

Research paper

Effect of freeze-drying and storage on β -carotene and ascorbic acid stability of mango milk shake

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Abstract

Freeze-drying is a process which retains the nutritional, sensorial and functional qualities of foods, together with extreme reduction in weight, high solubility, long shelflife at moderate temperature and the possibility to perform rehydration at any desired level. The application of this technique in the development of a Freeze dried mango milk shake powder using Badami mango (*Mangifera indica L*) pulp, milk (3.0g/100g fat, 8.5g/100g Solids Non Fat), nuts, flavourants and ascorbic acid to deliver RDA level of ascorbic acid and β -carotene was hence attempted. Optimized formulations were subjected to freeze drying for better storage stability. The water activity was found to be 0.22 and BET monolayer value 2.294g/100g solids. β -Carotene, ascorbic acid and oxidative rancidity profile monitored at ambient ($28\pm 2^\circ\text{C}$) and 37°C storage revealed no significant differences ($p>0.01$) at ambient temperature in ascorbic acid and thiobarbituric acid reactive substances (TBARS) but significant difference ($p<0.01$) was observed after storage at 37°C . Significant degradation of β -carotene was observed after 12 and 6 months of storage at ambient ($28\pm 2^\circ\text{C}$) and 37°C respectively. Total fatty acid profile by gas chromatography indicated the presence of saturated, monounsaturated and polyunsaturated fatty acids (PUFA) in the ratio of 1:0.51:0.28. PUFA like linoleic, linolenic and arachidonic were significantly ($p<0.01$) affected by 6 months of storage at 37°C . Organoleptically, the product was rated 7.60 ± 0.10 and 7.49 ± 0.09 under ambient and 37°C after 12 and 6 months storage respectively indicating good overall acceptability on a 9 point hedonic scale.

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Today foods are not intended to only satisfy hunger and to provide necessary nutrients for humans but, also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. The intake of dietary fiber and phytochemicals such as polyphenols, carotenoids, tocopherols and ascorbic acid have been related to the maintenance of health and protection from diseases such as cancer, cardiovascular diseases and many other degenerative diseases (Wang and Jiao, 2000). Recently, fruits and vegetables have received much attention as a source of biologically active substances because of their antioxidant, anticarcinogenic and

antimutagenic properties (Dillard and German, 2000). Hence, there has been a growing interest in functional foods, because of the health benefits imparted by them. The foods processed by conventional techniques could affect the food quality parameters like nutritive value and appearance (Ratti, 2001). In view of this, it was important to study the application of freeze drying process in the development of functional foods.

Mango (*Mangifera indica L*) is the most important fruit of Asia. India accounts for almost half of the world production. The nutritional importance of mango is mainly due to its β -carotene content which ranges from 800 (*Mulgoa*) to 13000 μg

(*Alphonso*) per 100 g depending on the cultivars (Hymavathi and Khader, 2005). It is also a rich source of ascorbic acid and dietary fibre. Vitamin A deficiency (VAD) is a public health problem in over 118 countries mostly in Africa and South East Asia. At any one time, up to 140 million children are at risk of clinical/subclinical VAD and over one million VAD-associated childhood deaths occur annually (World Health Organization, 2009). Thus, the recent attention towards the protective effect of β -carotene as an antioxidant is gaining momentum.

The importance of milk and other dairy products has been recognized by their inclusion in official dietary recommendations (Gopalan *et al.*, 1989). It can be considered as an ideal food in terms of nutritional value. At present, India ranks first in milk production in the world with annual production of 100.9 million tonnes in the year 2006-07 (Department of Animal Husbandry, Dairying and Fisheries, 2010).

Earlier the outlook of consumer was limited to taste, texture and cost but of late time constraints and health benefits are the major consumer concerns. Keeping these requirements, development of shelf-stable ready-to-serve or ready-to-reconstitute beverages with functional attributes need to be processed (German *et al.*, 2004). Freeze drying is reported to be a gentle technique as it retains the biological value of raw materials, their structure, flavour, aroma and colour and freeze dried foods can be reconstituted easily by simple addition of liquid. Further the consumer wants the food to be free from additives, preservatives, artificial colors etc. The consumer perception of an ideal product is based on its organoleptic properties among which colour is an important parameter (Henry, 1996). Freeze drying offers good colour retention and increase in functionality of foods (Soong and Barlow, 2004).

