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Effect of fat percentage on sensory attributes and growth characteristics of starter culture for development of whey-cereal based fermented beverage

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Abstract
Considering the popularity of cereal-based milk foods for their health benefits and excellent organoleptic qualities, the major task that lies ahead is to design these basic ingredients into products that help in alleviating the malnutrition and also appeal to the sophisticated palates of educated and health conscious consumers. So considering the need of the present day, present study was conducted to develop whey-cereal based fermented dairy products. Two different fat percentage (1 % and 4%) were used for development of the products and were evaluated for sensory acceptability as well as for growth indicators of NCDC-167 culture. It is depicted from the results that whey-cereal based fermented dairy beverage (Lassi) prepared using 4 % fat using germinated cereals and inoculation by NCDC-167 culture was found more suitable for preparation of whey pearl millet fermented dairy product (lassi) and whey moth bean (lassi). They scored higher and were more acceptable in comparison to product prepared using 1% fat.

Keywords: cereal, sensory, whey, beverage, fermented, dairy

Introduction
Fermented dairy foods have been an important part of human diet in many regions of the world since times immemorial. Evidences showing the use of fermented milks have been found in archeological research associated with the Sumerians and Babylonians of Mesopotamia, the Pharoes of Northeast Africa, and Indo-Aryans of the Indian subcontinent (Chandan, 2002; Tamime and Robinson, 1999) [1, 6, 21]. Also, the ancient Ayurveda system of medicine cites fermented milk (dadhi) for its health giving and disease-fighting properties (Aneja *et al.*, 2002) [1]. Buttermilk (lassi) is a popular lactic acid fermented milk beverage of the Indian subcontinent. It contains appreciable amounts of milk proteins and phospholipids along with various metabolites produced by lactic acid bacteria (Sharma *et al.*, 2016) [15]. Whey is a by-product of cheese, casein, paneer and channa manufacture and contains approximately 6% of whole milk solids. Whey contains almost all water soluble nutrients present in milk, particularly lactose, whey proteins, vitamins and minerals (Goyal and Gandhi, 2009) [11]. Dairy waste is major issue in the dairy industry. Most importantly, such huge volumes of whey represent a massive and growing protein resource available to the food and related industries of about 500×10^6 kg per year. Whey proteins have been known to have biological value superior to that of other naturally occurring proteins (e.g. egg, soya, beef, casein etc.).Considering the popularity of cereal-based milk foods for their health benefits and excellent organoleptic qualities, the major task that lies ahead is to design these basic ingredients into products that help in alleviating the malnutrition and also appeal to the sophisticated palates of educated and health conscious consumers. Today, the consumers demand products with functional properties which led to the promotion of added-value products such as probiotic and other functional yoghurts, reduced-fat and enriched milk products and fermented dairy drinks and organic cheese (Rudrello, 2004) [17]. Therefore, to make the best use of dairy whey and low cost grains, the present study was proposed to develop delicious and nutritious traditional cereals based fermented dairy products from the combinations of whey, skim milk powder and low cost grain (pearl millet and moth bean).

Materials and methods
The raw materials required for the product preparation viz. standardized milk (4.5% fat & 8.5% SNF), paneer whey and cream was procured from the Department of Livestock Products Technology, LUVAS, Hisar. Skim milk powder (SMP) of Nova brand, pearl millet and moth bean, cumin, black pepper and salt and glass bottles of 200 ml capacity were procured from the local market. Starter cultures i.e. NCDC-167 (*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* biovar. *diactetylactis* in 1:1:1 ratio) and NCDC-15 (*Lactobacillus acidophilus*) were obtained from Dairy Microbiology Division, National Collection of Dairy Cultures, ICAR-National Dairy Research Institute, Karnal (Haryana)-132001.

Other chemicals including pectin (degree of esterification 68-70 per cent) and nisin were procured from Hi Media Laboratories Pvt. Ltd 23, Vadhani Ind, Est., LBS Marg, Mumbai-400086, and India.

Preparation of Cereals and Whey

Two cereals (Pearl millet and Moth beans separately) @ 5% of base material, were germinated and then cereal slurry was prepared by grinding. The whey obtained was strained by passing through muslin cloth to remove suspended particles and then neutralization of fresh whey was done by using sodium bicarbonate (@0.5gm/100ml of whey) to adjust pH to 6.8±0.2. Then neutralized whey was standardized to 1 per cent fat and 18 per cent total solids level and 4 % fat and 18 % total solids levels using fresh cream and skim milk powder and then kept in refrigerator till further use.

Preparation of Control

Pearl millet control lassi was prepared as per procedure developed by Modha and Pal (2011) by using standardized milk (4.5% fat & 8.5% SNF) with some modifications.

