



Studies of microbiological effect on nutraceuticals based flavoured milk

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Abstract: The purpose of making flavoured milk is to put skim milk to a profitable use and possesses more nutritive value. The microbiological quality of the flavoured milk prepared from Gulkand, aniseed and carrot juice blended with cow milk was studied. The flavoured milk was prepared from cow milk standardized to three fat levels viz. A_1 , A_2 & A_3 as 2, 2.5 and 3.0% respectively, sugars B_1 , B_2 , B_3 & B_4 as 5%, 6%, 7% and 8% respectively, Gulkand (C_1), aniseed extract (C_2), carrot juice (C_3), and storage periods 0,3,6,9 and 12 days were used for the preparation of flavoured milk. The standard plate count /ml of flavoured milk were affected non-significantly by various fat levels. The maximum (7.42×10^4 / ml) and minimum plate count (7.39×10^4 /ml) was noted in A_3 and A_2 samples, respectively. The standard plate count of flavoured milk was affected significantly by various sugar levels. The maximum plate count (7.43×10^4 /ml) and minimum count (7.37×10^4 /ml) was noted in B_1 and B_2 samples, respectively. Similarly, the mean of plate count was minimum in C_1 (7.38×10^4 /ml). The maximum SPC count (19.65×10^4 /ml) was noted in fresh samples (D_1). The treatment combinations of ABCD had a non-significant on plate count of flavoured milk. The maximum count (19.73×10^4 /ml) was noted in $A_1B_1C_3D_3$ sample, while minimum (1.86×10^4 /ml) in case of $A_2B_1C_1D_1$ sample. The minimum SPC count was noted in case of gulkand flavoured combinations.

Keywords: Nutraceutical, flavoured milk, Gulkand, Aniseed extract, Carrot juice & SPC

Introduction

Milk production in the country is growing at the rate of 4 to 5 per cent per annum. India's share to world milk production at that time, about 15 per cent Rajorhia (2004). Indian economy dairying has benefited the poor maximum. Out of the 21 percent GDP of agriculture, 5 percent is from dairying Chandramogan (2006). Milk is nearly perfect food in nature. It supplies body building protein, bone-forming minerals, health giving vitamins and furnishes energy-giving lactose and milk fat. Milk slow down the biochemical processes leading to atherogenesis in rabbits fed on atherogenic diet Aggarwal and Kansal (1993). Flavoured milk is gaining popularity day by day because it is a cheaper cold drink than non-milk based drinks and possesses more nutritive value De (1993). Nutraceutical are products derived from food sources that provide extra health benefits, in addition to the basic nutritional value found in foods. Depending on the jurisdiction, products may claim to prevent chronic diseases, improve health, delay the aging process, increase life expectancy, or support the structure or function of the body reported Hasler *et al.* (2005). According to American Association of Nutritional Chemists the product that has been isolated or purified from food and generally sold in medicinal forms not usually associated with food named as nutraceutical Kalra (2003). The nutraceuticals are natural bioactive, chemical compounds that have health promoting, disease preventing or medicinal properties. Nutraceuticals may range from isolated

nutrients herbal products, dietary supplements and diets to genetically engineered "designs" foods Srividya *et al.* (2010).

Gulkand has been traditionally used as a cooling tonic to fight fatigue, lethargy, muscular aches, biliousness, itching, and heat-related conditions. It is good for memory and eyesight as well as a good blood purifier. It is also rich in calcium and has antioxidant properties. It can be used year-round by persons of all constitutions, especially Vata and Pitta. Indian Ayurvedic doctors, use it combined with other specific herbs for several types of cancer patients undergoing radiation or chemotherapies to counter the ill effects of these therapies Sundaram (2010). Gulkand includes reduction of pitta and heat in the body, reduction in eye inflammation and redness, strengthening of the teeth and gums, and the treatment of acidity NIAM (2008). Aniseed (*Pimpinella anisum L.*), an aromatic plant, belongs to the Umbelliferae family, which is native to the eastern Mediterranean region. Aniseed is used to add aroma and flavour to pastry, preserves, sauces, fish, and meat as well as in the manufacture of certain liqueurs and pharmaceutical products Garcia *et al.* (2007). Another important chemical constituent of anise is anethol which has estrogenic properties that help to increase milk in nursing mothers, promote delayed menstruation, and help prepare the body for childbirth. These hormonal actions may be the reason behind anise's ancient reputation as an aphrodisiac Grieve *et al.* (1931). Study of components of the whole plants and the seeds

