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Optimization of starter culture to develop healthy goat milk shrikhand

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Abstract
The present study was conducted to optimize the starter culture (NCDC-159) level (%) *i.e.* 2.0 (C1), 2.5(C2) and 3.0 (C3) % for preparation of goat milk shrikhand. The pH values decreased while titratable acidity increased significantly ($P<0.05$) with increased culture levels. Ash content of C1 whereas brix values of C2 were significantly ($P<0.05$) higher than other treatments. The values of all textural parameters values were significantly ($P<0.05$) higher in C2 than C1 and C3, whereas lightness values of C1 and C2 were significantly ($P<0.05$) than C3. Among the sensory attributes, flavour, texture, sweetness and overall acceptability scores of C2 were significantly ($P<0.05$) higher. Therefore, goat milk shrikhand prepared 2.5% NCDC-159 starter culture as found optimum.

Keywords: Goat milk shrikhand, starter culture level, optimization, textural and colour parameter, sensory evaluation

Introduction
Highest milk producer in world is India with 187.7 million tonnes of milk (NDDB, 2019) [13] due to advancement of technology, proper nutrition and appropriate managemental practices. Livestock contributes about 9.2% in gross value added (GVA) and 26.2% in agriculture sector in India. The livestock population in India includes 302.3 million bovines, 74.3 million sheep, 148.9 million goats, about 9.1 million pigs and 851.8 million poultry. The rural and urban population of goat is 129.081 million and 6.092 million respectively in India. Total goat milk production in India is 6.09 million tones, out of which Rajasthan and Uttar Pradesh produce 2.31 million tonnes and 1.34 million tonnes respectively (DAHD, 2019) [5]. Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of millions people worldwide and is an important part of the economy in India. Goat milk is having better digestibility, alkalinity, buffering capacity and certain therapeautic values in medicine and human nutrition (Park and Chukwu, 1989; Park, 1994) [15, 16] in comparison to cow's or human milk. The goat milk microbiota is also considered a good source of novel bacteriogenic *Lactic acid* bacteria (LAB) strains that can be exploited as an alternative for use as bio preservative in food (Perin and Nero, 2014) [17]. It is also rich source of amino acid, being 20-40 folds higher than cow milk (Mehaia and Al-Kanhal, 1992) [11] which is involved in bile salt formation, osmoregulation, antioxidation, calcium transport and in the central nervous system (Redmond *et al*., 1998) [18]. Minerals content such as calcium, potassium, magnesium and chloride as well as vitamin A, B, C, D, thiamin and niacin content of goat milk is higher than that of cow milk (Chandan *et al*., 1992) [3]. Goat milk also contains higher content of three characteristics fatty acids *i.e.* caproic acid, caprylic and capric acid which are having medicinal values for patients suffering from malabsorption, childhood epilepsy, cystic fibrosis and gallstones (Haenlin, 1992) [8]; however these are responsible for intense "goaty flavour" which limits the acceptability of goat milk products among the consumers.
The consumer's interest in fermented milk products is gaining momentum due to the development of new food processing techniques, changing social attitudes; scientific evidence of health benefits of certain ingredients. The shelf life and quality characteristics of fermented dairy products depend upon starter culture which is a carefully balanced blend of bacteria which consume lactose as energy source for their growth. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the commonly used as starter culture bacteria in manufacture of fermentative products. This blend of bacteria converts the lactose in milk to lactic acid, giving classic, deliciously tangy flavour. In recent years, it has been reported that lactic culture increases the vitamins and free amino acid content of dairy products. These microbes are capable of colonizing in lower intestine, improving gastrointestinal health and consequently

enhancing immune function and confer other health benefits on the host when consumed in adequate amount. Fermented milk products serve as the important delivery vehicles for these beneficial bacteria. Therefore, these microbes can play a dual role in transforming milk into a diverse array of fermented dairy products like Shrikhand, yoghurt, cheese, kefir, etc. and contribute for improvement of intestinal health by regulating the microbiota, immune system stimulation, reduction of lactose intolerance symptoms and risk of certain diseases (Oelschlaeger, 2010) [14].