Processing and preservation of fruits have an important role to play especially in India where the economy is predominantly agro-based. Under these conditions, there is an urgent need to produce carotene and ascorbic acid rich foods to process, preserve and supply them both to the rural and urban populations. Conventional types of mango products have been developed to a considerable extent but the mango industry is eager to develop new processed products (Hassan and Ahmed, 1998). A few studies have been conducted in India on development of mango powder. Spray drying of mango pulp produced good coloured powder but no pleasant flavour (Baldry *et al.*, 1976). Studies on freeze dehydration of juices (Ammu *et al.*, 1977; Jayathilakan *et al.*, 2003) have indicated the feasibility of this technology in developing high quality shelf-stable products.

Evidence exists on the effect of freeze drying (FD) on the food components. However, the data available regarding the effect

of freeze drying and storage on functional components is scanty. As such very few FD functional foods are available. Efforts need to be focussed on the development of a variety of high quality FD foods as well as understanding their interactions with other food components. So the present study was carried out to develop a FD mango milk shake powder by the combination of mango pulp and milk with nuts, flavourants, sugar and ascorbic acid. The paper aims to improve the retention of β -carotene and ascorbic acid during storage in paper foil pouches (PFP) since the intended use of the powder is to make it available in the off-season.

Materials and methods

Reagents and chemicals

All the reagents and chemicals used for the study were of Analar[®] grade and procured from M/s Sigma Chemicals, USA and M/s BDH Company. The study was conducted in the year 2010 and the duration of the study was nearly 12 months.

Sample material

Fresh fully ripened *Badami* mangoes were procured from the local market, washed thoroughly, peeled and cut into pieces and stones were removed manually. The cut pieces were fed into the pulper with a sieve size of 1/16". The extracted pulp was heated at 80 °C in a steam jacketed kettle for 10 min and cooled. Fresh pasteurized milk (3.0% fat, 8.5% Solids Non Fat) was procured from the local dairy (Nandini Dairy, Mysore). Sugar and good quality almonds (*Amygdalus communis*), cashew (*Anacardium occidentale*) and cardamom (*Amomum cardamomum*) were procured from the local market. Sugar was sterilized at 85 °C for 1h in a hot air oven.

Optimization of mango milk shakes for freeze drying

Different combinations of the product were prepared before freeze drying to formulate the most acceptable combination for shelf stability studies. 3:1, 3:2 and 3:3 combinations of mango pulp and milk were prepared. Ascorbic acid was incorporated at the rate of 0.7% of the pasteurized and cooled pulp. Sugar was added to the pulp so as to adjust the brix to 30 ° and total soluble solids were measured using a hand refractometer 0-32% (Erma, Japan). Acidity was adjusted to 1% by adding citric acid. Nuts (almonds and cashew) (4%) and cardamom (1%) were powdered coarsely and incorporated. All the 3 combinations were subjected for organoleptic evaluation by a panel of 14 judges and the parameters like colour, aroma, taste and overall acceptability were assessed on a 9-point hedonic scale (Murray *et al.*, 2001). Based on sensory evaluation data, the most suitable combination of mango pulp and milk was selected, so for freeze drying and further studies.

Freeze dehydration of mango milk shake

The formulated combination was initially subjected for blast freezing in a blast freezer (Hull Corp, USA) at $-50\text{ }^{\circ}\text{C}$ for 4 hours. After blast freezing, the frozen product was freeze dehydrated in a freeze drier (Hull Corp, USA) at $60\text{ }^{\circ}\text{C}$ with a vacuum of $100\text{ }\mu$ and a condenser temperature of $-60\text{ }^{\circ}\text{C}$. These were the critical parameters maintained during the freeze drying process. The whole operation took 15 hours to get the final product.

Packaging of freeze dried mango milk shake

The prepared powder was packed in pouches ($10 \times 11\text{ cm}^2$) made out of Paper foil polythene (45 gsm paper/20 Al. foil/37.5 μ LDPE) packaging containing 50 g of FD mango milk shake powder. All the pouches were sealed to make them leak proof with foot operated electric packing machine. The packed samples were kept for storage studies under ambient conditions ($28\pm 2\text{ }^{\circ}\text{C}$, 70-80% RH) and $37\text{ }^{\circ}\text{C}$ (85-87% RH) and analyses were carried out regular intervals of 0, 2, 4, 6, 8, 10 and 12 months.

Physico-chemical analysis

The proximate composition of freeze dried mango milk shake powder was determined as per AOAC (1984). The water activity (a_w) of the product was determined using water activity meter (Aqua Lab, Decagen devices Inc, Pullan, Washington, USA). BET monolayer value was determined using Hydrosorb (Quantachrome Instruments, USA).

