



Formulation of Millet Milk and Herb Extract Enriched Yogurt and to Assess Its Nutritional Characteristics

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Abstract: The dairy-based probiotic products dominate the functional food market primarily yogurts. Fibers derived from by-products of the fruit processing sector, herb extracts, and fresh spices have all been introduced to the milk and yogurt product portfolio. The main objective of this study was to investigate to what extent the addition of barnyard millet milk and different herb oleoresins in yogurts will improve its qualitative properties and antioxidant activity. The cow's milk, barnyard millet milk, and the oleoresins obtained from three other herbal plants are considered in this study, respectively, Sage (*Salvia Officinalis* L.), Oregano (*Origanum Vulgare* L.) and Rosemary (*Rosmarinus Officinalis*). On day 1, day 14 and day 28 after yogurt preparation, the effect of millet milk and oleoresins prepared from different herbs (0.5, 1, 1.5 percent) on yogurt qualitative features (pH, titratable acidity, syneresis, color parameters, viscosity total polyphenol and flavonoid and antioxidant activity) was evaluated. The final results demonstrated that after 28 days of storage, the physicochemical parameters of the yogurt with millet milk and herb oleoresins were improved over CM samples. As the proportion of herb extract increased, so did the total polyphenol and flavonoid values. Yogurt containing herb extract exhibited antioxidant activity that was notably higher than the standard and improved proportionately as herb extract concentration increased. When Oregano (*Origanum Vulgare* L.) oleoresin and barnyard millet milk were combined, the best antioxidant capability was obtained. As per the results, the apparent viscosity of the CM yogurt was higher than that of enriched yogurt samples, while the model with 0.5 percent Oregano (*Origanum Vulgare* L.) oleoresin addition seemed to have the best nutritional value. The findings of this study lead to the conclusion that yogurts enriched with barnyard millet milk and natural extracts could be used as functional foods with significant health benefits.

Keywords: Yogurt, Barnyard Millet Milk, Sage (*Salvia Officinalis* L.), Oregano (*Origanum Vulgare* L.), Rosemary (*Rosmarinus Officinalis*), Physicochemical Properties and Antioxidant Activity.

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1. INTRODUCTION

Yogurt is one of the most consumed dairy products on the globe¹. It's made by fermenting milk or reconstituted milk with lactic acid bacteria, and it's appealing to consumers since it improves the intestinal environment while also boosting immunity². Yogurt developed during fermentation is a good source of bioactive peptides; however, it has low antioxidant content. On the contrary, various initiatives to make yogurts fortified with natural antioxidants derived from natural sources have now piqued attention and represent a unique product development strategy^{3,4}. In this context, bioactive compound-rich extracts of various plants, herbs, fruits, and mushrooms are increasingly being utilized as a yogurt component to hiking nutritional and functional performance. Many researchers have employed crude artichoke extracts⁵, grape and grape callus extracts^{6,7}, tea infusions⁸, *Lycium barbarum* water extract⁹, seaweed extract¹⁰, spirulina¹¹, *Pleurotus ostreatus* aqueous extract¹², black tea extracts¹³ and *Garcinia mangostana* Lin. (mangosteen rind) extract¹⁴ to produce yogurt. Decades of research suggest that consuming fermented foods, especially fermented milk products, is associated with improved health outcomes. Although milk and dairy products are included in nearly every national dietary guideline, only a few of these specifically recommend fermented foods¹⁵⁻¹⁷. Several researchers have recently proposed that sufficient evidence now exists to consider yogurt and other fermented dairy products containing live bacteria when developing dietary strategies for improving health. The human gastrointestinal (G.I.) tract is colonized by a diverse and complex population of more than a trillion microbes. The gut microbiota performs many critical functions, including protecting the host against potential pathogens, extracting nutrients from dietary constituents, and modulating digestive and immune homeostasis. Although it is well established that the adult human microbiome is relatively stable, antibiotics, diet, disease, hygiene, and other factors can disturb the composition and function of this ecosystem. Both the microbes associated with the manufacture of fermented foods and microbes added as probiotics may influence the gut microbiota and other physiological functions. Some of the microbes found in fermented dairy foods have been shown to survive digestion and reach the distal G.I. tract¹⁸. Yogurt has the potential to be a vital player in the spectrum of food products that provide a wide range of health benefits to individuals through specific influences on their intestinal microbiota. However, important strides in scientific understanding and regulatory oversight must be made to reach this potential. The scientific understanding of intestinal microbiota is still being assembled. For yogurt, how much of the intestinal microbiota and its influence on whole-body health are alterable by diet¹⁹. For regulatory oversight, the scientific, industrial, and regulatory communities must agree on quantifiable measures of those microbiota-dependent health properties. Fermented milk products' role in human health has been the subject of extensive research, including epidemiological, observational, and clinical studies. Sage (*Salvia officinalis* L.), a medicinally valued plant and a widespread species from the Lamiaceae family, has roughly 900 species globally and has been characterized over several pharmaceutical plants, having identified radical scavenging properties^{20,21}. It has traditionally been consumed as a herbal tea, spice, and food flavoring component. It has also been identified as an aroma agent in cosmetics, perfumery, and the

pharmaceutical sector. Due to its immunomodulatory²², antimicrobial²³, preservative²⁴, antioxidant²⁵, anticarcinogenic properties²⁶, and wide range of biological activities, sage has been used to produce various pharmaceutical formulations. Moreover, sage is high in polyphenols, notably phenolic acids^{27,28} and flavonoids²⁹, characterized as bioactive components with excellent antioxidant activity. Oregano Oleoresin is isolated from the leaves of Oregano (*Origanum vulgare*), a Lamiaceae flowering plant extensively spread throughout the Mediterranean and Asia³⁰. Marjoram, known colloquially as Oregano, is a versatile herb that contains major components: carvacrol and thymol. Although it has traditionally been used in conventional healers as a diaphoretic, carminative, anti-inflammatory, and tonic, its antibacterial properties have just recently been discovered. It's been used as a considerable strategy for treating gastrointestinal problems, coughs, and pulmonary disorders^{31,32}. Oregano is used in mouthwashes to maintain gums healthy, and it may also be administered topically to alleviate cold symptoms, including nasal congestion. Several investigations revealed that oregano extract has potent antioxidant activity owing to the polyphenolic components present. This herb has recently drawn the interest of researchers due to its biological activities, which include antibacterial, antifungal, and antioxidant properties. It may have the highest potential for usage in commercial food products^{33,34}. Rosemarie *Officinalis*, popularly recognized as rosemary, is a member of the Labiatae family. Rosemary is a typical household plant prevalent in many regions of the world and can be seen blooming along the north and south shores of the Mediterranean Sea. Rosemary leaf extracts have been researched as potential therapeutic agents and antioxidants against various ailments^{35,36}. The rosemary extract yields a total of 16 phenolic compounds, including three significant compounds: carnosic acid, carnosol, and rosmarinic acid³⁷. The use of barnyard millet milk and medicinal herbs in the production of yogurt was proposed in this study. The objective of this research was to investigate the effect of adding natural components like herb extracts (concentration ranging from 0.50% to 1.5%) and barnyard millet milk (1:1 ratio) and also to examine whether it influenced the functioning and structural features of yogurt in contrast to a yogurt sample with no modifications. The manufacture of yogurt with antioxidant qualities can be a functional product. The novelty of this study is the combination of millet milk and herbal plant extracts to use as an addition to yogurts. This choice was made, considering the health benefits of the bioactive compounds present in millet milk and herbal plants.

