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Hydrogels for potential food application: Effect of sodium alginate and calcium chloride on physical and morphological properties

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Abstract
The purpose of present work was to study the effect of hydrocolloids and divalent ions on physical and morphological properties of beads (Imitated fruit pieces). Dropping method was followed to prepare alginate beads, involving cross linking of sodium alginate in calcium chloride solution. The effect of different concentration of alginate and divalent ion on the particle size, drying rate, color, gelling time and surface characteristics (SEM) was observed. The size and surface characteristics of the beads were significantly affected depending upon the sodium alginate and calcium chloride concentration. The beads with best characteristics were selected on the basis of physical and sensory evaluation. Based on the results the 2 % (w/v) sodium alginate and 3 % (w/v) calcium chloride concentration was used to prepare sugar loaded alginate beads. The sugar loaded beads were assessed for storage stability and found to be acceptable up to 30 days at ambient temperature. These imitated fruit can be used in various food products like dairy and bakery products or as topping in food products.

Keywords: Sodium alginate, Scanning Electron Microscopy (SEM), calcium chloride, alginate beads

1. Introduction

Gelled fruit products mainly jam and jellies are easily accessible in the market. Novel products like cherries, texturized fruits, bars, fabricated fruits pieces and desserts possessing desired texture along with functional and sensory characteristics, can be formulated by combination of polysaccharides and sugars with or without the addition of fruits [4, 15]. Several water-insoluble polymeric materials based encapsulation techniques required organic solvents to solubilize the polymers. However, high cost and safety issues related with these organic solvents make the requirement for organic solvent-free systems. Researchers focused on the development of technology for the use of polymeric carrier using various encapsulation techniques such as physical, chemical and mechanical depending on the encapsulating compounds and their applications in different systems [8].

Hydrocolloids are macromolcular hydrophilic substances and consist of heterogenous groups mainly polysaccharides and proteins. These are characterized by their property of forming colloidal solution or gels after dispersion in water [21]. However, some of the hydrocolloids are only to causes thickening of the media by swelling in water [7]. Because of the occurrence of large numbers of hydroxyl groups on them, increases their affinity for water. Hydrocolloids impart wide range of functional properties such as consistency, viscosity, thickness, coating, texture and binding of substances. Firstly, these are found from trees extrudates, plants or microorganisms [30]. Due to their hydrophilic properties they are also known as 'hydrophilic colloids' or 'hydrocolloids'. Different properties of food are influenced by different hydrocolloids. Depending upon the properties of hydrocolloids these provides food applications as in bakery products and in ice creams etc. Hydrocolloids are added in food products mainly because of two reasons- Firstly, to modify flow behavior and secondly to impart texture. Hydrocolloids provide stability to the food products and increase the overall acceptability of the food [12].

The applications of alginate in an encapsulation technique are a great interest nowadays because of their properties.Sodium alginate is a linear unbranced polysaccharide which consist of 1,4 linked β-D- mannuronic and 1,4 linked α-L-guluronic acid residue [26]. Firstly, Alginate was described Stanford in 1881. These residues are connected as homo or hetero polymers or alternatively. Alginates are produced by brown seaweeds mainly Laminaria [30].

Commercially alginate is extracted from seaweed, including giant *kelp Macrocystis pyrifera,* *Ascophyllum nodosum and Laminaria hyperborean.* Sodium alginate is natural, biodegrable, nontoxic polymer that can be used for entrapment of drugs and in food applications to form gels, to increase viscosity etc. It forms stable gel by forming cross links with divalent cations such as calcium and barium etc. [24].

Sodium alginate performs various functional properties such as it acts as a thickener, gelling agent, binder and stabilizer in food industries. It is used for encapsulation of volatile agents for drug delivery or flavor entrapment, due to its property of forming ionotropic gelation with calcium ion under mild conditions [13]. The binding of Ca^{2+} by polyguluronate proceeds until all the available binding sites becomes saturated and there is an increase in the concentration of Ca^{2+} ions [6]. Gelation of liquid alginate solutions occurs upon contact with polycation (Ca^{2+}), by binding and forming tight junctions between Ca^{2+} and guluronic acid blocks (G) in alginate [28]. This cross-linking between calcium ions and alginates to form gel is known as "the egg-box model" [16]. In this egg-box model, polymers of G G block comes close to each other after binding of the first cations, which enables the efficient and faster binding of the other molecules, an auto cooperative process [1]. As the concentration of alginate increases in formulation, number of the apparent cross-linking points also increases [13]. Calcium is the main divalent cation widely utilized because it is considered as clinically safe, easily accessible and economical [18].

