Research Article

Evaluating Shelf Life of Amaranth Date Flavoured Milk

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Abstract

Amaranth date flavoured milk was manufactured from milk which was standardized to 3.0 % fat and 8.5 % MSNF. The product contained date extract (@ 5.1 % w/w of milk), roasted amaranth flour @ 1.6 % w/w of milk), tri sodium citrate @ 0.15 % and sugar (6.0 % w/w of milk). The product was filled in glass bottles and subjected to sterilization at 121˚C for 15 min. During storage at 37±2˚C, the product was monitored for its compositional, physico-chemical, sensory and microbial properties after packing in glass bottles at every 15th day interval to evaluate its shelf-life. The initial value of L* (lightness) of fresh sample was 52.34, which decreased to 51.68 after 75th day of storage. It was found that there was a significant (P<0.05) increase in free fatty acid, tyrosine content, viscosity, sediment value throughout the period of storage. There was decrease in pH, total solid, colour value and sensory scores during storage. Throughout storage period SPC, spore count, yeast and mold count and coliform count was absent. The product was found to be acceptable up to 75 d when stored at 37±1˚C in glass bottles.

Keywords: Amaranth, Date, Flavoured Milk, Roasted Amaranth, Sensory quality, Storage

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Introduction

Amaranth, also known as rajgira (king seed) or ramdana (God's seed), is a highly nutritious pseudo-cereal with a far higher protein content than true cereals [1]. Lowering plasma cholesterol levels, activating the immune system, exerting anticancer activity, lowering blood glucose levels, and treating hypertension and anaemia are among the health benefits claimed. It also possesses anti-allergic and antioxidant properties, according to reports [2].

The date fruit has been recognized for its highly nutritious, well mineralized, immensely flavored and high calorific food value and is also a good source of dietary fiber [3]. According to Ayurveda certain food combination is beneficial for health, e.g. milk date. Date is a good source of iron and provides a practical method of supplementation during childhood, pregnancy and adolescence for iron deficiency anemia [4]. Thus, date offers a good source of rapid energy and good nutritional value. Dates are abundant in carbohydrate (44-88 percent), fat (0.2-0.5 percent), protein (2.3-5.6 percent), vitamins, and dietary fibre (6.4-11.5 percent) [5]. Date is known to have antioxidant, antimutagenic and antimicrobial properties [6].

The shelf life of food product is an important aspect that has to be taken into consideration, especially for retailing aspects. Any food product must have a fairly extended shelf life so that the product can with stand the long journey to the consumers and stay for a long time in the seller's shelf as well as in the consumer's household. A variety of deteriorative changes may be expected to occur in the sterilized flavoured milk during its storage period. These changes are influenced by a variety of factors like type of processing, hygiene maintained during processing, type of packaging material, humidity and environment within the product, storage temperature, etc. During the study, an attempt was made to evaluate the storage changes in the ADFM under ambient storage condition (37±2˚C). The aspects studied involved the physico-chemical properties, sensory quality and microbial quality of the sterilized flavoured milk.

Materials and Methods

Raw materials

Fresh, raw mixed (cow and buffalo) whole milk was procured from Anubhav Dairy, Anand and standardized to 3.0 % fat and 8.5 % SNF by mixing required quantity of skim milk and cream. The average composition of milk was 3.1+0.05 % fat and 8.6+0.05 % MSNF. Cane sugar (Madhur Brand, M/s Shree Renuka Sugars Ltd., Amaranth grain (Arena Organica, Arena Organica Pvt Ltd., Sikar, Rajasthan; Deseeded dates (Apis Brand, Apis India Ltd., New Delhi; was used for preparation of ADFM.

Preparation of ADFM

Milk standardized to 3.0 % fat and 8.5 % MSNF was heated to 60˚C prior to homogenization (2500+500 psi). Roasted amaranth flour was added in the form of a paste. Amaranth seeds were roasted at 110˚C for 10 to 12 min, cooled and ground to fine flour. The roasted amaranth flour and milk were mixed @ 1:20 parts for making the paste. The paste was heated at 70˚C and cool to room temperature. Date extract (@ 5.1 % w/w of milk), roasted amaranth flour @ 1.6 % w/w of milk), tri sodium citrate @ 0.15 % and sugar (6.0 % w/w of milk) were added and stirred for one min. The milk was blended by heating at 70˚C to 5 min. The product was filled in 200 ml sterilized glass bottles and sterilized at 121˚C for 15 min. The bottles were stored at room temperature i.e. 37˚C.

