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Comparative Analysis of Proximate Composition, Microbial, Antioxidant and Sensory Evaluation of Yoghurts Made from Chili Pepper and Freeze Dried Starter

Halima Suleiman[1], Gabriel O. Anyanwu[1], Gideon A. Gyebi[2]*

[1]*Department of Biochemistry, Faculty of life sciences, Bingham University Karu, Nigeria.*
[2]*Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, P.O. Box 1334, Durban 4000, South Africa.*

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ABSTRACT

Fermentation is an important process in yoghurt production. The precise role that red chilli peppers play in fermenting milk to make yoghurt is still unknown. The study examined the use of chili pepper as an alternative fermentation starter in yoghurt production. The pH, proximate composition, microbiological, antioxidant, phenolic, and sensory qualities of yoghurt produced from chili pepper starter (CPS) and freeze-dried starter (FDS) were compared. Results showed that the pH of milk decreases with an increase in chili pepper concentration. In terms of proximate composition, the protein (8% and 8.3%, respectively), fat (1.02% and 1.01%), and carbohydrate (16% and 17%) contents of the chili pepper and FDS yoghurts were similar. The microbial analysis verified the presence of Lactobacillus in both varieties of yoghurt, but antioxidant analysis reveals little antioxidant activity. Sensory analysis shows overall acceptability of the two yoghurts. The phenolic content of chili pepper yoghurt (572.30 ± 1.83 mg GAE/kg) was significantly higher in comparison to the FDS yoghurt (526.05 ± 0.77 mg GAE/kg). According to the study's findings, chili pepper can be utilized as a successful starter culture to produce yoghurt that is on par with yoghurt made with FDS, providing a novel approach to yoghurt production.

Keywords: Chili pepper, Freeze-dried starter, Yoghurt, *Lactobacillus*, Proximate composition, Sensory analysis.

Introduction

Yoghurt, a fermented milk product with a long history of consumption, is cherished for its nutritional value, sensory characteristics, and established health benefits.[1] Yoghurt is traditionally produced by fermenting milk with a consortium of bacteria, primarily *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Yoghurt boasts a unique flavour profile, high protein content, and the presence of beneficial lactic acid bacteria (LAB).[2] These LAB contribute significantly to human health by promoting a balanced gut microbiome, aiding digestion, and potentially offering protection against various gastrointestinal disorders.[3] Consumer demand for functional foods, which provide both necessary nutrition and extra health advantages, has increased in recent years.[4] Significant research into novel starting cultures and functional additives has been spurred by this change in customer expectations, especially in the yoghurt market. Because of its probiotic qualities, yoghurt has gained attention for increasing its ability to promote health.[5]In this regard, there has been a surge in interest towards exploring novel starter cultures for yoghurt production.[4] Novel starter cultures with probiotic properties or the ability to enhance specific health benefits like immune function or gut health hold significant potential in this market segment.[6]

*Corresponding author. Email:gideonagyebi@gmail.com
Tel: 27 736369403

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Traditional starter cultures may limit the sensory and functional diversity of yoghurt products. Exploring novel cultures derived from various sources, including vegetables, fruits, and alternative milks (e.g., soy, almond), opens doors to the development of yogurts with unique flavours, textures, and potentially enhanced functionalities like prebiotic effects.[7] The vast microbial diversity present in nature offers a treasure trove of potential starter cultures with unique functionalities. Research efforts directed towards isolating and characterizing novel strains from various ecological niches can lead to the discovery of cultures with superior properties for yoghurt production.[8] This growing interest is seen in the increasing number of scientific studies investigating the application of novel starter cultures in yoghurt production. For instance, research by Lee *et al.*[9] explored the use of *Lactobacillus plantarum* cultures isolated from kimchi for yoghurt fermentation, demonstrating their ability to produce yogurt with desirable sensory characteristics and potential probiotic properties. Red chili peppers (*Capsicum annuum*), a rich source of bioactive compounds, have emerged as a promising ingredient for yoghurt innovation due to their potential health benefits and unique sensory properties.[10] The primary bioactive compound in red chili peppers is capsaicin, responsible for the characteristic pungency. Capsaicin has been linked to a range of potential health benefits, including anti-inflammatory, antioxidant, and analgesic properties.[10] Additionally, recent research suggests capsaicin may possess prebiotic properties, selectively stimulating the growth of beneficial gut bacteria while inhibiting the growth of pathogenic bacteria.[11] These potential health benefits associated with capsaicin make red chili peppers an intriguing ingredient for incorporation into yoghurt fermentation. However, the potential application of red chili pepper in yoghurt production remains relatively unexplored.