The potential of the alginate to cause gelation upon contact with multivalent ions has been employed for the preparation of beads [26]. However, formation of the beads with a desired size and shape often needs trial and modifications of the properties of liquid (polymer) and cross linking agents. The main objective of the work is to identify the possibility of alginate to form cherry like product and to examine properties of the resultant product before and after formation along with their use in different products.

2. Material and Methods

2.1. Raw material

2.1.1 Chemicals

The Sodium alginate possessing medium viscosity ($C_6H_7O_6Na$, Molecular weight 85000) and Calcium chloride dehydrate AR grade used in this work was supplied by S D Fine Chem Limited (SDFCL), Mumbai, Maharashtra, India,. Honey, sugar and glucose were procured from the local market. Flavour (Orange-4378B) and colour (orange red) were procured from International Flavours and Fragrances India Private Limited (IFF), Chennai, India and Ajanta Food Products Company, Solan, India, respectively.

2.2 Method of preparation

2.2.1 Preparation of alginate beads

The different concentration of alginate and calcium chloride solution was taken in order to examine the effect of selected parameters on the size and shape of the beads. Different concentrations of sodium alginate solution from 1.0, 1.5, 2.0, 2.5 and 3.0 % (w/v) and calcium chloride solution as a gelling bath from 2, 3 and 4% (w/v) were used for the preparation of alginate beads. A drop wise addition of sodium alginate solution of different concentration (1.0, 1.5, 2.0, 2.5 and 3.0 %, w/v) into the solution of calcium chloride (2, 3 and 4 %, w/v) using syringe was done followed by stirring for specified period of time (15- 20 mins) and then separated by filtration. The alginate beads were washed with distilled water and dried on a filter paper [13]. The detailed formulation for the preparation of alginate beads used is mentioned in Table 1.

2.2.2 Preparation of sugar loaded beads

Best level of calcium chloride that is 3 percent was selected from the preparation of alginate beads to prepare sugar loaded beads. Sugar loaded beads were prepared by incorporation of mixture of Sucrose (40%), Glucose (40%) and Honey (20%) into sodium alginate (1.5%, 2.0%, 2.5% and 3.0%, w/v) solution. Colour (Orange- red) and flavour (Orange) were added to the resulting dispersion and then dropped through syringe into 3 percent w/v aqueous calcium chloride solution followed by stirring at 100 rpm. After stirring for required time, the formed beads were separated by filtration, washed with distilled water and dried at ambient temperature [14].

2.3. Evaluation of alginate beads

2.3.1 Gelling time of alginate beads

Gelling time is the time required by the alginate beads to form gel. The beads were prepared by dropping the dispersion into calcium chloride solution, allowed to harden for specified time and air-dried, in order to examine the time taken to form gel (1, 3, 5, 10 and 20 min) [26].

2.3.2 Measurement of alginate bead size

The alginate beads were spread over the flat surface using spatula. The diameter of the ten beads were measured by using calibrated scale and the diameter of one beads was calculated (cm) by dividing diameter of beads to total number of beads [25]. The size of beads was calculated by using the following equation 1:

$$Diameter\ of\ one\ bead\ (cm) = \frac{Diameter\ of\ 10\ beads}{Total\ number\ of\ beads} \longleftarrow \text{Equation 1}$$

2.3.3 Swelling index of alginate beads

The known number of pre weighed beads was placed in petri plate containing phosphate buffer (7.4) for 15 min, removed and blotted with filter paper. The swelling index was calculated by measuring the change in weight of beads after 15 min. [14]. Swelling index was calculated as given in equation 2:

$$Swelling\ Index\ (SI) = [\{Wt - W0\}/W0] \longleftarrow \text{Equation 2}$$

Where, Wt= Mass of swollen beads at time t
W0= Mass of dry beads at t=0

2.3.4 Drying behaviour of alginate beads

Prepared alginate beads were placed in pre weighed petri plates and kept in hot air oven at 50 ºC. The beads were removed at short interval of time (10-50 mins up to constant weight).These measurements were continued until attainment of constant mass and note down the temperature and time for the complete drying of beads [25].