of *Pimpinella anisum* from Alberta showed that the major oil constituent (*trans*-anethole) was 57.4% of whole plant and 75.2% of seed oil. The other constituents of plant oil, present in amounts of 1–5% were *cis*-anethole, carvone, β -caryophyllene, dihydrocarvyl acetate, estragole and limonene Shojai *et al.* (2012).

Carrots (*Daucus carota*) belong to the Umbelliferae family. Carrots originated in the central Asia. Carrot botanically biennial plants which were first used as medicines and then became popular as food crops. Carrot (*Daucus carota*) is highly valued for its nutritional and therapeutic properties and carotenoids content. The high intake of carotenoids helps in decreasing the incidence of some diseases like cancer, muscular degeneration, cardio vascular and pathological processes in human health. Carrot juice is also helpful for growth of Bifido bacterium bifidium in the infant's digestive system. There is a need to develop milk products based on carrots because when the latter combines with milk it gives a healthy, tasty, safe, and nutritious food. This study was under taken in order to incorporate all these merits in a single product, the natural colour and flavoure of carrot eliminates the addition of artificial colour and flavor to the flavoured milk Bandyopadhyay *et al.* (2012). Carrot juice with two cups of milk & 5-6 almonds in the morning sharpens memory. Slices of raw carrot and beetroot with lemon juice sprinkled on it cures anaemia. Juice of carrot, beetroot & cucumber eliminates headache & cures rheumatism. Juice of carrot & spinach after meals cures constipation. Juice of carrot & parsley, taken twice a day, reduces inflammation of joint Carrot juice mixed with lettuce juice removes extra fat Singh (2011).

The nutritional and potentially therapeutic value of food is a key characteristic in the development of new value added products manufactured for health conscious consumers Singh *et al.* (2013). The various three types of nutraceuticals were used for preparation of flavoured milk namely Gulkand, Aniseed (Saunf), and Carrot. The consumer is shifting toward 'light' foods with low calories and adopting 'functional foods' that is going to improve their health and well being too. Infact blending functional ingredients into dairy based foods helps increased sale of dairy foods Berry (2002). The purpose of making flavoured milk is to put skim milk to a profitable use and possesses more nutritive value; flavoured milk is to make milk more palatable to those who do not relish it as such to stimulate the sale of milk, changes in consumer behavior and to put skim milk to a profitable use. With this perspective in mind and with a view to serve better the interests of beverage industry. The objective of this work was to evaluate the effect of different type of nutraceuticals on the microbiological quality at various storage period and increased the shelf life of the nutraceuticals based flavoured milk. Hence, there is a need to merge non- dairy ingredients with dairy based ingredients and product to attained the previous mentioned objectives with attained saving in cost, enhance appearance, taste, flavoure, sweetness and even functionality.

Material and Methods

The details of materials and various methods used for manufacture of Nutraceuticals based flavoured milk and its analysis in the laboratory.

Materials: Milk, Skimmed Milk, Sugar, Stabilizer, Preservative, Nutraceuticals (Gulkand, Aniseed and Carrot) and Flavour

Procedure for carrot juice preparation: Fresh and good quality indigenous varieties of carrot procured from local market of Varanasi. They were washed in running water, peeled and cut in the form of slices with the help of knife and then cut slices place in the high speed booty mixie. When the mixie was start and slices was crushed and produced pulp then the pulp is squeeze by the muslin cloth and after received carrot juice. At last the carrot juice filtered and removed the rest matter from the juice and gives clean carrot juice take in the glass beaker and store in freezer before used Sharib (2013).

