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DEVELOPMENT OF *ALOE VERA* (*ALOE BARBADENSIS* MILLER) PROBIOTIC *LASSI* USING *LACTOBACILLUS FERMENTUM* BACTERIA

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The aim of this research was to investigate the effect of *Aloe vera* gel on the quality of probiotic *lassi*. Probiotic *lassi* was prepared by adding 15% of *Aloe vera* gel and inoculating with probiotic *Lactobacillus fermentum* strain. Results showed that physicochemical properties of fortified probiotic *lassi* were not affected by these modifications during the storage period ($P>0.05$), however, antioxidant activities, texture and aroma sensory parameters differed significantly ($P<0.05$). *Lactobacillus* and *Streptococcus* counts exhibited same behavior in *Aloe vera* probiotic *lassi* products as in control ($P>0.05$) after 21 days of storage. *Aloe vera* probiotic *lassi* has a beneficial effect on human health.

Keywords: *Aloe vera*, *lassi*, probiotic, quality, *Lactobacillus fermentum*

Today, consumers are more interested in functional foods that improve their immune systems than just basic diet. Essentially probiotic dairy products are an important part of the global functional food market. Probiotics are highly beneficial to the host by reducing lactose intolerance, suppressing cancer, lowering blood cholesterol levels, improving gastrointestinal immunity, preventing diabetes, etc. (Chapman et al., 2011). However, maintaining the viability of probiotics above 10^6 CFU ml^{-1} until consumption is an important criterion for achieving the desired health benefits. Thus, several factors promoting growth have been proposed to ensure the viability of probiotics in sufficient number before their consumption. In this context, fermented milk products are considered as the most accepted food carriers for the delivery of viable probiotic cultures to the human gastrointestinal tract (Hussain et al., 2015).

Aloe barbadensis Miller (*Aloe vera*) is a plant of the family *Aloaceae*, and it is considered the most biologically active of several species of *Aloe vera*. This species contains many biologically active constituents, such as vitamins, minerals, amino acids, phytosterols, and salicylic acids (Foster et al., 2011). *Aloe vera* also has several health benefits, such as antiobesity, antidiabetic, antimicrobial properties and so on (Pothuraju et al., 2016). Recently, the prebiotic effect of *Aloe vera* gel has also been demonstrated (Basannavar et al., 2014), which encourages its use in probiotic foods.

Lassi is a fermented milk beverage widely consumed (Behare et al., 2010). Good quality *lassi* should have a creamy consistency, smooth texture, glossy sheen, white colour with yellowish tinge, and a sweet rich aroma with mild to high acidic taste. It is flavoured either with salt or sugar and other spices, taking into account the preferences of the country.

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Lassi, being a fermented milk product, can serve as an excellent medium for probiotics and the incorporation of *Aloe vera* gel, which is rich in bioactive compounds.

To the best of our knowledge, addition of both *Aloe vera* gel and *Lactobacillus fermetum* to *lassi* has not been studied. Therefore, the main objective of the present work was to produce a novel functional fermented *lassi* with Aloe vera gel and probiotic strain *Lactobacillus fermetum*. Aloe vera gel was supplemented before or after fermentation to investigate its effect on yogurt quality. We measured physiochemical properties, sensory attributes, and antioxidant activitiy of *lassi* during storage.

1. Materials and methods

1.1. Culture propagation

Lactobacillus fermentum is among the best probiotic strains that have been isolated from camel milk (Mahmoudi et al., 2016). An overnight culture in MRS broth (Biokar Diagnostics, France) of *Lactobacillus fermentum* strain was used for *lassi* fermentation. Thereafter, yogurt culture (YF-L812; Chr. Hansen, France) was added as standard mixed fermenter. It contains *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

1.2. Aloe vera *gel extraction*

Aloe vera leaves (*barbadensis* miller species) were purchased from Aloé Mediteranée in Tunisia. The gel was extracted from the leaves into a clean container (Ramachandra & Rao, 2008).

1.3. Lassi *preparation*

Non-fat milk was pasteurised at 95 °C for 3 min, then it was rapidly cooled down to 43±1 °C. The milk was divided into three equal batches as follows: 1) control without gel, inoculated with starter culture YF-L812 (2%) and probiotic strain *Lactobacillus fermentum* (1%) corresponding to an initial count of about 8 log CFU ml^{-1} and 8.3 and 8 log CFU ml^{-1}, respectively; 2) batch supplemented with *Aloe vera* gel at 15% level before fermentation; 3) supplemented with *Aloe vera* gel at 15% level after fermentation, followed by incubation at 43 °C until pH reached 4.6 and acidity reached 75 °D. With these compositions firm *Aloe vera* probiotic *lassi* was obtained. The curd was broken down with a mixer for 2 min, and sugar syrup was added at 14% (w/v) (53 °Bx), followed by thorough homogenisation to obtain *Aloe vera* supplemented probiotic *lassi* with uniform consistency (Nripendra, 2012).

