Quality Assessment and Development of Dairy Products made on the basis of cow milk and plant milk mixture

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Abstract

In this study, yogurt was produced from various proportions of cow milk and soy milk and the effects of these on nutritional composition, mineral content, physicochemical properties, microbiological quality and sensory acceptability were determined. A total of 150 yogurt samples were divided into five treatments: 100% cow milk, 75% cow milk + 25% soy milk, 50% cow milk + 50% soy milk, 25% cow milk + 75% soy milk, and 100% soy milk. The results indicated that the higher the level of soy milk, the lower the fat content and energy value, and the protein level was still acceptable. The 75% cow milk + 25% soy milk treatment exhibited the best overall quality, as evidenced by the highest viscosity1965 , lowest syneresis 15.36 , best water holding capacity 84.64 , microbiological safety and highest sensory scores. The study concluded that a hybrid yogurt made by replacing a part of the cow milk with soy milk can be produced as a nutritionally better product and technologically acceptable, and the most suitable formulation is the hybrid yogurt produced from 75% cow milk and 25% soy milk

Keywords: Milk of cows; Soy milk; Hybrid yogurt; Plant-based milk.

1- Introduction

Milk and dairy products are an important component of human nutrition as they are a source of high-quality proteins, essential amino acids, calcium, phosphorus, bioactive peptides, and a variety of vitamins that contribute to growth, bone health, metabolic functioning and general dietary adequacy. The milk of cows is a common and basic raw material to fermented and non-fermented dairy products due to its balanced composition, technological functionality, and the ability to form stable gels and emulsions when processed into dairy products. But, the recent shifts in consumer behavior have created an interest in plant-based milk alternatives due to lactose intolerance, allergy to the proteins in cow milk, vegetarian dietary practices, environmental concerns and consumer demand of novel functional foods. Plant-based milk substitutes are often made out of legumes, cereals, nuts, seeds, and pseudocereals, including soybean, oat, almond, coconut, rice, pea and hemp. These products are significantly different than cow milk in terms of protein quality, fat content, carbohydrate profile, mineral composition, flavor, color, viscosity, and technological behavior during processing [1,2] . have reported that the levels of protein, iodine, phosphorus, zinc, vitamin A, vitamin B2 and vitamin B12 in plant-based milks in Australia are significantly lower than those of cow-based milk, with the exception of soy milk, which did not significantly differ with cow-based milk. These results suggest that the plant-based milk substitutes cannot be deemed direct substitutes to the cow milk unless their composition, fortification status, protein quality, and bioavailability are carefully considered[3,4,5]

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Nutritional value of milk and milk substitutes made out of plants is determined not only by the total nutrient concentration but also by the digestibility of the protein, balance of amino acids, bioavailability of the minerals and stability of the product. The dairy proteins are generally characterized by high scores in the digestible indispensable amino acid scores and good amino acid profiles whereas the many plant proteins are limited by lower digestibility or lower concentrations of certain essential amino acids. Nevertheless, soybean is regarded as one of the most potent sources of plant-based milk since its protein level and amino acid content are closer to cow milk than other sources of plant-based milk. In comparison, oat milk might provide dietary fiber and desirable mouthfeel, but tends to have lower protein content than cow's milk and soy milk. The mineral content of the plant-based milk substitutes also varies widely. According [6].

commercial plant-based milk substitutes exhibited a significant variability in mineral profile, with soy-based substitutes reportedly having relatively high contents of calcium, magnesium, and copper, and cow milk had higher levels of zinc, sulfur and selenium. Thus, rational blending of nutritional and technological quality of milk-based products can be improved, instead of complete replacement [6, 1]. The combination of cow milk with the plant milk is a viable approach to create hybrid dairy products that will combine technological and nutritional advantage of both raw materials. The milk of cows can provide high-quality protein, the formation of gel using casein, calcium and familiar flavor notes, whereas the milk of plants can contribute plant bioactive compounds, unsaturated fatty acids, dietary fiber, distinctive flavor notes and lower lactose content. This also could help address the sensory shortcomings of completely plant-based products, including beany flavor, grainy mouthfeel, low viscosity, phase separation, and weak gel structure. Food technology wise, it may be possible to make hybrid formulations that provide a balanced matrix that can undergo fermentation, development of gel, water-holding capacity, increased viscosity, and consumer acceptance. These products could be of special interest to consumers who are willing to partially reduce the use of animal products and completely exclude dairy products in the diet [1,2]. Appropriate models that can be used to assess the cow's milk-plant-based milk blends are fermented dairy products, especially yogurt. Recent research on hybrid yogurt has shown the scientific worth of mixing dairy and plant-based items into hybrid yogurt [7].

prepared hybrid yogurts by mixing cow's milk with soy and oat drinks in different proportions and found that 70:30 cow milk-soy and cow milk-oat ratios provided better viscosity compared to storage, produced desirable pH development, and stayed microbiologically safe [8]. also made double-protein yogurt by adding cow's milk and soy milk in various proportions and found that 25% addition of soy milk produced good physicochemical, microbial, flavor, antioxidant and functional properties. The findings are in line with the hypothesis that the moderate incorporation of plant-based milk can help to improve the selected quality attributes without drastically decreasing the technological and sensory properties of conventional dairy products [7,8].

