Chemical Composition and Microbial quality Assessment of Conventional Yoghurts within Awka Metropolis

Abstract

Evaluations of nutritive and microbiological significances of two conventional yoghurts are investigated in this study. Chemical components of the different yoghurt drinks showed the presence of: antioxidants of polyphenols and tannin; phytate, lectin, residual sugars and inhibitors of trypsin protein concentrations (mg/ml) of: 11.34, 10.14; 10.34, 11.21; 37.56, 38.77; 32.15, 30.28; 11.12, 8.92 and 11.58, 10.19, respectively. Total viable counts (TVC) of: 4.6 X 10^3 and 2.9 x 10^2 CFU/ml for the sample A and B yoghurt drinks respectively at day 0 of the microbial assessment were obtained. Coliform counts (CC) of 8.0 X 10^2, 7.8 x 10^3 and 1.3 X 10^3 CFU/ml were observed for the sample A and B yoghurt drinks respectively at day 0 of the microbial counting. Total fermenting of: 6.8 X 10^6, 5.33 x 10^5 and 5.6 X 10^4 CFU/ml were observed for the sample A and B yoghurt drinks respectively at day 0 of the counting. There was differential growth in the total population of the organisms as the day progresses from 0-14. *Enterococci* and airborne *Bacilli* were ubiquitous in the yoghurt drinks respectively. The present study has paved a way quality assurance of probiotics commonly sold within our metropolis and for upkeeping by nutritionist in maintaining stringent policies for manufacturing probiotics companies.

Keywords: Evaluation, chemical composition, microbial, yoghurts.

1.0 Introduction

Yoghurt is one of the oldest fermented milk products, tremendously popular all over the world. It is a very rich source of protein, calcium, vitamins among other phytochemical minerals and enzymes of clinical implications (13) Yoghurt is fermented by lactic acid producing bacteria, *S. thermophilus* and *L. bulgaricus* or some additional bacteria having mutual complementing

metabolism (95). The natural yoghurt is characterized by a smooth and viscous gel like texture and has a delicate walnutty flavor (26).

Fermentation of lactose by lactic acid bacteria results in the production of lactic acid, carbon dioxide, acetic acid, diacetyl, acetaldehyde and several other components giving a characteristic flavor to yoghurt (91). However very careful processing is required for the production of safe and good quality yoghurt. In a wider sense spectrum, little contamination may deteriorate the quality of yoghurt and may have very negative effects on consumer health (13).

Overall, quality of yoghurt is governed by number of factors: inferior milk quality, unhygienic conditions and the use of “wild type” of starter culture give rise to poor grade locally made yoghurt, having lower shelf life. In addition, microbiological aspect is one of the most important factors (19). The microbial quality of yoghurt reflects towards the quality and acceptability of the yoghurt. Due to unhygienic conditions there is possibility of microbial contamination (pathogens), which may have serious impact on the health of consumers. Further, unhygienic vending conditions, open packs (higher contamination) also deteriorate the keeping quality of yoghurt (4).

Aside the microbial load factorials in quality marker evidence, proportionate phytochemical compositions are likened to beneficial dairy products. This improves the nutritive components and health benefits of fermented dairy foods (4).

The present study took it wholesomely to identify comparatively the microbial loading index and the chemical compositions of yoghurt drinks from conventional brands within a municipal town in Anambra state known as Awka.

2.0 MATERIALS AND METHODS

2.1 Materials

All the reagents, equipment used in the present study were of analytical grade and products of BDh, May and Baker, Sigma Alrich. The equipment is calibrated at each use.

2.2 Methods

2.2.1 Collection of Brand Yoghurt

Conventional yoghurt drinks purchased from Awka market were taken to the laboratory under stable storage working condition as described by the manufacturers.

2.2.2 Chemical Analysis of the Yoghurt Samples

The following chemical components were determined and they include:

- Phytate
- Lectin
- Polyphenol
- Trypsin inhibitor
- Total acidity
- Tannin contents

2.2.2.1 Phytate Contents

This was determined as described by Price and Butler. (75).

Absorbance was taken at 520 nm using the UV-VIS spectrophotometer.

2.2.2.2 Lectin

This was determined as described by A.O.A.C. (1).

Absorbance was taken at 540 nm using the UV-VIS spectrophotometer.

2.2.2.3 Trysin Inhibitor

This was determined as described by A.O.A.C, (1).

Absorbance was taken at 410 nm using the UV-VIS spectrophotometer.

2.2.2.4 Total Acidity

This was determined as described by A.O.A.C. (1) using titrimetry method in the presence of organic indicator. 2ml of each of the yoghurt samples pipette into conical flasks, these was diluted with 20 ml of water and allow to stand for 20 min. one ml of phenolphthalein indicator was dropped into the solutions and titrated against 0.1M NaOH inside the buirrette.

2.2.2.5 Determination of Polyphenolic Contents

Total phenolics were determined using Folin-Ciocalteau Reagent (FCR) as described by A.O.A.C. (2), with slight modifications. FCR consist of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungsticheteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces FCR forming a blue coloured molybdenum oxide whose colour intensity is directly proportional to the phenolic contents.

The absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

2.2.2.6 Determination of Tannin Contents

Tannin content in each sample was determined using insoluble Potassium hexacyanoferrate, which binds tannins as described by Butler and Price. (76). Absorbance was read off at 720 nm.

2.2.2.7 Estimation of Residual Sugar Contents

This was achieved by measuring the glucose remaining (residual) of the yoghurt drinks samples using a modification of the 3, 5-dinitrosalicylic acid (DNS) reagent assay method described by Miller (70). The reaction mixture was allowed to cool and then the absorbance read at 540 nm.

2.2.3 Microbial Isolations and Quantifications

Both the prepared yoghurt sample and the industrially purchased yoghurt drinks were separately diluted serially into test tubes numbering ten (10) and containing 9ml of sterile water each. A suitable diluent (10^{-2} to 10^{-4}) was selected and cultured on three different media namely Nutrient, De Man, Rogosa and Sharpe (MRS) agar and MacConkey agar using pour plate techniques as described by Ezeonu *et al*. (2013).

2.2.3.1 Media Preparation

All media used in this study were prepared under sterile conditions and according to the manufacture's specifications. Each of them was mathematically calculated and dissolved in distilled water with respect to the desired quantity, heated to homogenize on a bunsen burner and sterilized in an autoclave at 121°C for 15 min, after which they were dispensed aseptically into sterile Petri dishes, bijou bottles and test tubes depending on which apparatus is appropriate for the intending test, and allowed to cool to gelling.

2.2.3.2 Determination of the Total Viable Count (TVC) of the Yoghurt Samples

The cultured nutrient media plates for the two samples were incubated aerobically for 24 h at 37 °C afterward were incubated anaerobically for 48 h at 37 °C. Incubation under aerobic condition was done to allow growth for bacteria that require oxygen while the latter anaerobic condition was to allow the unknown fermenters to grow also. After incubation, TVC was calculated in

CFU/ml (colony forming unit per ml) as CFU/ml = Number of cells

Colony forming units counts (CFU/ml) was calculated using the formular:

TOTAL HETEROTROPHIC COUNTS X RECIPROCAL OF VOL. OF INOCULUM X RECIPROCAL OF DILUT

2.2.3.3 Coliform Counts of the Samples

Samples cultured on MacConkey agar plates were incubated aerobically at 37 °C for 24 h. The total number of coliforms present in the samples was determined in CFU/ml as shown in section 2.2.7.2.

2.2.3.3.1 Fermenters' Count of the Yoghurt Sample

The colony count of the supposed fermenting organism of the yoghurt samples was done by incubating the culture on de-Man Ragoshie sharpie (MRS) plates anaerobically for 48 h at 37 °C and count taken as previously illustrated in section 2.2.7.2

2.2.3.4 Identification and Characterization of Bacterial Isolates

Morphological characterization of isolates

Discreet colonies from MRS, MacConkey and Nutrient agar plates were selected at random and sub-cultured on freshly prepared plates of the same isolation media. Further sub-culturing was done until a pure culture was obtained. The morphology of the isolates ranged from white, creamy, raise, flat, transparent, opaque to slimy depending on the nature of the possible organism present in the milk sample.

2.2.4. Biochemical Identification

Biochemical tests such as: catalase, oxidase, citrate/indole utilization, sugar fermentation, methyl red voges proskauer test, motility/ hydrogen sulphide were carried out on each of the isolates as described by Robinson and Tamine (78) and Robinson (79).

3.0 Results

Chemical Compositions of the Yoghurt Drinks

Table 1 showed the chemical compositions of the conventional yoghurt drinks against the control experiment. The table showed variation in compositions of each of the conventional yoghurt

drinks in chemical components such as phytate, polyphenols, residual sugar, inhibitors of trysin and lectin contents respectively. Results were expressed in equivalents of their standards respectively.

Heterotrophic Counts of Microbes from the Yoghurt Drinks

Table 2, 3 and 4 showed the heterotrophic counts of organisms from the yoghurt samples (both the commercial retailed and the tiger nut prepared yoghurt drinks). The table showed the differential counts of the organisms comprising: Total viable counts (TVC), Coliform counts and Fermenting bacteria counts from day 0 to 14 days.

Morphology and Biochemical Characteristics of the Microbes

Table 4 and 5 showed the morphology and biochemical significance of each of the isolates from the prepared and commercial yoghurt drinks respectively. From the table, Probiotics gram positive short rods Lactobacilli was found much in abundance from all the sample yoghurt drinks; *E.coli* were found in addition to the probiotics in the yoghurts while gram negative rods Proteus was found only in the control sample.

Statistical Analysis

Data obtained shall be expressed as mean ± SD and tests of statistical significance will be carried out using two-way analysis of variance (ANOVA). Mean values with $p < 0.05$ i.e 95% confidence interval were considered as significant.

Results and Discussion

Yoghurt is one of the oldest fermented milk products, tremendously popular all over the world. It is a very rich source of protein, calcium, vitamins among other chemical minerals and enzymes of clinical implications (Cutrim *et al*., 2017). There is an increasing demand for tasteful, cheap,

quality, stable and long lasting yoghurt. As stated earlier, majority of these dairy foods are processed locally in the society. The conventional yoghurt (the elite delights) in the market upon having scanty information about their compositions is not at reach for low income consumers (83,84,85).