This present study was aimed at conducting a comparative study on yoghurt produced with a red chili pepper starter culture against plain yoghurt prepared using freeze-dried starter culture. Thes study delved into the complex interplay between red chili pepper incorporation and the final yoghurt product, encompassing a detailed analysis of its physicochemical properties (pH, titratable acidity, sugar content, fat

content, total solids), microbiological profile (starter culture viability, potential presence of additional microbial communities), and sensory attributes (taste, aroma, texture, the study assessed consumer acceptability through sensory panels. Furthermore, the in vitro functionality of red chili pepper yoghurt will be compared to plain yoghurt, investigating potential prebiotic effects and other health-promoting properties relevant to gut health.

By undertaking this comparative study, this research seeks to contribute valuable knowledge to the field of functional yoghurt development. The findings will not only elucidate the impact of red chili pepper starter cultures on the microbiological, sensory, and physicochemical characteristics of yoghurt but also provide insights into the potential health benefits associated with the consumption of yoghurt. Ultimately, this research has the potential to pave the way for the production of novel yoghurt products with enhanced health functionality and consumer appeal, enriching the landscape of functional foods.

Materials and Methods

Freeze dried yoghurt starter (Yogourmet Lallemand special culture) and Lactorich powdered milk was purchased from central market, Kaduna state, located on longitude 10.516224 and latitude 7.423251 while the Red chili pepper was purchased from kado biko market, Abuja located on longitude 9.099602 and latitude 7.417738, Nigeria.

Chili samples and treatments
Capsicum annuum (red chili) was 2% sodium hypochlorite solution was used to disinfect the surface. chili was submerged in sodium hypochlorite solution for 2 to 3 minute, rinsed thoroughly with sterile water 3 to 4 times, and dried in laminar flow hood.

Chili starter preparation
The samples were prepared in triplicate using 2 g, 5 g and 10 g of chili pepper. Thirty milliliters of milk was pasteurized at 85 degree Celsius and allowed to cool to 43 degree Celsius, place into 3 different containers, then the different grams of Chili pepper with stalk which is the 2 g, 5 g, and 10 g was added placed in an air-sealed container and kept in a warm dark room for 20hours to allow the growth of desired bacteria (*LAC bacterial*) after which it was cooled to +6℃ to stop fermentation.

Yoghurt preparation
Yoghurt was prepared according to Ning *et al.*[12] procedure with slight modification. Powdered milk was mixed with water with the ratio 2:1 of water and milk, were filtered and the milk was pasteurized at 85 ºC for 30 min and rapidly cooled to 43 ºC. Then the yoghurt starter culture (chili starter culture and freeze-dried starter culture was added to 2 different containers containing the milk at the rate of 3% and blended thoroughly. After that it was packed in plastic container and left for a final fermentation at 42 ± 1 °C. Yoghurt samples were cooled to +4 °C and held at this temperature overnight in other to end the fermentation process.

Microbial Analysis
All glassware were autoclaved at 121ºC for 15 minutes. Each yoghurt samples were serially diluted in (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) dilution. All media were prepared based to the manufacturer's instructions.

Lactobacillus sp. was grown in MRS agar and was used for the growth of pure culture. MRS agar was mixed with distilled water and autoclaved for 15 minutes at 121 psi. The MRS agar was aseptically poured into a single sterile glass Petri dish. The chosen yogurt samples were inoculated aseptically into a Petri dish via pour plate method with a flame and incubated at 37^0C for 24-48 hours.[13] The incubated bacteria from the yogurt samples undergoes biochemical tests and microscopy to confirm its identification. The tests include catalase test, coagulase test, sugar fermentation test (glucose, lactose), and Gram staining.[14] A preliminary morphological examination of the plate identified and defined the colonies obtained. The colonies appearance in the plates was examined. Macroscopic examination of colonies on plates for colony shape, size, elevation, shape, border, consistency, colour, odour, transparency, haemolysis, and coloration was used to characterise and classify the colony isolation.[15] Glucose and lactose broth was used as sugar indicator broths in the sugar fermentation test. This test is used to determine which sugars an organism can ferment, produce acid and gas from. The test organisms were Aseptically inoculated into test tubes with the test microorganism using an inoculating needle or loop. A Durham tube is added to each test tube and 1-3 drops of phenol red were also added to each test tube. The results were obtained after 24-48 hours at 37 degrees.