2.3.5 Morphology and size determination of alginate and sugar loaded alginate beads

Shape and size of alginate and sugar loaded alginate beads prepared with different concentration of polymer in 3% (w/v) calcium chloride were evaluated by using SEM (Scanning Electron Microscopy) at 15KV. Prior to estimation the samples were placed on stub and coated with carbon. Sputtering is done under vacuum to render them electrically conductive.

2.3.6 Sensory quality evaluation

To assess the stability and strength of the sugar loaded beads, the product was examined at ambient temperature for 45 days at the interval of 7 days and evaluated for overall acceptability in terms of appearance, size and texture using semi-trained panel members on 9-point hedonic scale [11].

2.3.7 Statistical analysis

Data collected from aforesaid experiments was subjected to statistical analysis with the help of factorial completely randomized design. The critical difference (CD value) was used as the test of significance [5].

3. Result and Discussion

3.1 Effect of sodium alginate and calcium chloride concentration on the gelling time of alginate beads

The effect of alginate and divalent ion concentration on gelling time of beads is codifying in the Table 2. The empty alginate beads showed increase in gelling time as the concentration of polymer increased from 1.0 to 2.0 percent followed by decline (after 2.0%). Gelation occurs as the formation of three dimensional structures due to interchange ionic bonding between divalent ions and G blocks of polymer chain. The divalent ions stabilizes the three dimensional structure by cross linking [19]. The gelation occurs mainly due to the exchange of sodium ions from the guluronic acids of polymer with the divalent cations (Calcium ions) was reported [22].

The gelation rate increases as the presence of more substrate bind with calcium ions. However, high concentration of polymer leads to slow rearrangement of polymeric chains to form cross links with calcium [10]. It was also reported that an increase in viscosity causes hindrance and friction between the polymer chain and cause less gelation time.

3.2 Effect of sodium alginate and calcium chloride concentration on the alginate bead size

Table 3 displayed the average size of the alginate beads is within the range of 0.36 to 0.58 cm. The beads size was increased from 0.41 to 0.58 cm, 0.37 to 0.56 cm and 0.36 to 0.53 cm as concentration of sodium alginate increases from 1-3% (w/v) in 2%, 3% and 4% (w/v) calcium chloride solution, respectively. The results revealed that the increase in the concentration of sodium alginate increase the size of the beads based on the fact that sodium alginate binds more calcium chloride by cross linking. These observations are in accordance with the research study which described that higher viscosity resulted from increase in the alginate concentration causes development of larger microspheres and greater drug entrapment due to high degree of crosslinking [2]. The high polymer concentration makes viscous dispersion and form large droplets which lead to large alginate beads [27]. However, increase in concentration of calcium chloride results in bead size reduction. The data presented in the Table 3 showed that the size of the beads prepared from 1%, 1.5%, 2%, 2.5% and 3.0% (w/v) sodium alginate in 2%, 3% and 4% (w/v) calcium chloride solution was decreased from 0.41 to 0.36 cm, 0.47 to 0.42 cm, 0.50 to 0.48 cm, and 0.55 to 0.51cm, 0.58 to 0.53 cm, respectively. This can be explained by the fact that calcium (divalent ions) penetrates into the inner space of droplets which causes expulsion of water molecules and results in the formation of compact beads. This can be described by the fact that an increase in the cross linking is mainly attributed to sodium alginate concentration [13, 20].

3.3 Effect of sodium alginate and calcium chloride concentration on the swelling index of alginate beads

Table 4 illustrated the effect of concentration of sodium alginate and calcium ions on swelling index. The results presents that the beads prepared from 1-1.5% (w/v) alginate in 2 and 3 % (w/v) solution of calcium chloride, burst out and as the proportion of polymer and calcium divalent ions increased, the swelling ratio of beads increased as shown in the Table 4. Presence of hydrophilic groups on the polymer chain interacts with the water molecule and hydrates the chain which increases the swelling rate. The inert pores present in the polymeric chains filled by water, which contributes to a greater degree of swelling [22]. The swelling of beads resulted from Ca^{2+} and Na^{+} ions exchange between polymer chain and phosphate buffer, respectively. In the beginning, electrostatic repulsion increases between negatively charged COO^{-} groups as Ca^{+} present with the COO^{-} groups of mannuronic blocks exchange with Na^{2+} ions present in phosphate buffer which results in swelling. The formation of insoluble calcium phosphate precipitates causes the turbidity of solution (Phosphate buffer). This diffusion of Ca^{2+} (bound to polyguluronate blocks) in the phosphate buffer empowers the intake of swelling media into the beads due to the structure loosening [9]. An increase in amount of polymer proportionally increases the Ca content in beads [17]. The presence of salt such as K, Na etc disrupts the dimer–dimer associations and cause swelling of gel beads [31]. The increase in the calcium chloride concentration firstly increases followed by decline in swelling index. This can be explained by the fact that at low, medium and high concentration of calcium chloride solution loose, more structured and rigid gel is formed, respectively. The strong gel formed at high concentration of calcium chloride results in slow penetration of dissolution medium into the matix [32].