500 ml standardized milk was preheated to 40°C and then 25 gm cereal slurry (@ 5 %) prepared from germinated pearl millet was added. The contents were heated to 90°C for 5 min and then immediately cooled to 37°C. It was inoculated by adding starter culture @ 3% (NCDC-167) followed by incubation at 37°C for 6-8 h to obtain desirable acidity (0.6-0.8 % lactic acid).

In a separate pan 350 ml water heated to 60°C, 5.20 gm pectin (@ 3 per cent) was added while continue stirring followed by addition of 7.35gm salt (@ 0.85%), 0.75gm black pepper (@ 0.05%) and 2.45 gm roasted cumin (@ 0.25%) and heating continued to raise the temperature to 80°C and then immediate cooled to 30°C. To prepare pearl millet beverage (lassi), curd obtained was transferred to electric mixer jar having pectin and spiced water. The mixture was blended for 2 minutes. The final products (lassi) was cooled to 5 °C and stored in refrigerator (5–7 °C) for 2 hours before offered for sensory evaluation. Similarly, control of moth bean beverage (lassi) was prepared by replacing pearl millet with moth bean.

Préparation of Whey-Cereal Beverage (Lassi)

Likewise, whey cereal beverage was prepared substituting milk with neutralized and standardized whey with two different fat % separately (1% & 4%) was preheated to 40°C and pectin @ 0.1 per cent of whey was added and then converted into two types of whey - pearl millet beverage (lassi). Similarly, total two types whey-moth bean beverage (lassi) were prepared by replacing pearl millet with moth bean.

Selection Protocol

Each type of beverage (lassi) along with control was offered for sensory evaluation (using 9 point hedonic scale) to six judges selected from faculty and post graduate students of Livestock Products Technology Department separately for each parameter studied. Each type of beverage (lassi) along with control was also assessed for growth indicators (pH by pH meter, titrable acidity by AOAC (2007) [3], per cent soluble nitrogen AOAC (2005) [2] and acetaldehyde by Robinson *et al.* (1977) [16] of culture. Out of 2 treatments of each cereal with two different fat %, one best fat % was selected based upon sensory evaluation and growth indicators of culture.

Sensory evaluation

A semi trained panel consisting of faculty members and post graduate students evaluated the sensory attributes viz. colour and appearance, flavor, consistency and overall acceptability using a 9-point Hedonic scale (Stone *et al*. 1974) [20]. The test samples were presented to the panelists after assigning the suitable codes. The water was served for rinsing the mouth between the samples.

Results & Discussion

Whey composition

Fresh whey was obtained from Experimental Dairy Plant of Livestock Products Technology Department, it was assessed for its composition and results are depicted in table 1.

TA and pH observations were similar to Bund and Pandit (2005) who also observed paneer whey titrable acidity ranges between 0.4-0.6, and pH ranges from 4.0-5.0 respectively.

Table 1: Composition of whey

Parameters	Per cent
Moisture (%)	93.30
Total solids (%)	6.70
Lactose (%)*	4.88
Protein (%)	0.68
Fat (%)	0.65
Ash (%)	0.49

*Calculated by difference (T.S.-(Fat+Protein+Ash)

Selection of Suitable Fat Per Cent for Whey-Germinated Pearl Millet Based Beverage (Lassi)

After optimizing the form of cereal processing and selection of suitable starter culture this experiment was subjected to identify desired fat per cent for preparation of whey-germinated pearl millet slurry lassi. Desirable fat per cent was identified by judging sensory attributes and growth indicators of whey-germinated pearl millet slurry lassi prepared by using NCDC-167 starter culture with different fat content.

Sensory Evaluation

Sensory scores of whey-germinated pearl millet slurry lassi prepared by using NCDC-167 starter culture have been presented in (table 2).

Sample prepared by using 1 per cent fat was liked moderately by the panelist in reference to flavour, consistency, colour and appearance and overall acceptability while sample prepared by using 4 per cent fat was preferred as liked very much on 9 point hedonic scale by the judges for all the sensory attributes and it was at par with control sample scores. Present study results are in accordance with Chawla and Balachandran (1993) [8] they reported that higher fat content in yoghurt gave yoghurt with superior flavor and appearance scores however, statistically flavor scores of yoghurts with 3, 4.0 and 6.0 per cent fat were not different. Similarly, Becker and Puhan (1989) [4] observed that yoghurt made from whole milk did not show any whey separation. However, in case of skim milk out

of 63 yoghurt samples, 15 showed a whey layer on the surface after 14 days of storage, especially those with low TS.

