Journal of Agriculture and Veterinary Science
ISSN: 2959-1198 (Print), 2959-1201 (Online)

Check for updates

Research Article

Assessment of Physico-Chemical and Functional Characteristics of Yogurt Enriched with Mint Extract and Probiotics

Muhammad Waqas[1], Saima Rafiq[1], Imran Hayat[1], Sheraz Hussain[1], Anees Murtaza[1], Aqsa Qayyum[2], Diya Khan[1]
[1]*Department of Food Science and Technology, Faculty of Agriculture, University of Poonch Rawalakot, AJK-12350, Pakistan.*
[2]*Food Science Research Institute, National Agriculture Research Center Islamabad 45320, Pakistan.*

ABSTRACT

Medicinal plants and herbs are good source of antioxidant and bioactive compounds; hence they are used to fortified food as preservatives, flavorings and therapeutic agents. In this context, wild mint (Mintha) is important herb which is widely grown in Azad Jammu and Kashmir, but it is not properly utilized and mostly wasted. Therefore, present research work is an effort to properly utilize mint for the value addition of yogurt. The mint-based yogurt was prepared by using different combination of mint extract such as 0%, 0.5%, 1%, 1.5% and 2%. The prepared product samples were analyzed for different parameters such as physic-chemical (pH, titratable acidity, total soluble solid and fat), functional (total phenol, antioxidant activity and antimicrobial) and sensory attributes (flavor, taste, color and overall acceptability) at five days interval for 25 days storing period at 4 °C. The data obtained was statistically analyzed by using two factorial completely randomized design (CRD) @ 5% level of significance and treatments were compared by using LSD value. Use of mint extract produced better results in terms of lowering syneresis and improving appearance, body and texture. Mint extract with 1% addition showed best result for overall sensory acceptability and yogurt shelf life was increased up to 25 days at 4 °C temperature.

Keywords: Functional Yoghurt, Mint Extract, Probiotics, Physico-Chemical Attributes, Buffalo Milk.

OPEN ACCESS

Correspondence
Saima Rafiq
saimaft2009@gmail.com

Article History
Received: April 03, 2024
Accepted: August 19, 2024
Published: October 16, 2024

INTRODUCTION

The yoghurt supplies good quality proteins, and rich source of important minerals (calcium, phosphorus, potassium) and vitamins. Yogurt is considered as healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended to the people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control (Mckinley, 2005). Additionally, yogurt contributes many other physiological functions due to presence of useful microflora known as probiotics. Therefore, Probiotics are increasingly utilized in the dairy products due to several potential health benefits. Probiotic products can have specific target function in the human alimentary tract, and also be useful in reducing the risk of specific diseases, mitigating both objective and subjective symptoms, enhancing all over immune system (Pacini and Ruggiero, 2017). Probiotic microorganisms are usually available as culture concentrates in dried or deepfreeze form to be added to a food matrix (Dabija et al., 2018). The yogurt can be value-added through the addition of probiotic and herbs which are low-cost and used medically for natural antioxidants, preventing cancer, diabetes, and cardiovascular diseases (Alenisan et al., 2017).

Natural herbs can be used in dairy products including yoghurt, cheeses, butter, and ice-cream. Behrad et al. (2009) added Cinnamon herb to yogurt which increased antioxidant and total phenolic content of yogurt and probiotic bacteria present in the yogurt inhibited the growth of *H. pylori in vitro*.

Currently, usage of different herbal extract in yogurt and fermented milk has been considered as new trend to improve the viability of probiotics (Michael et al., 2015). Mint (*Mentha* spp.) a kind of fragrant persistent herbs found in temperate and sub-temperate areas (Bhat et al., 2002). Spearmint usage is famous in cuisines, medicines, and cosmetics because of betterment on health of humans (Saeed et al., 2006). This herb gives aid from common disease like cold, fever, influenza and illness. Moreover, it is widely used in confectionary, pharmaceuticals, cosmetics, oral hygiene products, and used as a flavor enhancing mediator in beverages and many other products (Croteau et al., 2005). Yogurt is the most ideal product for the addition of mint extract as it has antioxidant potential and is used commonly by the people, hence this research study is being planned to develop mint extract yogurt to improve the health and nutritional status of the people. Keeping in view the importance of mint herb and probiotics, the yogurt was formulated by the addition of these ingredients and studied for different quality attributes during storage of 25 days at refrigerated temperature.

MATERIALS AND METHODS

The planned study was conducted at Food Science and Technology, Department, Faculty of Agriculture, University of Poonch Rawalakot, Azad Kashmir. The raw materials, chemicals or reagents and procedure involved during the study described here as;

Procurement of raw material

Fresh buffalo milk for yogurt manufacturing was procured from dairy farm of Rawalakot city, Azad Kashmir and, milk was stored at low temperature 4°C until used.

Physico-chemical analysis of milk

The buffalo milk used for mint probiotic-yogurt was studied for physicochemical composition such as pH, fat, SNF, total solids, moisture, protein and acidity to checked its suitability for milk before yogurt making (AOAC, 2016).

Preparation of mint extract

The dried leaves of mint were crushed in mixer and then extractions were achieved in 1000mL Erlenmeyer flask in contact (1gm to 20mL ethanol 80%). Extractions were complete in a water-bath at a boiling point of ethanol (78 °C) intended for two hours. Then the extract was evaporated in an oven at temperature of 50°C to get concentrate. The concentrate was dried up to powder form and stored at room temperature.

Preparation of yogurt

Yogurt was ready by the method of Tamime and Robinson (1999). Milk was analyzed and stored at 4 0C. For yogurt preparation, milk was heated at 90-95 0C for2 to 3 minutes and rapidly cooled at 42 0C. After the addition of (0.25g/L) gelatin, (0.5g/L) probiotic (Bifidobacterium bifidum strain no. PRBT-004) and mint extract (0%, 0.5%, 1%, 1.5% and 2%) then milk was inoculated with set starter culture (1g/L) and incubated at 40-42°C for 4 hours. After incubation, cooling was done at 4 °C and stored for 25 days. The physicochemical and functional characteristics of mint extract probiotic-based yogurt were assessed during 25 days of storage with an interval of 5 days at 4 °C. Figure 1 illustrated the flow of yogurt preparation and different treatment concentrations are given in Table 1.

