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Effect of whey protein isolate and β-cyclodextrin wall systems on stability of microencapsulated vanillin by spray–freeze drying method

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ABSTRACT

Vanillin flavour is highly volatile in nature and due to that application in food incorporation is limited; hence microencapsulation of vanillin is an ideal technique to increase its stability and functionality. In this study, vanillin was microencapsulated for the first time by non-thermal spray–freeze-drying (SFD) technique and its stability was compared with other conventional techniques such as spray drying (SD) and freeze-drying (FD). Different wall materials like β-cyclodextrin (β-cyd), whey protein isolate (WPI) and combinations of these wall materials (β-cyd + WPI) were used to encapsulate vanillin. SFD microencapsulated vanillin with WPI showed spherical shape with numerous fine pores on the surface, which in turn exhibited good rehydration ability. On the other hand, SD powder depicted spherical shape without pores and FD encapsulated powder yielded larger particle sizes with flaky structure. FTIR analysis confirmed that there was no interaction between vanillin and wall materials. Moreover, spray–freeze-dried vanillin + WPI sample exhibited better thermal stability than spray dried and freeze-dried microencapsulated samples.

1. Introduction

Vanilla is widely used as a flavouring compound in bakery, beverage, and ice-cream industries. Vanillin (3-methoxy-4-hydroxybenzaldehyde) is extracted from the pods of *Vanilla planifolia* through an expensive process. Approximately 50% of the world production of vanillin is used as an intermediate in the production of herbicides, antifoaming agents or drugs (Walton, Mayer, & Narbad, 2003). Sinha, Sharma, and Sharma (2008) reported the uses of vanillin as an antioxidant, anticarcinogenic, antimutagenic, and antisickling agent. Due to its phenolic character, it is also used as a chemical intermediate during the production of fine chemicals and pharmaceuticals (Claudio, Freire, Freire, Silvestre, & Coutinho, 2010).

Microencapsulation is a technique in which sensitive bioactive compounds (e.g., vanillin, probiotic bacteria, β-carotene and omega-3 fatty acids) are packed within a secondary material (e.g., protein, polysaccharide or lipids) to protect from oxygen, heat, light and to facilitate food fortification. The microencapsulated material is coined as core whereas the surrounding encapsulating agent is termed as wall material (Pillai, Prabhasankar, Jena, & Anandharamakrishnan, 2012). Whey Protein Isolate (WPI) is found to be a suitable wall material for microencapsulation of volatile compounds (Bae & Lee, 2008). WPI consists of three principal components such as β-lactoglobulin, α-lactalbumin and bovine serum albumin; moreover it exhibits excellent film forming abilities (Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003). However, WPI has never been employed for the microencapsulation of vanillin. The other wall material like β-cyclodextrin, has been widely used for encapsulation in more than decade (Bhandari et al., 1998). Cyclodextrins are a series of cyclic oligosaccharides that are enzymatically derived from the starch employing CyD transglycosylase. β-Cyclodextrin comprises of 7-D-glucose units which are connected by α-1, 4 linkages. Further β-cyd structure resembles a thick walled bucket with a hydrophobic cavity and hydrophilic exterior. Weak forces such as van der Waals force, dipole–dipole interaction and hydrogen bonding help them to form an inclusion complex by entrapping guest molecule inside its cavity. Vanillin and β-cyclodextrin inclusion complex formation has been studied by Karathanos, Mourtzinos, Yannakopoulou, and Andrikopoulos (2007). Pena, Casals, Torras, Gumi, and Garcia-Valls (2008) have also investigated the vanillin release from polysulfone macrocapsules. Similarly, vanillin was encapsulated using carnauba wax to study the kinetics of vanillin release mechanism (Stojakovic, Bugarski, & Rajic, 2012). Tari, Annapure, Singhal, and Kulkarni (2003) have microencapsulated vanillin in starch based spherical

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aggregates of size 7.5–45 μm and it was prepared from amarnath, quinoa, rice and colocasia in presence of bonding agents such as gum Arabic, carboxy methyl cellulose, and carrageenan. However, retaining thermal stability of vanillin is a problem due to its very high volatility nature (Kayaci & Uyar, 2012). Thus, microencapsulation of vanillin can be solution for increasing the thermal stability of vanillin.

Spray drying and freeze drying are widely used for microencapsulation of various bioactive compounds and flavouring materials (Anandharamakrishnan, 2014). Microencapsulation of PUFA oils by spray drying leads to increased rancidity problems. On the other hand, freeze drying requires high residence time that leads to higher operating cost, which is the main hindrance for the utilisation of this technique. Hence, spray–freeze drying (SFD) is an effective alternative to spray drying and freeze drying techniques. SFD is a two-step process where the first step involves spray freezing, and the second one involves freeze drying (Anandharamakrishnan, Rielly, & Stapley, 2010). Currently, there are three types of techniques being used for spray freezing: (1) spray freezing into vapour, (2) spray freezing into vapour over liquid, and (3) spray freezing into liquid (Karthik & Anandharamakrishnan, 2013). Powders with large surface-to-mass ratio can be produced by SFD; furthermore wettability and dispersibility are the key factors and are important for the powder reconstitution (Rogers, Wu, Saunders, & Chen, 2008). Highly soluble due to large number of pores along with lower drying time makes SFD a highly appropriate technique for microencapsulation of sensitive compounds. Bioactive compounds which are thermally sensitive such as DHA algae oil (Karthik & Anandharamakrishnan, 2013), Tolbutamide (Kondo, Niwa, Okamoto, & Danjo, 2009) and probiotics cells (Dolly, Anishaparvin, Joseph, & Anandharamakrishnan, 2011) were efficiently microencapsulated by SFD technique.

The objective of this study is to apply spray–freeze drying technique to produce microencapsulated vanillin using WPI and β-cyclodextrin as wall systems. Further, the effectiveness of SFD technique was compared with SD and FD vanillin based on their morphology, particle size, moisture content, thermal stability, microencapsulation efficiency, and core to wall interaction.

