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Development of phyto-protein enriched mango ready to serve beverage

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Abstract

The investigation was conducted to develop the protein fortified mango RTS. Various proportions of soya protein isolate (SPI), peanut protein isolates (PPI) and rice bran protein concentrate (RBPC) were added to the mango pulp to fortify mango RTS. It was observed that there was progressive decrease in the sensory scores of the beverage with increase in proportion of protein concentrate in it. The ready to serve (RTS) beverage prepared by addition of SPI @ 1% followed by RBPC @ 1% showed maximum overall acceptability. Minimum acceptability was for RTS prepared by PPI. There was an increase in TSS, acidity, and non-enzymatic browning, while a decrease was observed in pH, ascorbic acid and crude protein contents of beverages during storage. However, microbial spoilage was not detected in beverages during storage. The soya isolate/milk fortified RTS scored relatively lesser with respect to control in organoleptic evaluation. The sugar replacement with fructose showed improvement in overall acceptability

Keywords: Fortification, fructose, mango, peanut milk, protein isolate, RTS

Introduction

India is the second largest producer of fruits and vegetables in the world. Fruits and vegetables are important constituents of our diet and provide significant quantity of nutrients, especially vitamins, minerals, fiber and sugars. Mango (*Mangifera indica* L.) belongs to family Anacardiaceae. It is national fruit of India, Pakistan, Philippines and Bangladesh. It is known as 'King of Indian Fruits' due to its high palatability, excellent taste and exemplary nutritive value (Nakesone, 1998) [12]. Mango is considered indigenous to eastern Asia, Myanmar and Assam State of India. It is one of the most commonly eaten fruits in tropical countries and is gaining popularity in different parts of the World due to its wide adaptability, high yield, attractive fruit colour, excellent taste and high therapeutic value. Mango is considered to be a fruit with great potential for future (Ravani and Joshi, 2013) [15]. Ripe fruit of mango are soft with a pleasant aroma and has a flavour often described as a peach-pineapple combination (Lalel *et al.*, 2003) [8]. It has an excellent flavor, attractive fragrance, delicious taste and high nutritional value that have made it one of the best fruits. It is a good source of vitamin A, B and C and minerals.

Consumers of the present day are becoming increasingly conscious of the health and nutritional aspects of their food. Their tendency is to avoid chemical and synthetic food and choose therapy and nutrition through natural resources. Fruits ready to serve beverages (RTS), which are acidic and non-alcoholic in nature, have been increasingly gaining popularity throughout the world due to their nutritional, refreshing and easily digestible properties. However, these beverages are traditionally poor in protein due to inherent low protein content in fruits and the technological difficulties in its protein fortification of acidic beverages.

The majority of people in India and other developing countries suffer from the deficiency of protein intake in their diets due to scarcity and high cost of animal or dairy based sources of protein. This problem demands consumption of plant based protein with low cost and good quality. The production of a protein rich fruit juice beverage would thus improve the nutritional profile of fruit beverages and will also have a good commercial potential (Segall, 2009) [17].

Soy protein isolates are highly-refined and processed forms of soyabean. These are popular protein sources among vegetarians and vegans. Soy protein isolate are virtually flavorless since the natural bean flavors have also been removed during processing. Soy protein isolate

commonly used as nutrient additive, meat and milk replacers and emulsifier (Singh *et al.*, 2008) ^[18]. Soymilk as a base for production of beverages remained deprived of commercial exploitation because of its low acceptability associated with unpleasant beany flavor, astringent and bitter aftertaste. Traditionally processed soymilk is a stable emulsion of oil, water, protein resembling dairy milk in appearance and composition. Fortification of mango pulp in soymilk improves the nutritional as well as therapeutic value of beverage. Soymilk based fruit juice beverage would offer several distinct nutritional advantages over the plain fruit beverage to the consumer. Fruit pulp can be added to soymilk to enhance its vitamin A, C and mineral contents. It also provides sweetness and masks the beany flavor of soymilk to some extent (Lee *et al.*, 1990) ^[9].

