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Assessment of the Quality of Yoghurt Produced From Cow Milk Using Natural and Artificial Starter Culture as a Function of Storage Time

***Joseph Oluwole AYODELE**
Department of Chemistry, Faculty of Physical Sciences,
Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria.
jwoleayo@gmail.com
+2348030401512

Israel Kehinde OMONIYI
Department of Chemistry, Faculty of Sciences,
Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria.
kiomoniyi@abu.edu.ng
+2348036257789

Olukemi R. Agbeke Iyun (Mrs)
Department of Chemistry, Faculty of Sciences,
Ahmadu Bello University, P.M.B. 1044, Zaria, Nigeria.
skemiruthiyun@gmail.com

+2348179287797

Salisu ABUBAKAR
Department of Chemistry, School of Sciences,
Federal University of Education, P.M.B. 1041, Zaria, Nigeria.
salismabera@gmail.com or abubakarsalisu@abu.edu.ng
+2348038069967

Augustina Oyibo UGWOKE

Department of Chemistry, Nigeria Police Academy, P.M.B. 3474, Wudil, Kano State, Nigeria
augustinaaroh@yahoo.com
+234 902 047 1987

Abstract:

Yoghurt is globally regarded as a functional food which combines unique nutritional values with promotion of good health, heart functions, and natural immune defense in humans. This study aimed to assess the quality of yoghurt produced from cow milk using natural and artificial starter culture and determine its physicochemical properties, antioxidant and mineral composition using the standard storage time (28 days). Fresh milk was collected from cow farms at National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria, it was kept in an ice box immediately to protect it from fermentation. A 1000 mL milk sample was boiled, cooled, poured to a small bowl and a tea spoon of the lemon juicy was added to it, it was kept for 20 Hrs in a warm

place to thicken. The sample was heated to 63 °C for 30 minutes to destroy unwanted microorganisms and provide a cleaner medium in which the desired organism can grow. It was immersed in a basin of cold water to stabilize it to about 42^{o}C. The pasteurized milk samples was then mixed with 2.5% of a mixed starter culture (2:1 of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and stirred cautiously until the pH reached 4.7. It was then left to coagulate for 3 hours at 42 ^{o}C. Some physicochemical parameters were comparatively determined. The findings revealed that, yoghurts produced from cow milk using natural starters has the highest Total solids TS (13.95 %), Ash TAC (0.69 %), Oil (5.3 %), Protein (3.31 %), pH 4.96, and Lactic Acid (1.07 %) at day1. It also exhibited higher phenol content (0.125 g/L), phosphomolybdate (0.082 g/L) and Tannins (0.082 g/L). It also content higher % of macro nutrient (P: 0.234 g/L, K: 1.050 g/L, Na: 1.450 g/L, Zn: 0.101 g/L and Ca s0.697 g/L). On the other hand, the one with artificial starter culture had higher Vitamin C (7.56 g/L) at storage time (28) days. It was concluded that, yoghurts produced from natural starter culture content higher % of some macro nutrient. While the one from artificial starter culture exhibited better storage capability and stability of shelf life.

Keywords: Yoghurt, Quality, Starter culture, Phytochemicals, Minerals, Vitamins

Introduction

Yoghurt is globally regarded as a functional food which combines unique nutritional values with promotion of good health, heart functions, and natural immune defense in humans [1]. Today eDairy Market shows you the relevance of this essential dairy product, yogurt. It is widely consumed for several reasons, such as its health benefits, taste, versatility and long shelf life.

One of the main reasons for yogurt's popularity is its health benefits. Yogurt contains live bacterial cultures that are good for intestinal health and digestion. It is also a rich source of protein, calcium and other nutrients essential for maintaining a healthy body.

In addition, yogurt is a versatile food that can be consumed in many different ways. It can be eaten on its own as a snack, used as a topping for fruit or granola, or added to smoothies and other recipes. It has a long shelf life, making it an ideal food for storage and transport. In many cultures, yogurt is also a traditional food that has been consumed for centuries and is a staple in the local diet.

