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Research Article

EFFECT OF COLOUR INTENSITY AND MICROBIAL QUALITY OF LASSI PREPARED BY USING *PUDINA* EXTRACT

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Abstract- Lassi prepared by using by the traditional method of buffalo milk with *Pudina* leaves extract was prepared from different proportion of *Pudina* extract. The investigation was undertaken to explore the possibilities of utilizing *Pudina* extract in lassi manufacture to improve the health benefits and the product sensory. The level of *Pudina* extract was optimized on the sensory basis the score obtained and treatment T2 getting better result as compare to other and it was subjected for colour intensity and microbial analysis by the hunter lab and various method used. Mixed the culture of lactic acid bacteria @ 2 per cent was used to prepare lassi from buffalo milk containing 6.0 per cent fat. A 2.5 per cent *Pudina* extract level gave desirable result and acceptability by the panel of judges. Addition of *Pudina* extract into lassi was a significant effect on colour intensity and microbial analysis. The colour intensity of accepted lassi treatment T2 was observed that the score was 60.40, 1.55 and 2.18 to L, a and b respectively and the microbial score of acceptable lassi T2 were 2.38, 2.45, 1.11 and 0.00 of Lactobacillus, Lactococcus, Coliform and yeast and mould respectively.
Keywords- *Pudina extract, Pudina, Buffalo milk, Pudina Lassi*

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Introduction

Fermented milk has played important role in the human consumption as refreshing beverages and nutritious food in many parts of the world since earliest ages and possess various nutritional and therapeutic properties. Starter culture used in the dairy industry of mixtures of carefully selected lactic acid bacteria which are added to the milk to fulfill the desired fermentation and it plays a major role in determining the positive health effects of fermented milks and related products [1]. Cultured products sold with any claim of health benefits should meet the criteria of suggested minimum number of more than 106 cfu per gram at the time of consumption. It includes prevention of gastrointestinal infections, reduction of serum cholesterol levels and ant mutagenic activity. The fermented products are recommended for consumption by lactose intolerant individuals and patients suffering from atherosclerosis. Many eminent workers studied on Ayurvedic system of medicine 2nd century B.C. referred to the value of curd in promoting the appetite in increasing vitality and in curing of dyspepsia, diarrhea, dysentery, intermittent fever, and other diseases. It has been evidently proved that fermented milk has unique importance in the diet of human being. Thus, these fermented milk products have been known for "Cure all" and "Life extending" properties [2]. The lactic acid bacteria involved in preparation of fermented milks include selected species as like *lactobacilli* and streptococci. These microbes have also proved their worth in developing nutritional and therapeutic properties in fermented milks. The presence of *lactobacilli* in gut helps in maintaining better health of consumers. Now, the consumers are increasingly interested in their personal health and expect the food that they eat to be healthy or even capable of preventing illness. Gut health in general has shown to be the key sector for functional foods in the world [3].The consumption of fermented milk containing *lactobacilli* have improved lactose digestion which aids lactose intolerant individuals [4], decrease in serum cholesterol levels, contribute to the maintenance of the enterohepatic circulation of bile acids through deconjugation of bile acids, increases vitamin B content of food increases daily weight gain in test suggesting a more efficient utilization of nutrients [5]. Dairy products cultured with *lactobacilli* reported to possess anti carcinogenic properties [6]. Considering the above facts and importance of cow milk, fermented milk products and probiotic cultures in the human diet, the present investigation was planned. The use of herbs in combination with different food has become regular practice to conserve the functional as well as nutritional attributes from herb. Many food items in the market available by different company are popular due to their acceptability and functionality *viz*. Herbal beverages, Arjuna ghee, yoghurt [7]. Menthol (*Mentha arvensis*) belongs to the family *Libeaceae* is a common edible and aromatic perennial herb which is cultivated throughout the India. Common name is *Pudina*. The physical-chemical properties of menthol are melting point 43^{0}C (106-109^{0}F), freezing point is 27-28^{0}C, boiling point is 212^{0}C (414^{0}F). Molecular formula $C_{10}H_{20}O$ and molecular weight is 156.27 g/mol. The aromatic leaves widely used for flavoring foods and beverages. It is an erect aromatic herb that grows up to 60 cm height with suckers. The stem is cylindrical, and the leaves are simple and opposing type. It is used as a contraceptive, carminative, antiseptic ulcer agent and has been given to treat indigestion, skin diseases, cough, and colds in folk medicine. In beverages *Pudina* is used as a cooling and flavoring agent [8].

