Microencapsulation of flexirubin-type pigment by spray drying: Characterization and antioxidant activity

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Abstract

In this work we directed our inquiries to develop a stable flexirubin powder and to evaluate its properties and antioxidant activity. Flexirubin powders were produced by spray drying using two types of carrier agents: gum arabic (GA) and κ-carrageenan (KC) at different inlet air temperatures (140–220°C). The effect of encapsulation yield, moisture content, hygroscopicity, bulk density, solubility, particle morphology, storage stability and antioxidant activities were determined for both carrier agents. Encapsulation yield, moisture content and bulk densities were greatly influenced by the drying inlet air temperature. The stability test revealed that microencapsulation offered greater protection to flexirubin compared to its free form. Antioxidant activity is higher for microcapsules (687.5 g/kg wet basis) and the boosted properties of microcapsules with high colour stability indicate that this product, which currently has no commercial value, may be used as a natural pigment.

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1. Introduction

Flexirubin-type pigments (FTP) are specific polyene pigments produced by several genera of Bacteroidetes (Flexibacter, Cytophaga, Sporocytophaga, Chryseobacterium and relatives) (Jehlicka et al., 2013). Pigments produced by such Bacteroidetes range from yellow to orange in color, exhibit great potential as natural colourants having no toxic effects, and are proven to have anticancer and antimicrobial activities (Bej, 2011). These compounds may be considered interesting and can be used as food colourants and in the pharma industry (Venil et al., 2015). However, the stability of the pigment may be affected by several parameters, such as storage temperature, light, pH, oxygen, etc. This characteristic has instigated the search for novel and inexpensive processes aimed at increasing FTP shelf life and improving pigment stability. Hence, the stability of these bioactive compounds could be improved using the encapsulation technique which entraps a sensitive ingredient inside the coating material (Saenz et al., 2009).

Spray drying is the most commonly used encapsulation method, as it is economical and can preserve natural colourants by entrapping the ingredient in a coating material (Cai and Corke, 2000; Ahmed et al., 2010). It results in good quality powders with low water activity, which are easy to handle and store and that protect the bioactive material (Carneiro et al., 2013). A successful product must result in a powder with minimum surface oil and maximum retention of the active material, and that protects the product against deterioration (Carneiro et al., 2013; Krishnaiah et al., 2015). Spray drying is a continuous, simple and fast process which involves the atomization of the liquid in a compartment that receives a flow of hot air. Quick evaporation of the water keeps the temperature of the particles low (Souza et al., 2015). Therefore, this method enables drying of heat sensitive products (e.g. food, biological and pharmaceutical products) without affecting their quality (Re, 1998). However, the pigment powders obtained by spray drying have some inconvenient properties, like high stickiness and high hygroscopicity, making their use and storage considerably difficult (Cano-Chauca et al., 2005). Wall materials have been used to overcome these constraints because of their high solubility and low viscosity (Ahmed et al., 2010; Tonon et al., 2010; Berg et al., 2012).

Wall materials can be selected from a wide variety of natural and synthetic polymers based on the characteristics of the desired final product (Wu et al., 2014). The spray drying processes in the food industry are carried out from aqueous feed formation, the wall material must be soluble in water at an acceptable level (Gouin, 2004). The microencapsulation of food ingredients is often

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http://dx.doi.org/10.1016/j.ibiod.2016.01.014
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achieved using biopolymers such as maltodextrin (MD), gum arabic (GA), κ-carrageenan (KC) etc. (Gharsallaoui et al., 2007). GA, common wall material has been used to encapsulate lipids because of its efficient emulsifying properties (Kenyon, 1995) and provides volatile retention during the spray drying process (Bhosale and Singhal, 2006; Rascon et al., 2011). GA has a high molecular weight and is useful for increasing the product’s glass transition temperature and avoiding spray drying operational problems like stickiness to the drying chamber (Tonon et al., 2010). κ-carrageenan (KC) is a good choice of wall material due to its pseudoplastic properties. These properties promote the formation of spherical and smooth-surfaced microcapsules and enhance the adhesion force between the wall and core materials (Su et al., 2008). None of the published works reported the influence of wall materials on the encapsulation efficiency and antioxidant activity of FTP.

