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Development of Vitamin A fortified *lassi* using natural sources

Rekha Chawla and S Sivakumar

Abstract
Micronutrient deficiency diseases is one of the common and widespread problem which requires immediate attention. Amongst various deficiency issues, vitamin A deficiency as occupied a prominent role not only in rural population but amongst urban population as well. The reasons can be attributed to either changing lifestyle or ignorance towards health. Therefore, the remedial action should be such which requires minimum efforts to combat the same. Dairy products only provide about 6-9% of total vitamin A intake in Indian diet without any fortification. Hence, fortification of such kind of milk and milk based products with vitamin A will improve the nutritional status of such foods as functional foods. Considering the same, mango and beetroot powder were used for fortification and different ratios were assessed for overall acceptability.

Keywords: Vitamin A, Beetroot, Mango, Fortification, Deficiency

1. Introduction
India with 2.5 percent of the global land mass and 16 percent of the global population recognized the importance of human resources as the engines powering national development and gave high priority to improvement of the health and nutritional status of the population. Also, been in the forefront in developing national food and nutrition databases, research studies and surveys documenting the ongoing agriculture, food, nutrition and health transitions are going on. However, despite of all the efforts the major concern related to combating with the needs of micronutrients deficiencies are still can be said as half job done. Therefore, during the past decade, micronutrient deficiencies have been attracting attention of both academicians and administrators. In India, the micronutrient deficiencies of public health significance are vitamin A deficiency, iodine deficiency disorders and iron deficiency anaemia. Though the latter two deficiencies were being addressed on a national level using fortification studies at one level or the other. However, vitamin A deficiency is still being the concern for government and well as for policy makers as even mild deficiency of vitamin A, which is more widespread and is associated with the increased risk of morbidity and mortality of young children. Diet surveys have shown that the intake of vitamin A is significantly lower than the recommended daily allowance in young children, adolescent girls and pregnant women. In these vulnerable sub groups, multiple nutritional problems such as inadequate intake of energy and micronutrients other than vitamin A also coexist. The most recent survey data obtained from 8 state surveys in 2003 suggested that 62% of preschoolers in India are vitamin A deficient, having serum retinol concentrations lower than 20 ug/dL. The data obtained by various research publications, surfing a computerized bibliographic search via PubMed, Web of Science and Google Scholar etc, revealed that there is an urgent need to address this problem and providing solutions for the same.

Considering the above facts and figures along with severity of problem in mind, vitamin A fortification was planned using dairy based fermented beverages or products; like *lassi* and *Dahi*, as it is always accepted that dairy products are good sources for fortification strategies, not only due to worldwide consumption by all groups at risk of deficiency, but also because of the high nutritional value, along with added advantages of buffer effect in digestion and absorption process, and the positive effects on growth, cognition, and morbidity. Otherwise, dairy products only provide about 6-9% of total vitamin A intake in Indian diet without any fortification. The fortification of such kind of milk and milk based products with vitamin A helps to improve the nutritional status of such foods as functional foods.

Since fermented milk products are among highly consumed food in the world, they have been used to deliver nutritional components into human diet. Furthermore, fortification of these

products is a good way to improve nutrient intake in daily food products (Preedy *et al*., 2013) [1]. Fermented dairy products are good, and in some cases excellent, sources of nutrients namely calcium, protein and potassium (McGill *et al*., 2008) [2].

Fortification of fermented dairy products with nutritionally rich sources of vitamin A is often been considered and few categories like mango powder and beetroot powder stands at high priority as it improves the nutritional as well as therapeutic value of conventional product, when made in combinations. Also, fruit based fermented dairy products would offer several distinct nutritional advantages over the plain fermented dairy products to the consumer.

Mango is a seasonal fruit grown in tropical regions. The fruit is good source of phytochemicals and nutrients, and so as is the pulp. It is high in prebiotic dietary fiber, vitamin C, diverse polyphenols and provitamin A carotenoids (Ajila and Rao, 2008) [3]. The antioxidant, vitamins A and C, Vitamin B6 (pyridoxine), folate, other B vitamins and essential nutrients, such as potassium, copper and amino acids, are also present in good amounts in mango fruit pulp (USDA, 2010) [4]. Besides all, the carotenoid content of mango has been reported as 553 ug/100 g edible portions (Setiawan *et al*., 2001) [5] whereas it is 1.9 mg/100g in beetroot (Rebecca *et al*., 2014) [6]. Along with carotenoids, beetroot is an excellent source of iron and has been regarded as a laxative, a cure for bad breath, coughs and headaches and even as aphrodisiac (Yadav *et al*., 2016) [7]. Beetroot has large content of betanin i.e. 300-600 mg/kg and lower content of iso-betanin, betanidin and betaxanthins (Kanner *et al*., 2001) [8]. Betalains as natural antioxidants, may provide protection against oxidative stress related disorder (Kanner *et al*., 2001; Tesoriere *et al*., 2005) [8, 9]. Apart from this, beetroot is known to contain large amounts of soluble fibers, flavonoids and betacyanin. It helps to reduce the oxidation of LDL cholesterol and does not allow it to deposit on the walls of the artery.