Table 1. Different Concentrations of Mint Extract

Treatments	Yogurt(mL)	MintExtract(%)
T_0	100	0
T_1	100	0.5
T_2	100	1
T_3	100	1.5
T_4	100	2

Physicochemical analysis of mint extract yogurt

pH

The pH of yogurt was determined by using pH meter permitting to process as described in AOAC (2006) (Method 881.12). Firstly, pH meter was standardized by using 4.0 and 10 buffer solutions then the yogurt sample was taken in

beaker and electrode of pH meter was rinsed with distilled water, then electrode was dipped in yogurt sample to obtain reading.

Titratable acidity

The titratable acidity of yogurt sample was measured by process as reported in AOAC. (2006), Method (9720.21). Firstly 10 mL yogurt was added in 100 mL flask, add 2-3 drops in the flask and then that solution was titrated against sodium hydroxide of 0.1 N until light pink color 23 achieved. The formula which is given below was used to calculate percent acidity.

$$Acidity(\%) = \frac{Volume\ of\ 0.1N\ NaOH\ used\ (mL)\ \times 0.009}{Weight\ of\ sample} \times 100$$

Fat content

The Gerber method analysis was used to assess the fat content in yogurt (AOAC, 2000). For this purpose, yogurt sample (10mL) was taken and added it into butyrometer, then added 1mL of Iso amyl alcohol and shake it well. After this, butyrometer was put into Gerber machine for 2-3 minutes. Lower end of fat column on to a main graduation mark were brought by slightly withdrawing stopper of butyrometer. The difference between the two readings stretches the percentage by mass of fat in yogurt.

Total soluble solids

The total soluble solids (TSS) of samples were measured by a digital refractometer. The refractometer reading determined a drop of sample on the prism and the reading was recorded. TSS was measured as a Brix at 20 °C temperature (Kaur et al., 2015).

Functional characteristics of mint yogurt

Total phenols

The assay used to measure the total phenolic content was altered from that used by Zainoldin and Baba (2009). 1.0 ml of homogenized yogurt water extract was put in a test tube along with 5 ml of distilled water and one milliliter of 95% ethanol. 50% (v/v) Folin-Ciocalteu reagent (0.5 ml) was added to each sample and stirred. The reaction mixture was left to stand for 60 minutes after 1 ml of 5% Na_2CO_3 was added after 5 minutes. After measuring the absorbance at 725 nm, the results were translated into total phenolics, which were then reported as micrograms equivalent to gallic acid per gram (GAE/g) of sample. The standard was gallic acid.

Antioxidant activity

Antioxidant activity of yogurt sample was calculated by DPPH method (free radical scavenging activity) as reported through (Brand-Williams et al., 1995). For this resolution, different amounts of extract (100µ g/mL) of yogurt sample were prepared by centrifugation and 0.5mL sample and 1mL of freshly prepared DPPH solution (0.25mM) and 1mL ethanol. Individually samples were mixed carefully and put in the dark for 30 min at room temperature. Therefore, each mixture was tested for DPPH radical scavenging activity through reading the absorbance at 517nm on a spectrophotometer. The formula which is given below was used to calculate antioxidant activity.

Antioxidant activity (%) = (Ao –As/Ao) × 100

Microbiological analysis

Viability of probiotic bacteria

Yogurt samples (10g) were weighed and homogenized in 90mL of sterile water containing 1g/L peptone. Each sample was prepared in triplicate and homogenized by vortexing for 5 minutes and proper thinning were pour plated onto MRS agar plates containing 50µg/mL tetracycline and incubated aerobically at 37 °C for 48 hours. Probiotic count (viable numbers of species) was determined after 0, 5, 10 and 15 days of storage at 4 °C (Hekmat et al., 2009).

Textural analysis of yogurt

Syneresis of samples was examined through centrifugation technique as designated by Shekhar et al. (2012). 10mL of the yogurt 25 sample was allowed to gel in the test tubes and kept under the cold storage for 24 hours at 10 ºC, then subjected it for the centrifugation at 3000rpm for 5 minutes. Water holding capacity was determined by the method reported by Mahmoudi et al. (2016).

Organoleptic Analysis

The organoleptic test was conceded out on well conserved samples by using panel of 5 judges from 9-1 scale (Faisal and Mukhriza, 2019).

Statistical analysis

All collected data were subjected to statistical analysis by using two factor factorial CRD, ANOVA by using software statistics 8.1 at 5% level of significance whereas, the mean was matched by using LSD test as described by (Steel, 1997).

RESULTS AND DISCUSSION

The main objective of this research to make yogurt with addition of mint extract and probiotic *Bifidobacterium bifidum* with ratio (0.5g/L), thus partiality of yogurt can have enhanced due to mostly can't know about beneficial of mint yogurt. Mint yogurt was subjected to evaluation of physiochemical, functional and sensory for 25 days storage time. Sensory evaluation of mint yogurt was based on 9-points hedonic scales which consisted of color, flavor, taste, texture, after taste and overall acceptability.

Physicochemical analysis of milk

Before the yogurt production, milk was examined for various tests like fat, protein, pH, acidity, ash and moisture. The outcomes are showed in Table 2.

The milk composition is an important feature which impacts the quality of manufactured goods (Guinee et al., 2000). Purposely, samples of milk were exposed to altered composition parameters to determine the suitability of milk for yogurt manufacture. The results (Table2) specified that raw buffalo milk comprised of proteins (4.25 ± 0.07 %), solid-not-fat (10.09 ± 0.03%), fat (6.58 ± 0.02%), acidity (0.12±0.01), ash (0.87±0.021), moisture (85.34±1.02) and total solids (16.67± 0.05%) contents.

Table 2. Analysis of raw milk.