2. MATERIALS AND METHODS

2.1 Materials

The yogurt samples were prepared in a laboratory environment with the following ingredients: Fresh cow's milk with 4.5% fat and 8.5% SNF was purchased from nearby Aavin parlor; Barnyard millet was brought from the local market in Salem district; lactic bacteria cultures (*Lactobacillus delbrueckii* sub sp. *Bulgaricus* and *Streptococcus thermophilus*) were supplied by Yogurtbio (Lactina brand). The herb extracts used in the research experiments were provided by Synthite Industries Private Limited, Kerala, India: Oregano (*Origanum vulgare*) – OO, Rosemary (*Rosmarinus Officinalis*) – R.O. and Sage (*Salvia Officinalis*) – SO.

2.2 Millet Milk Preparation

The millet milk was extracted from the Barnyard millet by the following method:

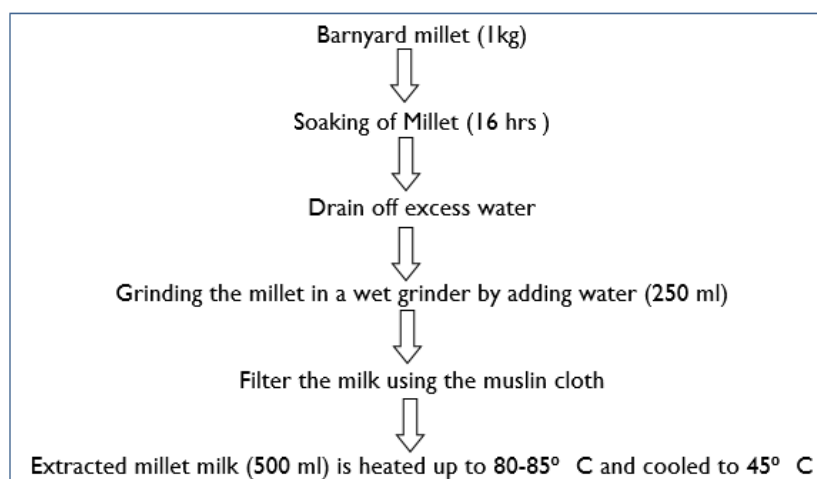


Fig 1: Processing flow chart of millet milk preparation

2.3 Yogurt Preparation

The manufacturing of yogurt with various formulations has been carried out using the traditional method: Milk was heated up to a boiling point and then cooled back to 45° C, both extracted barnyard millet milk and the cow's milk were mixed in a proportion of 1:1, then it is inoculated with a starter

culture, to this commix the herb extract (0.5%, 1% and 1.5% v/v) were added and mixed well. These commix were kept in incubation for 12 hours at 42° C. The finished yogurt samples were stored at 4° C for 24 hours, and the analyses were performed to determine the physicochemical and antioxidant properties of the pieces. The mixing ratios for yogurt fortified with different amounts of herbs are presented in (Table I)

Table I: Mixing ratio of fortified herbal yogurt					
Variations		Herb Extracts (ml)	Millet Milk (ml)	Cow's milk (ml)	Culture (g)
Oregano Oleo resin	OO1	0.5	50	50	5
	OO2	1	50	50	5
	OO3	1.5	50	50	5
Rosemary Oleo resin	RO1	0.5	50	50	5
	RO2	1	50	50	5
	RO3	1.5	50	50	5
Sage Oleo resin	SO1	0.5	50	50	5
	SO2	1	50	50	5
	SO3	1.5	50	50	5

2.4 Physicochemical Analysis

2.4.1 pH and Titratable Acidity of Yogurt Samples

A digital pH meter was carried out for the pH measurement (Testo 206 pH2 I-Kit). To determine titratable acidity, 10 mL of yogurt was titrated with 0.1 M sodium hydroxide solution. The titratable edge was expressed as a gram of lactic acid/100 g of yogurt and was calculated using the following equation:

$$\text{Total acidity} = \frac{V \times \text{NaOH factor} \times A \times D}{\text{Volume of Sample}} \times 100$$

V is the volume of NaOH added (mL), A is the conversion factor (0.009 for lactic acid), D is the dilution factor, and F is a factor of 0.1 N NaOH³⁸.

2.4.2 Susceptibility to Syneresis

Syneresis of the various yogurt samples were determined by centrifuging them at 1500 RPM for 12 minutes at 4° C, the

volume of whey separated was measured, and the following formula was used to calculate syneresis:³⁹.

$$\text{Syneresis (\%)} = W_s / W_y \times 100$$

Where, W_s = the supernatant after centrifuged
W_y = the yogurt sediments in the tube

2.4.3 Colour Evaluation

The color of the yogurt was determined using a tintometer (Lovibond color measurement group LC100 SV100 Kit). Measurements were taken directly after standardization in triplicates. Colour was expressed in L* (lightness/darker), a* (red/greenness), and b* (yellow/blueness) with illuminating D65.³⁹

2.4.4 Apparent Viscosity

The viscosity of various yogurt samples fortified with different herbs was determined using a rotational viscometer (Fungilab

Viscolead) equipped with an L4 spindle running at a 3 RPM rotation speed. The results were recorded in centipoise (cP).³⁹

