Asian Journal of Food Research and Nutrition

Volume 3, Issue 1, Page 148-156, 2024; Article no.AJFRN.113988

Mineral Composition and Presence of *Escherichia coli* in Selected Vended Sachet Yoghurts in Obio/ Akpor, Rivers State, Nigeria

Eruteya, O.C. [a*] and Obiaocha-Nwaogwugwu, C. N [a]

[a] *Department of Microbiology, Faculty of Science, University of Port Harcourt, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author ONCN designed the study, handled the laboratory work and wrote the draft. Author EOC modified and approved the design, supervised the research. Both authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113988

Original Research Article

Received: 08/01/2024
Accepted: 12/03/2024
Published: 16/03/2024

ABSTRACT

Aims: Sachet yoghurts as one of the ready-to-eat foods commonly available in the market mainly for pupils need to be microbiologically safe for consumption. The study was aimed at ascertaining the safety of retailed sachet yoghurt vended in Obio/Akpor in Rivers State, Nigeria.
Study Design: This work is based on completely randomized design with two replications and the average values calculated.
Place and Duration of Study: Emmadavistic Laboratory, Osak's house, East-West Road, Port Harcourt, between June and November 2022.
Methodology: A total fifty (50) samples from 10 brands (A-J) were examined for the presence of *Escherichia coli* (*E. coli*), their sensitivity to common antibiotics and possession of virulence genes [*E. coli* attaching and effucing (*eaeA*), aggregative adherence fimbriae (*aggR*) and antimicrobial sensitivity testing (*astA*)] using Eosin methylene blue agar. Mueller Hinton agar and virulent gene primer.

**Corresponding author: E-mail: onoriode.eruteya@uniport.edu.ng;*

Results: The results of the total heterotrophic plate count revealed range of 4.8×10^5 to 1.47×10^6 cfu/ml. The count of *E. coli* ranged from 4.4 to 7.5×10^2 cfu/ml from D and I products, respectively. The results of mineral content revealed the presence of magnesium (202.1 – 314 mg/100 g), calcium (117.81 to 176.31 mg/100g), potassium (30.14 to 48.62 mg/100g), phosphorus (8.62 to 12.91 mg/100g). The confirmed *E. coli* showed varying resistance to augmentin (70%), gentamicin (0%), cefuroxime (100%), cefixime (100%), ofloxacin (0%), nitrofurantoin (20%), ciprofloxacin (0%) and ceftazidime (100%). None of the confirmed *Escherichia coli* produced the expected bands of 106,248 and 254 base pair against *eaeA, astA* and *aggR*, respectively.
Conclusion: The presence of antibiotics resistant *Escherichia coli* in two samples portends danger for the consumers and is indicative of poor water treatment.

Keywords: Dairy products; Escherichia coli; mineral composition; sachet yoghurt.

1. INTRODUCTION

"Yoghurt is an end product of a controlled fermentation of high solids, whole, skimmed or fortified milk by a symbiotic mixture of lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus usually in the ratio of 1:1" [1-3]. "Milk is a well-known natural habitat for lactic acid bacteria (LAB), hence the LAB present in milk fermentation is known to be either spontaneous starter cultures or introduced certified strains" [4]. "The two genera play essential roles in production of yoghurt from milk product through acidification and synthesis of aromatic compounds, hence lactose intolerant individuals can tolerate yoghurt much easier than full milk since the LAB have improved lactose digestion, eliminating intolerance symptoms" [5-6]. "The lactic acid fermentation of milk is also a means of prolonging the shelf life of the nutrients in milk" [7-8]. "*Bifidobacterium bifidum* and *Lactobacillus acidophilus* are also frequently employed to provide the unique characteristics of the final products" [9].

"Yoghurt is one of the commonly available fermented milk products and has been consumed since the beginning of civilization" [10]. "With a mild acidic flavour, custard texture, high nutritive value, organoleptic and probiotic qualities yoghurt is the most, commonly relished foods among children and adults alike throughout the history of mankind" [11-12]. "Yogurts are regarded as ready-to-drink foods widely consumed to quench thirst; for vitality and for health globally being an excellent source of vitamins, minerals, and calcium necessary for healthy teeth, bones, and immune system" [13-15].