Materials and methods

The experiments were carried out in the Department of Livestock Products Technology, College of Veterinary Sciences and Animal Husbandry, U.P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura, 281001 (UP), India. Fresh clean wholesome milk of goat was procured from Department of Veterinary Physiology, DUVASU, Mathura. Starter culture (NCDC-159) was procured from NDRI, Karnal which contained mixed culture of *Lactococcus lactis*, *Lactococccus diacetylactis* and *Lactococcus cremoris.* The culture was activated to as per the standard method and the activated parent culture was maintained by sub culturing and stored under refrigeration. Clean crystalline sugar was procured from local market of Mathura. All the chemicals used in the study were of analytical grade and procured from Hi Media laboratories (P) Ltd, Mumbai.

Preparation of Shrikhand

The shrikhand was prepared as per method described by Gupta *et al*. (2018) [7] with slight modifications. Fresh goat milk was filtered through muslin cloth and then fat content was standardized using Pearson square method. Then milk was subjected to heat treatment at 85 °C for 30 minutes followed by cooling at 37±2°C. Milk was inoculated with NCDC-159 @ 2.5% by v/v of milk and incubated at 35-37 °C for 12-15 hours for proper curd setting. The curd thus obtained was transferred to clean muslin cloth and hanged for 16-18 hours in order to drain the whey to obtain chakka. The chakka was kneaded to have uniform consistency and then mixed with 30% ground sugar. Finally shrikhand was filled in pre sterilized thermorigid polypropylene cups and stored at under refrigeration at 4±2°C. In present study, following abbreviations were used for present experiment: F1- goat milk shrikhand prepared with 4.0% fat, F2- goat milk shrikhand prepared with 5.0% fat and F3- goat milk shrikhand prepared with 6.0% fat.

Fresh goat Milk
↓
Filtration through muslin cloth
↓
Fat standardization
↓
Heating of milk @ 85° C for 30 min
↓
Cooled to 37±2°C
↓
Inoculation with starter culture NCDC-159 @ 2.5% by v/v of milk
↓
Dahi
↓
Drainage of whey by hanging for16-18 hrs
↓
Chakka
↓
Addition of ground sugar (@ 30% of total weight)
↓
Mixing
↓
Shrikhand
↓
Filling of polypropylene cups
↓
Storage at refrigerator temperature (4±2°C)

Flow diagram 2: Preparation of shrikhand

Analytical methods

Physic-chemical properties

The pH of shrikhand was determined by using digital pH meter (WTW, Germany, model pH 330i) as per method given by Trout *et al*. (1992) [21]. Water activity of each sample was measured three times in duplicate using a water activity meter (AquaLab 3 TE, Inc. Pullman, WA) at Department of Goat Products Technology, CIRG, Makdhoom. Proximate parameters *viz*. moisture, protein, fat and ash content were estimated as per AOAC (1995) [1].

Textural and colour parameters

The texture profile analysis of shrikhand was done with the help of instrumental texture profile analyser (TA HD Plus Texture analyser) for firmness, consistency, cohesiveness and work of cohesiveness (Bourne, 1978) [2]. Texture analyzer equipped with 5 kg load cell and back extrusion test using 35 mm cylinder probe was used for texture profile analysis of the samples. Other conditions (test descriptions) set for analyses were as follows:

Mode	:	Measure force in compression
option	:	Return to start
Pre-test speed	:	1 mm/sec
Test speed	:	1mm/sec
Post-test speed	:	10mm/sec
Distance	:	30mm
Trigger type	:	Auto (F) -10g
Trigger force	:	0.04903 N
Tare mode	:	Auto
Data acquisition rate	:	400pps
Probe	:	Back extrusion cell (A/BE)

The colour parameters *i.e.* lightness (*L**), redness (*a**) and yellowness (*b**) of the shrikhand were measured using Hunter colourimeter of ColourTech PCM+ (Colour Tec Associates Inc. Clinton NJ, USA) at Department of Goat Products Technology, CIRG, Makdhoom.

Sensory evaluation

Sensory evaluation was conducted by experienced semi trained panellists using 8-point descriptive scale (where 1= extremely disliked and 8= extremely liked) (Keeton, 1983) [10] for colour and appearance, flavour, texture, sweetness, mouth coating and overall acceptability.

Statistical analysis

The data obtained in the study on various parameters were statistically analyzed on 'SPSS-16.0' software package as per standard methods of Snedecor and Cochran (1995) [20]. Duplicate samples were drawn for each parameter and the experiment was replicated thrice (n=6). Sensory evaluation was performed by a panel of seven member judges three times, so total observations being 21 (n=21) Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between samples.