2.4.5 Determination of Lactic Acid Bacteria (LAB) Count

Plating and isolation were done following^{40,41} the procedures. Enumeration was using pour plate technique. *S. thermophilus* and *L. bulgaricus* were enumerated on M17 and MRS agar, respectively. Serial dilutions were prepared using peptone diluents. One ml of thoroughly mixed yogurt sample was transferred to the first tube of 9 ml sterile diluent, representing the 10⁻¹ dilution. The diluted sample was blended for one minute by a vortex mixer. One ml of 10⁻¹ dilution was transferred to the second tube of 9 ml sterile diluent 10⁻² dilution. This operation was repeated until dilution was obtained by using fresh and sterile pipettes and diluents. For counting of *L. bulgaricus*, one ml of dilution was transferred into the Petri dishes in triplicates then 12 ml of MRS agar medium⁴² at 45°C was poured into each Petri dish with dilution. The content of was mixed carefully by rotating the five times clockwise and five times counter-clockwise, then allowed to solidify on a level surface. Plates were inverted and incubated anaerobically in a tightly sealed anaerobic jar at 37°C for 72 hours. For counting *S. thermophilus*, diluents were used for preparing serial dilutions. One ml of appropriate dilution was transferred into triplicates, then 12 to 15 ml of M17 agar at 45°C was added into each containing one ml of proper dilution. The content was mixed carefully by rotating the Petri dish five (5) times clockwise and five (5) times counter-clockwise, then allowed to solidify on the surface. Plates were then inverted and incubated aerobically at 37°C for 48 hours. Colonies in plate with 25-250 colonies were counted, and viable counts in CFU/ml were calculated.

$$N = \sum C / [(1.0 * n_1) + (0.1 * n_2)] d$$

Where;

N= number of colonies ml or gram of sample.

∑C=sum of all the colonies in all plates counted.

n1=number of plates in the lower dilution counted.

n2=number of plates in the next higher dilution counted.

d=dilution from which the first counts were obtained.

2.5 Determination of Antioxidant Activity

2.5.1 Determination of Total Phenolic Content (TPC)

Total Phenolic Content was determined by an assay proposed by Hernandez-Carranza et.al³³. The TPC results were expressed as mg gallic acid equivalent (GAE)/g of sample.

2.5.2 Determination of Total Flavonoid Content (TFC)

According to Hernández-Carranza et al., the TFC was assessed⁴³. Prior to standing for 10 minutes, 500µL of each sample were combined with 500µL of NaNO₂ (1.5%, 500 µL) in a vortex. After adding the AlCl₃ (3%), 1000 mL of NaOH 1N was added, stirred for 2 minutes, and read at 490 nm. The quercetin standard curve was then run. The outcome was given as milligrams of quercetin per 100 grams of yogurt.

2.5.3 DPPH Assay

PDF (2,2-diphenyl-1-picrylhydrazyl) assay of the various yogurt samples was determined using the method of Son and Lewis

(2002)⁴⁴. 0.5ml of the sample solution was mixed with 2.6mg of 0.066mM DPPH solution in methanol. The reaction mixture was incubated at 37° C for 30 minutes. The absorbance was measured at 516nm using a spectrophotometer. Methanol was used as a blank. The radical scavenging activity of the yogurt sample was expressed in percentage inhibition of the DPPH radical and was calculated by the following equation:

$$DPPH (\%) = \left[\frac{A_{control} - A_{sample}}{A_{control}} \right] \times 100$$

2.5.4 ABTS Identification

ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenger activity of yogurt samples was determined using the method of Re et al.⁴⁵ minor corrections. First, ABTS was dissolved in distilled water to a concentration of 7 mM. Next, ABTS radical cations were prepared by adding ABTS stock solution to 2.45 mM K₂S₂O₈ (2:1 ratio). Cover with aluminum foil and store in the dark for 24 hours before use. ABTS reagent was diluted in 94% ethanol to the appropriate absorbance (0.17±0.03) measured at 734 nm. ABTS reagent (950 µ l) was mixed with 50 µ l of test sample at the indicated concentration. The mixture was covered with aluminum foil and left in the dark for 10 minutes at room temperature. Absorbance at 734 nm was recorded with a microplate reader. Each sample was measured in triplicate and percent inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.5.5 Reducing Power Assay

The Fe³⁺ reducing capacity of yogurt samples was determined using a slightly modified method of Oyaizu⁴⁶. Different concentrations of samples (0.25 ml) were mixed with 0.25 ml of 0.2 M phosphate buffer (pH 6.6) and 0.25 ml of 1% (w/v) K₃Fe (C.N.)₆. The mixture was incubated in a 50° C. water bath for 20 minutes and the reaction was stopped by adding 0.25 ml of 10% (w/v) trichloroacetic acid solution. The solution was then centrifuged at 3000 rpm for 10 minutes. The supernatant (0.5 ml) was mixed with 0.5 ml distilled water and 0.1 ml 0.1% (w/v) FeCl₃ for 10 minutes. Reducing power was determined by measuring absorbance at 700 nm with a microplate reader. A standard curve for ascorbic acid was generated at concentrations ranging from 0 to 200 µg/mL.

2.6 Sensory Evaluation

An expert panel of judges on a 9-point hedonic scale evaluated the organoleptic quality of yogurt samples. The yogurt samples were served at 5°C and analyzed for sensory parameters like, colour and appearance, flavour, body and texture, sweetness, and overall acceptability, wherein a score of 1 represented "dislike extremely" and 9 represented "like extremely". The samples for evaluation were coded appropriately before serving the samples to the judges for sensory evaluation. Sensory evaluation of the samples was carried out in the sensory evaluation room under appropriate fluorescent lighting. The created items were organoleptically assessed by a semi-trained board of 150 judges from the Department of Nutrition and Dietetics, Periyar University, Salem, Tamil Nadu., using the acceptance test. As a criterion for research

participation, the consumers were asked about health problems, such as lactose intolerance and/or allergy to apicultural products. 9-point hedonic scale was used in the questionnaire for the evaluation of different attributes: colour, aroma, mouthfeel, consistency, flavour, taste, overall acceptability of the food yogurt.

2.7 Statistical Analysis

The statistical analysis was done using SPSS 16. at a significance level at $p < 0.05$. The graphical representation of the principal component analysis (PCA) allows the data to be analyzed on a two-dimensional F1/F2 map and to identify the trends between variables at a significance level $p < 0.05$.

3. RESULT AND DISCUSSION

3.1 pH, Titratable Acidity and Syneresis of Yogurt Samples

The yogurt samples were evaluated after three storage periods (1, 14 and 21 days) at 4°C. Table 2 shows the results, which are represented as the mean value of each assessed parameter of the samples, conducted in triplicate for the three storage periods investigated. The pH value of the yogurt samples (Table 2) during the preservation period in refrigerator (4°C) circumstances dropped collectively. The pH of all the yogurt samples declined with time; for example, the pH of the OO1 (Oregano oleoresin 0.5%) sample reduced from 4.59 on the first day to 4.37 on the 14th day and 4.28 on the last day of storage. When compared to the control sample, the addition of the herbs extracts and millet milk had no

