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Physicochemical, Sensory and Microbiological Characteristics of Yoghurt Produced from Cow's Milk Supplemented with *Aloe vera* Extract during Cooled Storage

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Abstract: The aim of the present study was to assess the effect of *Aloe vera* (AV) addition on yoghurt quality characteristics. The plain yoghurt was prepared using fresh cow's milk and starter culture. AV supplemented yoghurt was prepared containing four different concentrations of AV (5, 10, 15 and 20%). The yoghurt samples were analysed for total solids, fat, protein, ash, total phenolic content, pH and titratable acidity at day 1, 7 and 14 of cold storage (~6±1°C). Also, sensory evaluation and microbiological analysis were conducted. The physicochemical study showed that the pH was to be inversely proportional to the concentration of AV addition in yoghurt. Higher levels of AV decreased pH and increased titratable acidity during storage period. In addition, increased levels of AV decreased total solids, fat, protein and total phenolic contents over time. On the other hand, treatment with AV increased ash content. For sensory properties, the control and 5% AV supplemented yoghurts did not differ for all the sensory attributes evaluated and were more acceptable than 10%, 15% and 20% AV supplemented yoghurts. Based on the microbiological evaluation, the total count and lactic acid bacterial counts decreased in all AV supplemented yoghurts samples at day 7 then increased at day 14 for 5 and afterwards 10% AV supplemented yoghurts.

Key words: Yoghurt · Aloe vera · Cow's Milk

INTRODUCTION

Yoghurt is considered as a nutrient dense food that contains essential nutrients such as carbohydrates, protein, lipids, vitamin and minerals necessary for growth [1]. The nutritional composition of yoghurt varied according to the strains of starter culture used in the fermentation, type of milk used (Whole, semi, or skimmed milk), species that milk is obtained (Bovine, goat, sheep), type of milk solids, solid non-fat, sweeteners and fruits added before fermentation as well as the length of the fermentation process [2].

Apart from the nutritive constituents, consumers also can intake the active bacterial cultures/live microorganisms (Lactic acid bacteria) and the bioactive components formed during fermentation from yoghurt. Yoghurt is required to contain at least 10^7 cfu ml^{-1} of starter culture and a minimum of 10^6 cfu ml^{-1} of labeled microorganisms. A minimum of 0.6 % of titratable acidity is required of yoghurt to make sure the minimum requirements count of microorganisms and expressed as the percentage of lactic acid. However, the regulations are different from country to country [3].

The primary composition and unique live active cultures of yoghurts offer health benefits to humans such as reducing the risk of type 2 diabetes, weight management and prevention of cardiovascular disease. Additionally, lactic acid bacteria have positive effects on the immune and digestive systems and can relieve constipation, diarrheal disease, inflammatory bowel disease, Helicobacter pylori infection and colon cancer [2]. Lactic acid bacteria in yoghurt can also ameliorate lactose intolerance.

In fact, the European Food Safety Authority (EFSA) has suggested that yoghurt can have a positive effect on improving lactose malabsorption [4, 5]. Furthermore, the "Canadian Dairy Commission" emphasized that functional yoghurt, such as prebiotic and probiotic yoghurt, was the

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fastest growing sub-sectors within the yoghurt industry in 2009 [6]. Yoghurt was also regarded as a potential vehicle for probiotic [1-3]. Yoghurt consumption also has benefits in terms of enhancing immunity, controlling inflammation, modulating gut microbiota and improving cholesterol metabolism [2,3].

The use of *Aloe vera* (AV) as a food supplement can be a promising strategy as functional ingredients in the dairy foods. Little information is available regarding the use of AV extract in dairy products. Moreover, there is an increasing demand for taste, quality, stability and shelf life of the yoghurt from customers' side. Therefore, further research needed to investigate the biological activity and the development of *Aloe* constituents in milk products.

People are highly interested in consuming products with natural biological ingredients that have high content of bioactive compounds. Therefore, AV fortified yoghurt was developed to gain a better understanding of the effects of various levels of AV extract on the quality of yoghurt and health of rats after their consumption.

The present study aimed to investigate the effect of addition of AV extract on physicochemical, sensory and microbiological characteristics of yoghurt produced from Cow's milk during cooled storage (~6±1°C) for two weeks.

MATERIALS AND METHODS

Collection of Plant Specimens: Wild *Aloe vera* (AV) plants were collected freshly from El-Arish City, North Sinai, Egypt. The plant leaves used for extractions measured between 40 and 60 cm in length and taken from 3-year-old plants. To avoid biodegradation the AV leaf harvested and pulled carefully from the mother plant to avoid break of rind. Harvested leaves immediately kept in the ice box at 4±1°C to preserve their biological activity and transported to the laboratory.

Preparation of AV *Gel Aqueous* Extract: The fresh AV plant leaves was selected to derive crude gel. The gel collected as described in a previous study [7]. Briefly, the leaf surfaces were thoroughly washed with tap water and later with distilled water to remove traces of dirt and soil. The fleshy mass of the AV was carefully opened by cutting the sharp edges. The gel was funnelled into a sterile beaker. 10 and 20 g of AV gel were weighed using a digital microsensitive scale. Each of these was then diluted with 100 ml of distilled water to constitute 10 and 20 percentage (m/v) AV concentrations respectively. These were gently processed with a kitchen blender to achieve homogenous solution. This liquid was kept for 20 min to settle and later sieved using Whatman filter paper (No. 1) to obtain a particulate-free gel aqueous extract. The AV gel aqueous extract was freshly prepared every time before use. It contained all the functional ingredients of the crude gel in the same proportion as it appears in the leaf.

