

Development of Vitamin-enriched Pasteurized Milk Incorporated with Carrot (*Daucus carota* L.) and Determination of Its Quality Parameters

V.S. Jayamanne¹⁺, P.G. Kumudumali¹, M.P.K. Jayarathna², G.S.N. Fernando¹

¹ Department of Food Science and Technology, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

² Milco (Pvt) Ltd, No 45, Nawala Road, Narahenpita, Sri Lanka

Abstract. A novel carotene-enriched pasteurized milk drink was developed using cow milk, steamed and blended carrot and sugar. The product was further improved using cardamom, vanilla and mint flavours. The sensory, physico-chemical, microbiological properties and the shelf-life of the improved product were determined. The β -carotene content of the developed product was determined using the Open Column Chromatography (OCC) technique and the amount was 2.04 $\mu\text{g/g}$ which was 5.1 times higher than normal cow milk. The coliforms were absent in the final product whereas the total colony count was lower than 3.0×10^4 cfu/ml. The yeast and mould count was below the detection level after 5 days of cold storage (4 °C). The titratable acidity and the methylene blue reduction (MBR) time of the developed product were 0.13-0.15% and 6 h and 45min, respectively. The ash, total solid, fat and protein contents of the developed product were 0.92 %, 21.4 %, 3% and 3.5 %, respectively. The content of β -carotene was significantly ($P < 0.05$) higher in the developed product than the commercially available pasteurized milk packets. The shelf-life of the product was 5 days under refrigeration conditions (4 °C). It can be concluded that a carotene-incorporated pasteurized milk product can be produced as a nutritious drink.

Keywords: Carrot, Vitamin A deficiency, Carotenoids, Pasteurized milk, Chromatography, Shelf-life

1. Introduction

Vitamin A deficiency (VAD) is a major public health nutrition problem in the developing world. Vitamin A or retinol is an essential nutrient for human and all mammalian species since it cannot be synthesized within the body. Deficiency of the vitamin A results in adverse effects on growth, reproduction and resistance to infection. The most important manifestation of severe VAD is Xerophthalmia and further irreversible blindness may eventually occur in one or both eyes. VAD is still an important micronutrient deficiency problem in many developing countries affecting large numbers of pre-school children [1]. It is also becoming clear that VAD can extend through school age and adolescent years into adulthood. Although the health consequences of VAD are not well delineated beyond early childhood, recent data indicate that VAD in women of reproductive age may increase morbidity and mortality during pregnancy and the early postpartum period [1]. Severe maternal VAD may also disadvantage the newborn leading to increased mortality in the first months of life [2].

Beta-carotene is the main pro-vitamin in plant sources of vitamin A. Beta-carotene from fruits and from some yellow and orange color tubers contribute to the highest prevalence of VAD. Carrot (*Daucus carota* L.) is one of the most popularly consumed vegetables that provide many functional food components such as vitamins (A and D) and minerals (calcium sodium, iron). Carrot has a high concentration of β -carotene which is considered as the most essential micronutrient for human nutrition because of its antioxidant

⁺ Corresponding author: Tel: +940412293813, Fax: +9404192384.
E-mail address: vsjayamanne@fst.ruh.ac.lk

activity and property to act as a pro-vitamin A [3]. It has been reported that 100 g of carrot contains between 6 mg and 15 mg of carotenoids, mainly β -carotene (2-10 mg) [4]. Carotenoids and other antioxidants present in carrot play an important role in the inhibition of certain types of cancers and heart related diseases due to their ability to interruption of oxidation process and formation of free radicals. Moreover, carrot has a no allergic effect on humans [4].

Addition of carotenoids into foods can improve the nutritional value and may be a good solution to combat vitamin A deficiency in developing countries. Addition of carotenoids, the precursor of vitamin A, directly to the food is a practical solution than addition of vitamin A to foods. Many people in Sri Lanka consume milk which is an excellent source of nourishment for human beings. Thus, the development of foods that promote health and well-being is one of the major research priorities in the food industry [5]. Therefore, the objective of present study were to develop a pasteurized milk product enriched with vitamin A by adding carotenoids from Carrot and to determine its quality parameters.

2. Materials and Methods

2.1. Preparation of Pasteurized Milk with Incorporated of Carrot

Peeled and washed carrot was steamed using a steamer and blended to obtain carrot pulp. Then fat percentage of skimmed milk and cream was measured separately using Gerber method [6]. Fat percentage was standardized into 3% by adding the correct amounts of skim milk and cream together using Pearson square method. Nine different treatment combinations (T₁ -15g of carrot (C), 15g of sugar (S), T₂- 15g C, 25g S, T₃-15g C, 35g S, T₄- 25g C, 15g S, T₅- 25g C, 25g S, T₆- 25g C, 35g S, T₇- 35g C, 15g S, T₈- 35g C, 25g S and T₉- 35g C, 35g S) were prepared by changing carrot and sugar amount using 250ml of standardized milk for each treatment. Sugar was added to the milk and pasteurized at 71.7 °C for 15 sec and was allowed to cool up to room temperature. Finally developed product was stored under refrigerated conditions (4 °C).

