*	
Soft	Karish	C1	Local market	D	
		C2	Supermarket	B	
		C3	Local market	E	
	Domiati	C4	Supermarket	A	
		C5	Supermarket	B	
		C6	Supermarket	C	
	Tallaga	C7	Supermarket	A	
		C8	Local market	E	
		C9	Supermarket	B	
		C10	Supermarket	C	
		Mish	C11	Local market	A
			C12	Supermarket	C
			C13	Local market	E
	Istanbuli	C14	Supermarket	A	
		C15	Supermarket	B	
		C16	Local market	E	
Hard	Romy	C17	Supermarket	A	
		C18	Supermarket	B	
		C19	Supermarket	C	

*A: Down town of Cairo, B: Maadi/cairo, C: New Cairo, D: Helwan/Cairo, E: Kalyobia
Source: Authors

Measurement of cheese salt concentration

Salt concentration was measured using a digital salt meter (LAQUA Twin Salt-22, Horiba, Kyoto, Japan). Specifically, 10 g of each cheese sample was homogenized with 10 mL of distilled water in a stomacher bag. Thereafter, 1 mL of each homogenized cheese sample was used for the salt concentration measurement.

Enumeration of viable bacterial counts

In a stomacher bag, 10 g of each cheese sample was homogenized with 90 mL of 0.85% saline solution for 30 s and then serially diluted. Thereafter, 1 mL of each dilution series was inoculated onto appropriate Petrifilm count plates (3 M, St. Paul, MN, USA), and to obtain the total viable cell (TVC) and Enterobacteriaceae (EB) counts, 3M aerobic count (AC) plates and Enterobacteriaceae count plates (EB/VRBG) were used, respectively. Specifically, the plates were incubated at 37°C and viable bacteria were counted after 24 and 48 h for EB/VRBG and AC, respectively. Further, De Man Rogosa Sharp Media (MRS) agar plates (MRS, Oxoid, Hampshire, UK) were used to count lactic acid bacteria (LAB) using the spread-plate method after incubation for 72 h at 30°C.

Detection of pathogenic bacteria (*Listeria* spp. and *Salmonella* spp.)

Listeria spp. were isolated on PALCAM agar media (Merck KGaA, Darmstadt, Germany) in accordance with the ISO 11290 method with modifications. Briefly, 10 g of each cheese sample was primarily

enriched in 90 mL of half-Fraser broth (Oxoid) and incubated at 30°C for 24 h. This was followed by the transfer of 1 mL of this enriched solution into a 9-mL Fraser broth (Oxoid) and incubation at 30°C for another 24 h. The cultures were then plated on PALCAM agar solid media using spread-plate and streaking methods. Further, the plates were incubated at 30°C and examined after 48 h. For the detection of *Salmonella* spp., 10 g of each cheese sample was homogenized in 90 mL of Trypticase Soy Broth "TSB" (BD, Becton Dickinson, NJ, USA) as a pre-enrichment media and incubated overnight at 37°C. Thereafter, 1 mL of the enriched broth was transferred into a 9-mL Hajna Tetrathionate Broth (EIKEN, Tokyo, Japan) and incubated overnight at 37°C. Finally, the samples were streaked on deoxycholate hydrogen sulfide lactose agar (DHL) media (EIKEN) and incubated again at 37°C for 24 h.

Suspected colonies were then sub-cultured on the specified solid media for each strain under the same incubation conditions. Thereafter, single colonies were picked, purified, pelleted, and used for DNA extraction, which was performed using a Nucleo Spin DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Additionally, for the specific detection of *Listeria* spp. and *Salmonella* spp., a designed pair of forward and reverse primers, as described by Phraephaisarn et al. (2017) and a set of primers described by Rahn et al. (1992) were used, respectively. The PCR reaction was then performed in a GeneAmp thermal cycler (PCR System 9700, Applied Biosystems, Foster City, CA, USA). For *Listeria* spp., amplification was conducted for 40 cycles under the following conditions: 95°C for 5 min, 95°C for 10 s, 60°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 3 min. For *Salmonella* spp., 30 amplification cycles were run under the following cycling conditions: 95°C for 5 min, 95°C for 15 s, 65°C for 45 s, and 72°C for 20 s using a GeneAmp thermal cycler.

Table 2. Chemical and microbiological properties of cheese samples purchased from local market and supermarket in Cairo, Egypt.

