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Research Article

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Application of Microencapsulation Technology for the Production of Vitamin-C Fortified Flavoured Milk

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Abstract

A research study was carried out to develop vitamin-C enriched flavoured milk by using vitamin-C microcapsules. Vitamin-C was encapsulated through extrusion technique using sodium alginate (stabilizer) as coating material. Standardization of sodium alginate was done by the formation of beads. The beads were found hard and of good quality with 2 g sodium alginate for carrying the vitamin-C. The level of addition of vitamin-C powder was standardized based on the encapsulation efficiency (EE). In the present study, 71 percentage EE level was used to prepare vitamin-C fortified flavoured milk. The developed flavoured milk (F_1) and control flavoured milk (CF) were assessed for consumer acceptability through nine point's hedonic rating scale. Among the samples, F_1 had the highest rate of consumer acceptance next to the control sample (CF). The pH value of control (CF) and F_1 were observed to be 5.65 and 5.32 respectively. The sterilization efficiency of CF and F_1 samples was found positive. The vitamin-C encapsulated flavoured milk (F_1) sample was observed better up to 30 days with the retained quantity of 92 ± 0.50 mg of vitamin-C.

Keywords: Microencapsulation; Vitamin-C; Flavoured milk; Physicochemical; storage stability evaluation

Introduction

Encapsulation is the primary inexpensive packaging approach for active ingredients in food for maximum protection, targeted delivery and controlled release of nutrients [1]. Microencapsulation is described as a technique wherein a bioactive compound is encapsulated by a biopolymer thereby protecting it from oxygen, water, light or other conditions in order to improve its stability and liquid solution to powder for easier handling [2]. Developing products with active ingredients is more challenging in the food industry because of the necessity to natural appearance and viability in storage conditions and subsequent reprocessing or cooking before consumption [3].

Vitamins and minerals fortification has been used to improve nutrient content of foods. Many vitamins are relatively unstable and their activity in foods depends on pH and their stability to heat, light, oxygen, oxidizing agents and enzymes [4]. Encapsulation of vitamin-C provides higher shelf life. Stable vitamin-C shall be a good asset to fortify with foods. Flavoured milk is one of the good vehicles for supplying milk constituents to the milk consumption world and survives an ambient condition [5].

Hence, in this study, flavoured milk was fortified with vitamin-C through microencapsulation process by sodium alginate as a stabilizer and coating material. Alginate is useful as a matrix for immobilization of plant, animal and microbial cells as well as entrapment of bio active compounds and drugs [6,7].

Objective

- To develop flavoured milk with standardized vitamin-C microcapsules and
- To evaluate the physicochemical quality and storage stability of vitamin-C fortified flavoured milk.

Materials and Methods

The present study was carried out in the Dairy Technology laboratory of Faculty of Agriculture and Animal Husbandry, Gandhigram Rural Institute - Deemed University, Gandhigram, Dindigul District, TamilNadu, India.

Materials

Double toned milk (Raaj brand), Sugar and Cardamom were purchased from the local departmental store at Chinnalapatti Town, Dindigul district, TamilNadu. The L-Ascorbic acid (Vitamin-C) was purchased from HPLC Pvt Ltd, Mumbai and the sodium alginate (Molecular Biology Grade) was purchased from HiMedia Laboratories Pvt Ltd, Mumbai.

Methods

Preparation of microcapsules (Microencapsulation): Microcapsules were prepared using extrusion technique. Sodium alginate solution was prepared by mixing the dry powder of sodium alginate (matrix) (CAS No : 9005- 38-3/MB114-100G) in distilled water using magnetic stirrer (Cat No 5MLH, REMI instrument, Vasai, India) with mild heating at 30°C for 4 hours. L-Ascorbic acid (Vitamin-C) was added into the matrix of alginate solution. The aqueous solution of sodium alginate containing vitamin-C was delivered from burette at controlled flow rate (120 ml/min) into 0.2 M calcium chloride solution. The extrusion droplets fall in reaction vessel. Calcium chloride solution was continuously stirred at 1000 RPM during extrusion of alginate solution and was stirred for 30 minutes after extrusion for hardening. The hardened vitamin-C microcapsules were sieved and washed with deionized water. The representations of experimental set up are shown in Figure 1.

