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# Enhanced Microencapsulation of C-Phycocyanin from *Arthrospira* by Freeze-Drying with Different Wall Materials

Running title: C-phycocyanin microcapsule freeze-dried powder from *Arthrospira*

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## SUMMARY

*Research background*. C-phycocyanin (C-PC) as a water-soluble blue pigment was extracted from microalgal *Arthrospira*. C-PC could be a good substitute for synthetic pigments with high antioxidant activity. However, C-PC is unstable due to sensitivity to temperature, light, pH, and oxygen; therefore applications of C-PC in food and other products are limited. Microencapsulation of C-PC using freeze-drying is a solution to this problem and is considered a suitable method for drying heat-sensitive pigment.

*Experimental approach*. C-phycocyanin was extracted from *Arthrospira platensis*. C-phycocyanin microcapsules were modified by freeze-drying, with different ratios at 0-100 % of maltodextrin (MD) and gum Arabic (GA) used as microencapsulation wall materials. The powders produced were evaluated for physical properties including moisture content and water activity,

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solubility, hygroscopicity, bulk density, colour appearance, particle morphology and size distribution. Thermal stability and antioxidant activity of freeze-dried C-PC microencapsulated powders were also assessed.

*Results and conclusions*. Freeze-dried C-PC microencapsulated powders with maltodextrin and gum Arabic as wall materials gave high encapsulation efficiency of around 99%. At higher gum Arabic percentage, moisture content decreased and water activity improved. Maltodextrin gave higher solubility of C-PC powders whereas gum Arabic led to a similar colour of C-PC without microencapsulation. Freeze-dried C-PC microencapsulated powders were composed of different sized microparticles regardless of the combination of wall materials with amorphous glassy shapes. Thermal stability of encapsulated C-PC increased and also showed high antioxidant properties.

*Novelty and scientific contribution*. C-PC microcapsules that maintain colourant stability with high antioxidant levels and resistance to high temperatures can be applied in a wide variety of products and also in the food industry.

**Key words:** phycocyanin, *Arthrospira*, microencapsulation, freeze-drying, antioxidant properties

## INTRODUCTION

Colour is one of the most important attributes in the food industry and greatly influences product acceptability by consumers (*1*). Blue colours are rare in nature and bright blue food colours are often artificial (*2*). Increasing consumer health awareness has highlighted toxicity levels of synthetic colourants used in food (*3*). Seeking naturally derived blue-shaded colourants to replace artificial additives has recently become a major challenge for the food, pharmaceutical, and cosmetics industries (*2*,*4*).

The natural blue colour of C-phycocyanin (C-PC) is produced by the photoautotrophic cyanobacteria *Arthrospira platensis* (namely *Spirulina*). *Arthrospira* is considered as a nontoxic, non-carcinogenic natural blue colourant for food and cosmetic applications (*5*). Moreover, the US Food and Drug Administration (FDA) classified *Arthrospira* extract as a colour additive exempt from certification and approved its use for confectionery (including sweets and chewing gum), frostings, ice cream and frozen desserts, dessert coatings and topping, beverage mixers and powders, yoghurts, custards, puddings, cottage cheese, gelatin, breadcrumbs and ready-to-eat cereals. In the European Union, *Arthrospira* extract is classified as colouring foodstuff (*2*). Nowadays, food manufacturers are activity looking for natural additives (*6*). Protein content of *Arthrospira* ranges from 50 to 70 % dry mass with C-phycocyanin phycobiliprotein the major source (*7*).

C-phycocyanin (C-PC) is a water-soluble light harvesting pigment protein and offers many applications as a natural colourant for food and cosmetics (*8*). Interest in natural sources of C-PC has been growing because they may promote human health. Previous reports suggested various C-PC properties as antioxidant, anticancer, anti-inflammatory and other bioactivities which decrease reactive oxygen species (ROS), thereby promoting healthy cells with potential therapeutic applications in pulmonary diseases (*9*). C-PC is already used as a colourant; however, the natural blue colour is unstable in aqueous solution.

Microencapsulation is defined as a process of packaging solids, liquids, gases or sensitive ingredients, called core materials, in coating or wall materials to form capsules micrometres to millimetres in size based on a drying technique (*10*). The wall materials protect the sensitive ingredients from external influences, control the release of the ingredients and sometimes convert liquids into powders which are easier to handle (*11*). Various kinds of microencapsulation techniques such as emulsification, coacervation, spray drying, spray cooling, freeze-drying, fluid bed coating and extrusion have been developed (*12*). C-PC encapsulation was studied using alginate and chitosan following the extrusion method (*13*,*14*). However, the final product of C-PC encapsulation is required as a dry ingredient for ease of manufacture or consumption.

Among microencapsulation techniques, freeze-drying or lyophilisation is a process used to dehydrate heat-sensitive ingredients (*15*). However, the drying technique and material used as coating usually affect the retention capacity of ingredients within the matrix (*16*).