Name of cheese	Sample code	Salt concentration (%)	The number of bacteria (log CFU g ⁻¹)		
			TVC*	EB*	LAB*
Karish	C1	3.0	5.9	4.7	7.0
	C2	0.5	6.1	6.7	6.8
	C3	1.5	5.8	5.8	7.6
Domiat	C4	12.0	5.7	4.7	6.9
	C5	6.4	6.7	3.6	5.9
	C6	6.4	2.6	0.0	6.0
Tallaga	C7	1.2	6.8	5.1	7.5
	C8	1.5	6.9	5.8	8.3
	C9	1.3	6.9	5.0	8.0
	C10	1.5	6.4	2.5	7.5
Mish	C11	11.6	4.0	N.D.	3.6
	C12	3.8	2.5	2.9	N.D.
	C13	10.0	5.2	2.5	N.D.
Istanbuli	C14	1.2	4.9	2.0	7.4
	C15	8.0	2.5	5.7	N.D.
	C16	11.0	3.5	5.5	3.5
Romy	C17	1.3	6.3	2.4	5.9
	C18	5.0	7.4	5.9	6.5
	C19	2.2	6.6	2.1	6.6

*TVC: Total viable count, *EB: Enterobacteriaceae, *LAB: Lactic acid bacteria, N.D.: not detected. The values represent means of duplicates. Source: Authors

Additionally, to validate the results corresponding to the examined strains, 5- μ L aliquots of the PCR products were further analyzed via agarose gel electrophoresis.

16S rRNA gene amplicon sequencing analysis

To perform amplicon sequencing, 10 g of each cheese sample was added to 90 mL of 0.85% saline solution and homogenized for 30 s. Thereafter, 1-mL homogenized cheese samples were transferred into sterile Eppendorf tubes and centrifugation was performed at 15,000 $\times g$ for 3 min. After this step, the supernatant was discarded, and the pellets obtained were stored at -20°C for microbiota analysis. For efficient bacterial DNA extraction, the obtained pellets were first treated with 100 μ L of Achromopeptidase enzyme (Wako Pure Chemical Industries, Osaka, Japan), and the mixtures obtained were incubated at 55°C for 15 min. Finally, DNA extraction was performed using the NucleoSpin Tissue kit (Macherey-Nagel) in accordance with the manufacturer's protocol. Thereafter, to obtain 2 \times 300 bp paired-end sequences, amplicon sequencing analysis using the 16S rRNA V3/V4 region was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA). All the analyses were outsourced to Bioengineering Lab. Co. Ltd. (Sagamihara City, Japan).

Data analysis

After amplicon sequencing, chimeric and noise sequences were

removed, and representative sequences and operational taxonomic units (OTUs) were obtained. Further, to realize phylogenetic estimation, the feature-classifier plug-in (Greengene software version 13_8), which offers the possibility to compare representative sequences, was used for OTU identification at 97% similarity. Alpha diversities of the bacterial microflora in each sample were expressed using Shannon diversity index. PCA was conducted on OTUs and the calculations for PCA were done using the R "prcomp" function and plotted using the "plot" function.

RESULTS AND DISCUSSION

Physicochemical and microbiological analysis of cheese samples

In this study, the microbiological properties and salt concentrations corresponding to the 19 examined cheese samples, representing six different traditional Egyptian cheese types, including soft and hard cheeses, collected from different markets, were initially determined using conventional methods (culture-dependent methods).

As shown in Table 2, the different cheese types showed different salt concentrations, which varied from 0.5% (Karish cheese C2) to 12% (Domiat cheese C4). According to a previous study, salt concentration is a

common parameter used to distinguish different cheese types (Hammam et al. 2020).

Generally, microbial count results based on TVC and EB counts indicated an obviously high overall cheese microbial load (Table 2). Specifically, TVC exceeded $5.2 \log \text{CFU g}^{-1}$ in 13 out of the 19 cheese samples, and in a Romy cheese sample, it was up to $7.4 \log \text{CFU g}^{-1}$ (C18); the lowest value, $2.5 \log \text{CFU g}^{-1}$ (C12), corresponded to one of the Mish cheese samples. Nevertheless, the TVC values here reported were relatively lower than previously reported ones (Hassan and Gomaa, 2016; Heikal and Al-wakeel, 2014). However, they suggested that the marketed cheeses were of inferior quality.

Further, the EB counts showed high variability, ranging from 2.0 (sample C14) to 6.7 (sample C2) $\log \text{CFU g}^{-1}$ in 17 samples, while two samples (samples C6 and C11) were completely EB free. Furthermore, the EB count observed in this study was approximately two-fold greater than that reported for Karish cheese in a previous study, which showed a maximum EB count of $3.5 \log \text{CFU g}^{-1}$ (Awad, 2016). However, consistent with the results, in other studies involving Egyptian cheese samples, members of the EB group showed high counts (El-Etriby, 2017; Hassan and Gomaa, 2016; Heikal and Al-wakeel, 2014). Overall, this high range of EB count reflects a poor good manufacturing practice (GMP) from farm to fork, particularly, poor personal hygiene with possible fecal contamination. Further, this could also be attributed to the use of unpasteurized milk, which contains bacteria that may cause gastrointestinal illness as well as cheese spoilage (Baylis et al., 2011). Moreover, this high bacterial count possibly contributes to cheese defects, such as color change, gas production, and hole formation, which should not be ignored (Roberts et al., 1998).