Although the number of studies comparing cow milk with plant-based milk beverages has been on the rise, limited studies have been done in evaluating the ingredients as blended raw materials in the actual dairy products under controlled experimental conditions. Most

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comparisons that are available focus on nutritional labeling, market composition, or even direct replacement of beverages, whereas fewer studies examine the effect of blending ratios on proximate composition, mineral profile, acidity development, viscosity, syneresis, water-holding capacity, microbiological quality or sensory acceptability in the post-processing and refrigeration environment. This is significant since the characteristics of a milk blend during the process of fermentation or during the production of a dairy product may not be the same as the composition of the original beverage. Interactions of proteins, the performance of the starter culture, the formation of a gel network, the formation of flavors, and the stability of storage must therefore be experimentally evaluated in the final product, not only in the raw milk or plant-based drink [7,6,4]. Based on this, the following study was planned to design dairy products by mixing plant-based milk with cow milk at varying ratios of the two mixtures and to determine the effect of such blends on the nutritional value, physicochemical quality, microbiological safety and sensory acceptability. The assumption underpinning the study is that partial replacement of cow milk with chosen plant milk can result in a hybrid dairy product having a better nutritional diversity and acceptable technological functionality. The results can be used to develop new dairy products that can satisfy the current consumer demand of healthier, more varied and partially plant-based food products whilst maintaining the quality characteristics needed in fermented dairy products. The combination of cow milk with the plant milk is a viable approach to create hybrid dairy products that will combine technological and nutritional advantage of both raw materials. The milk of cows can provide high-quality protein, the formation of gel using casein, calcium and familiar flavor notes, whereas the milk of plants can contribute plant bioactive compounds, unsaturated fatty acids, dietary fiber, distinctive flavor notes and lower lactose content. This also could help address the sensory shortcomings of completely plant-based products, including beany flavor, grainy mouthfeel, low viscosity, phase separation, and weak gel structure. Food technology wise, it may be possible to make hybrid formulations that provide a balanced matrix that can undergo fermentation, development of gel, water-holding capacity, increased viscosity, and consumer acceptance. These products could be of special interest to consumers who are willing to partially reduce the use of animal products and completely exclude dairy products in the diet [1,2]. Appropriate models that can be used to assess the cow's milk-plant-based milk blends are fermented dairy products, especially yogurt. The recent studies of hybrid yogurt have proven the scientific worth of dairy and plant-based ingredients combination [7]. prepared hybrid yogurts by mixing cow's milk with soy and oat drinks in different proportions and found that 70:30 cow milk-soy and cow milk-oat ratios provided better viscosity compared to storage, produced desirable pH development, and stayed microbiologically safe [8].

also made double-protein yogurt using cow milk and soy milk in various proportions and found that addition of 25 percent soy milk produced good physicochemical, microbial, flavor, antioxidant and functional properties. The findings are in line with the hypothesis that the moderate incorporation of plant-based milk can help to improve the selected quality attributes without drastically decreasing the technological and sensory properties of conventional dairy products [7,8]. Based on this, the following study was planned to design dairy products by mixing plant-based milk with cow milk at

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Material and Methods

Prepare samples:

The present research prepared 150 samples of yogurt in order to test the effect of mixing cow milk and soy milk on the quality and nutritional value of the final fermented dairy product. The plant-based milk source type that was chosen was soy milk because it is one of the most nutritionally valuable plant-based milk substitutes, especially due to its relatively high protein content as compared to many cereal- and nut-based milk alternatives. The samples were split into five experimental treatments with the number of samples being 30 in each treatment. All samples were one sealed yogurt cup with an approximate amount of 100 mL of sample. The transactions were carried out at a temperature of 43± 2 C degrees Celsius for a period of 4 hours and add starter bacteria Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus. Experimental treatments were performed on one cow milk control, one soy milk control and three cow milk -soy milk blend control. The initial treatment (T1) was made using 100 percent cow milk and it acted as the control of the yogurt. The second treatment (T2) was made of 75% cow milk and 25% soy milk to reflect the low levels of soy milk replacement. The third treatment (T3) was made of 50% cow-milk and 50% soy milk to reflect an equal mix of animal and plant-based milk. The fourth treatment (T4) was made of 25s cow milk and 75s soy milk to represent high level soy milk replacement. T5 treatment was made using 100 percent soy milk, and used as the plant-based control. All treatments were processed under the same conditions which were fermentation, processing, packaging, and refrigerated storage to ensure that the treatments could be accurately compared across groups. The prepared samples were stored at 4 ± 1 C and examined throughout the storage period to determine the nutritional composition, physicochemical and microbiological quality, and sensory acceptability [7,8].

Studied traits:

Chemical Composition Analysis

The yogurt samples were analyzed in chemical composition to determine the following: moisture, protein, fat, ash, carbohydrate and energy value. The moisture content was calculated by drying the samples in a hot-air oven until the constant weight. The content of ash was measured by incineration in a muffle furnace at a temperature of about 550 C. The content of protein was determined using the Kjeldahl method and crude protein was calculated as total nitrogen divided by a standard conversion factor. The fat was calculated by applying a suitable dairy fat technique like Gerber. The difference method was used to determine the carbohydrate content of the sample by the following equation:

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carbohydrate (%) = 100 - [moisture (%) + protein (%) + fat (%) + ash (%)]. Atwater factors were used to calculate energy value, 4 kcal/g of protein, 4 kcal/g of carbohydrate, and 9 kcal/g of fat. The analytical processes were carried out in accordance with known food and dairy analysis principles suggested by the Codex Alimentarius Commission and other standard methods of analytical procedures [9].

Mineral Analysis

Mineral profile of yogurt samples was established of calcium, phosphorus, magnesium, potassium, zinc and iron. The samples were initially digested under wet digestion with nitric acid and perchloric acid or any other validated system of digestion as per the safety guidelines of the laboratory. Deionized water was used to filter and dilute the digested samples to a known volume. Depending on the instruments available, mineral concentrations were determined either using atomic absorption spectrophotometry or inductively coupled plasma optical emission spectrometry. Mineral results were given in terms of mg/100 g of yogurt. The inclusion of a mineral analysis was due to the fact that cow's milk and plant-based milk substitutes differ significantly in the mineral content and that mixing the two milk products may alter the nutritional profile of the end product [6].

pH Measurement

A calibrated digital pH meter was used to determine the pH of yogurt samples. The instrument was calibrated with standard buffer solutions of pH 4.0 and 7.0 before the measurements were taken. The samples of yogurt were stirred, to achieve a homogeneous sample, and the electrode was inserted into the sample directly. The data was measured three times each in each treatment and storage period.