3.4 Effect of sodium alginate and calcium chloride concentration on drying behaviour of alginate beads

Fig 1 displays the data for drying rate of alginate beads. As the drying progresses, the weight of the formulated beads was decreased. Drying significantly influences the size and shape of alginate beads. Surface of empty beads become smooth and spherical. Drying significantly change the shape of beads as it causes shrinkage of beads due removal of water [26]. Drying induces the change in the egg box model from egg box dimers to egg box multimers because they grow into larger size and consist of more than two polymeric chains. Drying also increase the calcium concentration of the beads which promotes the association of egg boxes [31]. The water loss during drying results in a variation in structure composed of small micronuclei due to the decrease in distance between the polymeric chains and also leads to the change in the dimensions and surface characteristics calcium Alginate-chitosan beads containing celecoxib as it become rough and wrinkled [23].

3.5 SEM analysis of alginate beads and sugar loaded alginate beads

According to the observed characteristics of alginate beads, beads with best characters were selected for the preparation of sugar loaded beads using 1.5-3% (w/v) sodium alginate in 3% (w/v) calcium chloride solution. Scanning Electron Microscopy (SEM) analysis of beads was done to examine size and surface morphology characterization (Fig 1). The results revealed that the particle size of empty beads showed more difference than loaded samples as their size significantly decreases to greater extent after air drying. This can be concluded that the presence of loading mixture (sugar-honey and glucose) inside the beads causes the binding of water molecule and does not allow the release of water molecules. Fig 2 illustrate the size of empty and loaded beads as 0.78mm (A), 1.10 mm (B), 1.44mm (C) and 1.53 mm (D) and 2.39mm (E), 2.58mm(F), 2.77mm(G), 2.93mm (H) respectively. These results exhibited that the size of sugar loaded beads was approximately double than the alginate beads. The shape of microbeads was found to be more spherical than drug and solid dispersion loaded beads as loading mixture lead to formation of rough and irregular surface which can results in an increase in surface area [3].

The surface of alginate beads was found smoother and harder than loaded beads. The surface morphology of the selected beads is presented in Fig 3. The alginate beads were found to be more spherical. The surface of the loaded beads becomes crystalline as due to the presence of loading mixture. The presence of sugar and polysaccharides together results in the formation of glassy structure [4].

3.6 Sensory quality evaluation of sugar loaded alginate beads

The sugar loaded beads prepared by using sodium alginate at different concentration (1.5- 3.0%, w/v) into 3% (w/v) calcium chloride solution were examined for stability and strength for 30 days under environmental conditions (Table 5). The high overall acceptability was found to in beads prepared with sodium alginate (2%), Sucrose (20%), Glucose (20%) and honey (15%) along with color and flavor in 3 % (w/v) calcium chloride**.** However, empty beads become harder due to the evaporation of water. Sucrose incorporation affects the strength and water holding capacity of gels. As the strength of mixed gels decreases and alginate gels increases. In case of whey protein gels, sucrose inclusion increased the water holding capacity but reduced the strength [29]. These beads can be used as fruit like substitute as topping on ice creams or bakery products (Fig. 4).

Table 1: Effect of sodium alginate and calcium chloride concentration on the gelling time, bead size of alginate beads

Treatments	Sodium Alginate (%, w/v)	Calcium Chloride (%, w/v)	Gelling Time (min)	Bead Size (cm)	Swelling Index
1	1.0	2	11:00	0.41	Burst
2	1.5	2	11:05	0.47	0.095
3	2.0	2	11:55	0.50	0.108
4	2.5	2	11:33	0.55	0.119
5	3.0	2	11:20	0.58	0.136
6	1.0	3	09:00	0.37	Burst
7	1.5	3	10:15	0.45	0.102
8	2.0	3	11:40	0.51	0.136
9	2.5	3	11:16	0.54	0.158
10	3.0	3	11:02	0.56	0.193
11	1.0	4	08:50	0.36	Burst
12	1.5	4	09:40	0.42	0.089
13	2.0	4	11:15	0.48	0.098
14	2.5	4	10:40	0.51	0.133
15	3.0	4	10:30	0.53	0.180
CD (5%)	Sodium Alginate (A)		11.1	0.13	0.017
	Calcium Chloride (B)		14.4	0.17	0.022
	A x B		25.0	NS	0.039