2. Materials and methods

2.1. Preparation of β-cyclodextrin/whey protein isolate and vanillin mixtures for microencapsulation

Total solid content was considered for preparation of microencapsulating feed mixture for SFD, FD and SD methods. Hence, β-Cyclodextrin and vanillin complexation was taken on weight (g) basis. β-Cyclodextrin (10 g, HiMedia Laboratories Pvt. Ltd., Mumbai, India) was mixed with deionized water (80 g) in a beaker and gently stirred using a magnetic stirrer for 5 min at 800 rpm (SLM-MGS, Genei, Bangalore Genei Pvt. Ltd., India). Vanillin (10 g, Mane India Pvt. Ltd., Qutbullapur Nandal, Hyderabad, India) was added to this mixture and homogenised using high-speed homogeniser (IKA Ultra-Turrax T 18 basic, Bangalore, India) at 10,000 rpm for 5 min. This mixture of solution contains 20% of solid content. Whey protein isolate (10 g, British Nutritions Pvt. Ltd., Bangalore, India) and vanillin mixture were prepared in a similar fashion. During the combination of wall materials, β-cyclodextrin (5 g) and whey protein isolate (5 g) were used in the same quantity with equal weight of core material (10 g).

2.2. Spray drying

Spray drying operation was performed in a tall form co-current lab-scale dryer (Spray Mate, JISL, Navi Mumbai, India). Twin fluid nozzle atomiser was used for atomisation of feed liquid using compressed air at 24 psi pressure. The inlet and outlet temperature of the air was controlled with the use of an electric heater. The inlet temperature was maintained at 110 ± 2 °C and outlet temperature was kept at 60 ± 2 °C. The feed solution was fed into the spray chamber only when the desired outlet temperature was achieved. The feed liquid flow rate was maintained at 20 ml/min. The spray dried microencapsulated vanillin powders were collected from the outlet chamber. Finally, the powder was packed in screw capped tube and sealed in aluminium bags and stored at refrigerated condition (4 ± 1 °C) until further analysis.

2.3. Freeze drying

The vanillin with β-cyclodextrin and whey protein isolate were freeze dried using LSI Lyophilization Systems. INC (USA). The freeze drying operation temperature was maintained at −24 °C for a drying period of about 16 h. The microencapsulated vanillin powders obtained were packed in a screw cap tube and sealed in aluminium bags and stored at refrigerated condition until further analysis.

2.4. Spray–freeze drying

SFD was performed in a two-step process. Spray freezing process was the first step followed by conventional freeze drying. During spray freezing, vanillin with β-Cyclodextrin and whey protein isolate mixture was atomised (twin fluid nozzle atomiser) into cold vapour over liquid nitrogen system (locally fabricated unit). Compressed air at 24 psi pressure was used for atomisation of the feed. Feed liquid flow rate was maintained at 20 ml/min with the help of a peristaltic pump. The resultant frozen particles were transferred into a steel petri-plate, and freeze-drying process employed for the sublimation of ice. The temperature of the freeze drying process was maintained at −24 °C. By the end of the drying period (about 4 h), the dried microencapsulated vanillin powders were collected and packed in a screw capped tube and sealed in aluminium bags and stored at refrigerated condition until further analysis.

2.5. Moisture content

The moisture content of microencapsulated vanillin power was determined gravimetrically on wet basis (Bae & Lee, 2008). Approximately 0.5 g of microencapsulated powder were placed in an aluminium pan and dried in hot air oven at 105 °C for 12 h. The moisture content was calculated by the following Eq. (1):

$$\text{Moisture } (\%) = \frac{(W_0 - W_1)}{(W_0)} \times 100 \quad (1)$$

where W_0 is the weight of original dry status of microencapsulated sample, and W_1 is the weight of the sample after the heat treatment. The experiment was carried out in triplicates, and average values were taken for the calculation.

2.6. Morphology studies of microencapsulated vanillin

Scanning electron microscope (Leo 435 VP, Leo Electronic Systems, Cambridge, UK) was used to study the morphology of microencapsulated (SD, FD, and SFD) vanillin powder. The samples were mounted on the specimen holder and sputter-coated with gold (2 min, 2 mbar) and observed at 15 kV in vacuum of 9.75×10^{-5} torr.

Table 1
Moisture content, mean particle diameter, sphericity, compactness, convexity and span values of the microencapsulated vanillin powders.

Drying methods	Type of samples	Moisture content (%)	Mean particle diameter (μm)	Compactness	Span	Sphericity	Convexity
SFD	Vanillin + WPI	6.63 ± 0.02^{c}	24.76	0.81	0.931	0.91	0.99
SFD	Vanillin + β-cyd	4.15 ± 0.13^{a,b}	165.40	0.80	2.953	0.91	0.99
FD	Vanillin + WPI	6.70 ± 0.86^{c}	36.91	0.84	2.105	0.93	0.99
FD	Vanillin + β-cyd	6.99 ± 0.27^{c}	120.1	0.78	1.340	0.87	0.98
SD	Vanillin + WPI	6.40 ± 0.01^{c}	14.18	0.87	1.778	0.94	0.99
SD	Vanillin + β-cyd	3.56 ± 0.53^{a}	42.58	0.85	3.025	0.89	0.99
SD	Vanillin + WPI + β-cyd	4.32 ± 0.05^{b}	41.18	0.87	2.495	0.94	0.99

All the moisture content values are expressed as mean ± SD of three measurements ($n = 3$). Different letters are significantly different at $P < 0.05$ levels according to the Duncan's multiple range test.

2.7. Particle size and shape distribution

The particle size distribution of the microencapsulated vanillin powders were measured using a laser light diffraction particle size analyser (S3500, Microtrac Inc., USA). A small quantity of microencapsulated vanillin powder was dispersed in methanol. The mixture was stirred to attain proper obscuration and analyses were done in triplicates. Width distribution of microencapsulated vanillin particle sizes was determined through span value described by Tonon, Pedro, Grosso, and Hubinger (2012).

2.8. Core and wall interaction

The core and wall interaction were examined by Fourier transform infrared (FTIR) spectroscopy (Nicolet 5700, M/S. Thermoelectron Corporation, Round Rock, TX) for all the SFD, FD and SD microencapsulated vanillin samples. Similarly, unencapsulated vanillin, β-cyd and WPI were also analysed. FTIR-KBr pure compound was used to pelletize all the samples in this experiment. The scanning was kept in the range of 500–4000 cm^{-1}.

