

ORIGINAL ARTICLE

Nanoencapsulation of *Lactobacillus casei* in Bitter Gourd Juice using Spray Drying

Archana Y Kalal^{1*}, Sharangouda Hiregoudar², Udaykumar Nidoni³, Ramachandra C. T.², Nagaraj Naik² and Roopabai. R. S²

¹M. Tech, Dept. of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences Raichur, Raichur-584 104, India

²Assistant Professor, Dept. of Processing and Food Engineering, College of Agricultural Engineering, University of Agricultural Sciences, Raichur

¹*email: archuyk.17@gmail.com

ABSTRACT

Probiotics are the microorganisms which offer many of the therapeutic roles on its consumption. Encapsulation is a technique for protection against oxidation and other undesirable reactions and helps in maintaining stability of the product. In this, *L. casei* was encapsulated in bitter gourd juice at three different inlet air temperatures of spray dryer with three different additives concentration. The resultant powders were analyzed for different physical properties. The encapsulated *L. casei* was assessed for viability in the bitter gourd powder over a storage period of 4 weeks. The results showed that, by increasing the inlet air temperature, the moisture content, water activity and bulk density of the encapsulated product were decreased, while solubility was increased. The highest viable counts of *L. casei* were found in powders encapsulated with maltodextrin over a storage period of four weeks. The colour values were higher for powders with maltodextrin and lower for gum arabic.

Keywords: Bitter gourd, Spray drying, Gum arabic, *L. casei*, Maltodextrin and Probiotics.

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INTRODUCTION

"Probiotics" are the "live microorganisms which when administered in adequate amounts confer a health benefit on the host". They have been reported to offer many of the therapeutic roles by modulating immunity, lowering cholesterol, improving lactose tolerance and preventing cancer [19]. Their microbial behaviour (growth, survival and death) in foods is largely governed by properties of the food (water availability, pH and buffering capacity) in addition to the storage conditions (temperature, relative humidity and atmosphere) [25]. Nanoencapsulation is a process which can provide the necessary protection against oxidation and other undesirable reactions [32], for extending the shelf life, enhancing bioavailability and controlling the release of the bioactive ingredients. It is a technique, which involves the incorporation of a chemically sensitive compound in a matrix or sealed capsule, protecting it against adverse reactions, preventing its degradation and increasing its shelf life [11].

Lactobacillus casei is a species of bacteria belonging to the genus *Lactobacillus* usually found in raw and fermented dairy products, intestinal tracts and reproductive systems of humans and animals. The lactic acid produced by *L. casei* through fermentation is very important to make cheese and yoghurt, reduce cholesterol level, enhance immune response, control diarrhoea, alleviate lactose intolerance, and inhibit intestinal pathogens as well as to serve as a probiotic. A new advanced approach has been made to protect the probiotic microorganisms from various undesirable environmental conditions (*i.e.*, temperature, relative humidity, pH, moisture, acidity and alkalinity) to increase their viability, just by encapsulating them in any of the food ingredients. Vegetables are rich sources of the biologically active compounds which have beneficial effects in the prevention of some of the diseases and certain types of

cancer. To increase the uptake of vegetables and their by-products researchers are involved in the development of functional foods fortified with probiotics.

Momordica charantia Linn, known as bitter melon or *karela*, is a cucurbit vine native to Asia [41]. It is a revolutionary plant for its versatility as foodstuff and therapeutic applications. It is highly nutritious due to presence of higher contents of protein, ascorbic acid, calcium, iron and phosphorus [23, 6]. Several phytochemicals with health benefits of bitter gourd have been isolated and studied [26]. Charantins, a mixture of steroidal saponins that are abundant in the fruit of bitter gourd, have been proposed to contribute to the hypoglycemic and antihyperglycemic activity of bitter gourd [17].

Though the bitter gourd contains numerous compounds and provides many of the benefits on its intake, many people avoid its intake due to its sour and bitter taste. So there is a need for technology which may help in binding or masking the bitter and sour taste of bitter gourd by adding some of the coating materials such as maltodextrin, gum arabic, starch, etc. The encapsulation processes commonly used in industries are spray drying and extrusion [7, 14]. Spray drying has become the most important commercial process for making dry flavourings. It is an economical, as well as an effective method for encapsulating vitamins, minerals, colorants, fat and oil flavour, aroma compounds, oleoresins and enzymes. Therefore, the present study was taken up with the objective to use the spray drying for encapsulating the probiotics in bitter gourd juice by using selective additives for masking its bitter taste and thereby increasing its consumption.