Using different wall materials resulted in different chemical properties of the microencapsulated powders such as moisture content, water activity, hygroscopicity and shelf life depending on the structure and characteristics of each wall material (*17*). Water plays a vital role as a major component of food products and influences food safety, stability, quality and physical properties (*18*). The solubility parameter is associated with reconstitution of powder, while hygroscopicity is essential for powder stability and storage (*19*). Colour is an important factor for dried products used as colourant ingredients (*20*). Colour is defined in terms of luminosity ($L^*$) red versus green ($a^*$) and yellow versus blue ($b^*$). Thermal analyses of DSC and TGA are important tools in determining the behaviour of microencapsulated natural colourants and their potential use in food (*21*).

It is important to consider the type of wall material used in the microencapsulation process because this may influence encapsulation efficiency and stability of the capsules (*22*). Maltodextrins (MD) with different molecular masses are products of hydrolysed starch and these compounds are commonly used as wall materials for microencapsulation. MD offers advantages due to its low cost, high water solubility, neutral aroma and taste, low viscosity at high solids concentration and low sugar

content (*23*). MD offers advantages due to its low cost, high water solubility, neutral aroma and taste, low viscosity at high solids concentration and low sugar content (*24*). MD with dextrose equivalent (DE) between 10 and 20 is widely used as a wall material in the encapsulation technique. Anthocyanin-rich blackberry microcapsules showed better results using maltodextrin 10 than 20DE (*20*). Moreover, gum Arabic (GA) is a hetero-polysaccharide with unique properties of emulsification, low cost and high solubility (*25*). Combination of different types of wall materials can increase encapsulation efficiency (*26*).

Selection of suitable wall materials is important to enhance the efficiency and properties of C-PC microcapsules as a coloured bioactive compound in food applications. Here, C-PC extracted from *A. platensis* was selected to generate microencapsulated powders by freeze-drying using different ratios of maltodextrin 10DE (MD) and gum Arabic (GA) as wall materials. Physical properties and thermal analysis of the C-PC microcapsules were evaluated.

## MATERIALS AND METHODS

### *Arthrospira microalgal preparation*

*Arthrospira platensis* IFRPD 1182 microalgae were sourced from the Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand. *Arthrospira* biomass production was generated in 500 L open raceway ponds (IFRPD, Kasetsart University, Thailand) of working volume 200 L in Zarrouk medium. The biomass was grown to exponential phase, harvested by nylon filtration and then cleaned with tap water to remove residual culture medium. *A. platensis* biomass was dried in a hot air oven (UT6760 Double Door, Thermo Scientific Heraeus Heating and Drying Ovens, Thermo Fisher Scientific Inc., Thermo Scientific, Germany) at 60 °C for 4-6 h and then milled to 0.5 mm particle size.

### *Extraction of C-phycocyanin*

C-phycocyanin (C-PC) was extracted from *Arthrospira* oven-dried biomass suspended in distilled water at a ratio of 0.06 g/mL and incubated under a controlled temperature at 25 °C for 24 h in the dark. The suspension was then centrifuged at 22,000x*g* for 30 min (Sorvall RC6 Plus Superspeed Centrifuge, Thermo Fisher Scientific Inc., Thermo Scientific, Germany) at 25 °C and C-phycocyanin was concentrated using a vacuum evaporator (R215, Buchi Ltd., Switzerland) to reduce to 1/3 of the initial volume and then stored in the dark at 4 °C until required for further experiments.

*Microencapsulation procedure*

Wall materials including maltodextrin 10DE (MD) (GB/T20884, food grade powder, Thai Food and Chemical Co., Thailand) and gum Arabic (GA) (KB-120, food grade powder, MT Instruments Co., Thailand) were mixed and dissolved in distilled water at room temperature. Combinations of wall materials at five different ratios were studied including MD:GA 0:100, 25:75, 50:50, 75:25 and 100:0 % (*m*/*m*). Wall material solutions were prepared at 40 % (*m*/*m*) solid and kept at 4 °C for 24 h to complete hydration. Solutions of concentrated C-phycocyanin extracted from *Arthrospira* and wall materials were mixed in a weight ratio (*m*/*m*) of 1:3 (C-PC:wall material). C-PC concentrate without wall material was used as a control (Free C-PC). The solutions were mixed with a high-speed homogeniser (Ultra Turrax, IKA, USA) at 12,000 rpm for 3 min with temperature controlled by cool water in an outer jacket to not exceed 25 °C. The mixture was then frozen at -20 °C for 24 h, followed by freeze-drying in a pilot-scale freeze drier (VFD-12SH, Grisrianthon Co., Thailand) at pressure ranging 30-60 Pa for 20 h. The dried samples were ground using a mortar and pestle and the powders were packed in polyethylene bags and stored in the dark until required for further analysis. All experiments were performed in triplicate.