discernible effect on the pH of the tested samples. The results provided above are comparable to those reported in early studies in which it was found that the pH of control yogurt was similar to that of yogurts containing *Pleurotus ostreatus* aqueous extract and wild plant extract^{12,47}. Furthermore, the Examination of this characteristic in yogurts, is particularly relevant in terms of product safety⁴. All yogurt samples had a titratable acidity of 0.73 to 1.15 during the first day of storage, 0.79 to 1.19 on the 14th day and 0.88 to 1.22 after 28th day of refrigeration storage for all the samples analyzed (Table 2). Similarly, in a study it was found that adding more *Spirulina platensis* enhanced the buffering capacity, requiring additional acid production by starter cultures throughout the duration of the 28th day storage period at 4°C¹¹. The result demonstrate that all herb extract and millet milk yogurt samples had lower pH values ($p < 0.05$) and higher acidity ($p < 0.05$) than the CM sample. One interpretation for this is that the herb extracts help the bacteria in the yogurt samples grow faster the result showed (Table 2). Higher whey separation is related to gel instability, which is also related to the pH of the yogurt system; thus, syneresis provides an indication of non-homogeneities in the gel system of the yogurt; thus, higher whey separation is associated to gel instability, which is also related to the pH of the yogurt system⁴⁸. From (Table 2) it is clear that, the longer the storage time, the higher the syneresis in all of the trial groups. The addition of herb oleoresins was associated with increased whey separation compared to CM sample in all storage periods, due to the rearrangement of the gel matrix which may be associated with the millet milk addition. Titratable acidity has a direct impact on syneresis, which is consistent with the notion that it is inversely proportional to pH⁴⁹.

Table 2: pH, titratable acidity and syneresis of the yogurt with different herb extract				
Storage day	Sample	Characteristics		
		pH	Titratable Acidity	Syneresis
Day 1	CM	4.25 ± 0.05	0.74 ± 0.03	54.40 ± 0.29
	CM + MM	4.64 ± 0.02	0.73 ± 0.02	56.20 ± 0.76
	SO1	4.47 ± 0.01	1.12 ± 0.01	72.50 ± 0.50
	SO2	4.45 ± 0.01	0.87 ± 0.01	74.83 ± 0.29
	SO3	4.48 ± 0.01	0.95 ± 0.01	74.83 ± 0.76
	OO1	4.59 ± 0.00	1.01 ± 0.01	70.33 ± 0.29
	OO2	4.62 ± 0.01	1.15 ± 0.01	70.00 ± 0.00
	OO3	4.58 ± 0.00	1.04 ± 0.01	75.17 ± 0.29
	RO1	4.35 ± 0.05	1.11 ± 0.01	75.17 ± 0.29
	RO2	4.57 ± 0.01	1.08 ± 0.01	76.83 ± 0.76
Day 14	RO3	4.61 ± 0.01	1.04 ± 0.01	72.50 ± 0.50
	CM	4.24 ± 0.06	0.79 ± 0.02	56.80 ± 0.76
	CM + MM	4.46 ± 0.02	0.83 ± 0.02	57.17 ± 0.58
	SO1	4.26 ± 0.02	1.16 ± 0.01	75.00 ± 0.50
	SO2	4.49 ± 0.02	0.92 ± 0.01	77.00 ± 0.50
	SO3	4.51 ± 0.03	0.98 ± 0.01	78.00 ± 0.50
	OO1	4.37 ± 0.01	1.07 ± 0.01	71.33 ± 0.29
	OO2	4.39 ± 0.01	1.19 ± 0.01	71.67 ± 0.29
	OO3	4.33 ± 0.00	1.08 ± 0.01	76.33 ± 0.29
	RO1	4.30 ± 0.02	1.15 ± 0.01	77.67 ± 0.29
	RO2	4.46 ± 0.01	1.11 ± 0.01	78.67 ± 0.29
	RO3	4.48 ± 0.01	1.08 ± 0.01	76.33 ± 0.76
	CM	4.06 ± 0.02	0.88 ± 0.01	58.40 ± 1.00
	CM + MM	4.30 ± 0.01	0.89 ± 0.01	59.00 ± 0.87
	SO1	4.17 ± 0.02	1.19 ± 0.01	76.33 ± 0.29
	SO2	4.25 ± 0.03	0.99 ± 0.01	79.50 ± 0.50
	SO3	4.34 ± 0.05	1.05 ± 0.01	79.67 ± 0.29

Day 28	OO1	4.28 ± 0.04	1.11 ± 0.01	72.83 ± 0.29
	OO2	4.27 ± 0.03	1.22 ± 0.02	73.50 ± 0.50
	OO3	4.27 ± 0.01	1.13 ± 0.01	77.33 ± 0.29
	RO1	4.16 ± 0.02	1.19 ± 0.01	78.83 ± 0.29
	RO2	4.29 ± 0.03	1.17 ± 0.01	81.50 ± 0.50
	RO3	3.35 ± 0.02	1.14 ± 0.02	79.50 ± 0.50

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.2 Color Values of Yogurt Samples

The CIELAB scale was used to determine the color of the yogurt. Zero value corresponded to white color. The (Table 3) shows that on the initial day, the L* (darkness/lightness) of all the yogurt samples was significantly greater ($p < 0.05$) and there was no significant difference noticed among the yogurt after 28 days of storage period. Even though many of the yogurt samples were greener/yellower, the intensity was substantially different ($p < 0.05$), almost similar results were reported in recent research⁵⁰.