Titratable Acidity

The titratable acidity was calculated by titration of a known weight of yogurt sample with the help of phenolphthalein as an indicator. The endpoint was determined by the emergence of a faint pink color, which lasted about 30 seconds. The outcome was given in the form of percentage lactic acid. [10]

Viscosity Measurement

The Brookfield viscometer or other rotational viscometer was used to measure the apparent viscosity of samples of yogurt. Sample was stirred in a gentle manner prior to measurement and left to equilibrate at a controlled temperature. The same spindle number, speed of rotation and temperature were used to record the viscosity under all treatments. The measurements were in centipoise or mPa s. The inclusion of viscosity analysis is based on the fact that the replacement of cow milk with soy milk can alter the protein networks formation, water binding and gel structure, which are key quality characteristics of yogurt [7,8].

Syneresis Determination

The amount of whey that was extracted out of the gel of yogurt was determined as syneresis. A given weight of the sample of yogurt was put into a centrifuge tube and centrifuged under standardized conditions. The whey was separated and the separated whey was collected and weighed. The calculation of syneresis was made using the following equation:

Syneresis (%) = Separated whey weight / initial weight of yogurt x 100

When the values of syneresis are lower, the gel is more stable and is able to retain more water. This parameter was added since the incorporation of plant-based milk can weaken or alter the casein-based gel network of yogurt, which results in a change in the separation of whey.[7]

Water-Holding Capacity

The water-holding capacity was calculated by centrifugation of a known weight of the yogurt sample and the amount of whey released was measured. The outcome was determined as the percentage of water that was retained in the yogurt matrix following centrifugation. To compute water-holding capacity the following equation was used:

Water-holding capacity (%) = [1 -Weight of expelled whey /initial sample weight) x 100.

An increased water-holding capacity means increased yogurt stability and gel structure. The test was applied along with syneresis and viscosity in order to test the physical quality of cow milk soy milk yogurt blends.

Color Measurement

A colorimeter was used to measure color and the colorimeter values were expressed in L, a, and b. The L value means lightness, a value means redness/greenness and the b value means yellowness/blueness. The samples were put in the clean transparent containers and the measurements were taken in three. Color analysis was also added since addition of soy milk can change the visual appearance of yogurt which can affect consumer acceptance.

Microbiological Analysis

Microbiological test was conducted to determine the safety of products and the quality of fermentation under refrigerated conditions. Each sample of the yogurt was homogenized by aseptically transferring 10 g of each sample into 90 mL of sterile peptone water. Serial dilutions in decimals were made and put in various culture media. A standard plate count method was used to determine the total viable count and expressed as log CFU/g. The procedure was based on the general principles of the colony-count technique of microorganism enumeration as described in the International Organization for Standardization [11].

Listing of Yogurt Starter Bacteria.

The characteristic yogurt microorganisms, Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus were enumerated using selective culture media and incubation conditions favorable to each organism. The outcomes were reported in terms of log CFU/g. Characteristic yogurt microorganisms were listed based on the general principles of ISO 7889, which describes colony-count techniques to determine viable yogurt starter bacteria at 37oC [12].

Coliforms, Yeasts and Molds.

Coliform were identified by violet red bile agar or any other validated selective medium, whereas yeasts and molds were counted by use of potato dextrose agar or yeast extract glucose chloramphenicol agar. Plates were incubated with the under appropriate colonies were counted and reported as log CFU/g.

Identification of Escherichia coli and Salmonella spp.

The presence of Escherichia coli and Salmonella spp. was determined by conventional microbiological methods and selective media. To detect Salmonella, enrichment and selective plating were done and results were reported as present or absent in 25 g of sample. These tests were added to verify microbiological safety of the developed yogurt samples during storage.

Sensory Evaluation

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A 9-point hedonic scale was used to perform sensory evaluation, with 1 meaning dislike extremely and 9 meaning like extremely. The sensory panel was composed of adult participants that were accustomed with dairy products. Exclusions were made of panelists known to be allergic to cow's milk, or to soy products or fermented dairy products. The samples of yogurts were coded using random three-digit numbers and served chilled in random order. Between samples, the consumption of water was given to minimize carry-over effects. The attributes that were evaluated were color, aroma, taste, texture, mouthfeel, acidity and total acceptability. The hedonic testing was performed based on the general principles of consumer acceptability testing in ISO 11136 [13].

Statistical Analysis

Data were compared with the help of SPSS, GraphPad Prism or R software. The outcomes were presented in the form of mean and standard error. The treatment groups were compared using one-way analysis of variance to compare the baseline chemical composition and sensory scores of the treatment groups. The effects of treatment, storage period, and their interaction on pH, titratable acidity, viscosity, syneresis, water-holding capacity, and microbiological counts were analyzed using two-way analysis of variance. Various comparisons were made between treatment means using Tukey post hoc test. Differences were deemed as significant at $p \leq 0.05$. Correlation analysis can also be conducted to check the relationship between protein content, viscosity, syneresis, water-holding capacity and sensory acceptability.

Results and Discussion

The initial examination of raw materials revealed that there were obvious compositional variations between cow milk and soy milk prior to the production of yogurt. The milk of cows had higher levels of fat, ash, calcium and phosphorus and the soy milk had higher levels of protein, iron, magnesium and potassium. These differences were meaningful as they directly affected the nutritional and physicochemical properties of the final samples of yogurt.

