Comparative Investigation of Nutritional Values of Tiger Nut (*Cyprus esculentus*) and Selected Conventional Yoghurts in Enugu, Enugu State, Nigeria

Abstract

This study evaluated the physicochemical, chemical and microbial compositions of tiger nut and selected conventional yoghurts. During the study proximate, mineral, vitamins, chemical and physicochemical and bacterial analysis were done accordingly. The results of proximate analysis show that the fat and crude fibre compositions of tiger nut differed significantly ($p < 0.05$) with the values of conventional yoghurts, while no significant difference ($p > 0.05$) was observed in the concentration values of ash. Tiger nut yoghurts recorded highest values of 8.39±0.04 mg/l (vitamin C), while Cd, Ni and Pb were below detectable limit in all the yoghurts. The concentration values of Cu in the conventional and tiger nut yoghurts varied significantly ($p < 0.5$). Tiger nut yoghurt concentration values of polyphenol, total tannin and phytate are significantly different ($p < 0.05$). Results show that pH, TTA and conductivity values in sample A, B and tiger nut yoghurts are not significantly different ($p > 0.05$), while it differed significantly ($p < 0.05$) with reference to viscosity, TSS, TS and magnesium. The microbial cell count of *Lactobacillus acidophilus* was dependent on innoculum size and number of days. At day 7 conventional yoghurt A, tiger nut yoghurt and conventional yoghurt B recorded the highest loads of TVC (3.8×10^2), TCC (4.33×10^2) and TBC (6.12×10^7), while the highest loads of 3.3×10^5 (TVC), 5.1×10^3 (TCC) and 4.1×10^6 (TBC) are observed in the tiger nut yoghurt, yoghurts A and B at day 14 respectively. The study recommends production and promotion of plant based yoghurts drink for improvement of healthy and unhealthy individuals.

Keywords: Yoghurt, Tiger nut, Conventional, Bacterial, Nutrient, Drink

Introduction

Yoghurt is a diary food produced during the fermentation reaction of lactose a disaccharide of glucose and galactose in the milk and fermenting bacterial enzymes under certain conditions (Philip *et al*., 2017). Yoghurt takes the advantages of the demerits of milk especially from animal origins and provides an opportunity to increase the shelf life of milk and preserve its nutritional constituents for human consumption owing to its relatively low pH values. It contains probiotics, prebiotics and symbiotic, antioxidants, vitamins, linolenic acid, essential fatty acids, soluble fibres and vitamins (Elsanhoty *et al*., 2009).

In Nigeria, tiger nut is locally called “Aki awusa”, “ofio” and “Ayaya” in Igbo, Yoruba and Hausa respectively. Tiger nut is a creeping perennial plant that belongs to the family of *Cyperaceae* (Muhammad *et al*., 2011). It is usually grown in rough tufts (Arafat *et al*., 2009) and commonly proliferates in West Africa and South Europe (Abiola and Mutiu, 2020). Brown, black and yellow varieties are the three main varieties grown in Nigeria (Abiola and Mutiu, 2020). Attractive color, big size, and fleshier body are one of the inherent properties that make yellow tiger nut the most preferred (Idoia *et al*., 2014). Tiger nut is valued for the high starch dietary fibre, carbohydrate, mineral and oil content. Mason (2008) reported that tiger nut is recognized as one of the crops with remarkable health benefit due to its high content of fibre, protein, natural, sugar, rich in minerals (phosphorus and potassium) and vitamins E and C. The oil is gold brown in color and has a rich, nutty taste (Sanchez-Zapata *et al*., 2010 and Adejuyitan, 2011). The high content of oil was implicated as lauric acid grade oil and non acidic stable (El-Naggar, 2016). Tiger nut is a healthy food that helps to prevent cancer, due to high content of soluble glucose and reduces the risk of colon cancer (Muhammud *et al*., 2011 and Imam, Aliyu and Umar, 2013). It is known to have more prospective usage as food and industrial materials due to its high oil yield and milk content (Achoribo and Ong, 2017), and is believed to contain functional compounds needed for a balanced diet (Chukwuma *et al*., 2010 and Manek *et al*., 2012). Earlier study confirmed that tiger nut enhances blood circulation, prevents heart disease, and reduces risk of colon cancer (Adejuyitan, 2011). Tiger nut is an age long underutilized minor food crop in Nigeria mainly consumed as raw, roasted and baked snacks at public places such as market, park, picnic, swimming pool and gardens. Improving the nutritive qualities of yoghurt drinks can be achieved through harnessing the potentials of some dairy composite plant samples as a cheap alternative source of yoghurt starter milk and maintenance of bioprocess sterility during the production. Tiger nut has long been recognized for its health benefits due to high contents of fibre, protein and natural sugars (Manson, 2008). Tiger nut is believed to be beneficial to diabetics and those seeking to reduce cholesterol or lose weight (Sanful, 2009). With its nutritional and therapeutic advantage could serve as good alternative to cow milk in the production of yoghurt. Also, production of tiger nut yoghurt could reduce the price; make it to be more affordable. Similar study done by Abdeldaiem *et al*. (2023) confirmed reduction in production cost of plant based yoghurt albeit its abundance nutritive benefits.