Overall, the combination of health benefits, versatility and convenience has made yogurt a popular food all over the world [2].

It is manufactured from milk fermented by the symbiotic action of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. Bulgaricus.* During the fermentation of milk, the lactic acid bacteria (LAB) utilizes lactose and nitrogenous compounds leading to production of extracellular lactic acid and various other primary metabolites that provoke acidification of the medium [3]. This causes several chemical changes which include decrease of pH, increased nutrient bioavailability, formation of flavor compounds, coagulation of proteins, and the subsequent gel formation [4].

Several health benefits including strengthening the immune system, improvement of the digestive function, and protection against colon cancer and Helicobacter pylori infection [5]. In recent years, there is an increasing demand for taste, quality, stability and shelf life of yoghurt [6]. However, it has been discovered that nutrient consumption does not commensurate with the recommended intake by nutritionists [7]. To ameliorate this problem of low-level protein intake, especially from cheap dairy sources, there is the need for concerted effort to bring about the massive production and utilization of protein based food items from diary milk and at competitive costs so that they would be affordable and accessible.

According to recent statistics the country that consumes the most yogurt per capita is Turkey. The average Turk consumes an estimated 27 kg of yogurt per year. Other countries with high yogurt consumption are Armenia, Lebanon, Bulgaria and Greece. However, yogurt is also consumed in many other countries around the world, such as the United States, Canada and several European countries [8]

In general, yogurt is considered as a nutritionally dense food. It is rich in available protein, calcium, milk fat, potassium, magnesium, vitamin B_2, B_6, and B_{12} [9]. In addition, it provides milk proteins with a higher biological value and provides almost all the essential amino acids necessary to maintain good health. Yogurt is considered as a probiotic carrier food that can deliver significant amounts of probiotic bacteria in the body and that can provide specific health benefits once ingested [10].

While yogurt is able to bring some benefits to humans from the nutritional perspective, it also has some potential hazards that are threat to human health. It is well known that yogurt is made by fermenting milk, from milk to yogurt, the production process is also very complicated, and there are many spoilage and pathogenic organisms in both milk and yogurt. Many potential food safety biological hazards associated with yogurt. The potential biological hazards with yogurt may come from both intrinsic and extrinsic. The internal biohazard mainly comes from the ingredients such as milk and fruit preparations while the external biohazard of yogurt comes from the manufacturing processing and packaging [11].

There are many microorganisms present in yogurt and most of them are thermophilic and bile-sensitive. Most yogurt on the market is made with some fruit preparations to make yogurts more varied and tastier, but these fruit preparations also harbor many microorganisms that make the yogurt spoilage. The Bacteria in yogurt do not usually live in the intestine; however, bacteria migrating along the gastrointestinal tract can positively affect the digestive system by interacting with the host and resident bacteria [12].

In this study therefore, yoghurts from cow milk was prepared with natural and artificial starter cultures and assessed for their physicochemical characteristics, anti-oxidants and mineral composition, nutritional values and storage life.

Therefore, the objectives of this research is to prepare cow milk yoghurt using natural and artificial culture starter and assess the nutritional values of the yoghurt produced using natural and artificial starter culture.

Materials and Methods

All the reagents used are of analytical grade obtained from good chemical vendors. They include; Conc. H_2SO_4, 40% NaOH, 2% H_3BO_3, HCl, Na_2SO_4, $CuSO_4$, methyl red, n – Amyl alcohol, Folincalteous reagent, 17% Na_2CO_3, and Garlic acid.

Milk collection and preservation

Fresh milk was collected from cow farms of National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Zaria, Nigeria and it was kept in an ice box immediately after to protect it from fermentation.

Preparation of the natural starter culture

A 1000 mL of the milk sample was boiled, cooled and poured to a small bowl. A tea spoon of lemon juicy was added to the milk and the mixture was kept for (15-20 hours) in a warm place until it thickened [8].