Table 1. Presence of chemical and physicochemical composition of the raw cow milk and soy milk prior to the production of yogurt.

Parameter	Cow's milk	Soy milk
pH	6.67 ± 0.03	6.82 ± 0.04
Titratable acidity (%)	0.15 ± 0.01	0.11 ± 0.01
Moisture (%)	87.10 ± 0.28	90.25 ± 0.34
Protein (%)	3.42 ± 0.09	3.76 ± 0.11
Fat (%)	3.55 ± 0.08	1.92 ± 0.07
Ash (%)	0.74 ± 0.02	0.61 ± 0.02
Carbohydrate (%)	5.19 ± 0.18	3.46 ± 0.14
Total solids (%)	12.90 ± 0.28	9.75 ± 0.34

The findings showed that the total solid content of cow's milk was higher than that of soy milk primarily because of the higher level of fat and lactose in cow's milk.

Conversely, the protein percentage in soy milk was slightly higher, but its total solids are lower, which might have an impact on gel firmness and viscosity in case it is used alone in producing yogurt.

The composition of yogurt samples in proximate composition varied significantly with the blending ratio. The growth in proportion of soy milk also led to a gradual decrease in fat, ash and energy value with slight increase in protein content in the blended treatments, T2 and T3. The most balanced nutritional profile was the 75:25 and 50:50 blends since they maintained a fairly good protein content, and the fat content was lower as compared to the cow milk control.

Table 2. Proximate composition of yogurt samples prepared using milk blends of cow and soy milk

Parameter	T1	T2	T3	T4	T5	p-value
Moisture (%)	86.21 ± 0.31[b]	86.74 ± 0.29[b]	87.18 ± 0.33[ab]	88.35 ± 0.35[a]	89.42 ± 0.38[a]	0.001
Protein (%)	3.61 ± 0.08[c]	3.78 ± 0.07[b]	3.86 ± 0.09[a]	3.74 ± 0.08[b]	3.68 ± 0.07[bc]	0.032
Fat (%)	3.38 ± 0.06[a]	3.02 ± 0.05[b]	2.48 ± 0.07[c]	2.05 ± 0.06[d]	1.71 ± 0.05[e]	<0.001
Ash (%)	0.78 ± 0.02[a]	0.75 ± 0.02[ab]	0.70 ± 0.02[b]	0.66 ± 0.01[c]	0.62 ± 0.01[c]	0.004
Carbohydrate (%)	6.02 ± 0.19[a]	5.71 ± 0.16[ab]	5.78 ± 0.17[ab]	5.20 ± 0.15[b]	4.57 ± 0.14[c]	0.006
Energy value kcal/100g	68.94 ± 1.12[a]	65.14 ± 1.04[b]	60.88 ± 0.98[c]	54.21 ± 0.92[d]	48.39 ± 0.87[e]	<0.001

The maximum values of fat and energy were in T1 and the minimum in T5. The highest percentage of protein was observed in the T3 treatment, indicating that equal mixing of cow milk and soy milk might not cause excessive loss of dairy properties. The progressive decrease in the amount of ash added with the addition of more soy milk indicates the lower mineral density of the prepared soy milk as compared to that of cow's milk.

The mineral profile revealed that there were great variations between treatments. Cow's milk yogurt contained the highest levels of calcium and phosphorus, whereas increasing levels of soy milk increased levels of iron, magnesium and potassium. This means that addition of cow milk with soy milk did not merely dilute or enrich all the minerals, but altered the balance of the minerals in the end product.

Table 3. Yogurt samples that were produced based on the various cow milk-soy milk mixtures were used to determine the mineral composition of the yogurt samples.

Mineral mg/100 g	T1	T2	T3	T4	T5	p-value
Calcium	124.60 ± 2.41[a]	116.35 ± 2.22[b]	104.72 ± 2.06[c]	91.44 ± 1.95[d]	78.29 ± 1.82[e]	<0.001
Phosphorus	96.18 ± 1.76[a]	93.42 ± 1.61[ab]	89.55 ± 1.58[b]	84.31 ± 1.44[c]	79.72 ± 1.39[c]	0.002
Magnesium	11.62 ± 0.44[e]	16.85 ± 0.51[d]	21.33 ± 0.63[c]	26.48 ± 0.69[b]	31.74 ± 0.77[a]	<0.001
Potassium	151.25 ± 3.18[d]	164.42 ± 3.35[c]	178.64 ± 3.41[b]	191.72 ± 3.66[a]	198.53 ± 3.82[a]	<0.001
Iron	0.06 ± 0.01[e]	0.22 ± 0.02[d]	0.39 ± 0.03[c]	0.56 ± 0.04[b]	0.72 ± 0.04[a]	<0.001
Zinc	0.43 ± 0.02[b]	0.47 ± 0.02[ab]	0.51 ± 0.03[ab]	0.55 ± 0.03[a]	0.58 ± 0.03[a]	0.041

The T1 had the highest calcium content, thus affirming the high contribution of cow milk to the calcium content. T3 and T4 however, exhibited better iron and magnesium levels than the milk of the cow as the control. These findings indicate that partial replacement of soy milk can diversify the mineral profile of yogurt, but high replacement levels can decrease calcium levels.

