DEVELOPMENT, SENSORY EVALUATION AND PROXIMATE ANALYSIS OF FERMENTED BUFFALO MILK PRODUCT PREPARED FROM DATE PALM (*PHOENIX DACTYLIFERA*) SYRUP[#]

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ABSTRACT

Date palm (*Phoenix dactylifera*) is one of the most suitable and popular tree grown in arid and semi-arid regions of Rajasthan which is characterized by long and hot summers, no (or almost low) rainfall, and very low relative humidity level. Dates palms also have numerous health benefits along with great taste. In view of this, an attempt was made to utilize Dates (*Phoenix dactylifera*) in form of Date Palm syrup @ 5% W/W and sugar @ 5% W/W basis incorporated in the milk samples fermented with *Lactobacillus helveticus* of buffalo milk used for production of fermented buffalo milk products at the time period of fermentation, where it show highest anti oxidant activity (both ABTS and DPPH basis) (8 hours of fermentation of buffalo milk) to develop low cost fibre rich products i.e. fermented buffalo milk product (yogurt) prepared from date palm (*Phoenix dactylifera*) extraction, for people suffering from micronutrient deficiency and to assess the sensory quality of developed products. A study on sensory evaluation of fermented buffalo milk product was take place at the time of production using a group of 10 panelists using 8 point hedonic scale. Thus, the proximate analysis and sensory evaluation data shows that the yoghurt made up of fermented buffalo milk had acceptable flavor, texture, colour and overall acceptability.

Key word: Date palm, buffalo milk, sensory evaluation, milk product, yogurt

Introduction

Milk and milk products are greatest blessing for new born mammals and human as it serve as an almost complete food with full of nutrients (Nema *et al.,* 2015). Milk fermentation by proteolytic lactic acid bacteria (LAB) is one of the economical and practical methods for the production of fermented dairy products enriched in bioactive peptides (Hayes *et al.,* 2007). A number of natural antioxidative agents have been produced from plants, and some dietary proteins have also been reported to have antioxidant activity (Okada and Okada, 1998).Several food protein hydrolysates have been found to exhibit antioxidant activity (Saiga *et al.,* 2003 and Davalos *et al.,* 2004). India has first rank in buffalo population in the world and Murrah is one of the most economical breed of buffalo (Malhotra *et al.,* 2015).

Date palm is cultivated in arid and semi-arid regions which are characterized by long and hot summers, no (or at most low) rainfall, and very low relative humidity level during the ripening period.

Exceptional high temperatures (± 56°C) are well endured by a date palm for several days under irrigation. During winters, temperatures below 0°C are also endured. The zero vegetation point of a date palm is 7°C, above this level growth is active and reaches its optimum at about 32°C; the growth will continue at a stable rate until the temperature reaches 38°C/ 40°C when it will start decreasing. Even though date palm is a thermopile species, it withstands large temperature fluctuations. The *Phoenix dactylifera* commonly known as Date or Date Palm is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. The species is widely cultivated and is naturalized in many tropical and subtropical regions worldwide. Dates provide a wide range of essential nutrients, and are a very good source of dietary potassium. The high fibre content of the date fruit prevents LDL cholesterol absorption in the gut. Additionally, the fibre works as a bulk laxative. They contain health benefiting flavonoid polyphenolic antioxidants known as tannins. They are moderate sources of vitamin A, which is known to have antioxidant properties and essential for vision. Additionally, it is also required maintaining healthy mucus membranes and skin. Consumption of natural fruits rich in vitamin A is known to help protect from lung and oral cavity cancers. They compose antioxidant flavonoids such as ß-carotene, lutein, and zeaxanthin. These antioxidants found to have the ability to protect cells and other structures in the body from harmful effects of oxygen-free radicals. Thus, eating dates found to offer some protection from colon, prostate, breast, endometrial, lung, and pancreatic cancers.