Sample preparation

Artificial and natural starter culture were used for production of yoghurt according to the method described by [13]. The milk was heated at a temperature of 63°C for at about 30 minutes to deaerate it, denature the proteins which increase the viscosity of the product and destroy pathogens so as to provide a cleaner medium to grow desired organism.

The hot milk was then immersed in a basin of cold water to bring the temperature to 42 ^oC for pasteurization and it was mixed with 2.5% starter culture (2:1 of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*). The mixture was stirred to a pH of 4.7 and it was left to coagulate for 3 hours at 42 ^oC. The products were labeled as COYA, COYN for artificial starter culture and natural starter culture respectively. They were stored in a refrigerator for 28 days at 4°C.

Physicochemical Analysis

Determination of total solid

Total solid in the samples were determined by the method described by [14].

$$\% \text{ Total Solid} = \frac{W2 - W1}{W}$$

W_1 = the weight in gram of empty crucible

W_2 = the weight in gram of crucible + residue

W = the weight in gram of the sample used

Determination of total ash content

The amount of ash content in the samples were similarly determined using the gravimetric method according to [15].

$$\% \text{ Ash} = \frac{W2-W1}{W} \times 100$$

W_1 = the weight in grams of empty crucible

W_2 = the weight in grams of crucible + ash

W = the weight in grams of the sample

Determination of protein content

Protein content was determined according to [16]. The amount of nitrogen in the samples culture was determined according to the Kjeldahl method and the values obtained multiplied by the factor of 6.38 to determine the composition of protein.

Total nitrogen, per cent by weight (on moisture – free basis)

$$\% \text{ Nitrogen} = \frac{(T-B)\times N\times 14.007}{Volume\ of\ sample\ (ml)}$$ Where;

T – Titration volume of sample (ml)

B - Titration volume of blank

N – Normality of acid to 4 place decimals

14.007 = Relative molecular weight.

% Crude Protein = % Nitrogen × 6.38

Where: 6.38 = Nitrogen conversion factor.

Determination of fat content

The fat contents of the samples were determined by using the Rose Gottlieb method described by AOAC, [16].

$$\% \text{ Fat} = \frac{Weight\ of\ extracted\ fat\ (g)}{Original\ weight\ of\ sample\ used\ (g)}$$

Determination of pH

The pH meter (Mettler Toledo seven2go, Germany) was used to measure of pH of the samples as described by [17].

Determination of lactic acid of yoghurt sample

Lactic acid of the samples was determined by the method described by [17]. The percentage of titratable acid as lactic acid was calculated using the formula below;

$$\% \text{ Lactic acid} = \frac{9\times A\times N}{\text{Sample weight}} \times 100$$

Antioxidants Analysis

Determination of total phenolic content

The total phenolic content of yoghurt produced from cow, goat and sheep milk using natural and artificial starter culture was determined by the spectrophotometric method.

A 10 cm^3 portion of the milk sample was placed in a beaker; 10 ml of distilled water was added. The mixture was heated up on a hot plate for 5 minutes. The mixture was filtered into 50 cm^3 volumetric flask with the help of filter paper; filtrate was made up to the mark with distilled water. This filtrate was then reserved for phenol analysis.

Determination Ascorbic Acid Analysis (Vitamin C) by Iodine Titration

Vitamin C content was determined using the titrimetric method. The ascorbic acid content in the test samples was titrated with 2,6-dichlorophenolindophenol until a light pink color was developed [18].

Mineral Composition Analysis

The phosphorus composition of the samples was determined by colourimetry. Potassium, and Sodium composition were determined using atomic emission spectroscopy, while Calcium and

Zinc composition of the milk and yoghurt samples were determined using atomic adsorption spectrophotometry.

