Optimizing microencapsulation of α -tocopherol with pectin and sodium alginate

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Abstract α -Tocopherol is a well-known fat-soluble antioxidant and is widely used in the food industry for stabilizing free radicals. Incorporation and stability of it into food is another challenge as directly added α -tocopherol is prone to inactivation by food constituents. This study was aimed at optimizing conditions for encapsulation of α -tocopherol using combination of sodium alginate (0.5, 1.0, 1.5 and 2.0%) as primary wall material and pectin (2.0%) as filler. The optimum conditions were selected on the basis of encapsulation efficiency, shape, size, bulk density, yield and swelling index with syringe method. The encapsulation efficiency of α -tocopherol in microencapsules produced under optimal conditions was 52.91% using sodium alginate 1.5% w/v and pectin 2.0% w/v. α -Tocopherol was encapsulated with encapsulator using standard conditions and was compared with syringe method. The encapsulation efficiency was found more (55.97%) in microencapsules prepared with encapsulator and 52.11% in microencapsules prepared with syringe.

Keywords α -Tocopherol · Pectin · Sodium alginate · Microencapsulation · Antioxidant

Introduction

α -Tocopherol is accepted as the major lipid-soluble antioxidant and is widely used in the food industry (Cervantes and Ulatowski 2017). It is called antioxidant because of its ability of quenching or stabilizing free radicals that lead with time to degenerative diseases, including cancer and cardiovascular disease (Yoo et al. 2006). Vitamin E can be degraded rapidly in the presence of oxygen and free-radical mediated oxidative processes. These obstructions of vitamin E can be partially overcome by applying microencapsulation technology to protect α -tocopherol from unfavourable environment and to solubilise it in aqueous environment. Encapsulation is a process by which small droplets or particles of solid or liquid core material are coated with a continuous film of polymeric material. Encapsulation improves the performance of biologically active substances and enhances its shelf life. It offers a means to convert liquid to solids, to change colloidal and surface properties, to provide environmental protection and to control the release characteristics or availability of core materials. Exclusivity of microencapsulation is the compactness of the coated particles and their subsequent use and adaptation to a wide variety of dose forms (Bansode et al. 2010). Microencapsulation is a process in which sensitive ingredients or ‘core’ materials are entrapped in a protective polymer encapsulating agent or wall material (Hogan et al. 2001). It is a process of building a functional barrier between core and wall material to prevent any chemical or physical reactions (Yusop et al. 2017). According to the US Food and Drug Administration (FDA), antioxidants are defined as “substances used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation. Antioxidants have become an indispensable group of food additives mainly because of

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their unique properties of enhancing the shelf life of food products without any damage to sensory or nutritional qualities. They include tocopherols and derived compounds in baked, fried products and vegetable oils (Nanditha and Prabhasankar 2008). Encapsulation of vitamin E has also been reported to improve its physicochemical stability during storage, in addition to maintain its biological activity after consumption. Vitamin E microcapsules were prepared using spray drying, freeze-drying and spray freeze-drying with whey protein isolate as encapsulating agent. The spray freeze-drying technique was found to be best to enhance oral bioavailability of vitamin E (Parthasarathi and Anandharamakrishnan 2016).

Pectin is a plant based polysaccharide having strong film forming and binding abilities which make pectin an ideal wall material for encapsulation applications (Liu et al. 2007). Pectin is not readily digested by human digestive enzymes and therefore is used to increase transit time or to obtain site specific delivery of bioactive compounds (Wong et al. 2011). Pectin with a degree of esterification (DE) > 50% is known as high methoxyl (HM) pectin. HM pectin gels are stable at acidic pH < 4.0 but dissolved at pH 7.0 or above. HM pectin is often used in pH dependent delivery systems, due to its pH-sensitive behavior (Liu et al. 2003). Pectin has the property for microencapsulation by spray drying, especially forming stable emulsions at low concentrations, which is critical for encapsulation of hydrophobic ingredients. Due to relatively low cost, pectin can be used as a substitute for expensive wall materials, such as proteins and gum Arabic (Drusch 2007). The gelling ability of pectin makes it an ideal agent in making biodegradable hydrogel beads, films and coatings for microencapsulation purposes (Humblet-Hua et al. 2011).