Several studies have shown that tiger nut is rich in carbohydrate and fibre (Adejuyitan, 2011 and Ekeanyanwu and Ononogbu, 2010), calcium, magnesium, sodium, phosphorous, potassium, vitamins A, C, and E as well as amino acids (Ekeanyanwu and Onogbugbu, 2010) required for metabolic processes in the body. Other pharmacological properties of tiger nut according to Abiola and Mutiu (2020) are hepatoprotective, anti-sickling, aphrodisiac, antioxidant, antimicrobial, anti-atherosclerotic and anti-inflammatory and anti-arthritic. The anti-inflammatory, anti-convulsion and anti-arthritic potential of tiger nut confirms tiger nut for the treatment of gastrointestinal (Abiola and Mutiu, 2020). In addition, Adeniyi *et al.* (2014) and Hasan *et al*. (2013) confirmed that tiger nut extracts inhibit the activities of *Salmonella typhi* and *Escherichia coli*. Health challenges, such as stomach discomfort flatulence, bloating, colon diseases such as colorectal cancer, diarrhea, and cramp due to poor and unhealthy nutritive qualities have been linked to consumption of yoghurt (Mlichova, 2006). Similar work done by Silanikoye *et al*. (2015) confirms increase in lactose-intolerance individuals most especially in developing countries.

However, adequate treatment and proper fermentation of yoghurt during the production of yoghurt may enhance its nutritive qualities (Dekker *et al*., 2019). Recent studies done by Schimidt *et al*. (2016), Karnyaczki and Csanadi (2017) and Skryplonk *et al*. (2017) stress the influence of adequate fermentation in the nutritive properties of yoghurt. Despite the health challenges associated to dearth of knowledge of physical, chemical and microbial properties of yoghurt, the demand has continued to soar by leaps and bounds (Eri *et al*., 2021).

Nutritive and probiotics benefits composition identify yoghurt as one of the choicest consumed diary foods (Aryana and Olson, 2017). Assessment of the relationship between nutrition and health significance have increased huge benefit on consumers' approach towards nutritious foods. Most yoghurt producing companies have little knowledge of the effect of various nutritive components of yoghurt. Commercial yoghurt manufacturers especially in countries where regulation on food processing and production are compromised use various sweetened and sugar alcohols as preservatives to increase the shelf life. Lack of ethical focus, empirical production recipes and profit enthusiasts by the producers have kept the nutritive standard of the commercial yoghurt below standard and the falling effects are evident on the health statues of the consumers. Knowledge of the nutritive qualities of yoghurt is usually left for detrimental assumptions and mirage affirmations of gullible consumers. Knowledge of nutritional qualities of the yoghurt produce from tiger nut and the conventional yoghurt may not only be of immense economic and health values for healthy and immuno-compromised individuals but may help to improve nutritive qualities of yoghurt and also alleviate food crises in Nigeria. Information with regard to nutritive compositions of the conventional yoghurts that are available in the market is not only very scanty but most at time not within the reach of low income consumers. Also, the increasing demand for tasteful, cheap, quality, stable and long lasting yoghurt in Enugu make this study critical. This study examined physicochemical, chemical and microbial properties of tiger nut and selected conventional yoghurts.

1.2: Materials and Methods

1.2.1: Source of Tiger Nut

The Tiger nut (*Cyprus esculentus*) used for this study is the yellow colored species (Plate 1). It was bought from Ogige Market Nsukka, in Nsukka LGA of Enugu state, Nigeria. The tiger nut was identified in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Aqua Rapha yoghurt (conventional yoghurt A) and Nosco yoghurt (conventional yoghurt B) were purchased from Ogige and Ogbete main markets in Nsukka and Enugu metropolis, respectively. The choice of selection was based on sample survey which reveals that consumers prefer the selected yoghurts due to the assumed nutritional contents, palatability of the taste, aesthetic delight and high demand by the consumers in the local communities. The conventional yoghurts were stored at room temperature (36^{o}C)

Plate 1: The Tiger Nut (*Cyprus esculentus*)

1.2.2: Production of Yoghurt from the Tiger Nut

This was done as described by Lee and Lucey (2010) using standard operational techniques and procedures. The flow chart for the tiger nut yoghurt production is shown in Figure 1.