MATERIALS AND METHODS

Materials

The bitter gourd or bitter melons were procured from the local market of Raichur, Karnataka. Probiotic bacterium (*Lactobacillus casei*) and other chemicals were obtained from the MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh, India to carry out the microbiological analysis. The additives or wall materials, viz., maltodextrin (DE=20), gum arabic and the other related chemicals and standards were procured from M/s Himedia, Mumbai and M/s, SD Fine, Bangalore. All the chemicals used were of analytical grade.

Nanoencapsulation by spray drying

Preparation of drying media

Three different feed solutions of bitter gourd concentrate were prepared by adding each with 30% maltodextrin, 30% gum arabic and 30% mixture of maltodextrin (15%) and gum arabic (15%). All the media were homogenized at 16,000 rpm for 5 min by using a high speed blender (Kenstar) and were heat treated at 80 °C for 30 min.

Preparation of bacterial suspension

Freeze-dried probiotic cells of *L. casei* were grown in nutrient broth solution to increase its number placed over a shaker at ambient temperature for a period of 24 h. This was considered as stock solution and was kept under refrigerated condition (4 °C) in sterile glass bottles. Before spray drying, the probiotic stock solution was incubated at 37 °C for 24 h after inoculated (@ 1%) into the feed solution.

Spray drying

The spray drying was carried out with a laboratory spray dryer (Labultima LU-222 advanced, Labultima, Mumbai) at three different inlet air temperatures of 140, 150 and 160 °C and at constant outlet air temperature of 85 °C with three different combinations of encapsulating agents: M1 (30% maltodextrin), M2 (30% gum arabic), M3 (30% mixture of maltodextrin (15%) and gum arabic (15%)). The feed solutions containing *L. casei* were kept under magnetic agitator at room temperature and fed into the main chamber through a peristaltic pump, with a feed flow rate of 2 ml/min. The nanoencapsulated bitter gourd powder samples were collected from the cyclone. The samples were packed in polypropylene pouches and stored at room temperature.

The powder properties were analysed in relation to their particle size, morphology, moisture content, water activity, bulk density, colour, and solubility. All the experiments were performed in triplicate, except those for morphology and particle size. The obtained spray dried powder samples were also analysed to determine the viable cell counts of *L. casei* in the nanoencapsulated probiotic bitter gourd juice powder during its storage per 4 weeks at room temperature.

Physical properties of the nanoencapsulated probiotic bitter gourd juice powder

Particle size and morphology

The size and morphology of the particles produced with different treatment combinations were analysed. The samples with different encapsulating agents were evaluated for size and morphology by scanning electron microscopy (SEM) according to the modified methodology described by Shu *et al.* [35]. The small

amount of samples *i.e.*, nanoencapsulated probiotic bitter gourd juice powder were placed on the surface of a double-sided carbon tape fixed to stubs. The samples were then coated with a thin layer of gold-palladium under vacuum with the help of a sputter coater. The coating of the sample was carried out using plasma current of 10 mA for a period of 90 s. A Zeiss scanning electron microscope (EVO LS 10, Carl Zeiss company, Germany) was used for analyzing the morphology of samples which were systematically observed at 3000X and 7000X magnification.

Moisture

The moisture content of spray dried powders was determined using a hot air oven (Swastik Electric and Scientific works, Ambalacant) method (945.43) according to AOAC, [5].

Water activity

The water activity (a_w) of spray dried powder samples was determined using a water activity meter (Rotronic, Germany) at a temperature of 24.5 ± 0.5 °C during all the experiments [20].

Bulk density

The bulk density of spray dried powder samples (g/ml) were determined by freely pouring 1 g of powdered sample into a 5 ml glass graduated cylinder (readable at 1 ml). The samples were repeatedly tapped manually by lifting and dropping the measuring cylinder under its own weight at a vertical distance of 14 ± 2 mm over a rubber mat for 25 times until negligible difference in volumes between succeeding measurements was observed [9, 21]. The ratio of powder mass and the volume occupied in the cylinder determined the bulk density of the powder.