2.9. Microencapsulation efficiency (MEE) and surface vanillin efficiency (SVE)

The surface oil content or superficial oil content was calculated as per the method stated by Tan, Chan, and Heng (2005) with some slight modifications. 5 g of microencapsulated powder was weighed in a beaker, and 50 ml of hexane (s d fine-chem Ltd., Mumbai, India) was added. This mixture was slightly shaken for 15 s to extort the surface vanillin. The solvent mixture was then filtered through filter paper to collect the unencapsulated vanillin via vacuum hexane evaporation. The determination of encapsulated vanillin was performed by soxhlet extraction of the same powders for 4 h. Hexane was evaporated to calculate the encapsulated vanillin and microencapsulation efficiency was calculated by Eq. (2):

$$\mathrm{MEE} = \frac{(\text{Total oil} - \text{Surface oil})}{(\text{Total oil})} \times 100 \quad (2)$$

Similarly, loading efficiency (LE) was calculated using the Eq. (3) given by Wang et al. (2012). Encapsulated oil was calculated as per the method given by Tan et al. (2005) with slight modifications. Loading efficiency was calculated from the above experiment as per the following equation where, W_t stands for weight.

$$\mathrm{LE} = \frac{(W_t \text{ of encapsulated oil})}{(W_t \text{ of microcapsules})} \times 100 \quad (3)$$

2.10. Thermogravimetric analysis (TGA)

Thermal stability of the microencapsulated samples and wall materials were analysed using TGA analyser (SII 6300 EXSTAR). The thermogram was recorded from 30 °C to 500 °C at a heating rate of 20 °C/min under nitrogen atmosphere.

2.11. Statistical analysis

Results were expressed as the mean value ± standard deviation of three independent experiments ($n = 3$). Statistical analysis was carried out by analysis of variance (ANOVA) using SPSS statistical software version 16. Comparison of means was performed by Duncan's multiple range analyses at $P < 0.05$.

3. Results and discussion

3.1. Moisture content

The average moisture content of microencapsulated spray–freeze dried, freeze dried and spray dried powders are given in the Table 1. SFD powders showed lower moisture content as compared to FD samples, particularly, WPI showed significant difference ($P < 0.05$). However, SFD and FD microencapsulated powder yielded more moisture than spray dried powders due to higher temperature involved in SD operations. There was a significant difference ($P < 0.05$) of moisture content observed between SD (WPI) in terms of FD (WPI), whereas no significant difference was observed with respect to SFD (WPI). During atomisation, exposure of larger surface area enhances the heat and mass transfer rates and thus lowered the moisture content in SD powders (Kuriakose & Anandharamakrishnan, 2010). The main driving force during drying is the temperature difference, hence higher the temperature difference greater the extent of drying (Anandharamakrishnan, Rielly, & Stapley, 2008). However, microencapsulated vanillin + WPI by spray drying showed slightly higher moisture content due to the crust formation that impedes the water vapour diffusion (Anandharamakrishnan, Rielly, & Stapley, 2007). Furthermore, there was no significant difference between SFD vanillin + WPI and SD vanillin + WPI. Malecki, Shinde, Morgan, and Farkas, (1970), Heldman and Hohner (1974) reported that 'diffusion of water' is the rate controlling factor in freeze drying and hence reduction in the size of the particle could speed up the process. This is because, drying times vary approximately with the square of the sample thickness, and this can be reduced by decreasing the dimensions of the sample (Anandharamakrishnan et al., 2010). Thus, lower moisture content is observed in SFD powders due to large surface area as compared to FD samples.

3.2. Morphology studies

Microencapsulated vanillin morphology is showed in Fig. 1 (by SEM). The spray–freeze dried microencapsulated samples are given in Fig. 1a–d. The process of SFD technique is similar to SD, the only major difference is the spraying of liquid feed into cryogenic liquid (liquid nitrogen). Whey protein isolate SFD microencapsulated