2.2. Sensory Evaluation and Further Improvement of the Developed Product

Sensory evaluation was carried out with 15 trained sensory panelists of the Research and Development Division, Milco (PVT) Ltd. Narahenpita, Sri Lanka to find out the best sample mixture from nine different treatment combinations. Taste, sweetness, color, thickness and overall acceptability of the developed product were evaluated using a five-point hedonic scale on the date of manufacture. Three different flavors (cardamom, vanilla and mint) were used for the improvements of the product. A volume of 0.05 ml of each flavor was added into 250 ml of the best carrot and sugar mixture separately and another round of sensory evaluation was conducted to determine the best treatment.

2.3. Determination of Physicochemical, Microbiological Properties and the Shelf-life

Finalized recipe selected by sensory evaluations were subjected to proximate, chemical, physicochemical and microbiological analysis. Protein (Kjeldhal method), fat (Gerber method), ash (Dry ashing method) and fiber (Gravimetric method) contents were determined using the methods prescribed by AOAC [6]. The pH, titratable acidity % [7], total solid percentage, Methylene blue reduction time, Phosphates test and Alcohol test (68% and 70% alcohol) of the selected product were also determined [6]. Moreover, Carotenoids (pro-vitamin A) content of the developed product was determined using Open Column Chromatography method [8] and enumeration of *Coliform* and *E. coli*, yeast and mould count and Total Colony Count (TCC) of the developed product were also determined. The Shelf-life of the developed product was determined using changes in pH, titratable acidity and microbiological properties.

2.4. Analysis of Data

Completely Randomized Design (CRD) was used as the experimental design and samples were completely randomized across all panelists in order to avoid or minimize the effect for results occurring due to the order of samples. Sensory data were analyzed using Kruskal-Wallis non parametric one way ANOVA using STATISTIX software (Ver 2.0) for Windows. The Duncan's Multiple Range Test (DMRT) was used to analyze the differences between the individual means at a 5% significance level.

3. Results and Discussion

3.1. Properties of the Carotene-enriched Pasteurized Milk

The results show (Table 1) that treatment 5 and 6 were not significantly different ($P>0.05$) from each other for taste but sweetness, color, thickness and overall acceptability were significantly different ($P<0.05$) from each other at 5% significance level. Considering the highest mean rank value for all sensory parameters (taste-108.47, sweetness-115.3, colour-116.3, thickness-115.8 and overall acceptability-122.8), treatment 5 (carrot:sugar, 25g:25g) was selected as the best combination for preparation of carotene-enriched pasteurized milk incorporated with carrot through sensory evaluation. Moreover, it was observed that addition of carrot increased the consumer preference of the colour of the final product. The observations of the present study are in agreement with the findings of a similar study carried out on the study of yoghurt incorporated with carrot juice [9]. The improved pasteurized milk product with cardamom flavor was the best improved sample and recorded the highest sensory scores for taste (25.7), flavor (32.0), sweetness (36.1) and overall acceptability (32.5). Further, the thickness and color of the all treatments were observed to be the same in all the treatments.

Table 1. Estimated mean rank values of carotene-enriched pasteurized milk samples

Treatment	Carrot: Sugar Ratio	Taste	Sweetness	Color	Thickness	Overall
1	15:15	4.37 ^c	16.1 ^c	0.87 ^d	36.1 ^c	5.7 ^d
2	15:25	7.57 ^{bc}	77.0 ^{7ab}	2.1 ^{cd}	38.9 ^c	0.7 ^{cd}
3	15:35	8.77 ^{bc}	59.3 ^{3bc}	9.1 ^d	36.1 ^c	5.1 ^{bc}
4	15:15	2.47 ^{ab}	62.1 ^{7b}	6.1 ^{bcd}	71.3 ^{3abc}	4.1 ^{bc}
5	15:25	08.47 ^a	115.3 ^{3a}	16.3 ^a	115.8 ^{87a}	22.8 ^a
6	15:35	08.17 ^a	77.0 ^{7ab}	7.57 ^{ab}	101.3 ^{37ab}	6.2 ^{ab}
7	15:15	2.7 ^{ab}	56.5 ^{bc}	3.7 ^{abc}	86.8 ^{7ab}	7.7 ^{bc}
8	15:25	2.47 ^{ab}	77.4 ^{3ab}	8.7 ^{bcd}	62.7 ^{bc}	4.8 ^{bc}
9	15:35	7.04 ^{ab}	71.0 ^{3ab}	7.57 ^{abc}	62.7 ^{bc}	4.8 ^{bc}

Means with different superscripts are significantly different at 0.05 significance level.