The protein, crude fibre, ash, fat, moisture and carbohydrate composition of the yoghurts were determined as described by AOAC (1997), while vitamins, minerals, phytochemical and physicochemical analysis were carried out accordingly

Media Preparation for Bacteria Isolations

All the media used in this study were prepared under septic 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. Dispensing was done aseptically into sterile Petri dishes, bijou bottles and test tubes depending on the apparatus appropriate for the intending test, and allowed to cool to gelling.

Microbial Analysis of the Yoghurts

Conventional and tiger nut yoghurts were diluted serially into test tubes numbering 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 McConkey agar using pour plate techniques.

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

The cultured nutrient media plates for the samples were incubated aerobically for 24 h at 37 °C afterward was 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 allowed in the unknown fermenters to grow also. After incubation, TVC was calculated in CFU/ML (colony forming unit per ml) as CFU/ML = a/v x D, Where a = number of colonies counted, V= volume plated; D= dilution factor

Colony Counts of the Microbes from the Yoghurt Samples

Samples cultured on McConkey agar plates were incubated aerobically at 37 °C for 24 h. The total number of colony present in the samples was determined in CFU/ML

Fermenting Organism Counts of the Yoghurt Sample

The colony count of the organism fermenting the yoghurt samples was done by incubating the culture MRS plates anaerobically for 48 h at 37 °C and count taken.

Heterotrophic Counting and Standardization of the Bacteria

Total colonies from both the nutrient media and the differential media were counted from the grown media plate ie TCFU/ml= microbial colonies observed X innoculum dilution factor X innoculum volume pippetted. TFCU/ml is total coliform unit per volume of the innoculum in millilitres.

Statistical Analysis: Data collected were subjected to one way analysis of variance to establish significant difference between means, and the result were subjected to *pos hoc* multiples test with least significant limit at $p < 0.05$.

Results and Discussion

The composition values of tiger nut with regards to moisture (47.41±0.50 mg/ml), protein (9.62±0.22 mg/ml) and carbohydrate (18.02±0.16 mg/ml) tiger nut differed significantly ($p < 0.05$) when compared with the values conventional yoghurts A and B (Table 1).

However, the highest values of fat obtained in the conventional yoghurts differed significantly ($p < 0.05$) with the values obtained in the produce tiger nut yoghurt.

Table 1: Proximate Compositions of Tiger Nut Yoghurt (mg/ml)

Proximate Compositions	Conventional Yoghurt A	Conventional Yoghurt B	Tiger nut Yoghurt

Ash content	1.02 ± 0.01^{a}	1.09 ± 0.12^{a}	1.14 ± 0.35^{a}
Fat	9.81 ± 0.70^{a}	10.32 ± 0.20^{a}	8.60 ± 0.56^{b}
Moisture (Aw)	35.67 ± 0.34^{a}	52.78 ± 0.11^{b}	47.41 ± 0.50^{c}
Protein	7.2 ± 0.15^{b}	6.79 ± 0.09^{a}	9.62 ± 0.22^{c}
Carbohydrate	14.43 ± 0.06^{c}	12.12 ± 0.57^{b}	18.02 ± 0.16^{a}
Crude fibre	19.12 ± 0.11^{a}	21.23 ± 0.42^{a}	28.02 ± 0.77^{b}

Results are expressed as mean±SD (n=2) Values with different superscripts on the same row are significantly different ($p < 0.05$).

The results of this study are in line with the findings of Suleiman *et al*. (2018) on proximate composition, mineral and some vitamin contents of tiger nut. They reported high moisture content while among the macromolecules, carbohydrate was relatively higher when compared with other compounds. Higher amount of moisture as available water in root crops is one of the significant characteristics (Suleiman *et al*., 2018). High concentration values of ash, carbohydrate, protein and crude fiber observed in the produced tiger nut may be due to plant based sources. Table 2 illustrates the vitamins composition of tiger nut and conventional yoghurts A and B. The table shows that conventional yoghurts A and B recorded 6.01±0.21 mg/l and 5.32±0.12 mg/l (vitamin C) respectively. This indicates that their concentration values differed significantly ($p < 0.05$) when compared to tiger nut derived yoghurt with values of 8.39±0.04 mg/l. Vitamins A concentration values in the tiger nut yoghurt (0.302±0.014 mg/l) is higher than the values obtained in the conventional yoghurts though their values are not significantly difference ($p > 0.05$).