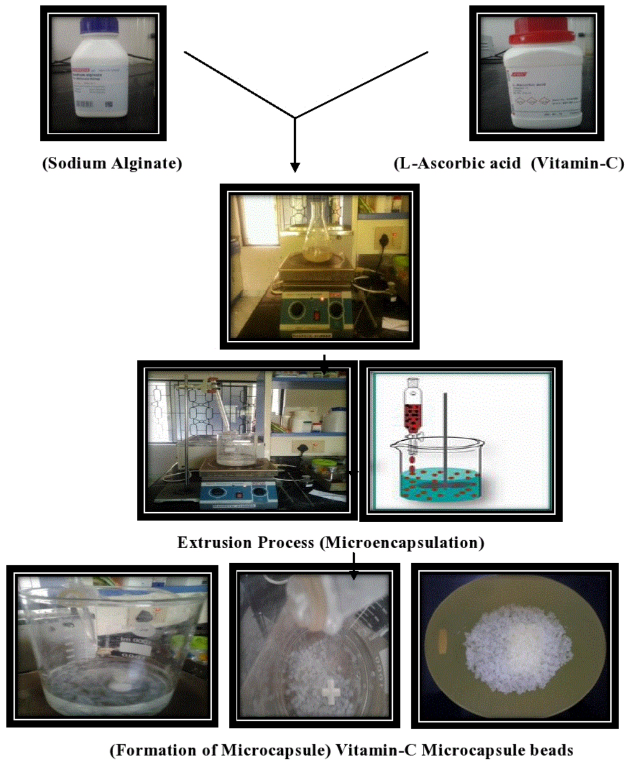

Figure 1: Formation of vitamin-C microcapsule.

This method was as per the Karim Narsaiah et al. [8] who found that the divalent calcium ions in reaction vessel replaced sodium ions and cross linked alginate polymer chain and formed calcium alginate microcapsules by ionotropic gelification.

Alginate beads may be easily produced by dropping an alginate solution in a calcium chloride bath. Alginate has been used in many encapsulation applications, including various fields, such as biomedical, bioprocess, pharmaceutical, and food and feed [9].

Encapsulation efficiency (EE): Encapsulation efficiency of vitamin-C content was estimated by 2,6 dichloro indophenols fibrimetric method as described in the AOAC manual [10]. Microcapsules were macerated in phosphate buffer (0.2 M/7.4 pH) and centrifuged at 1400 rpm for 30 minutes. Vitamin-C content of supernatant was estimated. Vitamin-C content of matrix solution of sodium alginate before extrusion was also estimated. EE was calculated using the following formula

$$EE=\frac{\text{Vitamin-C content in capsules}}{\text{Vitamin-C in alginate solution}}\times 100$$

Standardization of sodium alginate and vitamin C: Standardization of sodium alginate was done by the formation of beads. The standardization with sodium alginate as coating material, in the present study was undertaken in the range of 0.5 to 2.5 g (Table 1). Between these levels, the beads were found to be sufficiently hard and of good quality with 2 g, that has been further utilized for carrying vitamin-C. The results also found to corroborate with the findings of Sukumar De [11].

The standardization of vitamin-C was done based on the EE. For encapsulation, vitamin-C powder (L-Ascorbic acid) was used at various levels from 100 mg to 1000 mg in 2 g sodium alginate and 0.2 M $CaCl_2$ bath. The EE was calculated using the formula and results are given in Table 2. The results revealed that the EE was high in 100 mg vitamin-C powder with the value of 78 percentage, whereas it was 71 percentage under 500 mg of vitamin-C powder. In the present study, 71 percentage EE level was taken as standard value, since the storage loss was compensated at this level and also it is sufficient to deliver the daily requirement rate (RDA). The levels of vitamin decrease during processing and storage periods that are in line with the findings of Lachance [12].