In this study, 16 out of the 19 cheese samples showed high lactic acid bacteria (LAB) counts, in the range 3.5 to $8.3 \log \text{CFU g}^{-1}$, whereas the three remaining samples were LAB free. In a previous study, an average LAB count of $7.8 \log \text{CFU g}^{-1}$ was reported for Egyptian cheese (Awad, 2016). In another study, different cheese types showed different LAB counts, with the maximum value ($7.4 \log \text{CFU g}^{-1}$) corresponding to Domiati cheese (El-Baradei et al., 2007). Reportedly, LAB, which are responsible for the organoleptic characteristics of cheeses, show health benefits via various mechanisms owing to their probiotic nature. The high LAB count in Egyptian cheeses, which is also frequently observed in fermented products, possibly plays an important role in the control of pathogenic bacteria, such as *Listeria* spp. and *Salmonella* spp. (Losito et al., 2014). In this study, based on culturing on their specific media, eight and four typical of *Listeria* spp. and *Salmonella* spp. colonies were observed, respectively; however, PCR-based analyses confirmed their absence in all the examined samples (data not shown). The negative PCR-based results for these two species contradict the results of previous studies, which reported the presence of pathogenic bacteria, including

Listeria spp. (Ismail et al., 2014) and *Salmonella* spp. in Egyptian cheese (El-Baz et al., 2017). This implies that, in some cases, the use of traditional culture-dependent methods may generate misleading results given the possibility of the co-existence of microorganisms with similar growth conditions. Thus, to obtain accurate results, culture-independent PCR analysis is required.

Bacterial diversity of cheeses

To determine the bacterial communities, cheese samples were analyzed via 16S rRNA gene amplicon sequencing. Thus, large numbers of sequence reads with similarity were clustered into OTUs. As shown in Table 3, the observed OTUs, read numbers, and Shannon diversity indices varied among the cheese types. Specifically, the lowest OTU number (101) corresponded to Karish cheese sample C1, while the highest recorded value (875) corresponded to Istanbuli cheese sample C15. Further, the Shannon diversity indices (mean = 2.3), which ranged from 0.30 (sample C2) to 3.27 (sample C14), indicated the presence of a diverse microbiota in the Egyptian traditional cheese samples. These findings contradict those of some previous studies, which showed only moderate variation between the diversity indices corresponding to different cheese types (Quigley et al., 2012; Riquelme et al., 2015).

Overall comparison of each cheese types

In this study, 25 out of the 70 detected microbiota families had relative abundances above 1% in at least one of the cheese samples. The most abundant families, which showed varied relative abundances in the examined cheese samples, included Streptococcaceae, Lactobacillaceae, Leuconostocaceae, Enterococcaceae, Staphylococcaceae, Xanthomonadaceae, Pseudomonadaceae, Oceanospirillaceae, Vibrionaceae and Enterobacteriaceae (data not shown). Minor families, such as Bifidobacteriaceae were also frequently observed. Thus, the Egyptian cheese samples seemingly contain a higher abundance of bacterial families than Italian (Marino et al., 2017) and Pico Portuguese (Riquelme et al., 2015) cheese samples. Moreover, Bifidobacteriaceae, which reportedly, cannot survive at salt concentrations $\geq 5\%$ (De Castro-Cislaghi et al., 2012), was observed in four of the Egyptian cheese samples with high salt concentrations (6.4 to 12%), suggesting that some of the strains had developed high-salinity tolerance. This also suggests the possibility for their application as probiotic candidates for foods with high salt contents, such as cheese (Alegría et al., 2012; Marino et al., 2017).

Considering a lower taxonomic level, a total of 101 different genera were identified, 39 of which were present at $\geq 1\%$ relative abundance in at least one cheese

Table 3. Total raw read numbers, operational taxonomic units (OTUs) and Shannon index of cheese samples sequencing with NGS.

Sample number	Raw read number	Observed OTUs	Shannon index
C1	43,157	101	0.78
C2	63,240	167	0.30
C3	60,936	287	1.97
C4	62,267	732	2.83
C5	67,989	529	3.08
C6	75,962	384	1.87
C7	67,065	112	1.05
C8	58,890	196	2.08
C9	72,169	190	1.53
C10	74,589	210	1.67
C11	59,146	399	2.83
C12	70,726	403	2.65
C13	67,905	583	3.00
C14	61,873	468	3.27
C15	70,114	875	2.46
C16	63,296	603	2.57
C17	59,096	320	2.15
C18	62,775	421	3.19
C19	65,082	495	2.82

