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Ingredient formulation effects on physico-chemical, sensory, textural properties and probiotic count of *Aloe vera* probiotic *dahi*

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ABSTRACT

In this study, central composite rotatable design of response surface methodology (RSM) was used to investigate the combined effects of milk fat (2–4 g/100 g), milk solids-not-fat (MSNF; 8–10 g/100 g) and *Aloe vera* juice (AVJ; 16–20 g/100 g) on probiotic count, physico-chemical, sensory and textural properties of *A. vera* supplemented probiotic *dahi* (APD). Analysis of variance indicated that experimental data was well explained by the quadratic model with high efficiency check values ($R^2 > 0.78$) and the lack-of-fit tests were not significant. The results showed that with increasing AVJ content, pH and water holding capacity (WHC) of APD decreased and consequently syneresis increased. Increasing milk fat and MSNF levels significantly improved WHC and prevented syneresis. Sensory quality was improved by raising the level of milk fat. Improvement in probiotic count was noticed with increasing MSNF and AVJ content probably due to increase in amount and diversity of nutrients. Firmness and work of shear values were increased significantly with milk fat and MSNF levels. AVJ addition decreased the work of adhesion values. Levels of the ingredients in optimized APD with maximum overall acceptability were found to be 4 g/100 g milk fat, 10 g/100 g MSNF and 16 g/100 g AVJ.

1. Introduction

Fermented milks endowed with many therapeutic ingredients are an excellent medium to generate an array of products that fit into the current consumer demand for health-based foods. *Dahi* is a fermented milk product widely consumed in South-Asian countries. Traditionally, it is prepared by fermentation of milk using mixed strains of mesophilic lactic acid bacteria (LAB). *Dahi* is consumed as such regularly in households and also used as base material for the preparation of a variety of culinary products including *lassi*, *shrikhand*, *rabdi*, *raita* and *dahi-bhalle*. In the recent past, several innovations were made to enhance the nutritional value and physiological functionality of *dahi* by incorporating fruits, exopolysaccharides (EPS) cultures, probiotics and cereals.

Aloe barbadensis Miller (*Aloe vera*) is considered to be the most biologically active of the approximately 420 *Aloe* species identified and characterized till date. *A. vera* contains several biologically active constituents including vitamins, minerals, polysaccharides, amino acids, anthraquinones, saponins, phytosterols and salicylic acids etc. Several research reports credit *A. vera* with health benefits viz. antitumour, antidiabetic, hypolipidemic wounds and burns healing activity, ulcer prevention, immunomodulatory and prebiotic properties (Hussain, Panjagari, Singh, & Patil, 2015). A considerable portion of today's functional food market consists of *A. vera* as a functional ingredient. Probiotics have been defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002). Probiotic cultures belonging to *Lactobacillus* group had a long association with respect to the manufacture of dairy products. Among the *Lactobacilli*, species *Lactobacillus paracasei* is a common inhabitant of the human intestinal tract and also listed in the 'Inventory of microorganisms with documented history of use in human food'. Proven health benefits of *L. paracasei* include alleviation of lactose intolerance, minimization of the tumor recurrence, relieve from fatigue, immunomodulatory and anti-diarrhoeal activity (Chapman, Gibson, & Rowland, 2011). Different strains of *L. paracasei* occurred naturally in fermented vegetables, milk and meat. Selected strains of this species have also been used in

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preparing cheese, yogurt, kefir and *lassi* (Hussain, Patil, Yadav, & Singh, 2015).

Addition of functional ingredients like *A. vera* and probiotics into *dahi* enhances its nutritional and physiological virtues. However, these functional ingredients could alter the sensory and textural characteristics of *dahi*, which thus influences its consumer acceptance. Moreover, it is necessary to maintain the viability of the probiotics above the therapeutic minimum ($>10^6$ cfu/g) to provide health benefits to the host, which is influenced by the composition of food matrix and processing conditions. Hence, it is necessary to study the effect of functional ingredients on quality characteristics of developed product. Response surface methodology (RSM) has been applied in recent years to optimize the new product formulations. RSM delineates the effect of the independent variables on responses of importance and is regarded as an effective method to optimize the new product formulations. In the present study, effect of independent variables viz. Milk fat, milk solids-not-fat (MSNF) and *A. vera* juice (AVJ) on various quality characteristics of *A. vera* supplemented probiotic *dahi* (APD) was investigated with an aim to optimize these responses in the light of product formulation.

2. Materials and methods

2.1. Materials

Freeze dried *dahi* culture viz. *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* (NCDC 60) and probiotic culture *L. paracasei* ssp. *paracasei* (NCDC 627) were procured from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal. Skim milk powder (medium heat classified) was procured from M/s Modern Dairies Ltd., Karnal. Food grade *A. vera* juice was procured from Mehta Herbs and Spices, Tamil Nadu, India. The juice had aloin content of 0.012%, mucopolysaccharides 20,000 molecules in length, pH 5.1 and total solids (TS) content of 0.98 g/100 g. Dehydrated media i.e. MRS agar was procured from Hi–Media Laboratories, Bombay.