Alginate is perhaps the most widely used material for bio encapsulation (Chan et al. 2011). Alginate is a linear polysaccharide extracted from brown seaweed. It is composed of variable proportions of β -D mannuronic acid and α -L-guluronic acid linked by 1–4 glycosidic bonds (Fu et al. 2011). Sodium alginate is a polyelectrolyte with negative charges on its backbone (Zhong et al. 2011). Alginate forms a thermally stable and biocompatible hydrogel in the presence of di- or tri-cations. Alginate beads can be easily produced when dropping in a calcium chloride solution. Sodium Alginate has been used in many encapsulation applications, including various fields such as food industry, feed, pharmaceutical, biomedical and bioprocess. Alginate is useful as a matrix for immobilization of microbial cells, animal, plant as well as entrapment of bioactive compounds and drugs (Goh et al. 2012). Present study was aimed at optimising the process parameters of encapsulation of α -tocopherol to achieve maximum encapsulation efficiency in calcium alginate as wall material and pectin as filler. The focus was on development of

oil-in-water emulsions suitable for incorporating vitamin E in food products as antioxidant.

Materials and methods

Materials

Vitamin E (α -tocopherol) was procured from the Sigma Aldrich, Bangalore. Tween 80 (emulsifier), Calcium chloride, Pectin (DE > 50) and Sodium alginate were procured from SD Fine Chemicals, India.

Formulation of pectin/pectin–alginate microencapsules using ionotropic gelation technique

Microspheres containing α -tocopherol were prepared using pectin alone and pectin sodium alginate (PSA) together in different ratios as depicted in Table 1. The formulation process consisted of preparing a 2.0% w/v solution of pectin in distilled water containing 1.0% v/v of glycerol as dispersing aid. Solution of sodium alginate in distilled water was prepared depending upon the formulation to be made. The two solutions were then mixed under continuous agitation to form a homogenous solution of the two polymers. 0.1% w/v of Tween 80 (emulsifier) and 1.0% w/v α -tocopherol was then added drop wise to the above mixture to obtain Mix II. The homogenous mixture was subjected to further homogenization for a period of 30 min on portable electric lab scale homogenizer to produce o/w emulsion at 3000 rpm. The stable emulsion was then dropped with (0.6 mm $\times$ 25 mm) 25 G needle into a 5% w/v calcium chloride solution. The microcapsules were then thoroughly washed with ice cold water and air dried for 24 h at room temperature.

Table 1 Composition of pectin/pectin–sodium alginate microencapsules

Formulation code	P	PSA1	PSA2	PSA3	PSA4
<i>Composition</i>					
α -Tocopherol (%)	1.0	1.0	1.0	1.0	1.0
Tween 80 (%)	0.1	0.1	0.1	0.1	0.1
Pectin (g)	2.0	2.0	2.0	2.0	2.0
Sodium alginate (g)	–	0.5	1.0	1.5	2.0
Glycerol (%)	1.0	1.0	1.0	1.0	1.0
Distilled water (ml)	100	100	100	100	100

P pectin, PSA pectin sodium alginate

Encapsulation by encapsulator

Optimization of encapsulation was done with syringe. Selected formulation was followed for preparation of microencapsules by encapsulator. Microcapsules were prepared using air atomization technique with an in house developed encapsulator (Narsaiah et al. 2014) in the laboratory of Agricultural Structures and Environmental Control, CIPHET, Ludhiana.

Characterization of microencapsules

The prepared microencapsules were characterized for the following parameters:

Particle size, shape and surface

The particle size of microencapsules was evaluated under optical microscopy and to determine the average particle size, the size of minimum 20 particles was measured using vernier caliper. The shape and surface morphology of microencapsules was evaluated using Scanning Electron microscopy (SEM) analysis in Nano Technology Lab PAU, Ludhiana. The samples for SEM were obtained by lightly sprinkling the microencapsules on a double adhesive tape, which was stuck on to an aluminum stub. The stubs were then coated with gold to a thickness of 300 Å using a sputter coater and then viewed under SEM. SEM Photomicrographs of suitable magnification were obtained.