Table 3: Colour parameters of the yogurt with different herb extracts.							
Storage day		L*	a*	b*	C*	h*	Δ E
Day 1	CM	83.63 ± 0.57	0.43 ± 0.15	9.07 ± 0.12	9.00 ± 0.00	87.43 ± 1.06	3.10 ± 0.23
	CM + MM	80.23 ± 0.06	1.47 ± 0.06	9.43 ± 0.06	9.53 ± 0.06	81.20 ± 0.17	1.90 ± 0.10
	SO1	75.90 ± 0.46	-2.07 ± 0.25	18.90 ± 0.1	19.03 ± 0.15	96.23 ± 0.60	12.50 ± 0.20
	SO2	70.70 ± 0.35	-1.80 ± 0.00	19.90 ± 0.36	20.00 ± 0.36	95.17 ± 0.06	16.23 ± 0.47
	SO3	68.57 ± 0.59	-1.87 ± 0.06	22.40 ± 0.69	22.50 ± 0.69	94.83 ± 0.06	19.47 ± 0.15
	OO1	81.23 ± 0.71	-0.10 ± 0.10	7.90 ± 0.20	7.90 ± 0.20	89.83 ± 1.07	1.27 ± 0.06
	OO2	80.97 ± 0.74	0.03 ± 0.06	7.73 ± 0.58	7.77 ± 0.12	89.67 ± 0.49	1.40 ± 0.26
	OO3	81.23 ± 0.32	0.03 ± 0.06	8.30 ± 0.10	8.30 ± 0.10	89.73 ± 0.06	1.10 ± 0.10
	RO1	78.50 ± 0.70	0.63 ± 0.42	16.13 ± 0.06	16.17 ± 0.06	87.77 ± 1.52	8.57 ± 0.31
	RO2	77.10 ± 0.56	1.10 ± 0.10	15.50 ± 0.96	15.53 ± 0.91	85.87 ± 0.58	8.53 ± 0.81
	RO3	79.20 ± 1.73	0.73 ± 0.40	14.67 ± 0.23	14.70 ± 0.17	87.13 ± 1.67	7.03 ± 0.49
Day 14	CM	82.23 ± 0.93	0.77 ± 0.15	9.23 ± 0.59	9.30 ± 0.70	83.17 ± 3.97	1.87 ± 0.55
	CM + MM	78.00 ± 0.10	1.87 ± 0.06	16.17 ± 0.06	16.27 ± 0.06	83.43 ± 0.15	8.77 ± 0.06
	SO1	72.83 ± 2.20	-1.27 ± 0.35	18.67 ± 1.01	18.73 ± 1.07	93.90 ± 0.90	14.03 ± 0.71
	SO2	71.20 ± 1.76	-1.97 ± 0.35	20.23 ± 0.32	20.30 ± 0.35	95.53 ± 0.80	16.23 ± 0.83
	SO3	69.30 ± 0.78	-2.07 ± 0.12	22.97 ± 0.23	23.07 ± 0.23	95.17 ± 0.29	19.43 ± 0.32
	OO1	79.53 ± 0.31	0.27 ± 0.12	8.40 ± 0.46	8.40 ± 0.46	88.23 ± 0.76	2.27 ± 0.31
	OO2	79.87 ± 0.83	0.20 ± 0.00	8.17 ± 0.15	8.17 ± 0.15	88.70 ± 0.26	2.00 ± 0.72
	OO3	79.33 ± 0.23	0.27 ± 0.12	7.90 ± 0.46	7.90 ± 0.46	88.03 ± 1.09	2.47 ± 0.23
	RO1	75.90 ± 0.96	0.77 ± 0.59	16.13 ± 0.45	16.16 ± 0.40	87.20 ± 2.02	9.87 ± 0.25
	RO2	75.83 ± 0.71	1.00 ± 0.00	16.70 ± 1.56	16.73 ± 1.53	86.57 ± 0.38	10.27 ± 1.39
	RO3	76.47 ± 1.72	0.93 ± 0.38	15.80 ± 1.06	15.83 ± 1.09	86.73 ± 1.18	9.33 ± 0.38
Day 28	CM	81.80 ± 0.44	0.80 ± 0.35	9.63 ± 0.49	9.67 ± 0.55	85.37 ± 1.97	1.00 ± 0.20
	CM + MM	75.67 ± 1.02	2.20 ± 0.00	15.70 ± 0.66	15.80 ± 0.66	82.33 ± 0.32	9.67 ± 0.32
	SO1	71.70 ± 1.31	-1.77 ± 0.55	19.90 ± 0.61	19.97 ± 0.64	95.03 ± 1.36	15.63 ± 0.31
	SO2	69.37 ± 0.40	-1.97 ± 0.21	23.20 ± 0.46	23.30 ± 0.46	94.93 ± 0.55	19.57 ± 0.40
	SO3	69.07 ± 0.70	-2.03 ± 0.15	22.87 ± 0.21	22.97 ± 0.21	95.13 ± 0.32	19.50 ± 0.30
	OO1	76.93 ± 0.93	0.47 ± 0.29	8.97 ± 0.25	8.97 ± 0.25	87.17 ± 1.64	4.73 ± 0.89
	OO2	75.37 ± 1.01	0.50 ± 0.10	9.17 ± 0.21	9.17 ± 0.21	86.83 ± 0.65	6.27 ± 0.96
	OO3	74.93 ± 0.60	0.53 ± 0.06	9.00 ± 0.10	9.00 ± 0.10	86.57 ± 0.32	6.67 ± 0.60
	RO1	74.20 ± 2.02	0.67 ± 0.58	17.93 ± 1.53	17.93 ± 1.53	88.03 ± 1.79	12.37 ± 0.49
	RO2	75.00 ± 0.10	1.00 ± 0.00	19.07 ± 0.55	19.10 ± 0.60	87.03 ± 0.06	12.60 ± 0.75
	RO3	74.67 ± 0.49	0.87 ± 0.23	18.63 ± 1.27	18.67 ± 1.31	87.40 ± 0.61	12.57 ± 0.81

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin;

RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.3 Apparent Viscosity

Apparent viscosity (Figure 2) is influenced by the strength and number of bonds between casein micelles in yogurt, as well as their structure and spatial distribution⁵¹. The apparent viscosity of yogurt samples after 28 days of storage at 4°C. The

apparent viscosity of fortified yogurt is lower than the control, as shown in the figure. Since yogurt is a gel/matrix of casein micelles with entrapped water, this is the case. The addition of the millet milk and herb extract to the enhanced yogurt sample may cause the gel structure to be disturbed. During storage,

the viscosity of fortified yogurt dropped moderately, but the viscosity of the cow's milk-based yogurt, cow's milk and millet milk incorporated yogurt had a significant decline. It was concluded in a previous study that apparent viscosity of yogurt reduces with storage period^{52,53}.

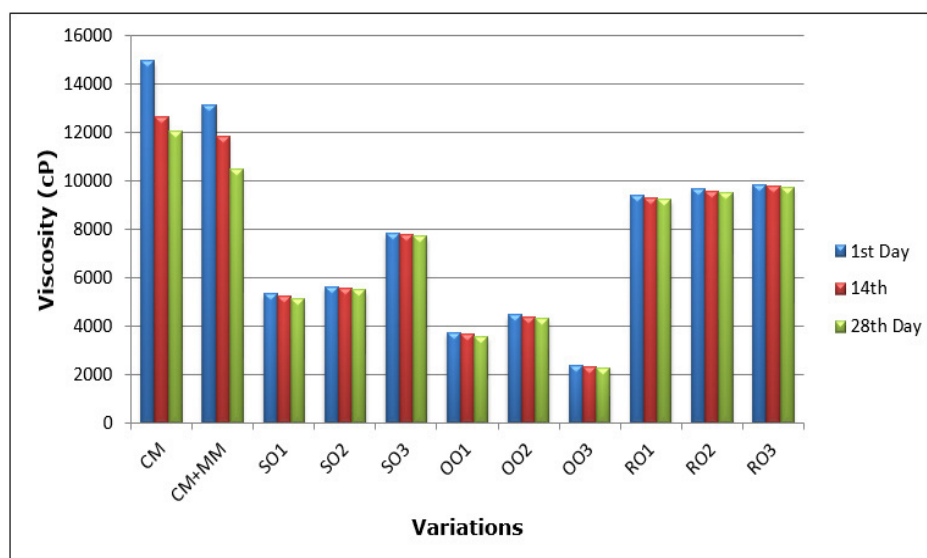


Fig 2: Apparent Viscosity of yogurt during 28 days of storage at 4°C

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.4 LAB Count of Yogurt Samples

The result of (Lactic Acid Bacteria) LAB count of various yogurt. *L. bulgaricus* mean viable counts ranged from 7.48 log₁₀ CFU/ml to 8.65 log₁₀ CFU/ml with SO2 and CM having the lowest and highest viable counts, respectively (Table 4).