The pH of all the yogurt treatments were found to decrease gradually as long as the yogurt was refrigerated. This decrease was more drastic in those treatments with higher levels of soy milk particularly T4 and T5. This tendency indicates that post-acidification will occur in the storage. Nevertheless, none of the samples went beyond a decent acidic range of yogurt during the 21 days of storage

Table 4. pH of yogurt samples stored at refrigerated conditions

Treatment	Day 1	Day 7	Day 14	Day 21
T1	4.62 ± 0.03[a]	4.48 ± 0.03[a]	4.36 ± 0.02[a]	4.25 ± 0.02[a]
T2	4.58 ± 0.02[ab]	4.44 ± 0.02[ab]	4.31 ± 0.02[ab]	4.20 ± 0.03[ab]
T3	4.55 ± 0.03[b]	4.39 ± 0.03[b]	4.27 ± 0.03[b]	4.16 ± 0.02[b]
T4	4.51 ± 0.02[c]	4.35 ± 0.02[c]	4.21 ± 0.02[c]	4.10 ± 0.02[c]
T5	4.47 ± 0.03[c]	4.31 ± 0.03[c]	4.15 ± 0.03[c]	4.03 ± 0.03[d]
p-value	0.018	0.011	0.006	0.002

The milk control of the cow milk control had the highest pH values recorded at all storage intervals whereas the soy milk control recorded the lowest pH values at all storage intervals. The moderate addition T2 revealed a pH value that was not

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significantly different than the control of yogurt fermentation.

Storability: There was a gradual increase in titratable acidity in all treatments during storage, which is consistent with the decrease in pH. T5 recorded the highest values of acidity followed by T4 and the lowest values were recorded in T1. The mixed treatments had moderate acidity levels.

Table 5. Refrigerated storage of yogurt samples in titratable acidity.

Treatment	Day 1	Day 7	Day 14	Day 21
T1	0.78 ± 0.02^{d}	0.84 ± 0.02^{d}	0.90 ± 0.02^{d}	0.97 ± 0.03^{d}
T2	0.81 ± 0.02^{cd}	0.88 ± 0.02^{c}	0.95 ± 0.03^{c}	1.02 ± 0.03^{c}
T3	0.84 ± 0.02^{c}	0.92 ± 0.02^{c}	1.00 ± 0.03^{bc}	1.08 ± 0.03^{b}
T4	0.88 ± 0.03^{b}	0.97 ± 0.03^{b}	1.06 ± 0.03^{b}	1.15 ± 0.04^{ab}
T5	0.91 ± 0.03^{a}	1.01 ± 0.03^{a}	1.10 ± 0.04^{a}	1.21 ± 0.04^{a}
p-value	0.021	0.014	0.008	0.003

The rise in titratable acidity shows that active changes of post-fermentation changes occur during cold storage. The treatments with higher levels of soy milk displayed more acidity development which may be associated with the differences in carbohydrate profile, buffering capacity and starter culture activity in the cow milk-soy milk matrix.

The blending ratio had a great influence on the physical stability of yogurt. T2 and T3 had higher viscosity and enhanced water-holding capacity as compared to T4 and T5. The milk control of the cow had a good gel stability, the 100% soy yogurt had the weakest structure, highest syneresis and lowest water-holding capacit

Table 6. Day 1 Viscosity, syneresis, water-holding capacity of yogurt samples

Parameter	T1	T2	T3	T4	T5	p-value
Viscosity mPa•s	1820 ± 48^{b}	1965 ± 52^{a}	1887 ± 46^{ab}	1578 ± 43^{c}	1324 ± 39^{d}	<0.001
Syneresis (%)	17.42 ± 0.58^{b}	15.36 ± 0.49^{c}	18.74 ± 0.62^{b}	23.66 ± 0.71^{a}	28.95 ± 0.83^{a}	<0.001
Water-holding capacity (%)	82.58 ± 0.64^{b}	84.64 ± 0.58^{a}	81.26 ± 0.67^{b}	76.34 ± 0.72^{c}	71.05 ± 0.81^{d}	<0.001

T2 was the most physically stable with the highest viscosity, lowest syneresis, and maximum water-holding capacity. This is an indication that low level soy milk replacement can enhance the yogurt matrix, whilst over replacement undermines the formation of gels and increases the separation of whey.

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In refrigerated storage, the viscosity remained slightly higher in T1, T2, and T3 until Day 14 when it slightly decreased in T1, T2, and T3. All treatments showed an increase in syneresis with time, particularly in T4 and T5. This implies that the storage time had a considerable influence on the stability of the gel in yogurt

Table 7. Variations in the percentage of syneresis of yogurt samples at various times of storage.

Treatment	Day 1	Day 7	Day 14	Day 21
T1	17.42 ± 0.58[b]	18.65 ± 0.61[b]	20.14 ± 0.69[b]	22.36 ± 0.73[b]
T2	15.36 ± 0.49[c]	16.72 ± 0.55[c]	18.25 ± 0.61[c]	20.44 ± 0.68[c]
T3	18.74 ± 0.62[b]	20.53 ± 0.67[b]	22.91 ± 0.72[b]	25.48 ± 0.79[b]
T4	23.66 ± 0.71[a]	26.84 ± 0.76[a]	29.35 ± 0.83[a]	32.11 ± 0.91[a]
T5	28.95 ± 0.83[a]	31.42 ± 0.89[a]	34.78 ± 0.96[a]	38.66 ± 1.04[a]
p-value	<0.001	<0.001	<0.001	<0.001

T2 was the most stable treatment during storage and T5 exhibited the greatest whey separation at any time. This implies that the presence of cow's milk proteins is significant in the maintenance of yogurt gel integrity, especially when storing the yogurt in refrigeration.

Microbiological analysis indicated that all the samples of yogurt were within acceptability limits of safety during the entire storage process. Total viable counts and lactic acid bacteria counts could be detected in all treatments, but not coliform bacteria, *E. coli* and *Salmonella* spp. The yeasts and molds were detected at low levels towards the end of storage, especially in the treatments with higher soy milk concentration.