Procedure for saunf extracts preparation: The cleaned aniseed (saunf) was soaked in water (water to saunf ratio 3:1) for 5-6 hours at 25-30°C temperature. The soaked water was then drained off. And put the soaked saunf in the high speed booty mixie. When the mixie was start and soaked saunf was crushed and produced semi dry matter then the matter was squeezed by the muslin cloth and after received aniseed extract. At last the aniseed extract filtered and removed the rest matter from the juice and give clean aniseed extract and take in the glass beaker and store in freezer before used Shahat *et al.* (2011).

Procedure for Gulkand preparation: Rose petals are used in making Gulkand. In a wide mouthed glass jar, arrange the layers of rose petals and sugar alternately using sugar twice the weight of petals. Close the mouth of jar and keep it in sunlight daily 10 AM to 4 PM, for three to four weeks and Gulkand become ready. The time varies depending on sunlight availability, season etc. one can add silver foil, praval pishti, cardamom seeds, pearl pishti (muktapishti) etc. to increase the cooling or pitta pacifying properties.

Preparation of flavoured milk

Preheating: Standardized milk was heated to 35 to 40°C in a double packed stainless steel vat with constant stirring by a stainless steel ladle and sugar, gulkand, aniseed extracts carrot juice, stabilizer and preservative was mixed. To prepare the Nutraceutical based milk the following formulations were used- Gulkand, Carrot juice & Aniseed extract 5 - 7% by w/v of milk, Sugar 5, 6, 7 and 8% by w/v of milk, Stabilizer-Sodium alginate- 0.2% by w/v of milk and Preservative- Sodium bi carbonate @ 2.5% by w/v of milk

To prepare Nutraceuticals based flavoured milk containing 5%, 6%, 7% and 8% sugar, 50, 60, 70 and 80 gram of sugar per liter of milk was dissolved in some amount of warmed milk in a beaker and mixed well to the milk used for the preparation of Nutraceuticals based flavoured milk. To dissolve the sugar, the milk was taken from the same lot used to prepare the Nutraceuticals based milk. Separately the required amount of gulkand, carrot and aniseed extract at the rate of 50 and 70 gram per litre milk was taken in a beaker and made to a homogenous liquid with 50 ml warmed milk and mixed well, the milk was taken from the same lot used to make the Nutraceuticals based milk. Like sugar and Nutraceuticals (gulkand, carrot juice and aniseed extract) 2gms of sodium alginate was weighed separately and transferred to a beaker, made a solution with 100 ml warmed milk (35- 40°C) and heated until the sodium alginate was completely dissolved in the milk and mixed well in the boiling milk with constant stirring.

Pasteurization: After through mixing, the milk was heated at temperature of 71°C for 30 minutes, cooled to 25°C. Immediately after manufacturing, the products was filled in 200 ml glass bottles which were properly cleaned and sterilized before filling the bottles were filled to the neck with Nutraceuticals based flavoured milk and sealed with crown cork using corking machine. All bottles were sterilized in a autoclave at a pressure of 15 lbs/inch at a temperature of 121°C/15 minutes and transferred to room for storage at refrigeration temperature (5 to 10°C) for various length of time to observe its period and analysis for various parameters. Flavoured milk was prepared by adopting the procedure as given in the Fig. 1.

Statistical Analysis: In order to study the effect of different levels of fat, sugar, gulkand, carrot juice, aniseed extract and storage periods on different character of flavoured milk, the data regarding microbial quality of Nutraceutical based flavoured milk, a laboratory experiment was conducted and desired data were collected. Analysis of variance of these data was worked out on the basis of factorial completely randomized design (Gupta and Kapoor, 2007).

