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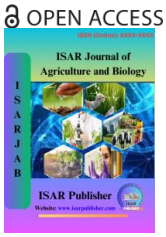

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PROXIMATE, MICROBIAL AND SENSORY PROPERTIES OF YOGHURT PRODUCED FROM COW MILK AND SOYMILK

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Abstract: This study was undertaken to assess quality of yoghurt made from cow milk and soy milk blend, the proximate, microbial, physicochemical, sensory and vitamin properties were analyzed. The proximate (moisture content, ash content, crude fiber, fat, protein and carbohydrate) Microbial (total viable count, coliform count, fungal count) and sensory (colour, texture, aroma, taste and over all acceptability). The yoghurt sample had a moisture range of 72-75% there is a significant difference between the samples P<0.05. Protein range of 4.51-7.69% and the sample made from 50% cow milk and 50% soymilk had the highest value. The fat content ranged from 1.95-3.80%, the fibre ranged from 0.74-0.97%. The ash content ranged from 1.23-1.41% and carbohydrate content ranged from 12-17.13 .The result of the microbial analysis showed that yoghurt sample made from 100% cow milk had the highest total viable count $1.70x10^4$ CFU/ml and the sample made from 72.5% cow milk and 27.5% soymilk had the least viable count 1.2×10^4 CFU/ml. the control sample had the highest value of coliform count 4.71×10^2 and when compared with others, Sample made from 65% cow milk and 35% soymilk and sample made from 57.5% cow milk and 42.5% soymilk had the least value for total coliform count. The sensory parameters revealed that sample made from 65% cow milk and 35% soymilk was the preferred, had the highest rate for color, texture, aroma, taste and over all acceptability. There vitamin A and B2 content in the samples ranged from 1.32-5.55 (%) and sample made from 65% and 35% cow milk and soymilk respectively had the highest amount vitamin A and B2 and there is a significant difference between the samples P<0.05.

Keywords: Quality Soymilk, Yoghurt, Fermentation, cow milk, Food science.

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Introductıon

Yoghurt is the Turkish word for fermented milk. It was developed by early nomadic herders, particularly in Asia, Southern and Eastern Europe. Yoghurt is prepared by combining a culture of acid-producing bacteria with homogenized, pasteurized, and fermented milk. Yoghurt is a fermented milk product that originated empirically years ago by letting naturally contaminated milk to sour at a warm temperature in the 40-50 °C range (Katz, S. E. (2012). The microorganisms that are traditionally utilized in this process are referred to as "Starter Culture." *Lactobacillus delbrueckii subsp*. *Bulgaricus and Streptococcus thermophilus* are among them. *Lactobacillus bulgaricus* has a diameter of 0.8 to 1.0m on average (Sanfu, 2009).

During fermentation, milk proteins are hydrolyzed, the pH drops, the viscosity increases, and bacterial metabolites are generated, which contribute to the flavour and probably the health-promoting characteristics of yoghurt. The bacteria ferment the sugars into lactic acid, which results in the creation of the distinctive curd. The acid lowers the pH of the yoghurt and inhibits the growth of germs that cause food poisoning (putrefactive or pathogenic). Yoghurt is not only a delightful quick, simple, and healthy snack, but research suggests that milk and yoghurt may potentially prolong life, as seen in some nations where fermented dairy products are a dietary staple Traditional yoghurt has been linked to several health advantages, and adding probiotic bacteria to it enhances this positive perception (Magenis, *et al.*, 2006).

Soymilk is an aqueous extract of soya beans (*Glycine max*) and is quiet similar in appearance to cow milk. It is commonly characterized as having a beany, grassy or soy flavour, which reportedly can be improved by lactic acid fermentation, as in yoghurt-like products Microorganisms possess endogenous β-

glucosidases which can be utilized to hydrolyze predominant isoflavone glucosides in soymilk to improve biological activity. It has been reported that probiotic organisms including *Bifidobacteria* and some other lactic acid bacteria hydrolyze isoflavone glycosides into corresponding a glycones (Cheng, 2010).