Source: Authors

sample (Figure 1A to E). Further, the results indicated that 10 genera (*Streptococcus*, *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Enterococcus*, *Staphylococcus*, *Lysobacter*, Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter*) constituted the core microbiome of the cheese samples. They were observed in at least 14 of the 19 examined cheese samples even though their proportions varied between samples (the Karish cheese samples did not show the presence of *Enterococcus*). Furthermore, the first five genera of LAB constituted the majority of the core microbiota in the cheese samples, indicating that they are responsible for the distinctive taste of the cheese samples, despite their varying relative abundances among the different cheese types. Unfortunately, the other five observed LAB are considered to be problematic in cheese environments. For example, consistent with our microbiological count results, the presence of Enterobacteriaceae is indicative of poor hygienic conditions (Baylis et al., 2011). Additionally, *Pseudomonas*, which usually results from the use of raw milk for cheese production, is responsible for various kinds of cheese defects and spoilage, as well as biofilm formation (Johnson and Sommer, 2020). In this study, *Macrococcus* and *Chryseobacterium* were also frequently detected; their presence was confirmed in 12 out of 19 examined cheese samples. Minor genera, including *Tetragenococcus*, were also observed in three of the cheese samples examined in this study (relative abundances in the range 28 to 29.6%). Further, given that this study is the first, to the best of the researchers'

knowledge, in which Egyptian cheese samples are analyzed using a robust technique; the presence of several genera, such as *Lysobacter*, *Chryseobacterium*, *Marinilactibacillus*, *Weissella*, *Enhydrobacter*, Planococcaceae, *Tetragenococcus*, and *Halanaerobium* was reported, in Egyptian cheese samples for the first time. Interestingly, four genera that have not been previously associated with cheese were observed in this study. These included *Lysobacter*, which constituted part of the core microbial composition of all the 19 examined samples, as well as *Salinivibrio*, *Alteromonadales* and *Candidatus*.

The relative abundances of different bacteria genera showed obvious variations among the different cheese types, and in some cases, varied between samples of the same cheese type. Specifically, *Lactobacillus* spp. and *Enterococcus* species were more prevalent in Romy cheese samples (hard cheese); however, their relative abundances in soft cheese samples were much lower. *Streptococcus* showed a higher relative abundance in Karish cheese samples; however, its relative abundance in Tallaga cheese samples was lower than that observed in the other cheese samples. Additionally, *Lactococcus* species showed greater predominance in all the five soft cheese samples than in the hard one. Our results also indicated that Enterobacteriaceae were widespread in all the cheese types, showing a higher relative abundance in Karish cheese, which is a soft-type cheese (sample C3; 11.8%). Further, *Streptococcus* spp. showed a high relative abundance in Karish cheese samples C1 and C2