Parameter	Results
Moisture (%)	85.34±1.02
Ash (%)	0.87±0.021
Protein (%)	4.25±0.07
Fat (%)	6.58±0.02
pH	6.60±0.05
Acidity(%)	0.12±0.01
Total Solid(%)	16.67±0.05
SNF (%)	10.09±0.03

Physiochemical analysis of yogurt

pH

Theoretically definition of pH is negative log of ion of hydrogen (H+) activity in a medium. Range of pH value is 1-14. It is obvious from the results that pH was significantly affected by different treatments, their interaction also has significant effect during storage intervals. The results related to analysis of inconsistency for the pH of yogurt samples. It is clear from the results of table 3, that pH of the mint based yogurt samples increase with increasing the concentration of mint extract. Highest value of (4.89) is observed in T_4 (with 2% mint extract) at 0 day, while minimum pH value (4.20) was recorded in T_0 (0% mint extract) at 25th day. This increase in pH with increasing the concentration of mint extract in yogurt samples may be due to the presence of basic compounds such as menthyl acetate, 1,8-cineole, limonene, beta-pinene and beta caryophyllene by Koca et al. (2008) and increase the basicity which increase the pH by increasing the concentration of mint extract.

Table 3. Effect of treatment and storage interval on pH value of yogurt.

Treatments (pH)	Days of storage						Means
	0	5	10	15	20	25	
T0	4.66±0.01gh	4.58±0.16 jk	4.44±0.15 n	4.38±0.17op	4.29±0.15 q	4.20±0.14 r	4.43E
T1	4.73±0.02de	4.69±0.14fg	4.66±0.14 h	4.52±0.17lm	4.40±0.13 o	4.28±0.13 q	4.53D
T2	4.78±0.01 d	4.71±0.04 ef	4.69±0.17 f	4.60±0.17 j	4.50±0.18 m	4.36±0.17 p	4.60C
T3	4.82±0.01 b	4.74±0.03 d	4.69±0.23 f	4.56±0.17 k	4.53±0.23 l	4.38±0.22op	4.62B
T4	4.89±0.07 a	4.81±0.06 b	4.74±0.11 d	4.63±0.17 i	4.57±0.11 k	4.44±0.11 n	4.68A
Means	4.78A	4.71B	4.62C	4.54D	4.46E	4.33±0.17F	

Fat

Fat plays a key role in nutritional value of the product as it gives high amount of energy than all the other ingredients. Results regarding mean values related to fat content of mint-based yogurt samples are given in Table 4. The results

clearly showed that highest value (3.80%) of fat content was observed in T_0 (yogurt without mint extract) followed by (3.78%) T_1 (yogurt with 0.5% mint extract) and lowest fat content (3.69%) was observed in T_4 (yogurt with 2% mint extract).

During the storage the highest value (3.80%) of fat content was observed at 0 days, while the lowest value (3.47 %) of fat calculation at 25 days of storage given in Table 4, showing that as the storage period increase the fat level of yogurt samples decreased although the percentage of decreasing trend is smaller. Mechanism behind this is the breakdown of casein structure because of the production of high acidity. It is concluded that fat% decreased non-significantly during the storage. Similar research study showed decreasing of fat level by the addition of different herbs into yogurt and Roy et al. (2015) concluded that addition of different tea extracts in yogurt decreased the fat level.

Table 4. Effect of treatment and storage interval on Fat (%) value of yogurt.

Treatments (Fat)	Days of storage						Means
	0	5	10	15	20	25	
T0	3.80±0.10	3.79±0.08	3.77±0.08	3.75±0.08	3.72±0.08	3.70±0.08	3.75A
T1	3.78±0.08	3.76±0.08	3.71±0.07	3.69±0.07	3.61±0.07	3.58±0.08	3.68B
T2	3.75±0.06	3.72±0.07	3.71±0.07	3.68±0.08	3.62±0.09	3.56±0.05	3.67C
T3	3.73±0.04	3.71±0.09	3.70±0.08	3.66±0.09	3.60±0.06	3.54±0.08	3.65D
T4	3.69±0.04	3.66±0.06	3.60±0.09	3.56±0.08	3.50±0.08	3.47±0.07	3.58E
Means	3.75A	3.70B	3.70AB	3.66AB	3.63AB	3.60C	

Titratable acidity

Acidity in yogurt is related to its yogurt production as lactic acid percentage. The results for the acidity of diverse treatments are showed in Table 5. It is clear from the results that acidity of the mint-based yogurt samples decreased with increasing the concentration of mint extract. Highest value of (1.19%) was observed in T_0 (with 0% mint extract) at 25th day, while lowest acidity value (0.57%) was recorded in T_4 (2% mint pulp) at 0 day. This decrease in acidity with increasing the concentration mint extract in yogurt samples may be due to the presence of basic constituents which were present in mint such as menthyl acetate, 1,8-cineole, limonene, beta-pinene and beta- caryophyllene by Koca et al. (2008) and decrease the acidity. The result of Rezaei et al. (2011) represented that reduction of lactic acid bacteria to produce the lactic acid in yoghurt so that reduced the microorganism activity then acidity of yoghurt in storage period decreases. Results reported in this study were reliable with the result of previous studies directed by Mosiyani et al. (2017).

Table 5. Effect of treatment and storage interval on acidity (%) value of yogurt.

Treatments (Acidity)	Days of storage						Means
	0	5	10	15	20	25	
T0	0.83±0.01	0.88±0.01	0.94±0.01	1±0.02	1.1±0.02	1.19±0.02	0.99A
T1	0.79±0.01	0.83±0.01	0.88±0.01	0.95±0.02	1.06±0.03	1.1±0.03	0.93B
T2	0.73±0.02	0.76±0.02	0.8±0.02	0.89±0.02	0.97±0.03	1.01±0.04	0.86C
T3	0.64±0.02	0.69±0.02	0.75±0.03	0.82±0.04	0.89±0.04	0.97±0.04	0.79D
T4	0.57±0.02	0.62±0.02	0.68±0.02	0.74±0.04	0.85±0.04	0.93±0.04	0.73E
Means	0.71F	0.75E	0.81D	0.88C	0.97B	1.04A	

Total solids

It is number of solids dissolved within a substance. It is showed from the results (appendix 4) that different treatments have significant effect during storage intervals, while their interactions have non-significant effect ($P \leq 0.05$).The results for the total solids of different treatments are showed in Table 6. It is noticeable from the results that total solids of the mint-based yogurt samples increase when concentrations of mint extract increases. Highest value of (15.85) was observed in T_4 (with 2% mint extract) at 0 day, while lowest TSS value (11.51%) was recorded in T_0 (0% mint pulp) at 25th day. This increase in total solid with increasing the concentration mint extract in yogurt samples due to the existence of solids fillings existing in mint extract as similar results reported by Gaglio et al. (2019).