Collection of Milk: Fresh cow's milk, obtained from private farm, in El-Arish City, North Sinai, Egypt.

Yoghurt's Starter Culture: Commercial yoghurt culture (YC-X11, Thermophilic yoghurt culture - YoFlex®), containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus,* was obtained from CHR-Hansen, Horsholm, Denmark.

Manufacturing of Yoghurt: Fresh cow's milk pasteurized at 90°C for 20 min then cooled to 42°C and inoculated with 3% (v/v) Yoghurt's starter culture (YC-X11). The plain yoghurt was prepared without addition of AV gel aqueous extract and was used as a control (0% *Aloe vera*). *Aloe vera* yoghurts were prepared by mixing milk and starter with different concentrations (5%, 10%, 15% and 20%) of AV extract, which was previously pasteurized (90°C for 20 min). Yoghurt samples were incubated at 42°C for 4 h until complete coagulation, cooled to refrigerator temperature (~6±1°C) and then stored for 14 days. Analyses were conducted at 7-day intervals.

Yoghurt Samples Analyses

Physicochemical Analyses

Determination of the Total Solids Content: Total solids were determined according to the method no. 952.23 described in AOAC [8]. The standard Gerber method for determination of yoghurt fat was used [9]. Protein content of yoghurt samples was determined by Kjeldahl method 991.20 of AOAC [8]. The ash content was analyzed based on the modified AOAC method 940.26 [8]. The total phenolic content of yoghurt samples was determined by Folin-Ciocalteu method Ivanova *et al.* [10]. Electronic digital type of pH meter (Jenway 3505, Italy) was used for pH determination according to method given in AOAC [8]. Titratable acidity was determined by direct titration method no. 947.05 of AOAC [8].

Sensory Evaluation: Sensory evaluation of produced yoghurt was carried out at first days of storage using ten-point (For flavour) and five-points (For consistency and appearance) hedonic scale method as described previously [11].

Microbiological Analysis: All media were obtained in a dehydrated form stored in hygroscopic environment in a cool dry place, away from light and prepared according to the manufactures instructions. Lactic Agar was used for counting of lactic *Streptococci* and *Lactobacilli* by pour plate technique [12].

RESULTS

Effect of *Aloe vera* Addition on Physicochemical Characteristics of Yoghurt

Total Solids Content: Table 1 shows the means for total solids content of control (T_0) and those supplemented with different concentrations of *Aloe vera* (AV) (5, 10, 15 and 20%) during storage period (14 days) at ~6±1°C. Total solids content ranged from 10.53 to 14.49 percent during storage period. The highest total solids content was observed in control treatment (14.49%) and the lowest total solids content was observed in treatment T_4 (10.53%). All yoghurts were significantly ($P< 0.05$) differed from each other. The results revealed that as the addition of different levels AV increases the total solids content of product goes on decrease. In addition, the total solids content decreased throughout the days of monitoring for both control and AV supplemented yoghurts.

The statistical results indicates the total solids content in yoghurt supplemented with different AV concentrations varied significantly ($P < 0.05$) compared to the control yoghurt at 7 and 14 days of storage. The effect of AV addition and their interaction with storage days found to be significant ($P < 0.05$) on total solids contents in various treatments compared to those treatments after 7 days of storage. As the storage time increased, the percentage of total solids content was decreasing, because the total solids content was very lower in 10% AV yoghurt samples at 14 days of storage.

In addition, the results exhibited that initial total solids content in yoghurt samples supplemented with different AV concentrations decreased with a significant effect during storage up to 14 days compared to the control yoghurt. It is clear that increasing the AV level significantly decreased total solids content of all yoghurt samples. The result was in agreement with the result of the Mukhekar and Desale [13] study, which found that the total solids content of product decreased gradually with increase in concentration of AV.

Fat Content: Table 2 presents the obtained results for the fat content of yoghurt supplemented with concentrations of AV during storage period of 14 days. The average fat content of yoghurt samples ranged from 2.65 to 3.55 percent during storage. Control yoghurt had the highest fat content (3.55% at 7 days of storage), while 10% AV supplemented yoghurt had the lowest fat content (2.65% at 14 days of storage). The fat content in control yoghurt samples appeared to be higher than that observed in AV supplemented yoghurt samples within the same storage conditions.

The statistical results indicates the fat content in yoghurt treated with different AV concentrations varied significantly ($P < 0.05$) compared to the control yoghurt at 7 and 14 days of storage. The effect of AV addition and their interaction with storage days found to be significant ($P < 0.05$) on fat contents in various treatments compared to those treatments after 7 days of storage. As the storage

Table 1: Mean values for total solids content of yoghurt during cold storage (14 days).

