ENCAPSULATION OF VITAMIN C (ASCORBIC ACID AND AMLA PULP) TO DEVELOP VITAMIN C FORTIFIED ICE CREAM

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ABSTRACT
In this research study, two percentage of alginate as wall material and 0.1m cacl₂ as hardening bath were optimized for encapsulation (bead formation) through extrusion process. The encapsulation efficiency (ee) at 500mg of ascorbic acid was highest (74%) compared to 750 mg and 1000mg. Ee was highest (95%) at 40g for amla pulp. Encapsulated ascorbic acid beads had significantly higher the content of vitamin c with 354±0.5mg to 318±0.8mg per 100g of beads at both ambient and refrigeration stage of storage, followed by the amla pulp encapsulated beads which were hold the content of vitamin c with 306±0.5mg to 286±0.6mg / 100g of beads. Based on the result of sensory evaluation, 30% of eaai-t₁, 10% of ueaai-t₁, 30% of eafi- t₁ and 10% of ueafi- t₁ were selected by its good sensory appealing characteristics with highest score among the experimental samples. Throughout the storage period unencapsulated vitamin c formulated ice cream samples had a decreasing manner of vitamin c 45.5±0.5 to 1.8±0.8mg (ueaai-t₁) and was 27.6±0.5 to 1.9±0.5mg in ueafi-t₁. The stabilized vitamin c was found in eaai-t₁ with 88.25±0.5 to 80.6±0.7mg and 95.5±0.5 to 93±0.8mg in eafi-t₁.

Keywords: Amla Pulp, Ascorbic Acid, Encapsulation, Stabilization of Vitamin C, Ice Cream

INTRODUCTION
Ice cream is rich in carbohydrates, fats, proteins some vitamins (A, D & E) and mineral calcium (Cam et al., 2013). However, the Ice cream currently available commercially is generally poor in natural antioxidants like vitamin C and phenolics (Waterhouse et al., 2013). There is a definite consumer trend towards the purchase of improved products with strong inclination on health aspects. Blending of Ice cream with vitamin C will provide product diversification and benefits to the health conscious consumers which could not be provided by Ice cream alone. Increasing preference of consumers towards natural ingredients has tempted the Ice cream manufactures to search for new innovations in components having favorable health effects.

Scientific studies on the utilization of Amla in combination with other foods are scanty. Amla (Emblica Officinalis) is a gift of nature to mankind. Amla was used as natural source of vitamin C (ascorbic acid) having approximately 720 to 900mg per fruit which is economical and locally available. It has a sour and astringent taste because of its high acidity and astringency as well as high content of vitamin C. Amla is a seasonal fruit and thus, various preservation techniques have been developed such as freezing and drying. However, there are concerns that there may be changes for reduction or loss of vitamin C content during the drying process (Thankitsunthorn et al., 2009).

Astringent taste and discolouration during processing of amla can be prevented by encapsulation technology which simultaneously found to improve the stability of vitamin C; the activity of microencapsulated vitamins remains stable for longer period. Encapsulation of vitamins provides really a great deal of benefit at industrial level. Encapsulated products often present new challenges to food product developers.

Considering the above facts, an attempt has been made to prepare ice cream using encapsulated vitamin C with good sensory and nutritional properties. The main objective of this work was to optimize the level of amla pulp and ascorbic acid for encapsulation and to compare the developed ice cream with optimized level of amla pulp and ascorbic acid encapsulates and to evaluate the physiochemical quality and storage stability of vitamin C in the developed ice cream.
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: Standardized milk, skimmed milk powder, unsalted butter; sugar was purchased from the local departmental store at Chinnalapatti Town, Dindigul district, Tamilnadu. Vannila flavor, stabilizer and emulsifier purchased from Dindigul essence stores, Dindigul. The L-Ascorbic acid was purchased from HPLC Pvt Ltd, Mumbai and the sodium alginate (Molecular Biology Grade) was purchased from HiMedia Laboratories Pvt Ltd, Mumbai. Amla fruits (Emblica Officinalis) were obtained from Orchard of Faculty of Agriculture and Animal Husbandry, Gandhigram Rural Institute - Deemed University, Gandhigram, Dindigul District, Tamilnadu, India.

Methods

Method of Preparation of Microcapsules (Microencapsulation)
Microcapsules were prepared using extrusion technique. Both synthetic and natural source of vitamin C was added into the matrix of alginate solution separately. The aqueous solution of sodium alginate containing vitamin-C was delivered from burette at controlled flow rate (120ml/min) into 0.1M calcium chloride solution.