Total vitamin E content

HPLC was used to determine the content of vitamin E in microencapsules. The Chromatographic conditions needed to be maintained were as follows: Mobile phase: Methanol, Column: stainless steel, length 25 cm, diameter 4 mm, stationary phase: ODS, mobile phase: methyl alcohol, Flow rate: 1.0 ml/min, Temperature: Ambient, Detector: UV (Shimadzu SDP- 6AV) at 294 nm, Inj. Vol.: 100 µl, Run time: 20 min. For standard preparation 10 mg of Vitamin E standard was dissolved in 25 ml methanol.

Free vitamin E content

The prepared microencapsules were weighed to 50 mg and dispersed in 50 ml of methanol. The dispersion was agitated for 10 min and then got filtered. The filtrate was analyzed using HPLC to estimate the content of vitamin E.

Encapsulation efficiency

Encapsulation efficiency was determined by:

Encapsulation efficiency

$$= \frac{\text{Vitamin E content in microencapsules}}{\text{Vitamin E content in polymer solution}} \times 100$$

Density

Bulk and tapped densities were measured as a measure of packability of the microencapsules. Around 10 g of microencapsules were taken in a 25 ml measuring cylinder and the volume was recorded as bulk volume. The bulk density was calculated using the formula as weight of microencapsules/bulk volume. The tapped volume and tapped density was determined by tapping the cylinder 100 times. The Carr's Index was calculated by using the following formula:

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped Density}}$$

Yield

The percent yield of microencapsules was estimated as follows:

$$\% \text{Yield} = \frac{\text{Wt. of microencapsules}}{\text{wt. of mix}} \times 100.$$

Moisture content and swelling index

Standard AOAC procedure (AOAC 2000) given under 44.15 A was followed to measure the moisture content.

For the calculation of swelling index, 100 mg of microencapsules were placed in water and then allowed to swell up to a constant weight. The microencapsules were removed, blotted with filter paper and weighed. The degree of swelling (a) was calculated from the formula:

$$(a) = (W_g - W_o) / W_o$$

where W_o is the initial weight of the microencapsules and W_g is the weight of the microencapsules at equilibrium swelling in purified water.

Statistical analysis of data

The data obtained was analyzed by using analysis of variance (ANOVA) using SAS software version 9.1 (SAS Institute Inc., NC, USA). To test the significant difference between the control and experimental samples, Duncan's

multiple comparison test was used for comparisons ($p \leq 0.05$). All the results were the average of triplicates.

Results and discussion

Microspheres containing α -tocopherol were prepared using pectin alone and pectin sodium alginate (PSA) together in different ratios. The results obtained for characterization of the microencapsules are presented in Table 2. In one formulation pectin (P) was taken alone at 2.0% w/v and other formulations were taken with sodium alginate (PSA1, PSA 2, PSA3 and PSA4) in increasing concentration from 0.5 to 2% w/v. The results revealed the change in shape of the microcapsules from disc shaped to spherical shaped till the concentration of sodium alginate was increased to 1.5% w/v. Sodium alginate was added as wall material and pectin was taken as filler. Pectin and alginate belong to the group of polyuronates and are the characteristic example of natural ionic polysaccharides undergoing chain–chain association and form hydrogels upon addition of divalent cations like Ca^{2+} (Fang et al. 2008). Narsaiah et al. (2011) discussed that alginate was useful as a matrix for entrapment of bioactive compounds. Shalaka et al. (2009) reported that pectin at 5% and sodium alginate at 1.5% has wide potential for formulation microspheres of vitamin E for cosmetic applications.

Average particle size, shape and surface morphology

Results of the study revealed that pectin/pectin alginate microencapsules showed an average particle size varied between 0.46 and 0.62 mm. Similar results have been found by Nochos et al. (2008) in alginate beads. With increase in the concentration of sodium alginate from 0 to 2%, it was found that the shape of microencapsules changed from disc shaped for pectin alone to spherical shape for

1.5% w/v sodium alginate. The sphericity was maintained in PSA2 to PSA4 formulations. However better sphericity was observed in PSA3 microspheres. SEM Photomicrograph of microencapsules prepared with syringe are shown in Fig. 1 and that prepared with encapsulator are shown in Fig. 2. The SEM photomicrographs of microencapsules prepared with syringe showed a round and smooth surface however those prepared with encapsulator were rough and not round in shape. This may be due to high pressure used for spraying the mix or may be due to shrinkage during air drying. The particle size distribution by laser light scattering method was determined and found that in vitro release capacity greatly affected by a droplet diameter and the average diameter for developed nano emulsions was found 86.45 nm. The shape of nano emulsion was observed spherical under the examination of atomic force microscope and for use in food industry the spherical shape has an importance (Dasgupta et al. 2016). The surface morphology of the microcapsules was observed by a scanning electron microscopy method and revealed that spray dried microcapsules has dents on the outer surface and these may be due to shrinkage of the particles during drying and cooling (Farias et al. 2007).