Table 2: Evaluation of the Vitamins Composition of Tiger Nut Yoghurt and Conventional Yoghurt Samples

Vitamin (Mg/L)	**Conventional Yoghurt A**	**Conventional Yoghurt B**	**Tiger Nut Yoghurt**
Vitamin C	6.01 ± 0.21^{a}	5.32 ± 0.12^{a}	8.39 ± 0.04^{b}
Vitamin A	0.22 ± 0.13^{a}	0.18 ± 0.22^{a}	0.302 ± 0.014^{a}
Vitamin E	BDL	BDL	BDL

Values are expressed as mean±SD (n=2) Values with different superscripts on the same row are significantly different ($p < 0.05$) BDL Below Detection Limit

The vitamins content according to table 2 shows that conventional yoghurt B has values of 5.32±0.12mg/l (vitamin C), while vitamin A and E recorded the values of 0.18±0.22 mg/l and below detectable limit respectively. In addition, the produced tiger nut yoghurt has values of 8.39±0.04 mg/l (vitamin C), 0.302±0.014 mg/l (vitamin A) and below detection limit (vitamin E). High concentration values of vitamin C in the produced tiger nut yoghurt differed significantly ($p < 0.05$) with conventional yoghurts A and B.

Table 3 shows the heavy metal analysis of tiger nut yoghurt compared to conventional yoghurt samples. The analysis revealed that Cd, Ni, and Pb are below detention limit in the three different yoghurts samples. Cr was within the below detectable limit in the sample B and tiger nut yoghurt while the concentration values of 0.072±0.02 mg/ml was observed in sample A yoghurt. It was deduced that the value of Cu in tiger nut yoghurt (0.443± 0.01 mg/ml), sample A (0.161±0.02 mg/ml) and B (0.214±0.04 mg/ml) yoghurts differed significantly ($p < 0.05$). From the table, Fe recorded higher values of 6.23±0.01 in the tiger nut yoghurt sample A yoghurt when compared to conventional yoghurt A and B with values of 4.56±0.04 mg/ml and 1.65±0.05 mg/ml respectively. Their concentration values however differed significantly ($p > 0.05$). A marked significant different ($p > 0.05$) with reference to Zn is observed in the concentration values of the studied yoghurts, though the tiger nut yoghurt recorded the highest values of Zn. This however may place the produced tiger nut yoghurt as choice yoghurt in the local market most especially for bone strengthening among adult population group.

Table 3: Mineral Composition of Tiger Nut and Conventional Yoghurts

Metals (mg/kg)	**Conventional Yoghurt A**	**Conventional Yoghurt B**	**Tiger nut Yoghurt**
Cd	BDL	BDL	BDL
Cr	0.072±0.02^{a}	BDL	BDL
Cu	0.161±0.02^{a}	0.214±0.04^{b}	0.443±0.01^{ab}
Fe	4.56±0.04^{c}	1.65±0.05^{a}	6.23±0.01^{b}
Ni	BDL	BDL	BDL
Pb	BDL	BDL	BDL
Zn	0.213±0.02^{a}	0.411±0.03^{b}	0.631±0.012^{c}

Values are expressed as mean±SD (n=2) Values with different superscripts on the same row are significantly different ($p < 0.05$) BDL Below Detectable Limit

Arsenic, beryllium, cadmium, chromium, lead, mercury, nickel, manganese and aluminum are metals with relatively high atomic mass and thus which reflect in their atomic weights (Osei, 2008). They are called heavy metals due to their high relative atomic mass. Heavy metals concentration analyzed using atomic absorption spectrophotometer machine showed below detection limits for trace metals such as Cd, Ni and Pb in all the analyzed samples (Table 3). Cr was within the un-detection limit in the tiger nut derived sample and conventional yoghurt A. Zn showed highest bioaccumulation concentration in all the analyzed samples while Cu and Fe were relatively abundance. The concentration values of Fe varied significantly ($p < 0.05$) in all the yoghurts. Cr was seen in conventional yoghurt B. Heavy metals were seen in the conventional yoghurt samples than in the tiger nut derived sample.