The objective of this work is to evaluate the potential of wall materials (GA and KC) for encapsulation of FTP by spray drying. The encapsulated pigments were characterized for encapsulation yield, moisture content, hygroscopicity, bulk density, solubility, morphology, storage stability and antioxidant activity.

2. Materials and methods

2.1. Chemicals

Nutrient broth, gum arabic, κ-carrageenan, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium sulphate (Na2SO4) and magnesium chloride (MgCl2) were purchased from Sigma–Aldrich, Malaysia. Acetone (Laboratory) and methanol (HPLC) grade were used. All other reagents were of analytical grade.

2.2. Culture conditions and extraction of pigment

Chryseobacterium artocarpi CECT 8497, a yellowish-orange pigment producing bacteria used in this study was isolated by our research group (Venil et al., 2014). The bacteria was maintained in nutrient agar and sub-cultured every month. The bacteria was grown in nutrient broth and incubated at 30 °C and 200 rpm for 24 h. The culture broth was centrifuged at 10,000 rpm for 10 min and the resulting pellet (pigment) was extracted with 5% acetone. Then, the extract was sonicated (QSonica, USA) for 20 s twice to break the cells, releasing the left over pigments. The extract was further centrifuged at 8000 rpm for 10 min and the supernatant containing the pigment was concentrated using a rotary evaporator.

2.3. Preparation of feed mixture

Encapsulation in both gum arabic (GA) and κ-carrageenan (KC) was prepared as follows: the carrier agent (GA) was combined with pigment concentrate (the ratio of the carrier agent to pigment was 2:1) at room temperature under magnetic agitation until dissolution. The concentration was selected in a preliminary study. The same procedure was adopted for KC as the carrier agent.

2.4. Spray drying

The spray drying process was performed in a laboratory-scale spray dryer (Lab Ultima LU 222) with a single standard nozzle (0.7 mm). The solutions were fed into the main chamber through a peristaltic pump with a drying air flow rate of 60 m³/h and a feeding rate of 6 g/min. The spray dryer was operated in a co-current air flow mode. The inlet temperature of drying air was set in the range of 140–220 °C, and its outlet temperature was set at 85 °C. The feed mixtures were stirred occasionally to ensure feed homogeneity during spray drying. Dried powders were collected from the base of the cyclone separator of the drier. A schematic representation for the spray drying procedure is shown in Fig. 1.

2.5. Characterization of pigment microcapsules

2.5.1. Encapsulation yield

The encapsulation yield was calculated as the ratio of the mass of the microcapsules obtained at the end of the process to the mass of the initial substances added (pigment and wall material) (Su et al., 2008; Wu et al., 2014).

2.5.2. Moisture and hygroscopicity

The moisture content (%) of pigment powder was determined gravimetrically (air oven method) according to AOAC (1990). Samples (2 g) were placed in an air tight plastic container filled with Na2SO4 (saturated) to determine hygroscopicity. After 1 week, the hygroscopic moisture was weighed and expressed as grams of moisture per 100 g of dry solids (Cai and Corke, 2000).

2.5.3. Bulk density

Bulk density of the pigment powder was measured by weighing 1 g of sample and placing it in a 10 mL graduated cylinder (Chegini and Ghobadian, 2007; Martinez et al., 2010). This was tapped 10 times onto a rubber mat from a height of 10 cm. The volume was then recorded and the bulk density was calculated in terms of g/mL.