At every 15th day of storage at ambient temperature, the free fatty acid (FFA, expressed as percent oleic acid), tyrosine content (mg/100g), sensory characteristics, physico-chemical properties (viscosity, pH, sediment value, total solid, colour analysis), and microbial quality (standard plate count, spore count, yeast and mould count, and coliform count) of stored samples of flavoured milk were monitored until the product was rejected on basis of sensory acceptability.

Sensory evaluation

The flavoured milk subjected to sensory evaluation by a panel of 10 members comprising of faculty members from the College. The sensory attributes evaluated were colour and appearance, body (mouth feel), flavour, sweetness and overall acceptability score, based on 9-point hedonic scale. The flavoured milk samples were served in 50 ml glass beakers. The containers were labelled with random digit codes. The order of presentation of samples to the judges was randomized. In a sensory evaluation lab kept at 23.20˚C, product sensory evaluation was conducted in isolated booths lighted with incandescent light. The panelists were instructed to rinse their hands with lukewarm water as needed between samples.

Physico-chemical analysis

Gerber method was used to estimate the fat content of flavoured milk [7]. The total solids (TS) of flavoured milk was analyzed using Mojonnier Milk Tester (Model-D, Mojonnier Brothers Co., Chicago, USA) [9]. The protein content was determined using semi-micro Kjeldahl method [9]. The ash content was determined by method described in [8]. Free fatty acid (FFA) of samples was determined by the method described by [10]. Tyrosine content of samples was determined by the method described by Juffs [11]. The pH values of flavoured milk samples were measured using electronic digital pH meter (M/s. Mettler Toledo AG, Schwerzenbach, Model CH-8603). The viscosity of flavoured milk was measured at 20˚C using a 'Brookfield' viscometer (DV II + Pro Viscometer, Model- LVDV- II + P, USA). Sediment in the flavoured milk was measured by centrifugation method, using calibrated centrifuge tubes (50 mL). The weight of centrifuge tube was recorded and milk was weighted in them. The samples were placed in a centrifuge (Remi laboratory centrifuge machine) operated at 3000 rpm for 15 min. After centrifugation, the solid sediments were separated from the supernatant by decanting. The tubes were then placed in an oven at 120˚C for 36 h and the dry weight of sediment was noted. The results were expressed as g of sediment/100g of milk [12]. Colour of flavoured milk was measured using Mini scan Xe Plus (Hunter Colour Lab) in the Hunter Colour mode and expressed as 'L', 'a' and 'b' values.

Microbiological analysis

Eleven gram of flavoured milk sample was taken aseptically in a sterile phosphate buffer flask to prepare 1:10 dilution. After thorough mixing, 1 ml of diluents from this dilution was transferred to 9 ml of sterile phosphate buffer to obtain 1:100 dilutions. It was further diluted using 9 ml of sterile phosphate buffer to prepare requisite dilutions. One ml of diluents, from suitable dilutions was poured aseptically into sterile petri plates in duplicate. For SPC, plates were prepared using standard plate count agar as per the procedure described in [13]. For coliform count, plates were prepared using Violet Red Bile Agar (VRBA) as per the procedure described in [14]. For yeast and mold count plates were prepared using Potato Dextrose Agar (PDA) as per the procedure described in [15]. For spore count plates were prepared using Spore Count Agar as per the procedure described by [16].

Statistical analysis

Statistical analysis of data was carried out using Completely Randomized Design (CRD).