Table 4 shows the phytochemical composition of tiger nut yoghurt compared to conventional yoghurt samples. The table reveals that all the samples contain varying quantities of phytate, lectin, polyphenols, total tannins, residual sugars and trypsin inhibitors. Tiger nut yoghurt concentration values of polyphenol (40.39±0.14 mg/l), total tannin (39.44±0.12 mg/l) and phytate (17. 01±0.41 mg/l) are significantly different ($p < 0.05$) when compared to values of 28.32±0.32 mg/l and 25.09±0.01 mg/l (polyphenol), 32.67±1.11 mg/l and 30.14±0.32 mg/l (total tannin) and 5.01±0.10 mg/l and 6.27±2.21 mg/l (phytate) obtained in conventional yoghurts A and B respectively. Contrastingly, the values of lectin in the tiger nut and conventional yoghurts A and B are not significantly different ($p > 0.05$). However, there is a marked significant different ($p < 0.05$) in concentration values of residual sugar in all the studied yoghurts, while concentration values of trypsin inhibitors in sample A (14.26±0.30 mg/ml) and B yoghurts (12.09±0.12 mg/ml) differed significantly ($p < 0.05$) with the observed values in tiger nut (10.80±0.02) yoghurts.

Table 4: Phytochemical Composition of Tiger Nut and Conventional Yoghurts

Phytochemical Components (Mg/L)	Conventional Yoghurt A	Conventional Yoghurt B	Tiger Nut Yoghurt
Phytate	$5.01±0.10^{a}$	$6.27±2.21^{a}$	$17.01±0.41^{b}$
Lectin	$11.20±0.41^{a}$	$13.11±0.30^{a}$	$9.76±0.21^{a}$
Polyphenols	$28.32±0.32^{a}$	$25.09±0.01^{a}$	$40.39±0.14^{b}$
Total Tannin	$32.67±1.11^{a}$	$30.14±0.32^{a}$	$39.44±0.12^{b}$
Residual sugars	$21.09±0.20^{c}$	$17.21±0.42^{b}$	$8.81±0.21^{a}$
Trypsin inhibitors	$14.26±0.30^{b}$	$12.09±0.12^{b}$	$10.80±0.02^{a}$

Values are expressed as mean±SD (n=2) Values with different superscripts on the same row are significant different ($p < 0.05$)

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 (Table 4). The results of concentration in tiger nut yoghurt show substantial concentration in phytate, polyphenol and total tynnin when compared with values obtained from conventional yoghurts A and B. In addition, there is no significant different ($p > 0.05$) in the concentration of lectin and trypsin inhibitors in all the studied yoghurts. Earlier study reported a relatively low level of phytate on dairy produced from tiger nut yoghurt with high concentration of antioxidant polyphenols and flavonoid (Suleiman *et al.*, 2018). Anti-nutrient phytate according to their study is scavenging agent in plant materials especially root crops and vegetables. Table 5 shows the physicochemical properties of tiger nut yoghurt compared to conventional yoghurt samples. The table shows that TTA concentration values of sample A (1.16±0.08 mg/ml), B (1.13±2.01 mg/ml) and tiger nut yoghurts (1.01±0.03 mg/ml) are not significantly different ($p > 0.05$). There is observed significant different ($p < 0.05$) with reference to concentration values of conductivity, TS, Mg and calcium in the sample A, B, and tiger nut yoghurts. The highest concentration values of viscosity (102.32±7.04 mg/ml) and total organic matter (187.25±0.05 mg/ml) was recorded in the untreated tiger nut sample B. The values of the two physicochemical parameters in the tiger nut yogurt varied significantly ($p < 0.05$) with concentration values in sample A and B yoghurts. Contrastingly, there is a significant different in the values of TSS in the sample A and B. The two yoghurts recorded highest values of 762±0.05 mg/ml and 755.6±0.41 mg/ml respectively. With reference to pH and TDS the concentration values differed significantly ($p < 0.05$) with the values observed in sample B and tiger nut yoghurt.

Table 5: Physicochemical Properties of Tiger Nut Yoghurt Compared to Conventional Yoghurt Samples

