Journal of Food Science and Agricultural Technology

International peer-reviewed scientific online journal

Published online: http://rs.mfu.ac.th/ojs/index.php/jfat

## Original Research Article

# Effect of inclusion conditions on characteristics of spray dried whey protein hydrolysate/γ-cyclodextrin complexes

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**ARTICLE INFO**

*Article history:*
Received 31 July 2018
Received in revised form 31 December 2018
Accepted 08 January 2019

*Keywords:*
Protein-carbohydrate inclusion
Whey protein hydrolysate
Taste masking

**ABSTRACT**

Whey protein hydrolysate (WPH) has functional food potential. However, the bitter taste and high hygroscopicity of WPH may limit its application to use as food ingredients. To reduce the shortcomings of WPH, it was encapsulated by γ-cyclodextrin (γ-CD) as inclusion complexes. The aim of this work was to study the effect of inclusion conditions on the various properties of spray dried inclusion complex of WPH/γ-cyclodextrin. Effects of incubation temperature (20-50 °C), incubation time (3-9 hr) and mixing ratio between γ-CD and WPH (20:80, 40:60 and 60:40) were studied. Spray drying of WPH/γ-CD inclusion complexes were operated at an inlet temperature of 180°C to produce the encapsulated WPH powder. The properties of encapsulated WPH powder including moisture content, bulk density, particle size distribution, solubility, morphology, color values, surface hydrophobicity, bitterness and hygroscopicity were investigated. It was found that all of encapsulated WPH showed the better properties than non-encapsulated WPH, especially hygroscopicity and bitterness. The hygroscopicity of encapsulated WPH reduced in the range of 27.42% to 57.67%, bitterness reduced in the range of 51.61% to 89.78% compared to that of non-encapsulated WPH. For the incubation time effect, moisture content and bitterness score of encapsulated WPH decreased with decreasing of incubation time. The incubation temperature did not significantly affect on various properties of encapsulated WPH. For the effect of ratio between WPH and γ-CD, higher content of γ-CD led to lower values of hygroscopicity and bitterness score. From the results in this study, encapsulation of WPH by inclusion method and spray drying improve the characteristics of whey protein hydrolysate used as food ingredients in a variety of food products.



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Published by School of Agro-Industry, Mae Fah Luang University

ISSN: 2408-1736

## INTRODUCTION

Protein hydrolysate is bioactive peptides that are breakdown products using enzymatic hydrolysis of protein into smaller peptides fragments and free amino acids. They are low molecular weight, so they are easily absorbed and high digested in the human body. The amino acids are source of various physiological functions of the human body (Neklyudov *et al*., 2000). They are ingredients for functional food production due to their health-promoting properties, such as antioxidative and immunomodulating properties (Udenigwe and Aluko, 2012). However, the major problems are bitter taste and high hygroscopicity of protein hydrolysates because releasing of hydrophobic and hygroscopic amino acid residues, which hinder their direct use in food systems. It is remarked that hydrophobicity of peptides is an important key for the bitterness. The potential carrier can be used for encapsulation of protein hydrolysate for its bitterness taste masking (Masahiro *et al*., 1990).

Cyclodextrin is a hollow truncated cone shaped cyclic oligosaccharides with exterior is hydrophilic and their cavity is hydrophobic, and they form specific inclusion complexes with many organic compounds. Because of special structure, cyclodextrin is considered as potential carrier for encapsulation. They have been used as flavour carriers, protectant of food ingredients, undesired taste masking and food packaging materials (Szente and Szejtli, 2004; Hedges, 1998). The inclusion complexes of cyclodextrin and protein hydrolysate can be prepared by a precipitation (Bhandari and Arcy, 1998) and kneading (Yoshii *et al*., 1998).