During this extrusion process sodium ions are bonded into calcium ions, due to this reaction added ascorbic acid and amla pulp gets automatically encapsulated which protect the vitamin C content. 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 beads were sieved and washed with deionized water separately. This method was as per the Karim et al., (2012) 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 can 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 (Chan et al., 2011).

Method of Preparation of Vitamin C Fortified Ice Cream
Accordingly, calculated quantity of standardized milk and butter was taken in open pan and heated at 65°C. The mix was then heated to 75°C, followed by addition of the skimmed milk powder, Sugar, Stabilizer and Emulsifier with constant stirring so as to dissolve the constituents completely, then pasteurized at 85°C for 30 minutes.

After cooling vitamin C encapsules were added separately except for the control. Aging was done at 5°C for overnight. After ageing the ice cream mix was frozen using a batch freezer. Ice cream was filled in 100ml food grade paper cups, covered with food grade lids and stored in deep freezer at -23°C to -18°C for hardening. Then the hardened ice cream samples were used for analysis. Detailed processing steps designed in the Flowchart-1 (Table 1a & 1b).

Sample Description
In the present study, encapsulated form of natural (amla paste) and synthetic (ascorbic acid) source vitamin C added ice cream was compared with unencapsulated and control ice cream. The samples were coded as, addition of encapsulated ascorbic acid (EAAI), addition of unencapsulated ascorbic acid as UEAAI, addition of encapsulated amla pulp as EAFI and addition of unencapsulated amla pulp as UEAFI.

Sensory Evaluation of Ice Cream
The nine point’s hedonic rating scale was used to measure the sensory evaluation of ice cream. All the samples of ice cream 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 Vitamin-C
Vitamin-C content was estimated by 2,6 di-chloro indophenols fibrimetric method as described in the procedure 967.20, AOAC manual, 16th Ed, Vol II, 1995.

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Experimental Design

The experimental process was conducted in a Completely Randomized Design (CRD) with different factorial combinations. All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Standardization of Wall Material (Alginate)

Standardization of alginate was done by the formation of beads. The standardization of alginate as coating material, in the present study was undertaken in the range of 0.5 to 2.5 g. Among these levels, the beads were found to be sufficiently hard and of good quality with 2g, which has been further utilized for carrying vitamin-C (Table-2). The results also found to corroborate with the findings of Sukumar (1991).

Optimization of Core Materials (Ascorbic Acid and Amla Pulp)

The optimization of ascorbic acid was done based on the EE. Alginate used as a coating material. For encapsulation, ascorbic acid was used at various levels from 100mg to 1000mg in 2 g alginate and 0.1M CaCl₂ bath.

The results revealed that the EE was high in 100mg ascorbic acid with the value of 78 percentage, whereas it was 71 percentage under 500mg of ascorbic acid. 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, which are in line with the findings of Lachance (2000). The optimization of amla pulp level was done based on the quality and formation of beads (encapsules). Amla pulp was optimized at various levels at 10g to 50g loaded in 2 g alginate and 0.1M CaCl₂ bath. In the amla pulp + alginate matrix was extruded for encapsulation in 0.1M of CaCl₂ solution. The best beads were assessed by its shape, surface, hardening time and encapsulation efficiency (EE). EE was highest (95%) at 40g.

The diameter of the beads ranged between 2 to 4mm. Based on the pay load, amla pulp (core content) 40g was selected for fortification of ice cream which has higher level of core content with 94.59 percentages at 5.40 percentages of wall content. Also it has the good quality of encapsulated beads. An upto 40 percentage of amla pulp was sufficient to encapsulate in 2 percentage alginate solution.

Encapsulation Efficiency

In the present study, ascorbic acid was encapsulated and retained 71 percentage of EE in total weight of 500mg ascorbic acid. Amla pulp (40g) having 306±0.5 mg of vitamin C which was achieved at the rate of EE was 96 percentages.

Since 10, 20 and 30 percentages of amla pulp encpapsules could deliver 306±0.5, 612.01±0.8 and 918.1±0.2mg of vitamin-C per 1000g of ice cream mix respectively. Amla pulp had the higher level of EE than the ascorbic acid during encapsulation (Table 3).

Pay Load

Pay load was expressed (Figure-1) to higher efficient encapsules of ascorbic acid (71% EE / 500mg) and amla pulp (96%EE/ 40g).