Ascorbic acid was estimated based on the reduction of 2, 6 dichlorophenol indophenol by ascorbic acid as per the method described by Ranganna (1995). β -carotene from the samples was determined using acetone and hexane as solvents for extraction of carotene and measurement of colour absorbance at 450 nm (AOAC, 1990) using a UV-visible spectrophotometer Model 160, (Chemito Instruments, India). Thiobarbituric acid reactive substances (TBARS) values were expressed as mg malonaldehyde/kg sample and estimated colorimetrically using 2-thiobarbituric acid (Taraldgis *et al.*, 1960) with a UV-visible spectrophotometer, Model 160, (Chemito Instruments, India). For 20 grams of the blended sample, 2.5 ml of concentrated HCl and 97.5 ml of distilled water were added and the pH was adjusted to 1.5. Then the sample was allowed for distillation from which 20 ml of distillate was collected and treated with 5 ml of TBA reagent. After boiling for 35 min in the water bath the optical density was measured at 538 nm. Along with the samples, separate aliquots of standard tetraethoxy propane were taken. These were treated in the same manner as above and TBARS values were calculated using a standard curve.

Total fatty acid profile of samples by Gas Chromatographic method

Fatty acid composition of the lipid extracts was determined by gas chromatography as fatty acid methyl esters using standard esters of fatty acids.

Esterification of fatty acids: The samples were esterified as per the procedure of Metcalf *et al.*, (1966) with slight modifications.

About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. Four milliliters of 0.5 N alcoholic sodium hydroxide solutions was added and heated for 5 min over a water bath at $90\text{ }^{\circ}\text{C}$. On cooling, 5 ml of boron tri-fluoride methanol reagent was added and heated for 5 min at $90\text{ }^{\circ}\text{C}$ over a water bath, followed by addition of 10 ml of saturated sodium chloride solution. The samples were thoroughly cooled to room temperature and 5 ml hexane was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate to remove the traces of moisture, if any. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

Total fatty acid analysis by Gas Chromatography: Analysis of total fatty acids was carried out by a Ceres-800, Chemito model gas chromatograph fitted with BPX 70 column (25 m, 0.32 mm ID) and flame ionization detector. Temperature gradient programming was employed from 150 to 220°C . Split ratio was adjusted to 1:25 and capillary flow of carrier gas to 2.0 ml/min. Injector and detector port temperatures were adjusted as 230°C and 240°C respectively. For FID, hydrogen and oxygen were used and the flow was adjusted as 45 ml/min and 450 ml/min respectively. Along with samples, standard esters of fatty acids (Sigma chemical company, St. Louis, USA.) were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by comparing with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris-32 software was used to integrate and evaluate the chromatogram in the analysis.

Sensory analysis

The sensory characteristics of the mango milk shake were evaluated during storage by subjecting these samples to an overall acceptability score on a 9 point hedonic scale by a panel of 12 judges, using the procedure of Murray *et al.*, (2001).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA)

and Duncan’s multiple range test to evaluate the statistical significance of the treatments and significance was established at $p < 0.01$. Four replicate experiments were carried out in statistical analysis.

Results and discussion

Proximate composition

The proximate compositional data of the freeze dried (FD) mango milk shake powder are shown in Table 1. The moisture was found to be 2.16% which is very nearer to the Brunauer-Emmett-Teller (BET) monolayer value of the product (2.09/100 g solids) where the product shows the maximum stability (Fabra *et al.*, 2009). The protein content was 12.28%, fat 9.36% and total carbohydrate 73.36%. The sample had a moderate percentage of fat, protein and considerable amount of carbohydrate and a calorific value of 427 kcal/100g.

Table 1: Proximate composition^a of the freeze dried mango milk shake

Parameter	% Composition
Moisture	2.16 ± 0.43
Protein	12.28 ± 1.04
Fat	9.36 ± 0.91
Total carbohydrate	73.36 ± 1.38
Total ash	2.89 ± 0.38
Calories	425 kcal

^a Values are expressed as Mean ± SD (n=5)

Initial a_w of mango milk shake before freeze drying was 0.96. The lowest value of a_w needed for the growth of normal bacteria, molds and salt tolerant bacteria is 0.90, 0.80 and 0.75 respectively (Potter and Hotchkiss, 1996). Therefore a_w must be reduced to below critical a_w to preserve the food and this was achieved by subjecting the product to freeze dehydration and the a_w of FD mango milk shake powder was found to be 0.22.

