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Use of tukhm-e-balangu (*Lallemantia royleana*) as a Stabilizer in set type Yogurt

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Abstract

Gums and mucilage's possess numerous food industrial applications. *Lallemantia royleana* seeds contain about 31% of crude fiber that offers it to be used as a suitable stabilizer in many foods. The present study was aimed to introduce *L. royleana* seeds as yogurt stabilizer instead of gelatin. The yogurt was prepared by the standard method. The tukh malanga in the powder form, was added to the milk after pasteurization @ 0.15%, 0.2% and 0.25% concentration. The yogurt was stored at 4±2°C for 20 days and analyzed for different physiochemical (pH, titratable acidity, synersis, water holding capacity, total solids, viscosity, hardness, fat, protein and ash), microbiological and sensory attributes at specified day intervals. The utilization of stabilizer and its rate of incorporation affected the given attributes. Among different concentrations of *L. royleana*, Y3 (with 0.25% *L. royleana*) gave best results for physical and chemical parameters but Y2 (with 0.20% *L. royleana*) attained highest score for overall sensory acceptability throughout the storage period. It was also committed from data incorporation of *L. royleana* into yogurt promoted an overall effective outcomes for sensory evaluation although physicochemical and microbiological results were almost comparable with yogurt containing gelatin.

Keywords: *Lallemantia royleana*, physicochemical, synersis, Storage period, stabilizers

1. Introduction

Fermented milk products are gaining popularity and new varieties are entering in the consumer market regularly. Fermented products that are most commonly consumed are cheese, butter milk, sour cream, acidophilus milk, ropy milk and yogurt. Among these, yogurt is probably the most popular and ideal fermented product due to its thick creamy consistency and pleasant aromatic flavour [1].

It is manufactured in a range of compositional variations which includes changing the dry matter content and fat of milk. It may be plain or either formed from addition of sugars, fruits or gelling agents. It contains microbial culture of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophillus*. These are vital culture flora for almost all types of yogurts and for the production of an acceptable yogurt flavour, presence of both of these microbes should be ensured [2].

The medication characteristics related to yogurt have enhanced both its consumption and manufacturing throughout the world. Medication properties related to health include decreasing plasma cholesterol level, preventing from gastrointestinal distresses, modify lactose to improve the digestibility and increased

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immunity due to the existence of viable microbial culture in the yogurt. It is also considered as healthy food as it contains a well proportion of protein and calcium [3]. Major attributes of yogurt quality are synersis, acidity, hardness, sourness, shelf life and overall texture. Synersis is considered as main problem that greatly influence the shelf life of yogurt and can be reduced by increasing the milk casein contents, reduction in acidification rate or by reducing the temperature of incubation. It can also be reduced by adding stabilizers that increase the consistency of yogurt and ultimately reduce the synersis [4]. Additives that are normally used as yogurt stabilizer are starches, pectin, gelatin, locust bean gum, carrageenan, guar gum, alginate, tragacanth, gum arabic, karaya gum, methylcellulose derivatives and xanthan gum. These types of stabilizing agents are used to modify the dispersion properties and consistency of yogurt powder while adding it into water [5].

Locust bean gum, CMC, guar gum and alginate are considered as primary stabilizers and are often used in combination with carrageenan (secondary stabilizer) for controlling synersis [6]. Mucilages and gums of natural origin are also extensively used as binders, thickeners, suspending and emulsifying agents. These possess benefits more than artificial ones since they're more affordable, non-toxic and less costly as well as readily available [7].

Lallemantia royleana is also naturally occurring mucilaginous vegetation which is vernacularly known as "tukhme balangu". It belongs to family Labiatae, genus *Lallemantia* and tribe *Stachyoideae*. Its genus is widely grown in different parts of Afghanistan, Turkestan, Pakistan and India. In Pakistan, it is locally known as tukhme balangu or tukh malanga and extensively cultivated in Bhakkar, Layyah, Hasilpur, Chishtian and Bahawalpur etc.[8].

Lallemantia royleana seeds have certain medicinal potential and are commonly employed for the relief of joint inflammation, rheumatism, joint pain, abscesses inflammations and osteoarthritis [9]. These are also used in the treatment of fever, common cold and as expectorant during. On the other hand, *L. royleana* mucilage is used in abscesses, inflammations and gastrointestinal problems and also in the treatment of disorders, such as nervous, hepatic and renal diseases Naghibi*, et al*., (2005) [10]. Macquet *et al*. (2007) [11] conducted a study and found that swelling of *L. royleana* mucilage powder is pH-dependent that matches with pH of human bowel and ultimately promotes its activity.