Due to continuous increase in the population and inadequate supply of animal milk protein leading to malnutrition in Nigeria many research works have been geared finding alternative protein source from legume. Soymilk can serve as a very good alternative to the expensive cow milk as it contains all the essential amino acid even though some are in a low concentration It is well known from experiment that diets containing casein or other animal protein could induce elevation of plasma total LDL cholesterol concentration but this can be prevented by vegetable protein such as soy protein. The Food and Drug Administration concluded in 1999 that including soy protein in a diet low in saturated fat and cholesterol, hence reduce the risk of coronary heart disease by reducing blood cholesterol levels. Based on this fact, the FDA recommends including 25 grams of soy protein in a daily meal, which is why soy is regarded as a new hero in the food sector and nutrition world.

Materials and Methods

The materials used in this study were Cow's milk powder (Peak Milk brand), soybeans (*Glycine max L.*), sugar, vanilla flavour (Foster Clark) and milk flavour, commercial freeze-dried yoghurt starter all was sourced from the local market Eke Awka Awka South Local Government and Onitsha main market Onitsha South Local Government Anambra State.

Production of Yoghurt Samples

Research Design; Completely Randomized method was used for the sampling.

Table1. Experimental Design

Run	Cow Milk (%)	Soymilk (%)
CMY	100	-
CSYM	57.5	42.5
CSYA	80.0	20.0
CSYN	65.0	35.0
CSYH	50.0	50.0
CSYR	72.5	27.5

Keywords: CMY = Made from 100% Cow Milk (Control Sample), CSYM = 57.5% Cow Milk42.5% Soy Milk; CSYA = 80.0% Cow Milk20.0% Soy Milk CSYN = 65.0% Cow Milk35.0% Soy Milk; CSYH = 50.0% Cow Milk50.0% Soy Milk

CSYR = 72.5% Cow Milk27.5% Soy Milk.

Soymilk Preparation

Soybeans (2 kg) was sorted and soaked for (24 hours.) at room temperature (28-30 °C) in 10liters. of water; the water was changed every 6 hours throughout the soaking time. The grains were drained, washed with new water, and de-hulled by manual rubbing between the hand palms; the de-hulled beans were wet-milled with a blending machine. For milk extraction, the resulting slurry was diluted with 4cups of water at 80 °C and filtered through muslin cloth. The separated soymilk was cooked for 25 minutes on low heat on a gas burner to concentrate the milk and inactivate the trypsin inhibitor to minimize the beany off-flavour and so improve the flavour then allowed to cool at room temperature (26-30 °C). Traditional techniques for removing beany flavour from soymilk, in particular, comprise soaking, grinding, filtering, and heating procedures and different heat treatments before grinding. The technique that Jimoh (2020) reported with minor adjustments was used in the current work to extract soymilk (Fig. 1)