The present study has shown the various nutritive components of different yoghurt drinks through its chemical, proximate and microorganismal sheer properties.

Analysis of the phytochemical components of the different yoghurt drinks showed the presence of: antioxidant polyphenols and tannin; phytate, lectin, residual sugars and inhibitors of trypsin protein. Tigernut derived yoghurt drink showed polyphenols, tannin, lectin and trypsin inhibitor concentrations (mg/ml) of: 40.09, 38.71, 9.14 and 11.23 respectively. Conventional yoghurt drinks (sample 1 and sample 2) showed corresponding phytochemical presence as follows: 11.34, 10.14; 10.34, 11.21; 37.56, 38.77; 32.15, 30.28 and 11.58, 10.19 mg/ml for phytate, lectin, polyphenols, total tannin and trypsin inhibitors.

Table 1: Chemical Compositions of the Conventional Yoghurt Samples Respectively.

Chemical components (mg/L)	**Conventional yoghurt A**	**Conventional yoghurt B**	**Control sampler nut)**
Phytate	11.34±0.01[a]	10.54±0.21 [a]	12.34±0.02 [b]
Lectin	10.34±0.04[b]	11.21±0.34 [b]	9.14±0.21 [b]
Polyphenols	37.56±0.25 [a]	38.77±0.52 [a]	40.09±0.3 [c]
Total Tannin	32.15±0.05 [b]	30.28±0.06 [b]	38.71±0.41 [c]
Residual sugars	11.12±0.2 [b]	08.92±0.03 [a]	8.72±0.52 [a]
Trypsin inhibitors	11.58±0.1 [b]	10.19±0.28 [b]	11.23±0.31 [b]

Results are expressed as mean values n=2

Suleiman *et al*. (88) reported a relatively low level of phytate on dairy produced from tiger nut. He went further to state that anti-nutrient phytate is a scavenging agent in plant materials

especially roots crops and vegetables. Their study revealed a high concentration of antioxidant polyphenols and flavonoid.

Microbial isolations, counting and identification of the inhabitant microbes from the different yoghurt drinks showed wide spectrum of microbial load from each of the yoghurt samples.

Total viable counts (TVC)which shows the entirety of whole organismal (using nutrient media) consortium from the 10^{-2} dilution factor showed heterotrophic counts of: 4.6 X 10^3, 2.9 x 10^2 and 5.4 X 10^3 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 0 of the counting.

Coliform counts (CC) which reflects the presence of pathogenic bacteria (isolated with Mackonkey media) of the organisms plated out from the 10^{-2} dilution factor showed heterotrophic counts of 8.0 X 10^2, 7.8 x 10^3 and 1.3 X 10^3 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 0 of the microbial counting. Total fermenting counts which reflects the multiplicity of desired bacteria i.e the starter cultures need for fermentation of the dairy for yoghurt production (isolated using *MRS-DeManRagoshie sharpie*) from the 10^{-1} dilution factor showed heterotrophic counts of: 6.8 X 10^6, 5.33 x 10^5 and 5.6 X 10^4 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 0 of the counting.

Table 2: Heterotrophic Counts of Bacteria from the Yoghurt Drink Samples

Heterotrophic Counts (CFU/ml)	**Yoghurt A,**	**Yoghurt B**	**Control Yoghurt**
Total Viable Counts (10^{-2})	4.6 X 10^3,	2.9 x 10^2	5.4 X 10^3
Total coliform Counts (10^{-2})	8.0 x10^2,	7.8 x 10^3	1.3x10^3
Fermenting bacteria (10^{-2})	6.8 x 10^6,	5.33 x 10^5	5.6 x 10^4

Day 0

Table 3: Heterotrophic Counts of Bacteria from the Yoghurt Drink Samples

Heterotrophic Counts (CFU/ml)	**Yoghurt A,**	**Yoghurt B**	**Control Yoghurt**

Total Viable Counts (10^{-2})	3.8 X 10^2,	2.9 x 10^4	1.9 X 10^3
Total coliform Counts (10^{-2})	1.40 x10^7,	1.09 x 10^6	4.33 x 10^2
Fermenting bacteria (10^{-2})	5.2 x 10^7,	6.12 x 10^7	5.55 x 10^6

Day 7

Table 4: Heterotrophic Counts of Bacteria from the Yoghurt Drink Samples

Heterotrophic Counts (CFU/ml)	Yoghurt A,	Yoghurt B	Control Yoghurt
Total Viable Counts (10^{-2})	2.2 X 10^4,	2.5 X 10^3	3.3 X 10^5
Total coliform Counts (10^{-2})	5.1 x10^3,	3.9 X 10^5	2.3x10^4
Fermenting bacteria (10^{-2})	3.3 x 10^7,	4.1 X 10^6	1.6 x 10^5

Day 14

Allam *et al.* (1) in their study on production of β-Galactosidase enzyme from *Lactobacillus acidophilus* RK isolated from different sources of milk and dairy products stated the heterotrophic dynamics of microbial consortium implicated in dairy and dairy products. They stated the seasonal fluctuation of these organisms as various physiologic factors such as pH, incubation periods, and temperature impacts on microbial proliferation in these dairies. There was differential growth in the total population of the organisms as the day progressed from 0-14. TVC showed heterotrophic counts of: 3.8 X 10^2, 2.9 x 10^4 and 1.9 X 10^3 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 7 of the counting; total coliform counts (TCC) showed heterotrophic counts of: 1.4 X 10^7, 1.09 x 10^6 and 4.33 X 10^2 CFU/ml for the Yoghurt A, B and the tiger nut processed yoghurt drinks respectively at day 7 of the counting and total fermenting bacteria counts (10^{-1}) showed heterotrophic counts of : 5.2 X 10^7, 6.12 x 10^7 and 5.55 X 10^6 CFU/ml for the sample A, B yoghurt drinks and the control yoghurt drinks respectively at day 7 of the counting respectively.