		Total Solids %		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	14.49±1.84	14.31±0.59	14.26±0.80
T1 (AV 5%)	+ *Aloe vera* 5%	11.96±0.87	14.04±0.59	11.86±0.85
T2 (AV 10%)	+ *Aloe vera* 10%	12.49±0.55	12.55±0.97	12.47±0.59
T3 (AV 15%)	+ *Aloe vera* 15%	11.42±0.48	11.96±0.60	12.08±0.94
T4 (AV 20%)	+ *Aloe vera* 20%	10.63 ±0.39	10.53±0.16	12.84±0.50

Values are means ± SEM for 3-5 samples per treatment.

Table 2:Mean values for fat content of yoghurt during cold storage (14 days).

		Fat %		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	3.20±0.12	3.55±0.03	3.47±0.03
T1 (AV 5%)	+ *Aloe vera* 5%	2.90±0.21	3.40±0.00	3.15±0.03
T2 (AV 10%)	+ *Aloe vera* 10%	2.90±0.12	3.00±0.00	2.65±0.03
T3 (AV 15%)	+ *Aloe vera* 15%	3.00±0.12	3.30±0.06	3.05±0.03
T4 (AV 20%)	+ *Aloe vera* 20%	2.90 ±0.06	3.33±0.06	2.85±0.03

Values are means ± SEM for 3 samples per treatment.

Table 3: Mean values for protein percentage of the final yoghurt product during 14 days storage.

		Protein %		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	5.10	5.52	4.31
T1 (AV 5%)	+ *Aloe vera* 5%	3.97	4.88	3.35
T2 (AV 10%)	+ *Aloe vera* 10%	3.42	4.13	3.79
T3 (AV 15%)	+ *Aloe vera* 15%	3.28	3.60	3.46
T4 (AV 20%)	+ *Aloe vera* 20%	3.44	4.67	3.10

Table 4: Ash content of yoghurt product over a 14-day storage period.

		Ash %		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	1.01±0.01	0.94±0.00	0.93±0.00
T1 (AV 5%)	+ *Aloe vera* 5%	1.04±0.01	0.97±0.00	0.96±0.03
T2 (AV 10%)	+ *Aloe vera* 10%	1.31±0.33	1.02±0.01	0.97±0.02
T3 (AV 15%)	+ *Aloe vera* 15%	1.51±0.24	1.04±0.01	0.98±0.03
T4 (AV 20%)	+ *Aloe vera* 20%	1.59±0.34	1.04±0.01	1.00±0.03

Values are means ± SEM for 3 samples per treatment.

time increased, the percentage of fat content was decreasing, because fat content was very lower in 10% AV yoghurt samples at 14 days of storage.

In addition, the results showed that initial fat content in yoghurt samples supplemented with different AV concentrations decreased with a significant effect during storage up to 14 days compared to the control yoghurt. It is clear that increasing the addition level of AV significantly decreased fat content of all yoghurt. This might be due to the higher moisture content in AV [13]. Yadav and Shukla [14] reported similar trend of decreasing fat percentage in herbal yoghurt.

Protein Content: Table 3 presents the obtained results for the protein content of the AV supplemented yoghurt samples during storage period of 14 days. The average protein content of yoghurt samples ranged from 3.10 to 5.52 percent during storage. Control yoghurt had the highest protein content (5.52% at 7 days of storage), while 20% AV yoghurt had the lowest protein content (3.10% at 14 days of storage). The protein content in control yoghurt samples appeared to be higher than that observed for AV yoghurt samples within the same storage conditions.

The results indicates the protein content in yoghurt supplemented with different AV concentrations numerically decreased compared to the control yoghurt at 7 and 14 days of storage. The effect of AV addition and their interaction with storage days found to be apparently increasing the protein contents in various treatments compared to those treatments after 7 days of storage. As the storage time increased, the percentage of protein content was decreasing, so that the lowest protein content was observed in 20% AV yoghurt samples with 14 days of storage.

In addition, the results exhibited that initial protein content in yoghurt samples treated with different AV concentrations reduced with an adverse effect during storage up to 14 days compared to the control yoghurt. It was clear that increasing the AV level significantly decreased protein content of all yoghurt samples as shown in the figure below.

Ash Content: Ash content in yoghurts (Control, 5, 10, 15 and 20% AV) were as shown on Table 4. The ash content was examined on regular intervals of 7 days up to 14 days storage. Ash content ranged from 0.93 to 1.59. Initial ash content was 1.01, 1.04, 1.31, 1.51 and 1.59 percent for control, 5, 10, 15 and 20% AV respectively. As the addition of different levels AV increases, the ash content of yoghurt goes on increase.

On 7th day, the control and *Aloe vera* supplemented yoghurt samples showed decline in ash content values up to 0.94, 0.97, 1.02, 1.04 and 1.04 percent for control, 5, 10, 15 and 20% AV respectively. The AV supplemented yoghurt samples showed the highest ash content values compared to control yoghurts. The effect of AV addition found to be significant ($P < 0.05$) on ash contents in various concentrations compared to control yoghurt.

Compared to 7 days storage, ash content was lower for control, 5, 10, 15 and 20% AV gradually (0.93, 0.96, 0.97, 0.98 and 1.00 percent) respectively at 14th day storage. However, high levels of AV resulted in higher ash content.