2.5.4. Solubility

Solubility was determined following the method of Eastman and Moore (1984) with slight modifications. 0.5 g of the sample was dissolved in 50 mL of distilled water and centrifuged at 10,000 rpm for 10 min. 25 mL of the supernatant was removed and placed in an incubator (100 °C) until a constant weight was obtained. Solubility was calculated based on the initial mass of the sample, and the result was expressed as a percentage.

2.5.5. Particle morphology

Field emission scanning electron microscopy (FESEM) images were obtained for the pigment powders encapsulated in GA and KC to examine the particle’s morphology. The samples were coated with platinum using an auto fine coater JFC-1600 and examined using JEOL JSM 7600F FESEM (Japan). Representative images were taken at ×1500,×2500 and ×5000 magnifications.

2.5.6. Fourier transform infrared spectroscopy

The spectra for non-encapsulated pigment (FTP) and encapsulated pigment powders (GA and KC) were obtained with a PerkinElmer FTIR spectrometer (USA) using a transformation of 20 scans. The spectra were observed in the mid infrared region between 4000 and 500 cm⁻¹.

2.5.7. Storage stability

The storage stability of the pigment powders was determined following the method of Souza et al. (2015) with slight modifications. The pigment powders were placed in a Petri dish for maximum surface exposure and stored in desiccators containing MgCl2 saturated solution (relative humidity 32.8%). The desiccator was kept at 25 °C, as recommended by Labuza (1984), for 120 days and analysed every 30 days for colour parameters using the CIELAB colour system (L*, a*, b*). The colour attributes were measured using a Color Flex EZ meter (Hunter Associates Laboratory, USA). Calibration was done using black and white tiles.
2.6. DPPH radical scavenging activity

The antioxidant capacity as the radical scavenging activity of samples was measured using the method of Luo et al. (2009) with slight modifications. 2 mL of the samples (encapsulated pigment powders and non-encapsulated pigment) were mixed with 2 mL of methanolic solution containing 1 mM DPPH. The mixture was shaken well and incubated for 30 min in the dark. The absorbance was measured at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. The DPPH radical scavenging activity was calculated according to the following equation:

\[
\text{DPPH radical scavenging activity} (\%) = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100
\]

2.7. Statistical analysis

All experiments were done in triplicate and the results were expressed as mean values. The analysis of variance (p < 0.05) and Turkey’s range test (p < 0.05) were used to assess significant differences between the samples. The statistical analysis was carried out using SPSS statistical package (version 10.1).

3. Results and discussion

3.1. Effect of the carrier agent

The pigments were spray dried with an inlet temperature 140–220 °C and carrier agent/pigment at a ratio of 2:1. Two different carrier agents were tested: GA and KC. The encapsulation yield, moisture content, hygroscopicity, bulk density and solubility of the pigment in the microcapsules prepared with different carrier agents is shown in Table 1. The successful encapsulation of pigment should result in an encapsulated powder with the maximum retention of core material inside the particles (Jafari et al., 2008; Wu et al., 2014). The encapsulation yield was influenced by the type of wall material used. The challenge was to encapsulate pigment particles with excellent stability, higher encapsulation yield, practical scale-up capacity and control over the morphology of the encapsulated pigments. This paper addresses these challenges for encapsulating bacterial pigments.

The pigment powder stability was greatly influenced by the choice of the carrier agent. The powder yield and stability were significantly greater when encapsulated with GA than with KC. GA is generally used as a thickening agent, showing a ramified structure with long chains, which may be responsible for its higher yield (Carneiro et al., 2013). GA is a polymer consisting of D-glucuronic acid, L-rhamnose, D-galactose, L-arabinose and 2% protein. The good emulsification properties and high retention of volatile compounds of GA is attributed to the presence of the protein fraction (Dickinson et al., 2003; Domain and Wasak, 2008) and it was found to be the better carrier agent for encapsulating bacterial pigments. Additionally, GA has been used to overcome the expensive cost of some commonly used carrier agents (Gharsallaoui et al., 2007).