TVC showed heterotrophic counts of: 2.2 X 10^4, 2.5 x 10^3 and 3.3 X 10^5 CFU/ml for the sample A, B yoghurt drinks and the control yoghurt drinks respectively at day 14 of the counting; total coliform counts (TCC) showed heterotrophic counts of: 5.1 X 10^3, 3.9 x 10^5 and 2.3 X 10^4 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 14 of the counting and total fermenting bacteria (TFB) counts showed heterotrophic counts of : 3.3 X 10^7, 4.1 x 10^6 and 1.6 X 10^5 CFU/ml for the sample A, B yoghurt drinks and the control yoghurt drinks respectively at day 14 of the counting respectively.

Probiotics gram positive short rods *Lactobacilli* was found much in abundance from all the sample yoghurt drinks; *E.coli* were found in addition to the probiotics in the yoghurts while gram negative rods Proteus was found only in the yoghurt produced from control milk.

Table 5: Morphology features of the bacteria isolates from the prepared and commercial brand yoghurt drinks.

Sample	Isolates	Cell morphology	Colour	Motility	Gram stain
Control Yoghurt	A1	Round, smooth, raised, Short rod	Whitish	+ve	+ve
	A2	Round, smooth, flat, Short rod	Brilliant whitish	-ve	+ve
	A3	Round, smooth, raised, Cocci like rods	Yellowish white-brilliant	+ve	-ve
Sample yoghurt 1	H1	Round, rough, flat, Short cocci like rods	Whitish	+ve	-ve
	H2	Round, smooth, drop-like, Short rod	Whitish	-ve	+ve
	H3	Round, smooth, flat, Cocci like rod	Yellowish	+ve	-ve
Sample yoghurt 2	E1	Round, smooth, flat, Short rods in pairs	Whitish	-ve	+ve
	E2	Round, smooth, raised, Cocci like rod	Whitish brilliant	+ve	-ve
	E3	Round, smooth,	Yellow	-ve	+ve

		drop-like, pairs	Cocci in	whitish	

Key –ve= negative

+ve= positive

Table 6: Biochemical Characterization of the Bacteria Isolates

Sample	Isolates	Catalase, indole	H_2S, VP.	Citrate, MR.	Suspected organism
Control Yoghurt	A1	+ve, -ve	-ve, -ve	+ve, +ve	*E.coli*
	A2	-ve, -ve	-ve, -ve	-ve, -ve	*L.bacillus*
	A3	+ve, +ve	+ve, -ve	+ve, +ve	*Proteus*
Sample yoghurt 1	H1	+ve, -ve	-ve, -ve	+ve, +ve	*E.coli*
	H2	+ve, -ve	-ve, -ve	+ve, +ve	*E.coli*
	H3	-ve, -ve	-ve, -ve	-ve, -ve	*L.bacillus*
Sample yoghurt 2	E1	+ve, -ve	-ve, -ve	+ve, +ve	*E.coli*
	E2	-ve, -ve	-ve, -ve	-ve, -ve	*L.bacillus*
	E3	-ve, -ve	-ve, -ve	-ve, -ve	*L.bacillus*

Key –ve= negative

+ve= positive

Ezeonu *et al.* (2013) reported that predominance of gram negative organisms of *entrococci* and other *entero bacteriaciae* in food and related items is a mark of fecal contamination.

Conclusion

Overall picture of yoghurt (both conventional and locally made) on quality assessment needs emphasis on quality control during processing and storage. Also standardization of milk for yoghurt manufacture should be observed to meet legal standards and adjustment of yoghurt mix should approach the standard of the yoghurt package label. This study has shown that there are variations in the quality of yoghurt drinks made from milk derived wholly from plant source in terms of proximate, chemical and microbiological relevance when compared with conventional yoghurts in terms of quality and nutritive implications.

Funding Information

This work was solely funded by the research group of Dr. Ezenwelu Chijioke, O.

Author's Contributions

Ezenwelu, C.**:** Conceived and designed the experiments, performed the experiment and processed the data, analyzed the data and wrote the manuscript.

Agu, K.C: Co-supervised the research and revised the manuscript.

Duruamaku, P.U: Analyzed the research design and methodology, interpreted the data.

Oparaji E. Henry: Guided the experimental design, supervised the research, performed the experiment interpreted the data, revised the manuscript and processed the data.