*C-phycocyanin concentration*

Absorbance (*A*) of C-phycocyanin was performed at 615 and 652 nm using a UV-visible spectrophotometer (SP-8001, Metertech Inc., Taiwan). C-PC concentration was calculated by the following equation:

$$\text{C-PC (mg/mL)} = \frac{A_{615} - 0.474\,A_{652}}{5.34} \quad /1/$$

where $A_{615}$ is the absorbance of the sample at 615 nm, and $A_{652}$ is the absorbance at 652 nm.

*Determination of microencapsulation efficiency*

To evaluate the effectiveness of C-PC microencapsulation, C-PC content (C-PC) and surface C-PC (SC-PC) of the microcapsules were determined following the modified method of Laokuldilok & Kanha (*27*). For the determination of C-PC, the samples were reconstituted by adding distilled water 10 mL and continuously vibrated using a vortex mixer for 3 min. Then, the mixture was centrifuged at 22,000x*g* for 10 min at 25 °C (Sorvall RC6 Plus Superspeed Centrifuge, Thermo Fisher Scientific Inc., Thermo Scientific, Germany). The clear supernatant was collected and filtered through a 0.45 mm pore-sized Millipore membrane to measure C-PC content.

To determine SC-PC, 100 mg of samples were directly extracted with 10 mL of 95 % (*V*/*V*) ethanol solution. The mixture was performed by continuously vibrating with a vortex for 30 min, followed by centrifugation at 10,000 rpm for 10 min at 25 °C. After phase separation, the clear supernatant was collected and filtered through a 0.45 mm pore-sized Millipore membrane and C-PC content was determined by absorbance. Microencapsulation efficiency was calculated by the following equation (*26*):

$$\text{EE (\%)} = \frac{\text{(C-PC)} - \text{(SC-PC)}}{\text{(C-PC)}} \times 100 \qquad /2/$$

where (C-PC) is the C-phycocyanin concentration calculated using equation 1, and (SC-PC) is the surface C-phycocyanin concentration.

*Moisture content and water activity*

Moisture content of the C-PC microcapsule powder was determined gravimetrically. Samples and aluminium cans were pre-weighed and dried in an oven at 105 °C for 24 h. Water activity ($a_w$) was measured using the principle resistive electrolytic humidity measuring system at 25 °C (LabMaster- $a_w$, Novasina AG., Switzerland).

*Solubility*

C-PC microcapsule powder solubility was evaluated following the method of Yamashita *et al.* (*20*). Briefly, samples were dissolved in distilled water and then stirred at room temperature for 30 min. The suspension was then centrifuged at 11,000x*g* for 5 min (Sorvall RC6 Plus Superspeed Centrifuge, Thermo Fisher Scientific Inc., Thermo Scientific, Germany). The aliquot supernatant was transferred to a pre-weighed aluminium can and dried at 105 °C in an oven until constant weight. Dry mass of the soluble solid was measured and percentage solubility of the powder product was calculated.

*Hygroscopicity*

Hygroscopicity of C-PC microencapsulated powder was determined as the tendency of a product to absorb moisture from the surrounding atmosphere. Samples were storage under 20 °C in desiccators which contained saturated sodium chloride solution at 75 % relative humidity and $a_w$ 0.75. The samples were weighed before storage and again after 1 week. The hygroscopicity was calculated as grams of absorbed moisture per 100 g of dry solids (g/100g) (*28*).

*Bulk density*

Ten grammes of C-PC microcapsules were poured into a 10 mL graduated cylinder. Bulk density was calculated by dividing powder weight by volume in the cylinder (g/cm$^3$) (*29*).

*Color measurement*

Colour of the C-PC microencapsulated powder was measured using a Datacolour Spectraflash Spectrophotometer (SF 600 plus, Datacolour International Co., USA). Colour measurements were expressed in terms of lightness (*L**) from 0 (black) to 100 (white) with chromaticity parameters (*a**) as green colour (-) to red (+) and *b** as blue (-) to yellow (+).

*Particle morphology and size distribution*

Particle microstructure of C-PC freeze-dried powders was evaluated using a Scanning Electron Microscope (SEM) (SU8020, Hitachi High-Technologies Corporation, Japan). Samples were placed in a carbon layer support and coated with a layer of platinum. The SEM was operated using an acceleration voltage of 5 kV with 5,000x and 1,000x magnification. Particle size was measured using a laser light diffraction instrument (Mastersizer 2000, Malvern Panalytical Ltd., UK). A small quantity of C-PC microcapsule powder was suspended in isopropanol under magnetic agitation using a sample dispersion unit connected to the equipment. Particle size distribution was observed until successive readings became constant and expressed as D [4,3], the De Brouckere mean diameter used to characterise a particle (*20*).

*Thermal stability*

Thermal stability of C-phycocyanin microencapsulated powders was evaluated using a differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA). In both analyses, a small sample of around 4-6 mg was loaded in a silver pan and crucible for DSC and TGA respectively. An empty pan and crucible were used as reference material. For DSC analyses (DSC3+, STAR$^e$ system, Mettler Toledo, Switzerland), the pans were sealed and scans were run at a heating rate of 10 °C/min, under nitrogen flow at 50 mL/min from 15 to 250 °C (*30*). Dynamic assays of TGA were performed using a thermobalance (TGA/DSA3+, STAR$^e$ system, Mettler Toledo, Switzerland). Temperature programmes for the assays were performed from 25 to 800 °C at a heating rate of 10 °C/min under nitrogen flow at 50 mL/min (*31*).