Treatment details: Fat levels viz. A₁, A₂ & A₃ as 2, 2.5 and 3.0% respectively, sugars B₁, B₂, B₃ & B₄ as 5%, 6%, 7% and 8% respectively, gulkand (C₁), aniseed extract (C₂) & carrot juice (C₃) and storage periods 0,3,6,9 and 12 days as D₁, D₂, D₃, D₄ & D₅.

Results and Discussion

Standard plate count: The comparison between different combinations in respect of standard plate count/ml of flavoured milk have been presented in the table-1, 2 and its analysis of variance in table-3 and represented by fig. 1, which revealed the following facts: table 1 represented the mean of different levels of all factors with regard to standard plate count/ml of flavoured milk. It was observed that maximum count (7.42 x 10⁴/ml) in case of A₃ samples followed by A₁, while minimum count (7.39 x 10⁴/ml) was noted in A₂ samples. The mean differences varied significantly when compared with CD at 5% level of significance. While comparing the average score of standard plate count/ml of flavoured milk on account of different sugar levels, it was observed that highest count (7.43x 10⁴/ml) was found in B₁ samples followed by B₂, while lowest (7.37x 10⁴/ml) was noted in B₄ samples. The results varied significantly from one another when compared with CD at 5%. The effect of different Nutraceuticals (C) on standard plate count/ml of flavoured milk indicated that highest count (7.40x 10⁴/ml) in case of

C₃ (carrot juice) samples followed by C₂, while lowest count (7.38x 10⁴/ml) was noted in C₁ samples. The result varied significantly. By comparing the average standard plate count /ml of flavoured milk on account of different storage periods (D) was

Table-1: Effect of fat levels (A), sugar levels (B), Nutraceuticals (C) and storage periods (D) on standard plate count (10⁴/ml) of Nutraceuticals based flavoured milk

	B ₁	B ₂	B ₃	B ₄	C ₁	C ₂	C ₃	D ₁	D ₂	D ₃	D ₄	D ₅	Mean
A ₁	7.45	7.43	7.33	7.30	7.40	7.44	7.42	1.90	2.31	4.82	8.32	19.66	7.40
A ₂	7.41	7.40	7.40	7.35	7.34	7.42	7.41	1.88	2.29	4.74	8.41	19.63	7.39
A ₃	7.44	7.43	7.41	7.40	7.40	7.44	7.43	1.89	2.31	4.81	8.43	19.66	7.42
B ₁					7.40	7.44	7.43	1.90	2.32	4.82	8.44	19.66	7.43
B ₂					7.40	7.44	7.43	1.89	2.31	4.82	8.43	19.67	7.42
B ₃					7.39	7.43	7.33	1.88	2.30	4.81	8.27	19.65	7.38
B ₄					7.33	7.41	7.30	1.88	2.29	4.72	8.40	19.62	7.37
C ₁								1.88	2.27	4.73	8.41	19.59	7.38
C ₂								1.89	2.33	4.82	8.44	19.68	7.43
C ₃								1.89	2.31	4.82	8.31	19.68	7.40
Mean								1.89	2.31	4.79	8.39	19.25	
SE (diff.)			A	B	C	D	AB	AC	AD	BC	BD	CD	
CD at (5%)			0.037	0.043	0.037	0.048	0.074	0.064	0.083	0.074	0.096	0.083	
			NS	0.070	0.061	0.079	NS	NS	NS	NS	NS	NS	

Table-2: Mean of standard plate count (10⁴/ml) of Nutraceuticals based flavoured milk as affected by different treatment combinations of ABCD