L. royleana seeds are considered to be good source of fiber, polysaccharides, protein and oil and also have nutritional, human health improvement and medicinal potential Naghibi *et al.,* (2005). [10]

In addition to other commercially available gums, balangu seeds also have rheological behavior, flowing action and viscosity improving potential and hence can be utilized as stabilizer, water holding agent and thickening agent [12].

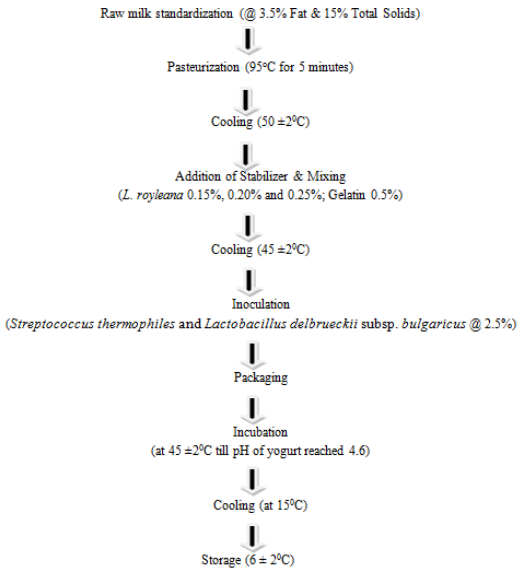
Keeping in view the high fiber content and stabilization property, it was planned to use *Lallemantia royleana* as stabilizing agent in set type yogurt instead of gelatin, to evaluate its influence on the composition, texture, sensory characteristics and shelf life of yogurt. It was hoped that this research would help the companies to improve their yogurt quality and also help the food technologist/scientists to conduct further research on its other related aspects.

2.Material and method

2.1 Procurement of raw material: Buffalo milk was procured from dairy farm, University of Agriculture, Faisalabad for yogurt manufacturing in a clean and pre-sterilized container and stored at 4°C. Food grade stabilizers; *Lallemantia royleana* and Gelatin were obtained from the local market. Commercial freeze dried starter culture of *Streptococcus thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was used. Chemicals used in this research was analytical grade. They were obtained from National Institute of Food Science and Technology, University of Agriculture Faisalabad.

2.2 Experimental plan: Fresh buffalo milk used for yogurt preparation was first standardized for fat (3.5%) and total solids (15%). After standardization, milk was divided into four equal portions and stabilizers were added. *Lallemantia royleana was* added @ 0.15%, 0.20% and 0.25% while gelatin @ 0.5% as a controlled sample.

2.3. Flow diagram of yogurt



2.4. Physicochemical analysis of yogurt: The yogurt samples were directly measured by using the pH meter (Model; pH-720 WTW series) according to AOAC (2000) [13] method No. 981.12 and acidity of yogurt samples were determined by direct acid-base titration according to AOAC (2000) [13] method No. 947.05. Fat was determined according to Kirk and Sawyer (1991) [14] Gerber method. Synersis was measured by the method described by Rodarte *et al.* (2004). [15] Water-holding capacity (WHC) of yogurt was determined by the procedure of Spasenija *et al.* (2007).[16] 5 g of experimental yogurt was centrifuged at 4500 rpm for 30 minutes at 10 ºC. After centrifugation, the supernatant was removed and the pellet was collected and weighed. The water holding capacity was calculated as follows:

$$WHC = [1- Wt/ Wi] \times 100$$

Where "Wt" is weight (g) of the pellet and "Wi" is the initial weight (g) of the yogurt sample.

Viscosity of the yogurt samples was measured according to method described by Gassem and Frank (1991) [17] by means of a Brookfield viscometer (model LVDVE 230, serial number E 5896, Brookfield Engineering Laboratories, Middleboro, MA).

Hardness of yogurt gel was measured in the fermenting container by texture profile analyzer (Model: TA-XT2i, Stable Microsystems, Goaldming, UK) using a cylinder plunger (16 mm), a compression rate of 5 mm per sec-1 and 75% (22 mm) deformation of set yogurt gel at 10 ºC. Force (in gram) applied on the yogurt sample by the probe was determined and calculated as yogurt's hardness (Gustaw, 2007). [18] Protein was determined by using Kjekdhal method according to method No. 991.20 (AOAC, 2000).[13] Moisture and total solids (percent residues) were determined by drying the sample in hot air oven according to method described in AOAC (2000) [13]. Ash content was analyzed according to AOAC (2000) [13] method.