References

1. A.O.A.C. (1997). The Official Methods of Analysis of AOAC International.16th edition, *The Association of Official Analytical Chemists, Arlington, USA.*

2. A.O.A.C. (2006). The Official Methods of Analysis of AOAC International.18th edition, *The Association of Official Analytical Chemists, Arlington, USA.*

3. Allam, R., Aly, M., El-zhrany, K. and Shafei, M. (2016). Production of β-Galactosidase enzyme from *Lactobacillus acidophilus* RK isolated from different sources of milk and dairy products. *International Journal of ChemTech Research*, **9** (10): 218-231.

4. Aziz, S., Oboh, G., Ademiluyi, A. O., & Akindahunsi, A. A. (2010). The effect of roasting on the nutritional and antioxidant properties of yellow and white maize varieties. *International Journal of Food Science & Technology*, 45(6), 1236–1242

5. Bassaneze, V., Miyakawa, A. and Krieger, J. (2008). A quantitative chemiluminescent method for studying replicative and stress-induced premature senescence in cell cultures. *Analytical Biochemistry,* **372 (2):** 198–203.

6. Belewu, M.A. and Abodunrin, O.A. (2006). Preparation of Kunnu from unexploited rich food source: Tiger Nut (Cyperus esculentus). *World Journal of Dairy Food Science*, **1:** 19- 21.

7. Belewu, M.A. and Belewu, K.Y. (2007). Comparative physico-chemical evaluation of tigernut, soybean and coconut milk sources. *International Journal of Agriculture and Biology*, **9:**785- 787.

8. Cantalejo, M.J. (1997). Analysis of volatile components derived from raw and roasted earth – almond (*Cyperus esculentus*). *Journal of Agricultural and Food Chemistry*, **45:** 1853 – 1860.

9. Champagne, C. P., Cruz, A. G., & Daga, M. (1994). Strategies to improve the functionality of probiotics in supplements and foods. *Current Opinion in Food Science*, **22:** 160–166.

10. Chukwuma, E.R., Obiama, N. and Christopher, O.I. (2010). The phytochemical composition and some Biochemical effect of Nigerian Tigernut *(Cyperus esculentus*) tuber. *Pakistan Journal of Nutrition*, **9(7):** 709-715.

11. Corbishley, D. and Miller, W. (1984). Tapioca, arrow Root and Sago Starches Production Production. *In Starch Chemistry and Technology, Academic Press, New York.* 469-476.

12. Cousin, M.A. (1982). Presence and activity of psychrotrophic microorganisms in milk and dairy products: *a review. Journal of Food Protection,* **45:** 172-207.

13. Crawford, E.M. (1962). The physical characteristics of polyoma virus: I. Two types of particle, *Virology* **2:** 170-176.

14. Cutrim, C., Barros, R., Franco, R. and Cortez, M. (2017). *Escherichia coli* O157:H7 Survival in traditional and low lactose Yogurt during fermentation and cooling periods. *Ciência Animal Brasileira*, **18**:1-9.

15. Davis, J.G. (1970). Laboratory control of yogurt. *Dairy Industries,* **35:** 139-145.

16. Davis, J.G. (1975). The microbiology of yogurt. In Lactic Acid Bacteria in Beverages and Food. *London: Academic Press.* 245- 266.

17. De Bruyn, C. and Yde, M. (2007). Binding of alkyl 1-thio-β-d-galactopyranosides to β-d-galactosidase from *E. coli*. *Carbohydrate Research*, **56:** 153–164.
18. Devries, F. and Feuke, T. (1999). Chufa (*Cyperus esculentus*) A weedy cultivar or cultivated weed? *Economic Botany*, **45:** 27- 37.
19. Dimri, G., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E., Linskens, M., Rubelj, I. and Pereira-Smith, O. (2015). A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proceedings from National Academy of Science* U.S.A, **92 (20):** 9363–7.
20. Ding, W. and Shah, N. (2010). Enhancing the biotransformation of isoflavones in soymilk supplemented with lactose using probiotic bacteria during extended fermentation. *Journal of Food Science*, **75**:140- 149.
21. Ezeonu, M., Okafor, J. and Ogbonna, J. (2013). Laboratory Exercise in Microbiology. *Ist edition Ephrata Publishing and Printing Company, Nsukka.* 100-117.
22. Farinde, E.O.,Obatolu, V.A., Fasoyiro, S.B., Adeniran, A.H.and Agboola, E.R (2008). Use of alternative raw materials for yoghurt production. *African Journal of Biotechnology* **7:**3339-3345
23. Fiordaliso, M., Kok, N., Desager, J. Goethals, F., Deboyser, D., Robertfroid, M. and Delzenne, N. (1995). Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids*, **30:**163-167.
24. Fleet, G.H. (1990). Yeasts in dairy products. *Journal of Applied Bacteriology*, **68:**199-211.
25. Fontaine, N., Meslin, J. C., Lory, S. and Andrieux, C. (1996). Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: Effects of inulin in the diet. *British Journal of Nutrition,* **75:** 881-892.
26. Friend, B. A., and Shahani, K. (1984).Nutritional and therapeutic aspects of lactobacilli. *Journal of Applied Nutrition,* **36:** 125-153.
27. Fuquay, J., Fox, P. and McSweeney, P. (2011). Encyclopedia of Dairy Sciences. *2nd Edition, Academic Press imprint of Elsevier, London, UK.*
28. Gibson, G. (1998). Dietary modulation of the human gut microflorausing prebiotics. *British Journal of Nutrition*, **80**: S209-12.
29. Gibson, G. (1999). Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *Journal of Nutrition*, **129**(7 Suppl):1438S-41S.
30. Gibson, G. and Roberfroid, M. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, **125:**1401-1412.
31. Gibson, G. R., Saavedra, J. M., and MacFarlane, S. 1997. Probiotics and intestinal infections. Pages 10-39 in: Probiotics: Therapeutic and Other Beneficial Effects. R. Fuller, ed. Chapman & Hall: London.
32. Goldin, B. R., Adlercreutz, H., Gorbach, S. L., Warram, J. H., Dwyer, J. T., Swenson, L., and Woods, M. N. 1982. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. *New England Journal of Medicine,* **307:** 1542-1547.
33. Goodenough, E.R. and Kleyn, D.H. (1976). Influence of viable yogurt microflora on digestion of lactose by the rat. *Journal of Dairy Science*. **59:** 601–606.
34. Green, M.D. & Ibe, S.N. 1987 Yeasts as primary contaminants in yogurts produced commercially in Lagos. *Nigeria. Journal of Food Protection,* **50:** 193- 198.