Table 6. Effect of treatment and storage interval on Total Solids (%) value of yogurt.

Treatments (Total Solid)	Days of storage						Means
	0	5	10	15	20	25	
T0	12.38±0.02	12.36±0.02	12.21±0.01	11.89±0.20	11.67±0.10	11.51±0.02	12.0E
T1	13.42±0.10	13.21±0.02	12.91±0.10	12.96±0.02	12.65±0.06	12.31±0.10	12.91D
T2	14.4±0.20	14.11±0.07	13.67±0.01	13.61±0.06	13.43±0.09	13.15±0.19	13.72C
T3	14.88±0.09	14.66±0.02	14.55±0.02	14.3±0.02	14.21±0.02	14.14±0.07	14.45B
T4	15.85±0.06	15.39±0.07	14.85±0.02	14.78±0.02	14.61±0.07	14.34±0.09	14.97A
Means	14.18A	13.94A	13.64B	13.51BC	13.31CD	13.09D	

Antioxidant activity

Antioxidant is a fragment that delays the corrosion of further particles. Basically, oxidation is a biochemical reaction which produced free spinner uncontrolled cell divisions that can loss cells. It is concluded from results that antioxidant activity was significantly affected by different treatments and storage pauses while their interaction has non-significant effect. Results regarding mean values related to antioxidant activity of mint-based yogurt samples are given in Table 7. The results clearly showed that highest value (65.2%) of antioxidant activity was observed in T_4 (yogurt with 2% mint extract) and lowest antioxidant activity (23.5%) was observed in T_0 (yogurt without mint extract). It is showed that antioxidant activity percentage increased by increasing mint extract concentration into yogurt samples. This increasing trend is may be due to the fact that mint extract has higher antioxidant include lycopene vitamin C, flavones and hydroxyl benzoic acids (Kumar et al., 2015) Because of these compounds in mint results increase in antioxidant activity when added it into yogurt. These results are in line with the research of Karaaslan et al. (2011) that added mint leave extract into yogurt and reported similar increasing trend.

Total phenols

Phenolic contents are extra nutritional bioactive compounds which are found in fruits, herbs and other food products. It is shown from the statistical results that are highly significant effect between treatments, storage and their interaction. The effects of different treatments of yogurt samples on total phenolic are presented in Table 8. It is noted from results that total phenolic increased by increasing the deliberation of mint extract in yogurt .The results indicated that highest value (35.29 mg/100g) of phenolic content was observed in T_4 (yogurt with 2% mint extract) followed by (30.71%) in T_3 (yogurt with 1.5% mint extract) and lowest phenolic content (22.4mg/100g) was observed in T_0 (yogurt without mint extract). It is showed that phenolic content increased by increasing mint extract concentration into yogurt samples. The increasing trend of total phenol in yogurt samples by the addition of mint extract is because of higher phenolic content founds in mint. These results were reinforced by Sengul et al. (2012), who also found the increasing trend of phenols by the addition of sour cherry pulp into yogurt, similarly by Kumar et al. (2015) also observed the increasing values of phenolic content in grape peel extract-based yogurt.

Syneresis

Syneresis in yogurt occurs when a liquid such as water is expelled or extracted from a gel. It is highly unattractive in yogurt lower syneresis improve the quality and also the acceptability of yogurt. It is shown from the results shown that treatments and storage mean have significant while their interaction have non-significant effect on syneresis level. Results regarding mean values related to syneresis of mint-based yogurt samples are given in Table 9. The results clearly showed that highest value (24.20%) of syneresis was observed in T_0 (yogurt without mint extract) followed by (21.24%) T_1 (yogurt with 0.5% mint extract) and lowest syneresis (15.10%) was observed in T_4 (yogurt with 2% mint extract). It is showed that syneresis percentage decreased by increasing mint extract concentration into yogurt samples. This decreasing trend is may be due to the fact that addition of mint extract bind water firmly to gel of yogurt due to presence of protein content that is why when the extract percentage increased the syneresis became lower.

During the storage the highest value (25.58%) of syneresis was observed at 25^{th} days, while the lowest value (16.03%) of syneresis percentage at 25^{th} day of storage given in Table 9, showing that as the storage period increase the syneresis of yogurt samples increase. The results of this work equal by the way of Zare et al. (2011) and stated that in storage time syneresis increases.

Water holding capacity

Water holding capacity WHC is allied to the capability of proteins to hold water within the yogurt structure. It is clear from the statistical result that is highly significant effect between the treatments and storage but their interaction has

non-significant effect. The effects of altered treatments of yogurt samples on WHC are presented in Table 10. It is noted from results that WHC amplified by growing the deliberation of mint extract in yogurt. The results indicated that highest value (38.33%) of phenolic content was observed in T_4 (yogurt with 2% mint extract) while lowest WHC (30.92 %) was observed in T_0 (yogurt without mint extract). It is showed that WHC increased by increasing mint extract concentration into yogurt samples. The increasing trend of WHC in yogurt samples by the addition of mint extract is because of the acidification process of the yogurts enhanced plant extracts allowed obtaining gels with increased firmness, lower permeability, finer protein networks and improved whey drainage hence increased WHC).

Table 7. Effect of treatment and storage interval on antioxidant.