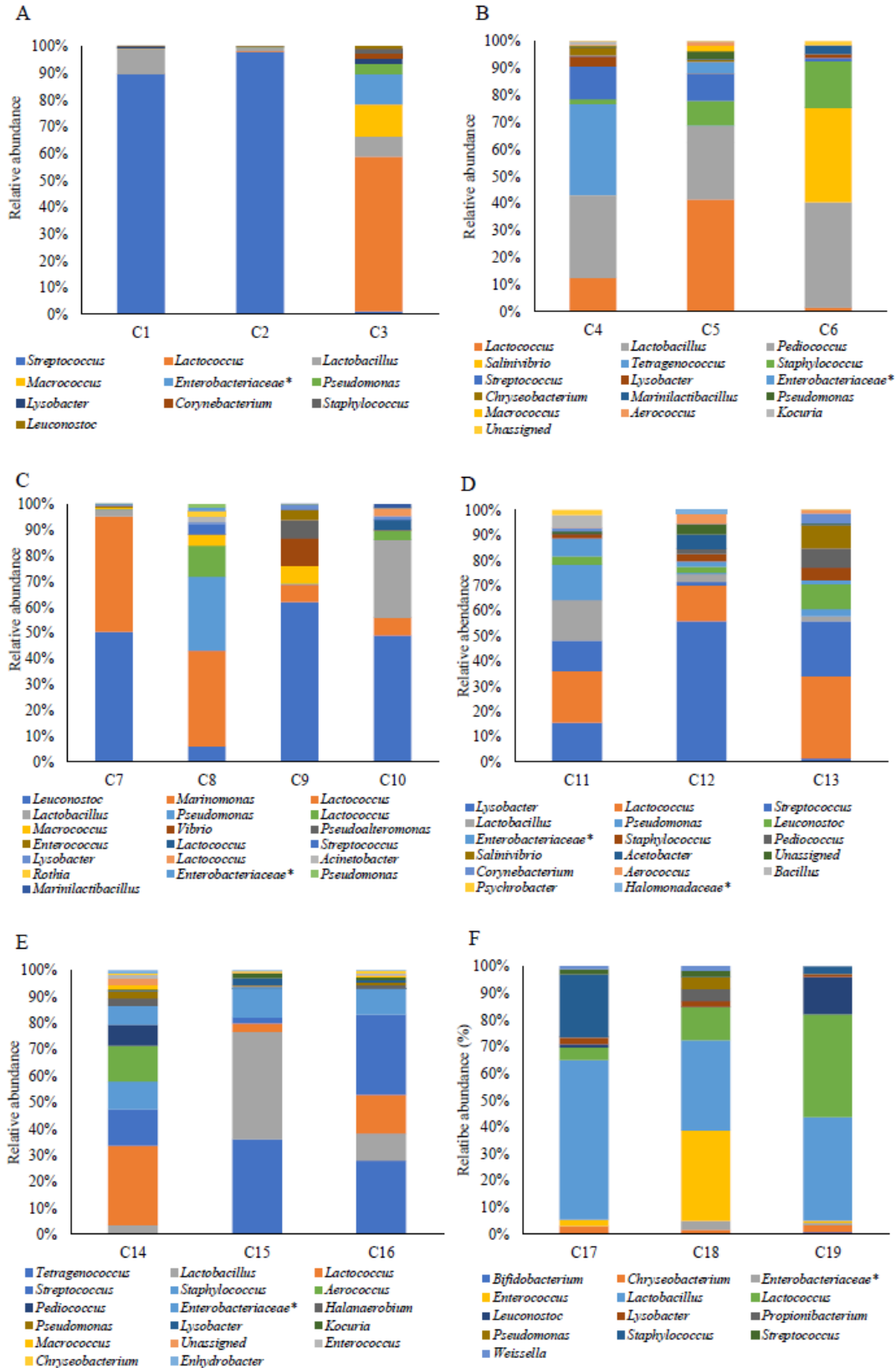


Figure 1. Relative abundance of OTUs at the genus level in Karish (A), Domiati (B), Tallaga (C), Mish (D), Istanbuli (E), and Romy (F) cheese; only OTUs with relative abundance $\geq 1\%$ in at least one sample are shown.

Source: Authors

(88.9 and 96.2%, respectively). The result also confirmed the presence of *Tetragenococcus* in Domiati and Istanbuli cheese samples, while *Leuconostoc* and *Marinomonas* showed very high relative abundances in Tallaga cheese samples.

Karish cheese

Considering the microbial communities in the individual cheese samples observed in this study, it was evident that Karish cheese samples predominantly contained LAB, including *Streptococcus*, *Lactobacillus*, and *Lactococcus* (Figure 1A), which were also the major part of the bacterial profile obtained via the TTGE analysis of Karish cheese (El-Baradei et al., 2005). This is consistent with the findings corresponding to raw milk cheese, such as Danish cheese (Masoud et al., 2011). Further, in this study, *Streptococcus* showed distinctive abundance and dominance in two of the Karish cheese samples, C1 and C2 (88.9 and 96.2%, respectively), while *Lactococcus* showed predominance in the third Karish cheese sample (sample C3, 53.1%). Thus, *Streptococcus* possibly contributes more to the fermentation of Karish cheese than *Lactobacillus* and *Lactococcus* species. Interestingly, in sample C3, with a lower *Streptococcus* relative abundance (2.2%), a high abundance of Staphylococcaceae (13.8%), including *Staphylococcus* and *Macrococcus*, was observed, suggesting that either *Streptococcus* is not a good competitor with Staphylococcaceae (Janek et al., 2016), or that the three Karish samples were not produced under the same standardized conditions possibly owing to the starter cultures that were used (Hammam et al., 2020).

Domiati cheese

In this study, Domiati cheese showed a complex and unique profile that reflected extensive diversity (Figure 1B). Generally, Domiati cheese was dominated by LAB genera (that is, *Streptococcus*, *Lactococcus*, *Lactobacillus*, *Tetragenococcus* and *Pediococcus*) as well as non-LAB genera (*Staphylococcus* and *Salinivibrio*). However, the three Domiati cheese samples showed differences in the dominant bacteria. Specifically, in sample C4, *Tetragenococcus*, *Lactobacillus*, *Streptococcus*, and *Lactococcus* were dominant (29.4, 26.9, 11.2 and 7.9%, respectively). Further, in sample C5, *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Staphylococcus* were dominant (31.6, 19.2, 9.2 and 7.7%, respectively), and in sample C6, *Pediococcus*, *Salinivibrio*, and *Staphylococcus* were dominant (37, 33.3 and 16.8%, respectively). The first study of the bacterial profile of Domiati cheese via TTGE and DGGE suggested that its bacterial content can be classified under three main groups, namely, dominant, frequently encountered, and