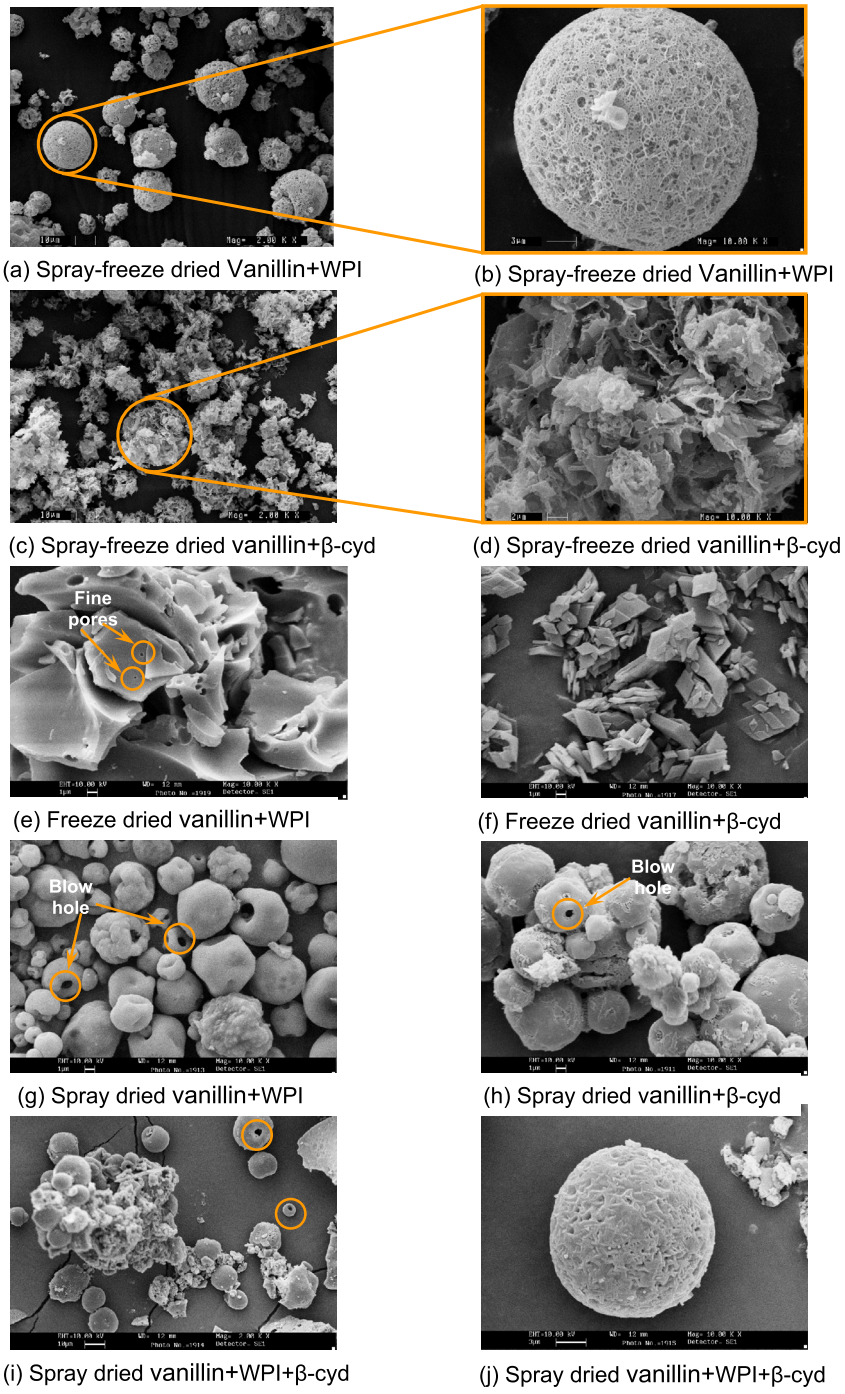

Fig. 1. Powder morphology of SFD, FD and SD vanillin microencapsulated samples with different magnification (Mag). (a) SFD vanillin + WPI, Mag-2000×; (b) SFD vanillin + WPI, Mag-10,000×; (c) SFD vanillin + β-cyd, Mag-2000×; (d) SFD vanillin + β-cyd, Mag-10000×. (e) FD vanillin + WPI, Mag-2000×; (f) FD vanillin + β-cyd, Mag-10000×. (g) SD vanillin + WPI, Mag-10,000×; (h) SD vanillin + β-cyd, Mag-10,000×. (i) SD vanillin + WPI + β-cyd, Mag-2000×; (j) SD vanillin + WPI + β-cyd, Mag-10,000×.

vanillin showed spherical shape with small fine pores on it due to the sublimation of ice crystals during secondary drying (Fig. 1a and b). Similarly, Karthik and Anandharamakrishnan (2013), Dolly et al. (2011) also observed the same type of morphology in their spray–freeze dried DHA and probiotics encapsulated samples, respectively. SFD powder exhibited dual advantages, such as the formation of spherical structure with large surface area like SD and the presence of high porous structure like FD that resulted in lower processing time. On the other hand, β-cyclodextrin did not result in spherical structure and it shows the structural collapse for the spray-freeze drying operation (Fig. 1c and d). However, the spray dried microencapsulated powder (Fig. 1g–j) showed spherical shape which is characteristic of spray dried powders. However, most of the particles have shown blow-holes on the surface of spray dried particles. Similar structure was also observed by Al-Hakim and Stapley (2004) and Rajam, Karthik, Parthasarathi, Joseph, and Anandharamakrishnan (2012). The presence of blow-holes on the surface indicates microencapsulated structure could be shell type. WPI wall material shows smooth, skin forming behaviour which is a typical characteristic of whey protein due

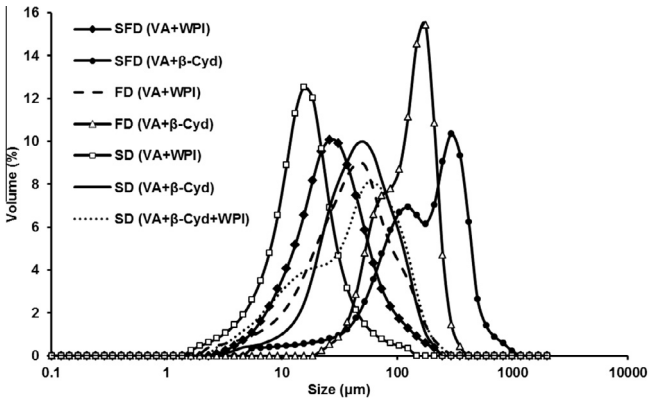

Fig. 2. Particle size distribution of SFD, FD and SD microencapsulated vanillin (VA).

to the principal component β-lactoglobulin, that possess interesting emulsifying and foaming properties (Jouenne & Crouzet, 2000), as compared to β-cyd, which shows striations, irregularities and rough surface. Moreover, wall material of β-cyd (Fig. 1h) and WPI + β-cyd (Fig. 1i and j) used microencapsulated powder exhibited agglomerated structure. Similar results were also reported by Peng, Li, Guan, and Zhao (2013). Agglomeration and structural collapse have been linked with the glass transition of amorphous carbohydrate matrices (Bae & Lee, 2008) and high amount of surface oil content (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001). The freeze dried microencapsulated particles (Fig. 1e and f) showed irregular, flaky, porous and crystalline structures. Similar morphology was reported earlier in freeze dried microencapsulated DHA algae oil (Karthik & Anandharamakrishnan, 2013).