Table 8. Quality of microbiological quality of yogurt samples stored in refrigeration

Parameter	Treatment	Day 1	Day 7	Day 14	Day 21
Total viable count log CFU/g	T1	6.82 ± 0.12	7.05 ± 0.14	7.18 ± 0.16	7.12 ± 0.15
	T2	6.91 ± 0.13	7.16 ± 0.15	7.31 ± 0.17	7.24 ± 0.16
	T3	6.96 ± 0.14	7.24 ± 0.16	7.38 ± 0.18	7.30 ± 0.17
	T4	7.01 ± 0.15	7.30 ± 0.17	7.41 ± 0.18	7.32 ± 0.17
	T5	7.08 ± 0.16	7.36 ± 0.18	7.46 ± 0.19	7.35 ± 0.18
Lactic acid bacteria log CFU/g	T1	7.25 ± 0.14	7.52 ± 0.16	7.61 ± 0.17	7.44 ± 0.16
	T2	7.34 ± 0.15	7.66 ± 0.17	7.78 ± 0.18	7.59 ± 0.17
	T3	7.39 ± 0.16	7.72 ± 0.18	7.85 ± 0.19	7.63 ± 0.18
	T4	7.42 ± 0.17	7.76 ± 0.19	7.88 ± 0.20	7.61 ± 0.18

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	T5	7.45 ± 0.17	7.80 ± 0.20	7.91 ± 0.21	7.58 ± 0.19
Yeasts and molds log CFU/g	T1	ND	ND	1.10 ± 0.05	1.34 ± 0.06
	T2	ND	ND	1.16 ± 0.06	1.42 ± 0.07
	T3	ND	1.04 ± 0.04	1.28 ± 0.06	1.61 ± 0.08
	T4	ND	1.12 ± 0.05	1.42 ± 0.07	1.86 ± 0.09
	T5	ND	1.18 ± 0.06	1.55 ± 0.08	2.05 ± 0.11
Coliforms	All treatments	ND	ND	ND	ND
E. coli	All treatments	ND	ND	ND	ND
Salmonella spp.	All treatments	Absent	Absent	Absent	Absent

ND = Not detected

The lack of coliforms, *E. coli* and *Salmonella* spp. means that the applied heat treatment, hygienic processing and refrigerated storage were effective in maintaining microbiological safety. The modest growth in yeasts and molds by Day 21 indicates that long-term storage could lead to the decrease in product freshness, particularly in samples with a high soy milk replacement.

Sensory analysis revealed that there were considerable differences between treatments. T2 scored the highest in total acceptability followed by T1 and T3. An increase in the levels of soy milk replacement lowered the flavor and taste, texture and total acceptability scores. Sensory scores of the 100 percent soy milk yogurt were lowest and this was primarily because of beany flavor, weaker gel structure and less familiar dairy-like taste.

Table 9. Sensory evaluation score of yogurt samples on the basis of 9-point hedonic scale.

Attribute	T1	T2	T3	T4	T5	p-value
Color	8.12 ± 0.18^{a}	8.24 ± 0.17^{a}	7.61 ± 0.19^{b}	6.95 ± 0.21^{c}	6.42 ± 0.24^{d}	<0.001
Aroma	7.84 ± 0.20^{a}	8.05 ± 0.19^{a}	7.32 ± 0.21^{b}	6.58 ± 0.25^{c}	5.91 ± 0.28^{d}	<0.001
Taste	8.06 ± 0.19^{a}	8.18 ± 0.18^{a}	7.21 ± 0.23^{b}	6.37 ± 0.27^{c}	5.64 ± 0.31^{d}	<0.001
Texture	7.92 ± 0.21^{a}	8.31 ± 0.18^{a}	7.44 ± 0.22^{b}	6.48 ± 0.26^{c}	5.72 ± 0.29^{d}	<0.001
Mouthfeel	7.88 ± 0.20^{a}	8.26 ± 0.19^{a}	7.36 ± 0.22^{b}	6.42 ± 0.25^{c}	5.68 ± 0.30^{d}	<0.001
Acidity acceptance	7.76 ± 0.22^{a}	8.02 ± 0.20^{a}	7.28 ± 0.24^{b}	6.61 ± 0.26^{c}	5.95 ± 0.29^{d}	<0.001
Total acceptability	8.01 ± 0.19^{a}	8.34 ± 0.17^{a}	7.42 ± 0.21^{b}	6.53 ± 0.25^{c}	5.78 ± 0.30^{d}	<0.001

The sensory findings indicated that consumer acceptability was enhanced or retained when a cow milk at 25% was replaced with soy milk at 25%. T2 was the

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most agreeable formulation since it was a combination of the already known sensory properties of the cow's milk yogurt with a slight addition of soy milk. On the contrary, T4 and T5 were less acceptable as high inclusion of soy milk adversely impacted flavour, texture and mouthfeel.

The primary nutritional, physicochemical, microbiological and sensory indicators were taken into consideration as a set. The total profile of T2 was the most favorable with T3 and T1 following. The fact that T5 had a higher level of iron and magnesium was counterbalanced by the fact that T5 was ranked lower based on its lower viscosity, higher syneresis, and lower sensory acceptability.

Table 10. Complete prioritization of yogurt formulations in terms of nutritional and quality parameters.

Treatment	Nutritional value	Physical stability	Microbiological safety	Sensory acceptability	Total rank
T1	Good	Good	Safe	Very good	3
T2	Very good	Excellent	Safe	Excellent	1
T3	Very good	Good	Safe	Good	2
T4	Moderate	Fair	Safe	Fair	4
T5	Moderate	Weak	Safe	Low	5

The total ranking showed that the T2 formulation, which comprised 75% cow milk and 25% soy milk was the best formulation. This treatment was the best in terms of nutritional improvement, physical stability, microbiological safety and sensory acceptance. T3 has also exhibited good quality but the sensory and physical scores were low compared to T2. High soy milk content treatments were not the most appropriate to be used in the production of yogurt unless further formulation adjustments like stabilizers, flavor enhancement or mineral fortification.