occasionally encountered bacterial species (El-Baradei et al., 2007), and in this study, all these three main groups, with different species categories was basically observed. Furthermore, via NGS, new genera in Domiati cheese that had not been previously identified were observed. Some of these newly reported genera (*Lysobacter*, *Pseudomonas*, *Chryseobacterium*, and *Marinilactibacillus*) were present in the three Domiati cheese samples; another (*Tetragenococcus*) was present in two of the Domiati cheese samples, while two others (*Salinivibrio* and *Alteromonadales*) were present in one of the Domiati cheese samples. Most likely, *Tetragenococcus* spp., which showed predominance in sample C4, with the highest salt concentration (12%), considering all the six cheese types, survived owing to its halotolerant nature. Further, this bacterial genus, together with *Pediococcus* spp., which was also observed in Domiati cheese samples, could confer possible health benefits owing to their probiotic function (Marino et al., 2017). In accordance with a previous study (El-Baradei et al., 2007), we could conclude that the microbiome of most of the Domiati cheese samples consisted of salt-tolerant and marine bacteria, owing to their high salt contents, which probably contributed to the ripening process of the Domiati cheese samples.

Tallaga cheese

Regarding the Tallaga cheese samples, three (C7, C9, and C10) predominantly consisted of *Leuconostoc* spp. (50.1, 60.9 and 47.1%, respectively), followed by *Marinomonas* in sample C7 (43.9%), *Vibrio* in sample C9 (10.3%), and Lactobacillaceae in sample C10 (29.3%). Conversely, Tallaga cheese sample C8 showed a different dominance pattern with *Lactococcus* showing predominance (46.1%), followed by *Pseudomonas* (29.6%) (Figure 1C). Interestingly, considering all the cheese types examined in this study, *Marinomonas* and *Vibrio* were only observed in the Tallaga cheese samples. Specifically, *Marinomonas* was identified as one of the dominant bacteria in sample C7; however, its relative abundances in the other Tallaga cheese samples (samples C9 and C10) were lower (6.6%), while *Vibrio* represented 10.3% of the total genera in sample C9 and 0.1% in sample C10. Based on these findings regarding the bacterial ecosystem in Tallaga cheese, which we report for the first time, it could be concluded that the process of Tallaga cheese manufacturing is primarily a *Leuconostoc*-driven process, with contributions from other LAB. Findings also suggested that except for two genera (*Marinomonas* and *Bacillus*), the bacterial profile of Tallaga cheese samples was part of that of Domiati cheese samples; this may be attributed to the similarities between their manufacturing process (except for salinity and ripening time) (Ismail et al., 2014). Nevertheless, these two cheese types showed obvious differences with

respect to the survival of some salt-tolerant bacteria, such as *Tetragenococcus*, *Corynebacterium*, *Kocuria* and *Salinivibrio* in the high-salt-containing Domiati cheese samples.

Mish cheese

Considering all the examined Egyptian cheese samples, Mish cheese appeared to have the highest bacterial diversity given that it harbored almost all the detected bacterial genera so far reported in this study (except *Marinomonas* and *Vibrio*) (Figure 1D). Specifically, the bacterial profile of the Mish cheese samples was predominated by LAB, and genera such as *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc*, and *Enterococcus*, with different abundances, were detected in all the samples. Further, other genera, including LAB and non-LAB were detected in two and one samples, respectively. In particular, sample C11 was dominated by *Lactococcus* spp. (18.6%), *Lactobacillus* (14.9%), non-LAB (*Lysobacter* and *Pseudomonas*, 14.1 and 13%, respectively), and *Streptococcus* (11.1%). Other subdominant bacterial genera included Enterobacteriaceae, *Bacillus*, *Leuconostoc*, *Corynebacterium*, *Staphylococcus*, and *Psychrobacter* (7.7, 4.6, 3.0, 2.7, 2.0 and 2.0%, respectively). Furthermore, in sample C12, *Lysobacter* spp. was predominant and showed its highest relative abundance (46.7%), considering all the 19 cheese samples examined in this study; the next abundant genera were *Lactococcus* (10.3%) and *Acetobacter* (5.9%). Additionally, bacterial genera, including *Lactococcus*, *Streptococcus*, *Leuconostoc*, and *Salinivibrio* showed predominance in sample C13 (27.7, 21.3, 8.7 and 8.1%, respectively), followed by *Pediococcus* (6.7%), *Corynebacterium* (5.2%), and *Staphylococcus* (4.9%), and given that Mish cheese is primarily made from Karish cheese, it contained all the bacterial genera observed in the Karish cheese samples. LAB was also identified as the dominant bacteria that possibly played a primary functional role in Mish cheese ripening owing to the favorable microaerophilic conditions as well as the high salt concentration, even though this was not consistent with the results obtained using the culture-dependent method, which did not show LAB growth (Table 2). LAB either entered the viable but not-culturable state (Millet and Lonvaud-Funel, 2000) or most probably were affected by acid stress, which is a self-imposed stress that leads to cell death owing to prolonged exposure to an acidic environment resulting from the accumulation of lactic acid by LAB during the over 1-year ripening of Mish cheese (Even et al., 2002).