Encapsulation technique is a processes in which the ingredients are coated with or embedded within a protective matrix, are regarded as an effective approach to overcome the aforementioned limitations and have been successfully used for the preservation of biologically active ingredients in food systems (Jiménez-Martín *et al.,* 2014; Vaslin *et al*., 2006; Munin and Edwards, 2011; Santhanam *et al*., 2015). Spray drying is usually used for encapsulation because it is efficient, inexpensive, and leads to the formation of dry powder (Shiga *et al*., 2001, Jafari *et al*., 2008, Furuta *et al*., 2010; Soottitantawa *et al*., 2015). In the previous study, cyclodextrin were used to encapsulate whey protein hydrolysate for reduced the bitterness and hygroscopicity by Yang *et al*. (2012). They studied the effect of spray-drying encapsulation process using maltodextrin and maltodextrin/β-cyclodextrin mixture as wall material compared with nonencapsulated WPH. Lixia *et al.* (2013) studied optimization of debittering of soybean hydrolysates with β-cyclodextrins using response surface methodology. The optimum conditions with the minimum bitterness and the maximum reducing power were: temperature 38.50 °C, the mass fraction of β-cyclodextrin 2.00%, and incubation time 12 min. Deshaware *et al.* (2018) reduced bitterness of the flesh bitter gourd juice by addition 0.25 to 2 % of β-cyclodextrin that addition of β-CD resulted in juice with better sensory quality in terms of reduction in bitterness, higher total phenolics, and higher total antioxidant activity than control. Although the reduction of bitterness of whey protein hydrolysate has been studied, the studies of the effect of inclusion parameters such as incubation temperature and time on properties of spray-dried encapsulated whey protein hydrolysate with cyclodextrin are limited.

The aim of this work was to study the effect of inclusion conditions on bitterness and other properties of spray dried inclusion complex of WPH/γ-cyclodextrin.

## MATERIALS AND METHODS

### Chemicals and reagents

For the preparation of whey protein hydrolysate, whey protein isolate was purchased from Mullin Whey (Marathon County, US), Alcalase from *Bacillus licheniformis* with a declared activity of 2.4 Anson units (AU) per gram was purchased from Sigma-Aldrich (St. Louis, US), γ-cyclodextrin was obtained from Wacker Chemie AG (Munich, Germany), 8-Anilino-1-naphthalenesulfonic acid (ANS) was purchased from Sigma-Aldrich (St. Louis, US). Dibasic sodium phosphate, monobasic sodium phosphate and methanol were purchased from Ajax Thermo Fisher Scientific (Melbourne, Australia).

### Preparation of whey protein hydrolysate

Whey protein hydrolysate was prepared by enzymatic hydrolysis method using Alcalase according to the method of Ma *et al*., 2014. Whey protein isolate was solubilized in deionized water (50 g/L). The pH of the protein solution was adjusted to 8.0 with 1 mol/L NaOH and then mixed with 6.0 AU of Alcalase (enzyme to substrate ratio of 1:20 w/w). In the hydrolysis conditions, The solution was stirred at 50°C for 3 hr and maintained at pH 8.0 by the addition of 1 mol/L NaOH because alcalase showed the maximum activity at alkaline pHs. The hydrolysate solutions were collected and heated in an 85°C water bath for 20 min to inactivate the protease and stopped the hydrolysis reaction. After cooling to room temperature, the hydrolysates were centrifuged at 4000×g (Cence, H1850 Tabletop High Speed Centrifuge, Hunan, China) for 15 min. Then the supernatants were dried using spray dryer (Buchi, B-290, Flawil, Switzerland).