Moreover, the data showed that the mean LAB counts of all variable yogurt samples were significantly higher ($P \leq 0.05$) than CM. The table (Table 4) represented the viable lactic acid bacteria cell for initial day, 14th day and 28th day of storage where *L.bulgaricus* and *S.thermophilus* were determined in yogurts supplemented with barnyard millet milk and herb extracts. The microbial counts of *L.bulgaricus* and *S.thermophilus* on the first day of storage were significantly different ($p < 0.05$), ranging from 7.48 to 8.65 Log CFU/g and 7.39 to 9.20 Log CFU/g, respectively. The viable bacterial counts (*L.bulgaricus* and *S.thermophilus*) increased till the 14th day and then began to decline for all yogurt samples during the period of storage of 28 days. Lactic acid bacteria count in all yogurt samples exceeded the Codex minimum threshold of 7.0 Log CFU/g. The addition of barnyard millet milk and herbal extract to yogurt did not adversely influence the growth of LAB⁵⁴.

Table 4: Changes in lactic acid bacteria count during incubation of yogurt with different herb extract			
Days		L.bulgaricus	S.thermophilus
		Log cfu/g	Log cfu/g
CM	Initial Day	8.65	9.20
	14 th Day	8.45	9.03
	28 th Day	8.05	8.58
CM + MM	Initial Day	8.15	8.56
	14 th Day	8.25	8.60
	28 th Day	7.84	8.29
SO1	Initial Day	7.87	7.60
	14 th Day	7.97	7.70
	28 th Day	7.89	7.64
SO2	Initial Day	7.48	7.48
	14 th Day	7.69	7.59
	28 th Day	7.58	7.51
SO3	Initial Day	7.58	7.58
	14 th Day	7.69	7.71
	28 th Day	7.60	7.62
OO1	Initial Day	7.86	7.54
	14 th Day	7.96	7.62

OO2	28 th Day	7.89	7.58
	Initial Day	7.79	7.45
	14 th Day	7.89	7.54
	28 th Day	7.82	7.49
OO3	Initial Day	7.97	7.73
	14 th Day	8.08	7.82
	28 th Day	7.96	7.75
	Initial Day	7.94	7.39
RO1	14 th Day	8.05	7.59
	28 th Day	7.97	7.48
	Initial Day	7.99	7.45
	14 th Day	8.04	7.55
RO2	28 th Day	7.96	7.49
	Initial Day	7.98	7.48
	14 th Day	8.11	7.59
	28 th Day	8.02	7.51

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.5 Total Phenolic and Flavonoid Content of Yogurt Samples

(Table 5) shows the total phenolic contents and flavonoid

contents of various yogurt samples the seed pulp powders used in ice cream production. The yogurt with oregano oleoresin addition yielded the best outcome, with TPC levels reaching 5.81 mg (GAE)/g in this study. The highest flavonoid content was observed 53.31 ± 2.02 . cow's+ milk and millet milk incorporated yogurt obtained total content of phenol was 0.99 ± 0.01 and flavonoid value observed 9.99 ± 0.12 , the lowest count of total phenol observed in RO1 variation it contained 1.07 ± 0.01 . The level of total phenolic content in yogurt samples varied based on the type of herb extract administered. The incorporation of bioactive components into yogurt can improve the health benefits of this dairy product. Several studies have evaluated yogurt as a potential carrier for antioxidant compounds⁵⁵

Table 5: Antioxidant Activity of the yogurt with different herb extract		
Variations	TPCmg (GAE)/g	Flavonoid mg quercetin/g
CM	0.98 ± 0.01	9.56 ± 0.56
CM + MM	0.99 ± 0.01	9.99 ± 0.12
SO1	3.16 ± 0.02	26.59 ± 0.99
SO2	3.55 ± 0.02	28.39 ± 0.83
SO3	4.72 ± 0.01	30.28 ± 0.79
OO1	5.81 ± 0.02	46.47 ± 3.15
OO2	5.02 ± 0.02	53.07 ± 2.99
OO3	5.56 ± 0.02	53.31 ± 2.02
RO1	2.36 ± 0.02	23.07 ± 1.69
RO2	1.07 ± 0.01	23.35 ± 2.56
RO3	2.69 ± 0.08	23.88 ± 2.41

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.6 DPPH, ABTS and Reducing Power of Yogurt Samples

(Table 6) Results obtained using the DPPH (2, 2, diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid), and Reducing power methods confirmed the presence of antioxidant activity in each variation. The plain yogurt had a DPPH radical scavenging activity of 10.56 percent,

and the DPPH radical scavenging activity of yogurt increased with millet milk and herb extract incorporation in the yogurt sample. In yogurt containing 0.5 percent oregano oleoresin, the average inhibition of DPPH radical formation was 35.18 percent respectively. The DPPH activity of the yogurt sample containing sage extract was also high with a value of 21.57 percent for the yogurt sample containing 1% sage extract. The lowest ABTS and Reducing power found in the plain yogurt was 8.81 percent and 5.02% respectively, the highest amount of ABTS Founded in 32 percent was oregano oleoresin variation three. In a recent study, it was concluded that adding herb extract to milk preceding bacterial fermentation significantly improved antioxidant activity ($p < 0.05$) compared to milk alone¹³. This might be due to the high flavonoid concentration of plant extract. Natural extracts were proven to increase the antioxidant activity of yogurt in similar investigations^{4,27,28,30,36,37,47}.