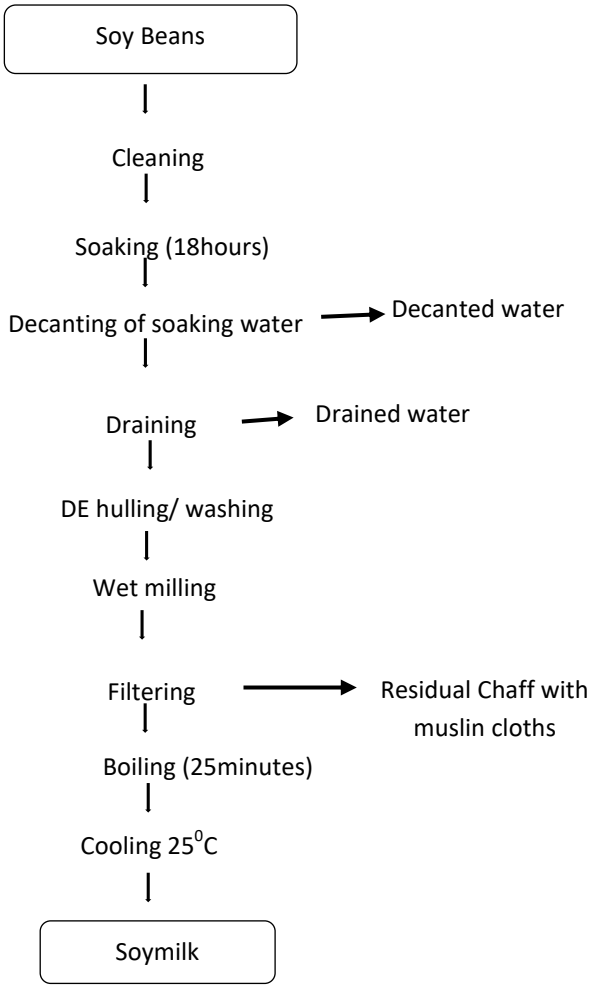

Figure 1: Soy milk Production

Source: Jimoh (2020)

Production of Yoghurt

The powdered milk was reconstituted at a 100 g to 1liter. Water ratio, and four samples of yoghurt prepared from cow and soy milk mixes was made. Four liters of each milk blend combination was pasteurized for 15 minutes at 85 °c, chilled to 40-44 °c, and inoculated with 120 ml of the prepared activated commercial freeze-dried "yogourmet" starter culture (prepared according to the manufacturer specification as presented in figure. 2) then, incubated at about 35 °C overnight. The formed yoghurt was blended for a smooth consistency using a multifunction blender,

sugar, milk flavour, and vanilla essence was added to improve the flavour of the yoghurt products, bottled and refrigerated pending the analyses. The flow chart for the production is presented in Figure 2 .

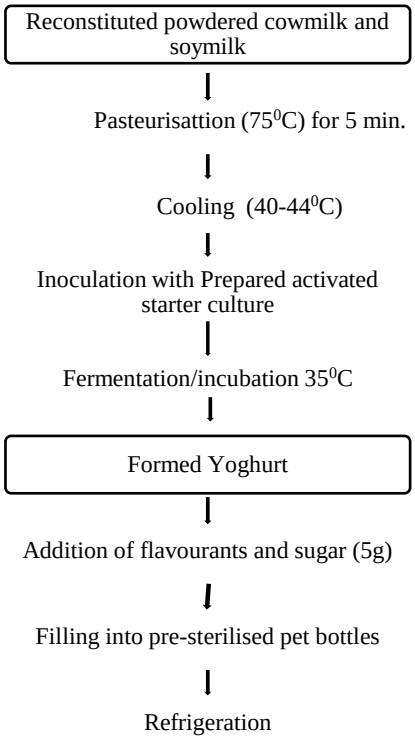

Figure 2. Production of yoghurt

Source: Otolowo *et. al.*, (2022)

Method of Analysis:

Proximate Analysis

This was determined using methods described by Analysis of Association of Official Analytical Chemist, AOAC (2010).

Determination of protein content: According to published research by Johann Kjeldahl, the macro Kjeldahl technique was used to determine the crude proteins. This is how the protein is calculated:

$$\%\text{Crude protein} = \%\text{nitrogen} \times 6.38$$

$$\%\text{Nitrogen} = \frac{(\text{ml standard acid} - \text{ml blank})\text{N of acid x1.4007}}{\text{sample in gram}} \ldots\ldots\ldots\ldots equation\ 1$$

Ash content determination: The ash content was determined by direct heating method as described by Analysis of Association of Official Analytical Chemist, AOAC (2010). The percentage ash content was calculated by the following formula.

$$\%\text{ash} = \left(\frac{z - x}{y - x}\right) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation } 2$$

where; X=weight of empty crucible; Y=weight of crucible + sample; Z=weight of crucible + ash

Determination of moisture content: The percentage of moisture content was determined by oven method as described by Analysis of Association of Official Analytical Chemist, AOAC (2010). Briefly, 2g of yoghurt samples was dried in the oven for 24 hours at 100^{0}C. The percentage moisture content was calculated by the following formula.

$$\%\ \text{Moisture} = W1 - \frac{W2 \times 100}{W1} \ldots\ldots\ldots\ldots\ldots\text{equation } 3$$

where, W_1=initial weight of sample; W_2=weight of the dried sample.