Istanbuli cheese

Regarding Istanbuli cheese, a vast diversity of bacterial microbiota was reported for the first time; *Lactococcus*,

Streptococcus, *Lactobacillus*, and *Tetragenococcus* showed high relative abundances among the identified LAB. Possibly they represented the major contributors to cheese flavor and the cheese ripening process; other LAB and non-LAB, with different relative abundances, were also frequently detected (Figure 1E). For individual samples, *Lactococcus*, *Streptococcus*, *Aerococcus*, and *Staphylococcus* were identified as the dominant genera in sample C14 (18.8, 14.4, 11.6 and 8.2%, respectively), followed by others; Streptococcaceae (7.5%), *Pediococcus* (6.9%), and Enterobacteriaceae (6.8%). Further, minor genera, with relative abundance below 5%, including, but not limited to *Lactobacillus*, *Pseudomonas*, *Enterococcus*, *Macrococcus*, *Chryseobacteria*, *Acinetobacter*, *Marinilactibacillus*, *Halanaerobium*, *Enhydrobacter*, and *Alteromonadalis*, were also detected. In sample C15, *Lactobacillus*, *Tetragenococcus*, and *Staphylococcus* showed predominance (35.9, 29.6, and 17%, respectively), whereas sample C16 primarily consisted of *Streptococcus* (31.6%), *Tetragenococcus* (28%), *Lactococcus* (12.6%), and *Lactobacillus* (11.6%). Interestingly, Istanbuli cheese samples C15 and C16 showed the highest prevalence of *Tetragenococcus* (28-29.6%) and along with Domiati cheese sample C4, the highest prevalence of *Kocuria*, a minor genus (1.1-1.6%). In general, the genus level bacterial profile and diversity corresponding to Istanbuli and Domiati cheeses showed several similarities, possibly owing to their high salinities, which allowed the survival of salt-tolerant and marine bacteria. Reportedly, *Kocuria* spp., which have been isolated from milk and fermented dairy products in India, is associated with Domiati cheese (El-Baradei et al., 2007), and their psychrotropic and salt-resistant nature may be the reason for their growth in cheese (Patil, 2019).

Romy cheese

Contrary to the variation of bacterial communities among soft cheese samples of the same type, the hard cheese samples (Romy cheese samples) showed great similarity (Fig 1F). Specifically, Romy cheese samples C17, C18, and C19, were dominated by *Lactobacillus* spp. (54.8, 31.3, and 32.2%, respectively), followed by *Staphylococcus* (22.6%) in sample C17, *Enterococcus* (30.7%) in sample C18, and Streptococcaceae (19.4%), *Lactococcus* (16.3%), and *Leuconostoc* (13.3%) in sample C19. Further, minor genera, such as *Pseudomonas*, Enterobacteriaceae, *Chryseobacterium*, *Weissella*, and *Propionibacterium*, were also detected in all these three samples; however, with different relative abundances. Further, the latter two species showed more notable relative abundances in the Romy cheese samples than in the other cheese types. The results here reported are similar to those obtained after the first systematic profiling of Romy cheese using NGS, which identified *Lactobacillus*, *Staphylococcus*, *Streptococcus*, and