Colour analysis

The colour analysis was performed with Hunter colourlab flex meter (Colour Flex EZ) for the nanoencapsulated probiotic bitter gourd juice powder. The colour was measured by using CIELAB scale at 10° observer at D65 illuminant. It works on the principle of focusing the light and measuring energy reflected from the sample across the entire visible spectrum. It provides reading in terms of L^* , a^* and b^* . Where, luminance (L^*) forms the vertical axis, which indicates whiteness (+) to darkness (-). In the same way a^* indicates redness (+) to greenness (-) and b^* indicates yellowness (+) to blueness (-). The sample was filled in the sample cup ensuring that there was no void space at the bottom while filling the powder. The deviation of the colour of the sample to standard were also observed and recorded in the computer interface [42].

Solubility

The solubility of the sample was measured according to the method reported by Zhang *et al.* [43] and Shittu and Lawal [33]. One gram of sample was taken into the 50 ml beaker and then 10 ml of distilled water (30 ± 2 °C) was added to it. The suspension was stirred intermittently for 30 min before it was finally centrifuged at 9000 rpm for 10 min and then the supernatant was completely drained into an evaporating dish and dried to constant weight in a hot air oven at 105 ± 2 °C for 4 h. The final powder weight on the dish was used for determination of the water solubility of the product (g of powder per 100 g of water).

Survival of nanoencapsulated *L. casei* after spray drying during storage period

The viable cell counts (*L. casei*) in the developed nanoencapsulated probiotic bitter gourd juice powder were enumerated over the storage period. The enumeration was carried out by following spread plate method with *Lactobacillus* MRS agar as a growth medium [28]. One gram of the sample was serially diluted in sterile distilled water until 10^{-6} dilutions were reached. About 0.1 ml aliquot from 10^{-6} dilution was transferred to the sterile petriplates containing solidified MRS agar media. Then the plates were incubated for a period of 48 h in an incubator maintained at a temperature of 32 °C. After the incubation period, the colonies were counted and the number of cfu/g of sample was calculated by applying the following formula;

$$\begin{aligned} & \text{Number of colony forming units (cfu) per g of the sample} \\ & = \frac{\text{Mean number of cfu} \times \text{Dilution factor}}{\text{Volume or weight of sample}} \end{aligned}$$

Statistical analysis

The experimental data obtained was statistically analyzed using statistical software, Design Expert Version 7.7.0 trial version (State-Ease, Minneapolis, MN). The models generated to represent the responses were evaluated in terms of F-ratio. The effects of the independent variables on the physical properties of the powders were studied. The statistical analysis was carried out using factorial completely randomized block design (FCRD).

RESULTS AND DISCUSSION

Physical properties of nanoencapsulated probiotic bitter gourd juice powder

Fig. 2 shows the SEM micrographs of powder samples produced with different encapsulating agents. The absence of free bacteria confirmed the formation of encapsulated powder particles containing *L. casei* for all the encapsulating agents. The particles showed a smooth and spherical shape of various sizes, produced by spray drying. Saenz, *et al.* [31] reported that the formation of indentations on the surface of atomized particles could be attributed to the shrinkage of the particles during the drying process because of the rapid evaporation of the liquid drops. It was seen that the powder obtained with maltodextrin as a carrier agent resulted in the formation of more homogeneous capsules. The spheres had less wrinkles on their surfaces, particularly at 3000X magnification. The particles obtained with gum arabic and maltodextrin showed similar structures to those obtained with maltodextrin with few wrinkles and smooth surfaces whereas particles obtained with the gum arabic encapsulant, however, exhibited irregular surfaces of angular shapes with several indentations.

Allamilla-Beltran *et al.* [3] observed that particles of maltodextrin produced under higher drying temperatures (170-200°C) presented a spherical and smooth surface format, while particles produced under lower temperature (110°C) presented a wrinkled appearance, similar to that of the atomized umbu particles produced at 133 °C. Nijdam and Langrish [27] explained that when the drying temperature was high enough, the moisture was rapidly evaporated with a subsequent formation of a dry and hard envelope, avoiding emptying the particle. However, when the drying temperature was low, the envelope remained moist and supple for longer periods, allowing the particle to deflate and wither during cooling. As for agglomeration, the behaviour of particles produced by atomization was closely related to the origin and concentration of the carrier agent used during the process. Cano-Chauca *et al.* [8] showed that the treatment of atomized mangoes with only 12% maltodextrin resulted in the formation of larger, agglomerated, and amorphous particles. Fazaeli *et al.* [12] observed similar results when using 8% of maltodextrin in the process of drying blackberries by atomization.