3.3. Particle size and shape distribution

The mean particle diameter and shape distribution of the microencapsulated vanillin samples are shown in Table 1. The particle mean diameter of the samples ranges from 14.2 μm to 165.4 μm. Fig. 2 illustrates the particle size distribution of all the microencapsulated vanillin powders. The spray–freeze dried vanillin + WPI showed low mean particle diameter of 24.8 μm. But, vanillin + β-cyd exhibits slightly higher particle size due to the agglomeration of ice crystals during freezing stage. Freeze dried particles of vanillin + β-cyd showed higher average mean particle diameter (120.1 μm), whereas vanillin + WPI depicted lower mean particle diameter (36.9 μm) which indicate that WPI is better wall material. Moreover, very little amount of vanillin was present on the surface of FD WPI microcapsules. Thus, this lower amount of surface vanillin leads to less agglomeration of microencapsulated powder which reduces particle mean diameter effectively. On the other hand, β-cyd showed higher agglomerated structure thus resulting in higher mean particle diameter. Hence, β-cyd spray dried vanillin powder showed slightly lower mean particle diameter (42.5 μm) as compared to spray–freeze dried and freeze dried powder. Compactness of the microencapsulated vanillin powders is given in Table 1. The overall compactness of the microencapsulated vanillin powder was observed in the range of 0.78–0.87. Spray–freeze dried powder (vanillin + WPI and vanillin + β-cyd) exhibited almost similar compactness values (0.81 and 0.80), whereas freeze dried vanillin + β-cyd powder showed slightly lower (0.78) compactness. This may be due to the rate of slow freezing that leads to lesser dense of FD powders. On the contrary, WPI microencapsulated FD vanillin showed slightly higher value (0.84). Further, span values were also calculated and given in Table 1. The span values of the samples were in the range of 0.931–3.025 (Table 1). The span value depicted the wider distribution of the microencapsulated vanillin samples. Tonon et al. (2012) indicated that higher span value is a sign of wider particle size distribution and *vice versa*. Spray–freeze dried vanillin + WPI showed the lowest span value that illustrates similar sized particle distribution. These results clearly indicates that wall materials and microencapsulation technique significantly affects the shape, size and overall structure of the microencapsulated vanillin samples.

3.3.1. Sphericity and convexity

Sphericity and convexity are used to predict the characteristics of particles which suggested that smaller sphericity value is an indication of irregular shaped particle (Gaiani et al., 2011). Sphericity values of the microencapsulated vanillin samples are given in Table 1. Sphericity value of a particle is comprised between 0 and 1. The β-cyd microencapsulated powder produces low sphericity values, whereas WPI powder produces higher sphericity values. Freeze dried microencapsulated vanillin samples showed more number of irregular shaped particles as compared to spray dried and spray–freeze dried samples. Spray dried particles showed highest sphericity value which indicates spherical shaped particles and it also in line with the SEM results.

3.4. Core to wall interaction study

Core to wall interaction analysis was performed to identify the interaction between vanillin and wall materials in the microencapsulated samples by Fourier transform infrared (FTIR) spectroscopy. Aldehyde, ether and phenol groups are the functional groups present in vanillin, which are used to confirm the presence of vanillin in the encapsulated samples. Fig. 3a shows the FTIR spectra of vanillin wherein absorbance at 1756.6 cm^{-1} and 1100.8 cm^{-1} shows the presence of the aldehyde group and ether group, respectively. The aldehyde group and ether group peaks were present in all the microencapsulated samples that indicated the presence of vanillin (Fig. 3d–j). A comparative study of the FTIR spectra of all the microencapsulated samples with the standard vanillin were shown similar results and it clearly suggested that there was no chemical interactions between the wall and the core (vanillin) materials.

3.5. Microencapsulation efficiency (MEE) and surface vanillin efficiency (SVE)

Microencapsulation and loading efficiency of vanillin by SFD, FD and SD techniques are depicted in Fig. 4. Spray–freeze-dried and freeze dried samples yielded almost equal amount of microencapsulation efficiency whilst using WPI as wall material and there was not much significant difference observed. Whereas, SD microencapsulated samples gave highest microencapsulation efficiency ($P < 0.05$) as compared to SFD and FD in all wall materials. This was due to the smooth surface (without pores) during spray drying operation. In contrast, SFD and FD microencapsulated process produced pores on the surface of the particles (due to ice sublimation during drying). Moreover, microencapsulation efficiency is dependent on surface oil and the presence of surface oil affects the physical properties of powder such as flowability, bulk density, dispersibility and induces rapid lipid oxidation (Keogh et al., 2001). The combination of wall materials showed higher significant differences ($P < 0.05$) when compared to β-cyd wall material alone. In preliminary study, combination (WPI + β-cyd) of wall material was used to find out their effects of encapsulation properties on encapsulation efficiency, thermal stability and morphology by spray drying technique. From this study, it was observed that combination of wall materials yielded 10% lesser encapsulation efficiency than the individual wall materials. Hence, individual wall materials were used to prepare microencapsulation of vanillin by SFD and FD techniques.