1.4. pH, acidity, and syneresis

pH and Dornic acidity (expressed as degree Dornic) of each sample were measured. Then, syneresis was determined according to the method reported by Rinaldoni and co-workers (2009). Briefly, 10 ml of *lassi* was centrifuged (80 000 r.p.m., 12 min, 4 °C) and the supernatant was recovered and weighed, thereafter, syneresis was calculated as follows:

$$\text{Syneresis (\%)} = (W_1/W_2)/100,$$

where: W_1=weight of supernatant and W_2 =weight of *lassi* sample.

1.5. Bacterial enumeration

Streptococcus thermophilus was enumerated on M17 agar (Biokar Diagnostics, France) after aerobic incubation at 44 °C for 48 h. The viable counts of *Lactobacillus fermentum* and *Lactobacillus bulgaricus* were determined on MRS agar after incubation at 37 °C for 48 h.

1.6. Antioxidant activity

Radical scavenging activity was determined by DPPH assayed according to procedure stated by Turkmen and co-workers (2007).

1.7. Colour

The measurement of colorimetric parameters L^*, a^*, and b^* of *lassi* products was carried out using a colorimeter (Minolta Chroma Meter CR-300, Tokyo, Japan).

1.8. Sensory analysis

The *lassi* samples were subjected to sensory analysis after 24 h, 14 days, and 28 days of storage at +4 °C. This study was carried out to determine the effect of *Aloe vera* gel supplementation before and after fermentation with probiotic *L. fermentum* and mixed fermenter (YF-L812) on the organoleptic properties of fermented *lassi*. The sensory evaluation panel consisted of 30 qualified panellists with sufficient knowledge of sensory analytical methods and product characteristics. Panellists were requested to judge each sample on the basis of aroma, flavour, texture, colour, appearance, and whey quantity. The score had to be indicated on a 9-point scale ranging from 1 (the least, the lowest) to 9 (the most, the highest) (Tamjidi et al., 2012).

1.9. Statistical analysis

The data were analysed using SPSS 20.0 software by means of independent one-way ANOVA tests at each sampling point. The differences among the three values were assessed by means of the Least Significant Difference (LSD) multiple comparison test ($P<0.05$).

2. Results and discussion

2.1. pH, acidity, and syneresis

The pH, lactic acidity, and syneresis values of different probiotic *lassi* products through 28 days of storage at 4 °C are presented in Table 1. The pH of *Aloe vera* probiotic *lassi* samples decreased to 4 registered on the 28th day of storage ($P<0.05$), but there were no significant ($P>0.05$) differences between samples. Also, lactic acidity increased ($P<0.05$) during storage to reach up to 101 °D for AVAF sample. The metabolic activity of lactic acid bacteria results in the accumulation of organic acids and causes reduction in pH of fermented milk. In fact, the prebiotic polysaccharides and other growth promoting substances of *Aloe vera* might have stimulated the metabolic activity of lactic acid bacteria, especially the probiotic strain, leading to a decrease in the pH of probiotic *lassi* products during storage (Vega et al., 2005).

Table 1. Changes in pH, acidity, and syneresis parameters during *Aloe vera* probiotic *lassi* products storage

Storage (days)	Samples	Parameters		
		pH	Acidity (°D)	Syneresis (%)
1	Control	4.24±0.01[a]	81±0.05[a]	38.65±0.007[a]
	AVBF	4.21±0.02[a]	80±0.02[a]	53.5±0.07[b]
	AVAF	4.25±0.01[a]	81.5±0.02[a]	50.3±0.002[b]
7	Control	4.16±0.015[a]	85.5±0.001[a]	49.6±0.02[a]
	AVBF	4.19±0.006[a]	85±0.022[a]	50±0.03[a]
	AVAF	4. 2±0.011[a]	86.5±0.014[b]	66.3±0.01[b]
14	Control	4.14±0.001[a]	90±0.002[a]	59.2±0.01[a]
	AVBF	4.17±0.007[a]	90±0.017[a]	60.25±0.01[a]
	AVAF	4.12±0.01[a]	91±0.008[a]	64±0.03[b]
21	Control	4.08±0.01[a]	98±0.007[a]	71.5±0.019[a]
	AVBF	4.12±0.02[a]	97.5±0.009[a]	73.2±0.004[b]
	AVAF	4.1±0.07[a]	97.5±0.003[a]	65±0.002[c]
28	Control	4.04±0.035[a]	99±0.006[a]	57.5±0.001[a]
	AVBF	4.02±0.025[a]	100±0.001[b]	54.6±0.002[b]
	AVAF	4±0.004[a]	101±0.001[c]	64.7±0.005[c]