Level of sodium alginate beads					
Requirements	0.5 g	1 g	1.5 g	2 g	2.5 g
Water	100 ml	100 ml	100 ml	100 ml	100 ml
$CaCl_2$ (0.1 M)	Not Appeared	Not Appeared	Not Appeared	Not Appeared	Not Appeared
$CaCl_2$ (0.2 M)	Not Appeared	Appeared	Appeared	Appeared	Appeared
Beads Texture	-	Loose	Semi hard	Hard	Very hard

Table 1: Development of Microcapsules by different proportions of sodium alginate at 0.1 and 0.2 M $CaCl_2$ bath.

Microencapsulated vitamin-C fortified flavoured milk: The preparation method of microencapsulated vitamin-C fortified flavoured milk was designed and shown in the flow diagram (Figure 2). Vitamin-C microcapsules are added after pre-heating (35 to 40°C) and clarifying the good quality of double toned milk (1000 ml). 10 percentage of sugar and one percentage of cardamom to the total quantity of double toned milk (w/v) are slowly added and stirred so as to dissolve them properly. The mixture was then pasteurized at 71°C for 30 minutes and cooled rapidly to 5°C. The prepared flavoured milk was sterilized at 121°C for 15 minutes after bottling. It was then stored at ambient condition (30°C) until analysis.

Sample description

In the present study, three types of flavoured milk were prepared viz., encapsulated, un-encapsulated and a control (without addition of vitamin-C) and necessary coding as F1, F2 and CF were given to these samples respectively.

Sl.no	Parameter	Vitamin-C Level (mg)				
		100	250	500	750	1000
1.	Capsules	78 ± 0.50	189 ± 0.21	355 ± 0.17	510 ± 0.14	652 ± 0.08
2.	$CaCl_2$	22 ± 0.23	61 ± 0.55	145 ± 0.14	240 ± 0.23	348 ± 0.14
3.	EE (%)	78	75	71	68	65

Table 2: Encapsulation efficiency (EE) of vitamin-C.

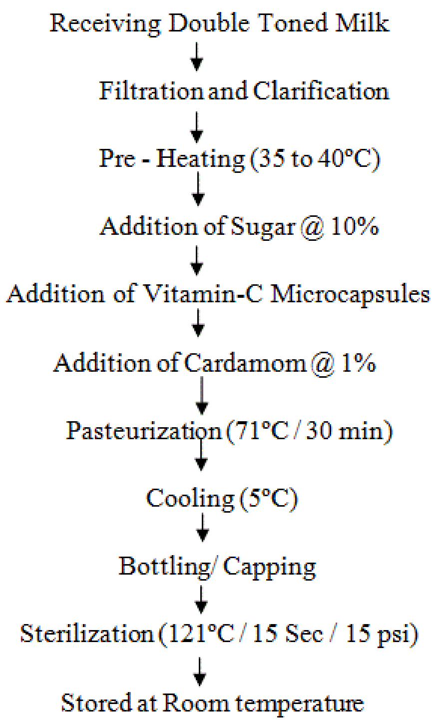

Figure 2: Preparation of microencapsulated vitamin-C fortified flavoured milk.

Consumer acceptability of flavoured milk: The results of encapsulation study revealed that the un-encapsulated (free) vitamin-C powder added sample (F_2) developed curdles during pasteurization at 71°C (for 30 minutes), resulted in sour taste and hence, rejected for further sterilization process and analysis. The sample F_1 was taken for further physicochemical and storage stability studies and the results were comparing with control (CF).

The nine point's hedonic rating scale was used to measure the consumer's acceptability of flavoured milk. Both the samples of flavoured milk (CF and F1) were served to the semi trained panelist, and the members were asked to rate the acceptability of the products ranging from like extremely to dislike extremely.

Estimation of pH: The pH value of flavoured milk samples was determined by ELICO digital pH meter.

Estimation of vitamin-C: The vitamin-C content was estimated by 2,6-di-chloro indophenols fibrimetric method as described in the procedure 967.20, AOAC manual [10].

Estimation of sterilization efficiency (turbidity test): The sterilization efficiency of flavoured milk was determined by turbidity test. Weighed about 20 ml of the flavoured milk sample with 4 g of ammonium sulphate and mixed the contents gently and kept it undisturbed condition for a minute. Then, it was filtered during the time that the filtrate placed in to water both for 10 minutes. The clear solution determines positiveness of the test.