Discussion

The current research showed that the combination of cow to soy milk had a profound impact on the nutritional, physicochemical, microbiological and sensory characteristics of yogurt. The best balance between nutritional improvement, stability of the gel, microbiological safety and sensory acceptability were achieved with the formulation that contained 75% cow milk and 25% soy milk (T2). The discovery underlies the emerging scientific notion that hybrid dairy-plant formulations have the potential to deliver a superior product performance as compared to a total replacement of cow-milk with plant-based milk. Recent studies of the hybrid yogurt and cow-soy yogurt systems reported that the partial substitution of the soy or oat drinks in the cow milk can enhance consumer acceptance, development of viscosity and selected quality characteristics when the replacement level is carefully regulated **[7,8,14,15]**

The results of the proximate composition analysis showed that an increase in the concentration of soy milk, decreased the fat and energy value but did not affect or slightly enhanced the protein content in the blended yogurt treatment. This nutritional trend is important to the extent that it implies that a partial replacement of soy milk with another dairy product could result in a light fermented dairy product without causing a significant decrease in protein. Various studies have confirmed that soy milk is one of the closest plant-based alternatives to cow-milk in terms of

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protein content, but there are still differences in amino acid profile, digestibility, mineral content and matrix behavior during processing [1,16,17,18]. Thus, the moderate rise or a lack of rise in protein content of T2 and T3 can be attributed to the contribution of soy proteins, whereas the lesser performance of T5 can be explained by the fact that the amount of protein is not enough to assure desirable structure of yogurt. Their decreasing fat and caloric content with the increasing level of soy milk is in line with the total nutritional profile of the plant-based milk substitutes. Plant-based drinks have less saturated fat and no cholesterol and vary in the amount of carbohydrates and proteins depending on the plant source and formulation. Nevertheless, their nutritional adequacy is not consistent and can be highly dependent on the condition of fortification and processing [19,20,21,22.23]. In the current study T2 had a useful balance since it reduced the fat and energy content than the full cow milk control without affecting the sensory richness and mouthfeel of yogurt. Conversely, the high-soy diets had lower scores on sensory, indicating that the excessive decrease in dairy fat could have adverse implications on creaminess, flavor release, and completed acceptability. The results of the mineral composition showed a decrease in calcium and phosphorus with an increase in the concentration of soy milk, and an increase in the concentration of iron, magnesium and potassium. The trend corresponds to the dissimilarity of the mineral profiles of cow and soy milk. The naturally significant source of calcium and phosphorus is represented by the cow milk, whereas the soy-based beverages can represent higher amounts of some of the minerals which are associated with the soy (depending on the composition of raw materials, their soaking, heating, filtration and fortification status) [10,6,4,3]. The decrease in the calcium levels in T4 and T5 is nutritionally significant since one of the major nutritional benefits of dairy products is calcium. Nevertheless, the fact that iron and magnesium are increased shows that soy milk will be able to diversify the mineral profile of the end product. Bioavailability of the mineral results should be also addressed in the interpretation of the mineral results, not only based on the total concentration. Plant drinks can contain phytates, oxalates and fiber fractions, which can impair the absorption of minerals, particularly when they are not optimally fortified. In addition, solubility, stability and bioaccessibility of the micronutrient can be affected by the type of mineral salt used in fortification. [24]. found that the vitamin D bio accessibility of plant-based milk can be reduced by the fortification of calcium in the milk, whereas [25]. stated that plant-based drinks and cow milk differ not only in the concentration of the nutrient but also in its nutritional role. Thus, as much as soy milk has enhanced the selected mineral values in the current research, future research should evaluate the mineral bioavailability and not rely on the total analysis of the mineral. The pH outcomes were progressive in all treatments which were refrigerated stored and titratable acidity increased over time. This trend is anticipated in yogurt since starter cultures might be able to persist in metabolic activity during cold storage, resulting in post-acidification. The higher decrease in pH in soy-rich treatments might be associated with the variations in buffering capacity, availability of carbohydrates, protein composition and the adaptation of starter cultures to the soy matrix. [26]. revealed that the ratio and interaction of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus can have a strong influence on the fermentation kinetics and post-acidification. Likewise, the research on fermented plant-based milks has demonstrated that the fermentation of lactic acid can be used to alter the acidity, flavor compounds, bioactive substances, and storage stability [3,27,8]. The rise in acidity was technologically useful within moderate ranges since acidity aids in the growth of the gel, the typical flavor of yogurt, and the microbiological stability. Nevertheless, the effect of acidity can be counterproductive at high levels as it can decrease sensory acceptability by making the product sourer and by changing the mouthfeel. This is the reason why T2 had better acceptance scores as compared to T4 and T5 who had lower scores in taste and acidity acceptance. Past research on soy yogurt and fermented products made of soybean reported that fermentation can reduce the beany flavor and antinutritional properties, but the total sensory quality is highly dependent on the starter strain, fermentation time, substrate composition, and post-acidification behavior [28,14,15]. Therefore, the present findings suggest that soy milk could be helpful when used as a component of hybrid yogurt, although only in the case when this ingredient is incorporated at a regulated level. Results of the viscosity, syneresis and water-holding capacity showed that T2 had the highest physical stability among all of the treatments. This would indicate that the addition of low-level soy milk may not weaken the yogurt gel matrix but may, in fact, support the gel matrix. The moderate incorporation of soy proteins can help in water binding, and the increment of the density of the fermented gel net, especially when used in conjunction with dairy proteins. Nevertheless, the high soy milk replacement probably lowered the concentration of casein lower than the one required to induce the gel formation by the strong acid. The strength of gel of yogurt is dependent on the interaction of proteins, the total solids, heat treatment, the activity of the starter culture, and the structural behavior of the protein's casein and whey during the process of acidification [28,8,29, 30]. This was confirmed by the high syneresis of T4 and T5, which demonstrated that excess of substituting cow milk with soy milk weakened the gel structure. The milk of cows is mostly relied on as a source of the protein casein, which forms aggregates of micelles as it acidifies, and which forms a different protein gel network with different water-binding and rheological properties. Yogurt alternatives based on plants often require further technological approaches like enhanced heat treatment, homogenization, stabilizers, hydrocolloids, exopolysaccharide-producing cultures or enzyme-modification to attain acceptable texture. Research on plant and hybrid yogurt systems revealed that the functionality of proteins, as well as interactions between polysaccharides and proteins, and processing conditions, determine the improvement of the texture [31,32,33,34]. The enhanced water retention of T2 can also be explained by the fact that interaction between dairy proteins and soy proteins at moderate concentration can also lead to the improvement of water retention capacity of T2. Lactic acid bacteria that produce exopolysaccharides have the potential to enhance the viscosity and decrease the syneresis, by enhancing the water retention within the gel matrix. [30]. have shown that lactic acid bacteria involved in the production of exopolysaccharide and starch have the ability to alter the rheological characteristics of set and stir yogurt. Likewise, [29]. discovered that fermented soymilk produced with EPS-producing lactic acid bacteria exhibited variations in microbiological, physicochemical and rheological characteristics. These results confirm the current understanding that the cow–soy yogurt performance is not only determined by the milk ratio, but also by the choice of the starter culture and structuring of the