### Preparation of the inclusion complex powders

Effects of incubation temperature (20-50°C), incubation time (3-9 hr) and mixing ratio between γ-CD and WPH (20:80, 40:60 and 60:40) were studied in this work. The method of inclusion complex was determined as described by Nguyen and Yoshii (2018). Firstly, γ-cyclodextrin was mixed with 80 wt% of deionized water using magnetic stirrer (IKA, C-Mag HS7, Staufen, Germany) for 5 min. WPH was added in the solution and stirred for 5 min (20% the total solid). Then the mixtures were incubated and continuously stirred. The ratio of the mixture, incubation temperature and time are shown in the Table 1. The agitating speed was 250 rpm using overhead stirrer (IKA, RW 20D, Staufen Germany). The mixtures were fed into spray dryer (Buchi, B-290, Flawil, Switzerland) at a feed rate of 3 mL/min and air flow rate was 35 $m^3$/hr. The inlet and outlet temperatures were maintained at 180°C and 127 ± 4°C, respectively. All the spray-dried powders were stored at -40°C in vacuum foil bags until analysis.

**Table 1.** The ratio between WPH and γ-CD and incubation conditions of WPH/γ-CD inclusion

| Conditions | E1 | E2 | E3 | E4 | E5 | E6 | E7 |
|---|---|---|---|---|---|---|---|
| γ-CD: WPH | 40:60 | 40:60 | 40:60 | 20:80 | 60:40 | 40:60 | 40:60 |
| Temperature (°C) | 35 | 35 | 35 | 35 | 35 | 20 | 50 |
| Time (hr) | 3 | 6 | 9 | 6 | 6 | 6 | 6 |

**Degree of hydrolysis (DH)**

For hydrolysis, NaOH was added in the solution to maintain pH. Volume of solution related to degree of hydrolysis. It was recorded every 30 min for determination of degree of hydrolysis using pH-stat method (Adler-Nissen *et al*., 1986).

$$\%DH = [(B \times N_b)/(\alpha \times h_{tot} \times M_p)] \times 100 \quad (1)$$

where B is the base consumption in mL, $N_b$ is the normality of the base, $M_p$ is the mass of protein being hydrolysed (g), α is the average degree of dissociation of the α-$NH_2$ groups was calculated as follows:

$$\alpha = [10 \exp(pH - pK)] / [1 + 10 \exp(pH - pK)] \quad (2)$$

where pK is average dissociation value for the α-amino groups liberated during hydrolysis and is dependent on temperature, peptide chain length and the nature of terminal amino acid. The average pK value at 50°C (used in this work) is 7.1. The parameter $h_{tot}$ is the total number of peptide bonds in the protein. For whey protein, the $h_{tot}$ is 8.8 meqV/g protein (Adler-Nissen et al., 1986).

**Moisture content**

The moisture content of encapsulated WPH was determined using AOAC method 984.25 (AOAC, 2000). Briefly, the samples were dried using hot-air drying at 105°C in duplicate, and an average value is reported.

**Hygroscopicity**

Two gram of encapsulated WPH was placed in dry Petri dishes, which stored in humidity and temperature-controllable chamber (Dae Yang, TH-180G, Gyeongbuk, Korea). The chamber was maintained temperature of 25°C and 81% of relative humidity for 7 days. The hygroscopicity was calculated as follows:

$$\text{Hygroscopicity (g/100g)} = [(\text{Saturated sp.} - \text{Original sp.}) / \text{Original sp.})] \times 100 \quad (3)$$

where Saturated sp is weight of sample (gram) after incubated in humidity and temperature-controllable chamber for 7 days and Original sp is initial weight (gram) of samples.

**Water solubility**

The solubility in water of the encapsulated WPH was determined as described by Costa *et al*. (2015). Briefly, 1.25 g of samples was reconstituted in 15 mL of deionized water and stirred with a magnetic bar at 25°C for 30 min. The solution was centrifuged at 7600×g for 30 min. Then 10 mL of supernatant was placed in a Petri dish and dried at 105°C for 5 h in a hot air oven. Solubility in water was calculated by the following equations:

$$\text{Water solubility(g/100g)} = (1.5 \times \text{dried supernatant in 10 mL/dried sample}) \times 100 \quad (4)$$