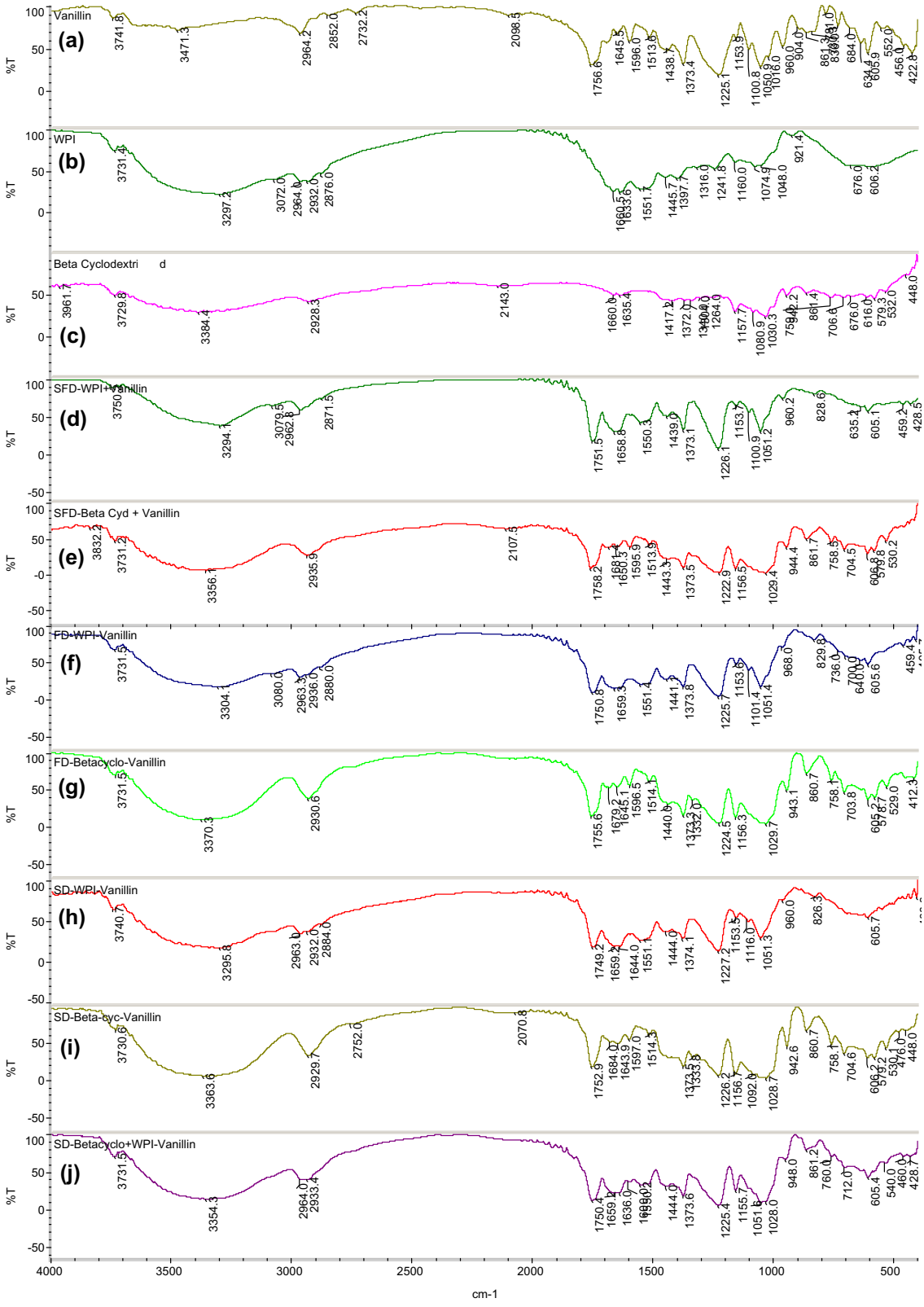

Fig. 3. FTIR spectrogram: (a) vanillin; (b) WPI; (c) β-cyclodextrin; (d) SFD-whey protein isolate + vanillin; (e) SFD-β-cyclodextrin + vanillin; (f) FD-whey protein isolate + vanillin; (g) FD-β-cyclodextrin + vanillin; (h) SD-whey protein isolate + vanillin; (i) SD-β-cyclodextrin + vanillin; (j) SD-whey protein isolate + β-cyclodextrin + vanillin.

Fig. 4 showed a higher level of surface vanillin efficiency (SVE) in SFD microencapsulation technique and it shows significant difference ($P < 0.05$). Whereas, slightly lower level of surface vanillin was observed for other encapsulated powders (SD and FD) with no significant differences. The lower amount of surface oil provides storage stability to microencapsulated materials (Anandaraman &

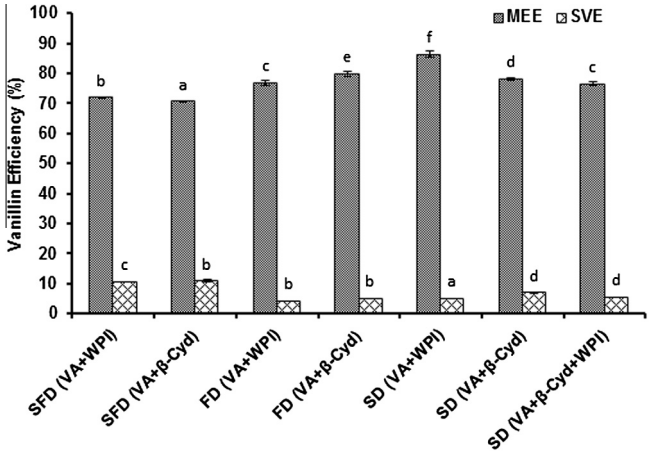

Fig. 4. Microencapsulation efficiency (MEE) and surface vanillin efficiency (SVE) of SFD, FD and SD microencapsulated vanillin (VA). All the MEE and SVE values are expressed as mean ± SD of three measurements ($n = 3$). Different letters are significantly different at $P < 0.05$ levels according to the Duncan's multiple range test.

Reineccius, 1987). During the extraction of surface oil, encapsulated oil near the surface can also be extracted and it gives higher surface oil (Buma, 1971). In addition, amongst all the microencapsulated samples, the WPI SD microencapsulated vanillin sample exhibited lower percentage of surface vanillin and higher percentage of MEE. Thus, WPI showed better retention of the core material because of its highly efficient skin forming behaviour without any pores and cracks on the surface that resulted in lower amounts of surface oil. WPI showed highest loading efficiency (39.22%) with spray–freeze drying technique as compared to FD WPI (34.30%), SD WPI (31.55%), SD β-cyd (31.76%), FD β-cyd (35.31), SFD β-cyd (37.71) and SD combination wall material (32.94%).

3.6. Thermogravimetric analysis

The thermal stability of microencapsulated vanillin can be easily explicated by TGA as it determines the weight loss due to the release of vanillin by increasing the temperature. Fig. 5a and b showed the thermal stability of all the microencapsulated vanillin samples along with the wall materials. The TGA thermogram of wall materials such as whey protein isolate and β-cyclodextrin showed complete degradation at around 450 °C and 400 °C, respectively (Fig. 5a). The TGA thermogram of pure vanillin depicts melting point at 80 °C and onset around at 150 °C, proving vanillin is volatile in nature (Kayaci & Uyar, 2012). Spray–freeze dried and freeze dried samples showed the delayed vanillin release than spray dried samples (Fig. 5b). The spray–freeze dried samples showed vanillin melting at around 155 °C for vanillin + WPI