Table 2: Sensory quality of sugar loaded alginate beads

Sodium Alginate (%, w/v) Time (days)	1.5	2.0	2.5	3.0
7	6.0	8.5	7.0	7.0
14	5.5	8.0	6.0	6.5
21	5.0	7.5	6.5	6.5
28	5.0	7.5	6.0	6.0

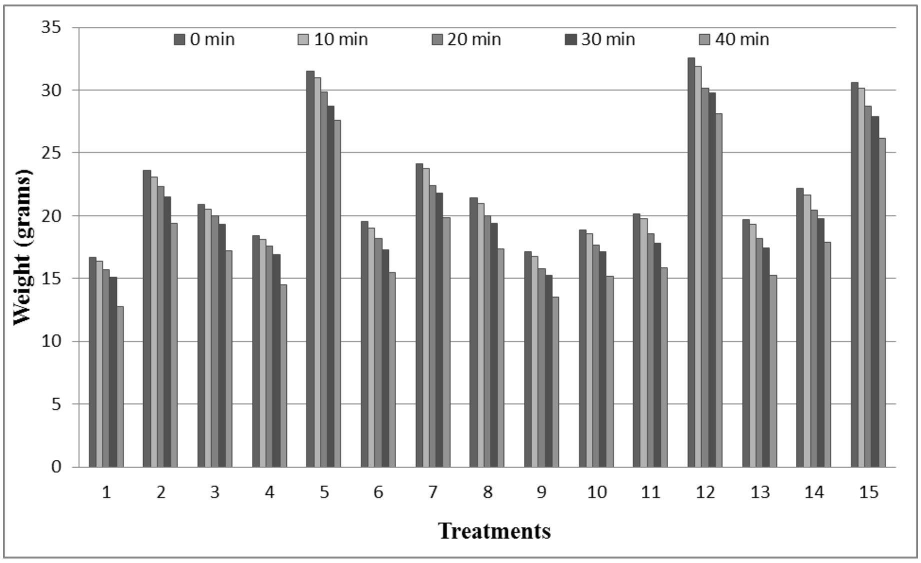


Fig 1: Effects of sodium alginate and calcium chloride concentration on the drying behaviour of alginate beads

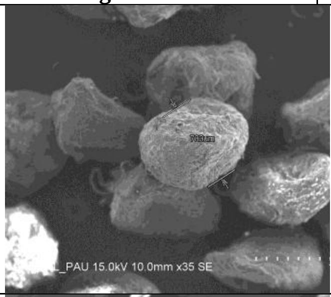
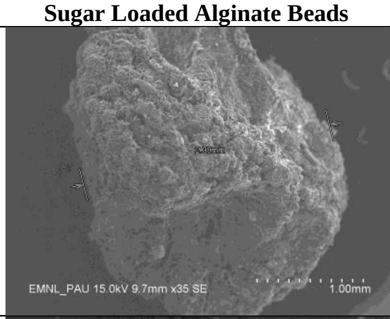
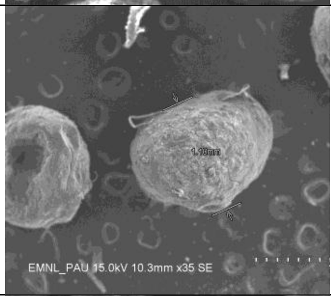
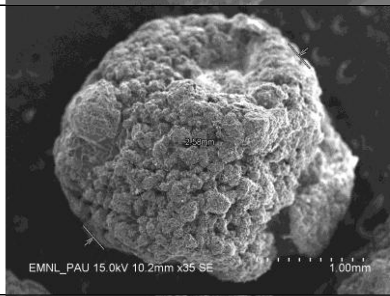
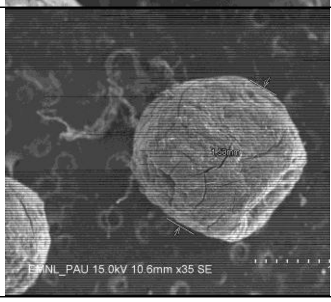
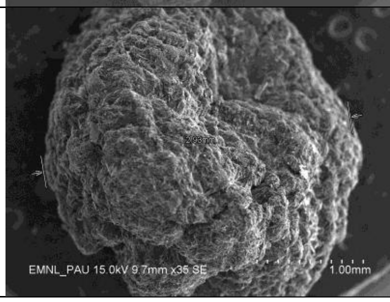
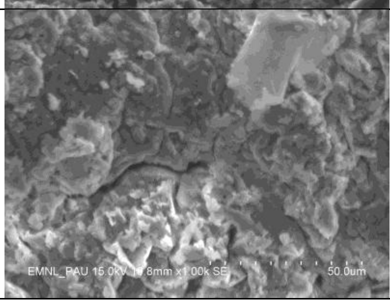
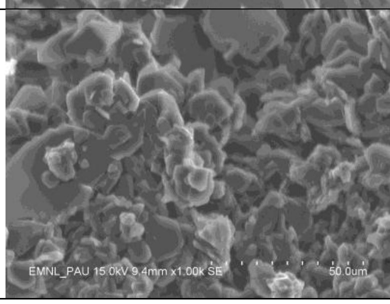
	Sodium Alginate (%)	Alginate Beads		Sugar Loaded Alginate Beads
A	1.5%		E	
B	2.0%		F	
C	2.5%		G	
D	3.0%		H	