Table 6: DPPH, ABTS and Reducing power activity of yogurt with different herb extract

Variations	DPPH Assay (%)	ABTS (%)	Reducing power
CM	10.56 ± 0.01	8.81 ± 0.29	5.02 ± 0.38
CM + MM	12.80 ± 0.01	10.66 ± 0.59	5.24 ± 0.71
SO1	20.15 ± 0.02	17.36 ± 1.28	11.58 ± 0.56
SO2	20.72 ± 0.02	17.75 ± 1.26	12.33 ± 1.19
SO3	21.57 ± 0.03	18.82 ± 2.15	13.47 ± 0.71
OO1	35.02 ± 0.01	30.90 ± 0.42	21.76 ± 0.83
OO2	35.18 ± 0.02	31.63 ± 0.65	22.87 ± 2.02
OO3	35.54 ± 0.01	32.42 ± 0.58	24.47 ± 0.54
RO1	11.08 ± 0.02	9.34 ± 1.07	5.74 ± 0.37
RO2	12.36 ± 0.02	10.81 ± 0.32	6.16 ± 0.28
RO3	12.65 ± 0.02	11.15 ± 0.67	6.49 ± 0.52

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

3.7 Sensory Evaluation

The organoleptic evaluation reported that the sensory properties of food are strongly related to consumer preferences and determine product acceptability this phenomenon can help to optimize a recipe. The results of the sensory evaluation of yogurt samples are shown (Table 7). Sensory perception of the yogurt samples and addition of CM had a positive effect on sensory. Consumer acceptance varied for the different formulations: the highly accepted SO2 was obtained 8.20 ± 0.44 and another variation OO2 highly accepted 8.65 ± 0.65. The lowest acceptability RO3 variation was 7.50 ± 0.54.

Table 7: Sensory evaluation of yogurt with different herb extract

	Colour	Aroma	Mouthfeel	Consistency	Flavour	Taste	Overall Acceptability
CM	8.30 ± 0.48	8.50 ± 0.53	8.10 ± 0.74	8.20 ± 0.42	8.40 ± 0.52	8.30 ± 0.48	8.30 ± 0.48
CM+MM	7.90 ± 0.74	7.80 ± 0.63	8.40 ± 0.52	8.20 ± 0.79	7.70 ± 0.48	7.60 ± 0.97	7.70 ± 0.67
SO1	8.80 ± 0.7	8.35 ± 0.74	8.00 ± 0.7	8.47 ± 0.51	8.00 ± 1.00	8.00 ± 0.00	8.20 ± 0.44
SO2	8.00 ± 0.83	7.80 ± 0.44	7.6 ± 0.54	7.20 ± 0.83	7.40 ± 0.54	7.60 ± 0.54	8.00 ± 0.70
SO3	7.89 ± 0.66	7.67 ± 0.94	7.62 ± 0.68	7.14 ± 0.76	7.49 ± 0.65	7.39 ± 0.79	7.89 ± 0.79
OO1	9.00 ± 0.00	8.47 ± 0.61	8.48 ± 0.69	8.10 ± 0.32	8.24 ± 0.58	8.36 ± 0.84	8.28 ± 0.84
OO2	8.80 ± 0.44	8.60 ± 0.54	8.28 ± 0.85	8.25 ± 0.58	8.10 ± 0.32	8.15 ± 0.63	8.65 ± 0.65
OO3	8.65 ± 0.56	7.75 ± 0.87	8.34 ± 0.74	7.55 ± 0.63	7.55 ± 0.79	7.52 ± 0.63	8.35 ± 0.84
RO1	7.80 ± 0.83	7.80 ± 0.44	7.60 ± 0.54	7.08 ± 0.63	7.49 ± 0.80	7.46 ± 0.78	7.71 ± 0.59
RO2	7.60 ± 0.54	7.20 ± 0.83	7.47 ± 0.63	7.54 ± 0.63	7.56 ± 0.79	7.11 ± 0.72	7.53 ± 0.63
RO3	7.24 ± 0.81	7.23 ± 0.53	7.29 ± 0.61	7.42 ± 0.63	7.44 ± 0.83	7.10 ± 0.54	7.50 ± 0.54

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

4. STATISTICAL ANALYSIS

The relationship between pH, titratable acidity, syneresis

and viscosity in yogurt sample was visualized using principal component analysis (PCA). Figures 3,4 and 5 illustrate the PCA findings that were obtained. As compared to titratable acidity and syneresis, the pH value of yogurt after a day of manufacturing is lower. During the first day of testing, the viscosity was positive. The PCA chart at 14 days demonstrates that yogurt manufacturing has increased viscosity and pH while acidity has decreased during storage. After looking at the data of the 28th day of storage, viscosity reduces to a negative value. During the storage period, syneresis and titratable acidity both reduced.