Results and Discussion

Effect of storage period on sensory characteristics

The results pertaining to the changes in the sensory quality (i.e. flavour, colour and appearance, mouth feel, sweetness and overall acceptability scores) of flavoured milks are collated in **Table 1**. There was a progressive decline in the flavour score of amaranth date flavoured milk (ADFM) throughout the storage period. The initial flavour score of the ADFM was 8.62, which was found to decrease significantly ($P<0.05$) to 8.47 after storage period of 15 d and it further decreased significantly ($P<0.05$) during storage. The flavour score declined significantly ($P\leq0.05$) at 15 d of storage. A significant decline in flavour score was noted at all the periods of storage studied.

Table 1 Sensory score of amaranth date flavoured milk during storage at 37 ± 2^0C

Storage period (in Days)	Sensory score				
	Flavour	**Colour and appearance**	**Mouthfeel**	**Sweetness**	**Overall acceptability**
T1 (0 d)	$8.62^{a}\pm0.07$	$8.49^{a}\pm0.08$	$8.46^{a}\pm0.10$	$8.47^{a}\pm0.12$	$8.48^{a}\pm0.10$
T2 (15 d)	$8.47^{b}\pm0.10$	$8.42^{ab}\pm0.12$	$8.38^{ab}\pm0.14$	$8.36^{ab}\pm0.12$	$8.38^{ab}\pm0.11$
T3 (30 d)	$8.38^{bc}\pm0.09$	$8.35^{ab}\pm0.11$	$8.29^{ab}\pm0.11$	$8.25^{b}\pm0.04$	$8.26^{b}\pm0.01$
T4 (45 d)	$8.32^{c}\pm0.10$	$8.25^{b}\pm0.10$	$8.26^{b}\pm0.08$	$8.24^{b}\pm0.06$	$8.25^{b}\pm0.13$
T5 (60 d)	$8.25^{c}\pm0.08$	$8.24^{b}\pm0.06$	$8.24^{b}\pm0.06$	$8.22^{b}\pm0.08$	$8.23^{bc}\pm0.06$
T6 (75 d)	$8.19^{c}\pm0.11$	$8.19^{b}\pm0.16$	$8.15^{b}\pm0.17$	$8.14^{b}\pm0.02$	$8.11^{c}\pm0.07$
SEm	0.05	0.06	0.06	0.04	0.05
CD(0.05)	0.14	0.17	0.18	0.13	0.14
CV (%)	1.15	1.38	1.46	1.03	1.15

Each observation is a mean ± SD of 4 replicate experiments; a-c: Different superscripts in each column indicate significant differences at 5 % level of significance

The trend reported by Anandh *et al*. for retort sterilized rose flavoured milk during storage up to 90 d under ambient storage conditions was similar to the trend noticed in the present investigation [17]. Similar results were observed by Shukla *et al.*, who reported that the flavour score sterilized mango beverage decreased after the storage of 75 d [18]. Kumar reported a significant decline in flavour score of chocolate flavoured milk beverage only from 3rd month of storage and thereafter the score remained constant during the remaining period of storage period up to 4.5 months [19].

The colour and appearance score of fresh of ADFM was 8.49 and such score decreased to 8.19 at 75 d of storage. The colour and appearance score showed significant ($P\leq0.05$) decline during each interval of 15 d, till the end of storage. There was a significant ($P\leq0.05$) decline in colour and appearance score at 45 d of storage (Table 1) and thereafter the score remained constant throughout storage period. Similar observations were reported in a study in which it was reported that the colour lightness of UHT treated chocolate flavoured peanut beverage decreased during first 6 weeks of storage and thereafter fairly constant during 25 weeks of storage at 35˚C [20]. In another study it was reported that the colour and appearance score of experimental chocolate beverage, there was a decline in colour and appearance score at 1 month of storage; the score remained fairly constant during subsequent 3 months of storage and then showed decline at 4th month and remained constant till the end of storage [19].

Figure 1 Amaranth date flavoured milk

The mouthfeel score of fresh (0 d) and 75 d stored ADFM was 8.46 and 8.15 respectively. Mouth feel score of flavoured milk showed significant ($P\leq0.05$) decline in score during other periods of storage (Table 1). The ADFM showed significant ($P\leq0.05$) decline in mouth feel score from 45d of storage and thereafter the score remained constant throughout storage period. Similar decline in mouthfeel score of the chocolate flavoured milk was also observed at 2 months of storage [19].