"It is a balanced food which contains virtually almost all the nutrients present in milk and in more absolvable form. There is also the wide range of flavors spicing it up [8,16]. Overall, yogurt quality can be impacted by changing the process parameters, adding ingredients, and using different starting cultures" [17].

Consuming commercial yoghurt has also been shown to increase immune system function [15], reduce the risk of overweight or obesity and metabolic syndrome [18-19], improve gut microbiota and gut function [20], and improve lipid profile [21]. Given that yoghurt has many health benefits, a number of companies have ventured into the yoghurt production market. However, in most of these businesses, the environmental conditions involved in the production, storage, and distribution of many yoghurts are not strictly monitored, which negatively impacts the microbial and sensory qualities of the product [22].

Yoghurt is not expected to contain other microorganisms beyond certain tolerable limits, besides the starter or fermenting organism [23] but being a product of fermented milk from fresh milk can be easily contaminated. According to Oyeleke [8], fungi (molds and yeast) is the dominant microbial contaminants detected in commercially produced yoghurt in Nigeria. As a result of these microorganisms using up some of the acid in the yoghurt, the acidity of the yoghurt decreases, which may encourage the growth of putrefactive bacteria [24]. A number of authors have isolated *Bacillus* spp., *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Listeria* spp., *Serratia* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp., *Escherichia coli*, *Aspergillus* spp., *Candida* spp., *Saccharomyces* spp., and *Mucor* spp. from vended yoghurt [14,22,24-27]. Microbiological parameters especially, general coliforms, *Escherichia coli* and *Enterococcus* bacteria counts are commonly used to ascertain levels of

contamination [28]. In view of the above background this study attempts to assess nutritional quality and presence of *E. coli* in selected vended sachet yoghurt in Obio/Akpor, Rivers State.

2. MATERIALS AND METHODS

2.1 Source of the Samples

A total of 50 sachets yoghurt, comprising 5 each of ten different products labelled A-J samples were randomly procured from different vendors in Choba, Rumuagholu Ozuoba Rumuokoro, Rukpokwu in Obio/Akpor Local Government Area, Rivers State and were brought to Microbiology Laboratory in ice packed cooler for analysis. One of the yoghurts examined was not registered by National Agency for Food Drug Administration and Control (NAFDAC).

2.2 Mineral Composition Content of Yoghurt

The techniques described by Lawal and Adedeji [29] was employed in determining the presence of mineral elements such as magnesium (Mg), calcium (Ca), potassium (K), and phosphorus (P) in the yoghurt samples. From each sample, 10ml was burned for 3 hours at 550^{o}C in a muffle furnace, yielding two milliliters (2 ml) of white ash. The ash was then allowed to cool and washed into a 250 mL beaker filled with 30ml of concentrated trioxonitrate (v) acid evaporated to dryness on steam bath, the residue was further heated for 30 minutes, thereafter the sample was dissolved in 40ml of hydrochloric acid at ratio 1:1 and digested about 2 hours on a hot plate magnetic stirrer.1ml of dilute hydrochloric acid was further added to the sample and boiled for about 1hour,filtered while hot with Whatman no 4 filter paper, washed with HCl and the volume made up to 100ml with distilled water. The components of the minerals were ascertained through the use of the Buck Scientific Atomic Absorption Spectrophotometer (Buck 205) spectrometry method. Samples were extracted, and for each extraction, the mean signal response was recorded at specific wavelength corresponding to the mineral being analyzed. The concentration of each element was calculated as follows: Concentration (mg/100g): Standard concentration x sample absorbance x100/Standard absorbance x weight of sample.

2.3 Enumeration of total Heterotrophic Bacteria and *E. coli*

Ten millilitres (10ml) of each yoghurt sample were homogenized in 90ml of sterile distilled water and this one was used as stock solution for serial dilution. Then dilutions were made up to 10^{-6} from the stock solution using dilution method as used by Taiwo et al. [26]. Aliquot (0.1 ml) of each dilution was seeded on Nutrient agar (NA) and Eosin methylene blue agar using pour plate method following which the plates were incubated at 37^{o}C and 44.5^{o}C for 24 h. Total colonies on the surface of the plates were counted and expressed as colony forming unit per millilitre (cfu/ml) of the yoghurt sample. Discrete colonies were purified on freshly prepared NA and stored on NA slants pending confirmation.