The nanoencapsulated probiotic bitter gourd juice powder produced with maltodextrin at lower inlet air temperature showed higher ($P < 0.05$) moisture content than that produced with the gum arabic and mixture of maltodextrin and gum arabic at the same inlet air temperature (Table 1). This was due to higher drying rate and decreased amount of water introduced to the dryer [24, 29]. Similar results were obtained by Al-Asheeh *et al.* [2], Goula and Adamopoulos [15] for tomato pulp powder; Abadio *et al.* [1] and Chegini and Ghobadian [10] for pineapple and orange juice powder, respectively and by Hong and Choi [18] for protein-bound-polysaccharide powder. Moreover, the moisture content of all the encapsulated samples was within the values (*i.e.*, < 5%) described by Simpson *et al.* [37] to guarantee microbiological stability.

All the powder samples showed water activity values below 0.4 (Table 1), which, according to Tonon *et al.* [39], was very positive for powder stability since it represented less free water available for biochemical reactions and hence longer shelf life. The increase in drying air temperature, regardless of the mass feeding flow and carrier agent concentration, led to product water activity reduction. Thus, greater temperature gradient between the atomized product and drying air led to higher heat transfer and, consequently, higher evaporation of water from the product resulting in lower water activity. Solval *et al.* [38] and Fazaeli *et al.* [12] used spray drying technology and observed a decrease in the particle's water activity with increasing drying air temperature for melons and blackberries, respectively. However, the increase in mass feeding flow, independent of the carrier agent concentration used, led to increased product water activity values [36]. Therefore, faster processes resulted in shorter contact time between the product and the drying air, making the process of heat transfer less efficient.

The powder encapsulated with maltodextrin exhibited a lower bulk density than that obtained with mixture of maltodextrin and gum arabic (Table 1). There was effect of temperature and carrier concentration on bulk density. The bulk density decreased when maltodextrin concentration increased. Similar results were observed by Goula and Adamopoulos [15] and Abadio *et al.* [1], when tomato and pineapple pulp were dried using maltodextrin as the carrier in a spray dryer. They stated that the particle size of the powder increased when the feed concentration increased. Also, Shrestha *et al.* [34] showed that an increase in maltodextrin concentration caused a decrease in bulk density of orange juice powder. Increased inlet air temperature caused a reduction in bulk density, as evaporation rates were faster and products dried to a more porous structure. According to Walton [40] increasing the drying air temperature generally produced a decrease in bulk and particle densities, and there was a greater tendency for the particles to be hollow.

The solubility of the powders encapsulated with maltodextrin increased with increase in the inlet air temperature when compared to the solubility of powders encapsulated with gum arabic and mixture of maltodextrin and gum arabic. The solubility of powders with gum arabic was lower because of its gummy

nature. For consumers, quick and complete reconstitution of powdered products was one of the main quality indicators [13]. Increase inlet air temperature led to an increase in the solubility of spray dried powders. This might be due to the effect of inlet air temperature on residual moisture content leading to increase in the particle size and consequently decrease in the time required for the powder to dissolve [40]. Higher maltodextrin concentrations led to an increase in powder moisture content and a decrease in powder solubility. The actual amount depends on the temperature and moisture content during the drying period.

The colour attributes of the samples produced with different encapsulating agents are shown in Table 2. In relation to the L^* and b^* parameters, there were no significant ($P < 0.05$) differences between the samples. According to Aryana and McGrew [5], a factor influencing product colour was the colour of the ingredients used. The colour values of the particles produced with maltodextrin were higher indicating tendency to white *i.e.*, colourless nature of the maltodextrin powder when compared to the gum arabic and mixture of maltodextrin and gum arabic had slightly lower values *i.e.*, light brownish in colour.