Determination of yoghurt samples pH
The method outlined in the literature was used to measure the pH of yoghurt samples.[16] In brief, 100 mL of distilled water was used to dissolve 10 g of yoghurt sample. he mixture was allowed to stand at room temperature, after which the pH of the samples was determined using a pH meter.

Determination of titratable acidity
The technique outlined by Oladipo *et al.*[17] was used to determine titratable acidity. In short, Ten grams of the sample were dissolved and thoroughly mixed in thirty milliliters of distilled waterA small volume of phenolphthalein indicator was introduced into the mixture, followed by titration with a standardized 0.1N sodium hydroxide solution until a light pink coloration remained stable for approximately 10–15 seconds, indicating complete neutralization. The titratable acidity was subsequently expressed in terms of lactic acid, which is the predominant organic acid in yoghurt samples.

Determination of carbohydrate content
Carbohydrates were determined using a mathematical function below as described in Literature.[18]

$$\text{CHO} = 100 - \%\,(\text{ash} + \text{protein} + \text{fat} + \text{crude fibre} + \text{moisture}) \quad \text{Equation 1}$$

Determination of fat content
The determination of fat content was carried out by measuring 20 ml of each yoghurt sample and diluting the sample with 20 ml of distilled water. Each measured yoghurt sample was poured into a thimble and placed into the Soxhlet extractor. N-hexane was added to the boiling flax of the Soxhlet extractor. At a boiling point of 200 degree celsius, this was left to extract for a minimum of 8 hours. After extraction, the solvent collected in the boiling flax was poured into a rotary evaporator for further extraction. After the fat is extracted using the rotary evaporator, the sample was put in an oven to dry for weighing and calculation of the end product.[18]

Determination of protein content
The macro Kjeldahl method, as detailed in the literature, was used to determine the crude proteins.[19] In brief, in a Kjeldahl digestion flask, 2g of the sample was combined with 10g of sodium sulfate and copper sulfate in a 5:1 ratio. The digestion flask was then filled with 25 mL of concentrated sulfuric acid, and it was carried out at approximately 1500°C in the fume closet until a clear and light blue coloring appeared and the frothing ceased. After cooling, distilled water was added to dilute the digest. to the appropriate level in a 100 mL volumetric flask of water. 18 mL of 40% sodium hydroxide was added to the distillation apparatus along with 10 mL of the diluted mixture. Two drops of a mixed indicator of bromocresol green and methyl red were introduced to the receiving conical flask along with 25 milliliters of 2% boric acid. The boric acid solution was distilled till It turned yellowish green instead of pink. 0.1N hydrochloric acid was used to titrate the solution in the conical flask until the end point was achieved following the distillation. A blank was obtained using the same technique but using distilled water. The protein was computed as follows using equation 2 and 3:

$$\text{percentage crude protein} = \text{percentage nitrogen} \times 6.38$$

Equation 2

percentage nitrogen = (ml standard acid - ml blank) x N of acid x 1.4007

Equation 3

Determination of moisture content

A modified technique of the oven drying method of Okunlola *et al*.[18] was adapted. Using a pair of tongs, the cleaned and dried petri dish was moved into a desiccator to chill after 30 minutes in the oven. After that, the weight of the empty dish was measured and noted as W1. The dish was reweighed and designated as W2 after two grams (2 g) of the yoghurt samples were added. To dry the sample, the oven was set to 10,000 degrees Celsius, and a steady weight was noted. After weighing the dish and its dried contents, the result was noted as W3. The equation below was used to determine the yoghurt sample's moisture content based on the recorded weights that signify W1, W2, and W3.

%mosture = (W2 - W3)/(W2 - W1) x 100

Equation 4

Sensory evaluation

Yogurt's organoleptic qualities—such as texture, taste, flavour, appearance, aroma and overall acceptability of yoghurt were assessed. The fifteen (15) panelists, who included four undergraduate and four graduate students, three lecturers, and four non-teaching staff members from Nile University of Nigeria and Bingham University Karu, assessed the samples. The samples were analysed using a nine-point hedonic scale.