The rapidly growing world protein requirement has directed major attention to plant proteins. Oilseeds are valuable sources of lipid and basically processed for their edible oils leaving behind a lot of protein-rich meal. Proteins are usually recovered from the meals and marketed as food ingredients in developed countries. Peanut proteins have good nutritional quality with high essential amino acid content (Basha & Pancholy, 1982) ^[1], which can easily be extracted to produce peanut protein isolate (PPI). Peanut protein isolate have higher purity of proteins and better functional properties than other peanut protein products, such as flour or concentrate (Wu *et al.* 2009) ^[22]. The major challenge to develop a protein fortified fruit beverage is to preserve protein functional properties and to prevent its sedimentation. The flavor challenges include overcoming the bitter/brothy flavor of protein and coagulation during pasteurization. Proteins undergo denaturation and discoloration due to disruption of food systems by heating and/or blending during processing. Temperature also affects food protein deterioration, which can affect storage time, sedimentation, pH, objectionable odors and off-flavors development. The protein enriched fruit- whey beverages have been reported to suffer from the problem of astringency also (Beecher *et al.*, 2008) ^[2].

Reactions of amines, amino acids, peptides, and proteins with reducing sugars and vitamin C results in non-enzymatic browning, often called Maillard reactions that cause deterioration of food during storage (Friedman, 1996). Replacement of sucrose with fructose in beverages improved overall acceptability and decreases the browning during storage. Sucrose causes browning and protein cross-linking at a rate 10 times greater than fructose (Knecht *et al.*, 1992) ^[7]. Ready-to-drink dairy- and soy-based beverages rely on stabilizers for suspending protein particles, improve viscosity and enhance mouthfeel. Also, stabilizers are employed as processing aids during high temperature short time (HTST) or ultra high temperature (UHT) processing. Common stabilizers used within the food industry are carrageenan, CMC and alginate, which are also known to improve the textural properties of beverages. The carrageenan creates a gel network in the product that surrounds both aggregates of proteins and fat droplets keeping them suspended (Syrbe *et*

al., 1998) ^[19]. Thus, keeping in mind the popularity of mango beverages and the need of its fortification with protein from plant sources, the present research work was undertaken.