Results and Discussion

Table1. : Physicochemical Composition of Cow milk and Yoghurt samples from Natural (COYN) and Artificial sources (COYA)

Parameter	TDS		TAC		Oil		Protein		pH	
	COYA	COYN	COYA	COYN	COYA	COYN	COYA	COYN	COYA	COYN
Milk	10.2	10.2	0.2	0.2	9.9	9.9	1.81	1.81	6.21	6.21
1st Day	13.94	13.95	0.68	0.69	3.4	5.3	2.1	3.31	5.2	4.96
7th Day	14.08	14.69	0.76	0.69	3.3	3	2.3	2.36	4.32	4.48
14th Day	12.46	11.96	0.52	0.52	2.3	2	2.23	2.1	4.13	4.24
28th Day	12	11.42	0.47	0.41	1.9	1.3	2.07	1.99	4	4.03
mean	12.536	12.444	0.526	0.502	4.16	4.3	2.102	2.314	4.772	4.784
SD	1.297762	1.507256	0.176975	0.168661	2.671828	2.838427	0.153775	0.48286	0.759768	0.709615

Key: COYA = Yoghurt s from Artificial source, **COYN =** Yoghurt s from Natural source.

The total solid and ash contents of the yoghurt samples were observed to increase after fermentation had taken place. According to [19], the fermentation process would produce metabolites in the form of lactic acid. These metabolites would be secreted from the cell and accumulate in the fermentation fluid, so the residue of total sugar, lactic acid, and organic acids are counted as the total dissolved solids.

The total solids and ash content of the yoghurt samples were also observed to increase after 7 days before dropping sharply after 14 days and continuously to 28 days. This significant increase in ash contents after 7 days could be due to the loss of CO_2 and water during charring of yoghurt samples. The COYN was observed to be slightly higher nutritional values than COYA, but decreased faster, as COYA was observed to have higher total solids after 28 days [20].

Oil content of the yoghurt samples decreased drastically after fermentation, while protein content was observed to slightly increase, when compared to milk sample. However, a steady decline of both properties continued during storage to 28 days. COYN exhibited a sharper decline than COYA. [20], noted that the hydrolysis of casein and the increase of soluble nitrogen during shelf life of bovine milk yoghurts could be consequential in the decrease in the total protein content after 28 days of storage. Protein content for natural and artificial cow milk yoghurt were consistent with results obtained by [21] in a similar study.

pH of cow milk sample declined sharply after fermentation. Artificial Cow Milk Yoghurt was the least acidic on the 1st day (pH = 5.2) but the most acidic after 28 days. This could be due to the conversion of lactose to lactic acid as observed by the increase in lactic acid values [22]. During fermentation, S. *thermophilus* produces lactic acid

and formic acid which activate the growth of *L.bulgaricus* that produces diacetyl and acetaldehyde. These compounds were reported to give the typical yogurt flavor [22].

Cow milk yoghurt samples showed higher pH values which was slightly higher than results obtained in a similar study [23] reported lower pH values for natural and artificial cow milk.

Table 2: Antioxidant Composition of Cow milk and Yoghurt samples from natural (COYN) and Artificial sources (COYA)

Duration	Phenol		Vitamin C		Tannin		Phosphomolybdate	
	COYA	COYN	COYA	COYN	COYA	COYN	COYA	COYN
Milk	0.343	0.343	2.047	2.047	0.072	0.072	0.061	0.061
1st Day	0.112	0.125	7.56	4.1	0.035	0.082	0.061	0.082
7th Day	0.036	0.066	7	3.79	0.03	0.074	0.063	0.075
14th Day	0.032	0.052	5.69	2.01	0.027	0.07	0.075	0.067
28th Day	0.016	0.024	1.62	1.91	0.024	0.065	0.077	0.069
mean	0.1078	0.122	4.7834	2.7714	0.0376	0.0726	0.0674	0.057
S.D	0.1222	0.115317	2.487558	0.964284	0.01758	0.005571	0.007088	0.007949

From the results in Table 2, phenolic content of the milk samples was observed to be higher in yoghurt samples. The degradation of milk proteins during the yogurt bacteria's proteolytic activity, which releases some phenolic compounds like phenolic acids, flavonoids, and isoflavonoids in components of the natural and artificial milk protein, could account for the differences in TPC between the milk yogurt samples [23]. The TPC of the yoghurt samples exhibited a sharp decrease after seven days and a constant decline through to 28 days. All yogurts' capacity to maintain phenolic compounds is significantly impacted by storage time, where they investigated the contents of phenolic compounds in storage in four grape varieties in yogurt, and where they examined yoghurts made from goat and cow milk, yielded similar findings [24].