Free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl)

The Okafor *et al*.[20] method was employed to measure the free radical scavenging activity. Briefly, 25 ul of test materials at different concentrations (78–1000 µg/ml) were mixed with 500 µl of a 0.3 mM alcoholic solution of DPPH. After 30 minutes of dark incubation, the samples were evaluated for absorbance at 518 nm using a PerkinElmer UV-visible spectrophotometer (Lambda 25; Perkin Elmer). The standard was ascorbic acid, while the control was pure water with no additional inhibitor. Equation 5 was used to express the scavenging activity as a percentage inhibition, and the experiments were conducted in triplicate.

% Scavenging = ([Abscontrol - Abssamples]/ Abscontrol)×100

Equation 5

Ferric Reducing Antioxidant Power (FRAP) Assay

A volume of 3.6 mL FRAP solution was mixed with 0.4 mL distilled water and incubated at 37 °C for 5 minutes. Subsequently, 80 µL of the yoghurt sample, prepared at concentrations of 7.8125, 15.635, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL, was added to the solution and incubated at 37 °C for 10 minutes. A calibration curve was prepared using $FeSO_4 \cdot 7H_2O$ at concentrations of 0.2, 0.4, 0.8, 1.0, 1.12, and 1.5 mM. The absorbance of the resulting reaction mixtures was then recorded at 593 nm using a spectrophotometer (Labomed Inc., Los Angeles, CA, USA). The absorbance values were calculated as for sample solutions.[21]

Determination of phenolic content

The Folin–Ciocalteu reagent was used in the spectrophotometric method to detect the phenolic content based on previous study with slight modification. In this investigation, 10 mL of (70% v/v) ethanol was combined with 0.25 g of yoghurt samples, and the mixture was extracted for 30 minutes in an ultrasonic bath. The extract was then centrifuged for 30 minutes at 3000 rpm. Next, 0.2 mL of the extract was mixed with 5 mL of distilled water, 0.8 mL of 7% sodium carbonate solution, and 1 mL of Folin–Ciocalteu reagent. The mixture was incubated in the dark at 20 °C for 60 minutes, after which the absorbance was measured at 760 nm using a spectrophotometer (Labomed Inc., Los Angeles, CA, USA).[22]

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) from triplicate measurements. The results from freeze-dried yoghurt and chili-pepper yoghurt analyses were exported into SPSS software (version 25) for statistical evaluation. An independent samples t-test was performed to compare the mean values, with statistical significance considered at $p < 0.05$.

Result and Discussion

pH Test result of chili starters

Yoghurt's qualities as an exceptional fermented milk product were known for its mild acidity and delightfully fresh flavour. In yoghurt production, increasing the amount of starter culture speeds up the fermentation process of milk. The pH test result shows the decrease in pH value of milk with the increase in chili pepper weight. The highest value of fermented milk is 4.51, which was observed in the sample with a chili pepper weight of 2 g, while the lowest value was 3.72, which was observed in the sample with a chili pepper weight of 10 g (Table 1). FDA in January 2024, modified the yoghurt standard acidity requirement to require products to have a pH of 4.6 or lower[24] By doing this, yoghurt's safety will be guaranteed while preserving its fundamental qualities. Yoghurt shelf life can be prolonged by the high level of acidity, which inhibits the growth of harmful and spoilage organisms.[25] Also, high acidity can reduce the yoghurt's acceptance by producing a sour taste that not all consumers will find appealing, particularly at a very low pH value.[26] In addition, extremely low pH values can cause milk proteins to coagulate excessively; this may cause curdling or other unwanted textural changes that compromise the consistency of the final product.[27]

Table 1: pH Test result of chili starters

Chili Pepper Weight (g)	pH of starter
2	4.51
5	4.15
10	3.72

Table 2: Gram staining reaction and microscopic description of selected yogurt samples

Selected Yogurt Sample	Gram-Staining	Microscopic description
FDS Yoghurt	Gram positive	Rod shaped
Chilli Yoghurt	Gram positive	Rod shaped