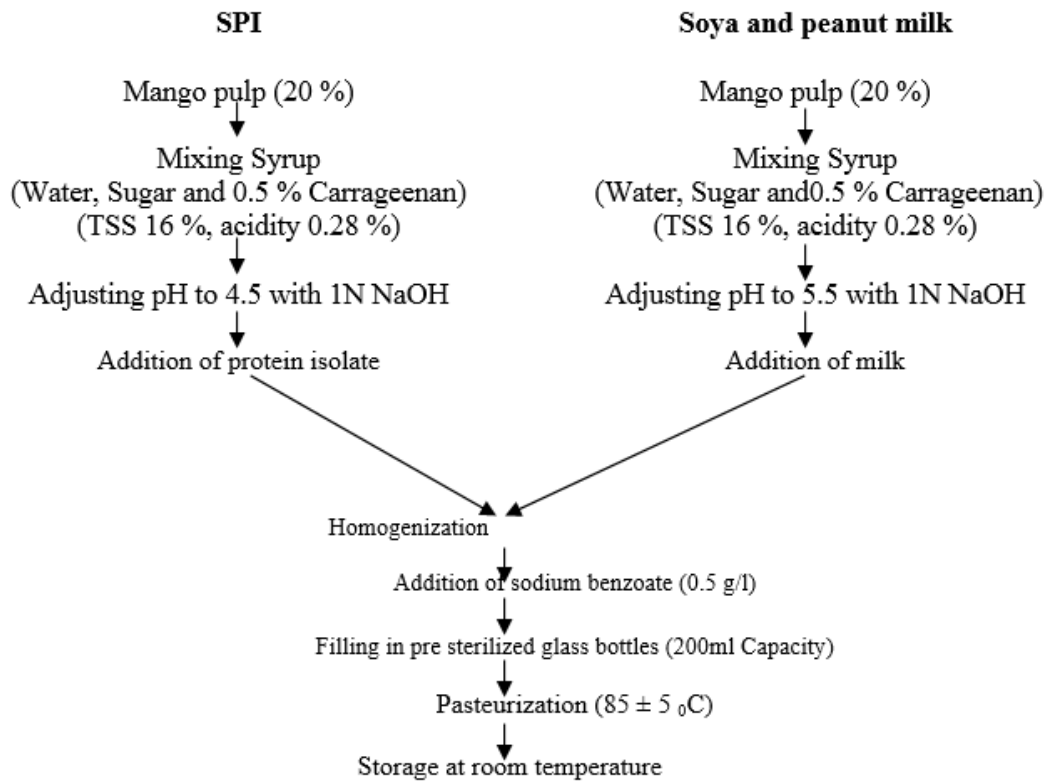
Materials and Methods

The present research work entitled, "Development and Evaluation of Plant Protein Fortified Mango Beverages" was carried out in Centre of Food Science and Technology, CCS HAU, Hisar. Ripe mango cv. Safeda fruits and soy and peanuts were procured from the local market, Hisar for extracting pulp and preparation of soya and peanut proteins. The recipe for RTS drink was standardized using 20% pulp, adjusting 16 % TSS and 0.20 to 0.30 % acidity. The squash recipe was standardized using 40% pulp, adjusting 50% TSS and 1.0 to 1.5 % acidity. For development of plant protein fortified beverages, following treatments were tried:

Soya, peanut protein isolates and rice bran protein concentrates @ 1, 2, 3, and 4 % for RTS and @ 2, 3 and 4 % for squash were added to the mango beverage. These protein isolates were mixed properly in water before adding in the beverages. The pH of the beverage was adjusted to 4.5 with 1 N NaOH before addition of protein source. The beverages prepared from various concentrations of protein sources were organoleptically evaluated to obtain the most acceptable treatment each for RTS and Squash. This treatment was only for RTS and not for squash. Soya and peanut milk blended with mango pulp in the ratio of 40, 50 and 60 %. For both soya and peanut milk blend with mango, sodium benzoate 0.5 percent was added and pH of pulp was adjusted to 5.5 with 1 N NaOH before mixing the pulp with milk in order to stabilize the beverage. The RTS prepared from various concentrations of milk were organoleptically evaluated to obtain the most acceptable treatment. In the best blend above obtained, to prevent browning during pasteurization and storage, the cane sugar (sucrose) was replaced with 0, 50 and 100 % keto group sugar fructose. The beverage blend with various proportions of fructose was then analyzed to obtain best treatment showing maximum organoleptic acceptability and minimum browning during storage upto 90 days at room temperature. The best combination of above prepared beverages with isolate and milk with replacement of sugar with or without fructose were separately bottled in 200 ml bottles, pasteurized, capped and stored at room temperature (35±5 °C) for further analysis and compared with control.

For preparing beverages, total soluble solids and acidity were first analyzed in mango pulp. On the basis of this analysis, requisite quantities of sugar and citric acid dissolved in water by heating were added to pulp for the adjustment of TSS and acidity in beverages (w/w basis).

The beverages were homogenized in colloid mill, strained, filled in pre-sterilized glass bottles (200 ml capacity) leaving 2.5 cm headspace and sealed with crown corks. The sealed bottles were processed in boiling water for 20 minutes. The bottles were then cooled in air, labelled and stored at room temperature for analysis during storage.



Flow sheet for preparation of plant protein fortified mango RTS drink

RTS beverages were analyzed for changes in chemical constituents and sensory quality at monthly interval for three months. Total soluble solids were estimated at ambient temperature by Abbe's refractometer (0-95%) or by hand refractometer (0-32%, Erma, Japan) and the values were expressed as per cent TSS. Acidity and ascorbic acid were analyzed by the methods of AOAC, 2005. Crude protein was estimated using Micro-Kjeldhal method with KELPLUS nitrogen estimation system. Non-enzymatic browning was recorded for stored product, by the procedure as described by Ranganna (1995) [14]. pH of the product was estimated by pH meter (Model: CL 54 digital Toshniwal Instruments Mfg. Pvt. Ltd., India). The sample (in case of pulp and squash) was diluted 1:10 for pH determination.