Material and Methods

Collection of buffalo milk

Fresh and standardized buffalo milk for fat 6 percent and SNF 9 per cent was procured from Natural Milk Pvt, Ltd, Latur.

Collection of *Pudina*

Fresh *Pudina* (*Mentha arvensis*) *i.e.,* Menthol leaves were purchased from local market of Latur city.

Propagation and maintenance of *lactobacillus* cultures

The NCDC-167 (BD4) culture was propagated in 10 ml sterile de Man-Rogosa-Sharpe (MRS) broth and maintained in litmus milk in refrigerator until use. These were periodically sub-cultured in the same medium once in a week. The culture was activated by sub-culturing before use and purity was always ascertained by Gram 's staining. One set of cultures was stored at -80°C in MRS broth containing 20% glycerol as a stock.

Microbial cultures

The standard dahi culture *i.e.,* Standard dahi contained *Streptococcus thermophilus* and *Lactococcus lactis* (NCDC-167) in this study was procured from National Dairy Research Institute (NDRI), Karnal.

Chemicals

Analytical (AR) or guaranteed grade (GR) reagents were used in the chemical analysis.

Preparation of *Pudina* lassi

The *Pudina* lassi was prepared by using dahi of buffalo milk having 6 percent fat and 9 percent SNF and *Pudina* leaves extract by method suggested by Sukumar De (2004) with minor modification in respect to quantity of *Pudina* extract and sugar [9].

Preparation of *Pudina* leaves extract :

The *Pudina* extract (*Mentha arvensis*) was prepared as shown in the following flow chart as per procedure fallowed by Satpute (2015) by using the fresh *Pudina* leaves [10].

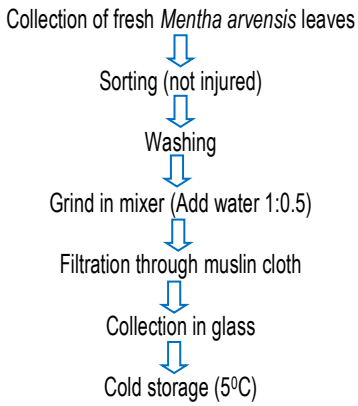

Fig-1 Flow chart for preparation of *Pudina* leaves extract

Two-liter buffalo milk was taken for each treatment and filtered through double layer muslin cloth and pasteurized on 63^{0}C for 30 min. Then milk was cooled at 37 ^{0}C and subsequently 2 per cent standard dahi culture was added in it and kept for fermentation for 10 hrs. Then *Pudina* extract was added as per treatments combination mentioned in section 3.2.1.1 after that equal quantity of potable water was added and churned it by using churner. Then 15 per cent sugar was mixed in it. The prepared lassi was packed in plastic bottles and stored at 5^{0}C until further study.

Treatment details: The *Pudina* leaves extract was optimized for the preparation of lassi by taking its proportion 2.5, 5.0 and 7.5 per cent as per following treatments combinations.

T_1 - 100 Parts of curd
T_2 - 97.5 Parts of curd + 2.5 Parts of *Mentha arvensis* extract
T_3 - 95.0 Parts of curd + 5.0 Parts of *Mentha arvensis* extract
T_4 - 92.5 Parts of curd + 7.5 Parts of *Mentha arvensis* extract

Preparation of *Pudina* lassi:

Pudina lassi was prepared as per the method used as per De, (2004) with some modification.