Results and Discussion

Consumer acceptability of flavouerd milk

The mean ± SD values of six trails of hedonic scores for colour and appearance, flavor, consistency, taste and overall acceptability of vitamin-C encapsulated flavoured milk as showed in Table 3 indicated that the overall acceptability score was high with control (CF) followed by F1 sample with a score of 8 ± 0.50 and 7.5 ± 0.40 respectively.

Physical attributes	CF	F1
Colour and Appearance	7.5 ± 0.25	7.3 ± 0.53
Flavor	7.8 ± 0.53	7.5 ± 0.25
Consistency	7.5 ± 0.63	7.2 ± 0.60
Taste	8 ± 0.21	7.5 ± 0.68
Overall Acceptability	8 ± 0.50	7.5 ± 0.40

Table 3: Mean value for consumer acceptability of vitamin-C encapsulated flavoured milk.

pH

The pH value of control sample was 5.65 which was followed by F1 (5.32) and the result was in accordance with the findings of Sukumar De [11] who found that the required pH for prevention of curdling as more than 5 in flavoured milk/milk-syrup mixtures.

Sterilization efficiency

The sterilization efficiency of flavoured milk was determined by turbidity test and the results for the both sample were positive. It denotes that both samples were sterilized well under 121°C for 30 minutes at 15 lbs pressure.

Stability for vitamin-C in flavoured milk

The stability of vitamin-C in flavoured milk was analyzed and presented in the Figure 3 and Table 4. For this stability study, 355 ± 0.17 mg i.e., 71 percentage of EE achieved capsules were fortified with flavoured milk (F1). The stability of vitamin-C content of the flavoured milk sample (F1) was assessed during storage under ambient temperature for one month.

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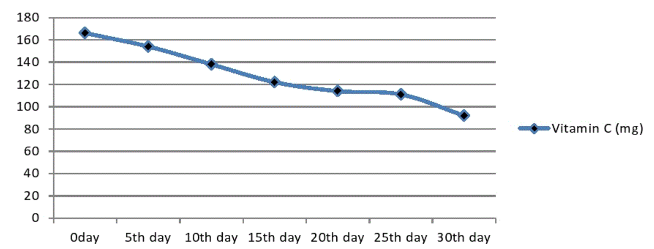

Figure 3: Stability of vitamin-C in flavoured milk during room temperature storage.

	Storage under room temperature condition						
DAYS	0	5nd	10th	15th	20th	25th	30th
Control	0	0	0	0	0	0	0
F2 (mg/120 ml)	166	154	138	122	114	111	92

Table 4: Stability of vitamin C in flavoured milk during storage period.

The results showed that the sample F1 retained 355 ± 0.17 mg and 92 ± 0.50 mg of vitamin-C content in 0 day and 30th day respectively. The results indicated that the sample F1 lost the vitamin-C content in descent manner during the storage period. This may be due to the effect of storage temperature. The vitamin-C encapsulated flavoured milk (F1) sample was good up to 30 days with the quantity of 92 ± 0.50 mg of vitamin-C. Hence it can be assumed that the quantity of vitamin-C may not be sufficient to deliver the daily requirement rate (RDA) after 30 days. The current RDA of 60 mg/d of vitamin-C is clearly far too low and the proposed new RDA of 200 mg/d while perhaps adequate for healthy age group people [13].

Results of the present study are similar to those of Desai et al. [14] who reported that the release rate of vitamin-C from immobilized particles and sodium alginate beads was sustained through an ion exchange process. A higher amount of stable vitamin C was recovered from the sodium alginate bead when compared to neat vitamin-C itself. Nakai et al. [15] and De Man et al. [16] stated that the use of microencapsulated forms for the delivery of vitamins to dairy and food products reduces loss of vitamins during storage. The method and stage of addition of microencapsulated ingredients into dairy and food products also affect the stability of vitamins during storage [17].

Conclusion

The study revealed the use of microencapsulation technologies for protection of health as achieved through high ingredient efficiency. The outcome of the study pointed out the possibility to stabilize the vitamin-C in flavoured milk by using microcapsules for even more than one-month storage at room temperature. The study also envisaged the need for replacing costly vitamin-C powder with alternate natural source and fortified in some other dairy products.

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