Result and discussion

Based on available literature, several preliminary trials were conducted to standardize the processing technology of goat milk shrikhand. The final formulation of goat milk shrikhand was optimized following the method prescribed by Gupta *et al*. (2018) [7] with slight modifications.

Physico-chemical properties

The pH values decreased while titratable acidity increased significantly ($P<0.05$) with increased culture levels, however there was no significant difference in titratable acidity of C2 and C3. Lower pH and higher titratable acidity values with increased culture level in shrikhand might be due to more lactic acid production during fermentation. The culture used for preparation of shrikhnad (NCDC 159) contained mixed culture of *Lactococcus lactis*, *Lactococcus diacetylactis* and *Lactococcus cremoris*. There was no significant difference in proximate parameters *i.e.* moisture, protein and fat content except ash content. C1 had significantly ($P<0.05$) higher ash content than C2; however ash content of C3 was comparable to C1 and C2. Lower ash content with higher culture levels might be due to leaching out of minerals along with whey due to improper setting of curd at higher culture level. Ronak *et al*. (2016) [19] developed mango flavoured steamed sweetened concentrated yoghurt (*Bhapa dahi*) with three different cultures *i.e*. Y-170F, Yo-Flex and Y-480F and observed no significant difference in proximate composition and physico-chemical properties except pH and titratable acidity. There was no significant difference in water activity, however brix values of C2 was significantly ($P<0.05$) higher than C1 and C3, which indicated firm, compact and smooth curd formation at 2.5% culture level.

Textural and colour parameters

All textural parameters *i.e*. firmness, consistency, cohesiveness and work of cohesiveness of C2 were significantly ($P<0.05$) higher than C1 and C3 due to proper setting of curd at 2.5% culture level leading to desirable firmness and smoothness. Curd formation was not appropriate at 2.0% and 3.0% culture level and shrikhand prepared by this curd was less viscous at 2.0% level and had custard like consistency at 3.0% level which was also observed by sensory panelists in sensory evaluation. Murti *et al*. (2019) [12] also observed that viscosity of fermented low fat-goat milk was significantly ($P<0.05$) higher in terms of brix values using culture containing *Streptococcus thermophilus* than mixed culture containing *Lactobacillus acidophilus, Bifidobacterium longum* and *Lactobacillus casei*. Lightness values of C2 were significantly ($P<0.05$) higher than C1 and C3. Whiteness in the fluid milk is result of the presence of colloidal particles such as milk fat globules and casein micelles capable of scattering light in visible spectrum (Fox and McSweeney, 1998) [6]. There was no significant difference in redness and yellowness values of shrikhand prepared by difference culture levels. Jeremia and Afam (2013) [9] also reported no definite pattern of colour values in commercial sour milk (amasi) products with decreasing pH.

Sensory evaluation

Flavour and texture scores of C2 were significantly ($P<0.05$) higher than C1 and C3; however there was no significant difference between C1 and C3. Higher flavour and texture scores of C2 were due to proper setting of curd providing desirable smoothness and firmness to the shrikhand. Courtin and Rul (2004) [4] reported that the association of *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* in yoghurt manufacture affected the production of volatile molecules involved in flavour development. There was no significant difference in colour and appearance as well as mouth coating scores. Sweetness scores of C1 and C2 were significantly ($P<0.05$) higher than C3; where no significant difference was observed between C1 and C2. Lower sweetness scores in C3 might be due to more lactic acid formation in curd resulting into sourness of product, which was also not liked by sensory panelists. Overall acceptability scores of C2 were significantly ($P<0.05$) higher than C1 followed by C3 due to proper curd setting, appropriate flavour and smooth texture of shrikhand at 2.5% culture level. Therefore, C2- goat milk shrikhand prepared with 5.0% fat and 2.5% NCDC-159 starter culture as found optimum.