Receiving of milk

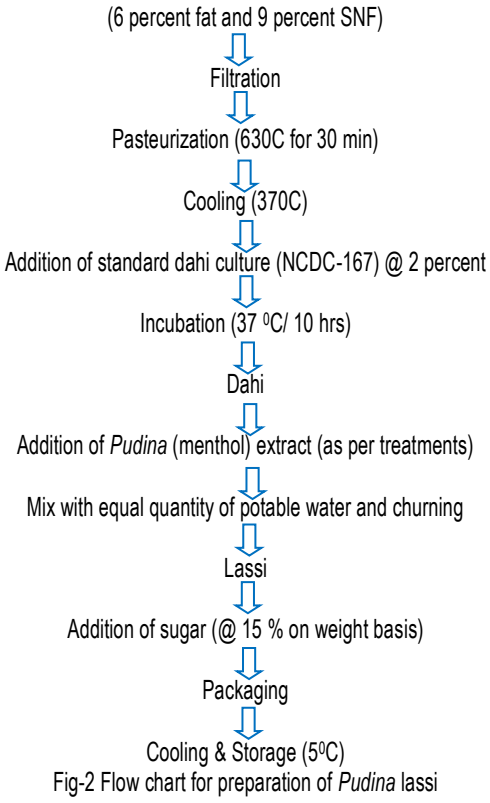

Fig-2 Flow chart for preparation of *Pudina* lassi

Determination of Color intensity

Colour intensity of lassi was measured by reflectance spectroscopy technique employing reflectance meter, colorflex (Hunter lab, Reston, Virginia, USA) along with the universal software. Before the test, the instrument was calibrated with standard black glass and white tile as specified by the manufacturer. The light source was duel beam xenon flash lamp. Data are received from the software in terms of L*[Lightness, ranges from 0 (black) to 100 (white)], a* [Redness, ranges from +60(red) to -60(green)] and b* [Yellowness, ranges from +60(yellow) to -60(blue)] in the value of the international color system.

Microbiological analysis of lassi

The lassi samples were examined for the *lactobacilli* count, *lactococcus* count, Coliform count, and yeast and mould count.

***Lactobacillus* count**

Enumeration of *lactobacilli* count of lassi was determined by using pour plate method employing MRS agar by Tharmaraj and Shah, (2003) [11].

***Lactococcus* count**

Enumeration of *lactococcus* of lassi was determined by using pour plate method employing M 17 agar as a medium agar [12].

Coliform count

Enumeration of Coliform count of lassi was determined by using pour plate method described by Hought, *et al.* (1992) employing Violet Red Bile Agar (pH 7.4 ± 0.1) [13].

Yeast and mold count

Lassi was ascertained for yeast and mold counts as per suggested by Marshall, (1993) using Potato Dextrose Agar and pH of media adjusted to 3.5 ± 0.1 using tartaric acid solution [14].

Statistical method

The data obtained in the present investigation was tabulated. The data were analyzed statistically by using Completely Randomized Design (CRD) as per Panse and Sukhatme (1967) [15]. The significance of the result was evaluated based on critical difference.

Table-1 Colour intensity of *Pudina* lassi

Treatment	Replication														
	R-I			R-II			R-III			R-IV			Mean		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
T_1	58.3	1.51	2.15	58.4	1.53	2.17	58.3	1.51	2.15	58.2	1.52	2.14	58.3	1.52	2.15
T_2	60.4	1.55	2.17	60.3	1.54	2.18	60.5	1.55	2.17	60.4	1.56	2.2	60.4	1.55	2.18
T_3	63.8	1.61	2.19	63.7	1.59	2.2	63.9	1.62	2.21	63.6	1.6	2.22	63.75	1.61	2.21
T_4	65.4	1.64	2.23	65.5	1.65	2.23	65.6	1.64	2.22	65.6	1.65	2.24	65.53	1.65	2.23
L*				S.E. ± 0.049						C.D. at 5% = 0.150					
a*				S.E. ± 0.004						C.D. at 5% = 0.014					
b*				S.E. ± 0.006						C.D. at 5% = 0.018					

Result and Discussion

Colour intensity of *Pudina* lassi

The values recorded in respect of viscosity content of the finished product are shown in [Table-1]. The colour intensity of the all lassi samples was measured by reflectance spectroscopy technique (Hunter lab, Reston, Virginia, USA) as per the result L* value shows lightness ranges from 0 (black) to 100 (white), a* value indicating the redness ranges from +60(red) to -60(green) and b* value showing yellowness of samples ranges from +60(yellow) to -60(blue). The results of colour intensity are presented in the [Table-1] and shows that, the L* value, a* value and b* value differs significantly ($p<0.05$) in all samples of lassi. The [Table-1] indicates that the average colour intensity of *Pudina* lassi was measure by three reading *viz*. L*, a* and b* as founded in samples were 58.30-65.53, 1.52-1.65 and 2.15-2.23 for treatment T1, T2, T3 and T4, respectively. The colour intensity towards the white colour *i.e.,* L* was recorded highest in treatment T4 *i.e.,* 65.53 and the lowest in treatment T1 *i.e.,* 58.30 both values indicate that whitist colour was prominent and not affected by addition of *Pudina* extract. As the same way the red to green colour intensity increased towards green values as the proportion of *Pudina* extract increased form T1 to T4 *i.e.,* highest in treatment T4 *i.e.,* 1.65 and lowest in treatment T1 *i.e.,* 1.52. The highest colour intensity was recorded for reading b* in treatment T4 *i.e.,* 2.23 and the lowest colour intensity was recorded for treatment T1 *i.e.,* 2.15. Above observations clearly indicate that, as the adding of *Pudina* extract in to the buffalo milk was increased then the colour intensity of the finished product also due to the chlorophyll contain in *Pudina* leaves.