Physiochemical Parameters	**Conventional Yoghurt A**	**Conventional Yoghurt B**	**Tiger Nut Yoghurt**
pH	$3.0±0.01^{a}$	$3.40±1.07^{b}$	$3.7±0.21^{c}$
TTA	$1.16±0.08^{a}$	$1.13±2.01^{a}$	$1.01±0.03^{a}$
Conductivity $\Omega^{-1}cm^{-1}$	$2360.54±0.05^{c}$	$2263±0.10^{b}$	$2204±1.12^{a}$
Viscosity	$84.11±2.06^{a}$	$87.70±0.14^{a}$	$102.32±7.04^{c}$
TDS (g/ml)	$1691.50±0.23^{c}$	$1623.9±1.00^{b}$	$1572.1±0.13^{a}$
TSS (g/ml)	$762.01±0.05^{c}$	$755.6±0.41^{b}$	$689.6±0.23^{a}$
TS (g/ml)	$2453.5±0.13^{d}$	$2379.5±0.33^{c}$	$2261.7±0.01^{a}$
Magnesium (mg/ml)	$12.42±0.09^{a}$	$17.61±0.22^{b}$	$33.72±0.11^{d}$
Calcium (mg/ml)	$25.16±1.01^{a}$	$30.04±0.20^{b}$	$42.43±0.13^{d}$
Total Organic Carbon mg/ml	$125.27±0.13^{a}$	$130.06±1.01^{b}$	$152.23±0.23^{c}$
Total Organic Matter mg/ml	$154.08±0.23^{b}$	$159.97±0.10^{b}$	$187.25±0.05^{c}$

Values are expressed as mean±SD (n=2) Values with different super scripts on the same row are significant different ($p < 0.05$)

The values of pH, TTA and conductivity are not significantly different ($p > 0.05$) in all the yoghurts but differed significantly ($p < 0.05$) with regard to TDS, calcium, TOC and TOM in the concentration values of conventional yoghurts. Low pH value and total acid concentrations of yoghurt drinks can be attributed to in situ compositions of dairy drinks and starter cultures used for the fermentations as most fermenting bacteria used during yoghurt production are obligate acidophile (Allam *et al.,* 2016). Conductivity of the yoghurt drinks shows the exchangeability of dissolved ions in the liquor; other physical attributes such as TS, TSS and TDS revealed the presence of dissolved solid substances. Hardness is a physical attribute that reveal the presence of dissolve minerals such as calcium and magnesium. The presence of higher concentrations of Ca and Mg relatively showed the conventional nutritive mark quality of dairies as good source of calcium for bone and teeth formation. Results of the previous studies indicates that pH values of 6.07 – 6.67 (Gemechu *et al.*, 2015) and 4.7 (Babatuyi *et al.*, 2019) obtained from animal based yoghurt drink are higher than the results obtained in this study. Interestingly, pH values of 3.7±0.21 obtained from tiger nut yoghurt relatively agree with pH values of 3.9 reported by Onyimba *et al*. (2022) and finding of previous study done by Sanful (2009). The study confirmed that pure tiger nut yoghurt had higher pH values when compared with milk yoghurt obtained from animal based yoghurt. This claim however validates claim that pH tilted to the left side is an ideal concentration values for yoghurt drinks.

Figure 1 illustrates the effect of incubation period on microbial growth rate at different innoculum sizes. The microbial cell count across the different innoculum sizes were found to be dependent on the incubation time such that a significant increase in cell count was observed between 12-24 hours while non-significant difference in microbial counts were observed between 24-48 hours. However, there was a decline in cell count for 1.5 and 2.0ml of innoculum after 36 hours, whereas cell count for 0.5ml, 1ml, 1.5ml and 2ml appeared to be highest at 48 and 36 hours of incubation respectively.