**Bulk density**

The bulk density (ρb) of the encapsulated WPH was determined by using the method of Yang *et al*. (2012). Approximately 2 g of powder were transferred to 10-mL graduated cylinder and recorded the volume. The bulk density was calculated as follow:

$$\text{Bulk density} = \text{Mass of dry powder (g)/Volume in the cylinder (mL)} \quad (5)$$

**Color values**

Color values were measured by CIE *L*a*b** Scale using Chroma meter (CR-400 Konica Minolta, Japan). The *L** for the lightness, the maximum is 100 and the minimum is 0 (represents black). The *a** for the green-red, the positive *a** is red and negative *a** is green. The *b** for blue-yellow, the positive *b** is yellow and negative *b** is blue. The total color different *ΔE** is a single values with takes into account the difference between the *L*, a** and b **of the encapsulated WPH and non-encapsulated WPH. The *ΔE** was calculated as follows:

$$\Delta E^* = \text{Square roots } (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}) \quad (6)$$

**Particle size distribution**

The size distribution of encapsulated WPH was measured by using a laser scattering particle size analyzer (Malvern PANalytical, Mastersizer 3000, Malvern, UK). The powder was dispersed in methanol for measuring the size distribution of particle. The volume-based diameter (D43) was regarded as the mean diameter that was calculated as follows;

$$D_{[4,3]} = \sum n_i d_i^4 / \sum n_i d_i^3 \quad (7)$$

where $n_i$ is the number of particles of diameter $d_i$

The particle size distribution of the powder was measure as the span that was calculated as follows;

$$\text{Span} = (d_{90} - d_{10})/d_{50} \quad (8)$$

where $d_{90}$, $d_{10}$, and $d_{50}$ are the equivalent volume diameters at 90, 10, and 50 % cumulative volume, respectively.

**Morphological analysis**

The surface morphologies of samples were evaluated using a scanning electron microscopy (Jeol, JSM-7600F Tokyo, Japan). The powder samples were coated with 5 nm platinum under vacuum by auto fine coater (Jeol, JFC-1600, Tokyo, Japan). Scanning electron microscopy was carried out at an accelerating voltage of 1.0 kV. The digital images were captured with magnifications of 2000X.

**Determination of the surface hydrophobicity**

The surface hydrophobicity ($S_0$) of encapsulated WPH was measured using 1-anilino-8-naphthalene sulfonate (ANS) as the hydrophobic fluorescence probe according to the method of Wang *et al*. (2018) with slightly modifications. The samples were dissolved in 10 mmol/L phosphate buffer (pH 7.0) and centrifuged at 10,000g for 10 min. The supernatant was diluted in phosphate buffer to obtain various concentrations ranging from 0.05 mg/mL to 0.5 mg/mL. Next, 10 mL of each diluted solution was mixed with 50 μL of 8 mmol/L ANS (in 10 mmol/L phosphate buffer at pH 7.0) at room temperature. The fluorescence intensity (FI) of protein was measured at excitation wavelength of 390 nm and emission wavelength of 470 nm using a spectrofluorometer. The initial slope ($S_0$) of the relative FI versus protein concentration (mg/mL) plot was calculated by linear regression analysis and used as the surface hydrophobicity ($S_0$).

### Sensory evaluation

The seven sensory panelists were trained by using caffeine standard solutions at concentration of 0.05, 0.08, 0.15 and 0.20% as standard references. Panel training included exercises using the 15 point spectrum intensity scale as outlined by Meilgaard *et al*. (2016) where 2 corresponds to a weak and 15 to a very strong intensity. All samples were dissolved in bottled deionized water (10% w/w) at room temperature for 20 sec. They were presented to assessors in opaque, plastic lidded 50 mL sample cups which were labelled with a corresponding 3-digit code. No more than 4 samples were assessed in a day to avoid palate fatigue. Before the sample was tasted, the mouth was rinsed with deionized water. The water and crackers were supplied for palate cleansing between samples. The panelists were asked to assign bitterness scores for each sample using the 15 point intensity scale; four bitter reference solutions of caffeine at intensities of 2 (0.05%), 5 (0.08%), 10 (0.15%) and 15 (0.2%) were supplied to panelists to aid scaling.