2.5. Sensory evaluation: Assessment of overall acceptability of *L. royleana* enriched yogurt was done by a panel of 5 judge's faculty and research scholars at NIFSAT, University of Agriculture, and Faisalabad. Panel constituted judges who were trained and familiar for yogurt's attributes and showed their willingness. Flavor, body and texture, acidity and appearance were given score as 45, 30, 15 and 10 respectively according to Tamime and Robinson (1985) [7], the attributes of flavor and body and texture were given priority over others. Total scores were calculated by adding the scores of all attributes. Yogurt samples were coded with numbers and presented together to panel members in day light. Water was provided for rinsing mouth after each sample. Yogurt sample were evaluated sensory during 0, 5^{th}, 10^{th}, 15^{th} and 20^{th} day of storage.

2.6 Microbiological analysis: Yogurt was tested for total viable and coliform counts by the methods determined by Marshal (1993) [19]

2.7. Statistical analysis: Data obtained was subjected to statistical analysis using two factorial Completely Randomized Design (CRD) and ANOVA techniques as described by Steel *et al.* (1997) [20] to evaluate the quality and acceptability of yogurt.

3. Results and discussions

3.1.Physicochemical analysis of yogurt. The results of analysis physiochemical attributes of four types of yoghurts are presented in Table 2. Statistical data showed that there was highly significant effect ($p \leq 0.01$) of storage period and different concentrations of stabilizers on pH of yogurt.

Data presented in Table. 2 dictates that the pH values of yoghurt progressively decreased (4.80 to 4.15) from 0 to 20th day which would be due to the conversion of lactose into lactic acid. Various scientists reported that the storage time has significant effect on pH value while pH of samples decreased due to the production of lactic acid as the storage time increased [21].

The titratable acidity values were significantly increased from 0 to 20th day (0.56% to 1.18%) which would be due to the production of more lactic acid. These results are according to the findings of Chougrani *et al*. (2008) and Khalifa *et al.* (2011) [22, 23] who reported that acidity increased as storage time increased. Synersis showed highly significant effect of storage of yoghurt in Table 2. Maximum synersis was executed by Y0 (2.03) and Y3 (2.07) at 20th day if storage. These results agreed with the findings of Guven *et al*. (2005) [24] who observed an increase in syneresis during storage period in all yogurt mixes.

Water holding capacity decreased during storage as shown in Table. 2 highly significant results were also found different concentration of stabilizers. Highest water holding capacity was observed in Y3 containing 0.25% *L. royleana* followed by the Y0. Maximum decrease in water holding capacity values was observed in Y1 at 15th and 20th day that was 12.33% and 11.97% respectively. Water holding capacity of yogurt is decreased as the storage time increased. The reason is that the interaction between polysaccharide stabilizers and casein aggregates became weaker as the lactose is converted into lactic acid. Lactic acid production leads to weakening of bond between casein micelles and stabilizer. These results are according to findings of Gassem and Frank (1991) [17].

Total solids were highly significant during storage. Therefore the Y3 yogurt showed the maximum total solids (19.98%). Minimum total solid content was observed in Y0 (13.58%) at 0 day while Y1 and Y2 exhibited same values of solid contents at 2nd, 11th and 20th day.

Increase in total solids with storage days was due to synersis in yogurt in which water content losses and solid content ratio increases. The results are parallel to the findings of Bahramparvar *et al.* (2009) [25] who investigated that as the concentration of palmate tuber salep increased, the total solids of the ice cream mixes also significantly increased. Table 2 shows that the viscosity of treated samples significantly decreased from 4789.3cP to 3808cP from 2nd to 11th day while it again increased significantly up to 20th day (4261cP. Maximum viscosity was observed in Y1 (5524cP). The total decrease in viscosity for Y0, Y1 and Y2 was observed as 361, 1919 and 11.6 respectively while total increase in viscosity for Y3 was 219cP. Interactive impact showed that Y3 (0.25% *L. royleana*) represented an overall minimum decrease in viscosity during storage. These results are according to the findings of Bahramparvar *et al.* (2009) [25]. The concentration of *L. royleana* increased, hardness of the treated samples also significantly increased. Maximum hardness values were observed in Y0 (5.80g) and Y3 (5.87g) at 0 and 20th day respectively. On the other hand, Y1 showed a minimum hardness value (5.13g) at 20th day. Interactive impact showed that Y3 demonstrated maximum stability towards hardness. These results are in accordance with the findings of Gustaw (2007) [18]. *L. royleana* significant impact on protein content of different treatments in Table 3. Minimum protein contents were observed in Y0 (4.55%) while Y3 showed maximum value of protein. Reason is that seeds of *L. royleana* exhibit about 26% crude protein contents and as the concentration of *L. royleana* increased, protein content of yogurt (except control) also increased. These results were matched with the findings of Qureshi *et al*. (2011) [29].