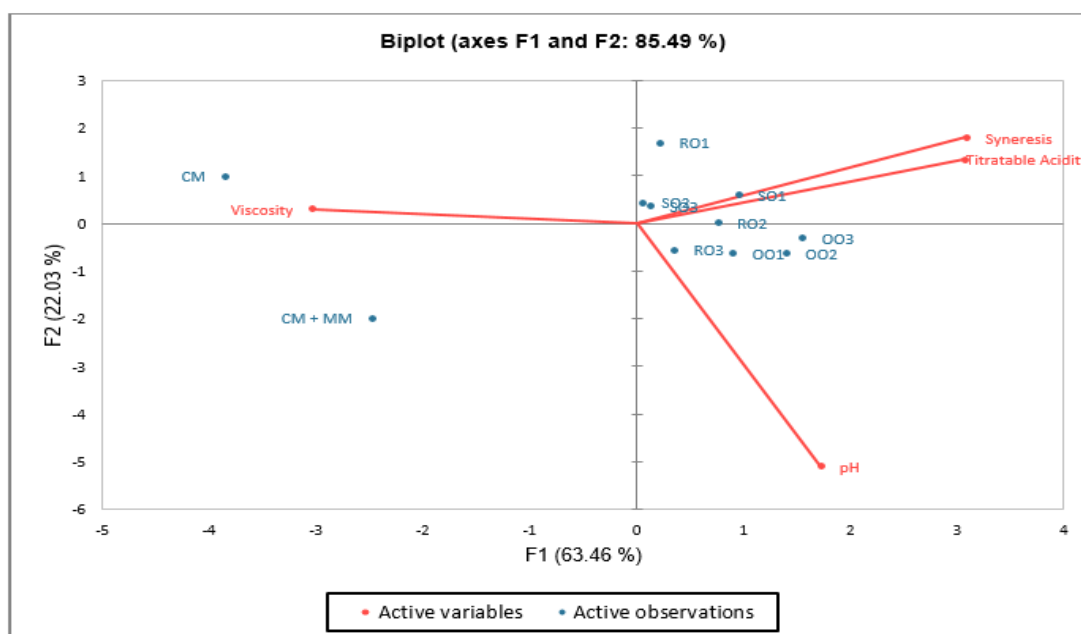


Fig 3: PCA loadings of the yogurt characteristics after initial day of storage

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

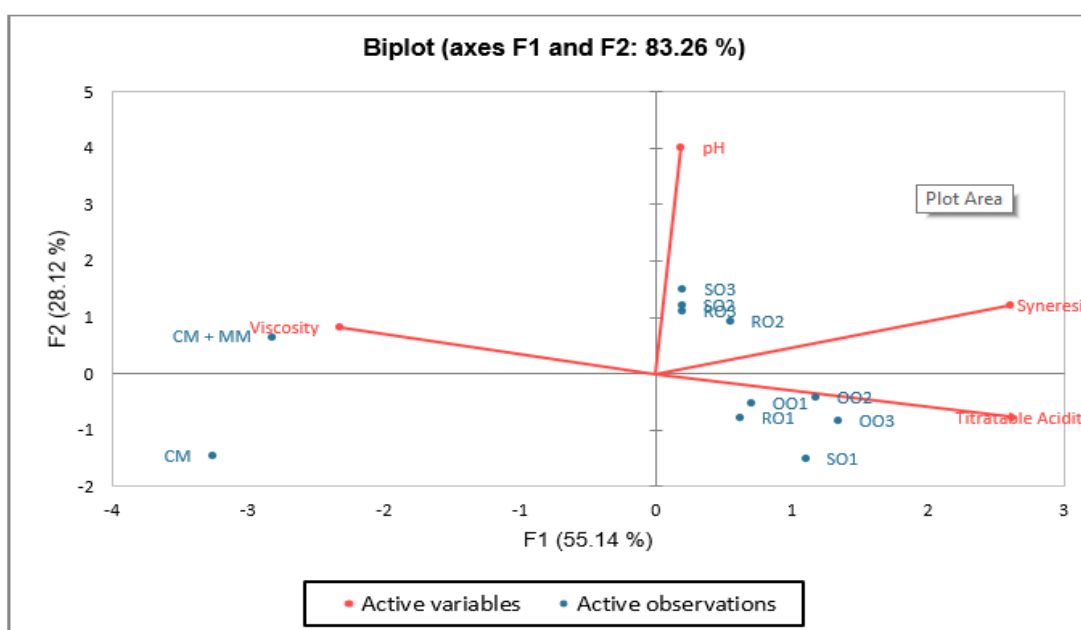


Fig 4: PCA loadings of the yogurt characteristics after 14th day of storage

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

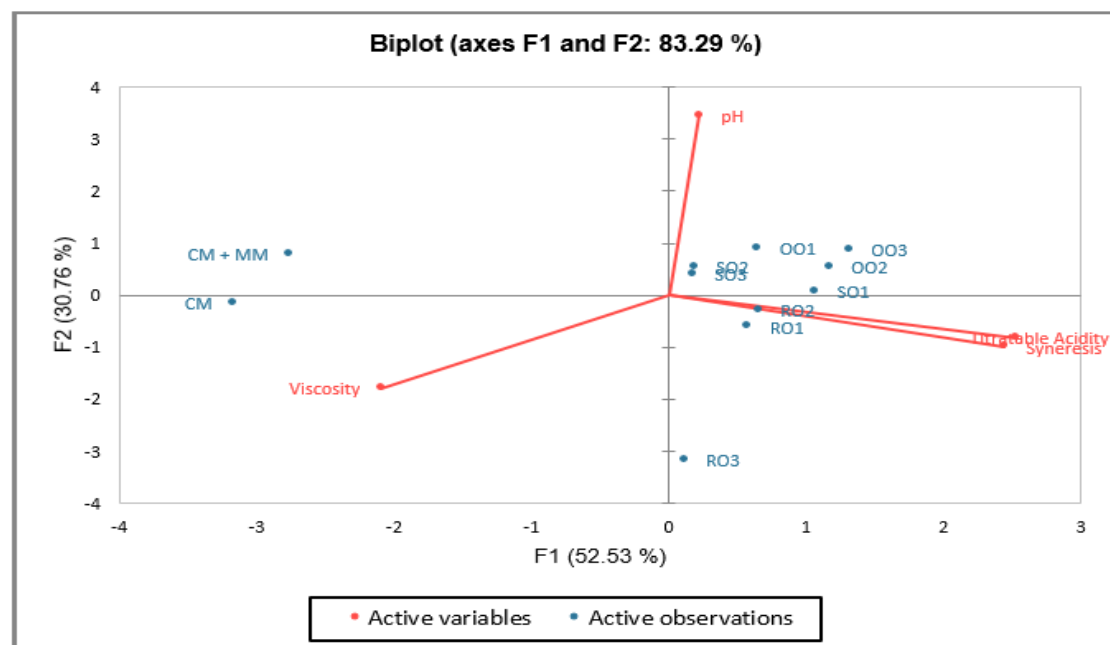


Fig 5: PCA loadings of the yogurt characteristics after 28th day of storage

CM: Plain Yogurt (cow's milk); CM + MM: Cow's Milk and Barnyard millet milk incorporated yogurt (1:1 ratio) without herbal extract; SO1: with 0.5% incorporation of sage oleoresin; SO2: with 1% incorporation of sage oleoresin; SO3: with 1.5% incorporation of sage oleoresin; OO1: with 0.5% incorporation of sage oleoresin; OO2: with 1% incorporation of sage oleoresin; OO3: with 1.5% incorporation of sage oleoresin; RO1: with 0.5% incorporation of sage oleoresin; RO2: with 1% incorporation of sage oleoresin; RO3: with 1.5% incorporation of sage oleoresin.

5. CONCLUSION

This study demonstrates how millet milk and extracts from various herbs can be successfully fortified into a fermented dairy product like yogurt. Milk contains all nutrients which is necessary to sustain life. It is a source of major nutrients that provides complete nourishment and offer a wide range of health benefits. Milk is highly unstable food which can be easily spoiled. To overcome this problem, preservation of milk by fermentation is widely practiced. For the research work we have chosen barnyard millet specifically because of its nutritional content, colour, and taste. Barnyard millet milk can be classified as functional food because it provides many health benefits beyond its nutritional content due to its calcium and fiber content. Barnyard millet is under-utilized, hence milk

extracted from these millets have more nutrient content than of cow milk in the production of yogurt, so they are incorporated in cow milk to enrich the nutritional value of yogurt. The commix of cow's milk, barnyard millet milk and incorporation of oleoresins from three different herbs was investigated in this research, sage (*Salvia officinalis* L.), Oregano (*Origanum Vulgare* L.) and rosemary (*Rosmarinus Officinalis*), have not been applied in the production of yogurt with commix of cow's milk and barnyard millet milk. After 28 days of storage period, the physicochemical and viscosity properties of the yogurt samples with barnyard millet milk and herb oleoresin addition were enhanced compared to the CM sample. According to the results, the sample with 0.5 percent oregano oleoresin (*Origanum vulgare* L.) added had the best nutritional value.

6. AUTHORS CONTRIBUTION STATEMENT

P.N. helped supervise the project and S.N. carried out the experiment. All authors discussed the results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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