2.4 Confirmation of *E. coli*

Characteristic discrete colonies displaying green metallic sheen on the Eosin methylene blue agar were confirmed on the basis of their physiological (Gram's staining) and biochemical characteristics involving indole, citrate, methyl-red, Vague Proskauer test [30-31].

2.5 Molecular Analysis

2.5.1 Deoxyribonucleic Acid (DNA) extraction

Deoxyribonucleic acid was extracted using the boiling method as described by Hitchins et al. [32]. Cells were harvested by centrifuging overnight pure culture of *E. coli* isolates in 2 ml

Table 1. Primers sequences described by Bisi-Johnson et al. [33]

Target gene	Primer Nucleotide Sequence (5'- 3')	Amplicon size (bp)
aggR	F 5' GTATACACAAAAGAAGGAAGC 3' R 5' ACAGAATCGTCAGCATCAGC 3'	256bp
eaeA	F 5' ATGCTTAGTGCTGGTTTAGG 3' R 5' GCCTTCATCATTTCGCTTTC 3'	248bp
astA	F 5' GCCATCAACACAGTATATCC 3' R 5' GAGTGACGGCTTTGTAGTCC 3'	106bp

Eppendorf tubes for 2 min at 10,000 rpm. The supernatants were discarded. Pellets were resuspended in 100 µl of sterile distilled water, boiled for 10 min, after which it was centrifuge at 10000 rpm. Supernatants were then transferred to fresh Eppendorf tubes and stored at -20^{o}C until further analysis.

2.5.2 Determination of virulent genes of *E. coli*

Oligonucleotide primers for *aggR*, *eae* and *ast* virulence genes described by Bisi-Johnson et al. [33] was employed (Table 1). Polymerase chain reaction (PCR) was conducted in thermocycler (Mastercycle-Eppendorf, Vapour Product, Germany) in a volume of 25 µl containing 2.5 10xPCR buffer, 2. 5 nM $MgCl_2$, 2.5 dNTPs, 0.1 each of appropriate primer, 0.1µl Taq polymerase, 1.0 µl of appropriate DNA preparation and 16.3 µl sterile distilled water. Amplification following an initial denaturation at 94^{o}C for 5 min was performed in 35 cycles at 94^{o}C for 15s, 55^{o}C for 20 s and 72^{o}C for 30 s. A final extension was done for 7 min at 72^{o}C. An 8 µl aliquot of the PCR product mixed with a loading dye (10mM, EDTA, 10% glycerol, 0.015% bromo phenol dye and 0.017% SDS, made up to 100 ml) were checked using Portable Gel hood built in Blue LED (470 nm) by Royal Biotech/Biolympics,1.5% agarose gel at a constant voltage and 1X TBE for approximately 1 h. They were visualized by Ethidium bromide staining and photographed under ultraviolet light. The ladder used is 1kb base pair ladder from thermo scientific.

2.6 Antibiotic Sensitivity Testing of *E. coli* Obtained from the Yoghurt Samples

Isolates from the yoghurt samples were tested for sensitivity against standard conventional antibiotic discs that is made up of cefuroxime (CRX) 30µg, gentamicin (GEN) 10µg, cefixime (CXM) 5µg, ofloxacin (OFL) 5µg, augmentin (AUG) 30µg, nitrofurantoin (NIT) 20µg, ciprofloxacin (CPR) 5µg, and ceftazidime (CAZ) 30µg. From 18 h cultures of *E. coli*, 0.5 MacFarland turbidity standard bacterial culture was prepared in sterile saline, from which 0.1 ml was inoculated onto Mueller Hinton agar. Thereafter, antibiotic discs were carefully and aseptically placed on the surface of the agar. The plates were incubated at 37^{o}C for 24 h. Zone of inhibition was measured in millimeter [34-35].