Table 5: Mean values for total phenolic content of yoghurt during storage (14 days).

		Total Phenolic Content (mg/g)		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	0.904	0.741	0.836
T1 (AV 5%)	+ *Aloe vera* 5%	0.895	0.638	0.649
T2 (AV 10%)	+ *Aloe vera* 10%	0.694	0.758	0.688
T3 (AV 15%)	+ *Aloe vera* 15%	0.695	0.454	0.448
T4 (AV 20%)	+ *Aloe vera* 20%	0.688	0.904	0.683

Table 6: pH of yoghurt made with AV extract during storage at 4±1°C for 14 days.

		pH		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	4.52±0.01	4.52±0.01	4.47±0.03
T1 (AV 5%)	+ *Aloe vera* 5%	4.51±0.01	4.51±0.01	4.43±0.01
T2 (AV 10%)	+ *Aloe vera* 10%	4.51±0.01	4.48±0.01	4.41±0.05
T3 (AV 15%)	+ *Aloe vera* 15%	4.50±0.00	4.47±0.00	4.39±0.04
T4 (AV 20%)	+ *Aloe vera* 20%	4.47±0.00	4.43±0.01	4.34±0.02

Values are means ± SEM for 3 samples per treatment.

Total Phenolic Content: Table 5 presents the results obtained for the total phenolic content of the different yoghurt samples during storage up to 14 days. The average total phenolic content of the prepared yoghurt samples ranged from 0.448 to 0.904 (Gallic acid equivalent mg/g) during storage. Control and 20% AV yoghurts had the highest total phenolic content (0.904 mg/g) at 1 and 7 days of storage respectively, while 15% AV yoghurt had the lowest total phenolic content (0.448 mg/g) at 14 days of storage. The total phenolic content in control yoghurt samples appeared to be higher than that recorded in AV yoghurt samples within the same storage conditions except after 7 days storage where 20% AV yoghurt had the higher value.

The results indicates the total phenolic content in yoghurt treated with different AV concentrations numerically decreased compared to the control yoghurt at 7 and 14 days of storage. The effect of AV addition and their interaction with storage days found to be apparently increasing the total phenolic contents in various treatments compared to those treatments after 7 days of storage. As the storage time increased, the content of total phenolic was decreasing, so that the lowest total phenolic content was observed in 15% AV yoghurt samples with 14 days of storage.

In addition, the results exhibited that initial total phenolic content in yoghurt samples treated with different AV concentrations reduced with an adverse effect during storage up to 14 days compared to the control yoghurt. It was clear that increasing the AV level significantly decreased total phenolic content of all yoghurt samples as shown in the figure below.

pH: Table 6 shows the average pH values for the control yoghurt and AV supplemented yoghurt samples over a 14-day storage period. The pH values of the examined yoghurt ranged between 4.34-4.52. Comparing pH values of samples revealed that the highest value observed with the control yoghurt samples while the samples supplemented with AV were lower in pH values than other samples. All treatments were significantly ($P< 0.05$) differed from each other.

The initial pH value for the control yoghurt samples were 4.52 while 4.47 was pH value observed at the end of storage period for the 14 days. These results indicate that there is a decline in pH for all the control yoghurt samples, as the storage time increased.

The average pH values of the control yoghurt samples remained almost unchanged for the 7 days of refrigerated storage and then declined slightly during cold storage, an effect commonly known as "Post acidification" [15]. This stability in pH was because of lower activities of starter culture microorganism during refrigeration storage.

Similarly, the average pH values of the 5% AV yoghurt remained almost unchanged for the seven days of refrigeration at ~6±1°C and then it decreased slightly at the end of storage period. The change in the AV yoghurt samples occurred at the day 7.

On the other hand, all yoghurt samples declined in pH units over storage periods indicating the development of acidity. However, the pH observed did not fall below levels that generally considered detrimental to the survival of starter culture microorganism for samples stored at ~6±1°C. Furthermore, sensitivity of starter culture to low pH is species and strain dependent.

Table 7: The titratable acidity of yoghurt supplemented with AV extract during storage (14 days).

		Titratable Acidity (%)		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	0.66±0.03	0.78±0.02	0.91±0.01
T1 (AV 5%)	+ *Aloe vera* 5%	0.73±0.03	0.81±0.01	0.96±0.02
T2 (AV 10%)	+ *Aloe vera* 10%	0.74±0.00	0.90±0.00	0.97±0.04
T3 (AV 15%)	+ *Aloe vera* 15%	0.75±0.05	0.91±0.00	1.00±0.06
T4 (AV 20%)	+ *Aloe vera* 20%	0.84±0.00	0.96±0.02	1.01±0.01

Values are means ± SEM for 3 samples per treatment.

Table 8: Sensory evaluation of fresh yoghurt samples made with AV extract.