Materials and Methods

Fermentation of buffalo milk by lactic acid bacteria (LAB)

Lactobacillus helveticus (one of the lactic acid bacteria) was used for a period of 12 hour fermentation to make anti oxidant rich fermented buffalo milk. *Lactobacillus helveticus* cleaved the native protein of buffalo milk and form peptides fragments. These peptide may work as bioactive peptide and show different types of functional properties, from which anti oxidant potential is one of them. For study of anti oxidant potential of fermented buffalo milk, about 2 litres of fresh buffalo milk was skimmed to bring the fat contents to below 0.5% using cream separator. Buffalo milk was heated to boil at least for 5 min to inactivate/kill the inherent microbial population present in milk, separately for the process of pasteurization. Then after

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cooling of milk at room temperature, *Lactobacillus helveticus* NCDC 288 (obtained from the National Collection of Dairy Cultures, Dairy Microbiology Division ICAR-National Dairy Research Institute, Karnal, India) were inoculated @ 1% in pasteurized buffalo milk and after proper mixing the samples were drawn at 0, 2, 4, 6, 8, 10, 12 hours and were inoculated at 37^0C for different time intermission and subjected to analyzed, for change in soluble protein concentration. The supernatant/ hydrolysate collected by centrifugation at 4ºC @ 10000 x g of Buffalo Milk during fermentation process from experiment further utilized for antioxidant assay (ABTS and DPPH activity determination).

DPPH (2, 22 -diphenyl-1-picrylhydrazyl) radical-scavenging activity

The ability to scavenge 2, 22 -diphenyl-1-picrylhydrazyl (DPPH) radical by added antioxidants in samples was estimated following the method of Brand-Williams *et al.* (1995), with slight modification. Two ml of DPPH reagent (100 ìM) was mixed with 0.50 ml of 0.1 M Tris-HCl buffer (pH 7.4) and 50 ìl of hydrolysate sample in test tubes. The content was gently mixed and the absorbency in zero minute (At_0) was measured at 517 nanometer (nm) using a spectrophotometer. The sample tubes were also incubated at room temperature under dark for measurement of absorbency in 20 minute (At_{20}). Ethanol was used as blank. The free radical-scavenging activity was calculated as decrease in absorbance from the following equation:

DPPH activity (% inhibition) = 100" [(At_{20}/At_0) x 100]

(Where, At_0 = absorbency in zero minute and At_{20} =absorbency in 20 minute)

ABTS (2, 22 -azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical-scavenging activity

The spectrophotometric analysis of ABTS radical-scavenging activity was determined according to method described by Salami *et al.* (2009). ABTS radical cation (ABTS+) was produced by reacting ABTS+ stock solution with equal volume of 2.45 mM potassium persulphate ($K_2S_2O_8$) and allowing the mixture to stand in the dark at room temperature for 16 hours before use. Prior to use, the stock solution was diluted with distilled water to get an absorbance of 0.70 at zero minute. About 4 ml of ABTS+ working standard solution was mixed with 40 ìl of hydrolysate and placed in dark to get the absorbance after twenty minutes. The absorbance was measured after 20 min (At_{20}) at 734 nanometer spectrophotometer. The ABTS+ activity was calculated by using the following formula:

ABTS activity (% inhibition) = [(0.7"At_{20})/ 0.7] x 100

(Where, At_{20} = absorbency in twenty minute)

Production and sensory evaluation of fermented buffalo milk product (buffalo milk yogurt formation)

About, 500 g of dry dates cleaned and the seeds were removed, then soaked with 1500 ml of warm distilled water overnight, then were good blended with the electrically laboratory blender, Then filtered through very fine sieve (0.5 mm), and the extract stored in a refrigerated temperature at 4°C. The syrup was diluted with distilled water until the total solid been 13-14%, then were taken 5% W/W of yoghurt and placed in the plastic cups for making yoghurt. The fermented buffalo milk was taken at the time of fermentation where highest anti oxidant property was observed and mixing of sugar and date syrup at a level of 5% W/W of yoghurt take place in a hygienic way. Then the mixture was blended with laboratory blender until all ingredients were dissolved in the fermented buffalo milk and further use for sensory evaluation. Colour serves as a preliminary parameter for the acceptance of food and indicates the fitness of milk products for consumption. The investigated samples were evaluated using a panel test at day one of storage at room temperature. Ten panelists consisting of worker and staff were included in sensory evaluation. Yoghurt samples were presented in transparent plastic cups under fluorescent light. All samples were marked with digital code, and the order of presentation of samples was randomized for each panelist. The panelists rated the buffalo milk product (yoghurt) as per 8 point hedonic scale performa. Flavour means an overall integrated perception of taste and aroma associated with the product.