3. RESULTS AND DISCUSSION

3.1 Mineral Composition

As a dairy product that is both highly nutritious and readily digested, yoghurt is an excellent source of over ten key elements, including specific minerals and vitamins [36]. The results of the mineral composition of composite samples are presented in Table 2. It revealed the predominance of calcium with values ranging from 117.81 to 176.31 mg/100g, followed by potassium (30.14 to 48.62 mg/100g), then magnesium (20.21 to 31.36 mg/100g) and lastly phosphorus (8.62 to 12.91 mg/100g). The findings of this study are not consistent with the report of higher values (ranges) of potassium compared to calcium by Kang et al. [17] of values ranging from 123.01±2.59 to 157.23±0.95mg/100g and 121.42±4.29 to 148.39±10.3mg/100g, respectively in commercial drinking yoghurt in Korea, and The Dairy Council [37] who reported ranges of 130 to 280mg/100g and 100 to 200mg/100g, respectively from varieties of yoghurt. The values reported by Ibhaze et al. [38] in artificial and naturally flavoured yoghurt in Akure, Ondo State revealed very low ranges for calcium (1.77±0.09 to 2.40±0.01mg/100g), magnesium (0.01±0.05 to 1.20±0.07 mg/100g) and phosphorus (0.21±0.01 to 0.37±0.01mg/100g) compared to the values obtained in this present study. The phosphorus values ranging from 84.45±3.71 to 112.28±0.39mg/100g and 81 to 170mg/100g reported by Kang et al. [17] and The Dairy Council [37] in yoghurt samples where higher than the values obtained in this study. These variation in values may not be unconnected with the type of milk used (whole, semi-skimmed, or skimmed), the species from which the milk is obtained (bovine, goat, or sheep), the type of milk solids, solid non-fat, sweeteners, and fruits added prior to fermentation, as well as the duration of the fermentation process [36].

3.2 Total Heterotrophic Bacteria and *E. coli* count

The heterotrophic bacteria count result is presented in Table 3. The values ranged from 4.8×10^5 to 1.47×10^6 cfu/ml (5.68 to 6.17$\log_{10}$cfu/ml) for samples E and I respectively. Samples D, J, C and A had similar higher counts of 8.3, 7.9, 7.8 and 7.4×10^5 cfu/ml, respectively. Samples E, F and G however, showed counts of 4.8 and 6.9×10^5cfu/ml respectively. The values

Table 2. Mineral composition of composite yoghurt samples (mg/100 g)

Sample Code	Ca	Mg	K	P
A	117.81	21.89	37.09	9.85
B	141.21	20.21	30.14	11.23
C	163.49	23.48	41.72	12.73
D	152.25	22.24	30.62	11.82
E	152.02	28.88	34.26	11.87
F	128.52	31.42	46.89	9.88
G	176.31	31.36	48.62	8.62
H	146.25	21.97	38.06	9.99
I	151.22	30.42	31.15	12.46
J	162.48	23.24	35.63	12.91

Table 3. The total heterotrophic bacteria count

Sample code	Dilution	Mean count	Colony forming unit/millilitre (cfu/ml)	Log cfu/ml
A	10^{-3}	74	7.4×10^{5}	5.86
B	10^{-3}	86	8.6×10^{5}	5.93
C	10^{-3}	78	7.8×10^{5}	5.87
D	10^{-3}	83	8.3×10^{5}	5.92
E	10^{-3}	48	4.8×10^{5}	5.68
F	10^{-3}	59	5.9×10^{5}	5.73
G	10^{-3}	69	6.9×10^{5}	5.83
H	10^{-3}	76	7.6×10^{5}	5.88
I	10^{-3}	147	1.47×10^{5}	6.17
J	10^{-3}	73	7.3×10^{5}	5.89

reported by previous authors are as varied as the authors and locations with counts generally ranging from 0 to 6.0×10^{9} cfu/ml, thereby accommodating the values obtained in this study [22,24-26,39-42]. *Escherichia coli* was detected only in two samples (D and I) with values of 4.4 and 7.5×10^{2}cfu/ml, respectively. *Escherichia coli* is a frequent inhabitant of the intestine of animals and human and an indicator of the presence of other enteric pathogens in raw milk constituting public health hazard to consumers [43-44]. It may be possible that the disposal of waste and type of water within the environment may have contributed to the contamination of the vended yoghurts [45].