Both active ingredients are encapsulated at two percentages of sodium alginate. From the study, amla pulp was encapsulated by lowest level of wall content (5.40%) was observed when compared to ascorbic acid (80%). It may unable to arised the textural queries in ice cream (Figure -1).

Stability of Vitamin C in Encapsules

Two types of selected encapsules were then subjected to a 40 days stability test under the controlled storage conditions of ambient and refrigeration. During the storage, vitamin C retention was followed. The results were compared with same level of unencapsulated sources of vitamin C. Ascorbic acid encapsulated beads had significantly positive effect with 354±0.5 mg to 318±0.8 mg of vitamin C, followed by the amla pulp encapsulated beads which were hold 306±0.5 mg to 286±0.6 mg of vitamin C. Over the storage period, large quantity vitamin C losses were observed in all unencapsulated samples (Figure 2a & 2b).

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Table 1a: Standardization of ingredients for 1000g of ice cream mix with two types of ascorbic acid

<table>
<thead>
<tr>
<th>Ingredient(s)</th>
<th>Control (g)</th>
<th>Unencapsulated ascorbic acid added</th>
<th>Encapsulated ascorbic acid added</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UEAAI - T1</td>
<td>UEAAI - T2</td>
<td>UEAAI - T3</td>
</tr>
<tr>
<td>Milk</td>
<td>711.67</td>
<td>701.67</td>
<td>691.67</td>
</tr>
<tr>
<td>SMP</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Butter</td>
<td>89</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Sugar</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>S&amp;E</td>
<td>3.33</td>
<td>3.33</td>
<td>3.33</td>
</tr>
<tr>
<td>UEAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Flowchart 1: Preparation steps of encapsulated vitamin C fortified Ice cream
Table 1b: Standardization of ingredients for 1000g of ice cream mix with two types of amla fruit

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control (C)</th>
<th>Unencapsulated amla fruit added ice cream</th>
<th>Encapsulated amla fruit added ice cream</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UEAF -T1</td>
<td>UEAF -T2</td>
<td>UEAF -T3</td>
</tr>
<tr>
<td>Milk</td>
<td>711.67</td>
<td>611.67</td>
<td>511.67</td>
</tr>
<tr>
<td>SMP</td>
<td>46</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Butter</td>
<td>89</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>Sugar</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>S&amp;E</td>
<td>3.33</td>
<td>3.33</td>
<td>3.33</td>
</tr>
<tr>
<td>UEAF</td>
<td>-</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>EAF</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Table 2: Optimization of wall material and beads forming bath for encapsulation

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>T1 (0.1M)</th>
<th>Presence of beads</th>
<th>Shape of beads (Surface)</th>
<th>Hardening Time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>W1L1H1T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>W1L1H2T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>W1L2H1T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>W1L2H2T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>W1L3H1T1</td>
<td>Positive</td>
<td>Mucus</td>
<td>10±3</td>
</tr>
<tr>
<td></td>
<td>W1L3H2T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>4.</td>
<td>W1L4H1T1</td>
<td>Positive</td>
<td>Mucus</td>
<td>20±2</td>
</tr>
<tr>
<td></td>
<td>W1L4H2T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>W1L5H1T1</td>
<td>Positive</td>
<td>Spherical</td>
<td>25±3</td>
</tr>
<tr>
<td></td>
<td>W1L5H2T1</td>
<td>Negative</td>
<td>Nil</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2 (0.2M)</th>
<th>Presence of beads</th>
<th>Shape of beads (Surface)</th>
<th>Hardening Time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>W1L1H1T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>W1L1H2T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td>7.</td>
<td>W1L2H1T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>W1L2H2T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td>8.</td>
<td>W1L3H1T2</td>
<td>Positive</td>
<td>Mucus</td>
</tr>
<tr>
<td></td>
<td>W1L3H2T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td>9.</td>
<td>W1L4H1T2</td>
<td>Positive</td>
<td>Spherical</td>
</tr>
<tr>
<td></td>
<td>W1L4H2T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
<tr>
<td>10.</td>
<td>W1L5H1T2</td>
<td>Positive</td>
<td>Spherical</td>
</tr>
<tr>
<td></td>
<td>W1L5H2T2</td>
<td>Negative</td>
<td>Nil</td>
</tr>
</tbody>
</table>