Evaluation of the oxidative deterioration of lipids in FD mango milk shake

Data on the changes in thiobarbituric acid reactive substances (TBARS) content at ambient (28±2°C) and 37°C of storage as a measure of the extent of lipid oxidation are presented in Figure 1. From the data, it is evident that the FD mango milk shake powder stored at ambient temperature exhibited good stability characteristics and was acceptable till one year of storage. There were no significant differences ($p > 0.01$) in the TBARS values. But the product stored at 37°C exhibited significant differences ($p < 0.01$) in oxidative rancidity values

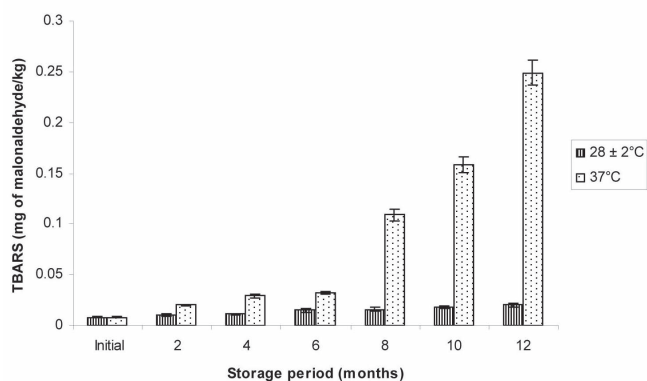


Figure 1: TBARS values of freeze dried mango milk shake during storage at ambient (28±2°C) and 37°C.

after 6 months of storage rendering the product unacceptable.

In the case of hot air dehydration and freeze drying, lipid oxidation has been reported to be the major problem limiting the acceptability of the products (Radhakrishna *et al.*, 1988). During ambient temperature storage, the product exhibited good oxidative stability as reflected in the TBARS values. In addition to this, incorporation of ascorbic acid which is a natural antioxidant also might have contributed to the oxidative stability (Verma and Sahoo, 2000). But on the other hand, the product stored at 37C was acceptable up to 6 months and there was an increase of 0.032 ± 0.006 to 0.109 ± 0.10 mg in malonaldehyde values which is significantly different ($p < 0.01$) and continued to increase during further storage. The polyunsaturated acids present in the sample undergo oxidation and this might contribute to the rancidity (Lai *et al.*, 1991). These values are in accordance with the profile of fatty acids as analyzed by GLC reflected in Table 2.

Effect of FD and storage on the β-carotene profile

The stability of carotene was monitored after freeze drying and storage at ambient and 37°C in FD mango milk shake and the data generated is depicted in Figure 2. Evaluation of the stability of β-carotene is important in food materials as it has got lot of functional characteristics. Apart from being precursors of provitamin A, carotenoid pigments exhibit functional properties as they act as antioxidants (Krinsky, 1988).

From the data presented in Figure 2, it is evident that the storage temperature has a clear impact on the degradation values of β-carotene. The β-carotene values of the samples did not show any significant difference ($p > 0.01$) up to 8 months of storage at ambient temperature (28 ± 2 C).

But the samples exhibited significant difference ($p < 0.01$) in a-

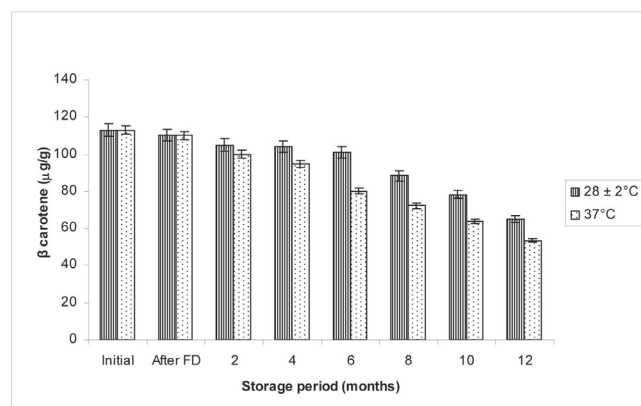


Figure 2: Effect of freeze drying and storage on the β -carotene profile of mango milk shake during storage at ambient ($28\pm 2^\circ\text{C}$) and 37°C .