Survival of nanoencapsulated *L. casei* after spray drying during storage period

The effect of different encapsulating agents on the viability of *L. casei* in nanoencapsulated probiotic bitter gourd powder throughout storage is shown in Fig. 1. The spray dried particles containing *L. casei* showed high survival up to its first week of storage encapsulated with maltodextrin than that when compared to gum arabic and mixture of maltodextrin and gum arabic. In addition, the counts of viable probiotic cells were above the recommended levels for probiotic food throughout the whole storage time, *i.e.*, equal to or greater than 6 log cfu/g of the product, according to Roy [30]. The capsules produced with maltodextrin and gum arabic showed higher ($P < 0.05$) count, when compared to the capsules produced with mixture of both maltodextrin and gum arabic. This suggested that maltodextrin had a positive effect on the protection of *L. casei* during the encapsulation process, probably because it acted as a thermoprotector for the cells undergoing the drying process. Lian *et al.* [22] reported that besides difference in chemical characteristics, the encapsulating agents had different physical properties. Therefore, it was reasonable to expect that these agents tested in this study might exert different degrees of protective effect on the entrapped cells of a test organism when subjected to heat inactivation during spray drying.

Table 1: Properties of nanoencapsulated probiotic bitter gourd juice powder

Additives (%)	Temperature (°C)	Properties			
		Moisture Content (%)	Water activity	Bulk density (g/cc)	Solubility (%)
M1	140	4.31	0.36	0.35	95.33
	150	4.25	0.35	0.34	95.97
	160	3.46	0.32	0.32	96.53
M2	140	4.24	0.37	0.36	93.33
	150	3.47	0.31	0.34	93.67
	160	3.39	0.29	0.32	94.73
M3	140	3.53	0.34	0.36	95.43
	150	3.28	0.32	0.32	95.93
	160	3.21	0.31	0.30	96.43

M1= 30% Maltodextrin; M2= 30% Gum arabic;
M3= 30% Mixture of maltodextrin and gum arabic

Table 2: The colour attributes of nanoencapsulated probiotic bitter gourd juice powder

Additives (%)	Temperature (°C)	Colour values		
		L^*	a^*	b^*
M1	140	87.89	0.65	8.56
	150	86.41	0.22	7.63
	160	87.88	0.18	10.6
M2	140	81.81	0.80	8.50
	150	87.25	0.01	9.39
	160	78.85	1.14	9.46
M3	140	69.10	0.19	8.47
	150	70.12	0.21	8.21
	160	79.16	0.19	9.10

M1= 30% Maltodextrin; M2= 30% Gum arabic;
M3= 30% Mixture of maltodextrin and gum arabic

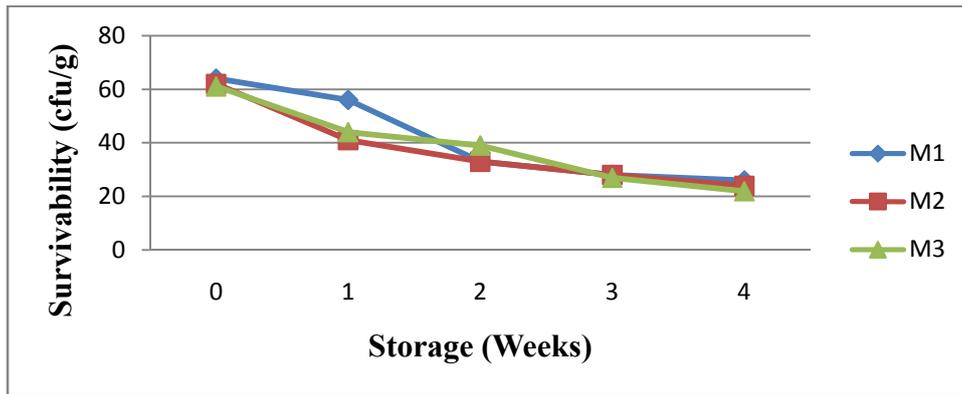


Figure.1: Survivability of *L. casei* encapsulated with different encapsulating agents.