*Antioxidant activity*

C-PC microencapsulated powders were evaluated for radical scavenging activity of different wall materials using DPPH antioxidant assay (2, 2-diphenyl-1-picrylhydrazyl). The samples were dissolved in distilled water. Two millilitres of sample solutions were mixed with 1 mL of 200 µM DPPH in an ethanol solution. The mixtures were incubated at room temperature for 30 min. Absorbance of the mixture was measured at 517 nm by a UV-vis spectrophotometer (SP-8001, Metertech Inc., Taiwan). Percentage of inhibition (%) was calculated by the following equation (*32*):

$$\text{Inhibition (\%)} = \frac{(A_{\text{blank}}) - (A_{\text{sample}})}{(A_{\text{blank}})} \times 100 \qquad /3/$$

where $A_{\text{blank}}$ is the absorbance of control and $A_{\text{sample}}$ is the absorbance of the sample.

*Statistical analysis*

Data were analysed by analysis of variance (ANOVA) using SPSS V.11.0 (*33*). Duncan's multiple range test (DMRT) was used to assess significant differences between the samples at $p < 0.05$. All experiments were performed in triplicate.

## RESULTS AND DISCUSSION

*Effect of wall materials on C-phycocyanin microcapsules*

Microencapsulation is an enduring technology for protection and controlled release of food ingredients (*34*). For microencapsulated loading of one or more bioactive ingredients, the key functional properties include encapsulation efficiency, size, morphology and also stability under storage (*35*). C-phycocyanin (C-PC), the blue colour from *Arthrospira* is a natural resource, generally recognised as safe (GRAS) for human consumption (*36*). C-PC was extracted from *Arthrospira*, followed by evaporation to increase total solid content from 1.25 ± 0.26 % (*m*/*m*) in the aqueous extract to 5.20 ± 0.23 % (*m*/*m*) in C-PC concentrate. C-PC content was 7.31 ± 0.76 mg/mL in the extract solution which increased to 18.89 ± 1.11 mg/mL after concentration by evaporation with volume reduced to 1/3 of the original amount.

After freeze-drying at different combinations of MD and GA, C-PC content in the microencapsulated powders and efficiency of encapsulation are shown in Table 1. C-PC content in microcapsules ranged 18.85-20.48 mg/g. A higher ratio of GA gave higher C-PC remaining in the microcapsules. Moreover, adding GA as the wall material showed better results than using MD for encapsulation efficiency. Our results determined high encapsulation efficiency of freeze-dried C-PC powders. Most commonly used wall materials are maltodextrin, gum Arabic, emulsifying starches and

whey protein (*34*). Moreover, modified starch and gelatin were used as wall materials in freeze-drying of turmeric microcapsules (*37*). Ezhilarasi *et al*. (*34*) found that wall materials of whey protein and maltodextrin had excellent encapsulation efficiency during freeze-drying of *Garcinia* fruit extract. Highest encapsulation efficiency of 78-97 % was obtained for freeze-dried *Averrhoa carambola* extract compared with spray-drying (*38*).

Table 1

*Physical properties*

After freeze-drying, moisture contents, water activity ($a_w$), solubility, hygroscopicity and bulk density of C-PC microencapsulated powders are shown in Table 2. Results showed decreasing moisture content with increasing ratio of GA in the wall materials combination. Moisture content was significantly lowest at 0.99 % with 100 % GA as the wall material. Moreover, $a_w$ values were not significantly different when using higher MD and $a_w$ decreased with increasing ratio of GA. However, moisture content and $a_w$ recorded in free C-PC as the control were higher than in C-PC microcapsules. Moisture content and water activity ranged at 0.99-3.38 % and 0.07-0.19 respectively for different wall material ratios. Moisture content of food affects its storage, packaging and processing (*20*), while water activity plays a major role in determining both quality change and microbial growth or survival as it indicates the amount of free water available for microbial growth and quality change. To prevent microbial growth, water activity below about 0.6 is needed (*39*). The higher the $a_w$, the more free water is available for biochemical reactions and shorter shelf life is predicted (*20*). Average $a_w$ for different wall materials was lower than $a_w$ for free C-PC. Therefore, C-PC microencapsulated powders were considered relatively more stable against microbial growth and hydrolytic and enzymatic reactions with $a_w$ values less than 0.6 (*40*). Moreover, freeze-drying of lemon by-product aqueous extract using maltodextrin and soybean protein determined microparticles with lower moisture content and water activity than those produced by spray-drying (*41*).