Considering the well-known health benefits of mango and beetroot and to eradicate difference in quality attributes, powders of both the commodities were tried and functional vitamin A enriched dairy drink –*lassi* was prepared.

2. Material and Methods

2.1 Preparation of curd

The milk was obtained from Experimental Plant of College of Dairy Science and Technology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana and standardization was done to 3% milk fat and 10% SNF. After heating to 75^0 C, the milk was cooled to 37^0 C and cultured with NCDC 167. The cultured milk was incubated at 37^0 C for 6 hours or until 0.6 to 0.8% acidity was achieved.

2.2 Processing of Raw Material

The mango powder and beetroot powder were purchased from Calensa Agro Biotech, Pune. For incorporation in fermented dairy product, mango powder was used in natural form whereas for beetroot, the juice was extracted from beetroot powder. In case of beetroot, direct addition was not found appropriate due to coarse nature of the same and ultimate sedimentation in *lassi*. Therefore, beetroot powder was immersed for 2 hours in hot water and extract thus obtained after straining through muslin cloth was further used for fortification.

2.3 Preparation of fermented dairy product-*lassi*

The curd and *lassi* were processed in the Post graduate laboratory of College of the Dairy Science and Technology. For *lassi* preparation, 50% dilution was made. The curd was churned with small quantity of water using a hand blender (Philips, 250 W) and made into *lassi.* To this, 7% sugar was dissolved in small quantity of water and further added into *lassi*.

2.4 Physico-chemical analysis

The samples were analysed in three replications for every parameter. The moisture content, protein, ash, acidity and total soluble solids were determined according to AOAC method (AOAC, 2000) [10]. The kjeldahl method was used for protein estimation and a conversion factor of 6.25 was used. The titratable acidity was determined by titrating the *lassi* with 0.1 N NaOH and expressed as percent lactic acid. The hand refractrometer (Erma, Japan) was used for total soluble solids at 20^0 C. Fat content of flavoured *lassi* was estimated using Rose- Gottlieb method. The weighed sample was extracted in ethyl ether and petroleum ether as per standard method. Mixed ethers were evaporated and residue obtained thus was fat available in the sample. The total sugars were estimated with Lane and Eynon method of BIS (BIS, 1981) [11]. The pyknometer was used for determination of specific gravity of flavoured *lassi* maintained at 20^0 C. The viscosity of all prepared samples was measured with Brookfield Viscometer (LV DV2T) using LV- 0 1 (61) spindle at 5^0 C. Brookfield viscometers employ the principle of rotational viscometry i.e. the torque required to turn an object, such as a spindle, in a fluid indicating the viscosity of the fluid.

2.5 Sensory evaluation

Samples of *lassi* containing different levels of mango powder and extracted beetroot juice were evaluated by 5 judges semi trained panel for attributes of colour/appearance, mouthfeel, richness, sweetness, flavour and overall acceptability scores on a 9- point hedonic scale (Larmond, 1982) [12].

2.6 Statistical analysis

The experimental data was analysed for analysis of variance (ANOVA) using SPSS software (Version 20) and expressed as values were average of three observations. The least significant difference (post hoc) was also calculated and superscripts with different alphabets showed difference amongst variables.

3. Results and Discussion

The fermented dairy product - *lassi* was prepared with varying levels of mango powder and beetroot powder. Different levels of powders were tried 2.5, 5, 7.5 and 10 percent for mango powder and 1, 2, 3 and 4 percent for beetroot powder (Table 1). Above ranges of these (5 percent for beetroot and more than 10 for mango were also tried) but were not found appropriate for sensory studies due to more viscosity than desired.