AVBF: *Aloe vera* before fermentation; AVAF: *Aloe vera* after fermentation. Values in each stage of storage with different letters are significantly different at $P<0.05$. Data means ± standard deviation (n=3).

For syneresis, the percentages increased for 21 days during storage ($P<0.05$). These results are in agreement with findings of Supavititpatana and co-workers (2010), which showed that the whey separation generally increases for 21 days of storage. In addition, *Aloe vera* gel favours the syneresis phenomenon during storage. In fact, the highest value (73.2±0.004) was obtained for AVBF sample. The water release in probiotic *lassi* products during storage could be directly ascribed to the bacterial growth inducing protein destabilisation, resulting in water outlet of the casein micelle, which was previously demonstrated by Peng and co-workers (2009). This whey separation is a sensory defect.

2.2. Bacterial enumeration

The counts of *Lactobacillus* and *Streptococcus* bacteria are shown in Table 2. In three probiotic *lassi* samples, the *Lactobacillus* number increased progressively for 21 days of storage. Then, a decrease was observed for all products with numbers of 11.54±0.035, 12.04±0.006, and 11.77±0.001 log CFU ml^{-1}, respectively, for the control, AVBF, and AVAF samples ($P>0.05$). These results were similar to those obtained by Hussain and co-workers (2015), who reported that *L. paracasei* ssp. *paracasei* probiotic strain survived during storage up to the recommended therapeutic level i.e. 6 log CFU ml^{-1}. The growth of *Lactobacillus,* especially *Lactobacillus fermentum,* could be attributed to the better resistance to acidic conditions. Also, prebiotic potential of *Aloe vera* could be responsible for stimulating the growth and the metabolic activity of probiotics (Ben Moussa et al., 2019). Thus, the product obtained could be considered as a symbiotic product beneficial to human health (Sudheendra et al., 2018).

For *Streptococcus thermophilus*, cell numbers increased until 21st day of storage in all samples. However, the content of *Streptococcus* remains higher than that required in fermented milk (>10^7 CFU ml^{-1}). The growth of *Streptococcus thermophilus* has a total synergy with *Lactobacillus bulgaricus* and *Lactobacillus fermentum* for the three probiotic *lassi* products during storage. Choi and co-workers (2016) reported similar trends in the counts of lactobacilli in symbiotic fermented milk containing *Lacobacillus sakei* as probiotic.

Table 2. Changes in viable bacteria counts during *Aloe vera* probiotic *lassi* products storage

Storage (days)	Samples	Viable counts (log CFU ml^{-1})		
		Control	AVBF	AVAF
1	*Lactobacillus*	11.54±0.01^{b}	10.7±0.05^{ab}	9.77±0.007^{a}
	Streptococcus	14.08±0.02^{b}	11.56±0.02^{a}	11.9±0.07^{a}
7	*Lactobacillus*	11.3±0.015^{a}	10.87±0.001^{a}	11.47±0.02^{a}
	Streptococcus	11.7±0.006^{a}	12.47±0.022^{b}	11.47±0.03^{a}
14	*Lactobacillus*	11.84±0.001^{b}	9.6±0.002^{a}	10.47±0.01^{a}
	Streptococcus	13.7±0.007^{a}	13.47±0.017^{a}	11.7±0.01^{b}
21	*Lactobacillus*	8.6±0.01^{a}	8.54±0.007^{a}	11.87±0.019^{b}
	Streptococcus	13.47±0.02^{a}	14.83±0.009^{b}	13.47±0.004^{a}
28	*Lactobacillus*	11.54±0.035^{a}	12.04±0.006^{a}	11.77±0.001^{a}
	Streptococcus	11.87±0.025^{a}	12.07±0.001^{a}	11.6±0.002^{a}

AVBF: *Aloe vera* before fermentation; AVAF: *Aloe vera* after fermentation. Values in each stage of storage with different letters are significantly different at P<0.05. Data means ± standard deviation (n=3).

