Process of Fortification of Cow Milk Curd with Beta (β) Carotene Producing Sweet Potato

Abstract
Sweet potato (SP) is the world’s seventh most important tropical food crop after wheat, rice, maize, potato, barley and cassava. Pigments present in sweet potato namely, β-carotene, anthocyanin and unidentified flavonoids are regarded as antioxidants. As SP roots are rich in starch, sugars, minerals, vitamins and dietary fiber, they have high potential for undergoing fermentation into value-added commodities like curd, yogurt, pickles, amongst others. Added β-carotene rich SP provided vitamin A, starch, dietary fiber that functioned as thickener and stabilizer. In the present study, β-carotene rich SP curd was prepared by fermenting (hyphenate these two words) 16% SP and cow’s milk. The samples were subjected to consumer evaluation that was modeled using logistic regression to identify which sensory attributes determine acceptability of the product. Our results showed that texture and color significantly influence consumer acceptability. Addition of SP enriched curd with dietary fiber and starch improved the firmness of the curd.

Keywords: Milk curd; Lactic acid bacteria; β Carotene; Sweet potato

Introduction
The sweet potato, Ipomoea batatas L. (Lam.), is a dicotyledonous plant belonging to Convolvulaceae family. It is an extremely important crop mainly seen in tropical countries. It ranks seventh in the world from the viewpoint of total production. It is also a storehouse of many important pigments like β-carotene, anthocyanin etc which act as good antioxidants [1]. In addition to its nutritional benefits, the crop’s easy adaptability to tropical climate and minimal growth requirements, make it a crop of high commercial importance [2]. Recently it has been found that sweet potato can control blood sugar levels and insulin resistance [3].

Some sweet potato varieties are rich in β-carotene and anthocyanin pigment. To get benefit from these naturally available antioxidants, sweet potato can be consumed either directly or can be incorporated into some other edible food [4]. One such novel idea is production of vegetable curd [5]. Curd or yoghurt is a rich source of proteins, essential vitamins, minerals etc. It is a product of lactic acid fermentation of milk and it is nutritionally more beneficial than milk. Yoghurt involves the use of specific symbiotic /mixed culture of Lactobacillus bulgaricus and Streptococcus thermophilus. It usually consists of heterogeneous mixture of lactic acid bacteria i.e. Lactobacillus bulgaricus, S. lactis, S. diacetylactis, S. elonoris, etc. Basically curd is produced due to fermentation of milk lactose into lactic acid by lactic acid bacteria. This curd can be further enriched by dietary fibres, starch, minerals, vitamins and important pigments by co-fermenting it with vegetables such as sweet potato, lemon etc [6]. This study aims to produce β-carotene rich sweet potato curd and its overall acceptability by the consumers.

Materials and Methods

Preparation of Starter Culture
300 ml of cow milk (Amul Dairy) was heated to boil for about 10-12 minutes. Then it was cooled to room temperature 28 ± 2 °C. After cooling, 2 ml of previously prepared homemade curd and 2 ml (v/v) inoculums of L. plantarum and 0.5:1.5 (v/v) ratio of S. thermophilus and L. plantarum [7] was inoculated into 100 ml each of three milk sample respectively. Then the three milk sample mixtures were kept in an incubator at 37 °C for 14 h to prepare curd starter culture. In our study, cow milk inoculated with 2% (v/v) L. plantarum was used as the control starter culture to form cow milk curd.

Preparation of β-Carotene Rich Sweet Potato (SP) Curd
Clean SP roots with skin intact were boiled for 10–15 min. After boiling, the skins were peeled off and the pulp was macerated into a puree. SP-milk mixtures were prepared by adding SP puree (15%) to previously pasteurized milk (200 ml). Then 2% (v/v) starter culture was added aseptically and fermented for 16 h at 37 °C to form sweet potato curd. 2% (v/v) starter culture of L. plantarum was added into 100 ml of boiled cow milk to form curd used as control.

Estimation of β carotene in sweet potato curd by solvent extraction method [8].

Procedure (Figure 1)
a) Extraction: 5 g of each of the curd samples was taken in a beaker, and to the sample 62.5 ml of diacetone alcohol was
added. The mixture was then allowed to macerate for 5 min into a blender. It was then filtered through a Buckner funnel. The residues were washed with a diacetone alcohol until washings were colorless.

b) Separation: The filtrate was transferred in a separating funnel and 25 ml of petroleum ether was added to it. The mixture was shaken well and was allowed the phases to separate. The lower layer was drawn off to a second separating funnel. To this, another 25 ml of petroleum ether was added, shaken well and the lower layer was drawn to a beaker and the petroleum ether extract was combined in the first separating funnel.

c) Purification: 12.5 ml of diacetone alcohol was added to the combined petroleum ether extracts and was shaken at least for 30 sec. The mixed layers were then allowed to separate and the lower layer was discarded. Then 12.5 ml of methanolic KOH was added, shaken for 1 min, and the phases were allowed to separate and the lower layer was discarded. Then, 50 ml of water was added to the petroleum ether extracts in a separating funnel. It was then shaken for 1 min, allowed the phases to separate and then the aqueous layer was discarded. Then, the separating layer was filtered through Na₂SO₄ contained in a funnel into volumetric flask. The sample was diluted and was read in a spectrophotometer at 450 nm.

d) Preparation of standard solution: 5 mg of standard β carotene was taken. It was dissolved in a 50 ml of petroleum ether. It was considered as the 'stock solution.' The stock solution was diluted to a concentration of 0.001 to 0.5 mg/ml for preparing the standard curve and then was estimated through spectrophotometer at 450 nm.

e) Calculation: Using the value obtained from the standard curve, the carotene present in the curd sample was calculated by the formula

\[
\mu g \text{ of carotene } / g = \frac{\text{concentration from the curve} \times \text{final volume} \times \text{dilution}}{\text{Weight of the sample}}
\]

Sensory analysis by consumer panel

Well trained panelists (both male and female faculties and research scholars- of total 30 males, 30 females and 25 research scholars) belonging to the age group of 26-40 years were chosen for the sensory study. Prior to analysis, panel members were questioned about the frequency of their curd consumption and only people who consume curd regularly were invited to participate in the preference test. Curd containing 15 % SP puree was selected for the study.