35. Gulcin, I., Beydemir, S., Elmastas, M. and Kufrevioglu, O.I. (2004). Comparison of antioxidant activity of clove (Eugenia caryophytaThunb) buds and lavender (Lavandulastoechas L.). *Food Chemistry*, **87:** 393-400.
36. Gyamfi, M. A., Yonamine, M. and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguine on experimentally-induced liver injuries. *General Pharmacology,* **32:** 661-667.

37. Halliwell, B. (2007). Oxidative stress and cancer: have we moved forward? *Biochemical Journal,* **401(1):** 1-11.

38. Halliwell, B. and Gutteridge, J. M. C. (1999).Free radicals, other reactive species and disease. In:free radical in Biology and Medicine. *Clarendon Press, Oxford,* 617-783.

39. Halliwell, B. and Gutteridge, J.M.C. (1989).Free radicals in Biologgy and Medicine. 2nd Edition *Clarendon Press. Oxford UK*.

40. Halliwell, B., Gutteridge, J. M. C and Aruoma, O. I (1987). The deoxyribose method: simple testube assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry*, **165:** 215-219.

41. Harbone, J.B. (1973). Phytochemical Methods: a Guide to Modern Technique of Plant analysis. *1st edition Chapman and Hall, London.* 107-150.

42. Harrigan, W.F. and McCance, M. (2016). Laboratory Methods in Food and Dairy Microbiology. *Academic Press, London, UK.*
43. Harvey (2000). A. Strategies for discovering drugs from previously unexplored natural products. *Drug Discovery Today, 5***:** 294–300.

44. Hennekens, C. H. and Goziano, J. M. (1993). Antioxidants and heart disease: epidemiology and clinical evidence. *Clinical Cardiology,* **16 (1):** 7-10.

45. Huber, R., Hakda, S., Cheng, C., Cupples, C. and Edwards, R. (2013). Trp-999 of β-galactosidase (*Escherichia coli*) is a key residue for binding, catalysis, and Synthesis of allolactose, the natural Lac operon inducer. *Biochemistry*, **42:** 1796–1803.
46. Imele H. (2001). Preliminary Study of the Utilisation of Coconut in Yoghurt Production. *Journal for Food Technology* **6:** 121-125.
47. Jachak, S.M.; Saklani, A. (2007). Challenges and opportunities in drug discovery from plants. *Curriculum. Science*, **92:** 1251–1257.

48. Jacob, R. A (1995). The integrated antioxidant system. *Nutrition Research,* **15 (5):** 755-766.

49. Jacobson, R., Zhang, X., Dubose, R. and Matthews, B. (2014). Three-dimensional structure of β-galactosidase from *E. Coli*. *Nature,* **369 (6483):** 761–766.