2.3. Antioxidant activity

The scavenging rates of *Aloe vera lassi* products are illustrated in Table 3. In general, the AVBF and AVAF samples have higher (P<0.05) antioxidant activities than the control, ranging from 20.46±0.01 to 46.18±0.20% throughout storage. Moreover, several studies reported that both *Aloe vera* and probiotics have many biological effects, especially antioxidant activities (Kumar & Kumar, 2016), so our research confirmed that the combination of the *Aloe vera* gel and probiotic strain possesses better antioxidant effect. Bioactive compounds in fermented dairy products such as peptides are able to donate electrons to neutralise free radicals and enhance antioxidant properties (Aluko, 2012).

Table 3. Changes in antioxidant activities by DPPH assay during *Aloe vera* probiotic *lassi* products storage

Samples	Storage (days)				
	1	7	14	21	28
Control	10.16±0.06^{c}	12.31±0.06^{c}	16.45±0.01^{d}	18.94±0.02$^{a.d}$	27.95±0.21^{b}
AVBF	20.46±0.01^{a}	22.03±0.01^{a}	32.49±0.02^{a}	42.20±0.02^{a}	45.33±0.03^{a}
AVAF	23.15±0.01^{b}	24.34±0.03^{a}	36.82±0.03^{c}	41.33±0.12$^{a.b}$	46.18±0.20$^{a.b}$

AVBF: *Aloe vera* before fermentation; AVAF: *Aloe vera* after fermentation. Values in each stage of storage with different letters are significantly different at P<0.05. Data means ± standard deviation (n=3).

2.4. Colour

The effect of *Aloe vera* gel adjunction on colour parameters of probiotic *lassi* products is shown in Table 4. Lightness (L*) values were significantly different ($P<0.05$), and the highest value (117.08±0.011) was obtained on the 7th day of storage for the AVAF sample. For redness intensity (a*), the control sample had the highest value of 6.32±0.01 after one week of storage. Generally, the values finally decreased to –1.77±0.006, –0.77±0.001, and –1.52±0.001, respectively, by the end of the storage. Moreover, *Aloe vera* gel contains a proportion of chlorophyll and starch within chlorophyll parenchyma, which is used for direct fermentation and contributes to more intense parameter values (a*) of AVBF than AVAF probiotic *lassi* samples (Kumar & Kumar, 2016). Regarding the yellowness parameter (b*), the values of *Aloe vera lassi* similarly changed during storage to reach 2.44±0.002 and 2.84±0.005, respectively, for AVAF and AVBF samples.

Table 4. Changes in colour parameters (L, a, and b) during *Aloe vera* probiotic *lassi* products storage

Storage (days)	Samples	Colour parameters		
		Lightness (L)	Redness (a)	Yellowness (b)
1	Control	76.02±0.01^{b}	2.61±0.05^{a}	9.48±0.007^{c}
	AVBF	71.93±0.02^{a}	2.11±0.02^{a}	6.43±0.07^{a}
	AVAF	70.17±0.01^{a}	3.38±0.02^{b}	7.55±0.002^{b}
7	Control	106.79±0.015^{a}	6.32±0.01^{b}	6.43±0.02^{a}
	AVBF	103.12±0.006^{b}	4.02±0.022^{a}	8.91±0.03^{b}
	AVAF	117.08±0.011^{c}	4.72±0.014^{a}	8.88±0.01^{b}
14	Control	80.07±0.001^{b}	–2.49±0.002^{b}	6.65±0.01^{a}
	AVBF	76.82±0.007^{a}	–1.25±0.017^{a}	6.26±0.01^{a}
	AVAF	76.31±0.01^{a}	–1.47±0.008^{a}	6.17±0.03^{a}
21	Control	79.54±0.01^{b}	–5.18±0.007^{c}	7.77±0.019^{b}
	AVBF	67.25±0.02^{a}	–2.51±0.009^{b}	3.88±0.004^{a}
	AVAF	64.41±0.07^{a}	–1.33±0.003^{a}	4.32±0.002^{a}
28	Control	65.29±0.035^{a}	–1.77±0.006^{b}	3.41±0.001^{b}
	AVBF	64.19±0.025^{a}	–0.77±0.001^{a}	2.44±0.002^{a}
	AVAF	61.26±0.004^{b}	–1.52±0.001^{b}	2.84±0.005^{ab}

AVBF: *Aloe vera* before fermentation; AVAF: *Aloe vera* after fermentation. Values in each stage of storage with different letters are significantly different at $P<0.05$. Data means ± standard deviation (n=3).

2.5. Sensory attributes

The results of sensory tests of *Aloe vera* probiotic *lassi* samples are shown in Figures 1A, 1B, and 1C.