Prior to evaluation, an introductory session was held to familiarize panelists with the product. After introductory session, the panelists were served 80 g of SP curd in 150 mL odorless plastic cups. Sample temperature at the time of serving was 20–22 °C. Panelists were instructed to rinse with water prior to evaluation. They evaluated SP curd on a specifically prepared sensory scale comprising of five major sensory attributes viz. color and appearance, body and texture, flavor, mouthfeelness, overall acceptability. These attributes were carefully selected after reviewing wide range of sensory evaluation studies of dairy products. The sensory attributes were quantified using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Consumers also indicated their acceptability on a nominal scale (1 = acceptable and 0 = not acceptable).

Results and Discussion

From Table 2, it has been shown that plain milk curd i.e. without addition of sweet potato carotene is present in insignificant amount whereas, sweet potato curd contains significant amount of β carotene [9]. Yet its nutritional profile makes the calories worth it, especially since they are fat-free. Its fibre alone is enough to make a sweet potato worth eating. Moreover, from the table it can also be seen that, only with the addition of Lactobacillus can enhance the amount of β carotene into the curd, hence the firmness of the curd was enhanced with the addition of mixed culture of S. thermophilus and L. plantarum.

Sensory Evaluation

The sensory evaluation of fermented dairy products was based on Hedonic analysis where different attributes namely, taste, flavor, color, acidity and overall acceptability had been considered and was tested by the panelists to provide the marks from 1 to 9 in the Hedonic Rating Scale where 1 stands for very poor and 9 stands for excellent. The sensory analysis was essential for enhancing the acceptance of sweet potato curd samples by the consumers. This sensory analysis was based on the Qualitative Descriptive Analysis (QDA) [10] of fermented dairy product and then was ranked by the trained panelists (n=30) according to the different parameters of the sweet potato curds. The results are reported in Table 1 & 2.

Where, S1: cow milk curd (without fortification with sweet potato) inoculated with 2 % (v/v) L. plantarum, S2: Sweet potato curd inoculated with 2 % (v/v) L. plantarum, S3: Sweet potato curd inoculated with 0.5:1.5 (v/v) S. thermophilus and L. plantarum. The experiment was done in triplicate. Here, S1, S2 and S3 are the mean values.
From the sensory evaluation it can be concluded that (table 3), sweet potato curd was accepted by the consumers; however, it has lower acceptability compared to the curd without fortification with sweet potato. It might be due to the lower consistency and the overall appearance of the sweet potato curd samples.

However, from the nutritional point of view and from the health benefit so far studied such types of fortified enriched fermented dairy products are well acceptable to the consumers (State the reference).

| Concentration (µg/ml) | Absorbance at 450 nm |
|----------------------|----------------------|
| 0                    | 0                    |
| 0.001                | 0.001                |
| 0.005                | 0.002                |
| 0.01                 | 0.004                |
| 0.05                 | 0.014                |
| 0.1                  | 0.028                |
| 0.5                  | 0.127                |

Table 1: Standard curve of β Carotene.

| Samples (Ensure Uniformity in Writing of Ratios) | β Carotene |
|--------------------------------------------------|------------|
| Cow milk curd inoculated with 2% (v/v) inoculum of S. thermophilus | Nil        |
| Sweet potato curd inoculated with 2% (v/v) L. plantarum | 4.8 µg/g   |
| Sweet potato curd inoculated with 0.5:1.5 (v/v) S. thermophilus & L. plantarum | 3.2 µg/g   |

Table 2: Estimation of β carotene in curd samples.

| Parameters                  | Sweet Potato Curd |
|-----------------------------|-------------------|
|                            | S1                |
|                            | S2                |
|                            | S3                |
| Color and appearance       | 7.1 ± 0.04        |
| Body and texture           | 7.3 ± 0.15        |
| Flavor                     | 7.4 ± 0.09        |
| Mouthfeelness              | 7.3 ± 0.11        |
| Acidity                    | 7.0 ± 0.12        |
| Overall acceptability      | 7.6 ± 0.06        |
|                            | 6.9 ± 0.05        |
|                            | 7.1 ± 0.10        |
|                            | 7.4 ± 0.09        |
|                            | 7.1 ± 0.13        |
|                            | 6.9 ± 0.08        |
|                            | 6.5 ± 0.06        |
|                            | 6.3 ± 0.13        |
|                            | 7.2 ± 0.08        |
|                            | 7.3 ± 0.05        |
|                            | 7.4 ± 0.08        |
|                            | 7.2 ± 0.06        |

Table 3: Sensory analysis of Sweet potato curd samples.

**Conclusion**

Fortification of the curd with beta carotene rich sweet potato can enhance the nutrient content as well as the culinary qualities (color and texture). Consumer evaluation is an important component in the product development process. In this study, β-carotene rich SP curd was prepared by co fermenting 16 % SP and cow’s milk. The samples were subjected to consumer evaluation to identify which sensory attribute determines acceptability of the product. Texture and color of the sweet potato significantly influence consumer acceptability. Moreover, it can also be concluded that, with the addition of lactic cultures along with the sweet potato puree enhance the content of β-carotene in the curd sample having consumer benefit.
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