Results and discussion

a. PH and TSS

There was a slight decrease in pH of RTS with increasing storage period. There was no significant difference in pH of the variants of RTS containing sucrose or fructose (Tables 1). Fruit juices have a low pH because they are comparatively rich in organic acids (Tasnim *et al.*, 2010) [20]. The pH is negative function of natural acidity, thus decrease in pH is accompanied with increase in acidity of fruit juice during storage (Rehman *et al.*, 2014) [16]. There was a slight but significant increase in TSS with increasing storage period of RTS. There was no significant effect observed in TSS of variants of RTS where sucrose was replaced by fructose (Tables 1). The increase in TSS might be due to hydrolysis of insoluble polysaccharides into simple and soluble sugars (Woodroof and Luh, 1975; Majumdar, 2011) [21, 10]

Table 1: Effect of different treatments and storage period on pH and TSS (%) of protein fortified mango RTS

Treatments	Storage period (days)									
	pH					TSS (%)				
	0	30	60	90	Mean	0	30	60	90	Mean
T ₀	3.9	3.9	3.7	3.7	3.8	16.0	16.2	16.3	16.4	16.2
T ₁	3.9	3.9	3.7	3.8	3.8	16.0	16.2	16.4	16.5	16.3
T ₂	4.5	4.4	4.3	4.1	4.3	16.0	16.3	16.5	16.5	16.3
T ₃	4.5	4.4	4.3	4.2	4.4	16.0	16.4	16.6	16.6	16.4
T ₄	5.5	5.5	5.4	5.4	5.5	16.0	16.1	16.3	16.7	16.3
T ₅	5.5	5.5	5.3	5.2	5.4	16.0	16.3	16.6	16.6	16.4
T ₆	5.5	5.5	5.4	5.2	5.4	16.0	16.2	16.4	16.7	16.3
T ₇	5.5	5.5	5.3	5.3	5.4	16.0	16.1	16.4	16.7	16.3
Mean	4.9	4.8	4.7	4.6		16.0	16.2	16.4	16.6	

CD at 5 % Treatment = 0.15.; Storage = 0.12; Treatment × Storage = 0.28 Treatment = NS; Storage = 0.17; Treatment × Storage = NS

T₀ (Control with Sucrose); T₁ (Control with Fructose); T₂ = T₀ + SPI; T₃ = T₁ + SPI; T₄ = T₀ + PM; T₅ = T₁ + PM; T₆ = T₀ + SM; T₇ = T₁ + SM; SPI = Soya Protein isolate; PM = Peanut milk; SM = Soya milk

b. Acidity and Ascorbic acid

There was a slight increase in acidity of RTS with increasing storage period (Tables 2). There were no significant differences in acidity of the variants of RTS containing sucrose or fructose. The increase in acidity of beverages during storage might also be due to formation of organic acids by ascorbic acid, degradation of polyphenols and conversion of proteins to amino acids, degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances and uronic acid (Iqbal *et al.*, 2001; Hussain *et al.*, 2008) [5, 4]. There was a significant decrease in ascorbic acid

content of protein fortified mango RTS with increasing storage period. Among the various treatments, no significant differences were observed in ascorbic acid content of control and protein fortified beverages (Tables 2). The, loss of ascorbic acid during storage may be due to oxidation of ascorbic acid to dehydro ascorbic acid or furfurals with the passage of time by both enzymatic and non-enzymatic catalyst (Mapson, 1970) [11]. Decrease in ascorbic acid content during storage in whey based mango beverage has been attributed to degradation of ascorbic acid to carboxylic acid under high acidic condition by Ismail *et al.*, (2011) [6].