Table 3 shows that significant effect of ash content with different concentration of *L. royleana*. Minimum ash contents was demonstrated by yogurt containing 0.5% gelatin (0.86%) while ash contents of *L. royleana* enriched yogurt was increased as the concentration of stabilizer increased. Reason is that seeds of *L. royleana* exhibit about 3.6% crude ash contents and as the concentration of *L. royleana* increased, ash contents of yogurt samples (except control) also increased. These results were matched with the findings of Hussein *et al*. (2011) [26].

Table 1. Treatment Plane

Yogurt samples	*Lallemantia royleana* (% w/v)	Gelatin (% w/v)
Y0	-	0.5
Y1	0.15%	-
Y2	0.20%	-
Y3	0.25%	-

Table 2. Effect Lallemantia *royleana* on yogurt's pH, Acidity, Total Solid, WHC, Synersis, Hardness, Fat (%)

		Y0	Y1	Y2	Y3	Mean
pH	0	4.84 a	4.75 b	4.80 ab	4.84 a	4.80 A
	5	4.62 c	4.50 d	4.54 d	4.55 d	4.55 B
	10	4.42 ef	4.38 f	4.44 e	4.44 e	4.42 C
	15	4.20 h	4.16 hi	4.28 g	4.28 g	4.23 D
	20	4.13 ij	4.10 j	4.18 hi	4.20 h	4.15 E
Means		4.44 A	4.38 B	4.45 A	4.46 A	
Acidity (%)	0	0.62 g	0.74 ef	0.51 h	0.38 i	0.56 E
	5	0.69 fg	0.86 cd	0.84 cd	0.82cd	0.80 D
	10	0.78 de	0.88 c	0.98 b	0.99 b	0.91 C
	15	1.02 b	1.01 b	1.04 b	1.04 b	1.03 B
	20	1.15 a	1.20 a	1.17 a	1.20 a	1.18 A
Means		0.85 C	0.94 A	0.91 AB	0.89 BC	
Total Solids	2	13.58 h	14.69 g	15.35 g	17.12 f	15.19 C
	11	17.67 ef	17.96 de	18.22 de	19.40 c	18.31 B
	20	18.62 d	20.46 b	20.81 b	23.42 a	20.83 A
Means		16.62 D	17.70 C	18.13 B	19.98 A	
WHC	0	23.84 e	25.41 d	35.92 a	30.32 c	28.87 A
	5	23.77 e	21.13 g	33.80 b	29.91 c	27.15 B
	10	24.00 e	19.45 i	22.49 f	14.02 l	19.99 C
	15	19.50 hi	18.09 j	20.48 gh	12.33 m	17.60 D
	20	19.10 i	15.59 k	19.09 ij	11.97 m	16.44 E

Means		22.04 B	19.93 C	26.35 A	19.71 C	
Synersis	0	0.63 hi	0.89 g	0.54 i	1.13 f	0.80 E
	5	0.66 h	0.93 g	0.63 hi	1.31 e	0.88 D
	10	0.96 g	0.93 g	0.93 g	1.33 e	1.04 C
	15	1.86 bc	1.80 c	1.77 c	1.64 d	1.77 B
	20	2.03 a	1.96 ab	1.96 ab	2.07 a	2.01 A
Means		1.23 C	1.31 B	1.17 D	1.50 A	
Hardness	0	5.80 a	5.47 cd	5.57 bcd	5.70 ab	5.63 A
	20	5.40 d	5.13 e	5.60 bc	5.87 a	5.50 B
Means		5.60 B	5.30 C	5.58 B	5.78 A	
Fat	0	4.37	5.57	5.70	5.87	5.37 A
	20	4.37	5.43	5.57	5.67	5.25 B
Means		4.37 D	5.50 C	5.63 B	5.77 A	
Viscosity	2	4683.7 b	5524.3 a	4450.3 c	4458.7 c	4789.3 A
	11	3635.3 f	3198.7 g	4173.3 e	4225 de	3808.3 C
	20	4322 cde	3605.3 f	4438.7 cd	4677.7 b	4260.9 B
Means		4213.7 B	4109.4 B	4354.1 A	4443.8 A	

Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

Table 3. Effect *Lallemantia royleana* on protein, ash content of yogurt's (%)

Treatments	Y0	Y1	Y2	Y3
Protein	4.55 b	4.72 ab	4.75 a	4.86 a
Ash	0.86 c	0.90 bc	0.94 ab	0.96 a

Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

Table 4. Effect of Lallemantia *royleana* on yogurt's total viable count and Coliform (CFU/mL)

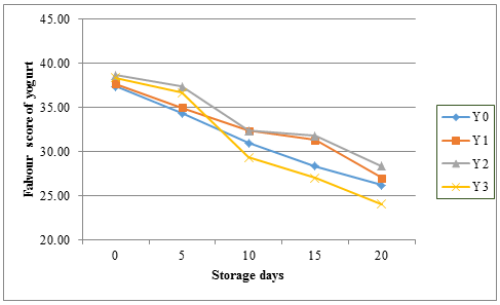
	Storage Days	Treatments				
TPC		Y0	Y1	Y2	Y3	Mean
	2	2.75×10^7 e	2.39×10^7 e	2.48×10^7 e	3.17×10^7 de	2.71×10^7 C
	11	4.46×10^7 c	3.92×10^7 cd	3.81×10^7 cd	4.54×10^7 c	4.18×10^7 B
	20	2.76×10^8 a	2.45×10^8 b	2.69×10^8 a	2.69×10^8 a	2.65×10^8 A
Means		11.59×10^7 A	10.27×10^7 C	11.05×10^7 B	11.55×10^7 AB	
Coliform(cfu/1oo ml)	2	-ve	-ve	-ve	-ve	-ve
	11	-ve	-ve	-ve	-ve	-ve
	20	-ve	-ve	-ve	-ve	-ve
Means		-ve	-ve	-ve	-ve	

Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

It is also cleared from the results in Table 3 shows that as the concentration of *L. royleana* increased, fat content of yogurt also significantly increased. Minimum fat content value was observed in Y0 (4.37%).Yogurt containing 0.25% *L. royleana* demonstrated an overall highest fat content values during storage. Reason is that *L. royleana* seeds contain about 19% of crude oil that results in enhancement of fat content of food. These results were in accordance with Ramirez-Santiago *et al.* (2010) [27].

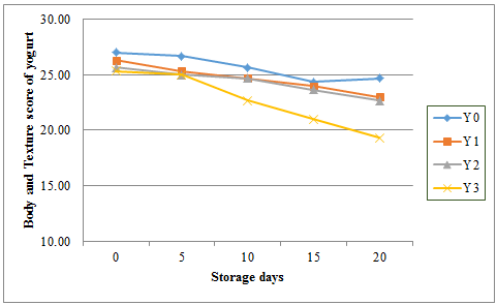
*3.2 Sensory evaluation:*Statistical data revealed that storage days and different concentrations of stabilizers highly significantly ($p \leq 0.01$) affected the flavour of yogurt. Figure 1 showed that the storage period negatively correlated with the flavour of yogurt and it decreased from 38 sensorial score to 26 sensorial score from 0 to 20th day. It is also obvious from the data that Y0 and Y3 got an overall same flavour score while minimum flavour score was attained by Y3 (24) at 20th day of storage. These results are agreed with the findings of Malik *et al.* (2012) [28].

Figure 2. also show that storage period, stabilizers concentrations and their interaction significantly ($p \leq 0.01$) affected the body and texture of yogurt.



Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

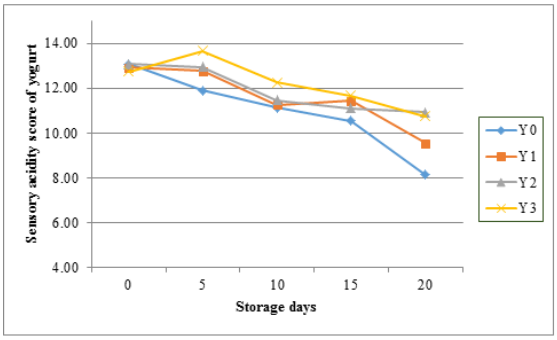
Figure 1. Effect of storage on flavour of yogurt



Y0 = Control (0.5% Gelatin) Y1=0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

Figure 2. Effect of storage on body and texture of yogurt

It indicates that Y0 got an overall maximum score for body and texture followed by Y1, Y2 and Y3. These results are in contrast to the findings of Bahramparvar *et al.* (2009) [25].