3.2. Proximate Composition and Carotene Content

Both the developed product and commercial pasteurized milk showed the same concentration of fat (3%). Commercially available pasteurized milk contains 3.29 g protein, 3% fat, 0.6 g fiber and 0.72g of ash. But carotene-enriched milk contains more proteins (3.5g), fiber (0.74) and ash (0.92g) compared to the commercially available products. Scientific evidence shows that β -carotene content of normal cow milk is 0.4 $\mu\text{g/g}$. When milk was enriched with carrot in the present study it increased the carotene content to 2.04 $\mu\text{g/g}$. Therefore, the developed product contains 5.1 times more β -carotene than normal cow milk. Daily requirement of vitamin A for the human body is 5000 IU and 250 ml of carotene enriched milk appears to provide 20% of the daily requirement of vitamin A. Accordingly, addition of carrot to milk products appears to be a good solution to prevent vitamin A deficiencies in a vulnerable population.

3.3. Shelf-life of the Developed Product

The developed product had a pH value of 6.8, a titratable acidity value of 0.13% and a total solid content of 21.42% on the day of manufacture. The activity of lactic acid bacteria results in the development of acidity of milk during the storage period thereby increasing the acidity of milk. This is due to the conversion of lactose into lactic acid by bacteria. The acidity of the developed product changed from 0.13% to 0.15% during cold storage (4 °C) period of 5 days. According to the Sri Lankan Standards, titratable acidity of pasteurized milk should be less than 0.15%. These results are in agreement with the similar study in which acidity increased over the storage period of fresh milk [10].

Methylene blue reduction (MBR) time for the developed product during 5 days cold storage was 6 h and 45 min. According to the observations in a similar study the control milk sample decolorized within one hour during the methylene blue reduction test and was classified as class 4 milk and treated samples did not decolorize and were ranked as class 1 products (class 1- excellent, not decolorized in 8h; class 2- good, decolorized in 6-8h; class 3- fair, decolorized in 2-6h; class 4- decolorized in less than 2h) [11]. The short time observed in the present study for decolorization of the methylene blue is an indication of the high microbial load present in milk. Alcohol test was negative for the developed product for five days storage period in the cold room (4 °C). The basis of alcohol test is that when milk contains more than 0.12% acid or when Ca and Mg are present in milk in amounts is greater than normal amount; it will coagulate on the addition of alcohol. The total solid content did not change in the developed product during the cold storage and was higher compared to the other pasteurized flavored milk (15-16 %) due to the addition of carrot.

3.4. Microbiological Properties of the Developed Product

Coliforms and *E. coli* colonies were not detected in the developed product during cold storage, thus, showing hygienic production procedures and acceptable for consumption. Coliform bacteria appeared to have been destroyed at the pasteurization temperature. Therefore, the possible presence of coliforms in pasteurized products indicates improper pasteurization techniques and/or post pasteurization contaminations. Further microbiological testing (standard plate count and coliform count) revealed that the microbiological results are highly correlated with the methylene blue reduction time. Total colony count (TCC) changed from 3.6 cfu/ml to 4.47 cfu/ml during five days. According to Sri Lanka Standard Institute (SLSI) standards, TCC in pasteurized milk packets should be maintained at a population lower 3.0×10^4 cfu/ml [12]. The TCC in the developed product was lower than 3.0×10^4 cfu/ml up to 5 days of storage at 10 °C. According to the findings of a similar study, pasteurization of freshly prepared carrot juice at 80 °C for 20 min decreased the microbial population below the detected limit (10 cfu/ml) [13]. The yeast and mould count was negative for three days and it was lower than <10 cfu/ml on the fifth day. According to the SLSI standards, yeast and mould count in pasteurized milk should be less than 1.0×10^1 cfu/ml. According to the shelf-life study and microbiological properties, it can be concluded that the developed product had 5 days of shelf-life. These results are in line with findings of a similar study in which based on the organoleptic analysis the shelf-life of whole pasteurized milk in Greece was 5 days [14].

3.5. Sensory Properties of the Developed Product in Comparison to the Commercial Products

Carotene enriched milk sample recorded significantly higher ($P < 0.05$) preference for each sensory attributes such as taste (108.4), sweetness (100.27), color (106.3), thickness (103.3) and overall acceptability (110.8). The highest preference recorded by panelists for the carotene-enriched pasteurized milk appears to be due to the addition of carrot and cardamom flavor. Color of the three samples was significantly different ($P < 0.05$) from each other and the highest preference for the color of carotene enriched milk could be due to the color of carotenoids of carrots. Further, sensory panelists observed that the carotene-enriched milk had an attractive flavor and odor due to addition of cardamom flavor.

4. Conclusions

Carrot can be effectively used for the development of carotene-enriched pasteurized milk. There was a significantly higher amount of β -carotene in the carotene-enriched pasteurized milk (2.04 $\mu\text{g/ml}$) than normal cow milk (0.4 $\mu\text{g/ml}$) and this was 5.1 times more β -carotene than normal cow milk. Carotene-enriched pasteurized milk could be effectively used to prevent vitamin A deficiencies in a vulnerable population. Carotene-enriched milk can be stored for five days under refrigeration conditions (4 °C) without changing its quality parameters.

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