		C ₁					C ₂					C ₃				
		D ₁	D ₂	D ₃	D ₄	D ₅	D ₁	D ₂	D ₃	D ₄	D ₅	D ₁	D ₂	D ₃	D ₄	D ₅
A ₁	B ₁	1.91	2.31	4.82	8.45	19.64	1.93	2.36	4.87	8.48	19.72	1.92	2.25	4.85	8.46	19.71
	B ₂	1.90	2.30	4.81	8.43	19.62	1.91	2.35	4.84	8.46	19.70	1.90	2.33	4.84	8.44	19.70
	B ₃	1.89	2.28	4.80	8.42	19.60	1.90	2.34	4.83	8.45	19.69	1.89	2.32	4.83	7.08	19.68
	B ₄	1.88	2.26	4.78	8.40	19.58	1.89	2.32	4.81	8.43	19.67	1.88	2.31	4.82	8.41	19.66
A ₂	B ₁	1.86	2.25	4.76	8.38	19.56	1.87	2.31	4.80	8.41	19.65	1.89	2.32	4.84	8.44	19.68
	B ₂	1.89	2.28	4.80	8.41	19.61	1.90	2.34	4.82	8.44	19.68	1.88	2.31	4.82	8.43	19.67
	B ₃	1.88	2.26	4.79	8.41	19.59	1.89	2.33	4.82	8.44	19.67	1.87	2.30	4.81	8.40	19.67
	B ₄	1.87	2.25	4.10	8.39	19.56	1.88	2.31	4.81	8.42	19.65	1.88	2.31	4.81	8.40	19.65
A ₃	B ₁	1.90	2.30	4.81	8.44	19.63	1.92	2.35	4.86	8.47	19.71	1.92	2.34	4.84	8.45	19.70
	B ₂	1.90	2.30	4.80	8.42	19.61	1.90	2.34	4.83	8.45	19.76	1.90	2.32	4.83	8.43	19.70
	B ₃	1.89	2.28	4.80	8.41	19.60	1.90	2.33	4.82	8.45	19.68	1.89	2.31	4.82	8.41	19.68
	B ₄	1.88	2.27	4.78	8.40	19.58	1.89	2.31	4.80	8.43	19.66	1.88	2.31	4.82	8.40	19.65

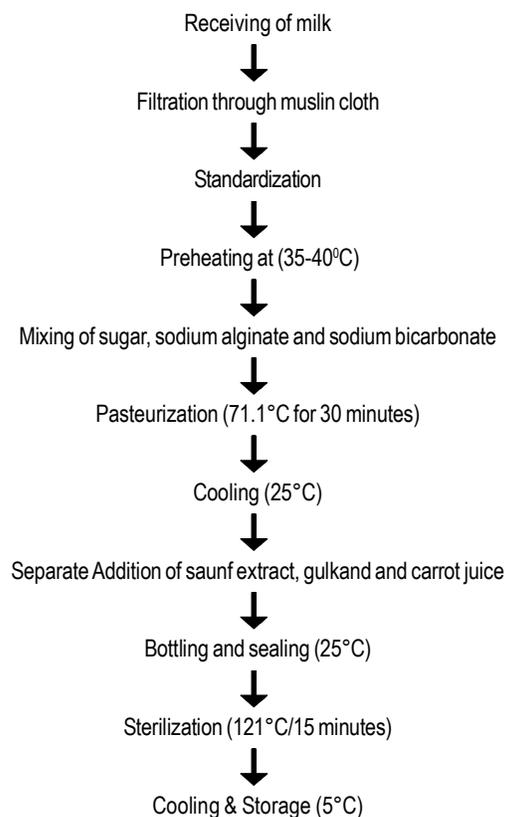


Fig. 1: Flow Diagram of Manufacture of Nutraceuticals based Flavoured milk

observed that maximum bacterial count ($19.65 \times 10^4/\text{ml}$) was found after 12 days storage (D_5), while minimum count ($1.89 \times 10^4/\text{ml}$) was noted in D_1 (fresh) samples. The mean differences varied significantly from one another, which may be due to growth of microorganism on storage periods. Table 10(A) denoted the mean interaction between fat and sugar levels (A&B) revealed that maximum count ($7.45 \times 10^4/\text{ml}$) was in case of A_1B_1 samples followed by A_3B_1 , while minimum count ($7.30 \times 10^4/\text{ml}$) was noted in A_1B_4 samples. The result varied non-significantly.