50. Jancewicz, L., Wheatley, R., Sutendra, G., Lee, M., Fraser, M. and Huber, R. (2012). Ser-796 of β-galactosidase (*Escherichia coli*) plays a key role in maintaining a balance between the opened and closed conformations of the catalytically important active site loop. *Archives of Biochemistry and Biophysics*, **517:** 111–122.
51. Jay, J. (2000). Modern Food Microbiology, 113-130, *Aspen Publishers.*
52. Jelkmann, W. (2004). Molecular biology of erythropoietin. *Internal Medicine,* **43:** 649-659.
53. Jialal, I. and Fuller, C. J. (1993). Oxidized Low Density Lipoprotein and antioxidants. *Clinical Cardiology,* **16 (1):** 1-6.
54. Juers, D., Heightman, T., Vasella, A., McCarter, J., Mackenzie, L., Withers, S. and Matthews, B. (2011). A structural view of the action of *Escherichia coli*lacZ β-galactosidase. *Biochemistry,* **40:** 14781–14794.
55. Juers, D., Matthews, B. and Huber, R. (2012). LacZ β-galactosidase: Structure and function of an enzyme of historical and molecular biological importance. *Protein Science,* **21(12):** 1792–1807.
56. Juers, D., Matthews, B. and Reuben, E. (2012). LacZ β-galactosidase: Structure and function of an enzyme of historical and molecular biological importance. *Protein Science,* **21 (12):** 1792–1807.
57. Juers, D., Hakda, S., Matthews, B. and Huber, R. (2013).Structural Basis for the Altered Activity of Gly794 Variants of Escherichia coli β-Galactosidase. *Biochemistry,* **42 (46):** 13505– 13511.
58. Krivtsov, A. and Armstrong, S. (2007). MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Reviews Cancer,* **7 (11):** 823–833.
59. Lederberg, J. (2010). The β -D-galactosidase of *Escherichia coli*, strain K-12. *Journal of Bacteriology,* **60:** 381–392.
60. Lee, W. and Lucey, J. (2010). Formation and physical properties of yogurt. *Asian-Australian Journal of Animal Science,* **23**:1127-1136.
61. Lindsay, R.C., Day, E.A., and Sandine, W.E. (1965). Identification of volatile flavor components of butter culture. *Journal of Dairy Science*. **48:** 1566–1574.
62. Lourens-Hattingh, A., Viljoen, B.C. (2002). Growth and survival of a probiotic yeast in dairy products. *Food Research International*, **34:**791-796.
63. Lucey, J. A., C. T. Teo, P. A. Munro and H. Singh. (1997). Rheological properties at small (dynamic) and large (yield) deformations of acid gels made from heated milk. *Journal of Dairy Research*. **64:** 591-600.
64. Lucey, J. A., P. A. Munro and H. Singh. (1998). Whey separation in acid skim milk gels made with glucono−δ−lactone:glucono−δ−lactone: Effects of heat treatment and gelation temperature. *Journal of Texture Studies.* **29:** 413-426.
65. Lucey, J.A., Singh, H. (1998). Formation and physical properties of acid milk gels: a review. *Food Research International*, **7:** 529-542.
66. Mara, J., Bassier, A., Joniau, M., & Baert, J. (2002). Effect of heatinduced association of whey proteins and casein micelles on yogurt texture. *Journal of Dairy Science,* **72:** 2247–2256.

67. Matthews, B. (2015). The structure of *E. coli* beta-galactosidase. *Comptes Rendus Biologies*, **328 (6):** 549–56.
68. Meydani, S.K and Ha, W. (2000). Immunologic effects of yogurt. *The American Journal of Clinical Nutrition*, **71:** 861–872.
69. Milks, W. R. (2001).High-resolution scanning electron microscopy of milk products: A new sample preparation procedure. *Food Structure,* **12:** 475–482.
70. Miller, G**.** (1959). Use of Dinitrosalicyclis Acid Reagent for Reducing Sugar. *AnalyticalChemistry,* **31**:426-442.
71. Mistry, V. V. (2001). Manufacture of nonfat yogurt from a high milk protein powder. *Journal of Dairy Science*, **75:** 947–957.
72. Okafor, J.N., Mordi, J.I., Ozumba, A.U., Solomon, H.M. and Olatunji, O. (2003). Preliminary studies on the characterisation of contaminants in tiger nut (yellow variety). *In: Proceedings of 27th Annual Conference and General Meeting of Nigerian Institute of Food Science and Technology Kano.* 210- 211.
73. Oladele, A.K., and. Aina, J.O. (2007). Chemical composition and Functional properties of Flour produced from two varieties of tiger nut. *African Journal of Biotechnology*, **6:** 2473- 2476.
74. Özer, B.H., Robinson, R.K. (1999). The Behaviour of Starter Cultures in Concentrated Yoghurt (Labneh) Produced by Different Techniques,LWT - *Food Science and Technology*,7:391- 395.
75. Price, N., and Stevens, L. (2000). Fundamentals of Enzymology. *3rd Edition Oxford Press, London.* 155-161.
76. Price, R. and Butler, T. (1977). Spectrophotometric analysis of total tannin content in brewery products. *Journal Food Science and Technology*, **14**:2754-2759.
77. Rita E. S, (2009). The use of tiger nut, (*Cyperus esculentus*), cow milk and their composite as substrates for yoghurt production. *Pakinstan Journal of Nutrition*, **8:** 751-58.
78. Robinson, R.K. & Tamine, A.Y. (1975). Yogurt-a review of the product and its manufacture. *Journal of the Science of Dairy Technology*, **28:** 149-163.
79. Robinson, R.K. (1981). *Dairy Microbiology* Vol. 2. London & New Jersey: Applied Science Publishers.
80. Saint-Eve, A.,.Lévy, C., Le Moignea, M.,Ducruetb, V.,Souchon, I. (2008). Quality changes in yogurt during storage in different packaging materials, *Food Chemistry* **2:** 285-293.
81. Salau, R. B., Ndamitso M. M., Paiko, Y. B., Jacob, J. O., Jolayemi, O. O., and Mustapha, S.(2012). Assessment of the proximate composition, food functionality and oil characterization of mixed varieties of Cyperus esculentus (tiger nut) rhizome flour. *Continental Journal of Food Science and Technology,* **6 (2):** 13 – 19.
82. Sandine, W. E., Daly C.; Elliker, P.R. & Vedamuthu, E. R. (1972). *Journal of Dairy Science*. 55, 1030.
83. Sanful, R.E. (2009). Promotion of coconut in the production of yoghurt. *African Journal of Food Science,* **3:**147-149.