		Sensory Scores (Points)			
Treatment	Yoghurt	Appearance	Consistency	Flavour	Total
T0 (Control, AV 0%)	+ *Aloe vera* 0%	5.00±0.00	5.00±0.00	9.50±0.55	19.50±0.32
T1 (AV 5%)	+ *Aloe vera* 5%	4.83±0.41	4.67±0.52	8.50±1.64	18.00±0.68
T2 (AV 10%)	+ *Aloe vera* 10%	4.83±0.41	5.00±0.00	8.67±1.37	18.50±0.70
T3 (AV 15%)	+ *Aloe vera* 15%	4.17±0.75	4.50±0.55	7.17±1.94	15.83±0.75
T4 (AV 20%)	+ *Aloe vera* 20%	3.50±1.38	3.38±1.17	6.17±1.72	13.50±0.28

Values are means ± SDM for 10 panelists for each.

Titratable Acidity: Table 7 presents the titratable acidity of yoghurts, which ranged from 0.66% to 1.01%. The control yoghurt samples (0% AV) had lower acidity values followed by 5, 10, 15 and 20% AV. All treatments were significantly ($P < 0.05$) differed from each other. AV fortified yoghurt had the highest value, when compared with the control yoghurt. Acidity increases with increase of the addition level of AV extract in yoghurt and over time.

The average acidity values of the control yoghurt remained almost unchanged for 7 days and then it declined slightly during cold storage, an effect commonly known as "Post acidification" [15]. This stability in acidity is due to lower activities of starter culture microorganisms during refrigerated storage for the 14 days at ~6±1°C.

Similarly, the average acidity values of the 5% AV supplemented yoghurt remained almost unchanged for seven days and then it decreased slightly at the end of storage period for the 14 days. The change in the AV supplemented yoghurt samples occurred in the day 7.

On the other hand, all yoghurt samples declined in acidity over storage periods indicating the development of acidity. However, the acidity observed did not fall below levels that generally considered detrimental to the survival of starter culture [16] for samples stored at ~6±1°C.

Effect of *Aloe Vera* Addition on Sensory Quality of Yoghurt: Table 8 shows the mean score of different sensory evaluation for all fresh developed yoghurt samples (After 1 day of manufacture). Appearance score obtained for control and four AV treatments ranged from 3.50 to 5.00. Control yoghurts obtained the highest score (5.00) for appearance while 20% yoghurts obtained the lowest score (3.50). The appearance score of control yoghurts was 5.00 on the 1th day of storage and it gradually decreased to reach 4.83, 4.83, 4.17 and 3.50 as AV added to yoghurts in concentration of 5, 10, 15 and 20% respectively. It was clear that as the addition level of AV increases the appearance score of AV yoghurts samples goes on decreasing.

The scores for consistency of all yoghurts ranged from 3.83 to 5.00. The control and 10% AV yoghurts had the maximum score for consistency (5.00) and 20% yoghurts samples showed the lowest scores (3.83). The consistency score of control and 10% AV yoghurts was 5.00 on the 1th day of storage and it gradually decreased to reach 4.67, 4.50 and 3.38 as AV added to yoghurt in concentration of 5, 15 and 20% respectively. It obvious that as the level of AV increases the consistency score of AV yoghurt goes on decreasing.

The scores for flavour ranged from 6.17 to 9.50. The highest score observed in control yoghurt samples (9.50) and the lowest score by the 20% yoghurts (6.17) for flavour. The flavour score of control yoghurts was 5.00 on the 1th day of storage and it gradually decreased to reach 4.83, 4.83, 4.17 and 3.50 as AV added to yoghurts in concentration of 5, 10, 15 and 20% respectively. This result suggested that as the level of AV increases the flavour score of AV yoghurts goes on decreasing.

Table 9: Total bacterial count (Log cfu ml^{-1}) of fresh yoghurt supplemented with AV extract.

		Total Bacterial Count (log cfu ml^{-1})		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	9.76	9.58	9.54
T1 (AV 5%)	+ *Aloe vera* 5%	9.54	8.51	9.64
T2 (AV 10%)	+ *Aloe vera* 10%	9.40	8.32	9.52
T3 (AV 15%)	+ *Aloe vera* 15%	9.43	8.32	8.49
T4 (AV 20%)	+ *Aloe vera* 20%	9.41	8.30	8.46

Table 10: Lactic acid bacteria count (Log cfu ml^{-1}) of fresh yoghurt supplemented with AV extract.

		Lactic Acid Bacteria (log cfu ml^{-1})		
Treatment	Yoghurt	Day 1	Day 7	Day 14
T0 (Control, AV 0%)	+ *Aloe vera* 0%	9.54	8.57	9.72
T1 (AV 5%)	+ *Aloe vera* 5%	9.30	8.43	8.77
T2 (AV 10%)	+ *Aloe vera* 10%	9.45	8.26	8.59
T3 (AV 15%)	+ *Aloe vera* 15%	9.43	8.28	8.54
T4 (AV 20%)	+ *Aloe vera* 20%	9.34	8.20	8.54

Effect of *Aloe vera* Addition on Microbiological Properties of Yoghurt

Total Bacterial Count (TBC): Table 9 shows the mean of the total bacterial count (TBC) for all yoghurts during storage for 14 days. TBC obtained for control and four treatments ranged from 8.30 to 9.76 log cfu ml^{-1} Control showed the highest mean (9.76) for TBC while T_4 obtained the lowest mean (8.30). It also observed that as the level of AV increases the TBC of yoghurt goes on decreasing.