Determination of carbohydrates content: Carbohydrates was determined using a mathematical function below as described by Analysis of Association of Official Analytical Chemist, AOAC (2010).

CHO=100 - % (ash + protein + fat + crude fibre + moisture).

Determination of Microbial Quality

Microbial analysis was carried out using the method described by Ijah *et al*. (2014); the media used include; Nutrient Agar (Sigma-Aldrich-70,148), McConkey Agar (Sigma-M7408), Agar and Potato Dextrose Agar (Sigma-Aldrich- 70,139).

Sensory Evaluation.

The sensory assessment of the yoghurt samples was performed a few hours after manufacturing and refrigeration and was examined for colour, consistency, scent, taste, and general acceptability. Twenty-five semi-trained panelists selected from the Department of Food Science and Technology at Nnamdi Azikiwe University would be given portable water and encouraged to clean their mouths before and after each evaluation. The panelists were selected based on their knowledge of yoghurt products, while those who are unfamiliar with the product being disqualified to avoid bias in the assessment. (Jimoh 2020). Additionally, lactose-intolerant people will not participate in exercise to prevent any potential health problems. Yoghurt with varying levels of soymilk substitutions was rated on a scale of 1 to 9 with 1 being extremely disliked, 8 being liked very much, 7 being liked moderately, 6 being liked slightly, 5 being neither liked nor disliked, 4 being disliked slightly, 3 being disliked moderately, 2 being disliked very much, and 9 being liked extremely.

Statistical Analyses

All the determinations were done in triplicates and a mean value was calculated in each case. Analysis of variance (ANOVA) was performed and separation of the means was by Duncan's Multiple Range Test, significant difference was accepted at $p < 0.05$ using SPSS version 21.0.

Result and Discussion

Proximate composition of cow milk and blend of soymilk yoghurt:

The proximate composition is shown in Table 2. Tthe average moisture content of the yoghurt ranged from 71.80 to 79.58 (%) . The sample CSYH made from 50.0% Cow Milk and 50.0% Soy Milk had the least moisture while the sample CYSN 65.0% Cow Milk and 35.0% Soy Milk had the highest and the control CMY had a moisture 72.90 there was a significant difference between the control sample and other produced samples (P>0.05). The moisture

content is required to be at a range of 70-85% as report by Ahmed *et al.*, (2004). Much water makes it less viscous and affect its mouth feel and texture Ahmend *et. al.*, (2004).

The protein content of the sample ranged from 4.51 to 7.69 (%) sample. The sample CSYA made from (80.0% Cow Milk and 20.0% Soy Milk) had the least protein and sample CSYH made from 50.0% Cow Milk and 50.0% Soy Milk had the highest protein content 7.69% when compared to the control CMY value 7.36% and there is a significant difference between the samples and the control (P> 0.05). According to USDA (2010) yoghurt should have a maximum of 2.8% the high protein could be due to soymilk content.

The fat content ranged from 1.95 to 3.8 (%) the control CMY made from 100% cow milk had a fat content of 2.61 % when compared to sample CSYH made from (50.0% Cow Milk and 50.0% Soy Milk) which had a value of 3.80%. there was a significant difference (P > 0.05), between the samples. Fat plays important roles in improving the consistency of the yoghurt and provide as much quality than protein and carbohydrate. according to United States Department of Agriculture (USDA). (2001). yoghurt samples with more than 3.25% of fat content should be labelled yoghurt; yoghurt with fat content in the range of 0.5-2.0% should be labelled as Low-Fat yoghurt and yoghurt with less than 0.5% fat content should be labelled Non-Fat yoghurt. The results from our studies showed that the fat content of the above six samples are comply with these standards.