Gram staining reaction and microscopic description of selected yogurt samples

The microscopic description and Gram staining reaction of the selected yoghurt sample used in these experiments show a detailed description of the microorganisms after staining. It also shows the shapes and arrangements when viewed under the microscope at magnifications of 40x and 20x. Lactobacillus was found in each yoghurt sample (Table 2); the FDS and chilli pepper yoghurt samples all gave a gram-positive result. All images observed using the magnification above are similar to the ones observed in the work of Oleksy and Klewicks.[28] Gram staining classifies bacteria according to the makeup of their cell walls: those with a thick peptidoglycan coating and those with thin peptidoglycan. In the cell wall, bacteria with a thick layer of peptidoglycan are able to retain primary dye (crystal violet) in the cell wall and are labelled purple (positive), while bacteria with a thin layer of peptidoglycan are unable to retain the primary dye and are stained reddish pink (negative) with secondary dye (safranin).[29-30]

Macroscopic Images of selected yogurt sample
The macroscopic images of the selected yoghurt samples used in this experiment when grown on MRS Agar (Table 3). These microorganisms displayed the same macroscopic features based on abundance of growth, size, pigmentation, optical characteristics, consistency, margin, and elevation (Figures 1 & 2). According to Yang *et al*.[31], MRS agar has nutrients and a pH of 6.5, which is ideal for *Lactobacillus* growth. Consequently, for MRS media, a Lactobacillus-specific medium was utilized for sample culture. However, other lactic acid bacteria, such as *Streptococcus* and *Pediococcus*, also grow in MRS media, per Renschler *et al*.[32] The microscopic description of *Lactobacillus* was similar to the work of Mehmood *et al*.[33]

Confirmatory biochemical test on selected yogurt sample
The various confirmatory biochemical tests that were carried out on the selected yogurt samples. This biochemical confirmatory test ensures that the yogurt samples contain the fundamental microorganism known as *lactobacillus* in each sample. The biochemical tests performed were sugar fermentation tests (glucose and lactose), catalase, and coagulase.

Table 3: Macroscopic images of selected yogurt sample

Macroscopic images	FDS yoghourt	Chilli yoghourt
Abundance of growth	Small, moderate, and large	Small, moderate, and large
Pigmentation	Nonchromogenic (Grayish white)	Nonchromogenic (Grayish white)
Optical Characteristics	Opaque (with little light transmission)	Opaque (no light transmission)
Consistency	Mucoid	Mucoid
Margin	Entire (Sharply defined and even)	Entire (Sharply defined and even)
Elevation	Convex	Convex

Table 4: Confirmatory biochemical test on selected yogurt sample

Biochemical Tests	FDS yoghurt	Chilli yoghurt
Glucose	Positive	Positive
Lactose	Positive	Positive
Catalase	Negative	Negative
Coagulase	Negative	Negative

Proximate analysis and Titratable acidity results
The various confirmatory biochemical tests that were carried out on the selected yoghurt samples. The sugar fermentation tests (glucose and lactose) were positive, and the *catalase* and *coagulase* tests were negative. The confirmatory tests validate the presence of *Lactobacillus* species, particularly the homofermentative *Lactobacillus bulgaricus*. *Lactobacillus* can use the Embdem-Meyerhof pathway (EMP) of glycolysis in a process called homofermentative metabolism. The process occurs in two stages, converting lactose to lactic acid. The acidification gives a low pH environment that helps in preserving the yoghurt by inhibiting spoilage bacteria.[34] A negative result of the *catalase* test means *Lactobacillus* lacks *catalase* enzymes, according to Ramzy and Weerasooriya.[35] Hydrogen peroxide (H_2O_2) is hydrolyzed into oxygen and water when the catalase enzyme is present. The evolution of O_2 bubbles in the catalase test will not be seen in bacterial isolates that do not generate catalase, indicating that the organism is anaerobic or microaerophilic. Yoghurt benefits from this catalase characteristic, which may cause unfavourable oxidation reactions that change the texture and flavour of the yoghurt. The negative coagulase result helps differentiate the bacterium from harmful bacteria that are linked to food-borne diseases, such as Staphylococcus aureus, by showing the absence of the enzyme coagulase, which causes blood plasma to clot.[36]