Consistency scores of whey-germinated pearl millet lassi with 4 per ecnt fat were at par with control, while sample with 1 per cent fat was scored significantly lowest. Magra *et al.* (2012) [12] observed samples with 3.5% fat were perceived as more viscous, less acidic and more acceptable than those with less fat %. Increased milk base fat content resulted in increased viscosity and reduced flow behavior index. Similar observations were made by several authors regarding the viscosity of yoghurt and how it's affected by the fat content of the milk used for its production (Becker and Puhan, 1989; Shaker *et al*., 2000) [4]. This increased viscosity is attributed to the formation of clusters from the fat globules of the homogenized milk, which are active due to the presence of phospholipids and proteins on their surface, and they interaction with each other even before they form cross links with whey proteins and casein micelles (Cho *et al*., 1999).

Table 2: Selection of suitable fat percentage for preparation of whey germinated pearl millet based fermented beverage (Lassi) with culture NCDC-167 based on sensory attributes

Sensory attributes	Control	Fat Levels	
		1%	4%
Colour &Appearance	8.00±0.72^{b}	7.70±0.83^{a}	8.00±0.94^{b}
Consistency	8.20±0.48^{b}	7.20±0.59^{a}	8.10±0.61^{b}
Flavour	8.20±0.19^{b}	7.50±0.28^{a}	8.10±0.37^{b}
Overall Acceptability	8.20±0.19^{b}	7.50±0.82^{a}	8.00±0.93^{b}

Mean ± SD, n=18

Means with different superscripts in a row differ significantly (P≤0.05)

Growth Indicators

Growth indicators of whey-germinated pearl millet slurry lassi prepared by using NCDC-167 starter culture with different fat content have been presented in table 3.

Significantly higher pH was observed in control sample followed by 4 per cent fat sample and then1 per cent fat. While, acidity has shown the reverse trend than pH.

Maghra *et al.* (2012) [12] observed 4.25, 4.21, 4.23 on day zero with 0, 1.5 and 3.5 fat % respectively. This may be because solid nonfat were 8.5 percent in control while being highest in 1 per cent fat i.e. 17 % and followed by 4 per cent fat i.e. 14 %. Higher solid nonfat % contributes to more acidity. (Chawla, 1985) [7].

Soluble nitrogen was higher in control sample but statistically it was at par with the lassi sample prepared by using 4 per cent fat as well as the lassi sample prepared by using 1 per cent fat. Acetaldehyde was recorded as statistically similar in all lassi samples.

On the basis of sensory scores and culture growth factors results it is concluded that high fat (4 per cent) had significant influence on flavour and overall acceptability of beverage. Therefore, 4 per cent fat was selected for further experiments.

Table 3: Selection of desirable fat per cent for preparation of whey-germinated pearl millet lassi prepared by using starter culture NCDC-167 based on growth indicators.

Growth indicators	Control	Fat Levels	
		1%	4%
pH	4.52±±0.01^{b}	4.43±±0.07^{a}	4.50±±0.02^{b}
Titrable acidity	0.67±±0.06^{a}	0.77±0.03^{b}	0.70±0.08^{a}
soluble nitrogen (per cent)	0.24±0.05	0.21±0.04	0.22±0.07
Acetaldehyde (ppm)	1.25±0.03	1.23±0.06	1.24±0.02

Mean ± SD, n=6

Means with different superscripts in a row differ significantly (P≤0.05

Selection of Suitable Fat Percent for Whey-Germinated Moth Bean Based Beverage (Lassi) Based On Sensory Attributes

After optimizing the form of cereal processing and culture, this experiment was subjected to standardize the process with respect to fat per cent (1 per cent and 4 per cent) for preparation of whey-germinated moth bean based Lassi.

Sensory Attributes

Sensory scores of whey-germinated moth bean slurry lassi prepared by using NCDC 167 starter culture have been presented in (table 4).

Judges scored whey-germinated moth bean based lassi sample prepared by using 4 per cent fat and control sample statistically at par for its color and appearance, consistency, flavor and overall acceptability, respectively, while whey-germinated moth bean based lassi sample prepared by using 1 per cent fat was scored significantly lower for its colour and appearance, consistency, flavor and overall acceptability. This may be because, the fat per cent was comparable between the two (control and sample with 4 per cent fat) and higher fat per cent contributes enhancement in color, consistency, flavor and further overall acceptability. Spasenija *et al.* (2009) [19] found out that fat content influenced textural properties of fermented milk beverages including firmness and consistency. And observed that higher fat content of beverage affects the firmness, consistency, cohesiveness and viscosity index, of samples during storage. Pandya and Ghodke (2007) [14] observed improvement in firmness of curd and reduced wheying off after increasing fat content in milk and also Low SNF leads to flat and watery product and increased wheying off and less stability (Driessen and Puhan, 1988) [10].