carotene values after 5 months of storage at 37°C as indicated in Figure 2. The stability of carotenoid pigments at normal temperature in FD samples of carrot pulp was reported by Tang and Chen (2000). Effect of drying treatments like freeze drying and hot air drying on the stability of carotenoids in Taiwanese mango was studied by Chen *et al.*, (2007) and they reported better retention of pigments in FD samples during normal temperature of storage. Vasquez-Caicedo *et al.*, (2007) in their report mentioned about 93% retention of β -carotene during storage of carrot powder. The loss of β -carotene that is occurring after 5 months of storage at 37°C , reported in this study may be due to autoxidation. The highly unsaturated chemical structure makes them very susceptible to thermal degradation and oxidation (Stefanovich and Karel, 1982). So from the data it was evident that the freeze drying process and storage at ambient temperature ($28\pm 2^\circ\text{C}$), the β -carotene exhibited good stability while at 37°C the degradation was very significant at the end of 5 months of storage. The positive effect of temperature and storage in the degradation of β -carotene in freeze dried samples was also reported by Cinar (2005).

Stability of ascorbic acid in the mango milk shake during FD and storage

Studies were carried out to evaluate the stability of ascorbic acid in the FD mango milk shake at two different temperatures of storage i.e., ambient and 37°C . Since the initial ascorbic acid content in the product was not meeting the RDA requirements, it was enriched with ascorbic acid at 70 mg/100 g and then subjected to FD and its stability was monitored during the study.

The data obtained with respect to ascorbic acid stability has been shown in Figure 3. From the data it could be ascertained

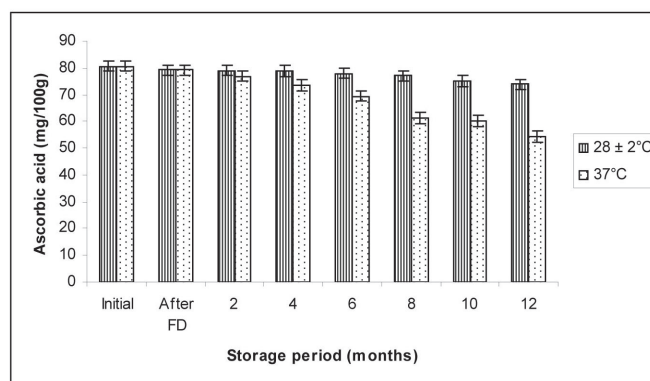


Figure 3: Effect of freeze drying and storage on the ascorbic acid stability of mango milk shake during storage at ambient ($28\pm 2^\circ\text{C}$) and 37°C .

that FD did not have any impact on the values of ascorbic acid, but temperature of storage had a positive effect in the degradation of ascorbic acid and it was clearly observed that significant difference ($p < 0.01$) was found in the levels of ascorbic acid after 6 months of storage at 37°C . But the ambient temperature storage as observed in the earlier findings of TBARS and β -carotene did not make any significant difference ($p > 0.01$) in ascorbic acid degradation during storage. Marfil *et al.*, (2008) studied the effect of different drying treatments and temperatures on the ascorbic acid degradation kinetics in tomatoes and reported an increase in Ascorbic acid degradation with respect to temperature. Hymavathi and Khader, (2005) carried out the effect of storage time and temperature on the stability of ascorbic acid in mango powder with various packaging materials and established the impact of storage and temperature on the degradation of ascorbic acid. The degradation of ascorbic acid at 37°C after 6 months of storage may be attributed to the presence of trace elements and increase in moisture content and the atmospheric temperature (Dennison and Kirk, 1982, Eison-Perchonok and Downes, 1982).

Total fatty acid profile by gas liquid chromatography

Fatty acid composition as % of total fatty acids in FD mango milk shake during storage at ambient and 37°C has been estimated and the effect of storage period and temperature on the saturated, monounsaturated and polyunsaturated fatty acids has been depicted in Table 2. From the data obtained it could be interpreted that saturated fatty acids like lauric, myristic, palmitic and stearic did not show any significant changes ($p > 0.01$) in the levels because of storage period and temperature. But the polyunsaturated fatty acids like linoleic, linolenic and arachidonic showed significant difference ($p < 0.01$) after 6m of storage at 37°C . The possible reason for

Table 2: Total fatty acid profile of FD mango milk shake^{a,b} during storage (as methyl ester % of total fatty acids)