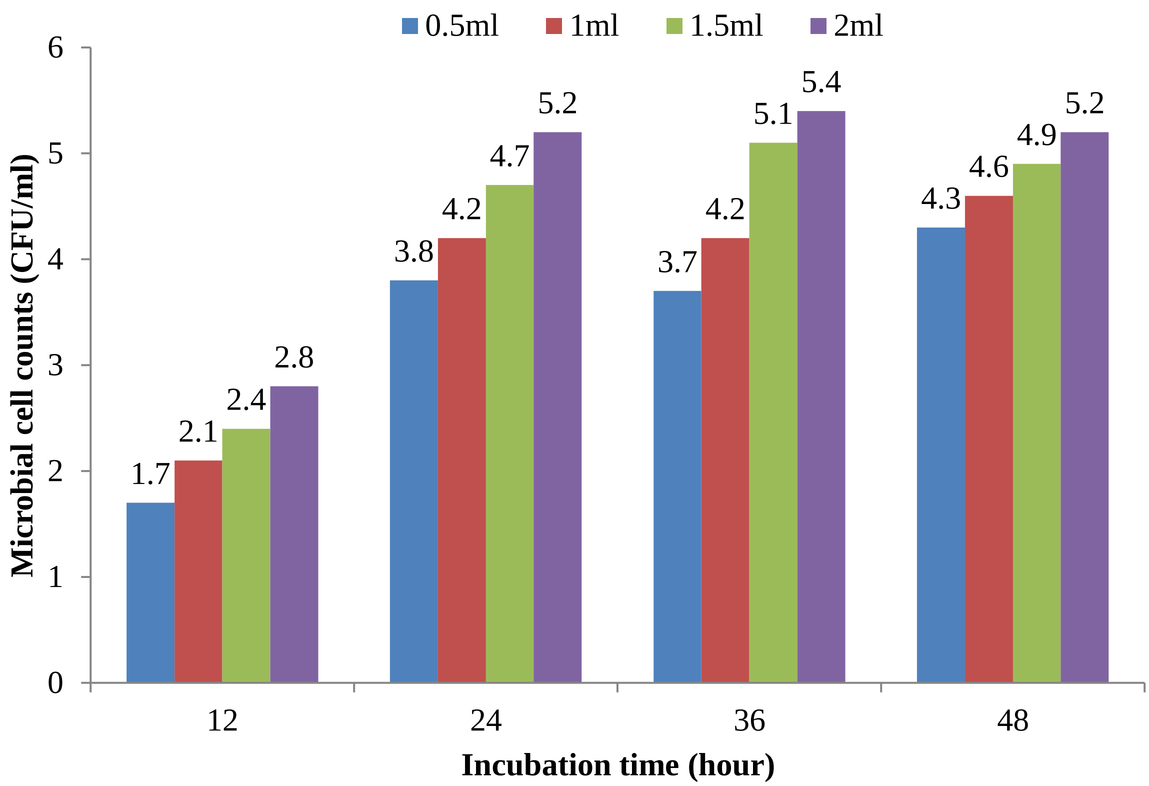

Figure 1: Effect of innoculum sizes and time on the growth of Bacteria

Studies on the effect of incubation period on microbial growth rate at different inoculum size are dependent on the incubation time. A significant increase in cell count was observed between 12-24 hours while non-significant difference in microbial counts was observed between 24-48 hours (Figure 1). However, there is a decline in cell count for 1.5 and 2.0ml of innoculum after 36 hours, whereas cell count for 0.5 and 1ml appeared to be highest after 48 hours of incubation. Ezeonu *et al*. (2013) reported that microbial population largely depend on the inoculum volume, they state that proliferation of microorganism is categorized in four distinct stages (lag phase, exponential phase, stationary phase and death phase). Decline in the microbial heterotrophic counts could be attributed to multiplicity of the microbes leading to deficient nutrient in the media (Ezeonu *et al*., 2013).

The microbial concentration of the conventional and produced tiger nut yoghurts is indicated in Table 6. The results reveal that the highest microbial loads of TVC and TCC and TBC are observed in tiger nut yoghurt and conventional yoghurt A, while the lowest microbial loads of TVC and TCC and TBC are observed in conventional yoghurt B and tiger nut yoghurt at day 0 respectively. At day 7 yoghurt A, tiger nut yoghurt and yoghurt B recorded the highest loads of TVC (3.8×10^2) and TCC (4.33×10^2) and TBC (6.12×10^7), while tiger nut, sample A and B yoghurts highest loads of 3.3×10^5 (TVC), 5.1×10^3 (TCC) and 4.1×10^6 (TBC) are observed at day 14 respectively.

Table 6: Microbial Concentration of Conventional and Tiger Nut Yoghurts (CFU/ml)

Yoghurt Sample	Day	Total Viable Count	Total Colony Count	Total Bacteria Count
Yoghurt A	0	4.6×10^3	8.0×10^2	6.8×10^6
Yoghurt B	0	2.9×10^2	7.8×10^3	5.33×10^5
Tiger Nut Yoghurt	0	5.4×10^3	1.3×10^3	5.6×10^4
Yoghurt A	7	3.8×10^2	1.4×10^7	5.2×10^7
Yoghurt B	7	2.9×10^4	1.09×10^6	6.12×10^7
Tiger Nut Yoghurt	7	1.9×10^3	4.33×10^2	5.55×10^6
Yoghurt A	14	2.2×10^4	5.1×10^3	3.3×10^7
Yoghurt B	14	2.5×10^3	3.9×10^5	4.1×10^6
Tiger Nut Yoghurt	14	3.3×10^5	2.3×10^4	1.6×10^5