Statistical analysis

All the experiments of fermentation study were repeated three times and samples were drawn in duplicate. Data collected during the present investigation were subjected to statistical analysis by using F-test and adopting appropriate methods of analysis of variance as described by Snedecor and Chochran (1994). Wherever, the variance ratio were found significant at 5 per cent and highly significant at 1 per cent levels of probability, the significance of mean differences were tested by Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1957).

Results and Discussion

Fermentation of buffalo milk by lactic acid bacteria (LAB)

ABTS and DPPH activity (% inhibition) of buffalo milk during lactic acid bacteria fermentation is shown in Table 1. Results obtained by both assay were showing highest anti oxidant potential at 8 hour of fermentation. The anti oxidant potential of buffalo milk during fermentation process showed a increasing nature up to 8 hour of fermentation then it declined. So, for production of fermented milk product, buffalo milk was fermented by *Lactobacillus helveticus* up to 8 hour and then it further utilized for production of yogurt.

Sensory evaluation of fermented buffalo milk product

The mean values of sensory evaluation scores of fresh buffalo milk yoghurt (day 1 of storage) fortified with date palm syrup are summarized in Table 2. Texture properties of buffalo milk yoghurt had the heights 7.3±0.11 score. The overall acceptability scores of the sensory evaluation revealed that the buffalo milk fermented by culture *L. helveticus* and fortified by date palm syrup of level 5 % was the most acceptable.

Proximate analysis of fermented buffalo milk product

Proximate analysis (Table 3) of buffalo milk product was done according to method described by AOAC (2000). Moisture content in buffalo milk product was showing average of 75.16 ± 0.09. The mean dry matter percentage of buffalo milk products was 24.44 ± 0.06%. Crude protein and ether extract of buffalo milk product were 11.72 ± 0.05 and 8.25 ± 0.05, respectively. The crude fibre percentage in buffalo milk was 0.90 ± 0.01%, due to source of availability; Date Palm. From the results of

Table 1: ABTS and DPPH activity (Mean ± SE) of buffalo milk during fermentation

Treatment by *L. helveticus*	ABTS (% inhibition)	DPPH (% inhibition)
Fresh	$1.14^{a} \pm 0.00003$	$0.54^{a} \pm 0.0002$
Hour2	$4.71^{b} \pm 0.001$	$1.38^{b} \pm 0.0010$
Hour4	$5.84^{c} \pm 0.010$	$1.70^{c} \pm 0.0020$
Hour6	$7.28^{d} \pm 0.002$	$3.89^{e} \pm 0.0010$
Hour8	$12.28^{g} \pm 0.001$	$6.14^{g} \pm 0.0020$
Hour10	$11.85^{f} \pm 0.004$	$4.81^{f} \pm 0.0010$
Hour12	$8.853^{e} \pm 0.001$	$3.68^{d} \pm 0.0030$

Note: Means bearing different superscripts differ significantly.

Table 2: Sensory evaluation (Mean ± SE) of fermented buffalo milk product

Attributes	Score (Mean ± SE) for buffalo milk product
Appearance/colour	7.4 ± 0.12
Flavour	7.35 ± 0.10
Texture/ Viscosity	7.3 ± 0.11
Overall Acceptability	7.32 ± 0.11

A 8-point hedonic rating scale (8= excellent; 1 = extremely poor) was used for sensory evaluation

sensory evaluation and proximate analysis; it may be concluded that fermented buffalo milk product prepared from date palm (*Phoenix dactylifera*) syrup is acceptable and nutritious.

Table 3: Proximate analysis (Mean ± SE) of fermented buffalo milk product

Constituent (%)	Buffalo milk product (Mean ± SE)
Moisture	75.16 ± 0.09
Dry matter	24.44 ± 0.06
Crude protein	11.72 ± 0.05
Crude fibre	0.90 ± 0.01
Ether extract	8.25 ± 0.05
Total ash	0.71 ± 0.02

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