At 7th day of study the TBC decreased in all yoghurts compared to those at 1st day then increased gradually after 14th day and showed good viability for 14th days.

Lactic Acid Bacteria (LAB): Table 10 shows the mean of the lactic acid bacteria (LAB) for all yoghurts during storage for 14 days. LAB obtained for control and treatments ranged from 8.20 to 9.72 log cfu ml^{-1} Control showed the highest mean (9.72) for LAB while T_4 showed the lowest mean (8.20). This suggests that the level of AV increases the LAB of yoghurt goes on decreasing.

At 7th day of study the LAB decreased in all yoghurt samples compared to those at 1st day then increased gradually after 14th day and showed good viability for 14th days.

DISCUSSION

The present research work aimed to evaluate the effect of the addition of *Aloe vera* (AV) gel aqueous extract on the physicochemical, organoleptic and microbiological properties of yoghurt prepared from Cows' milk. AV extract is rich in phytonutrients and reported to have various health benefits [17, 18]. However, when consumed, its flavour is bitter. Therefore, the objectives of the work were to test the *in vivo* and *in vitro* effects of different concentrations of the AV extract on various functional and quality parameters of fresh cow's milk yoghurt and during 14 days cold storage at ~6±1°C.

The results revealed that the total solid of yoghurt was decreased with increasing the percent of AV addition in a dose dependent manner. In addition, the total solids content decreased throughout the days of monitoring for both control and AV enriched yoghurts.

This result was in agreement with the result of the Mukhekar and Desale [13] study, which found that the total solids content of product decreased gradually with increase in concentration of AV. The significant decrease in total solids could be due to the constitution of the plant itself, since it consists mostly of water, as mentioned by Pandey and Singh [19].

Fat play an important role in improving the consistency of yoghurt and provide more energy (Over twice as much) than carbohydrate and protein [20]. The fat content, in the case of whole yoghurt, contributes to the viscosity and texture of the product and helps to avoid syneresis [21].

In this study, the fat percentage in control yoghurt samples appeared to be higher than recorded in AV yoghurt samples within the same storage conditions. As the storage time increased, the percentage of fat content was decreasing and the lowest fat content was observed in 10% AV yoghurt samples with 14 days of storage.

It became obvious that increasing the AV level significantly decreased fat content of all yoghurt samples. This might be due to the higher moisture content in AV [13]. Yadav and Shukla [14] reported similar trend of decreasing fat percentage in herbal yoghurt.

Jenness [22] reported that difference in animal breed, weather, type of feed, age of animal, stage of lactation are the major reasons of changing composition of raw milk and those changes may be affected on the nutritional composition of prepared drinking yoghurt.

The result obtained in the present experiment showed that ash content increased gradually with increase in AV concentrations during storage period. These results may be due to two reasons: First, there is a direct relationship between acidity and syneresis in yoghurt. Second, the longer fermentation time for yoghurts could lead to greater proteolysis and it lead to the more syneresis in produced yoghurt [23].

Ghadge *et al*. [24] observed similar results with respect to ash content of yoghurt and recorded 1.40 percent ash content in yogurt. The difference in ash content may be due to insoluble solids and fiber content that may contribute in increasing the ash content**.** Rajasekaran and Aathishsekar [25] reported similar results.

Appropriate pH and acidity is important factors to assure the quality of yoghurt [26]. In our study, increasing the AV concentration led to decreasing of the pH value. In contrast, the acidity of the final product is increased a result of increasing AV levels. This might be due to the microorganism's activity during fermentation in which residual enzymes produced by starter that decrease the pH of the final product [13]. The decline in pH can be an effective way to inhibit spoilage and pathogenic microorganisms [27].

The average pH and TA values of the control and 5% AV yoghurt samples remained almost unchanged at the end of storage period for the 7 days refrigeration at ~6±1°C and then declined slightly during cold storage, an effect commonly known as "Post acidification" [15]. This stability in pH and TA was because of lower activities of starter culture microorganism during refrigeration storage at 4°C.

On the other hand, all yoghurt samples declined in pH and TA units over storage periods indicating the development of acidity. However, the pH recorded did not fall below levels that generally considered detrimental to the survival of starter culture microorganism for samples stored at ~6±1°C. Furthermore, sensitivity of starter culture to low pH is species and strain specific [16].

These results were in agreement with the result of the Shah and Ravula [28] and the Panesar and Shinde [29] study, which found that post-acidification of the yoghurt due to the activities of lactic acid bacteria (LAB), decreased at 4°C.

The sensory attributes evaluated during the storage of the yoghurt samples include appearance, flavour, consistency and total acceptability. An optimum consistency is desirable in case of fermented products [30] to achieve maximum consumer acceptability. Flavour, a combination of smell and taste, changes with chemical or compositional characteristics of the final product. The consumers will not accept too thin or too thick consistency in yoghurts.

Among four sensory attributes, it also observed that as the level of AV increases the sensory quality of yoghurt goes on decreasing. The AV extract had a significant effect on appearance, consistency, flavor and total acceptability and this effect was more noticeable in higher concentration of AV extract. Addition of AV extract may decrease the formation of coherent and uniform consistency. In addition, milk fat level had an enhancing effect on flavour, texture, appearance and overall acceptability scores, whereas AV had a negative effect.