Microbial isolations, counting and identification of the inhabitant microbes from the different yoghurt drinks showed wide spectrum of microbial load from each of the yoghurts and tiger nut yoghurt samples. TVC which indicate organismal consortium from the 10^{-2} dilution factor showed heterotrophic counts of 4.6×10^3 CFU/ml, 2.9×10^2 CFU/ml and 5.4×10^3 CFU/ml for the yoghurts A, B and the tiger nut processed yoghurt respectively at day 0 of the counting. Colony counts which reflect the presence of bacteria of the organisms plated out from the 10^{-2} dilution factor showed heterotrophic counts of 8.0×10^2 CFU/ml, 7.8×10^3 CFU/ml and 1.3×10^3 CFU/ml for the yoghurts A, B and the tiger nut yoghurt respectively at day 0 of the microbial count. The microbial loads of TCC (1.3×10^3 cfu/ml) obtained in the recent study done by Onyimba *et al*. (2022) are similar to the result observed in the produced tiger nut yoghurt. The results of total bacterial counts which reflect the multiplicity of desired bacteria from the 10^{-1} dilution factor showed heterotrophic counts of 6.8×10^6 CFU/ml, 5.33×10^5 CFU/ml and 5.6 x

10^4 CFU/ml for the yoghurts A, B and the tiger nut derived yoghurt respectively at day 0. Allam *et al.* (2016) reported that the seasonal fluctuation of bacterial at various physiologic factors such as pH, incubation periods, and temperature impacts on microbial proliferation in dairies. There was differential growth in the total population of the microorganisms 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 yoghurts A, B and the tiger nut processed yoghurt drinks respectively at day 7, total colony counts (TCC) showed heterotrophic counts of 1.4 x 10^7 CFU/ml, 1.09 x 10^6 CFU/ml and 4.33 x 10^2 CFU/ml for the yoghurts A, B and the tiger nut processed yoghurts respectively at day 7 of the counting and total fermenting bacteria counts (10^{-1}) showed heterotrophic counts of 5.2 x 10^7 CFU/ml, 6.12 x 10^7 CFU/ml and 5.55 x 10^6 CFU/ml for the sample A, B yoghurt drinks and the tiger nut derived yoghurt respectively at day 7 of the counting respectively. TVC showed heterotrophic counts of 2.2 x 10^4 CFU/ml, 2.5 x 10^3 CFU/ml and 3.3 x 10^5 CFU/ml for the sample A, B yoghurt drinks and the tiger nut derived yoghurt respectively at day 14 of the counting. The results of microbial load of 3.5 x 10^1 CFU/ml in the produced tiger nut yoghurt earlier reported by Agwuna *et al*. (2019) are in consonance with the values of TVC. TCC showed heterotrophic counts of 5.1 x 10^3 CFU/ml, 3.9 x 10^5 CFU/ml and 2.3 x 10^4 CFU/ml for the yoghurt A, B and the tiger nut derived yoghurt drinks respectively at day 14 of the counting and total fermenting bacteria count (TBC) counts showed heterotrophic counts of 3.3 x 10^7 CFU/ml, 4.1 x 10^6 CFU/ml and 1.6 x 10^5 CFU/ml for the sample A, B yoghurt drinks and the tiger nut derived yoghurt respectively at day 14 of the counting respectively. Similar result obtained by Bristone *et al*. (2015) is in accordance with results of TBC obtained from conventional yoghurts A and B at day 0 and 7. The highest microbial concentration values of the produced tiger nut observed in TBC are comparatively similar with earlier report by Allam *et al.* (2016). Their study on production of β-galactosidase enzyme from *Lactobacillus acidophilus* RK isolated from different sources of milk and dairy products stated that lactic acid bacteria are predominant organism with highest heterotrophic counts in dairy and dairy products.

Conclusion

This examined the physicochemical, nutritive and microbial loading indexof tiger nut derived yoghurt and conventional yoghurts. The research output vividly show that the produced tiger nut has higher concentration values of proximate compounds of: ash, carbohydrate, protein and crude fiber when compared with values observed in the conventional yoghurts A and B. Similar concentration patterns are observed with regards to vitamins (A and C), minerals (Cu, Fe and Zn), phytochemical (phytate, polyphenols and total tanin) and physicochemical (pH, viscosity, magnesium, calcium, total organic carbon and total organic matter) contents of the studied yoghurts. Also, the study found that bacterial concentration increases with increase in inoculum size and time. The microbial concentration of TCC and TBC peaked at day 7 in the produced tiger nut yoghurts, while TVC and TBC concentration of conventional yoghurt A recorded minimum concentration at day 14.

The elevated concentration of essential nutrients in the tiger nut has shown that the produced yoghurt is healthier than the conventional yoghurts. The study recommends production and promotion of plant based yoghurt for healthy and unhealthy individuals in the study area.

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