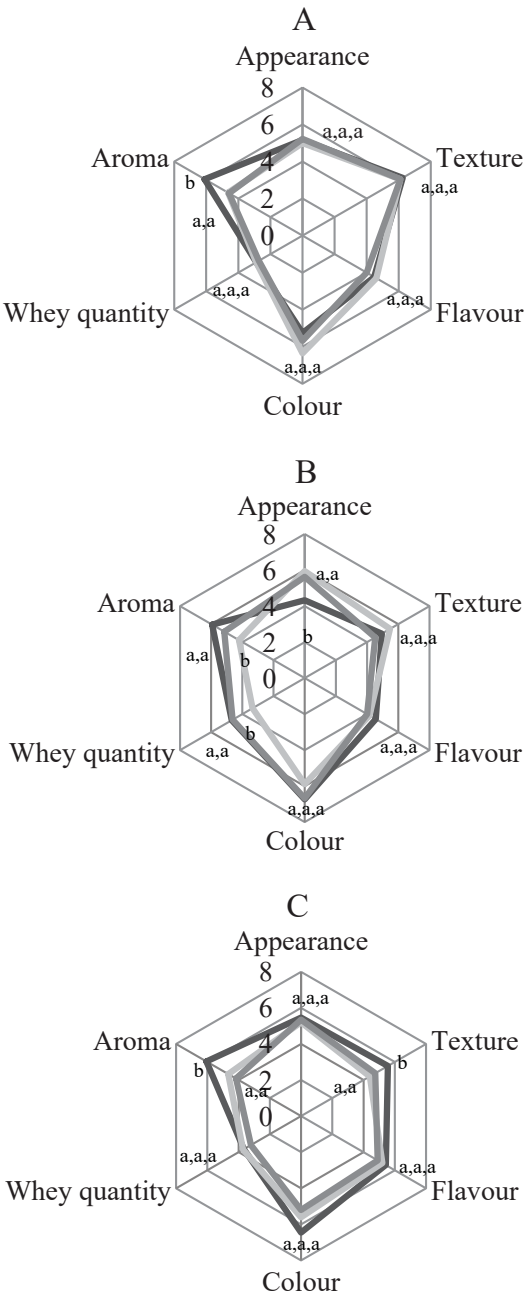

Fig. 1. Sensory profiles of *Aloe vera* probiotic *lassi* products after 24 h (A), 14 days (B), and 28 days (C) of storage

——: Control; ——: AVBF; ——: AVAF

AVBF: *Aloe vera* before fermentation; AVAF: *Aloe vera* after fermentation. Values in each stage of storage with different letters are significantly different at $P<0.05$. Data means (n=3).

After 24 h of storage, there was no significant difference (P>0.05) between samples regarding flavour, texture, colour, appearance, and whey quantity, while the taste of *Aloe vera* gel has negatively influenced the final aroma of fortified probiotic *lassi*. Furthermore, the AVBF *lassi* had after 14 days of storage better appearance, texture, odour, and a white colour, but with an off taste. This could be due to the effect of *Aloe vera* to stimulate the flavour producing activity of lactic acid bacteria during fermentation. These profiles were similar to those obtained by Hussain and co-workers (2015), who obtained the best consistency score of more than seven for *Aloe vera* fermented *lassi*.

Figure 1C shows similar sensory profiles at the end of storage, with the exception of texture and aroma attributes. The control sample had the most appreciated texture and aroma. These attributes show that the addition of *Aloe vera* gel affects both characteristics. Indeed, the gel is slightly bitter, of which arises the need to incorporate a flavour as additive. Regarding texture, the incorporation of *Aloe vera* gel improves the texture up to 14 days of storage, but weakens it afterwards.

3. Conclusions

This study showed that the *Aloe vera* adjunction during *lassi* fermentation has no influence on post-acidification. However, it enhanced the syneresis phenomenon during the storage period. Regarding lactobacilli cell counts of *lassi* products, the numbers increased progressively for 21 days and then decreased to the end of storage. The sensory characterisation showed the acceptance of *lassi* products by consumers, especially *Aloe vera* probiotic *lassi*. In addition, the sensory characteristics were similar, except for texture and aroma, which were affected by *Aloe vera* gel. So, it would be needed to flavour the product and to add a thickening agent such as starch. In fact, the addition of *Aloe vera* gel did not affect the overall appreciation of *lassi*, possessing similar qualities. Finally, these *Aloe vera* probiotic *lassi* have promising possibilities in the fermented dairy industry.

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