The sweetness score of fresh of ADFM was 8.47and such score decreased to 8.14 at 75 d of storage. The sweetness score showed significant (P≤0.05) decline during each interval of 15 d, till the end of storage. There was a significant (P≤0.05) decline in sweetness score at 30 d of storage (Table 1) and thereafter the score remained constant throughout storage period.

The overall acceptability score of fresh (0 d) and stored (45 d) ADFM was 8.48 and 8.14 respectively. There was a continuous decline in the overall acceptability scores of ADFM throughout storage period. The overall acceptability score remained highly acceptable till 45 d of storage; further storage led to significant (P<0.05) decline in the score (Table 1). Similar trend in overall acceptability score has been reported by Anandh *et al*. (2014) for retort sterilized (121°C/15 min) rose flavoured milk during 90 d storage period of 90 d at ambient conditions; the score for fresh and 90 d stored sample was 8.97 and 7.07 respectively [17]. In another study conducted by Kumar (2018), there was a continuous decline in the overall acceptability scores of control as well as experimental chocolate beverages throughout storage period up to 3 months of storage [19].

The average overall acceptability score of the product was 8.11 on the 75th day of storage. Thus, the product was liked very much (9 point hedonic scale) even on 75th day of storage.

Effect of Storage Period on Physico-Chemical Properties

The results pertaining to the storage changes in the physico-chemical properties (pH, viscosity, sediment, FFA, TS and tyrosine value) of flavoured milk samples are collated in **Table 2**.

Table 2 Physico-chemical properties of ADFM stored at 37±2^{0}C

Storage period (in Days)	Physico-chemical properties					
	pH	Viscosity (cP)	Sediment (g/100g)	Total solid (%)	FFA(%oleic acid)	Tyrosine (mg/100g)
T1 (0 d)	6.23^{a}±0.01	38.88^{e}±0.57	4.95^{c}±0.09	20.96^{a}±0.13	0.132^{d}±0.01	21.499^{f}±0.31
T2 (15 d)	6.21^{ab}±0.05	40.58^{d}±0.77	5.14^{b}±0.03	20.94^{a}±0.12	0.151^{d}±0.03	26.537^{e}±0.93
T3 (30 d)	6.18^{b}±0.01	41.55^{cd}±0.55	5.18^{b}±0.02	20.88^{b}±0.16	0.192^{c}±0.01	30.411^{d}±0.34
T4 (45 d)	6.15^{bc}±0.03	42.17^{c}±0.59	5.45^{a}±0.08	20.83^{c}±0.18	0.222^{b}±0.02	34.221^{c}±0.24
T5 (60 d)	6.13^{c}±0.02	44.79^{b}±0.25	5.48^{a}±0.13	20.82^{cd}±0.14	0.231^{ab}±0.04	40.445^{b}±0.63
T6 (75 d)	6.12^{c}±0.03	46.61^{a}±0.94	5.48^{a}±0.11	20.78^{d}±0.17	0.246^{a}±0.02	46.372^{a}±0.82
SEm	0.01	0.45	0.05	0.01	0.01	0.30
CD(0.05)	0.03	1.33	0.14	0.04	0.02	0.91
CV (%)	0.29	2.11	1.73	0.12	5.84	1.83

Each observation is a mean ± SD of 4 replicate experiments; a-f: Different superscripts in each column indicate significant differences at 5 % level of significance

pH

The values depicted in Table 2 indicates that the period of storage had a significant (P≤0.05) influence on the pH of flavoured milks. The pH of fresh ADFM which was 6.23 decreased to 6.12 at 75 d of storage. The pH of the fresh ADFM remained stable up to 15 d, while further storage (up to 45 d) led to significant lowering in the pH, extended storage period led to decrease in the pH value.

Iserliyska *et al.* also reported decrease in the pH of pasteurized chocolate beverage during storage at 4°C for 21 d [21]. The pH of drink containing 1.0 % cocoa powder tended to decline from initial pH 6.63 to 6.61 at the end of storage period. In another study, the initial pH of UHT processed chocolate flavoured peanut beverage of 7.68 decreased to 7.33 and 7.42 respectively at 25th week of storage at 35°C and 20°C [20]. On the other hand, Kumar (2018) reported that the pH of fresh chocolate beverage remained literally unchanged up to 3 months of storage [19]. However, extended storage period led to a significant decrease in the pH value.