### Statistical analysis

The statistical comparison was done in duplicate that the data in the table presented mean and standard deviation using one-way analysis of variance (ANOVA). Duncan Multiple Range Test used to separate the difference between the data at the significant level p=0.05.

## RESULTS AND DISCUSSION

### Degree of hydrolysis (DH)

The average DH value of WPH by pH-stat method was 19.68%. This value was similar to Ma *et al.* (2014) that the time for 3 hr have the highest DH value and optimal hydrolysis period to produce immunomodulatory peptides.

### Characterization of WPH and encapsulated WPH

Table 2 showed the values of color, moisture content, hygroscopicity, bulk density, water solubility and particle diameter of seven encapsulated WPH conditions compared with non-encapsulated WPH. The total color differences of encapsulated WPH were in the range of 4.06 to 6.08. The E3, E5 and E6 changed higher than other conditions that effected of *L**. The *L** trend to increased, the *a** tend to decreased that approach green and *b** trend to decreased that reduced yellow.

The moisture content of six encapsulated WPH conditions significantly were in the range of 1.01-3.44% (d.b.). The lowest value was presented in case of E1 condition (1.01 %), which was incubated for 3 hr. The higher incubation time led to increasing of the moisture content. On the other hand, the highest moisture content was presented in case of E5 condition (4.25 %, d.b.) that was similar to non-encapsulated WPH. Because this condition consisted of the highest wall material (γ-CD), which have high moisture content (5.75 %, d.b.).

The hygroscopicities of encapsulated WPH were in the range of 17.46 to 29.42 g/100g, which were decreased in the range of 27.42% to 57.687% compared to that of non-encapsulated WPH. The decreasing of ratio between WPH and γ-CD led to decreasing of hygroscopicity. It was because γ-CD had a low hygroscopicity. However, the incubation time and temperature did not significantly affect on hygroscopicity.

The bulk densities of all encapsulated WPH were in the range of 377.06-483.14 kg/m$^3$. The lowest bulk density was in case of E7 condition (377.06 kg/m$^3$). The highest value was in the case of E4 condition (483.14 kg/m$^3$) that close to the bulk density of the non-encapsulated WPH.

The water solubilities of encapsulated WPH were 86.28-96.45 $g_{samples}$/100 $g_{water}$. Most of them showed the highly dissolving in the

**Table 2.** Color, moisture content, hygroscopicity, bulk density, water solubility and particle diameter of the γ-CD, WPI, non-encapsulated WPH and encapsulated WPH