In contrast, Panesar and Shinde [29] found that increases in AV concentration lead to an acceptable range of consistency score in sensory evaluation test. Manoharan *et al.* [31] and Manoharan and Ramasamy [32] made herbal ice cream with different inclusion levels of AV pulp for organoleptic evaluation. The authors suggested that increased levels of AV upto 20% resulted in maximum sensory scores.

Fermented dairy foods are the most accepted food carriers for delivery of viable probiotic cultures to the human gastrointestinal tract [33]. However, the viability of probiotic bacteria in yoghurt or other fermented milk drinks can be unstable, depending on a number of factors that include the types of strains used, the interaction between the bacterial species present, the chemical composition of the fermentation medium and final acidity of the product [34].

Since, the present study involves the addition of AV extract as a functional ingredient. The acidic nature and compositional characteristics of AV extract may affect the growth characteristics of the Lactic Acid Bacteria (LAB) employed in this study. We have set out to study the influence of AV addition on the growth of LAB and total bacterial count (TBC) in the produced yoghurts.

The results of microbiological examination of all yoghurt samples show that the count of LAB and TBC decreased markedly in the 7th day compared to the 1st day of storage period. However, the control and 5% AV yoghurt samples showed a positive influence on the growth of counted bacteria in the 14th day and afterwards samples with 10% AV. In contrary to this, the yoghurt samples with 15% AV and 20% AV had an adverse effect on the growth of LAB and TBC in the 14th day of storage period. This effect, at 7th day of storage, may be due to either the acidic nature or the antimicrobial impact of AV that may inhibit the growth of yoghurt culture strains or total bacteria during the storage period. This finding is in agreement with the results of Panesar and Shinde [29] who also reported the viable counts of *L. acidophilus La-5* decreased significantly in fortified yoghurt with AV after 28 days of refrigerated storage.

At 14th day of storage, the low level of AV extract counteracted on the adverse effect seen in the first week of storage. This effect may be du the availability of nutrients and growth promoters present in AV extracts. However, in our study the high level of AV fail to counteract the adverse effect, there are several earlier studies indicated that increasing the density of AV has a positive influence on the growth of probioticbacteria in yoghurt [35, 36]. The reason may be due to the type of bacterial culture studied or the method of AV extract preparation.

CONCLUSION

From the obtained results addition of *Aloe vera* at level up to 5% to yoghurt could be recommended. On the other hand this ratio led to decreasing in the values of total solids, fat, protein, total phenolic and pH. But, this ratio led to increasing in the values of ash and acidity. Also, improved the microbiological quality and organoleptic properties of yoghurt.

REFERENCES

1. Tamime, A.Y. and R.K. Robisons, 2007a. Background to Manufacturing Practice. In Tamime and Robinson's Yogurt: Science and Technology; 3rd Edn.; Woodhead Publishing: Cambridge, UK, pp: 11-118.
2. Frias, J., C. Martinez-Villaluenga and E. Peñas, 2016. Fermented foods in health and disease prevention; Elsevier: London, UK.
3. Marette, A., E. Picard-Deland and M.A. Fernandez, 2017. Yogurt: Roles in nutrition and impacts on health; CRC Press: Boca Raton USA, pp: 192.
4. EFSA, 2010. Scientific Opinion on the substantiation of health claims related to yoghurt cultures and improving lactose digestion. EFSA Journal, 8: 1763-1781.
5. Zare, F., J.I. Boye, V. Orsat, C.P. Champagne and B.K. Simpson, 2011. Microbial, physical and sensory properties of yogurt supplemented with lentil flour. Food Research International, 44: 2482-2488.
6. CDC, 2009. Supply Management. http:// www.cdc.ca/ cdc/ index_en.asp?caId=812 (Accessed April 25, 2009).
7. Oyeyemi, M.O. and O.S. Ajani, 2015. Haematological parameters, semen characteristics and sperm morphology of male albino rat (Wistar strain) treated with *Aloe vera* gel. Journal of Medicinal Plants Research, 9: 510-514.
8. AOAC, 2000. Official Method of the Association of Official Analytical Chemists; 17th Edn.; AOAC: Washington, DC., USA, pp: 21-447.
9. Ling, E.R., 1963. A text book of Dairy chemistry; 3rd Edn.; Chapman and Hall Ltd.: London UK, pp: 16-80.
10. Ivanova, V., M. Stefova and F. Chinnici, 2010. Determination of the polyphenol contents in macedonian grapes and wines by standardized spectrophotometric methods. J. Serb. Chem. Soc., 75: 45-59.
11. Bodyfelt, F.W., J. Tobias and G.M. Trout, 1988. The Sensory Evaluation of Dairy Products; Van Nostrand Reinhold: New York, USA, pp: 598.
12. Elliker, P.R., A.W. Anderson and G. Hanesson, 1956. An agar culture medium for lactic acid *Streptococci* and *Lactobacilli*. J. Dairy Science, 39: 1611-1612.
13. Mukhekar, A. and R.J. Desale, 2018. Studies on Physicochemical Properties of Yogurt Fortified with *Aloe vera*. Multilogic in Science, 7: 141-142.
14. Yadav, K. and S. Shukla, 2014. Microbiological, physicochemical analysis and sensory evaluation of herbal yogurt. The Pharma Innovation Journal, 3: 1-4.
15. Ng, E.W., M. Yeung and P.S. Tong, 2011. Effects of yogurt starter cultures on the survival of *Lactobacillus acidophilus*. International Journal Food Microbiology, 145: 169-175.
16. Dave, R.I. and N.P. Shah, 1997. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. International Dairy Journal, 7: 31-41.