Previous authors have reported the non-detection of *E. coli* in yoghurt samples examined in Nsukka metropolis, Enugu State [39]; a range of 0 to 4.4×10^{3} cfu/ml in samples examined in Keffi, Nasarawa State [40] and in 34.00% of samples examined in Kano Metropolis, Kano State, in Nigeria. According to Tamine and Robison [28], microbiological parameters especially, general coliforms, *Escherichia coli* and *Enterococcus* bacteria counts are commonly used to ascertain the safety conditions of yoghurts. Consequently, majority of the examined ready-to-drink yoghurt are fit for human consumption notwithstanding the total heterotrophic bacteria count since the survival of probiotic bacteria are an essential factor for yoghurt. Moreso, Kang et al. [17] have reported a range of 6.12 to 8.13 $\log_{10}$cfu/ml and 4.54 to 7.93 $\log_{10}$cfu/ml of *Lactobacillus* and *Bifidobacterium*, respectively in yoghurt samples. This agrees with the suggestion that a probiotic product should contain at least 10^{6} cfu/mL at the expiry date [46-47].

3.3 Presence of Virulence Genes

The confirmed *E. coli* did not produce the expected bands of 106, 248 and 256 base pair (bp) for the *astA, eaeA* and *aggR* virulence genes. A number of authors have employed several virulence genes primers among them is *eaeA* for *E. coli* isolated from milk and fermented milk products, including yoghurt. Madani et al. [48] have also reported the non-detection of *eaeA* in *E. coli* isolated from milk and dairy products in Isfahan, Iran. Other authors, Dehkordi et al. [49], Elafify et al. [50] and Elzhraa et al. [51] have reported 36, 38 and 100%, respective occurrence of *eaeA* in *E. coli* strains from milk and dairy or heat-treated dairy

Table 4. Percentage antibiotics resistant pattern of confirmed *E. coli*

Organism	Number	Antibiotics							
		CRX	GEN	CXM	OFL	AUG	NIT	CPR	CAZ
E. coli	10	10 (100%)	0 (0%)	10 (100%)	0 (0%)	7 (70%)	2 (20%)	0 (0%)	10 (100%)

AUG= Augmentin, NIT= Nitrofurantoin, CPR= Ciprofloxacin, CAZ= Ceftazidime, GEN= Gentamicin, CXM= Cefixime, OFL= Ofloxacin and CRX= Cefuroxime

products. According to Madani et al. [48] the virulence genes were more predominantly found in raw milk compared to dairy products. Enteropathogenic *E. coli* (EPEC) strains are a prevalent source of potentially deadly diarrhea in newborns, and contains the *eaeA* and *bfpA*. [52-53]. The enterohaemorrhagic *E. coli* (EHEC) strains are also known to harbour all *stx1*, *eae* and *ehly* virulence genes [54]. Typical EPEC (tEPEC) isolates have both *bfpA* and *eaeA*, whereas isolates with negative *bfpA* are categorized as atypical EPEC (aEPEC) [53].

3.4 Antibiotic Sensitivity of Confirmed *E. coli* Isolated from the Yoghurt Samples

The results of the percentage resistance of the confirmed *E. coli* against commonly used antibiotics is presented in Table 4. All the isolates were resistant to cefuroxime, cefixime and ceftazidime but were all sensitive to gentamicin, ofloxacin and ciprofloxacin [55]. The level of resistance for *E. coli* in the present study with gentamicin, ofloxacin and ciprofloxacin is consistent with reports of 0% resistance of *E. coli* or *E. coli* 0157H7 isolated from yoghurt or foods of bovine origin, including yoghurt in Nigerian, Ethiopia and Burkina Faso [44,56-58]. Okwelle and Oha [59] however, reported a 5.1 and 10.1% resistant against ciprofloxacin and gentamicin of *E. coli* from branded yoghurt drink sold in Rumuolumeni, Rivers State, Nigeria. The 80% *E. coli* resistance against augmentin by Robinson et al. [56] in yoghurt drink sold in Port Harcourt metropolis is comparable to70% obtained in this study.

4. CONCLUSION

This study has provided an insight into the mineral content and safety of sachet yoghurt sold in the selected communities. They contain essential mineral for healthy living. The presence of *E. coli* in two of the sachet yoghurt samples indicates that some are not fit for consumption since *E. coli* is an indicator organism for the presence of especially intestinal organisms and pathogens. Notwithstanding the non-detection of the presence of virulence genes, their resistance to the selected antibiotics portends danger for the consumers and is indicative of poor water treatment.