Since the various three types of nutraceuticals were used for preparation of flavoured milk namely Gulkand, Aniseed (Saunf), and Carrot. The consumer is shifting toward 'light' foods with low calories and adopting 'functional foods' that is going to improve their health and well being too. Infact blending functional ingredients into dairy based foods helps increased sale of dairy foods Berry, (2002). Hence, there is a need to merge non- dairy ingredients with dairy based ingredients and product to attained the previous mentioned objectives with attained saving in cost, enhance quality and even functionality.

Among the treatment combinations of (A.C), it was observed that maximum plate count was noted in C_2 combinations (aniseed extract), while minimum count was noted in case of gulkand flavour combinations. The result varied non-significantly from one another. The effect of interaction between fat levels and storage periods (A.D) showed that maximum plate count was noted in D_5 combinations, while minimum in fresh samples. A non-significant and significant variation was noted between and within treatments,

Table-3: Analysis of variance for standard plate count ($10^4/\text{ml}$) of Nutraceuticals based flavoured milk

Source	D.F.	M.S.S.	F.
A	2	0.0385	1.038 ^{NS}
B	3	0.0841	2.268 ^{NS}
C	2	0.1350	3.640 [*]
D	4	5782.8301	155918.125 ^{***}
AB	6	0.0450	1.214 ^{NS}
AC	4	0.0501	1.351 ^{NS}
BC	6	0.0494	1.333 ^{NS}
AD	8	0.0388	1.0464 ^{NS}
BD	42	0.0397	1.069 ^{NS}
CD	8	0.0614	1.656 ^{NS}
ABC	42	0.0336	0.906 ^{NS}
ABD	24	0.0362	0.975 ^{NS}
ACD	16	0.0356	0.958 ^{NS}
BCD	24	0.0380	1.025 ^{NS}
ABCD	48	0.0375	1.011 ^{NS}
Error	360		

respectively. From the interactions between sugar levels and Nutraceuticals (B.C) revealed that the maximum ($7.44 \times 10^4/\text{ml}$) count was noted in C_2 combinations followed by C_3 (carrot juice), while C_1 (gulkand) showed minimum plate count. The effect of interaction between sugar levels and storage periods (B.D) and (C.D) revealed that maximum plate count was noted in D_5 samples followed by D_4 , while minimum count was noted in fresh samples. Sugar levels and Nutraceuticals had no any impact in plate count. It was further observed that bacterial count increased with increase in storage periods. The results varied significantly within treatments while between treatments did not. Table-2 revealed the effect of fat levels, sugar, Nutraceuticals and storage periods (ABCD) on standard plate count/ml of flavoured milk. The maximum counts were noted in all combinations of D_4 , while minimum count was in the combinations of D_1 (fresh sample). From analysis of variance for standard plate count/ml of flavoured milk, it was observed that main effect of C and D were found to be significant. The results are in conformity with the findings of Vijay Lakshmi and Tamararasi (2001) who reported that the product showed a decreasing trend during storage. Similar results were also found by Nilofer *et al.* (2012) reported shrikhand containing 2:1.5 gm of Gulkand and dried rose petal powder examined for storage efficiency. On the basis of sensory evaluation and microbial analysis the product was acceptable for a period of 21 days under refrigerated temperature, Notarj (1985) Studies on the effect of processing techniques and storage condition of the physico-chemical, microbiological and sensory evaluation of sterilized chocolate milk formulation. Observed that during storage period microbial count of sterilized chocolate milk increase one per ml initially and 5613 to 3589 per ml after 56 days at room temperature and 5 to 10°C respectively, Singh (2008) soymilk and chicory root contains soy fructooligosaccharide and inulin which are prebiotics, Lin *et al.* (2004) reported Soymilk is a suitable medium for growing lactic acid bacteria, Yusuf *et al.* (2002), Farrukh and Ahmad, (2003) Plant products have been shown to have side effect free, good therapeutic potential due to presence of active pharmacological important substances, such as terpenes, alkaloids, flavonoid and glycosides.

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