| Samples | | Color | | | | % Moisture content | Hygroscopicity (g/100g) | Bulk density (kg/ m$^3$) | Water solubility (g/100g) |
|---|---|---|---|---|---|---|---|---|---|
| | | *L** | *a** | *b** | *ΔE** | | | | |
| γ-CD | | 91.58 ± 0.40 $^{de}$ | -1.68 ± 0.29 $^{a}$ | -0.98 ± 0.05 $^{e}$ | - | 5.75 ± 0.25 $^{f}$ | 11.24 ± 0.79 $^{a}$ | 515.83 $^{j}$ | 23.20 $^{i}$ |
| WPI | | 87.28 ± 0.99 $^{f}$ | -3.52 ± 0.07 $^{de}$ | 6.23 ± 0.29 $^{c}$ | - | 4.50±0.20 $^{e}$ | 29.51 ± 1.09 $^{d}$ | 292.82 $^{a}$ | 87.69 $^{g}$ |
| Non-encapsulated WPH | | 91.37 ± 1.57 $^{e}$ | -3.70 ± 0.29 $^{e}$ | 9.33 ± 0.11 $^{a}$ | - | 3.71 ± 0.16 $^{de}$ | 41.25 ± 2.61 $^{e}$ | 484.17 $^{i}$ | 89.04 $^{f}$ |
| Encapsulated WPH | **Effect of time** | | | | | | | | |
| | E1 | 93.23 ± 0.44 $^{d}$ | -3.06 ± 0.04 $^{bc}$ | 5.70 ± 1.16 $^{c}$ | 4.18 ± 0.81 $^{a}$ | 1.01 ± 0.30 $^{a}$ | 23.58 ± 4.25 $^{c}$ | 426.62 $^{f}$ | 93.50 $^{b}$ |
| | E2 | 95.06 ± 0.26 $^{c}$ | -2.87 ± 0.00 $^{b}$ | 7.45 ± 0.13 $^{b}$ | 4.22 ± 0.17 $^{a}$ | 1.85 ± 0.26 $^{ab}$ | 27.61 ± 0.81 $^{cd}$ | 472.00 $^{g}$ | 92.80 $^{c}$ |
| | E3 | 97.23 ± 0.36 $^{ab}$ | -3.04 ± 0.03 $^{bc}$ | 8.27 ± 0.10 $^{b}$ | 5.99 ± 0.34 $^{b}$ | 3.34 ± 0.34 $^{cd}$ | 25.01 ± 0.27 $^{c}$ | 414.95 $^{d}$ | 92.55 $^{d}$ |
| | **Effect of wall material content** | | | | | | | | |
| | E4 | 95.41 ± 0.24 $^{bc}$ | -3.56 ± 0.02 $^{de}$ | 9.69 ± 0.04 $^{a}$ | 4.06 ± 0.24 $^{a}$ | 2.53 ± 0.32 $^{bc}$ | 29.94 ± 1.93 $^{d}$ | 483.14 $^{h}$ | 90.54 $^{e}$ |
| | E2 | 95.06 ± 0.26 $^{c}$ | -2.87 ± 0.00 $^{b}$ | 7.45 ± 0.13 $^{b}$ | 4.22 ± 0.17 $^{a}$ | 1.85 ± 0.26 $^{ab}$ | 27.61 ± 0.81 $^{cd}$ | 472 $^{g}$ | 92.80 $^{c}$ |
| | E5 | 95.60 ± 1.36 $^{abc}$ | -3.42 ± 0.11 $^{de}$ | 5.49 ± 0.28 $^{c}$ | 5.77 ± 0.80 $^{b}$ | 4.25 ± 0.71 $^{de}$ | 17.46 ± 0.71 $^{b}$ | 409.55 $^{c}$ | 86.28 $^{h}$ |
| | **Effect of temperature** | | | | | | | | |
| | E6 | 97.35 ± 0.01 $^{a}$ | -3.58 ± 0.01 $^{de}$ | 8.19 ± 0.09 $^{b}$ | 6.08 ± 0.01 $^{b}$ | 3.44 ± 1.07 $^{cde}$ | 25.21 ± 1.45 $^{c}$ | 416.24 $^{e}$ | 96.45 $^{a}$ |
| | E2 | 95.06 ± 0.26 $^{c}$ | -2.87 ± 0.00 $^{b}$ | 7.45 ± 0.13 $^{b}$ | 4.22 ± 0.17 $^{a}$ | 1.85 ± 0.26 $^{ab}$ | 27.61 ± 0.81 $^{cd}$ | 472 $^{g}$ | 92.80 $^{c}$ |
| | E7 | 95.75 ± 0.08 $^{abc}$ | -3.31 ± 0.01 $^{cd}$ | 8.08 ± 0.23 $^{b}$ | 4.57 ± 0.20 $^{a}$ | 1.89 ± 0.05 $^{ab}$ | 24.66 ± 0.60 $^{c}$ | 377.06 $^{b}$ | 92.64 $^{d}$ |

Mean values followed by different superscripts within the same raw indicate a statistically significant difference between the mean values (p<0.05).