Fatty acid as methyl ester	Ambient (28±2°C)			37°C	
	Initial	6m	12m	6m	12m
Lauric (C _{12:0})	2.28±0.59 ^a	2.19±0.68 ^a	2.08±0.39 ^a	2.09±0.19 ^a	2.04±0.13 ^a
Myristic (C _{14:0})	4.39±0.86 ^a	4.30±0.22 ^a	4.19±0.20 ^a	4.09±0.13 ^a	3.94±0.20 ^a
Palmitic (C _{16:0})	18.89±1.09 ^a	18.11±0.91 ^a	17.99±0.84 ^a	18.04±0.76 ^a	17.83±0.59 ^a
Stearic (C _{18:0})	18.96±1.35 ^a	18.54±1.40 ^a	17.86±0.79 ^a	18.64±0.63 ^a	17.77±0.55 ^a
Oleic (C _{18:1})	21.29±1.81 ^a	20.31±0.91 ^a	20.06±0.62 ^a	19.14±0.59 ^a	18.09±0.64 ^b
Linoleic (C _{18:2})	8.16±0.91 ^a	7.54±0.64 ^a	7.39±0.55 ^a	6.69±0.32 ^b	5.09±0.41 ^b
Linolenic (C _{18:3})	3.89±0.46 ^a	3.04±0.22 ^a	2.81±0.26 ^b	2.32±0.11 ^b	1.92±0.12 ^c
Arachidonic (C _{20:4})	1.85±0.12 ^a	1.69±0.20 ^a	1.51±0.10 ^a	1.09±0.08 ^b	0.79±0.06 ^c

^a Values are shown as Mean±SD (n=4)

^b Within the rows values superscripted with different letters differ significantly (p<0.01)

Values with same letters did not differ significantly (p>0.01)

the difference in saturated and polyunsaturated fatty acid results could be the deterioration of unsaturated fatty acids due to lipid peroxidation.

Oxidation of lipids is one of the primary causes of deterioration, leading to development of off flavour, decrease in nutritive value, loss of colour etc (Morrissey *et al.*, 1998). The substrate for the lipid oxidation reaction is mainly unsaturated fatty acids (Simic and Taylor, 1987). So the degradation observed in polyunsaturated fatty acids with respect to samples stored at 37°C after 6 months of storage may be attributed to the lipid oxidation. These findings are in positive correlation with the TBARS values reported in Figure 1, which is an indicator of oxidative rancidity.

Sensory characteristics of the product

Table 3 illustrates the sensory characteristics of the product stored at two different temperatures (ambient (28±2°C) and 37°C). The chemical evaluation pertaining to the stability in terms of TBARS, β-carotene, ascorbic acid and fatty acids of the product during storage was earlier discussed in Figures 1, 2 and 3 and Table 2 respectively. To support the findings of these studies, sensory profile of this product was obtained on a 9 point hedonic scale and the overall acceptability score is presented in Table 3.

From the table, it is observed that the samples stored at ambient temperature (28±2°C) exhibited good overall acceptability score and no significant difference (p>0.01) was found till 12 months of storage. But samples stored at 37°C exhibited slight rancidity after 6 months of storage and this is in accordance with the other chemical parameters studied like TBARS, β-carotene, ascorbic acid and fatty acids.

Conclusion

Freeze dried functional foods can be used to fetch premium for their use at high altitudes and arctic regions as well as for mountaineering and space expeditions. There are many potential research areas in freeze dried functional foods. Freeze drying technology has got great potential in developing a functional mango and milk based product which can deliver the RDA level of Vitamin C and β-carotene. Ambient temperature (28±2 °C) storage of the product did not affect the stability of functional ingredients like ascorbic acid, β-carotene and PUFA's and produced a product of one year shelf stability. Evaluation of the chemical markers clearly indicated 6 months stability of the product at elevated temperature of storage i.e., at 37 °C. The oxidative rancidity parameters assessed through TBARS values well coordinated with polyunsaturated fatty acids degradation pattern at elevated temperature of storage.

Table 3: Organoleptic characteristics of FD mango milk shake (overall acceptability score^a on a 9 point hedonic scale) during storage

Storage temp	Storage period (months)						
	Initial	2	4	6	8	10	12
Ambient (28±2°C)	8.25±0.14	8.16±0.09	8.09±0.08	8.00±0.11	7.83±0.21	7.68±0.14	7.60±0.10
37°C	8.25±0.14	8.09±0.13	7.90±0.06	7.49±0.09	7.20±0.10	6.39±0.12	6.04±0.13

^a Values are expressed as Mean ±SD (n=12)

The chemical parameters and sensory profile evaluation clearly established the overall stability and acceptability of the product. This ready-to-reconstitute freeze dried product, functional in terms of β -carotene, ascorbic acid and PUFA's has got potential in both service and civilian sectors.

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