Table 1: Treatments of mango powder and beetroot powder

Treatment	Mango powder	Treatment	Beetroot powder
Control	0	Control	0
T1	2.5	P1	1
T2	5.0	P2	2
T3	7.5	P3	3
T4	10	P4	4

3.1 Effect of addition of mango powder and beetroot powder on physico-chemical properties of *lassi*

The addition of both the powders in *lassi* caused a significant ($p< 0.05$) increase in various physico-chemical properties

(Table 2). The control sample had 11.51 percent total solids which increased to 19.77 percent with addition of 10 percent mango powder and 15.15 percent with 4 percent beetroot powder. This increase was due to high total solids in mango powder and extracted beetroot juice. Results of total solids are in concurrent with the studies undertaken by [13] Bajwa and Mittal, 2015 in which significant increase in total solids with mango pulp addition was observed. Yadav *et al*., 2016 [7] reported similar kind of results with respect to increase in total solids, when beetroot powder was added to yoghurt @ 6 - 10 percent. Also increasing trend was found in total soluble solids with augmented levels of mango powder and beetroot powder. It increased from 9.80 ^{0}B to 17.00 ^{0}B in mango powder and to 11.30 ^{0}B in beetroot powder, when added @ 10 and 4 percent, respectively. Comparative to mango powder, less amount of TSS was observed in beetroot powder and can be attributed to more sweet nature of mango and use of extract in *lassi* from beetroot. Also while evaluating acidity, mango based *lassi* was found to have more elevated levels of acidity compared to beetroot *lassi*. Similar findings were reported by [14] Raut *et al*., 2015, wherein researchers reported increase in acidity with increased level of mango pulp in yoghurt drink. Though the increase in acidity was high in higher percentages of mango based *lassi* but was not acceptable in sensory evaluation (Table 3). The mango powder and beetroot powder addition increased the total solids which ultimately increased weight of product. This caused the increment in specific gravity with augmented levels of mango powder and beetroot powder. The viscosity of prepared samples increased significantly due to increased solids in prepared mango samples. Similarly, findings for increased specific gravity, total solids and viscosity were reported by [15] Singh *et al*., 2005 in carrot juice flavoured milk.

Table 2: Effect of mango and beetroot powder on physico-chemical properties of *lassi*

Mango powder *lassi* (in percent)	**Control**	**T1**	**T2**	**T3**	**T4**
Total Solids, (%)	11.52^{a}±1.36	13.55^{b}±0.97	15.86^{c}±0.76	18.38^{d}±0.07	19.77^{d}±0.88
TSS, (^{0}B)	9.80^{a}±0.00	11.00^{b}±0.00	13.00^{c}±0.00	15.00^{d}±0.00	17.00^{e}±0.00
Acidity, (% lactic acid)	0.32^{a}±0.00	0.38^{ab}±0.02	0.41^{b}±0.05	0.49^{c}±0.05	0.54^{c}±0.00
Specific Gravity	1.03^{a}±0.001	1.04^{b}±0.001	1.05^{c}±0.001	1.06^{d}±0.001	1.07^{e}±0.001
Viscosity, (cp)	48^{a}±0.00	318^{b}±6.92	540^{c}±27.71	1218^{d}±96.24	1410^{e}±126.04
Beetroot powder *lassi*, (in percent)	Control	P1	P2	P3	P4
Total Solids, (%)	11.52^{A}±1.36	13.48^{B}±0.27	14.65^{BC}±0.22	14.72^{BC}±0.17	15.15^{C}±0.10
TSS,(^{0}B)	9.80^{A}±0.00	9.85^{A}±0.05	10.20^{B}±0.20	11.05^{C}±0.05	11.30^{D}±0.11
Acidity, (% lactic acid)	0.32^{A}±0.00	0.36^{B}±0.001	0.38^{B}±0.002	0.41^{C}±0.00	0.45^{D}±0.00
Specific Gravity	1.031^{A}±0.00	1.031^{A}±0.01	1.032^{A}±0.01	1.033^{B}±0.01	1.034^{C}±0.01
Viscosity, (cp)	48^{A}±0.00	48^{A}±0.00	54^{A}±6.92	60^{B}±0.00	72^{C}±0.00

n=3, Values are Mean ± Standard Deviation, superscripts as a,b,c,d and e represents statistical significance of mango *lassi* with the control whereas A, B,C,D and E for beetroot powder