Vitamin C was observed to be higher in yoghurt samples than the milk sample. This was accompanied by a steady decrease during storage. COYA showed higher values of Vitamin C than COYN. [25] reported higher values of Vitamin C than the values obtained for natural cultured yoghurt from cow milk in this study. Vitamin C aids in the body's absorption of iron and calcium and stimulates the oxidation process, collagen production, and adrenal function. In addition, it aids in vitamin E regeneration [26].

COYA exhibited extremely lower values of tannin after fermentation while COYN showed a slight increase. Both samples showed a steady decrease in tannin during storage.

Phosphomolybdate assay was also observed to slightly increase after fermentation. However, while phosphomolybdate assay for COYA increased slightly during storage, that of COYN decreased gradually during storage and increased slightly after 28 days. This implies that COYA would perform better than COYN during storage as phosphomolybdates help to prevent oxidation of fats and oils, and extend the shelf life of dairy products. The difference in phosphomolybdate concentration could be due to the fact that antioxidants can be affected by many factors such as enzymes, pH, and temperature.

Table 3: Mineral Compositions of Yoghurt from cow milk using Natural and Artificial Sources

	Phosphorus		**Potassium**		**Sodium**		**Calcium**		**Zinc**	
Description	**COYA**	**COYN**	**COYA**	**COYN**	**COYA**	**COYN**	**COYA**	**COYN**	**COYA**	**COYN**
Milk	0.232	0.232	0.15	0.15	0.058	0.058	0.11	0.11	0.053	0.053
1st Day	0.118	0.234	0.485	1.05	0.37	1.45	0.596	0.663	0.084	0.101
7th Day	0.118	0.234	0.435	1.029	0.361	1.449	0.386	0.622	0.084	0.098
14th Day	0.109	0.21	0.435	1.011	0.35	1.432	0.347	0.477	0.062	0.071
28th Day	0.099	0.093	0.411	0.992	0.3	1.4	0.342	0.411	0.06	0.067
mean	0.1352	0.2006	0.3832	0.8464	0.2878	1.1578	0.3562	0.4566	0.0686	0.0638
S.D	0.048906	0.054559	0.119067	0.348729	0.117423	0.550197	0.154462	0.196258	0.012924	0.020388

Results in Table 3, shows Potassium, Zinc, Calcium and Sodium observed to be higher than in yoghurt samples than milk samples except for phosphorus in COYA. The mineral composition was also observed to decrease gradually during storage. COYN contained higher mineral concentrations than the COYA. [27] reported a higher average value for Calcium (120.33 mg/), Phosphorus (85.33mg/L), Potassium (136.87 mg/L) but lower values for Zinc (0.37 mg/100g) for yoghurt from natural starter culture.

Conclusion

This study to assess the physicochemical, anti-oxidant properties, and mineral composition of cow milk yoghurt from natural and artificial starter. Results from the findings revealed that yoghurts produced from natural starter culture exhibited the high physicochemical properties and minerals, but less storage (shelf life). While yoghurts from artificial starter culture exhibited better storage capability and stability of shelf life with fewer physicochemical parameters.

Therefore all the null hypotheses were rejected. The acceptability of yoghurt products and their storage potential are very important factors to consider in the production of yoghurts. Certain additives can be employed to improve the shelf-life of yoghurt samples as well as their nutritional composition. It is recommended that, in order to ensure that the external factors of yogurt do not harm the quality and ensure the safety of the yogurt, workers should focus on the packaging and manufacturing processing.

Yoghurt producer need to pay attention to the growth of the harmful microorganisms in the ingredients and there is need to disinfect and sterilize every device used at each step to ensure and control yeasts and molds, which are the main causes of spoilage in yogurt.

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