Treatments (Antioxidant)	Days of storage						Means
	0	5	10	15	20	25	
T0	23.5±0.81	23±0.78	21.2±0.75	20.3±0.73	19.6±0.69	18±0.67	20.93E
T1	33.6±1.04	33±1.02	31.3±1.01	30.1±0.06	29.7±0.04	29±0.04	31.11D
T2	41.8±1.75	40.8±1.72	40.3±1.65	40±1.61	39.8±1.59	39.3±1.55	40.33C
T3	52.3±1.58	51.4±1.54	50.5±1.52	49.3±1.50	47.2±1.48	46.7±1.43	49.56B
T4	65.2±1.60	64.4±1.50	62.3±1.50	61.1±1.47	60.7±1.44	59±1.42	62.11A
Means	43.28A	42.52B	41.1C	40.0D	39.4E	38.4F	

Table 8. Effect of treatments and storage interval on total phenolic mg/100g of yogurt.

Treatments (Phenol)	Days of storage						Means
	0	5	10	15	20	25	
T0	22.40±0.83mn	21.25±0.83 b	20.13±0.82 p	18.91±0.81q	17.15±0.73 r	21.25±0.71 r	19.37E
T1	24.45±1.07 j	24.01±1.07jk	22.94±1.06lm	22.75±1.05lm	22.24±1.04m	21.5±1.03 op	22.99D
T2	27.38±1.89 h	26.25±1.79 i	24.94±1.77 j	24.88±1.71 j	24.10±1.69 j	23.36±1.64jk	25.15C
T3	30.71±1.70 cd	30.15±1.70de	29.34±1.60ef	28.56±1.58 fg	27.96±1.56gh	27.72±1.51gh	29.07B
T4	35.29±1.76 a	33.87±1.76 b	32.96±1.73cd	31.39±1.71cd	30.33±1.66 ef	30.46±1.62 ef	31.15A
Means	28.04A	27.10B	26.06C	24.57D	23.90E	23.61E	

Table 9. Effect of treatment and storage interval on Syneresis (%) value of yogurt.

Treatments (Syneresis)	Days of storage						Means
	0	5	10	15	20	25	
T0	24.20±0.48	24.71±0.47	25.01±0.45	25.08±0.45	25.34±0.42	25.58±0.41	24.98A
T1	21.24±0.58	20.46±0.56	20.55±0.55	20.78±0.52	20.96±0.48	20.99±0.46	20.79B
T2	19.13±0.65	19.36±0.63	19.43±0.62	19.66±0.60	17.73±0.55	19.88±0.53	19.53C
T3	16.86±0.62	17.13±0.60	17.41±0.57	17.45±0.55	17.66±0.51	17.97±0.50	17.41D
T4	15.10±0.63	15.28±0.63	15.32±0.60	15.62±0.55	15.80±0.53	16.03±0.50	15.53E
Means	19.30D	19.39B	19.47CD	19.72BC	19.92C	20.08A	

Table 10. Effect of treatment and storage interval on WHC value of yogurt.

Treatments (WCH)	Days of storage						Means
	0	5	10	15	20	25	
T0	30.92±0.13	28.21±0.12	27.04±0.11	25,33±0.10	23.21±0.10	21.23±0.09	25.99A
T1	32.21±0.14	31.22±0.13	30.06±0.13	29±0.12	28.19±0.12	25.22±0.11	29.28B
T2	34.45±0.17	33.1±0.16	32.13±0.15	30.21±0.13	29.21±0.12	27.21±0.12	31.05C
T3	36.44±0.18	35.22±0.17	34.28±0.17	32.35±0.16	30.34±0.14	26.42±0.13	32.50D
T4	38.33±0.23	37.23±0.22	36.04±0.22	34.55±0.21	33.54±0.21	29.31±0.20	34.79E
Means	34.47F	32.95E	31.91D	30.28C	28.89B	25.99A	

During storage there was reduction in water holding capacity that is after 5 days 37.23, after 10 days 36.04, after 15 days 35.55, after 20 days 33.54, after 25 days storage 29.31, because of interface between casein and polysaccharides stabilizers became weaker, it was because lactose was converted into lactic acid, that increased

acidity and resultantly interaction bonds between polysaccharides stabilizers and casein micelles become weaker. Due to that weaker interaction, water was not tightly bounded.

Sensory evaluation

Sensory characteristic is one of the important parameters that show about the quality of product. Yoghurt samples were introduced to panel of 7 judges and all sample were try out with 9 points hedonic scale that is 1 is used for extremely dislike and 9 for liked extremely. Yoghurt was organoleptically assessed at 0, 5th, 10th, 15th, 20th and 25th days of storing period, so this collected data was managed for statistical examination and result thus gotten for each determination.

Taste

Taste is a feeling observed by tongue and inclined by aroma, texture and conformation of diets. It is obvious from the results that highly significant results for different treatments and storing intervals showed in table 11 and also have significant effect for interaction. The evaluation of the treatment means value resulted T_2 (yogurt with 1 % mint extract) as top scorer (8.41) while minimum value (6.06) was noted in T_4 (yogurt with 2 % mint extract). The taste of yogurt increases as the mint extract rises up to 2 % shown from results. Yogurt taste increases due to the basis of numerous organic acids that contribute in increment of taste in yogurt samples. The results related the effect of different storage intervals on taste of yogurt depict that taste decreases with passage of time.

Table 11. Effect of treatments and storage interval on taste of yogurt.

Treatments (Taste)	Days of storage						Means
	0	5	10	15	20	25	
T0	7.55±0.26 cd	7.57±0.24 cd	7.46±0.223 de	7.30±0.23 ef	7.02±0.21 g	6.44±0.20 hi	7.22C
T1	7.89±0.26 b	7.79±0.26 bc	7.56±0.24 de	7.56±0.23 cd	7.44±0.21 de	7.15±0.20 ef	7.56B
T2	8.41±0.17 a	8.28±0.16 a	7.92±0.15 b	7.89±0.15 b	7.61±0.14 cd	7.39±0.13 de	7.91A
T3	6.99 ±0.16 g	6.69±0.15 h	6.61±0.14 h	6.47±0.13 h	6.47±0.11 j	5.94±0.10 j	6.47D
T4	6.06±0.13 d	6.65±0.13 cd	6.18±0.12 ij	6.18±0.11 j	5.46±0.10 k	4.95±0.10 ij	5.87E
Means	7.35A	7.38A	7.14B	7.03B	6.72C	6.37D	

Highest value (8.28) was observed after 5 days while minimum score (7.39) was noted after 25 days of storage. Taste is reduced potency due to the fact that rise in acidity and invention of lactic acid by lactose degradation by bacterial action which produce bad taste in yogurt as reported by Celik et al. (2006).