ACKNOWLEDGEMENTS

The authors are grateful to the Emmadavistic Laboratory, Osak's house, East-West Road, Port Harcourt for the use of their facilities and technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bourlioux P, Pochart P. Nutritional and health properties of yogurt. World Rev Nutr Diet. 1988;56:217-258.
2. Adams MR, Moss MO. Food microbiology. 3rd ed. Royal Society of Chemistry, Cambridge UK; 1999.
3. Courtin PO, Rul CA. Yogurt production and presentation. J Integrated Food Sci Technol Trop. 2003;24(3):221-230.
4. Delavenne E, Mounier J, Deniel F, Barbier G, Le Blay G. Biodiversity of antifungal lactic acid bacteria isolated from raw milk samples from cow, ewe and goat over one-year period. Int J Food Microbiol. 2012; 155(3):185-190.
5. Serra M, Trujillo JA, Guamis B, Ferragut V. Flavor profiles and survival of starter cultures of yoghurt produced from high pressure homogenized milk. Int Dairy J. 2009;19:106-109.
6. Saborido R, Leis R. Yogurt and dietary recommendations for lactose intolerance. Nutr Hosp. 2018;35:45–48.
7. Hui YH. Yoghurt manufacturing. Enc. Food Sci. Technol. 1992;4:2905- 2907.
8. Oyeleke SB. Microbial assessment of some commercially prepared yoghurt

retailed in Minna, Niger State. Afr.J. Microbiol. Res. 2009;3(5):245-248.
9. Perez-Cornago A, Sanchez-Villegas A, Bes-Rastrollo M, Gea A, Molero P, Lahortiga-Ramos F, Martinez-Gonzalez MA. Intake of high-fat yogurt, but not of low-fat yogurt or prebiotics, is related to lower risk of depression in women of the SUN cohort study. J Nutr 2016;146:1731-1739.
10. McKinley MC. The nutrition and health benefits of yogurt. Int J Dairy Technol. 2005;58:1-12.
11. Adams MR, Moss MO. Food microbiology, 2nd ed. The Royal Society of chemistry UK; 1995.
12. De N, Goodluck TM, Bobai M. Microbiological quality assessment of bottled yoghurt of different brands sold in Central Market, Kaduna Metropolis, Kaduna, Nigeria. Int J Current Microbiol Appl Sci. 2014;3:20-27.
13. Alfa-Lawal AB. Dairy and Food Engineering. Division Lund-Sweden; 1984.
14. Alli JA, Oluwadun A, Okonko IO, Fagade OE, Kolade AF, Ogunleye VO. Microbial assessment and microbiological quality of some commercially prepared yoghurt retailed in Ibadan, Oyo State, Southwestern Nigeria. British J Dairy Sci. 2010;1(2): 34-38.
15. El-Abbadi NH, Dao MC, Meydani SN. Yoghurt: Role in healthy and active aging. Am J Clin Nutr. 2014;99(5):1263S-1270S.
16. Anthar IH. Preparation of cheese and yogurt at household level. Pakistan Agricultural Research Council, Islamabad; 1986.
17. Kang S, Kim MK, Kim Y. Comprehensive evaluation of microbiological and physicochemical properties of commercial drinking yogurts in Korea. Food Sci. Anim. Resour. 2019;39(5):820~830.
18. Martinez-Gonzalez MA, Sayon-Orea C, Ruiz-CanelaM, de la Fuente C, Gea A, Bes-Rastrollo M. Yogurt consumption, weight change and risk of overweight/obesity: The SUN cohort study. Nutr Metab Cardiovasc Dis. 2014;24: 1189–96.
19. Babio N, Becerra-Tomas N, Martinez-Gonzalez MA, Corella D, Estruch R, Ros EF, et al. Consumption of yoghurt, low-fat milk, and other low-fat dairy products is associated with lower risk of metabolic syndrome incidence in an elderly Mediterranean population. J Nutr. 2015; 145:2308–16.
20. Adolfsson O, Meydani SN, Russell RM. Yogurt and gut function. Am J Clin Nutr. 2004;80:245-256.
21. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V, et al. Effect of probiotic yoghurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci. 2011;94:3288-394.
22. Adebisi OO, Oyetayo VO, Ajayi KO. Microbial quality and sensory assessment of yogurt marketed in Akure Metropolis, Nigeria. Int J Sci Res Pub. 2017;7(11):704-710.
23. FAO. Manuals of Food Quality Control for Microbiological Analysis FAO, Food and Nutrition paper, United Nations, Rome, Italy; 1979;14(4):A1-F10.
24. Agu KC, Archibong EJ, Anekwe DC, Ago CA, Okafor AC, Awah NS. Assessment of bacteria present in yoghurt sold on awka metropolis. Scholars J Appl Med Sci. 2014;2(6D):3071-3075.
25. Awah NS, Agu KC, Muokwe J, Irondi C, Okeke CB, Anaukwu CG, et al. Microbial assessment of yoghurts sold in Amawbia, Nigeria. Universal J Microbiol Res. 2016; 4(2):55-58.
26. Taiwo OS, Afolabi RO, Oranusi SU, Owolabi JB, Oloyede AR, Isibor PO, et al. Microbiological assessment of commercial yoghurt sold in Ota Metropolis, Ogun State, Nigeria. 8th International biotechnology conference, exhibition and workshop. IOP Conf. Series: Earth Environ Sci. 2018;210:1-19.
27. Nwaiwu O, Aduba CC, Igbokwe VC, Sam CE, Ukwuru MU. Traditional and artisanal beverages in Nigeria: Microbial diversity and safety issues. Beverages. 2020;6(53): 1-22.
28. Tamine AY, Robinson RK. Yoghurt: Science and technology. 3rd ed. Woodhead Publishing Limited. 2007;808.
29. Lawal AK, Adedeji OM. Nutritional and elemental analysis of warankasi (fermented milk product) sold in Lagos metropolis, Int Res J Biotechnol. 2013; 4(6):112-116.
30. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press. UK; 2000.
31. Holt GH, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's manual of