Fig 2: SEM analysis of alginate beads (A-D) and sugar loaded alginate beads (E-H):- Effect of loading mixture on size of beads prepared in 3% (w/v) calcium chloride solution

	Sodium Alginate (%, W/V)	Alginate Beads		Sugar Loaded Alginate Beads
1	1.5%		5	
2	2.0 %		6	

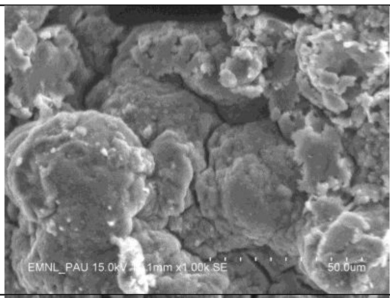
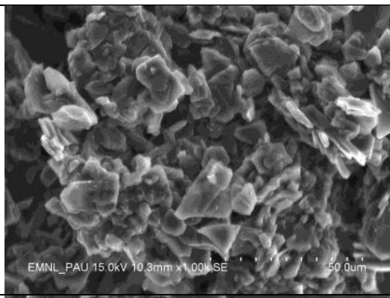
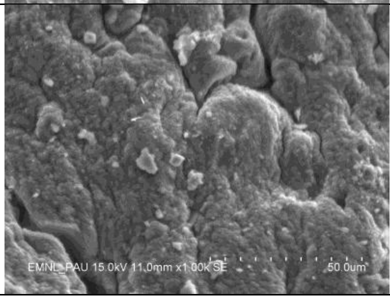
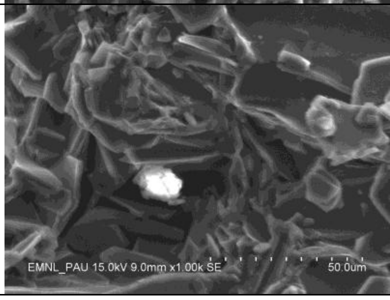
3	2.5%		7		
4	3.0%		8		

Fig 3: SEM analysis of Alginate beads (1-4) and Sugar loaded alginate beads (5-8):- Effect of loading mixture on surface morphology of beads prepared in 3% (w/v) calcium chloride solution

Fig 4: Cherry like product added on ice cream

4. Conclusion

This study is mainly concerned on the applicability of sodium alginate for preparation of imitated fruit product. The alginate beads were prepared by drop method. The results revealed that with an increase in concentration of the sodium alginate, an increase in particle size and swelling rate of alginate beads was observed. The gelling time firstly increased followed by decline after 2 percent. It was observed that alginate beads prepared from 2 % (w/v) sodium alginate and 3% (w/v) calcium chloride possessed best characteristics. Best sugar loaded alginate were prepared from sodium alginate (2%), Sucrose (20%), Glucose (20%), Honey (15%) in 3 % (w/v) calcium chloride solution.

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