Encapsulation efficiency

All the formulations of pectin/pectin–alginate microspheres showed good encapsulation efficiency (45.12–52.91%) irrespective of the core: coat ratios or variations in pectin alginate combinations as reflected from the HPLC analysis for vitamin E content. The observations for surface content (0.025–0.033%) also confirmed that the surface content of vitamin E was negligible. The encapsulation efficiency was assessed by determining the total tocopherol content of the surface and powder and the encapsulation efficiency ranged from 52 to 70%. They reported that encapsulation efficiency of vitamin E

Table 2 Analysis of pectin/pectin alginate microencapsules

Formulation code	P	PSA1	PSA2	PSA3	PSA4
<i>Parameters</i>					
Average particle size (mm)	0.59 ± 0.03^a	0.62 ± 0.02^b	0.54 ± 0.01^c	0.59 ± 0.03^a	0.46 ± 0.02^d
Free % vit E	0.025 ± 0.01^a	0.027 ± 0.02^b	0.030 ± 0.01^c	0.033 ± 0.02^d	0.031 ± 0.1^c
%Encapsulation efficiency	45.12 ± 0.41^a	48.91 ± 0.33^b	51.73 ± 0.26^c	52.91 ± 0.34^c	50.53 ± 0.13^d
Bulk density (mg/ml)	0.55 ± 0.01^a	0.49 ± 0.02^b	0.42 ± 0.01^c	0.40 ± 0.01^c	0.45 ± 0.01^b
Carr's index	12.36 ± 0.11^a	15.65 ± 0.02^b	14.23 ± 0.01^c	11.56 ± 0.21^d	12.31 ± 0.01^a
%Yield	90.39 ± 0.37^a	91.29 ± 0.26^b	91.89 ± 0.32^c	91.24 ± 0.15^b	90.45 ± 0.11^a
Moisture content %	4.51 ± 0.04^a	4.57 ± 0.09^a	4.29 ± 0.06^c	4.41 ± 0.02^b	4.73 ± 0.02^b
Swelling index	0.911 ± 0.013^a	0.919 ± 0.04^a	0.935 ± 0.09^b	0.947 ± 0.04^c	0.959 ± 0.02^d

All values are means with standard deviation ($n = 3$). Different letters within the same row differ significantly from each other ($p < 0.05$)

Fig. 1 SEM photomicrograph of microencapsules prepared with Syringe. **a** $\times 35$ SE, **b** $\times 50$ SE, **c** $\times 100$ SE

Fig. 2 SEM photomicrograph of microencapsules prepared with encapsulator. **a** $\times 35$ SE, **b** $\times 50$ SE, **c** $\times 100$ SE

microcapsules is affected by increasing the core and wall ratio from 0.6 to 1.0. This may be due to instability of the oil droplets in the emulsion before spray drying and may also be due to thin layers of wall material between droplets of encapsulated oil (Hogan et al. 2001). The conditions were optimized for encapsulation of nisin using sodium alginate and guar gum and reported the encapsulation efficiency of nisin as 36.65% (Narsaiah et al. 2014). Vitamin E was encapsulated in liposomes using supercritical fluid process coupled with vacuum-driven cargo loading and obtained 99.32% encapsulation efficiency of vitamin E (Tsai and Rizvi 2017).

Bulk density and Carr's index

Pectin/Pectin alginate microspheres showed excellent flow properties as indicated from the value of Carr's index. The Carr's index is used as an indicator of the flow ability of a powder. In free flowing powder, the bulk density and tapped density are close in value. Whereas, in poor flowing powder there are greater interparticle interactions and the difference in bulk and tapped density observed will be greater. A Carr's index below 15 shows maximum flowability (Bowker and Stahl 2008).