Table 1: Physico-chemical properties (Mean±SE) of goat milk shrikhand prepared with different culture levels

Parameters	C1	C2	C3	Treatment Mean
pH	4.78^{a}±0.03	4.51^{b}±0.04	4.14^{c}±0.03	4.48±0.03
Titratable acidity	0.47^{b}±0.01	0.53^{a}±0.01	0.56^{a}±0.01	0.52±0.01
Moisture (%)	47.11±0.58	45.60±0.44	45.56±0.71	46.09±0.36
Protein (%)	6.37±0.13	6.37±0.14	6.23±0.17	6.32±0.08
Fat (%)	10.44±0.14	10.95±0.24	10.62±0.45	10.67±0.17
Ash (%)	0.72^{a}±0.01	0.64^{b}±0.02	0.66^{ab}±0.02	0.67±0.01
Water activity	0.945±0.06	0.941±0.05	0.939±0.09	0.941±0.03
Brix value	29.13^{b}±0.02	30.11^{a}±0.02	27.34^{c}±0.04	28.86±0.02

- Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05)
- n=6
- C1- goat milk shrikhand prepared with 5.0% fat and 2.0% starter culture
- C2- goat milk shrikhand prepared with 5.0% fat and 2.5% starter culture
- C3- goat milk shrikhand prepared with 5.0% fat and 3.0% starter culture

Table 2: Texture profile analysis (Mean±SE) of goat milk shrikhand prepared with different culture levels

Parameters	C1	C2	C3	Treatment Mean
Firmness	64.20^{b}±0.05	68.41^{a}±0.14	61.28^{c}±0.35	64.63±0.71
Consistency	42.35^{b}±0.13	45.30^{a}±0.11	41.82^{c}±0.23	42.82±0.16
Cohesiveness	31.94^{b}±0.25	34.21^{a}±0.28	28.09^{c}±0.26	31.41±0.63
Work of cohesiveness	24.46^{b}±0.34	26.54^{a}±0.14	21.86^{c}±0.21	24.28±0.48

- Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05)
- n=6
- C1- goat milk shrikhand prepared with 5.0% fat and 2.0% starter culture
- C2- goat milk shrikhand prepared with 5.0% fat and 2.5% starter culture
- C3- goat milk shrikhand prepared with 5.0% fat and 3.0% starter culture

Table 3: Colour estimation (Mean±SE) of goat milk shrikhand prepared with different culture levels

Parameters	C1	C2	C3	Treatment Mean
Lightness *(L*)*	75.62^{c}±0.14	78.98^{a}±0.04	76.34^{b}±0.04	76.98±0.05
Redness *(a*)*	4.95±0.13	4.90±0.09	4.81±0.07	4.88±0.05
Yellowness *(b*)*	7.44±0.14	7.69±0.07	7.54±0.09	7.55±0.06

- Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05)
- n=6
- C1- goat milk shrikhand prepared with 5.0% fat and 2.0% starter culture
- C2- goat milk shrikhand prepared with 5.0% fat and 2.5% starter culture
- C3- goat milk shrikhand prepared with 5.0% fat and 3.0% starter culture

Table 4: Sensory evaluation (Mean±SE) of goat milk shrikhand prepared with different culture levels

Attributes	C1	C2	C3	Treatment Mean
Colour and appearance	6.95±0.04	7.01±0.14	6.98±0.08	6.98±0.06
Flavour	6.68^{b}±0.07	7.04^{a}±0.09	6.75^{b}±0.08	6.74±0.05
Texture	6.55^{b}±0.11	6.86^{a}±0.09	6.53^{b}±0.09	6.65±0.06
Sweetness	6.74^{a}±0.08	6.86^{a}±0.08	6.35^{b}±0.13	6.65±0.06
Mouth coating	6.70±0.13	6.84±0.08	6.68±0.08	6.74±0.06
Overall acceptability	6.79^{b}±0.03	7.04^{a}±0.03	6.56^{c}±0.05	6.79±0.02

- Overall means bearing different superscripts in a row (a, b, c, d.....) differ significantly (P<0.05)
- n=6
- C1- goat milk shrikhand prepared with 5.0% fat and 2.0% starter culture
- C2- goat milk shrikhand prepared with 5.0% fat and 2.5% starter culture
- C3- goat milk shrikhand prepared with 5.0% fat and 3.0% starter culture

Conclusion

Use of starter culture in preparing different fermented products is very common it enhances the overall value of the product and it is also good for our gut health. Therefore it was concluded that goat milk shrikhand prepared with 5.0% fat and 2.5% NCDC-159 starter culture as found optimum.

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