3.2. Effect of the inlet temperature

Pigment powders were encapsulated with both carrier agents under five different drying conditions (T_inlet: 140, 160, 180, 200, 220 °C). The data showed that the encapsulation yield of both carrier agents increased with the increase of drying inlet air temperature up to 180 °C and decreased further at increasing temperatures (Table 1). The improvement of pigment powder with the increase of drying inlet air temperature (180 °C) was able to increase the rate of film formation on the surface of the powder particles. This crust is firmer and acts as a protective layer that
limits core material migration of thermolabile and volatile compounds towards the surface (Rascon et al., 2011). However, the encapsulation yield was higher in GA than in KC. Low and high inlet temperatures may break the balance between the water evaporation rate and particle formation rate, leading to a break-down in the microcapsule wall system (Shu et al., 2006). The stability of pigment powder decreased beyond 180 °C, and we propose that 180 °C is the most suitable inlet temperature for the production of microcapsules.

3.3. Characterization of spray-dried pigment

3.3.1. Encapsulation yield

A higher drying temperature usually results in a faster drying rate and higher powder productivity (Cai and Corke, 2000; Tonon et al., 2009; Martinez et al., 2010). The yield was higher in GA, and this could be due to the higher drying air temperatures of GA. The powder productivity increased at increasing inlet temperatures up to 180 °C, and decreased when the inlet temperature was above 180 °C for both carrier agents (Table 1). Zakarian and King (1982) reported that high air inlet temperature causes an excessive evaporation and results in cracks in the membrane that induces premature release and degradation of the encapsulated ingredients.

3.3.2. Moisture content

Moisture content in the present study ranged from 2.96% to 3.12% for both samples at 180 °C. There was not much difference in moisture content between the samples. This may be due to the high temperatures used, which led to greater energy being transferred to the mixture and the evaporation of a higher amount of water (Souza et al., 2015). The moisture content of the pigment powder decreased with increasing temperatures (Table 1). The greater rate of heat transfer of the particle for higher inlet air temperature provided the greater driving force for moisture evaporation (Wu et al., 2014).

3.3.3. Bulk density

The bulk density of the pigment powders decreased with an increase in spray drying inlet temperature (Table 1) above 180 °C for both carrier agents. The higher drying rate obtained at higher temperatures produced the higher ratio of surface to volume for the spray-dried capsules, and caused the lower bulk density of the powders (Cai and Corke, 2000). This led to the formation of vapour impermeable films on the droplet surface, followed by the formation of vapour bubbles and, consequently, droplet expansion. Therefore, there is a greater tendency for the particles to be hollow, resulting in more occluded air within the powder, and there is a possibility for degradation of the pigments and reduced storage stability (Martinez et al., 2010). Chegini and Ghobadian (2007) observed that the effect could also be attributed to the fact that a product with a high moisture content would tend to have a higher bulk density caused by the presence of water, which is considerably denser than the dry solids.

The highest bulk density relates to the molecular weight of GA. GA consists of a mixture of a lower molecular weight polysaccharide (MW ~ 0.25 × 10^6, major component) and a higher molecular weight hydroxyl-proline-rich glycoprotein (MW ~ 2.5 × 10^6, minor component). Its glycoprotein is a high molecular weight hydroxyl-proline-rich arabinogalactan (~2% protein) containing a repetitive and almost symmetrical 19 residue consensus. The heavier the material, more easily it is accommodated into the spaces between the particles, thereby occupying less space and resulting in a high bulk density (Tonon et al., 2010).

3.3.4. Solubility

There was no significant variation in the solubility of the sample with a variation in inlet temperature for GA (Table 1). The solubility was greater in GA (95.29%) than in KC (90.81%). The presence of GA increases the solubility of atomized samples due to this carrier being highly soluble in water, making it one of the materials of interest in spray drying. However, the stability was maintained above 90% in pigment powders for both carrier agents. An increased inlet air temperature led to an increase in the solubility of pigment powders. This may be due to the effect of inlet air temperature on residual moisture content leading to an increase in the particle size and consequently decreasing the time required for the powder to dissolve (Walton, 2000; Sarabandi et al., 2014).