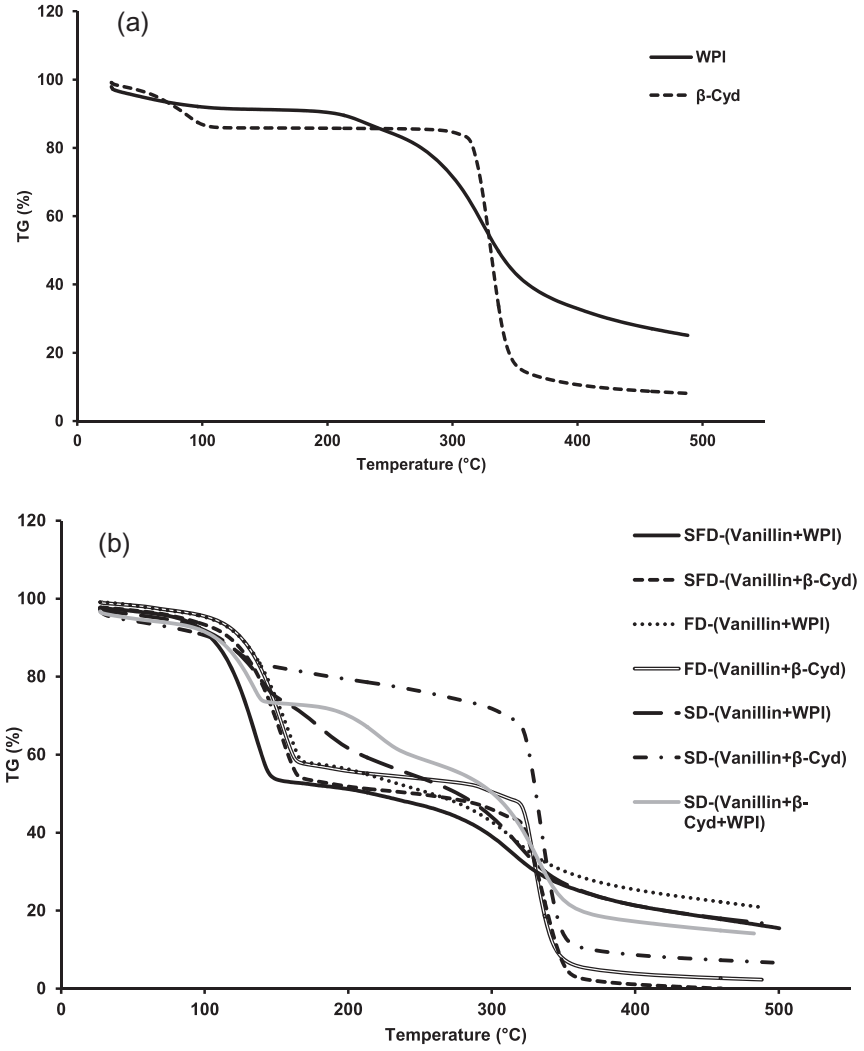

Fig. 5. (a) Thermogravimetric (TG) analysis of whey protein isolate (WPI) and β-cyclodextrin (β-Cyd) wall materials. (b) TG analysis of SFD, FD and SD microencapsulated vanillin.

whereas, vanillin + β-cyd showed at around 160 °C. However, according to the thermogram of vanillin + β-cyd, a steep decline indicates the faster degradation of sample, whereas vanillin + WPI depict gradual decrease representing slow, uniform and continuous release of vanillin. In freeze dried samples, the vanillin melting and subsequent release was achieved at 160 °C for both the wall materials. Moreover, the degradation was achieved at around 480–495 °C. Similar results were also obtained for spray dried microencapsulated vanillin samples, i.e., delayed melting and continuous release of vanillin starting from 135 °C to 350 °C, after which the wall materials starts to degrade. The wall materials decomposition fairly ends at around 480 °C for all the samples.

Closer analysis of the TGA thermogram of all microencapsulated samples showed similarities. At around 100–120 °C, there was some mass loss which can be attributed to the loss of moisture. The second major weight loss starts at around 150–350 °C, this may be due to the continual release of vanillin from the microencapsulated samples. The third loss of mass may be due to the degradation of both released vanillin and wall materials. Similar trends were also obtained by Levic et al. (2013) for microencapsulation of ethyl vanillin in calcium alginate and calcium alginate/poly (vinyl alcohol) beads. The spray–freeze dried samples showed better stability (Fig. 5b) than SD and FD based on TGA analysis. WPI fared complete degradation of the microencapsulated vanillin at around 460 °C temperature as compared to 400 °C of β-cyclodextrin. Thus, WPI encapsulated vanillin showed better sustainability as compared to β-cyd.

4. Conclusion

Vanillin was successfully microencapsulated using spray–freeze drying method. Spray–freeze dried methods yielded microencapsulated vanillin in spherical particles with numerous fine pores (exhibits good rehydration behaviour) whilst using WPI as wall material, whereas, β-cyd wall material did not yield spherical structure. FTIR study indicated that there was no interaction between the wall and core (vanillin) materials. Spray dried powder resulted in lower moisture content than spray–freeze dried and freeze dried powders due to its higher operating temperature. Amongst the three wall materials (WPI, β-cyd and WPI + β-cyd), the WPI yielded 86.2% of encapsulation efficiency by SD technique compared to SFD (72%) and FD (76.8%). Moreover, spray–freeze dried and spray dried samples demonstrated superior particle size distribution and mean particle diameter than freeze dried samples. Microencapsulated vanillin + WPI by spray–freeze drying technique yielded better thermal stability than spray dried and freeze-dried samples.

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References

Al-Hakim, K., & Stapley, A. G. F. (2004). Morphology of spray-dried and spray–freeze-dried whey powders. In *Proceedings of the 14th International Drying symposium (IDS 2004)*, Sao Paulo, Brazil.

Anandaraman, S., & Reineccius, G. A. (1987). Analysis of the encapsulated orange peel oil. *Perfumer & Flavorist, 12*, 33–39.

Anandharamakrishnan, C. (2014). *Techniques for nanoencapsulation of food ingredients*. New York: Springer.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2007). Effects of process variables on the denaturation of whey proteins during spray drying. *Drying Technology, 25*, 799–807.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2008). Loss of solubility of α-lactalbumin and β-lactoglobulin during the spray drying of whey proteins. *LWT – Food Science and Technology, 41*, 270–277.

Anandharamakrishnan, C., Rielly, C. D., & Stapley, A. G. F. (2010). Spray–freeze-drying of whey proteins at sub-atmospheric pressures. *Dairy Science & Technology, 90*, 321–334.

Bae, E. K., & Lee, S. J. (2008). Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of Microencapsulation, 25*, 549–560.

Bhandari, B. R., D'Arc, B. R., & Thi Bich, L. L. (1998). Lemon oil to β-cyclodextrin ratio effect on the inclusion efficiency of β-cyclodextrin and the retention of oil volatiles in the complex. *Journal of Agricultural and Food Chemistry, 46*, 1494–1499.

Buma, T. J. (1971). Free fat in spray-dried whole milk. 10. A final report with a physical model for free fat in spray-dried milk. *Nether Milk Dairy Journal, 25*, 159–174.