Table 2: Effect of different treatments and storage period on acidity (%) and ascorbic acid (mg/100 ml) of protein fortified mango RTS

Treatments	Storage period (days)									
	Acidity (%)					Ascorbic acid (mg/100 ml)				
	0	30	60	90	Mean	0	30	60	90	Mean
T ₀	0.41	0.41	0.43	0.43	0.42	1.2	1.0	0.8	0.4	0.9
T ₁	0.41	0.41	0.44	0.44	0.43	1.2	0.9	0.8	0.5	0.9
T ₂	0.28	0.29	0.30	0.32	0.30	1.3	1.1	0.8	0.5	0.9
T ₃	0.28	0.30	0.31	0.33	0.31	1.3	1.1	0.7	0.6	0.9
T ₄	0.23	0.23	0.24	0.25	0.24	1.1	0.9	0.7	0.6	0.8
T ₅	0.23	0.24	0.25	0.26	0.26	1.2	0.9	0.8	0.5	0.9
T ₆	0.24	0.25	0.26	0.26	0.25	1.1	0.8	0.8	0.4	0.8
T ₇	0.24	0.25	0.26	0.26	0.25	1.2	1.1	0.6	0.4	0.9
Mean	0.30	0.30	0.32	0.33		1.2	1.0	0.8	0.5	

CD at 5 % Treatment = 0.026; Storage = 0.021; Treatment × Storage = 0.048 Treatment = NS; Storage = 0.11; Treatment × Storage = NS
T₀ (Control with Sucrose); T₁ (Control with Fructose); T₂= T₀+ SPI; T₃= T₁+ SPI; T₄=T₀+ PM; T₅= T₁+PM; T₆= T₀+SM; T₇= T₁+SM; SPI= Soya Protein isolate: PM= Peanut milk: SM= Soya milk

c. Crude protein and non-enzymatic browning

There was a slight but significant decrease in crude protein of protein fortified mango RTS with increasing storage period. In the present investigation, significant increase in crude protein w.r.t. control was observed in different variants of protein fortified beverages. There was no significant effect observed in total carotenoids of variants of RTS and squash where sucrose was replaced by fructose (Tables 3). The decrease in protein content during storage of fruit products was attributed to denaturation and degradation of protein into amino acid (Paramita and Arora, 2015) [13]. There was a

progressive and significant increase in total non enzymatic browning of protein fortified mango RTS with increasing storage period. Among the various treatments, minimum NEB was observed in SMfortified RTS followed by PM fortified RTS, while it was maximum in SPI containing RTS (Tables 3). It was reported by Kuchi *et al.*, (2014) that degradation of ascorbic acid during storage followed by its further degradation to 2, 3-diketogulonic acid and finally to Maillard compounds such as furfural and 2-furonic acid to eventually form brown pigments.

Table 3: Effect of different treatments and storage period on crude protein (%) and non-enzymatic browning (O.D. at 440 nm) of protein fortified mango RTS

Treatments	Storage period (days)									
	Crude protein (%)					Non-enzymatic browning (O.D. at 440 nm)				
	0	30	60	90	Mean	0	30	60	90	Mean
T ₀	0.96	0.94	0.92	0.91	0.93	0.055	0.077	0.089	0.109	0.083
T ₁	0.99	0.97	0.91	0.89	0.94	0.046	0.063	0.072	0.089	0.068
T ₂	1.87	1.86	1.75	1.69	1.79	0.076	0.098	0.106	0.118	0.100
T ₃	1.90	1.80	1.80	1.70	1.80	0.075	0.078	0.081	0.087	0.080
T ₄	1.17	1.15	1.11	1.07	1.13	0.086	0.097	0.106	0.113	0.101
T ₅	1.18	1.16	1.12	1.08	1.14	0.085	0.089	0.091	0.101	0.092
T ₆	1.53	1.50	1.43	1.40	1.47	0.068	0.072	0.079	0.092	0.078
T ₇	1.50	1.52	1.45	1.46	1.48	0.066	0.069	0.072	0.076	0.071
Mean	1.37	1.34	1.29	1.25		0.070	0.080	0.087	0.098	

CD at 5 % Treatment = 0.075; Storage = 0.053; Treatment × Storage = 0.093 Treatment = 0.007; Storage = 0.009; Treatment × Storage = 0.016
T₀ (Control with Sucrose); T₁ (Control with Fructose); T₂= T₀+ SPI; T₃= T₁+ SPI; T₄=T₀+ PM; T₅= T₁+PM; T₆= T₀+SM; T₇= T₁+SM; SPI= Soya Protein isolate: PM= Peanut milk: SM= Soya milk

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