2.2. Methods

2.2.1. Preparation of APD

Fresh buffalo milk was standardized to desired fat and SNF levels. Standardized buffalo milk was heat treated to 90 °C for 15 min followed by immediate cooling to 37 °C and then added with sterilized (121 °C for 16 min) AVJ at 16 g/100 g level. This milk-AVJ mixture was inoculated with a culture combination comprising of NCDC 60 and NCDC 627 (1:1) at 2 g/100 g level, filled in pre-sterilized glass beakers followed by incubation at 37 °C for 10 h to obtain a firm curd. APD samples obtained were stored in refrigerator at 5 ± 1 °C until subjected for various analyses.

Starter cultures NCDC 60 and probiotic strain NCDC 627 were maintained in sterilized skim milk tubes. The propagation of the cultures was done at weekly intervals.

2.2.2. Physico-chemical analysis

2.2.2.1. pH. The pH of APD samples was measured using a digital pH meter (pH Tutor, EUTECH Instruments, Malaysia) at 20 °C using a combined glass electrode fitted in association with a temperature probe. Before use, the pH meter was calibrated using standard buffers of pH 4.0 and 9.0 at 20 °C.

2.2.2.2. Syneresis. A cup of APD removed from the refrigerator was stirred 20 times clockwise and anticlockwise with a glass rod. Approximately 30 g of the APD was transferred into 50 mL polypropylene conical centrifuge tube using 5 mL pipette and left for 2 h at 5 °C for stabilization. The stirred samples were then centrifuged at 3000 rpm for 15 min at 10 °C to observe whey separation. The syneresis was expressed as percent syneresis.

$$\textit{Percent Syneresis} = \frac{\text{Weight of separated whey}}{\text{Initial weight of sample}} \times 100$$

2.2.2.3. Water holding capacity (WHC). A 30 g of sample was centrifuged at 3000 rpm for 15 min at 10 °C. The supernatant was removed, and the pellet weight was recorded. Pellet weight divided by initial weight of APD and multiplied by hundred is taken as percent WHC.

2.2.3. Textural attributes

Textural attributes such as firmness, stickiness, work of shear and work of adhesion were determined by back extrusion method using a texture analyser, TA-XT2i (M/s Stable Micro Systems, UK) fitted with a 25 kg load cell, which was calibrated with 5 kg standard dead weight prior to use. For determining the textural attributes, the pasteurized and cooled APD mix was filled up to 5 cm (approx. 125 mL) in pre-sterilized glass beakers (10 cm height and 6 cm dia) and incubation (37 °C for 12 h) was carried out to obtain APD. Overnight refrigerated APD samples were drawn from the refrigerator (4–5 °C) and subjected for texture analysis. The probe (A/BE 35) was penetrated up to 10 mm into the set APD samples at cross head speed of 1.0 mm/s. The operational speed of the probe was kept at possible minimum level to obtain more precise data from the test. The pre and post test speed of the probe were adjusted as 2.0 mm/s. From the resulting force–time curves, firmness (N), stickiness (N), work of shear (N.s) and work of adhesion (N.s) were calculated using the Texture Expert Exceed software (v 2.55) supplied by the manufacturer. All measurements were done in quadruplicate for each sample. Typical force deformation curve obtained for APD is given in Fig. 1.

2.2.4. Sensory evaluation

Sensory evaluation of APD samples was carried out using 9-point Hedonic scale. The APD samples were drawn from the refrigerator immediately before serving to the panellists. Sensory evaluation panel consisted of ten judges having adequate knowledge about the sensory evaluation methods and product characteristics were chosen from the Dairy Technology Division of ICAR-NDRI, Karnal. Sensory attributes evaluated were, colour and appearance (look at the colour of APD and observe for any possible whey separation and layered appearance on its surface), flavour (place adequate amount of APD in mouth and perceive the taste and odours like acidity, off flavours if any), body and texture (place adequate amount of APD in mouth and perceive the firmness, ease of dissolving, adhesiveness to the tongue and palate) and overall acceptability (draw a conclusion based on the fore mentioned attributes). Sensory score card consisted of the following hedonic ratings viz. dislike extremely (1), dislike very much (2), dislike moderately (3), dislike slightly (4), neither like nor dislike (5), like slightly (6), like moderately (7), like very much (8) and like extremely (9).

2.2.5. Enumeration of probiotic bacteria

After homogenous mixing with a sterile glass rod, 1 g of APD sample was transferred aseptically to a test tube containing 9 mL of sterile normal saline (0.85 g NaCl in 100 g distilled water) and mixed well. Further, 1/10th serial dilutions were prepared in saline. Appropriate sample diluents were pour plated using MRS agar for the enumeration of probiotic *Lactobacilli*. The *Lactobacillus* colonies grown were counted after incubating the plates at 37 °C for 48 h. The results were expressed as log cfu/mL.