Viscosity

The viscosity of fresh (0 d) ADFM was 38.88 cP which tended to increase up to 46.61 cP at 75 d of storage. Table 2 indicates that the viscosity of ADFM increased throughout the 75 d of storage at ambient temperature. The period of storage had a significant (P≤0.05) influence on the viscosity of flavoured milks.

Anandh *et al.* established that the extent of proteolysis, age gelation, enzymatic activity and interaction of milk fat and milk protein could have an influence on the increase in viscosity of milk (The increase in viscosity observed during storage could be due to presence of heat resistant proteolytic enzymes which could be responsible for age

gelation) [17]. The findings related to the increase in the viscosity of experimental flavoured milks observed in the present study was similar to the one noted for UHT treated flavoured milk [22]. The viscosity of chocolate flavoured peanut drink increased from initial value of 15 cP to 42 cP at 21 d of storage at 4˚C [21]. Kumar (2018) reported that the viscosity of chocolate flavoured milk increase up to 2 months of storage; further storage led to decline in the viscosity [19].

Sedimentation

The sedimentation value of fresh (0 d) and 45 d ADFM was 4.95 g/100g and 5.48 g/100 g respectively. It can be seen from the Table 2 that there was a significant ($P \leq 0.05$) influence of the period of the storage on the sedimentation behavior of ADFM. There was a continuous increase in sedimentation value of ADFM throughout storage period.

Rustom *et al.* (1995) reported increase in the sedimentation rate in chocolate flavoured peanut beverage over storage period at 37˚C; the severity of the heat treatment to milk also led to increased sedimentation [20]. Kumar (2018) reported increase in the sedimentation rate in chocolate flavoured milk over storage period at 37˚C [19].

Total solids

The changes in Total solids of flavoured milk during storage at room temperature were presented Table 2. It was observed that the TS value of ADFM decreased gradually during the entire storage period. The total solids content of fresh (0 d) flavoured milk was 21.96 % which tended to decrease up to 21.78 % at 2.5 months of storage. The storage period showed significant ($P<0.05$) effect on TS content of ADFM samples. The decrease in TS during storage could be attributed to hydrolysis of components during storage period.

Mittal and Bajwa (2015) reported that TS content of the drinks decreased and TSS increased significantly with progress in storage [23]. Similarly, in another study it was reported that the TS content of sterilized mango beverage decreased after the storage of 60 d [18].

Free Fatty Acids

The data obtained for changes in free fatty acids content of ADFM during storage at 37±1˚C are presented in Table 2. The free fatty acid content of flavoured milk seemed to gradually increase during storage as seen from the FFA values (expressed as % lauric acid). It can be seen from the table that there was a significant ($P<0.05$) increase in FFA content throughout the storage period. The results indicated that the initial free fatty acids content of fresh samples and 75 d of ADFM was 0.132% oleic acid and 0.246% oleic acid respectively.

Eknath (2017) reported that initial FFA content of coconut flavoured milk was 0.40 – 043 % lauric acid, which significantly increased to 0.53% (PET bottle) and 0.60% lauric acid (glass bottle) at the end of 8th day [24].

Tyrosine value

Tyrosine value has been used to measure the degree of proteolysis in flavoured milk. The degree of proteolysis can be measured by observing changes in nitrogen levels or by estimating the free amino acids like tyrosine. Release of tyrosine leads to bitterness, unclean, sour and tenuous defects [11]. The data obtained for changes in tyrosine content of ADFM during storage at 37±1˚C were presented in Table 2. The tyrosine content of ADFM increased with increase in storage period. Fresh ADFM had tyrosine content 21.499 mg/100g which increased significantly ($P<0.05$) to 46.799 mg/100g after 75 d of storage.