W1- Alginate;  
L1- 0.5g; L2- 1g; L3- 1.5g; L4- 2g; L5- 2.5g  
H1- Calcium chloride; H2- Sodium Chloride  
T1 – 0.1M; T2-0.2M
Table 3: Encapsulation Efficiency Profile for vitamin C encapsules

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Ascorbic acid Encapsules</th>
<th>Vitamin C (mg) Matrix</th>
<th>Encapsule</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>P1C1B</td>
<td>78±1.8</td>
<td>355±0.5</td>
<td>71</td>
</tr>
<tr>
<td>2.</td>
<td>P1C2B</td>
<td>189±0.6</td>
<td>510±0.8</td>
<td>68</td>
</tr>
<tr>
<td>3.</td>
<td>P1C3B</td>
<td>355±0.5</td>
<td>652±1.5</td>
<td>65.2</td>
</tr>
<tr>
<td>4.</td>
<td>P1C4B</td>
<td>510±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>P1C5B</td>
<td>652±1.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Amla pulp Encapsules</th>
<th>Vitamin C (mg) Matrix</th>
<th>Encapsule</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>P2E1B</td>
<td>78±2.5</td>
<td>306.6±0.5</td>
<td>95.8</td>
</tr>
<tr>
<td>7.</td>
<td>P2E2B</td>
<td>151±1.8</td>
<td>95.2</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>P2E3B</td>
<td>228±1.5</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>P2E4B</td>
<td>306.6±0.5</td>
<td>95.8</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>P2E5B</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

P1- L-Ascorbic acid; P2- Amla Fruit
C1- 100mg; C2- 250mg; C3- 500mg; C4- 750mg; C5- 1000mg
E1- 10g; E2- 20g; E3- 30g; E4- 40g; E5- 50g
B- 2g Sodium alginate

Figure 1: Payload of encapsules

Figure 2a: Stability of vitamin C in encapsulated and unencapsulated ascorbic acid at different storage conditions
**Research Article**

Figure 2b: Stability of vitamin C in encapsulated and unencapsulated *amla* pulp at different storage conditions

Figure 3a: Sensory evaluation of encapsulated and unencapsulated ascorbic acid added Ice cream

Figure 3b: Sensory evaluation of encapsulated and unencapsulated *amla* pulp added Ice cream
Sensory Evaluation

The overall acceptability of the ice cream was analyzed by sensory evaluation test using nine point’s hedonic rating scale. Based on the result, 30% of EAAI-T3, 10% UEAAI-T1, 30% EAFI-T3 and 10% of UEAFI-T1 was selected by its good sensory appealing with highest score among the other samples. Recommended ice cream samples by sensory evaluation subjected to the further analysis (Figure 3a & 3b).

Stability of Vitamin C in Ice Cream

The stability of vitamin-C content of different encapsules added Icecream sample was assessed during refrigerated storage periods and the results presented in the Figure-4. Vitamin C was absent in control. The result revealed that the stability of vitamin C was assessed once in 5 days interval of 45 days storage period. Throughout the storage period unencapsulated vitamin C formulated ice cream samples has a decrease manner of vitamin C been showed in 45.5±0.5 to 1.8±0.8mg (UEAAI-T1) and was 27.6±0.5 to 1.9±0.5mg in UEAFI-T1. This may be due to the effect of processing and storage temperature. The stabilized vitamin C was found in EAAI-T3 with 88.25±0.5 to 80.6±0.7mg and 95.5±0.5 to 93±0.8mg in EAFI-T3. Nakai et al., (1983) and De Man et al., (1986) stated that the use of microencapsulated forms for the delivery of vitamins to 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.

Conclusion

It is feasible to incorporate vitamin C from natural and synthetic form into dairy product (Ice cream) using the encapsulation technology platform developed in this study. The applicability of the technology has been demonstrated in typical frozen dessert Ice cream. Extrusion and internal gelation of Ca and alginate promises found to be suitable technology for protecting vitamin C. Ice cream fortified with Ascorbic acid using the encapsulated form by extrusion based agglomerated process had the desired sensory properties and was stable for up to 45 days. A higher amount of stable vitamin C was recovered from the alginate bead when compared to unencapsulated vitamin C. Throughout the study conclude that employing naturally occurring vitamin C from amla pulp to encapsulation technique adopted ice cream found superior compared to other experimental ice cream samples, which has good barrier to vitamin C and stabilization of vitamin C as well as the economy along with good taste, which could preserve vitamin C and deliver them to the body in the form that is organoleptically acceptable, and is essentially transfer to the consumer.
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REFERENCE