Claudio, A. F. M., Freire, M. G., Freire, C. S. R., Silvestre, A. J. D., & Coutinho, J. A. P. (2010). Extraction of vanillin using ionic-liquid-based aqueous two-phase systems. *Separation and Purification Technology, 75*, 39–47.

Dolly, P., Anishaparvin, A., Joseph, G. S., & Anandharamakrishnan, C. (2011). Microencapsulation of *Lactobacillus plantarum* (mtcc 5422) by spray–freeze-drying method and evaluation of survival in simulated gastrointestinal conditions. *Journal of Microencapsulation, 28*, 568–574.

Gaiani, C., Boyanova, P., Hussain, R., MurrietaPazos, I., Karam, M. C., Burgain, J., et al. (2011). Morphological descriptors and colour as a tool to better understand rehydration properties of dairy properties. *International Dairy Journal, 21*, 462–469.

Heldman, D. R., & Hohner, G. A. (1974). An analysis of atmospheric freeze drying. *Journal of Food Science, 39*, 147–155.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal, 11*, 137–144.

Jouenne, E., & Crouzet, J. (2000). Determination of apparent binding constants for aroma compounds with β-lactoglobulin by dynamic coupled column liquid chromatography. *Journal of Agriculture and Food Chemistry, 48*, 5396–5400.

Karathanos, V. T., Mourtzinos, I., Yannakopoulou, K., & Andrikopoulos, N. K. (2007). Study of the solubility, antioxidant activity and structure of inclusion complex of vanillin with β-cyclodextrin. *Food Chemistry, 101*, 652–658.

Karthik, P., & Anandharamakrishnan, C. (2013). Microencapsulation of docosahexaenoic acid by spray–freeze-drying method and comparison of its stability with spray-drying and freeze-drying methods. *Food and Bioprocess Technology, 6*, 2780–2790.

Kayaci, F., & Uyar, T. (2012). Encapsulation of vanillin/cyclodextrin inclusion complex in electrospun polyvinyl alcohol (PVA) nanowebs: Prolonged shelf-life and high temperature stability of vanillin. *Food Chemistry, 133*, 641–649.

Keogh, M., O'Kennedy, B., Kelly, J., Auty, M. A., Kelly, P. M., Fureby, A., et al. (2001). Stability to oxidation of spray-dried fish oil powder microencapsulated using milk ingredients. *Journal of Food Science, 66*, 217–224.

Kondo, M., Niwa, T., Okamoto, H., & Danjo, K. (2009). Particle characterization of poorly water-soluble drugs using a spray freeze drying technique. *Chemical and Pharmaceutical Bulletin, 57*, 657–662.

Kuriakose, R., & Anandharamakrishnan, C. (2010). Computational fluid dynamics (CFD) applications in spray drying of food products. *Trends in Food Science & Technology, 21*, 383–398.

Levic, S., Djordjevic, V., Rajic, N., Milivojevic, M., Bugarski, B., & Nedovic, V. (2013). Entrapment of ethyl vanillin in calcium alginate and calcium alginate/poly (vinyl alcohol) beads. *Chemical Papers, 67*, 221–228.

Malecki, G. J., Shinde, P., Morgan, A. I., & Farkas, D. F. (1970). Atmospheric fluidized bed freeze drying. *Food Technology, 24*, 601–603.

Pena, B., Casals, M., Torras, C., Gumi, T., & Garcia-Valls, R. (2008). Vanillin release from polysulfone macrocapsules. *Industrial & Engineering Chemistry Research, 48*, 1562–1565.

Peng, Z., Li, J., Guan, Y., & Zhao, G. (2013). Effect of carriers on physicochemical properties, antioxidant activities and biological components of spray-dried purple sweet potato flours. *LWT – Food Science and Technology, 51*, 348–355.

Pillai, D. S., Prabhasankar, P., Jena, B. S., & Anandharamakrishnan, C. (2012). Microencapsulation of *Garcinia cowa* fruit extract and effect of its use on pasta process and quality. *International Journal of Food Properties, 15*, 590–604.

Rajam, R., Karthik, P., Parthasarathi, S., Joseph, G. S., & Anandharamakrishnan, C. (2012). Effect of whey protein – Alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. *Journal of Functional Foods, 4*, 891–898.

Rogers, S., Wu, W. D., Saunders, J., & Chen, X. D. (2008). Characteristics of milk powders produced by spray freeze drying. *Drying Technology, 26*, 404–412.

Sinha, A. K., Sharma, U. K., & Sharma, N. (2008). A comprehensive review on vanilla flavor: Extraction, isolation and quantification of vanillin and other constituents. *International Journal of Food Sciences and Nutrition, 59*, 299–326.

Sliwinski, E. L., Roubos, P. J., Zoet, F. D., van Boekel, M. A. J. S., & Wouters, J. T. M. (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. *Colloids and Surfaces B: Biointerfaces, 31*, 231–242.

Stojakovic, D., Bugarski, B., & Rajic, N. (2012). A kinetic study of the release of vanillin encapsulated in Carnauba wax microcapsules. *Journal of Food Engineering, 109*(3), 640–642.

Tan, L. H., Chan, L. W., & Heng, P. W. (2005). Effect of oil loading on microspheres produced by spray-drying. *Journal of Microencapsulation, 22*, 253–259.

Tari, T. A., Annapure, U. S., Singhal, R. S., & Kulkarni, P. R. (2003). Starch-based spherical aggregates: screening of small granule sized starches for entrapment of a model flavouring compound, vanillin. *Carbohydrate Polymers, 53*, 45–51.

Tonon, R. V., Pedro, R. B., Grosso, C. R., & Hubinger, M. D. (2012). Microencapsulation of flaxseed oil by spray drying: Effect of oil load and type of wall material. *Drying Technology, 30*, 1491–1501.

Walton, N. J., Mayer, M. J., & Narbad, A. (2003). Molecules of interest Vanillin. *Phytochemistry, 63*, 505–515.

Wang, Y., Ye, H., Zhou, C., Lv, F., Bie, X., & Lu, Z. (2012). Study on the spray-drying encapsulation of lutein in the porous starch and gelatin mixture. *European Food Research and Technology, 234*, 157–163.