The storage period showed significant ($P<0.05$) effect on tyrosine content of ADFM samples. TVs for milk freshly drawn from individual cows were extremely varied (0.31-0.92 mg/ml). TVs for samples of refrigerated milk from daily supply farms were in the range 0.40-0.58 mg/ml [25].

Effect of Storage Period on Optical Properties

The colour of food is one of the major attributes which affecting its quality and thus, consumer acceptability. Colour of any food is also an important tool for quality control and marketing. The change in colour characteristics of ADFM during storage was measured and the observations are given in **Table 3**.

As seen from the values quoted in Table 3, The L*, a* and b* value represent lightness, redness and yellowness. The values given in the Table 3 reveal that for ADFM, initial value of L* (lightness) 52.34 was decreased to 51.68 after 75 d of storage. The initial redness value of ADFM was 5.01, which decreased after 75 d to 4.63 and *b** values

of ADFM, corresponding to the position on the yellow (+)/blue (-)-axis, mainly varied between 17.90 and 16.72. The *c** values of ADFM, mainly varied between 18.60 to 17.81 and *h*° values of ADFM, mainly varied between 74.63 and 73.62. The result of present study are in agreement with those reported by [26]. These authors reported that change in colour could be mainly attributed to Maillard reactions, increasing storage temperature and non-enzymatic spoilages.

Table 3 Optical properties of ADFM stored at 37±2 °C.

Storage period	**Optical properties**				
(in Days)	***L****	***a****	***b****	**c***	**h°**
T1 (0 d)	52.34^{a}±0.03	5.01^{a}±0.04	17.90^{a}±0.05	18.60^{a}±0.04	74.63^{a}±0.06
T2 (15 d)	52.29^{a}±0.08	4.91^{b}±0.01	17.67^{b}±0.03	18.47^{b}±0.06	74.39^{b}±0.03
T3 (30 d)	51.94^{b}±0.04	4.84^{c}±0.03	17.39^{c}±0.03	18.24^{c}±0.07	74.20^{c}±0.08
T4 (45 d)	51.85^{b}±0.03	4.76^{d}±0.01	17.02^{d}±0.07	18.11^{d}±0.06	73.96^{d}±0.09
T5 (60 d)	51.75^{c}±0.06	4.65^{e}±0.06	16.83^{e}±0.06	17.98^{e}±0.05	73.81^{e}±0.09
T6 (75 d)	51.68^{c}±0.05	4.63^{e}±0.05	16.72^{e}±0.13	17.81^{f}±0.07	73.62^{f}±0.03
SEm	0.03	0.02	0.04	0.03	0.04
CD(0.05)	0.09	0.06	0.11	0.10	0.11
CV(%)	0.11	0.90	0.42	0.35	0.10

Each observation is a mean ± SD of 4 replicate experiments; a-f: Different superscripts in each column indicate significant differences at 5 % level of significance

Effect of Storage Period on Microbial Quality

Milk and milk products is an excellent growth medium for microorganisms since it is almost a complete food. The microbiological quality of any dairy products depends on the initial flora of raw milk, the processing conditions, contamination taking place after heat treatment and particularly on its storage conditions [27]. Coliforms which are mainly show their presence owing to unhygienic practices followed in food processing plant and they appear as post-pasteurization contaminants [28].

The microbial count (viz., SPC, YMC, coliform count and spore count) of fresh as well as stored sterilized ADFM, kept at 37±2˚C revealed that the product, whether fresh or stored (up to 75 d) did not show any count (per gram) for SPC, YMC, coliform and spores. Such absence of any sort of microbial count reveals adoption of strict hygienic practices during production, processing and packaging of amaranth date flavoured milk. Sabbir *et al.* (2015) also reported absence of viable bacteria and coliform in all the three samples of commercially available UHT processed chocolate milks in Bangladesh [29].

Conclusion

In conclusion, the product was found to be acceptable up to 75 d when stored at 37±1˚C in glass bottles. During storage there was a significant ($P<0.05$) increase in free fatty acid, tyrosine content, viscosity, sediment value. There was decrease in pH, total solid, colour value and sensory score during the storage. Throughout storage period SPC, spore count, yeast and mold count and coliform count were absent.

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