The results of the proximate analysis done of the two samples, one yoghurt made from CPS and another made from FDS, are summarised in Table 5. The protein content of chilli pepper yoghurt and FDS yoghurt was found to be 8% and 8.3% respectively (Table 4). This result showed that there was no significant difference in the protein content of both yogurts. Protein content was higher than the Codex Alimentarium minimum standard of 2.7 %.[37-38] The protein content of yoghurt influences its amino acid profile, essential for the body's synthesis of proteins and enzymes. Also, high nutritional composition of protein contains amino acids, which aid in muscle and immune-system repair[39]. Casein is a cluster of protein found in yoghurt; it digests slowly, which could be helpful for those looking for a consistent source of amino acids.[40]

The fat content of chilli yoghurt and FDS yoghurt were found to be 1.02% and 1.01%, respectively. The result showed both chilli pepper yoghurt and FDS yoghurt had similar results; however, FDS yoghurt had the lowest fat content. Fat in yoghurt aids the absorption of fat-soluble vitamins such as vitamins A, D, E, and K. Even small differences in fat content can influence the bioavailability of these vitamins.[41]

The lipid profile (ratio of unsaturated to saturated fats) plays important in cardiovascular health. A slight increase in fat content could slightly alter the ratio of beneficial unsaturated fats to saturated fats in the yogurt. However, the change from 1.02% to 1.01% is unlikely to have significant health consequences in this case. In Brazil, yoghurt ranges from 1.2% to 2.0 %. at contributes significantly to the consistency of yoghurt and supplies roughly twice the amount of energy per unit compared to carbohydrates and proteins.[42]

The carbohydrate content of FDS yoghurt and chilli pepper yoghurt is 17% and 16%, respectively. Freeze-dried yoghurt had the highest carbohydrate content than chilli yoghurt. The enzyme lactase (B-galactosidase) hydrolyzes the disaccharide " lactose" present in yoghurts, releasing glucose and galactose in the form of simple sugars. Because the body can easily absorb the broken simple sugars, yoghurt is a beneficial dairy food for those with lactose intolerance.[43] A slight variation in the yoghurt's carbohydrate content will affect the calorie intake by raising the carbohydrate level (for example, from 16% to 17%), which can have an impact on persons who are watching their carbohydrate intake, particularly those who have diabetes or are on low-carb diets. Yoghurt's carbs are beneficial for providing energy and promoting the absorption of certain minerals, such as calcium, for those who are not lactose intolerant.[44]

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Figure 1: FDS yoghurt under the microscope

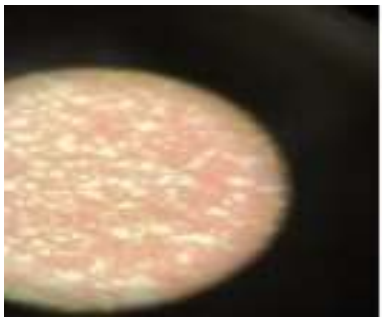

Figure 2: Chili pepper yoghurt under the microscope

The moisture content of chilli yoghurt was 74.78%, and the FDS yoghurt was 73.78%. This value corresponded with the report by Igbabul *et al*.[45] He said that yogurt should have a maximum moisture level of 84% because too much water reduces its viscosity, which alters its mouthfeel and texture.

Titratable acidity in the yoghurt samples was expressed as the percentage of lactic acid. The titratable acidity percentages for FDS yogurt and chilli yoghurt were determined to be 0.75% and 0.71%, respectively. The highest proportion of acidity is found in FDS yogurt, which may be because there are more fermenting bacteria available. According to the Codex standard for fermented milk[46], a minimum titratable acidity of 0.6% is recommended.[48] In comparison, the Brazilian Quality and Identity Standard (PIQ)[47] specifies a titratable acidity range of 0.6–1.5%, with observed values in Brazil falling between 0.83% and 1.06%. The titratable acidity results from this study were consistent with these reports. The acidity of yoghurt can influence both nutrient bioavailability and digestive processes. The acidic environment of yoghurt increases the milk proteins' digestion and facilitates their absorption by the body. Furthermore, the low pH can improve mineral intake by making calcium more soluble.[49] High acidity also helps probiotics, such as strains of Lactobacillus and Bifidobacterium, survive and offer gut health advantages like better digestion and immune system performance.