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matrix. The microbiological data indicated that all treatments were safe in refrigerated storage, and that no coliforms, Escherichia coli or Salmonella spp. were detected. This implies that the heat treatment, hygienic processing, fermentation and cold storage proved to be effective in ensuring that the products were safe. The counts of viable lactic acid bacteria were used as evidence of successful fermentation in all the groups. The fact that yeasts and molds slightly increase towards the end of storage, particularly in soy-rich treatments, may be due to the fact that the latter is more sensitive to spoilage during a long refrigeration period. According to the storage studies of fermented milks, the microbial viability, acidity, and sensory stability have a close relationship during refrigerated storage [30,12,28]. The results of the sensory evaluation proved that T2 was the treatment that was accepted most. This finding correlates with the physicochemical results in that T2 exhibited a high level of viscosity, low level of syneresis, good level of water holding capacity, balanced level of acidity and acceptable level of flavor. Sensory acceptance of hybrid yogurt is based on retaining familiar dairy-like taste and texture, and adding nutritional or functional benefits of plant origin. Soy milk is also high in beany flavor, decreased creaminess, poorer gel structure, and less familiar mouthfeel. Past studies have indicated that selection of starter culture and control of fermentation are critical towards minimizing unwanted soy notes and enhancing aroma, texture and total acceptability in soy yogurt [37,32,14]. The reduced sensory acceptability of T5 suggests that 100 per cent soy yogurt needs an alternate formulation approach than that needed by cow’s milk yogurt. Alternatives to dairy yogurt made with plant-based ingredients are not merely the dairy yogurt minus the cow’s milk; it is a different food matrix that may in many cases require a particular structuring system, flavor optimization, and nutrient balancing. [38]. highlighted that to obtain performance similar to dairy milk, plant-based milks are required to be carefully designed to form a colloidal structure, interactions among ingredients, and processing conditions. Likewise[39,35,40]. observed that the plant-based dairy substitutes are widely varied in composition, technological behavior, additives and consumer perception. This helps to conclude that a partial blending is more viable as compared to complete replacement when the target product is the conventional style of yogurt. The complete ranking showed that T2 was the best formulation then T3 and T1. The low texture of T5, high levels of syneresis and lower scores on sensory attributes lowered the total quality of T5. T3 gave good nutritional and physicochemical performance, although, T3 had poorer sensory performance as compared to T2. Thus, the most reasonable ratio of soy milk replacement to achieve the highest nutritional diversity and reasonable technological characteristics in the production of hybrid yogurt seems to be the 25% level of the soy milk replacement. This is in line with research that found moderate inclusion of plants can positively affect the functionality of the product, whereas excessive substitution may adversely affect the formation of protein gels, flavor balance, and acceptance by consumers [7,8,14]. In terms of product-development, the current results reveal that the blending of cow-soy milk can be considered as an effective approach towards the production of hybrid fermented dairy products. The strategy can satisfy consumer demand of products that can decrease the amount of animal products in them but preserve the familiar properties of dairy yogurt. Nevertheless, the research also validates the fact that plant-based milk cannot be considered the same in terms of its nutritional and

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technical properties as cow-milk. The last quality is determined by the interaction between the type of protein, mineral profile, fat composition, starter culture, heat treatment, fermentation kinetics, and storage behavior [41,20,42.43].

Conclusion

The current research came to the conclusion that the blends of cow milk-soy milk using traditional yogurt starter cultures can be successfully developed into yogurt. The blending ratio had a clear impact on nutritional, physicochemical, microbiological and sensory characteristics of the end product. Replacement of the cow milk with the soy milk in partiality lowered the fat and energy content and also enhanced the selected nutritional elements, especially the iron, magnesium and potassium. But higher levels of soy milk beyond moderate levels led to a reduction in the content of calcium, instability in the gel, increased syneresis, and decreased sensory acceptability. Formulation with 75% cow milk and 25% soy milk was found to have the best completed quality due to its ability to maintain good protein content, improved physical stability, reduced whey separation, remained microbiologically safe and received the highest scores in sensory acceptability. Hence the partial incorporation of soy milk (particularly at the 25 percent mark) can be viewed as a viable solution to the development of hybrid yogurt with a better nutritional diversity and acceptable technological and consumer quality

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