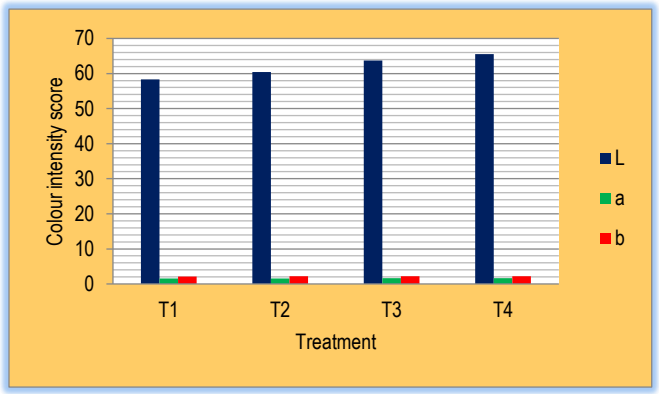

Fig-3 Graphical presentation of colour intensity of *Pudina* lassi

Deb and Seth, (2014) [16] studies on the physico-chemical and microbiological analysis of plain dahi of Assam, India and concluded that Colour measuring indices L*, a* and b* values ranged from 57.29-85.25, (0.33)-(1.69) and 1.90-2.39, respectively. Among these L* value only showed significant changes. Padghan (2012) studied on bio-functional properties of traditional Indian lassi prepared from buffalo milk and mentioned the colour intensity of lassi-L1 was more b* value (9.18 ± 0.11) and less L* value (83.59 ± 0.02), a* value (-4.45 ± 0.01) as compared to lassi-L0 and L2. More the L* value means samples showing more whiteness which is observed in lassi-L0 and L2 as compared lassi-L1.

Bacterial count of selected treatments of fresh lassi

The fresh product prepared was subjected to microbiological analysis with respect to *lactobacillus* count, *lactococcus* count, yeast and mould count and coliform count. The microbial load of fresh product was estimated and expressed in log10 (cfu/gm) in forthcoming tables.

Lactobacillus count

The *lactobacillus* count of lassi prepared from *Pudina* extract mixed with buffalo milk was estimated at 10-6 dilution as per the method of pour plate, by Tharmaraj and Shah (2003) is tabulated in [Table-2].

Table-2 *Lactobacillus* count for *Pudina* lassi [log10 (cfu/gm)]

Treatment	Replication				
	R-I	R-II	R-III	R-IV	Mean
T_1	2.45	2.41	2.43	2.46	2.44^{a}
T_2	2.41	2.36	2.37	2.39	2.38^{b}
T_3	2.40	2.36	2.38	2.35	2.37^{b}
T_4	2.41	2.30	2.34	2.36	2.35^{bc}
S.E. ± 0.014			C.D. at 5% 0.046		

The values with different small letters superscripts row wise differ significantly at 5 per cent level of significance.

From the [Table-2], it was observed that the average *lactobacillus* count of fresh sample of *Pudina* lassi were found to be 2.44, 2.38, 2.37 and 2.35 cfu per g for treatments T1, T¬2, T3 and T4, respectively. The mixed treatments were decreased for *lactobacillus* count as compared to control lassi but count T¬2, T3 and T4 was not significantly differed whereas control lassi T1 is significantly differ than all of the *Pudina* added samples. This might be due to the inhibitory action of (mentha arevensis) for the growth of *lactobacilli*. But all samples ensured claim of health benefits by fulfilled the criteria of suggested minimum number of more than 106 cfu/g at the time of consumption.

Lactococcus count

From the [Table-3] it was observed that the average *lactococcus* count of fresh sample of puduna lassi were found to be 2.47, 2.45, 2.37 and 2.29 cfu per g for treatments T1, T2, T3 and T4, respectively. When the *lactococcus* count was compared with *lactobacillus* count, it was observed that both bacilli and cocci were found near about in equal proportion in lassi. The treatments T1 and T2 at par with each other.