Sensory evaluation of yoghurt samples

The sensory characteristics of the two yoghurts—taste, aroma, flavor, appearance, and general acceptability—were assessed. A hedonic scale of nine points was used to evaluate the samples. There was a score of 9 for "like extremely," and a score of 1 for "dislike extremely." The most desirable attribute is represented by the lowest number, while the least desirable attribute is represented by the highest number. A score of five, then, indicates neither like nor dislike. The average point scores for "appearance" were found to be 7.1 and 6.9, respectively, for chilli yoghurt and FDS yoghurt. The average point scores for "aroma" were found to be 7.0 and 7.0; there was no significant difference for chilli yoghurt and FDS yoghurt (Table 6). The average point score for "flavour" was found to be 7.1 and 6.8, respectively, for chilli yoghurt and FDS yoghurt. Chilli yoghurt has the highest. It has been observed that the quality or integrity of a particular food sample can be determined by evaluating its sensory characteristics.[50] In this context, some of the yoghurt samples were found to meet the USDA[50] specifications. While colour, texture, and viscosity are important quality attributes, flavor and taste are generally regarded as the most critical determinants of consumer acceptance. As previously noted by Olugbuyiro and Oseh,[51] lower overall acceptability scores are typically linked to deficiencies in flavor, taste, and aroma. The observations from the chilli yoghurt in this study align with this conclusion.

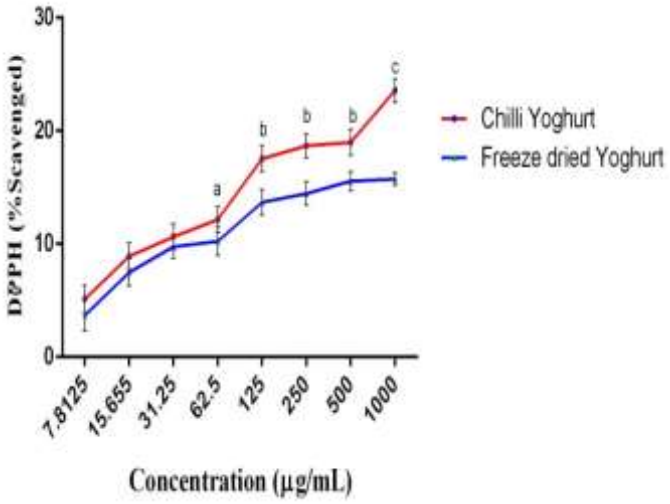

Figure 3: DPPH scavenging activity of sample yoghurt. Values are expressed as Mean ± Standard Error of Mean SEM of triplicate determination.

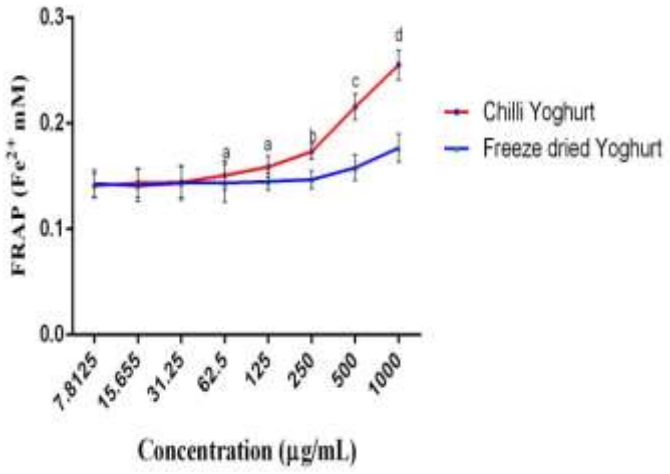

Figure 4: Ferric reducing antioxidant power of sample yoghurt Values are expressed as Mean ± Standard Error of Mean SEM of triplicate determinations

Antioxidant activity of sample yoghurt

The DPPH radical scavenging activity of the yoghurt samples was between 5-10% at low concentrations of 7.8, 15.65 and 31.25µg/mL and showed no significant difference between both yoghurt samples. However, CPS-yoghurt showed significantly higher DPPH scavenging capacity at concentrations of 62.5, 125, 250, 500, and 1000 µg/mL when compared to the FDS-yoghurt (Figure 3). FRAP of both yoghurt samples did not differ significantly at concentrations of 7.8, 15.6 and 31.2 µg/mL. CPS-Yoghurt, however, showed significantly higher FRAP when compared to the FDS-yoghurt at 62.5, 125, 250, 500 and 1000 µg/mL (Figure 4).