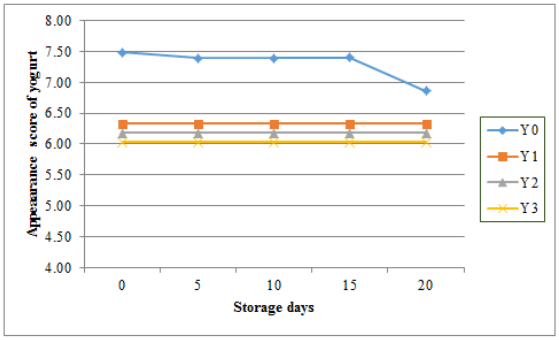


Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

Figure 3. Effect of stabilizer on the Sensory acidity of yoghurt during storage.

It is evident from the Figure 3 that yogurt enriched with 0.25% *L. royleana* got an overall highest sensory acidity score during storage followed by Y2, Y1 and Y0.These results are according to the findings of Ramirez-Santiago *et al*. (2010) [27].

Figure 4 demonstrated that the appearance of yogurt samples was non-significant ($p>0.05$) with respect to the storage time while interaction of storage days and treatments is significantly effect on the appearance of yogurt. In addition, appearance of all yogurt treatments is highly significantly ($p\leq0.01$) different from each other.



Y0 = Control (0.5% Gelatin) Y1 = 0.15% *L. royleana*
Y2 = 0.20% *L. royleana* Y3 = 0.25% *L. royleana*

Figure 4. Effect of stabilizer on the appearance of yoghurt during storage.

Maximum appearance score was perceived at 0 day (6.5) followed by 5th (6.48), 10th (6.48), 15th (6.48) and 20th (6.35) day. Maximum appearance score was given to Y0 at 0 day (7.5) that remained same (non-significant) up to 15th day and significantly decreased (6.86) at 20th day of storage. This showed that consumer perceiving towards *L. royleana* enriched yogurt is less therefore as the concentration of *L. royleana* increased and consumer perceiving towards sensory appearance decreased. Tarakci and Kucukoner (2003) [30] found a decrease in scores of appearance of yogurt during storage.

3.3.Microbiological analysis: Analysis of total viable count in Table 4. Showed that there was highly significant ($p\leq0.01$) effect of storage time, different concentrations of stabilizer and their interaction on yogurt.Y0 (11.59×10^{7}) demonstrated an overall maximum increase in total viable count during storage followed by Y3, Y2 and Y1. Increase in microbial count ultimately resulted in acidity enhancement due to proliferation of microbial activity. Results showed that coliform was absent in milk and it remained absent throughout the storage interval. These findings indicated that if raw material is of good quality, processing, storage conditions etc. are hygienic and packaging is proper then coliforms remain absent till 20th days of storage or more. It may also be due to enhancement in acidity values of yogurt with the passage of time. These results are according to the findings of Malik *et al.* (2012) [28].

4. Conclusion

Data regarding physico-chemical, microbiological and sensory analysis of yogurt revealed that *Lallemantia royleana* treated samples gave an overall acceptable results for pH, titratable acidity, synersis, water holding capacity, hardness, viscosity, microbial and sensory perception. Among different concentrations of *L. royleana*, Y3 (with 0.25% *L. royleana*) gave best results for physical and chemical parameters but Y2 (with 0.20% *L. royleana*) attained highest score for overall sensory acceptability throughout the storage period. Yogurt containing 0.25% *L. royleana* also demonstrated a more bulky flavour with the passage of time while *L. royleana* yogurt attained highest sensory score towards flavour as compare to control. Microbial count was also significantly affected by incorporation of *L. royleana* seeds and *L. royleana* enriched yogurt samples

demonstrated minimum microbial count as compare to control while coliform test remained negative throughout the storage study. At the end, it can be concluded that the usage level of *L. royleana* seeds is quite less (0.15%-0.25%) as compare to gelatin (0.5%) but still it gave better results for all parameters.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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