Table-3 *Lactococcus* count for *Pudina* lassi [log10 (cfu/gm)]

Treatment	Replication				
	R-I	R-II	R-III	R-IV	Mean
T_1	2.42	2.48	2.48	2.46	2.47^{a}
T_2	2.41	2.46	2.46	2.47	2.45^{a}
T_3	2.38	2.34	2.39	2.38	2.37^{b}
T_4	2.23	2.3	2.3	2.34	2.29^{c}
S.E. ± 0.0166			C.D. at 5% 0.049		

The values with different small letters superscripts row wise differ significantly at 5 per cent level of significance. The results recorded in the present investigation are comparable with the findings with following results. The difference for *lactococcus* count in *Pudina* lassi as compared to many references may be due to the different product type, culture rate and dilution factor varies in their respective studies.

Coliform count

[Table-4] indicates the coliform count of lassi prepared from *Pudina* leaves and buffalo milk. The values with different small letters superscripts row wise differ significantly at 5 per cent level of significance. The [Table-4] shows the coliform count of *Pudina* lassi. The range for coliform found in between 1 to 5 cfu per g. The higher count for coliform was observed in treatment T1 (1.14 cfu per gm) and lower in treatment T1 (1.06 cfu per gm). All the treatment was significantly different from each other.

Table 4 Coliform count of fresh *Pudina* lassi (cfu per gm)

Treatment	Replication				
	R-I	R-II	R-III	R-IV	Mean
T_1	1.12	1.15	1.14	1.13	1.14[a]
T_2	1.11	1.12	1.1	1.09	1.11[b]
T_3	1.08	1.09	1.09	1.1	1.09[c]
T_4	1.06	1.07	1.05	1.04	1.06[d]
S.E. ± 0.005			C.D. at 5% 0.018		

It was seen that the coliform count remained within the limit (maximum 100 c.f.u./gm) prescribed by IS: 9617 (1880) indicated that all treatments were prepared at hygienic condition and suitable for consumption.

Yeast and Mould count

[Table-5] indicates the yeast and mould count of lassi prepared from Buffalo milk with addition of *Pudina* extract. The [Table-5] shows the yeast and mould count of fresh *Pudina* lassi. The yeast and mould was found in control treatment only which had count 01 cfu/gm in each. The mixed lassi was blank for yeast and mould. It was seen that the yeast and mould count remained within the limit (maximum 100 c.f.u./gm) prescribed by IS: 9617 (1880). All treatments were prepared at hygienic condition and at par to each other.

Table-5 Yeast and mould count for fresh *Pudina* lassi (cfu per gm)

Treatment	Replication				
	R-I	R-II	R-III	R-IV	Mean
T_1	1	2	1	0	1.00[a]
T_2	0	0	0	0	0.00[b]
T_3	0	0	0	0	0.00[b]
T_4	0	0	0	0	0.00[b]
S.E. ± 0.204			C.D. at 5% 0.628		

The values with different small letters superscripts row wise differ significantly at 5 per cent level of significance.

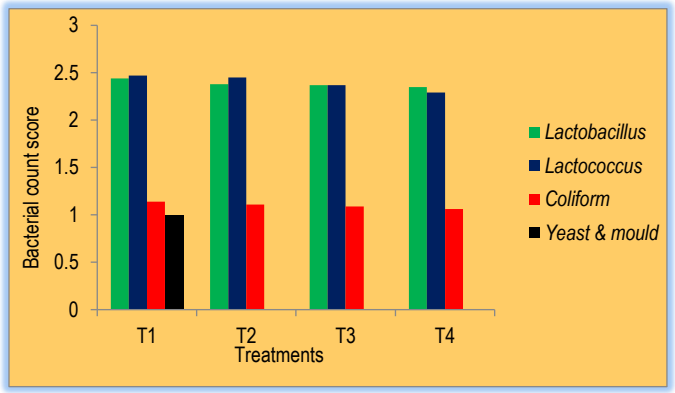

Fig-4 Graphical presentation for bacterial count of *Pudina* lassi

Conclusion

From the result of the present study, it may be concluded that the *Pudina* extract @ 2.5 per cent with 15 per cent sugar level has decrease the microbial count with increase the leval of *Pudina* extract and the colour intensity from the observations it is clearly indicate that, as the adding of *Pudina* extract in to the buffalo milk was increased the colour intensity of the finished product due to the chlorophyll contain in *Pudina* leaves, so it is not only used to increase colour intensity but also increase the self-life and provide a novelty product.

Application of research: The developed herbal lassi can provide the great opportunity to dairy industry as well as housewife for better utilization of medicinal attributes of pudina. This lassi will act as functional food for curative and preventing measures for cardiovascular, immune defense system and blood system related problems

Research Category: Animal Husbandry and Dairy Science

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