The crude fiber contributes to the health of gastro intestinal system of man according to Cheeseman and Lean 2002 yoghurt have fiber level because they are milk and water based product. Fiber content ranged from 0.85 to 0.97 (%) the control sample had the lowest fiber content 0.74% when compared to sample CSYR made from (72.5% Cow Milk and 27.5% Soy Milk) there was no significant difference (P<0.05) between control sample CMY 100% cow milk and other samples.

The literature value of percentage of crude fibre content of the samples is 0.21-0.51% (Igbabul B *et al*., 2014) However, the percentage crude fibre content of all six samples were found to be much higer than literature values this coude be due to the soymilk component. Fibre content improves textural properties and structure, reduce lipid retention and reduce caloric content by acting as acting as a bulking agent (Jambi, H. A. 2018)

The ash content of the yoghurt sample ranged from 1.23 to 1.41 (%) sample CSYN made from 65.0% Cow Milk and 35.0% Soy Milk had the highest ash content when compared to the control sample CMY 100% cow milk which had 1.32% there was a significant difference P> 0.05 and there was no significant difference when compared to the remaining samples. Ash content is the measure of the total amount of minerals present within a food (a reflection of the mineral element) (IUPAC, 1997). The carbohydrate sample ranged from 10.61 to 17.13 (%) the control sample CYM made from 100% cow milk had the highest value 14.75% There was no significant difference between the samples (P<00.5). The low rate of carbohydrate is attributed to the fermentation process which converted carbohydrate to lactic acid Younus *et al*.; (2002).

Table 2: Proximate composition analysis.

Sample	Moisture %	Protein %	Fat %	Fiber %	Ash %	Carbohydrate %
CMY	72.90 ^{c}±0.51	7.36 ^{ab}±0.11	2.61 ^{bc}±0.28	0.74 ^{c}±0.01	1.32 ^{a}±0.00	14.76 ^{b}±0.02
CSYM	75.44 ^{b}±0.24	6.53^{abc}±0.23	3.15 ^{ab}±0.02	0.95 ^{ab}±0.02	1.23 ^{b}±0.01	12.55 ^{d}±0.01
CSYA	79.58 ^{a}±0.13	4.51 ^{d}±0.65	3.26 ^{ab}±0.11	0.85 ^{b}±0.00	1.36 ^{a}±0.01	10.16 ^{f}±0.00
CSYN	72.40 ^{ab}±0.17	5.66 ^{c}±1.15	1.95^{c}±0.84	0.95 ^{a}±0.02	1.41 ^{a}±0.13	17.13 ^{a}±0.01
CSYH	71.80 ^{d}±0.17	7.69 ^{a}±0.72	3.80 ^{a}±0.34	0.85 ^{b}±0.01	1.23 ^{b}±0.01	14.36 ^{c}±0.00
CSYR	75.55 ^{b}±0.27	6.44 ^{bc}±0.19	3.15 ^{ab}±0.02	0.97 ^{a}±0.01	1.36 ^{a}±0.02	12.44 ^{e}±0.01

Values are mean ± standard deviation of three (3) replicate. Data in the same column bearing different super script differed significantly (P<0.05). CMY = Made from 100% Cow Milk (Control Sample), CSYM = 57.5% Cow Milk42.5% Soy Milk; CSYA = 80.0% Cow Milk20.0% Soy Milk CSYN = 65.0% Cow Milk35.0% Soy Milk; CSYH = 50.0% Cow Milk50.0% Soy Milk

CSYR = 72.5% Cow Milk27.5% Soy Milk.