Yield

The % yield of Pectin/pectin alginate microencapsules varied between 90.39 and 91.89% and is directly

proportional to the number of drops of the formulation which fall into the calcium chloride solution.

Moisture content and swelling index

Pectin/pectin alginate microspheres showed moisture content in the range between 4.29 and 4.73%. At this moisture content microencapsules could be kept for a long period.

Pectin/pectin alginate microspheres showed good swelling index (0.911–0.959), retained their sphericity and were found to restore their original size prior to drying. Similar results were reported by Shalaka et al. (2009), they reported that pectin/pectin alginate microspheres of vitamin E showed good swelling index, retained sphericity and size prior to drying.

Comparison of microencapsules prepared with encapsulator and syringe

Optimization of encapsulation of α -tocopherol was done with the help of syringe. PSA3 i.e. pectin 2.0% and sodium alginate 1.5% was selected based upon the highest encapsulation efficiency. Final encapsulation of α -tocopherol was done with the help of encapsulator. Microencapsules prepared with syringe were compared with microencapsules prepared by encapsulator, results are depicted in Table 3. Both the microencapsules were evaluated for various parameter such as average particle size, free % vitamin E, bulk density, % encapsulation efficiency, %

Table 3 Comparison of microencapsules prepared with syringe and encapsulator

Parameters standardized	Microencapsules prepared with encapsulator	Microencapsules prepared with syringe
Average particle size (mm)	0.49 ± 0.03 ^a	0.58 ± 0.12 ^b
Free % vit E	0.033 ± 0.01 ^a	0.04 ± 0.01 ^a
%Encapsulation efficiency	55.97 ± 0.34 ^a	52.11 ± 0.13 ^b
Bulk density (mg/ml)	0.40 ± 0.12 ^a	0.53 ± 0.01 ^a
Carr's index	9.87 ± 0.01 ^a	11.56 ± 6.1 ^b
%Yield	91.24 ± 0.15 ^a	93.20 ± 0.11 ^b
Moisture content %	4.41 ± 0.02 ^a	5.23 ± 2.1 ^b
Swelling index	0.947 ± 0.34 ^a	1.03 ± 0.92 ^b

All values are means with standard deviation (n = 3). Different letters within the same row differ significantly from each other ($p < 0.05$)

yield, moisture content and swelling index. The results of these parameters indicated that average particle size of microencapsules obtained by using encapsulator was found to be 0.49 mm size and that obtained by syringe to be 0.58 mm; thus size differed significantly from each other. Free % vitamin E was found to be more in microencapsules prepared with syringe i.e. 0.04% and less in microencapsules prepared with encapsulator i.e. 0.033%. The per cent encapsulation efficiency was found to be more i.e. 55.97% in microencapsules prepared with encapsulator and varied significantly with 52.11% in microencapsules prepared with syringe. The bulk density of microencapsules was found to be 0.40 mg/ml and 0.53 mg/ml of microencapsules prepared by using encapsulator and syringe respectively. Per cent yield and moisture content was found to be more in microencapsules prepared by using syringe i.e. 93.20 and 5.23% as compared to microencapsules prepared with the encapsulator that are 91.24 and 4.41% respectively. The swelling index represents significant variation and showed more value in microencapsules prepared with syringe i.e. 1.03 and low in microencapsules prepared with the encapsulator with the value of 0.947. From comparative study it was observed that encapsulation efficiency of α -tocopherol was more of microencapsules prepared with encapsulator as compared with syringe.

Conclusion

It was concluded that microencapsules can be prepared by using two encapsulation techniques such as syringe and encapsulator for the better handling of the liquid particles by converting them to solid form. α -tocopherol is one of the best antioxidants that is used in the food industry to enhance shelf life of fat based bakery products. The optimum conditions for encapsulation of α -tocopherol with maximum encapsulation efficiency were: sodium alginate (1.5% w/v) and pectin (2.0% w/v). The microencapsules

prepared by using encapsulator gives good parametric studies such as % yield, average particle size, % encapsulation efficiency, bulk density, moisture content and swelling index but the smooth and round shape of microencapsules was obtained with syringe method. These microencapsules containing α -tocopherol may be used as antioxidant in fat based bakery products to prevent auto oxidation and hence enhance shelf life. Encapsulation of such lipophilic substances facilitates its handling, enhance stability and maintain sustainable release.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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