Table 3: Effect of mango powder and beetroot powder on sensory scores of *lassi*

Mango Powder *lassi*, %	**0**	**T1**	**T2**	**T3**	**T4**
Colour	7.12±0.25	6.75±0.64	7.00±0.91	6.90±0.52	6.98±0.77
Mouthfeel	6.37±1.10	6.62±0.75	7.05±0.75	6.65±0.67	6.97±0.36
Richness	6.62±0.75	6.62±0.75	7.22±0.63	7.15±0.50	6.80±1.23
Sweetness	7.25±0.28	7.37±0.47	7.35±0.47	7.52±0.41	6.80±1.23
Flavour	7.00±0.40	7.12±0.25	7.10±0.20	6.90±0.66	6.67±1.16
Overall Acceptability	6.62±0.75	6.37±0.75	7.00±0.91	6.90±0.77	6.67±0.83
Beetroot Powder *lassi*, %	0	P1	P2	P3	P4
Colour	7.12±0.71	7.45±0.49	7.70±0.42	7.57±1.07	6.95±1.18
Mouthfeel	6.37±0.74	7.10±0.74	7.62±0.41	7.87±0.22	7.45±0.49
Richness	6.62±0.86	7.35±0.41	7.37±0.50	8.12±0.54	7.70±0.51
Sweetness	7.25±1.10	7.85±0.75	8.12±0.54	8.25±0.44	8.20±0.47
Flavour	7.00±1.09	7.35±1.09	7.12±0.75	8.50±0.83	7.20±0.35
Overall Acceptability	6.62±0.98	7.70±0.62	7.77±0.41	8.22±0.46	7.45±0.49

3.2 Effect of mango and beetroot powder on sensory scores of *lassi*

The data for effect of mango powder and beetroot powder on sensory scores of *lassi* has been presented in Table 3. The sensory score values differed non-significantly for all the attributes studied**.** The control sample had highest scores for color and appearance and inclusion of vitamin A enriched powder beyond 5 percent caused the decrease in sensory color values. This may be due to disliking associated with dark color, with more amounts of mango powder above some ranges in *lassi* and consequent fetched lower scores. The scores for mouthfeel was increased with augmented level and highest scores was observed to T2 and P3. The richness scores were observed to increased upto 5% level in mango *lassi* and 3% in beetroot *lassi* beyond which it decreased and did not result in acceptable product. High degree of acceptability in terms of flavour was perceived by panellists in T1 and T2 mango *lassi* whereas in case of beetroot *lassi*, maximum scores were fetched by P3 with 3% beetroot powder. Higher concentration more than this, led towards the decline in acceptability scores due to increased viscosity and total solids. Also increased amount of powders perhaps masked the flavour of the product and did not fetched consumer acceptance. [14] Raut *et al*., 2015 studied that 6 percent mango pulp added yoghurt drink had highest scores for flavour, body and texture, appearance and color. Similarly, flavoured milk drink prepared with 10% mango pulp had highest scores for overall acceptability (Bajwa and Mittal, 2015) [13]. Maximum overall acceptability was found in case of T2 and P3 fortified *lassi*. Similar findings were observed for mango juice supplemented soy milk beverage wherein different ratios were tried and combination of 50:50 ratio was found most acceptable (Sakhale *et al*., 2012) [16].

However, higher ratios of beetroot have been reported like yogurt incorporated with 8% beetroot powder scored higher acceptance for all attributes investigated (Yadav *et al*., 2016) [7].

3.3 Quality attributes of selective treatments

The proximate composition of *lassi* has been presented in Table 4. The five percent mango powder *lassi* had 16.02 percent total solids whereas beetroot *lassi* had been found to contain 14.65% compared to 11.82% in control and both the samples were significantly different from control. The fat content of both the fortified *lassi* was found similar and a minor variation can be attributed to least amount of fat contributed by the powders. The ash and protein content of both the samples was bit on higher side but was not found statically significant compared to control. However, sugar content of control and mango varied significantly ($p>0.05$).

Table 4: Quality attributes of selective treatments

Parameters	Control	Mango powder *lassi* (5%)	Beetroot powder *lassi* (3%)
Moisture, %	$88.17^{a\,A}$±1.36	83.97^{b}±0.76	85.35^{B}±0.17
Total solids, %	$11.82^{a\,A}$±1.36	16.02^{b}±0.12	14.65^{B}±0.17
Fat, %	1.46±0.05	1.43±0.05	1.45±0.17
Protein, %	1.74±0.08	1.83±0.08	1.92±0.09
Ash, %	0.286±0.09	0.438±0.02	0.402±0.04
Total sugars, %	12.19^{a}±0.16	13.03^{b}±0.06	12.49±0.30

n=3, Values are Mean ± Standard Deviation, superscripts as a,b,c,d and e represents statistical significance of mango *lassi* with the control whereas A, B,C,D and E for beetroot powder

4. Conclusion

Sensory evaluation revealed both the powders (mango and beetroot) acceptable at 5 and 3 percent, respectively. Percent moisture, total solids and total sugars were found significantly different in both the samples when compared with control ($p>0.05$). Thus, vitamin A fortified *lassi* can be prepared with a great degree of acceptability from consumer point of view along with its added benefits of enhanced nourishment and wellness.

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