The microbial load Properties of cow milk and blend of soymilk yoghurt:

The mean value of the total viable count is presented in table 4.2. the sample ranged from 1.23 x 10^4 to 1.70 x10^4. Yoghurt sample CSYR 1.70^{a}±0.01 made from (72.5% cow milk and 27.5% soymilk) has the highest value and sample CSYM 1.23^{b}±0.15 made from (57.5% cow milk and 42.5%) soymilk has the lowest value when compared to the control value (100% cow milk) which is at 1.3^{b}±0.05. There was no significant difference between the control sample CMY and sample CSYR (P<0.05) and samples (CSYM, CSYA, CSYN, CSYH) are significantly different (P>0.05) from the control sample CMY.

The value of the total mold count ranged from 2.63 - 5.60 x (10^4) CFU/ml with sample CSYR bearing the highest value 5.60^{a}±0.20 and sample CSYM having the lowest values when compared to the control CMY sample values. There was no significant difference between the control sample CMY and sample CSYH (P<0.05). Presence of yeasts or molds in yoghurt is also indicative for poor sanitary practices in manufacturing or packaging. Yoghurts made with added sugar is especially susceptible to yeast growth which can be a cause of higher results (Lourens-Hattingh, 2001).

The value of the total coliform count ranged from 1.01 - 4.7 x (10^2) CFU/ml with sample CSYR bearing the highest value 4.71^{a}±0.08 and sample CSYM and the CMY having the lowest values

1.01^{e}±0.01. There was a significant difference (P>0.05) between the control sample CMY and samples CSYH and CSYN. This was agreed with the range of other previous study (Younus *et al*., 2002). The studied result reflected highly poor hygienic conditions and improper sanitation during manufacturing of yoghurt.

Table 3: Microbial colony count (CFU/ml)

SAMPLE	Total viable count	Total fungal count	Total coliform count
CMY	1.3^{b}±0.05	3.10^{c}±0.01	1.01^{e}±0.01
CSYM	1.23^{b}±0.15	2.63^{d}±0.15	1.01^{e}±0.01
CSYA	1.36^{b}±0.15	4.16^{b}±0.15	2.08^{c}±0.01
CSYN	1.63^{a}±0.15	5.56^{a}±0.15	3.43^{b}±0.15
CSYH	1.20^{b}±0.10	4.40^{b}±0.02	1.58^{d}±0.15
CSYR	1.70^{a}±0.01	5.60^{a}±0.20	4.71^{a}±0.08

Values are mean ± standard deviation of three (3) replicate. Data in the same column bearing different super script differed significantly (P<0.05). CMY = Made from 100% Cow Milk (Control Sample) CSYM = 57.5% Cow Milk42.5% Soy Milk; CSYA = 80.0% Cow Milk20.0% Soy Milk, CSYN = 65.0% Cow Milk35.0% Soy Milk; CSYH = 50.0% Cow Milk50.0% Soy Milk

CSYR = 72.5% Cow Milk27.5% Soy Milk.

Sensory Properties of the Yoghurt Samples.

The mean value for sensory properties of yoghurt samples is present in Table 4. The mean value for colour ranged from 6.55 to 8.40. The control sample CMY made from (100% cow milk) had the value 7.75, yoghurt CSYH made the form (50% cow milk and 50 % soymilk) had the least value 6.55 while yoghurt CSYN made from (65.0% Cow Milk 35.0% Soy Milk) had the highest value 8.40. there is a significant difference (P>0.05) between the samples. According to Hiralal, L., colour may be due to the flavouring type used.

The texture ranged from 5.90 to 8.00 the control sample CMY (made from 100% cow milk) had the value 7.95 and sample CSYN made from (65.0% Cow Milk 35.0% Soy Milk) had the highest value 8.00 there was a significant difference between the sample (P>0.05).

Aroma of the yoghurt sample ranged from 6.55 to 7.80 the control sample CMY had the value 7.50 while the yoghurt with highest value 7.90 is CSYN made from (65.0% Cow Milk 35.0% Soy Milk) and the yoghurt with the least value 6.20, is sample CSYH made the form (50% cow milk and 50 % soymilk) there is a significant difference between the sample (P>0.05) jimoh, et al., (2020) states that in some cases, the context of formation of the aroma is based on the fermentation.