3.3.5. Particle morphology

Fig. 2 shows the scanning electron micrographs of the encapsulated pigment in GA and KC. Structural analysis revealed that microcapsules for GA were irregularly spherical in shape with more surface indentions and looked like smooth spheres with surface cracks in the wall systems. The appearance of cracks on the surface is attributed to rapid evaporation of drops of liquid during the drying process in the atomizer. The morphology for KC was also irregularly spherical shapes with dented surface. These dented surfaces were attributed to the shrinkage of particles during the spray drying process. The particles were observed to be sphere-like collapsed walls and agglomerate structures.

3.3.6. FTIR analysis

FTIR analysis provided information about the chemical bonds and structure of the compounds (Wu et al., 2014). FTIR spectra of the encapsulated pigment powders in GA and KC were recorded (Fig. 3). The spectrum of encapsulated pigment in GA shows bands at 3,454, 1,724, 1,642 and 1,381 cm⁻¹. The characteristic peak at 3,454 cm⁻¹ is due to O–H stretching; 1,724 and 1,642 cm⁻¹ are attributed to the asymmetrical and symmetrical stretching of the

<table>
<thead>
<tr>
<th>Carrying agent</th>
<th>Inlet temperature (°C)</th>
<th>Encapsulation yield (%)</th>
<th>Moisture content (%)</th>
<th>Hygroscopicity (g/100 g)</th>
<th>Bulk density (g/cm³)</th>
<th>Solubility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>140</td>
<td>56.03 ± 0.06</td>
<td>5.3 ± 0.04</td>
<td>46.8</td>
<td>0.43 ± 0.02</td>
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<td>160</td>
<td>63.18 ± 0.11</td>
<td>3.5 ± 0.02</td>
<td>46.2</td>
<td>0.49 ± 0.03</td>
<td>92.65 ± 0.06</td>
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<tr>
<td></td>
<td>180</td>
<td>70.06 ± 0.15</td>
<td>2.96 ± 0.02</td>
<td>44</td>
<td>0.62 ± 0.011</td>
<td>95.29 ± 0.02</td>
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<tr>
<td></td>
<td>200</td>
<td>59.51 ± 0.09</td>
<td>2.70 ± 0.06</td>
<td>44.6</td>
<td>0.64 ± 0.012</td>
<td>94.50 ± 0.06</td>
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<tr>
<td></td>
<td>220</td>
<td>34.7 ± 0.05</td>
<td>2.50 ± 0.01</td>
<td>45.4</td>
<td>0.61 ± 0.016</td>
<td>94.37 ± 0.01</td>
</tr>
<tr>
<td>KC</td>
<td>140</td>
<td>20.54 ± 0.13</td>
<td>6.01 ± 0.03</td>
<td>46</td>
<td>0.31 ± 0.013</td>
<td>89.75 ± 0.08</td>
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<tr>
<td></td>
<td>160</td>
<td>37.41 ± 0.09</td>
<td>5.8 ± 0.07</td>
<td>45</td>
<td>0.35 ± 0.017</td>
<td>90.04 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>54.87 ± 0.12</td>
<td>3.12 ± 0.01</td>
<td>44</td>
<td>0.377 ± 0.016</td>
<td>90.81 ± 0.83</td>
</tr>
<tr>
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<td>2.9 ± 0.01</td>
<td>46</td>
<td>0.41 ± 0.018</td>
<td>91.26 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>220</td>
<td>18.31 ± 0.17</td>
<td>2.4 ± 0.08</td>
<td>43</td>
<td>0.42 ± 0.013</td>
<td>91.62 ± 0.01</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.
C=O group; and 1381 cm\(^{-1}\) is attributed to the ester C=O group. The spectrum of encapsulated pigment in KC shows bands at 3,450, 1,727, 1,600 and 1,385 cm\(^{-1}\). The peaks were similar to those of encapsulated pigment powders in GA. The peaks observed with the encapsulated pigment powders in GA and KC were compared with non-encapsulated pigment (flexirubin) (Fig. 3). The peaks that appeared with flexirubin also appeared with the encapsulated pigment powders in GA and KC, and those characteristic peaks did not show any shifts, indicating there is no structural changes between flexirubin and encapsulated flexirubin. This shows that the spray drying did not affect the structure of the flexirubin.