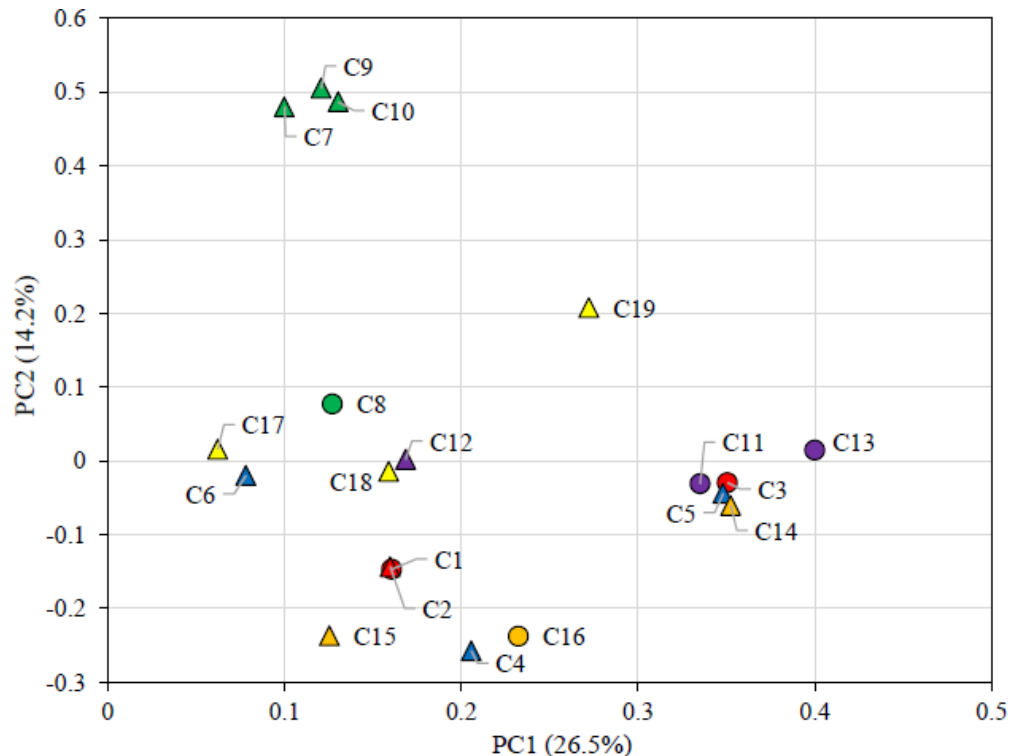


Figure 2. Principal component analysis (PCA) of cheese microbiota at the OTU level according to the cheese type. Each plot is indicated as a different shape and color depending on sampling location (supermarket [cone] and local market [circle]) and cheese types (Karish [red], Domiati [blue], Tallaga [green], Mish [purple], Istanbuli [orange] and Romy [yellow]).
Source: Authors

Lactococcus as the dominant bacterial genera, and possibly, these are primarily responsible for the sensory attributes and ripening process of Romy cheese. Furthermore, these bacterial genera have also shown dominance in Gouda cheese, which is manufactured via a similar process. However, the proportion of *Staphylococcus* in Romy cheese (1.5-22.6%) was found to be slightly higher than that in Gouda cheese (2.0-13.4%). However, the relative abundance of *Lactococcus* in the Romy cheese samples examined in this study (1.3-16.3%) was lower than that previously observed for Gouda cheese (40.1-49.1%) (Salazar et al., 2018). Differences between the commonly used milk or starter cultures employed may explain this variation.

Differences in microbial community structure by PCA

PCA was performed to investigate the possibility of clustering bacterial communities according to cheese type and salt concentration. The results thus obtained (Figure 2) indicated that most of the samples tended to show clustering according to cheese type, and the highest homology corresponded to Karish cheese samples C1 and C2, excluding sample C3. Further, Tallaga cheese

samples C7, C9, and C10, excluding sample C8, all of which were obtained from local markets, showed clustering. It was observed that for Mish, Istanbuli, and Romy cheese samples, two out of three samples showed clustering with relatively lower homology compared with the Karish and Tallaga cheese samples that showed clustering. However, in the case of Domiati cheese, the three samples, C4, C5 and C6 did not show any clustering, possibly because the high salt concentration of sample C4, which was approximately double those of samples C5 and C6, might have resulted in the clustering of this sample with other high-salt-containing samples (Istanbuli cheese samples C16 and C15). Further, the three cheese samples C11, C3, and C13 which were all obtained from the local market, showed another clustering despite them originating from different cheese types. Thus, it could be suggested that three factors, cheese type, salinity, and manufacturing technique/market type, are primarily responsible for the bacterial microbiota composition of traditional Egyptian cheeses. The majority of the cheese samples, excluding Domiati cheese samples, could also be clustered based on their type. Particularly, the greatest homology was observed in low-salt-containing cheese samples (Karish and Tallaga cheese samples), while high-salt-containing Domiati cheese samples did not show

any clustering based on cheese type. They rather showed clustering with other samples of different cheese types probably owing to their high salt concentration. Moreover, samples obtained from the local market showed a third clustering pattern. This finding highlights the importance of the standardization of processing techniques for the analysis of different dairy producers so that harmonized results can be obtained. In general, it is understood that no particular factor decisively determines the composition of the microflora of the complex cheese environment; a set of factors, from farm to fork, with relevance to industry have been previously discussed in this regard (Marino et al., 2017; Montel et al., 2014; Yeluri et al., 2018).

Conclusion

This study is the first in which the bacterial communities in traditional Egyptian cheese samples were investigated via 16S rRNA gene amplicon sequencing analysis. Based on NGS technology, various LAB known for their probiotic properties, constituted a major part of the bacterial profile of the cheese samples examined in this study. Unfortunately, the presence of pathogenic bacteria and bacteria with cheese spoilage ability was also confirmed. These results, in combination with those obtained using culture-dependent approaches, suggest a public health risk, the production of low-quality cheese with possible defects, and poor GMP. Regardless, the results of this study provide valuable information that can help cheese producers obtain specific and standardized characterization, minimize product variation, and enhanced cheese quality as well as safety.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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