Solubility, hygroscopicity and bulk density of the different wall materials used in the microencapsulation process are shown in Table 2. Results indicated that all microencapsulated powders had excellent solubility with values ranging from 93.3 to 97.1 % and higher than free C-PC. Highest solubility was obtained from C-PC microcapsules with 100 % MD wall material. Colourant powders used as ingredients for the food industry must exhibit good solubility. Our results showed that different ratios of maltodextrin and gum Arabic used as wall materials did not affect the solubility values. Hygroscopicity ranged from 8.1 to 13.7 %. Higher MD or MD equal with GA showed lower hygroscopicity but with no significant differences ($p > 0.05$). Low hygroscopicity of powders resulted

in lower adsorptions and thus lower molecular mobility (*42*), whereas higher GA ratio of wall materials showed higher significant hygroscopicity ($p < 0.05$).

Bulk densities of all C-PC microencapsulated powders and free C-PC were around 0.6 g/cm$^3$. Wall material composition showed no influence on bulk density. Bulk density of fat powder capsules containing fat and PUFA-rich oils using different wall materials and liquid oils showed no effect on bulk density and supported this result (*43*). However, bulk properties of food powder are highly dependent on particle size and its distribution (*44*). Moreover, bulk density decreased with increase in inlet air temperature for encapsulation of vegetable oil by spray drying. Gum Arabic is the most commonly used wall material due to its high soluble fibre content, prebiotic effect, highly digestive tolerance and low caloric value. Gum Arabic is also suitable for various formulations of functional foods as it is non-cariogenic (*45*).

Table 2

C-PC microcapsules are used as colourants in food products. Colour of C-PC microencapsulated powders and free C-PC revealed that different wall materials had no significant effect on lightness, whereas free C-PC was darker (lower *L** value) and deeper blue (*b**) (Table 3). Blue shade colours (*b**) for mixtures of wall materials from high ratio of maltodextrin to low ratio were deeper blue. Lightness (*L**) was lighter in C-PC microencapsulated powders with higher ratio of maltodextrin wall material mixture. Destruction of C-phycocyanin reduced pigment with lighter powders (*38*) that were less blue. Therefore, blue colour of C-PC microencapsulated powders using gum Arabic as wall material gave blue colour (*b**) comparable with the control (free C-PC).

Table 3

Fig. 1 shows the external morphology of freeze-dried C-PC microencapsulated powders with different wall materials and size particle distribution with free C-PC as a control. The microparticles showed a structure similar to a broken glass of variable sizes. Structural characteristics of freeze-dried powders were irregularly shaped like broken glass of various sizes (*46*). At low temperatures of freeze-drying the physical state is important for frozen food stability (*47*). A glassy structure with irregular shape might protect the bioactive compounds against heat and oxygen exposure (*20*). The micrographs showed that only 100 % maltodextrin used as wall material resulted in porous powders. Loss of porous structure was observed in microcapsules when gum Arabic was added as wall

material, possibly due to increased hygroscopicity. However, free C-PC did not show a porous structure.

Fig. 1

A size distribution graph of all experiments and free C-PC (control) particles is presented as Fig. 2. All experiments showed only one distinct peak with particle diameter varying from 1.5 to 316 μm. Mean particle diameters of C-PC microencapsulated powders at different wall material conditions and free C-PC were in the range 51 to 74 μm (Table 3). C-PC microencapsulated powder with higher maltodextrin in the wall material mixture had smaller particle size, while higher gum Arabic increased particle size ($p < 0.05$). Large particle size of freeze-dried samples was caused by the low temperature process and lack of strength necessary to break the frozen drops or to alter the surface during drying. Particle size is related to kinetic solubility which increases as particle size decreases. Moreover, solubility influenced particle size dissolution (*48*). Large particle size influences solubility and higher solubility is associated with smaller particle size because of the greater surface area available for hydration (*19*).

Fig. 2

*Thermal stability*

Table 4 shows DSC and TGA analysis results for characterisation and evaluation of the formation of C-PC microencapsulation natural blue colourant with different wall material ratios. DSC measures the physical properties of C-PC microcapsules and free C-PC powder change was determined for temperature against time. All experiments obtained one peak from the DSC thermogram caused by C-PC and wall materials combined as homogeneous with the microencapsulated powders. Glass transition temperature ($t_g$) was determined as the midpoint of the beginning ($t_0$) temperature and endpoint ($t_f$) temperature range of the endothermic peak. Results show that glass transition temperatures of C-PC microencapsulated powders were in the range 158 to 173 °C, whereas free C-PC gave a lower midpoint temperature at 152 °C. Therefore, freeze-dried C-PC microencapsulated powders had higher glass transition temperatures than free C-PC, especially in the high ratios of maltodextrin at 100:0 and 25:75 as high gum Arabic. Our results showed higher glass transition temperature than freeze-dried blueberry extract with maltodextrin DE 4.0-7.0 at 100.7 °C (*49*). Percentages of mass loss from thermogravimetric analysis (TGA) were in the range 73 to 82 % at temperature gradient from 25 to 800 °C. *Arthrospira* cells dried at various temperatures 80-110

°C had mass loss of 31.7-25.8 % at temperature gradients from 180 to 350 °C (*31*). C-PC as a natural pigment extract mixed with other components as wall materials showed high percentage mass loss at high temperatures.