3.3.7. Storage stability

Table 2 shows the variation in colour parameters L* (luminosity), a* (difference between red and green) and b* (difference between blue and yellow) during the storage period at 25 °C and 32.8% of relative humidity. The colour parameters were evaluated for pigment powders encapsulated in GA, KC and non-encapsulated pigment. As the encapsulating agent is white, a greater percentage of this material makes the sample lighter (Souza et al., 2015), and the values obtained for L* are higher. The darkest one was the non-encapsulated pigment, showing a low L* value, probably because it was the most concentrated pigment. The storage period affects the L* value, the value increases for non-encapsulated pigment at 120 days and there is only a minor variation in L* for the pigment in encapsulated forms. Parameter a*, which measures the difference between green (−) and red (+) is shown in Table 2. There is a decrease in a* starting from 60 days of storage in the non-encapsulated pigment. However, there is not much difference in the values of a* for pigment powders in the encapsulated forms. Parameter b* measures the difference between blue (−) and yellow (+). The values of b* start decreasing from 90 days of storage in the non-encapsulated form, and the encapsulated pigments showed stability until 120 days of storage. Colour characteristics were more preserved in the encapsulated samples than in the non-encapsulated pigment. Thus, the stability of the spray dried pigments may expand its applicability in the food and pharma industries.

3.4. Antioxidant activity

The antioxidant activity of all samples ranged from 341.4 to 687.5 g/kg wet basis (Table 3). The encapsulated pigment showed significantly higher activity compared to the non-encapsulated pigment. The antioxidant activity of the pigment can be attributed to the high phenolic OH groups present in the pigment. The

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![Image](image-url)
pigment has the elementary composition of C_{13}H_{26}O_{13}. The encapsulated pigment showed good protection against oxidation, which is attributed to the amphiphilic properties of the phenol constituents. It is assumed that an increase in the number of hydroxyl groups in a phenol enhances the hydrogen donor ability and the inhibition of oxidation (Evans and Miller, 1996). Thus, encapsulated pigment has higher antioxidant activity.

4. Conclusions

The microcapsules of FTP were successfully prepared using the spray drying method and it is possible to produce water-soluble pigment powders. The microcapsules showed good encapsulation yield and good product stability for GA in comparison to KC. The FTIR spectrum of microcapsules (GA and KC) is similar to the spectrum of flexirubin and did not show any shifts, indicating there is no structural change between the flexirubin and microcapsules. Antioxidant activity is higher for microcapsules in comparison to non-encapsulated pigment. According to the results, GA could be suggested as a good carrier agent for FTP, which results in good antioxidant activity and better encapsulation efficiency, and the data obtained could be useful to scale up the process. This study would be helpful to promote the application of FTP, which shows a remarkable potential for the food and pharma industries due to its low cost. However, further study and research is recommended over a longer and continuous time period to obtain reliable and conclusive results.

Acknowledgements

The authors are thankful to the Universiti Teknologi Malaysia for the ‘Visiting Researcher’ Fellowship (Q.J090000.21A4.00D20) to Dr. C.K. Venil. The authors would like to thank the Research University grants (Q.J130000.2526.07H03, Q.J130000.2526.10J38, RJ.130000.7826.4F454), Ministry of Agriculture, Malaysia for the Techno fund grant (TF0310F080) for financial support of research activities.

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