84. Shaker, M. A., Ahmed, M. G, Amany M. B and Shereen L. N, (2009). Chufa Tubers (*Cyperus esculentus L.*): *As a New Source of Food. World Applied Sciences Journal*, **7 (2):** 151- 156.
85. Shoemaker, G., Juers, D., Coombs, J., Matthews, B. and Craig, D. (2013). Crystallization of β- galactosidase does not reduce the range of activity of individual molecules. *Biochemistry*, **42:** 1707–1710.
86. Sinnott, M. and Withers, S. (2011). The β-galactosidase-catalysed hydrolyses of β-galactopyranosyl salts. Rate limiting generation of an enzyme-bound galacto-pyranosylcation in a process dependent only on aglycone acidity. *Biochemistry Journal*, **143:** 751–762.
87. Smits P., van Brouwershaven J. H. (1980).Heat-induced association of β-lactoglobulin and casein micelles. *Journal of Dairy Research* **47:** 313–325.
88. Suleiman, M., Olajide, J., Omale, J., Abba, O. and Ejembi, D. (2018). Proximate composition, mineral and some vitamin components of Tigernut (*Cyperus esculantus*). *Research Article-Clinical Investigation*, **8(4):** 2041-2054.
89. Suriyarachchi, V.R and Fleet, G.H.(1981). Occurrence and Growth of Yeasts in Yogurts. *Applied and Environmental Microbiology*, **42:** 574-579.
90. Tamime A Y, Robinson R K and Latrille E (1989) Yoghurt and other fermented milks. In Mechanization and Automation in Dairy Technology, 152–203. Tamime A Y, Law B A, eds. Sheffield, *UK: Sheffield Academic Press.*
91. Tamine, A. and Robinson, K. (2004). Yoghurt Science and Technology. *Published by Institute of Applied Science.*
92. Tenu, J., Viratelle, O. and Yon, J. (2012).Kinetic study of the activation process of β-galactosidase from *Escherichia coli* by Mg^{2+}. *European Journal of Biochemistry***, 26:** 112–118.
93. Umerie, S.C., E.P. Okafor and A.S. Uka, (1997). Evaluation of the tubers and oil of *Cyperus esculentus*. *Bioresource Technology,* **61:** 171- 173.
94. Vedamuthu, E. R. (1991). The yoghurt story-past, present and future. *Dairy Food and Environmental Sanitation.* **7:** 371-374.
95. Vinderola, R. and Reinheimer, T. (2010). Manufacture of Low Lactose Yoghurt by Simultaneous Lactose Hydrolysis and Bacterial Fermentation. *Cultured Dairy Products Journal*, **17**:18- 19, 2.
96. Warra A. A, Babatola L. J, Omodolapo A.A,. Basiru Dende Ibraheem Characterization of Oil Extracted from Two Varieties of Tiger Nut (*Cyperus esculentus* L.) Tubers. *American Journal of Heterocyclic Chemistry 2017;* **3(3):** 28-36.
97. Welply, J., Fowler, A. andZabin, I. (2008).β-Galactosidase α-complementation; effect of single amino acid substitutions. *Journal of Biological Chemistry*, **256:** 6811–6816.
98. Wentworth D. and Wolfenden, R. (2014).Slow binding of D-galactal, a "reversible" inhibitor of bacterial β-galactosidase. *Biochemistry*, **13:** 4715–4720.
99. Wheatley, R., Kappelhoff, J., Hahn, J., Dugdale, M., Dutkoski, M., Tamman, S., Fraser, M. and Huber R. (2012). Substitution for Asn460 cripples β-galactosidase (*E. coli*) by increasing substrate affinity and decreasing transition state stability. *Archives of Biochemistry and Biophysics*, **521:** 51–61.
100. Whey Lactose Hydrolysis with Immobilized β- galactosidase Enzyme from *Kluyveromyces lactis*. *Pakistan Journal of Nutrition*, **I0(7)**: 675-679.

101. Willett W.C, (2007). The role of dietary n-6 fatty acids in the prevention of cardiovascular disease. *Journal Cardiovascular Medicine (Hagerstown) 2007;* **8:** S42-5.
102. Yeboah S.O, Mitei Y.C, Ngila J.C, Wessjohann L, Schmidt J. (2011). Compositional and structural studies of the oils from two edible seeds: Tiger nut, *Cyperus esculentum*, and asiato, Pachira insignis, from Ghana. Food Research International doi:10.1016/j.foodres.2011.06.036.
103. Yeganehzad,S.,Mazaheri-Tehrani,M., and Shahidi, F. (2007). Studying microbial, physiochemical and sensory properties of directly concentrated probiotic yoghurt. *African Journal of Agricultural Research,* **2:** 366-369.
104. Zhang, H.Y., Hanna, M.A., Yusuf, A. and Nan, L. (1996). Yellow nut-sedge (*Cyperus esculentus L*) tuber oil as a fuel. *Industrial Crops and Products,* **5 (3):** 177-181.