Table 4

*Antioxidant activity*

The antioxidant activity of food is an expression of its capability to defend the human organism from the actions of free radicals and prevent degenerative disorders deriving from persistent oxidative stress. Use of natural antioxidants in the food industry is a promising alternative to synthetic antioxidants and highly compatible for dietary intake with no harmful effects inside the human body (*50*). C-phycocyanin has a high antioxidant capacity (*9*) and one of the important characteristics of natural blue C-PC colourants is their scavenging ability for free radicals of reactive oxygen species (ROS). The 50 % DPPH free radical scavenging ($IC_{50}$) results from all experiments are presented in Table 5 in the range 7.6 to 13.5 mg/mL. A higher or equal ratio of maltodextrin in the wall material mixtures showed lower $IC_{50}$ values, whereas free C-PC had the lowest $IC_{50}$ values with no significant difference. Increasing the gum Arabic ratio of the wall material increased $IC_{50}$ value.

Table 5

## CONCLUSIONS

Selection of suitable wall materials is crucial for the microencapsulation freeze-drying process of C-phycocyanin. Wall materials prevent changes due to chemical interaction and maximise retention of the C-PC blue colourant after the drying process is completed. A mixture of maltodextrin and gum Arabic was optimised at ratio 25:75 and provided the best condition for freeze-dried C-PC microencapsulation. Findings indicated that freeze-dried C-PC microcapsules using a combination of maltodextrin and gum Arabic as wall material offer an interesting alternative to maintaining C-PC colourant stability during encapsulation to produce a powder with high levels of antioxidant blue colourant.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## AUTHORS' CONTRIBUTION

W. Pan-utai and S. Iamtham conceived and designed the experiments. W. Pan-utai performed the experiments, analyzed the data, authored or reviewed draft of the manuscript. W. Pan-utai and S. Iamtham approved the final manuscript.

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**Figure legends**

Fig. 1. Scanning electron micrographs and particle size distributions of freeze-dried C-PC microencapsulated powders using different ratios of MD:GA wall materials, a) (100:0, 5000x), b) (75:25, 5000x), c) (50:50, 1000x), d) (25:75, 1000x), e) (0:100, 1000x), and f) (free C-PC, 1000x).

Fig. 2. Particle size distribution of freeze-dried C-PC microencapsulated powders using different wall material ratios.