Flavor

Flavor is a sensory impression of yogurt or other food material and resolute chiefly by the chemical intelligence of taste and smell. It is clear from the result treatments; storage and their intervals have highly significant effect. The results concerning effect of different treatments on flavor were showed in table 12. It is concluded from result that flavor of yogurt sample increased as concentration of mint extract increase. Maximum value (7.7) was reported in T_2 (yogurt with 1% mint extract) whereas lowest value (5.95) in T_4 (yogurt with 2% mint extract). It may be because of mint is the rich cause of acids that effect and delay the flavor of yogurts sample that is why judges were given minimum score after T_2 (yogurt with 1% mint extract).

Table 12. Effect of treatments and storage interval on flavor of yogurt.

Treatments (Flavor)	Days of storage						Means
	0	5	10	15	20	25	
T0	7.31±0.25	7.36±0.23	7.04±0.22	6.88±0.21	6.73±0.20	6.66±0.20	6.97C
T1	7.51±0.13	7.36±0.12	7.13±0.12	7.06±0.11	6.95±0.10	6.76±0.08	7.13B
T2	7.7±0.23	7.61±0.21	7.44±0.21	7.39±0.20	7.21±0.20	6.93±0.18	7.38A
T3	6.09±0.13	6±0.11	5.82±0.11	5.44±0.10	5.36±0.10	5.17±0.10	5.64E
T4	5.95±0.18	5.65±0.16	5.65±0.16	5.4±0.14	5.21±0.13	4.95±0.11	5.49D
Means	6.91A	6.79B	6.61C	6.38D	6.31D	6.13E	

Color

Color is usually human visual observation defined through color categories (red, blue yellow, green and orange) as well as acceptance of respective product. The results concerning effect of different treatment on color are presented

in Table 13. It is clear from result that color of yogurt increase by the addition of mint extract into yogurt. The highest value (8.14) recorded in T_4 (yogurt with 2% mint extract) while minimum value (6.50) recorded in T_0 (yogurt without mint extract). Increasing value of yogurt samples by the addition of mint extract is due to fact that mint contains higher percentage of anthocyanin (cyaniding, rutinoside, pelargonidi) which is a major pigment responsible for color in mint as reported by Koca et al. (2008).

Table 13. Effect of treatments and storage interval on color of yogurt.

Treatments (Color)	Days of storage						Means
	0	5	10	15	20	25	
T0	6.50±0.23 j	6.22±0.23 lm	5.74±0.21 op	5.42±0.20 qr	5.21±0.14rs	5.03±0.11st	5.68E
T1	7.25±0.21 def	6.94±0.19gh	6.35±0.17 kl	6.24±0.14lm	6.04±0.11 n	5.66±0.10 pq	6.41D
T2	7.40de±0.31c	7.10±0.30 fg	6.93±0.27gh	6.80±0.24 hi	6.40±0.21 kl	5.94±0.20 no	6.76C
T3	7.67±0.17 c	7.47±0.15 cd	7.32±0.15 ef	6.98±0.13gh	6.68±0.11 ij	4.25±0.10 jl	7.09B
T4	8.14±0.35 a	7.94±0.35ab	7.72±0.33 bc	7.35±0.31 de	7.15±0.30 fg	4.9±0.25st	7.21A
Means	7.39A	7.13B	6.81C	6.55D	6.29F	5.60E	

Body and texture

The second most imperative factor for customer liking or disliking is sensory evaluation, apparent body and texture. Texture is another significant quality parameter in various finish products of food and also being acceptability of customer to the end product. The highest scores (8.4) were awarded to T_2 and lowest score (6.8) to T_4 yoghurt. Texture of yogurt is exaggerated by numerous reasons (percent acidity, texture and body) reduced through increased acidity, and this acidity frequently improved with the way of time were according to the findings of Rehman (1987). Therefore, results are also contract by Ahmad (1999), Bilal (1995), and Gyawali et al. (2022) they stated that the yogurt texture decreased.

Table 14. Effects of treatment and storage interval on texture of yogurt.

Treatments (Texture)	Days of storage						Means
	0	5	10	15	20	25	
T0	7.6±0.26ac	7.0±0.25abc	6.7±0.23af	6.4±0.22cdf	6.2±0.21ac	5.4±0.19 def	7.16A
T1	7.8±0.24ad	7.3±0.23lmn	7.1±0.21lmn	6.9±0.20 pqr	6.6±0.19 pqr	5.7±0.18cdf	6.9 B
T2	8.4±0.34def	7.5±0.32rst	7.3±0.31 lm	7.0±0.29af	6.9±0.28acd	5.9±0.26cdf	7.16A
T3	6.9±0.23xy	6.8±0.21 efg	6.9±0.35cd	6.7±0.33ch	6.4±0.30 def	4.5±0.29 efg	6.38D
T4	6.8±0.17cdf	6.6±0.16 efg	6.4±0.15 de	6.2±0.13def	5.8±0.12 def	4.0±0.12rst	5.9 E
Means	7.23A	6.85 B	6.66 C	6.30D	6.00 E	5.52F	

Overall acceptability

The evaluation of diverse treatment values showed highest overall acceptability (8.05) for T_2 containing 1 % mint extract as best followed by T_1 while lowest value (6.06) reported in T_4 (yogurt with 2 % mint extract). The yogurt samples with 1 % mint extract showed best acceptable results. An overall acceptability increases as concentration of mint extract increase up to 1 % after that decreasing trend was observed. The treatments containing 1% mint extract gained best scoring via panel of judges since of acceptable flavor and taste, rest of actions in which deliberation of mint extract higher showed poor results because of increase acidity and tartness.

