

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: 4.6×10^3 and 2.9×10^2 CFU/ml for the sample A and B yoghurt drinks respectively at day 0 of the microbial assessment were obtained. Coliform counts (CC) of 8.0×10^2 , 7.8×10^3 and 1.3×10^3 CFU/ml were observed for the sample A and B yoghurt drinks respectively at day 0 of the microbial counting. Total fermenting of: 6.8×10^6 , 5.33×10^5 and 5.6×10^4 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 (Cutrim *et al.*, 2017). Yoghurt is fermented by lactic acid producing bacteria, *S. thermophilus* and *L. bulgaricus* or some additional bacteria having mutual complementing metabolism (Vinderola and Reinheimer, 2010). The natural yoghurt is characterized by a smooth and viscous gel like texture and has a delicate walnutty flavor (Fuquay *et al.*, 2011).

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 (Tamine and Robinson, 2004). 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 (Cutrim *et al.*, 2017).

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 (Ding and Shah, 2010). 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 (Aziz, 2015).

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 (Aziz, 2015).

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. (1977).

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. (1997).

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, (1997).

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. (1997) 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-Ciocalteu Reagent (FCR) as described by A.O.A.C. (1997), 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. (1977). 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 (1959). 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 $\text{CFU/ml} = \text{Number of cells}$

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 Chessbrough (2006) and Mamta *et al.* (2017).

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 \pm 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.

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 \pm 0.01 ^a	10.54 \pm 0.21 ^a	12.34 \pm 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

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})	8.0×10^2 ,	7.8×10^3	1.3×10^3
Fermenting bacteria (10^{-2})	6.8×10^6 ,	5.33×10^5	5.6×10^4

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})	3.8×10^2 ,	2.9×10^4	1.9×10^3
Total coliform Counts (10^{-2})	1.40×10^7 ,	1.09×10^6	4.33×10^2

Fermenting bacteria (10^{-2})

5.2×10^7 ,

6.12×10^7

5.55×10^6

Day 7

UNDER PEER REVIEW

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})	2.2×10^4 ,	2.5×10^3	3.3×10^5
Total coliform Counts (10^{-2})	5.1×10^3 ,	3.9×10^5	2.3×10^4
Fermenting bacteria (10^{-2})	3.3×10^7 ,	4.1×10^6	1.6×10^5

Day 14

UNDER PEER REVIEW

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, drop-like, Cocci in pairs	Yellow whitish	-ve	+ve

Key -ve= negative

+ve= positive

Table 6: Biochemical Characterization of the Bacteria Isolates

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

Key -ve= negative

+ve= positive

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.

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. Suleiman *et al.* (2018) 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: 4.6×10^3 , 2.9×10^2 and 5.4×10^3 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 8.0×10^2 , 7.8×10^3 and 1.3×10^3 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: 6.8×10^6 , 5.33×10^5 and 5.6×10^4 CFU/ml for the Yoghurt A, B and the control yoghurt drinks respectively at day 0 of the counting. Allam *et al.* (2016) 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: 3.8×10^2 , 2.9×10^4 and 1.9×10^3 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: 1.4×10^7 , 1.09×10^6 and 4.33×10^2 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 : 5.2×10^7 , 6.12×10^7 and 5.55×10^6 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: 2.2×10^4 , 2.5×10^3 and 3.3×10^5 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: 5.1×10^3 , 3.9×10^5 and 2.3×10^4 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 : 3.3×10^7 , 4.1×10^6 and 1.6×10^5 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. Ezeonu *et al.* (2013) reported that predominance of gram negative organisms of *enterococci* and other *enterobacteriaceae* 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.

Ethics

Authors declared no ethical issues that may arise after the publication of this manuscript.

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