The value for the taste of the yoghurt sample ranged from 6.2 to 7.8 the control CMY had the value 6.35 and the sample CSYR made the form (72.5% Cow Milk and 27.5% Soy Milk) had the highest value 7.80, the least value 6.20. is sample CSYH made the form (50% Cow Milk and 50% Soy Milk) there is significant difference in the samples the sourness is as a result of the astringency produced as a result of production of lactic acid by *lactobacillus lactics* on lactose in substrate, according to Sanful, (2009).

In other to access the quality of individual sample of the yoghurt, the general acceptability mean score was calculated as the composite of all sensory attribute evaluated. The most liked sample was the sample CSYN with the highest value 7.90 made from (65% cow milk and 35% soymilk) and the least liked sample is CSYH 6.35 made from (50% cow milk and 50% soymilk) there was a significant value between the samples (P>0.05).

The overall mean score for taste colour and texture are important quality characteristic for general acceptability of yoghurt. The aroma and taste are generally considered as the most critical and important indicator of consumer acceptance.

Table 4: Sensory analysis result

Sample	Colour	Texture	Aroma	Taste	Over All Acceptability
CMY	7.75^{ab}±1.33	7.95^{a}±1.14	7.50^{a}±1.53	6.35^{b}±1.92	7.00^{bc}±1.48
CSYM	7.20^{b}±0.95	6.60^{b}±1.84	6.55^{b}±1.173	6.75^{b}±1.37	6.90^{c}±1.37
CSYA	8.05^{a}±0.75	7.50^{a}±0.82	7.50^{a}±0.76	7.60^{a}±0.88	7.70^{ab} ±0.73
CSYN	8.40^{a}±0.82	8.00^{a}±1.02	7.90^{a}±0.85	7.70^{a}±1.03	7.90^{a}±0.73
CSYH	6.55^{c}±0.82	5.90^{b}±1.07	5.85^{b}±1.49	6.20^{b}±1.23	6.35^{c}±1.18
CSYR	8.05^{a}±0.94	7.70^{a}±0.92	7.65^{a}±0.81	7.80^{a}±0.95	7.80^{a}±1.05

Values are mean ±standard deviation of three (3) replicate. Data in the same column bearing different super script differed significantly (P<0.05). CMY = Made from 100% Cow Milk (Control Sample), CSYM = 57.5% Cow Milk42.5% Soy Milk; CSYA = 80.0% Cow Milk20.0% Soy Milk, CSYN = 65.0% Cow Milk35.0% Soy Milk; CSYH = 50.0% Cow Milk50.0% Soy Milk

CSYR = 72.5% Cow Milk27.5% Soy Milk.

Conclusion and Recommendation

This study has shown that blending cow milk and soymilk can affect the quality properties of the yoghurt. The proximate, microbial and sensory properties of the yoghurt was affected as seen from experiment analysis.

The yoghurt samples had a protein content higher than the minimum amount set by codex stand for yoghurts 2.7% protein. The sample CSYH made from 50% cow milk and 50% soymilk had the highest amount of protein 7.6 % which increased the product quality. The study also showed that samples (CSYM, CSYA, CSYN, CSYH, CSYR) had high range of coliform count at 1.01 to 4.7 (x 10^4 CFU/ml) but lower than the recommend allowance of 1.0 x10^5 CFU/ml set by the codex standard. However, there is need to employ good hygienic practices during the production process. The mean result of the sensory showed significant difference (p<0.05) between the samples (CSYM, CSYA, CSYN, CSYH, CSYR, CYM) in terms of colour, taste, aroma, texture, overall acceptability. Yoghurt CSYN 65% cow milk and 35% soymilk was the most accepted.

The analysis carried out revealed that soymilk can serve as a great substitute for cow milk to reduce cost of product and to produce yoghurt with more nutritive and acceptable values for both vegan and non-vegans and lactose tolerant patients.

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