17. Yagi, A., S. Hegazy, A. Kabbash and E. Abd-El Wahab, 2009. Possible hypoglycemic effect of *Aloe vera* L. high molecular weight fractions on type 2 diabetic patients. Saudi Pharm. J., 17: 209-215.
18. Radha, M.H. and N.P. Laxmipriya, 2014. Evaluation of biological properties and clinical effectiveness of *Aloe vera*: A systematic review. J. Tradit. Complement. Med., 5: 21-26.
19. Pandey, A. and S. Singh, 2016. *Aloe vera*: A Systematic Review of its Industrial and Ethno-Medicinal Efficacy. Int. J. Pharm. Res. Allied Sci., 5: 21-33.
20. Ehirim, F.N. and E.N. Onyeneke, 2013. Physicochemical and organoleptic properties of yoghurt manufactured with Cow milk and Goat milk. Nat. Appl. Sci., 4: 245-252.
21. Izadi, Z., A. Nasirpour, G.A. Garoosi and F. Tamjidi, 2014. Rheological and physical properties of yogurt enriched with phytosterol during storage. Journal of Food Science and Technology, 52: 5341-5346.
22. Jenness, R., 1980. Composition and characteristics of goat milk: review 1968-1979. Journal of Dairy Science, 63: 1605-1630.
23. Michael, M., R.K. Phebus and K.A. Schmidt, 2010. Impact of a plant extract on the viability of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in nonfat yogurt. International Dairy Journal, 20: 665-672.
24. Ghadge, P.N., K. Prasad and P.S. Kadam, 2008. Effect of fortification on the physico-chemical and sensory properties of buffalo milk yoghurt. Electron. J. Environ. Agric. Food Chem, 7: 2890-2899.
25. Rajasekaran, S. and D. Aathishsekar, 2007. Therapeutic evaluation of *Aloe vera* leaf gel extract on glycoprotein components in rats with streptozotocin diabetes. J Pharmacology Toxicology, 2: 380-385.
26. Azari-Anpar, M., H. Payeinmahali, A.D. Garmakhany and A.S. Mahounak, 2017. Physicochemical, microbial, antioxidant and sensory properties of probiotic stirred yoghurt enriched with *Aloe vera* foliar gel. J. Food Process Preserv, 41: e13209.
27. Garrote, G.L., A.G. Abraham and G.L. De Antoni, 2000. Inhibitory Power of Kefir: The Role of Organic Acids. J. Food Prot, 63: 364-369.
28. Shah, N. and R. Ravula, 2001. Freezing conditions frozen out. Dairy Industries International, 10: 22-26.
29. Panesar, P.S. and C. Shinde, 2011. **Efiect** of storage on syneresis, pH, *Lactobacillus acidophilus* count, *Bifdobacterium bifdum* count of *Aloe vera* fortified probiotic yoghurt. Current Research in Dairy Sciences, 4: 17-23.
30. Lee, E.H. and S.D. Choi, 1994. Studies on the manufacture of aloe yoghurt. J. Agric. Technol. Res. Institute Chinju University (Korea Republic), 7: 55-59
31. Manoharan, A.P., D. Ramasamy, C.N. Kumar, B. Dhanalashmi and V. Balakrishnan, 2012. Organoleptic evaluation of herbal ice creams prepared with different inclusion levels of *Aloe vera* pulp. Indian J. Med. HC., 1: 55-59.
32. Manoharan, A.P. and D. Ramasamy, 2013. Physicochemical, microbial and sensory analysis of *Aloe vera* (pulp) ice cream with natural colour in different artificial sweeteners. Indian J. Fundamental & Appl. Life Sci., 3: 114-121.
33. Kianoush, K.D. and K.M. Reza, 2008. Probiotics in milk and dairy products, Marzedanesh Press, First edition, pp: 12-1.
34. Shah, N.P., J.F. Ali and R.R. Ravula, 2000. Populations of *Lactobacillus acidophilus*, *Bifidobacterium* spp. and *Lactobacillus casei* in commercial fermented milk. Biosci. Microflora, 19: 35-39.
35. Parmjit, S.P. and C. Shinde, 2012. Effect of storage on Syneresis, pH, *Lactobacillus acidophilus* count, *Bifidobacteriumbifidum* count of *Aloe vera* fortified probiotic yoghurt. Curr. Res. Dairy. Sci., 4: 17-23.
36. Houshang, J., M.M. Hossein, Y. Mohaddese, K.J. Zahra and R. Elham, 2014. Studying the influence of *Aloe vera* on the growth of the *lactobacillus acidophilus* and *Bifidobacterium* Bifidum Probiotic bacterias in producing probiotic milk and yoghurt. UJPBS, 02: 16-20.