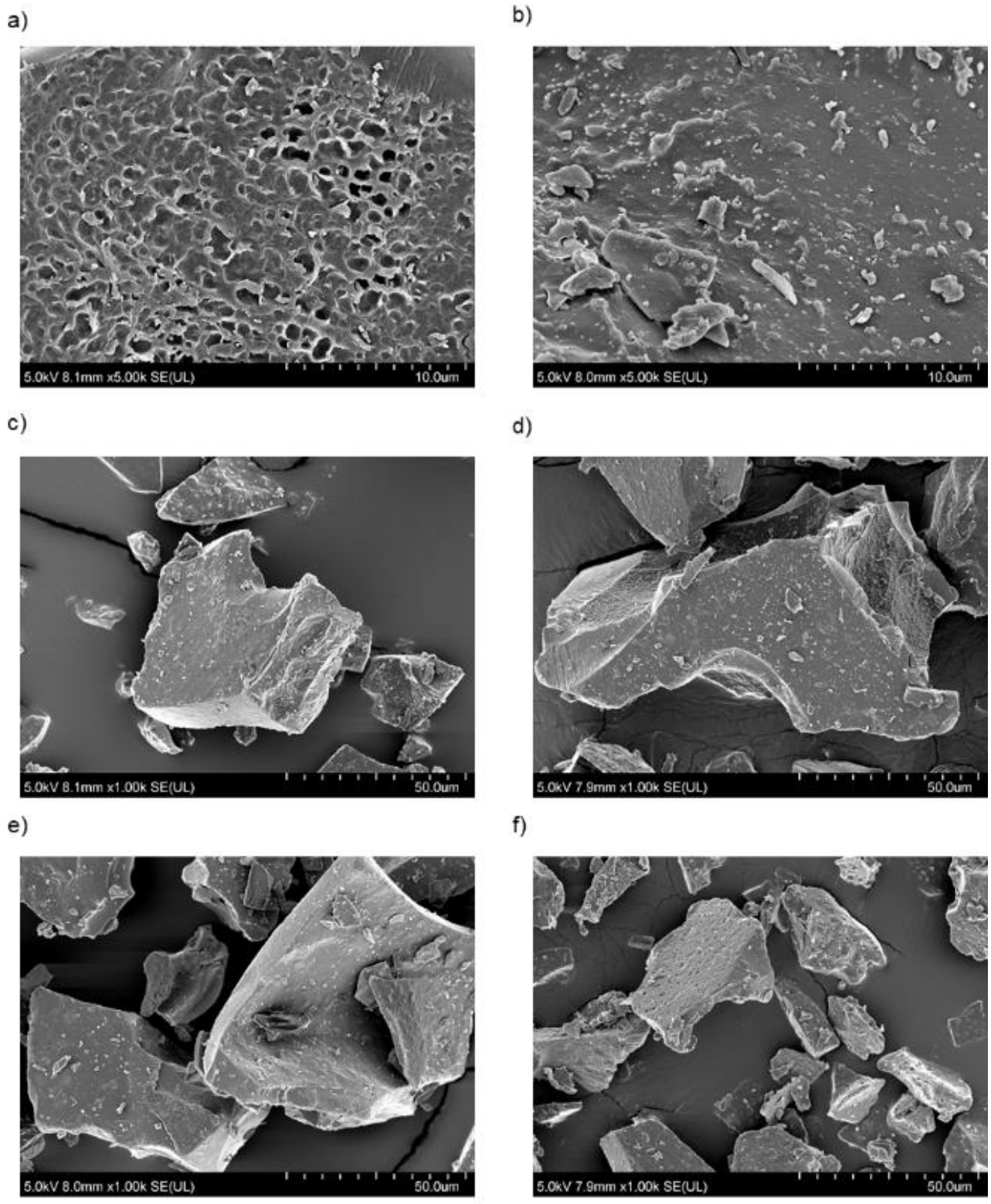


Fig. 1

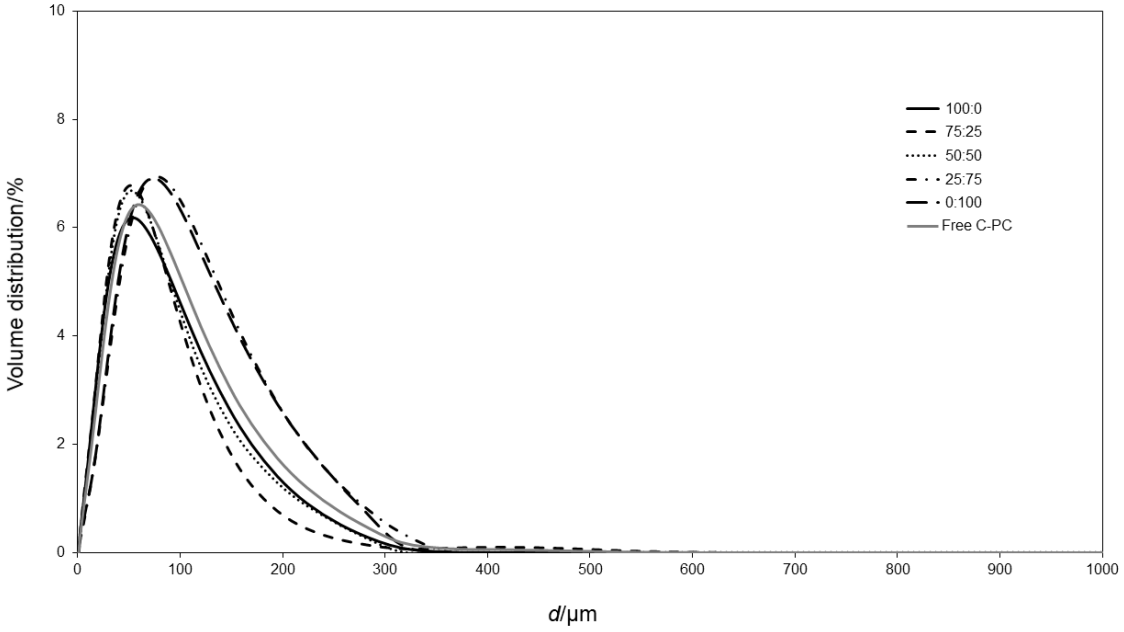


Fig. 2

Table 1. C-phycocyanin content in microencapsulated powders and encapsulation efficiency using different wall materials.

| Wall material (maltodextrin:gum Arabic) | *w*(C-phycocyanin)/(mg/g) | EE/% |
|---|---|---|
| 100 : 0 | (18.98±0.65)[a] | (99.75±0.14)[b] |
| 75 : 25 | (18.85±0.37)[a] | (99.95±0.00)[a] |
| 50 : 50 | (19.11±0.32)[a] | (99.92±0.05)[a] |
| 25 : 75 | (20.23±0.64)[b] | (99.94±0.10)[a] |
| 0 : 100 | (20.48±0.27)[b] | (99.90±0.06)[a] |

Values are expressed as mean ± standard deviation. Different letters indicate significant difference between treatments ($p < 0.05$).