Magra *et al.*, (2012) [12] observed samples with 3.5% fat were perceived as more viscous, less acidic and more acceptable than those with less.

Table 4: Selection of desirable fat level for preparation of whey germinated moth bean based fermented beverage (Lassi) with culture NCDC-167 based on sensory attributes

Sensory attributes	Control	Fat Levels	
		1%	4%
Colour &Appearance	8.00±0.09^{b}	7.70±0.09^{a}	8.00±0.09^{b}
Consistency	8.20±0.09^{b}	7.20±0.09^{a}	8.10±0.09^{b}
Flavour	8.10±0.09^{c}	7.40±0.09^{a}	7.90±0.09^{b}
Overall Acceptability	8.10±0.09^{b}	7.40±0.09^{a}	8.00±0.09^{b}

Mean ± SD, n=18

Means with different superscripts in a row differ significantly (P≤0.05) in each particular group

Growth indicators

Among growth indicators, pH results revealed that whey-germinated moth bean lassi prepared by using 1 per cent fat had significantly lowest pH than control beverage sample as well as lassi sample prepared by using 4 per cent fat which had significantly high pH. Titrable acidity was recorded significantly lower for control sample in comparison to whey-germinated moth bean lassi sample prepared by using 1 per cent fat as well as 4 per cent fat. However, there was significant difference among whey-germinated moth bean lassi sample prepared by using 1 per cent fat as well as 4 per cent fat. This may be because of solid non fat (SNF) difference between the samples. Similar results are also reported by Chawla (1985) [7] who observed high milk solid nonfat contributes to higher titrable acidity.

Table 5: Selection of suitable fat level for preparation of whey lassi prepared by using germinated moth bean with culture NCDC-167 based on growth indicators.

Growth factor	Control	Fat Levels	
		1%	4%
pH	4.76±0.01^{c}	4.52±0.02^{a}	4.62±0.03^{b}
Titrable acidity	0.67±0.04^{a}	0.85±0.07^{c}	0.75±0.09^{b}
Soluble nitrogen percent	0.21±0.02	0.18±0.04	0.20±0.06
Acetaldehyde (ppm)	1.23±0.08	1.20±0.03	1.21±0.05

Mean ± SD, n=6

Means with different superscripts (a,b, c) in a row differ significantly (P≤0.05)

Soluble nitrogen was higher in control sample but statistically it was at par with the beverage prepared by using 4 per cent fat as well as the beverage prepared by using 1 per cent fat. This may be because of similar protein content in all which contributes to soluble nitrogen per cent. Acetaldehyde was recorded as statistically similar in all the beverage samples.

Therefore, on the basis of sensory score and growth indicators, whey-germinated moth bean lassi prepared by using 4 per cent fat scores were comparable with control; therefore, 4 per cent fat was selected for further product development and for further studies.

Conclusions

The increasing awareness for nutrition, health and quality food consciousness of consumers and the keen competition in the market, compel the food industry to search for such ingredients, which can impart specific functionalities to food products, while preserving and enhancing the nutritional quality of foodstuffs, in order to sell their products profitably. The food manufacturing industry has come to realize that milk proteins in general and whey proteins in particular have potential to improve the quality of food products. To develop whey-cereal based beverage (lassi), pearl millet and moth bean were used and cereals were processed by germinating before making slurry of cereals. The slurry was mixed with standardized whey to develop whey-cereal based beverage (lassi).Whey fat content was adjusted to 1 percent by using fresh cream and total solids were enhanced to the tune of 18 percent by adding skim milk powder and inoculated with starter culture viz.NCDC-167 for making curd from the mixture of whey and cereals slurry. Then curd obtained was used for preparing whey-cereal based fermented beverage (lassi) by stirring with addition of pectin and spiced water. Selection of suitable fat % (1 % & 4%) was done on the basis of sensory attributes and growth indicators starter cultures. Present study results indicated that whey-cereal lassi samples prepared with 4 % fat were found most suitable in comparison to 1 % fat, because 4 % fat improved the sensory scores and growth indicators (depicting growth characteristics of starter culture). Sensory panelist scores (for flavor and overall acceptability) were recorded highest in samples inoculated with starter culture 4% fat. Overall acceptability scores of sensory attributes did not differ significantly among control sample and sample prepared from 4% fat. So, while keeping in view the acceptability, 4% fat was selected for the development of whey cereal based fermented beverages and for further study.

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