Mean values of storage intervals on overall acceptability are given in Table 15. Minimum score (5.24) regarding overall acceptability was noted after 25 days of storage that is as compared to first day storage, which showed maximum results i.e., 7.29. Color, flavor, taste and growth of certain organic acids like citric acid and lactic acid decreases with the passage of time and results in cost of overall suitability of the yogurt.

Viability of probiotics

Bacterial aspect displays the probiotic-bacterial calculations of yogurt samples in the refrigerated storing. The highest viability of Bifidobacterium shown in T_4 (sample containing 2% mint extract) after the storage of 25 days, was substantial in comparison to former conducts ($p < 0.05$) So, in controlled samples viability was lowest. These results presented that addition of mint extract to probiotic-yogurt containing Bifidobacterium spp significantly enlarged the viabilityof these bacteria linked to the control sample ($p < 0.05$).

The significant difference in treatment and storage interval while non-significant difference between their interactions. The effects of different treatments of yogurt samples on viability of probiotics are presented in table 16. The result indicates that highest value (5.0×10^7) was observed in T_4 (yogurt with 2 % mint extract) and lowest value (2.5×10^5) was observed in T_0 (yogurt with 0% mint extract), It is showed that viability was increased by increasing mint extract concentration into yogurt samples.

This strength exists due to the phenolic complexes (caffeic acid, rosmarinic acid and diosmin) and essential oils (menthol, menthone and limonene) of herbal extracts that boost the starter culture growth in yogurt by Oh et al. (2016) to probiotic bacteria by Marhamatizadeh et al. (2013). While the inhabitants of *Bifidobacterium* spp decreased considerably in last storage ($p < 0.05$), because of the growth of lactic acid by the starter culture, foremost to a decline in yogurt pH and an rise in the acidity by Joung et al. (2016).

Table 15. Effects of treatment and storage interval on overall acceptability of yogurt.

Treatments (Acceptability)	Days of storage						Means
	0	5	10	15	20	25	
T0	7.41±0.26	7.30±0.26	7.18±0.24	6.93±0.23	6.89±0.22	6.74±0.21	7.07C
T1	7.83±0.13	7.70±0.13	7.46±0.12	7.45±0.11	7.36±0.11	7.18±0.10	7.49B
T2	8.05±0.33	7.90±0.31	7.71±0.31	7.63±0.32	7.49±0.31	7.29±0.30	7.68A
T3	7.08±0.13	6.88±0.13	6.81±0.12	6.49±0.11	6.29±0.11	6.25±0.10	6.63D
T4	6.06±0.13	5.96±0.11	5.83±0.11	5.67±0.10	5.53±0.10	5.24±0.10	5.71E
Means	7.28A	7.15B	6.99C	6.83D	6.71E	6.54F	

Table 16. Effect of storage interval and treatments on viability of probiotic bacteria of mint yogurt.

Treatments (Viability)	Days of storage						Means
	0	5	10	15	20	25	
T0	2.5×10^5	1.5×10^7	1.3×10^5	1.0×10^5	0.52×10^5	0.2×10^5	1.3×10^5
T1	3×10^7	2.73×10^7	2.58×10^7	2.33×10^7	1.5×10^7	1.0×10^7	2.33×10^7
T2	4×10^7	3.9×10^7	3.0×10^7	2.7×10^7	2.2×10^7	2.0×10^7	3.0×10^7
T3	4.8×10^7	5.05×10^7	4.7×10^7	4.3×10^7	3.23×10^7	3.0×10^7	3.23×10^7
T4	5×10^7	4.23×10^7	4.0×10^7	3×10^7	2.5×10^7	1.5×10^7	4.0×10^7
Means	6.88×107	$6.22.\times10^7$	6.0×10^7	5.4×10^7	4.4×10^7	2.2×10^7	

CONCLUSION

Nutritious yogurt can be prepared by the addition of mint extract up to 2 % level, while higher concentration of mint extract results in syneresis as well as decline organoleptic scores. The mint-based yogurt can be stored (4ºC) and acceptable for 25 days.

AUTHOR CONTRIBUTIONS

Muhammad Waqas: execution of research work, Saima Rafiq: research planning, draft writing, Imran Hayat: research planning, data analysis, Sheraz Hussain, Aqsa Qayyum & Diya Khan: Article write up and proof reading, Anees Murtaza: data analysis and data interpretation.

COMPETING OF INTEREST

The authors declare no competing interests.

REFERENCES

Ahmad, M., 1999. Preparation and evaluation of mango fruit yoghurt. M. Sc.(Hons.) Thesis, Department of Food Science and Technology. University of Agriculture, Faisalabad.

Alenisan, M.A., Alqattan, H.H., Tolbah, L.S., Shori, A.B., 2017. Antioxidant properties of dairy products fortified with natural additives: A review. Journal of the Association of Arab Universities for Basic and Applied Sciences 24, 101-106.

AOAC., 2006. Association of official and analytical chemists. Official Methods of Analysis Washington, D. C. AOAC. 1990.

Behrad, S., Yusof, M., Goh, K., Baba, A., 2009. Manipulation of probiotics fermentation of yogurt by cinnamon and