Table 5: Proximate analysis and Titratable acidity results

Sample	Titratable acid	Protein (g)	Fat (g)	Carbohydrate (g)	Moisture content
CPS Yoghurt	0.71 ± 0.006	8.0 ± 0.29	1.02 ± 0.00	16.0 ± 0.40	74.78%±0.02
FDS yoghurt	0.75 ± 0.006	8.3 ± 0.29	1.01 ± 0.006	17.0 ± 0.17	73.78%±0.12

Values are expressed as Mean ± Standard Error of Mean SEM of triplicate determinations.

Table 6: Sensory Evaluation results of yoghurt samples

Sample	Appearance	Aroma	Flavour	Taste	Texture	Overall acceptability
CPS Yoghurt	7.1 ± 1.8	7.0 ± 1.2	7.1 ±1.6	7.3 ± 1.5	6.9 ±1.7	6.9 ± 1.7
FDS yoghurt	6.9 ± 1.7	7.0 ± 1.2	6.8 ±1.8	7.0 ± 2.2	7.3 ±1.4	6.9 ± 1.3

Values are expressed as Mean ± Standard Error of Mean SEM of triplicate determinations.

The total phenolic content of the selected yoghurt samples used in this experiment. Chili yogurt had (572.30 ±10.83 mg GAE/kg) and (526.05 ±13.77 mg GAE/kg) phenol content, respectively (Table 7). According to Rafieian *et al*.[52], antioxidants are compounds that have the ability to scavenge and deactivate free radicals, which damage cells and tissues and cause degenerative illnesses. Antioxidant capacity tests on both yoghurt samples revealed that they all had minimal antioxidant activity. Research suggests that antioxidants in food might shield cells from oxidative stress, and dairy products with increased antioxidant content may be beneficial to health.[53] As a result, yoghurts with low antioxidant content may not be as useful as those with higher antioxidant content.[54]

Antioxidants prevent lipid peroxidation. Thus, low-antioxidant yoghurts may be more prone to oxidative deterioration, which could impact their flavour, aroma, and shelf life. Exposure to light, heat, and oxygen might worsen this degradation because they all speed up oxidative processes.[55]

Table 7: Evaluation of total phenol content of yoghurt

Samples	Total phenolic (mg GAE/kg)
Chilli Yoghurt	572.30 ±10.83
Freeze dried yoghurt	526.05 ±13.77

Values are expressed as Mean ± Standard Error of Mean SEM of triplicate determinations.

Low antioxidant activity may be caused by pasteurization, according to Juncker *et al*.,[56] there was a reduction of antioxidant activity of human milk at a high pausterization temperature. Also, at pasteurization temperatures higher than 80 ^{0}C, vitamin C levels were found to decrease considerably in most cases. This is to be expected since vitamin C is a heat-labile micronutrient, meaning that it degrades more quickly at higher temperatures due to a chemical interaction.[57] Phenolic compounds (PCs) are widely occurring phytochemicals present in the tissues of most plants, including fruits and vegetables.[58] Both yoghurts have phenolic contents of 573.30 and 526.05 mg GAE/kg. The highest amount of phenolic content is seen in chilli pepper yoghurt, which may be due to the characteristics of chili peppers. Chili peppers are known for having a high phenolic content, comprising substances like phenolic acids.[59] Even though the phenolic content of the yoghurt is high, the antioxidant activity of the yoghurt was minimal; this may be due to interactions with other compounds. Phenolic molecules in yoghurt may bind to proteins, reducing the antioxidant capacity. For example, phenolic molecules interact with proteins in a reaction called phenolic-protein interactions (PPI), reducing the product's overall antioxidant activity by lowering its capacity to scavenge free radicals.[60]

Conclusion

In conclusion, the research study showed that even though the nutritional profiles of yoghurt made with freeze-dried starter and yoghurt made with chilli pepper were comparable, the phenolic content of the chilli yoghurt was noticeably higher. This suggests that adding chilli pepper to yoghurt as a starter not only preserves its nutritional value but also increases its phenolic richness, which is linked to several health advantages like anti-inflammatory properties. The result shows the potential of chili pepper yoghurt as a functional food, providing healthy advantages without affecting the nutritional content of the yoghurt. Future research can make findings on the level of capsaicin in the chili pepper yoghurt as well as explore further antioxidant tests.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors declare that the work presented in the article is original and that any liability for claims relating to the content of the article will be borne by them.

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