determinative bacteriology (9th ed.). Baltimore: Williams and Wilkins; 2004.
32. Hitchins AD, Jinneman KC, Yoshitomi KJ, Blackstone GMK, Thammasouk K, Johnson J et al. Multiplex real-time PCR to simultaneously detect *Listeria* spp. A and *L. monocytogenes* from a variety of food enrichments, Abstr. 49 XV International Symposium on Problems of Listeriosis (ISOPOL), Uppsala, Sweden; 2004.
33. Bisi-Johnson MA, Obi CL, Vasaikar SD, Baba KA, Hattori T. Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. Gut Path. 2011;3:1-8.
34. Bauer AWD, Perry DM, Kirby WMM. Single-disk antibiotic-sensitivity testing of staphylococci: An analysis of technique and results. A.M.A. Arch. Intern. Med. 1959;104:208-216.
35. Benson H. Microbiological applications 9th ed. McGraw hill higher education. New York; 2005.
36. Weerathilake WADV, Rasika DMD, Ruwanmali JKU, Munasinghe MADD. The evolution, processing, varieties and health benefits of yoghurt. Int J Sci Res Pub. 2014;4(4):1-11.
37. The dairy council. The nutritional composition of dairy products. London; 2013.
38. Ibhaze GA, Amanze UR, Onibi GE. Mineral, vitamin concentrations and sensory properties of artificial and naturally flavoured yoghurts. Nigerian J. Anim. Sci. 2020;22 (2):318-323.
39. Mbaeyi-Nwaoha IE, Egbuche NI. Microbiological evaluation of sachet water and street-vended yoghurt and Zobo drinks sold in Nsukka metropolis. Int. J. Biol. Chem. Sci. 2012;6(4):1703-1717.
40. Makut MD, Ogbonna AI, Dalami H. An assessment of the bacteriological quality of different brands of yoghurt sold in Keffi, Nasarawa State, Nigeria. J Nat Sci Res. 2014;4(4):19-22.
41. Omola EM, Kawo AH, Shamsudden U. Physico-chemical, sensory and microbiological qualities of yoghurt brands sold in Kano metropolis, Nigeria. Bayero J Pure Appl Sci. 2014;7(2):26-30.
42. Dirisu CG, Lily G, Igwe E. Microbiological load of yoghurt sold in Omoku schools, Rivers State, Nigeria. Afr J Microbiol Res. 2015;9(34):1960-1963.
43. Soomro AH, Arain MA, Khaskheli M, Bhutto B. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam. Pak. J. Nutr. 2002;1:151-152.
44. Bagré TS, Kagambèga A, Bawa HI, Tchamba GB, Dembélé R, Cheikna Zongo C, et al. Antibiotic susceptibility of *Escherichia coli* and *Salmonella* strains isolated from raw and curds milk consumed in Ouagadougou and Ziniaré, Burkina Faso. Afr J Microbiol Res. 2014;8(10): 1012-1016.
45. WHO. Essential safety requirements for street- vended foods. world health organization, Geneva; 1996.
Available:http://apps.who.int/iris/handle/10665/63265
Accessed on 29 April 2021.
46. Gueimonde M, Delgado S, Mayo B, Ruas-Madiedo P, Margolles A, de los Reyes-Gavilan CG. Viability and diversity of probiotic *Lactobacillus* and *Bifidobacterium* populations included in commercial fermented milks. Food Res Int. 2004;37:839-850.
47. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol. 2000;78:80-88.
48. Madani A, Esfandiari Z, Shoaei P, Ataei B. Evaluation of virulence factors, antibiotic resistance, and biofilm formation of *Escherichia coli* isolated from milk and dairy products in Isfahan, Iran. Foods. 2022;11(7):960.
49. Dehkordi FS, Yazdani F, Mozafari J, Valizadeh Y. Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. BMC Res Notes. 2014;7(217):1-8.
50. Elafify M, Khalifa HO, Al-Ashmawy M, Elsherbini M, Abd El Latif A, Okanda T, et al. Prevalence and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* in milk and dairy products in Egypt. J Environ Sci Health. Part B. 2019:1-8.
51. Elzhraa F, Al-Ashmawy M, El-Sherbini M, Abdelkhalek A. Critical occurrence of verotoxigenic *E. coli* and non-typhoidal *Salmonella* in some heat-treated dairy products. Italian J Food Safety. 2021;10: 9318.

52. Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. 2004;2:123-140.
53. Canizalez-Roman A, Gonzalez-Nuñez E, Vidal JE, Flores-Villaseñor H, León-Sicairos N. Prevalence and antibiotic resistance profiles of diarrheagenic *Escherichia coli* strains isolated from food items in northwestern Mexico. Int. J. Food Microbiol. 2013;164:36–45.
54. Ranjbar R, Safarpoor Dehkordi F, Sakhaei Shahreza MH, Rahimi E. Prevalence, identification of virulence factors, O-serogroups and antibiotic resistance properties of Shiga-toxin producing *Escherichia coli* strains isolated from raw milk and traditional dairy products. Antimicrob Resist Infect Contr. 2018;7(53): 1-11.
55. National committee on clinical laboratory standards performance standards for antimicrobial susceptibility testing: Sixteenth International Supplement CLSI document, M100-S11, Pennsylvania, USA; 2006.
56. Abebe E, Gugsa G, Ahmed M, Awol N, Tefera Y, Abegaz S, et al. Occurrence and antimicrobial resistance pattern of *E. coli* O157:H7 isolated from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. PLoS Negl Trop Dis. 2023; 17(1):1-22.
57. Robinson VK, Aleruchi O, Okafor AC, Ahuokpo HI, Ipalibo CH. Antibiogram and resistant gene (gentamicin and extended beta-lactam) profile of *Escherichia coli* isolated from yoghurt. Asian J Biochem Gen Mol Biol. 2023;14(2):1-11.
58. Sarba EJ, Wirtu W, Gebremedhin EZ, Borena BM, Marami LM. Occurrence and antimicrobial susceptibility patterns of *Escherichia coli* and *Escherichia coli* O157 isolated from cow milk and milk products, Ethiopia. Sci Reports. 2023;13(16018):1-13.
59. Okwelle AA. Antibiogram of some pathogenic bacteria species isolated from branded yoghurt drink sold in Rumuolumeni, Rivers State, Nigeria. IOSR J Pharm Biol Sci. 2021;16(5 Ser. I):34-39.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113988