Table 2. Physical properties of different wall material ratios for C-PC microencapsulated powders.

| Wall material (maltodextrin:gum Arabic) | *w*(moisture)/% | $a_w$ | Solubility/% | Hygroscopicity/% | $\rho_b$/(g/cm$^3$) |
|---|---|---|---|---|---|
| 100 : 0 | (2.41±0.73)$^{cd}$ | (0.15±0.00)$^{b}$ | (97.07±3.31)$^{c}$ | (8.06±0.55)$^{a}$ | (0.68±0.05)$^{c}$ |
| 75 : 25 | (2.16±0.59)$^{bc}$ | (0.18±0.02)$^{b}$ | (94.98±0.62)$^{bc}$ | (8.49±0.59)$^{a}$ | (0.68±0.03)$^{c}$ |
| 50 : 50 | (3.38±0.17)$^{d}$ | (0.19±0.01)$^{b}$ | (93.31±0.43)$^{b}$ | (8.89±0.80)$^{a}$ | (0.64±0.02)$^{ab}$ |
| 25 : 75 | (1.20±0.90)$^{ab}$ | (0.08±0.04)$^{a}$ | (93.95±1.54)$^{bc}$ | (12.82±1.40)$^{c}$ | (0.66±0.02)$^{bc}$ |
| 0 : 100 | (0.99±0.98)$^{a}$ | (0.07±0.01)$^{a}$ | (94.80±0.80)$^{bc}$ | (13.68±0.95)$^{c}$ | (0.68±0.02)$^{c}$ |
| Free C-PC | (7.94±0.26)$^{e}$ | (0.26±0.01)$^{c}$ | (87.32±4.63)$^{a}$ | (11.07±0.26)$^{b}$ | (0.62±0.02)$^{a}$ |

Values are expressed as mean ± standard deviation. Different letters indicate significant difference between treatments ($p < 0.05$).

Table 3. Colour indices and mean diameter particle size of different wall material ratios for C-PC microencapsulated powders.

| Wall materials (maltodextrin:gum Arabic) | Colour | | | D[4,3]/ μm |
|---|---|---|---|---|
| | *L** | *a** | *b** | |
| 100 : 0 | (59.58±1.26)$^{b}$ | (-16.45±0.53)$^{a}$ | (-24.55±0.53)$^{a}$ | (55.22±1.06)$^{ab}$ |
| 75 : 25 | (59.34±0.75)$^{b}$ | (-16.26±0.27)$^{ab}$ | (-24.34±0.49)$^{a}$ | (50.78±0.75)$^{a}$ |
| 50 : 50 | (59.91±3.52)$^{b}$ | (-15.18±0.64)$^{abc}$ | (-23.21±0.95)$^{ab}$ | (54.40±0.73)$^{ab}$ |
| 25 : 75 | (56.15±2.84)$^{b}$ | (-14.90±1.40)$^{bc}$ | (-21.34±1.89)$^{b}$ | (74.30±1.19)$^{c}$ |
| 0 : 100 | (57.27±2.60)$^{b}$ | (-14.33±1.04)$^{c}$ | (-19.37±1.40)$^{c}$ | (72.75±1.18)$^{c}$ |
| Free C-PC | (40.81±2.59)$^{a}$ | (-8.41±1.26)$^{d}$ | (-17.53±1.95)$^{c}$ | (59.34±5.89)$^{b}$ |

Values are expressed as mean ± standard deviation. Different letters indicate significant difference between treatments ($p < 0.05$).

Table 4. Thermal analysis of microencapsulated powders using different wall materials.

| Wall material | DSC* | | | | TGA** |
|---|---|---|---|---|---|
| (maltodextrin:gum Arabic) | $t_0$/°C | $t_g$/°C | $t_f$/°C | ΔH/(J/g) | Mass loss/% |
| 100 : 0 | 169.86 | 173.00 | 178.27 | -132.76 | 81.77 |
| 75 : 25 | 153.71 | 158.25 | 173.26 | -220.90 | 76.13 |
| 50 : 50 | 162.28 | 164.42 | 173.19 | -188.34 | 84.92 |
| 25 : 75 | 169.69 | 171.42 | 178.89 | -142.39 | 81.20 |
| 0 : 100 | 169.42 | 171.00 | 176.75 | -142.51 | 77.94 |
| Free C-PC | 149.70 | 152.34 | 170.96 | -170.73 | 73.20 |

*DSC: Differential scanning calorimetry

**TGA: Thermogravimetric analysis

Table 5. Antioxidant capacity of microencapsulated powders using different wall materials.

| Wall material (maltodextrin:gum Arabic) | $IC_{50}$/(mg/mL) |
|---|---|
| 100 : 0 | $(8.89\pm0.19)^{ab}$ |
| 75 : 25 | $(8.50\pm0.29)^{ab}$ |
| 50 : 50 | $(8.91\pm1.11)^{ab}$ |
| 25 : 75 | $(10.22\pm1.27)^{b}$ |
| 0 : 100 | $(13.50\pm1.07)^{c}$ |
| Free C-PC | $(7.57\pm0.14)^{a}$ |

Values are expressed as mean ± standard deviation. Different letters indicate significant difference between treatments ($p < 0.05$).