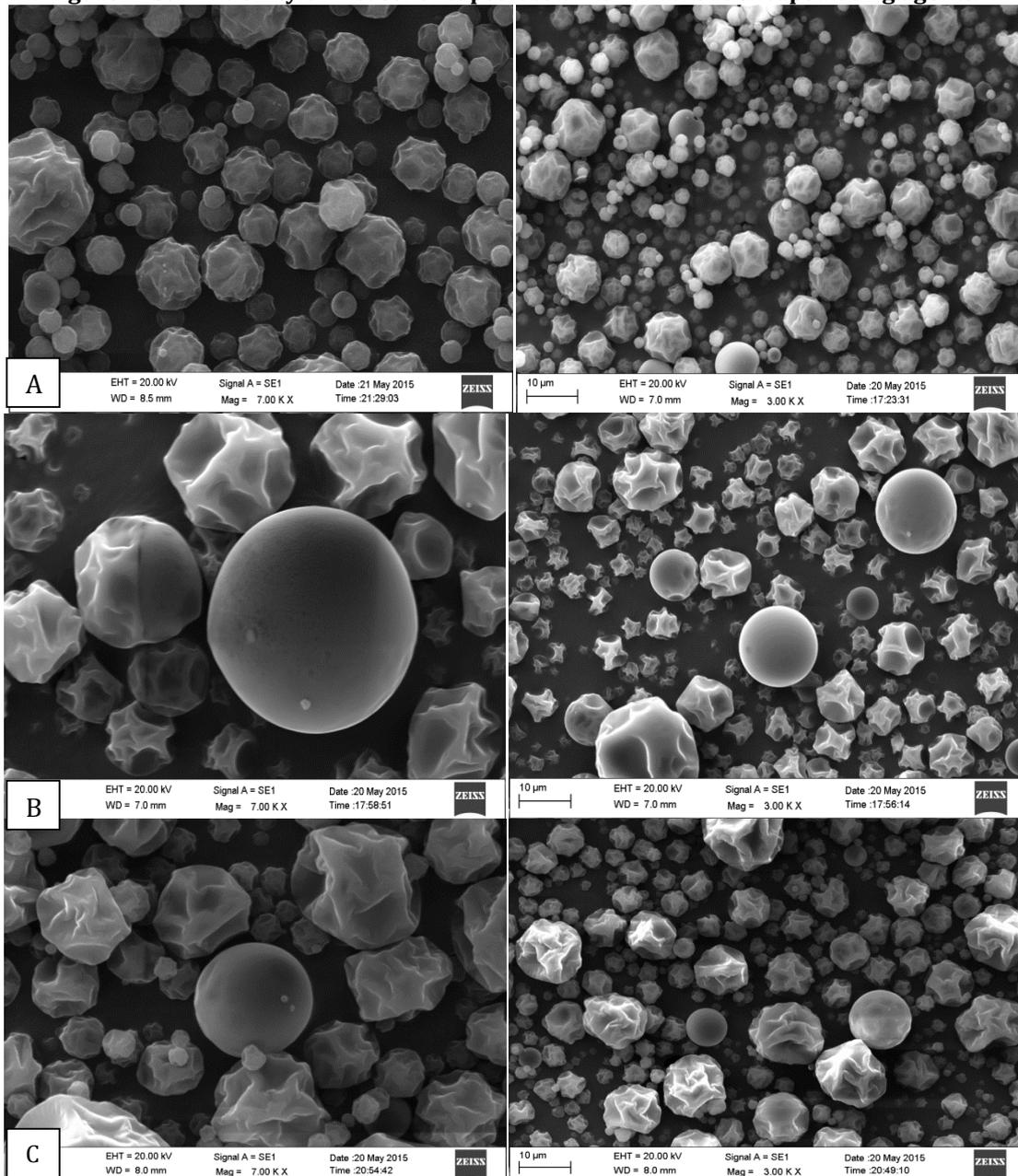


Figure 2: Photomicrographs of nanoencapsulated probiotic bitter gourd juice powder, formulated with different carrier agents at 140 °C. Magnification of 3,000X (left) and 7,000X (right). (A) Maltodextrin (B) Gum arabic and (C) Maltodextrin and gum arabic

CONCLUSIONS

The spray drying of bitter gourd juice using different encapsulating agents did not affect the morphology of the powdered particles. It shows that the powder obtained with maltodextrin and mixture of maltodextrin and gum arabic as a carrier agent resulted in the formation of more homogeneous capsules whereas with that using gum arabic resulted in several indentations. They also helped in maintaining stability of the powders during storage by decreasing the moisture content and water activity. The particles produced with gum arabic showed the lowest solubility while the particles produced with maltodextrin were highly soluble. The powders encapsulated with maltodextrin exhibited a lower bulk density than that obtained with mixture of maltodextrin and gum arabic. The L^* values of powders encapsulated with maltodextrin were higher compared to that encapsulated with gum arabic indicating its tendency towards whiteness. Finally, the results showed that the viability of the *L. casei* in the developed nanoencapsulated probiotic bitter gourd juice powder was considerably good when encapsulated with maltodextrin in turn retaining the powder properties.

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