licorice: effects on yogurt formation and inhibition of Helicobacter pylori growth in vitro. International Journal of Nutrition and Food Engineering 3, 563-567.
Bhat, S., Maheshwari, P., Kumar, S., Kumar, A., 2002. Mentha species: in vitro regeneration and genetic transformation. Mol Biol Today 3, 11-23.
Bilal, A., 1995. Effect of some hydrocolloids on the physio-chemical characteristics of yogurt. M. Sc.(Hons.) Thesis, Department of Food Science and Technology, University of Agriculture, Faisalabad.
Brand-Williams, W., Cuvelier, M.-E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology 28, 25-30.
Celik, S., Bakırcı, I., Şat, I., 2006. Physicochemical and organoleptic properties of yogurt with cornelian cherry paste. International Journal of Food Properties 9, 401-408.
Croteau, R.B., Davis, E.M., Ringer, K.L., Wildung, M.R., 2005. Menthol biosynthesis and molecular genetics. Naturwissenschaften 92, 562-577.
Dabija, A., Codină, G., Gâtlan, A., 2018. Influence of different commercial starter cultures on quality of yogurt. Journal of Environmental Science, Toxicology and Food Technology 12, 17-24.
Faisal, M., Mukhriza, T., 2019. Organoleptic analysis of yogurt with banana addition and stevia sweeteners. Rasayan Journal of Chemistry 12.
Gaglio, R., Gentile, C., Bonanno, A., Vintaloro, L., Perrone, A., Mazza, F., Barbaccia, P., Settanni, L., Di Grigoli, A., 2019. Effect of saffron addition on the microbiological, physicochemical, antioxidant and sensory characteristics of yoghurt. International Journal of Dairy Technology 72, 208-217.
Guinee, T.P., Auty, M.A., Fenelon, M.A., 2000. The effect of fat content on the rheology, microstructure and heat-induced functional characteristics of Cheddar cheese. International Dairy Journal 10, 277-288.
Gyawali, R., Feng, X., Chen, Y.P., Lorenzo, J.M., Ibrahim, S.A., 2022. A review of factors influencing the quality and sensory evaluation techniques applied to Greek yogurt. Journal of Dairy Research 89, 213-219.
Hekmat, S., Soltani, H., Reid, G., 2009. Growth and survival of Lactobacillus reuteri RC-14 and Lactobacillus rhamnosus GR-1 in yogurt for use as a functional food. Innovative Food Science & Emerging Technologies 10, 293-296.
Joung, J.Y., Lee, J.Y., Ha, Y.S., Shin, Y.K., Kim, Y., Kim, S.H., Oh, N.S., 2016. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. Korean Journal for Food Science of Animal Resources 36, 90.
Karaaslan, M., Ozden, M., Vardin, H., Turkoglu, H., 2011. Phenolic fortification of yogurt using grape and callus extracts. LWT-Food Science and Technology 44, 1065-1072.
Kaur, H., De Muyt, A., Lichten, M., 2015. Top3-Rmi1 DNA single-strand decatenase is integral to the formation and resolution of meiotic recombination intermediates. Molecular Cell 57, 583-594.
Koca, I., Ustun, N.S., Koca, A.F., Karadeniz, B., 2008. Chemical composition, antioxidant activity and anthocyanin profiles of purple mulberry (Morus rubra) fruits. Journal of Food Agriculture and Environment 6, 39.
Kumar, Y., Yadav, D.N., Ahmad, T., Narsaiah, K., 2015. Recent trends in the use of natural antioxidants for meat and meat products. Comprehensive Reviews in Food Science and Food Safety 14, 796-812.
Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., Baiti, I., 2016. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian Ficus carica L. varieties. Asian Pacific Journal of Tropical Biomedicine 6, 239-245.
Marhamatizadeh, M.H., Ehsandoost, E., Gholami, P., Mohaghegh, M.D., 2013. Effect of olive leaf extract on growth and viability of Lactobacillus acidophilus and Bifidobacterium bifidum for production of probiotic milk and yoghurt. International Journal of Farming and Allied Sciences 2, 572-578.
Mckinley, M.C., 2005. The nutrition and health benefits of yoghurt. International Journal of Dairy Technology 58, 1-12.
Michael, S.L., Merlo, C.L., Basch, C.E., Wentzel, K.R., Wechsler, H., 2015. Critical connections: health and academics. Journal of School Health 85, 740-758.
Mosiyani, D.T., Lozano, M., Mosmann, T., 2017. Phenolic compounds, antioxidant and anti-bacterial activities of Turkish endemic Origanumsipyleum leaf extract. Journal of Food Lipid 14, 157-169.
Oh, N.S., Lee, J.Y., Joung, J.Y., Kim, K.S., Shin, Y.K., Lee, K.-W., Kim, S.H., Oh, S., Kim, Y., 2016. Microbiological characterization and functionality of set-type yogurt fermented with potential prebiotic substrates Cudrania tricuspidata and Morus alba L. leaf extracts. Journal of Dairy Science 99, 6014-6025.
Pacini, S., Ruggiero, M., 2017. Description of a novel probiotic concept: implications for the modulation of the immune system. American Journal of Immunology 13, 107-113.
Rehman, A., 1987. Some microbiological aspects in relation to quality control of yogurt. M. Sc.(Hons.) Thesis, Department of Food Science and Technology, University of Agriculture, Faisalabad.
Rezaei, R., Khomeiri, M., Kashaninejad, M., Aalami, M., 2011. Effects of guar gum and arabic gum on the physicochemical, sensory and flow behaviour characteristics of frozen yoghurt. International Journal of Dairy Technology 64, 563-568.
Roy, D.K.D., Saha, T., Akter, M., Hosain, M., Khatun, H., Roy, M.C., 2015. Quality evaluation of yogurt supplemented with fruit pulp (banana, papaya, and water melon). International Journal of Nutrition and Food Sciences 4, 695-699.

Saeed, S., Naim, A., Tariq, P., 2006. In vitro antibacterial activity of peppermint. Pakistan Journal of Botany 38, 869.
Shekhar, S., Joe, J., Kumar, R., Jyothis, J., Kumar, K., Priya, Y., Rao, K., Pagote, C., 2012. Effect of heat treatment of milk on the sensory and rheological quality of dahi prepared from cow milk. Journal of Food Dairy Technology 1, 9-15.
Steel, R., 1997. Analysis of variance I: The one-way classification. Principles and procedures of statistics a biometrical approach, 139-203.
Tamime, A., Robinson, R., 1999. Yoghurt: science and technology.,(Woodhead Publishing Ltd: Cambridge, UK).
Zainoldin, K., Baba, A., 2009. The effect of Hylocereus polyrhizus and Hylocereus undatus on physicochemical, proteolysis, and antioxidant activity in yogurt. World Academy of Science, Engineering and Technology 60, 361-366.
Zare, F., Boye, J., Orsat, V., Champagne, C., Simpson, B., 2011. Microbial, physical and sensory properties of yogurt supplemented with lentil flour. Food Research International 44, 2482-2488.