water more than the non- encapsulated WPH (89.04 g/100g) but the E5condition dissolved in the water only 86.28 g/100g. It was the result of the over content of wall material (γ-CD) that dissolved in the water only 23.2 g/100g. As shown in Table 2, the ratio of core and wall material was very important. The highest water solubility was not occurred in case of the lowest wall material content. Therefore, the ratio between WPH and γ-CD should be optimized. In addition, the water solubility of encapsulated WPH decreased with an increase of incubation temperature. As can be seen in case of E6 condition, the lowest incubation temperature (20°C) demonstrated the highest water solubility. The solubility decreased with an increase of incubation temperature.

Table 3 showed the mean diameter, span and specific surface area of non-encapsulated WPH and encapsulated WPH. The microcapsules had mean diameter range from 19.04 to 22.13 µm, span value from 1.394 to 1.531 and specific surface area range from 384.3 to 584.7 $m^2$/kg. The mean diameters of non-encapsulated WPH were significantly lower than that of microcapsules. The E5 condition showed the smallest size (16 µm of diameter), the highest surface area (584.7 $m^2$/kg) and a narrow distribution (Figure 1). In addition, the surface area increased with the reduction of particle size. The highest temperature (E7 condition) presented the largest mean diameter (22.13 µm).

**Table 3.** Mean diameter D[4,3], span and specific surface area

| Samples | $D_{[4,3]}$ (µm) | Span | Specific surface area ($m^2$/kg) |
|---|---|---|---|
| **WPH** | 10.73±0.06$^a$ | 1.621$^f$ | 775.0 |
| **Effect of time** | | | |
| **E1** | 21.13±0.06$^e$ | 1.435$^b$ | 401.0 |
| **E2** | 21.03±0.06$^e$ | 1.459$^c$ | 407.5 |
| **E3** | 21.93±0.12$^f$ | 1.394$^a$ | 384.3 |
| **Effect of wall material content** | | | |
| **E4** | 19.40±0.20$^c$ | 1.531$^e$ | 466.8 |
| **E2** | 21.03±0.06$^e$ | 1.459$^c$ | 407.5 |
| **E5** | 16.05±0.09$^b$ | 1.475$^d$ | 584.7 |
| **Effect of temp** | | | |
| **E6** | 20.23±0.06$^d$ | 1.481$^d$ | 431.9 |
| **E2** | 21.03±0.06$^e$ | 1.459$^c$ | 407.5 |
| **E7** | 22.13±0.06$^g$ | 1.460$^c$ | 391.3 |

Mean values followed by different superscripts within the same raw indicate a statistically significant difference between the mean values ($p<0.05$).

### Morphological analysis

The morphology of non-encapsulated WPH and E1-E7 conditions of spray–dried encapsulated WPH using γ-CD as wall material were presented by SEM micrograph with magnification of 2000X are shown in Figure 3. Most of encapsulated samples exhibited a pseudo-spherical and rather smooth surface. Some particles of encapsulated samples distorted, cracked and roughened surface but less than non-encapsulated WPH. Various shapes can be normally observed for spray drying particles obtained from aqueous solutions (Cicco, D. *et al.*, 2014). In Figure 3A, the particles of non- encapsulated WPH strongly agglomerated. The particles of all encapsulated WPH had better distribute and bigger size compared to non-encapsulated WPH.

### Surface hydrophobicity

The surface hydrophobicity ($S_0$) of non-encapsulated WPH and encapsulated WPH are shown in Figure 2. The $S_0$ of WPH was significantly reduced with enzymatic hydrolysis compared to WPI. The WPI consisted of a high content of hydrophobic amino acid that not embedded inside the protein molecules at bulky structure. They might situate at the surface that led to high $S_0$. After enzymatic hydrolysis, the molecular weight of WPI reduced because it was changed to be WPH which led to decreasing of $S_0$. The results resembled to report of Chanikan *et al.* (2018) that reported about hydrolysed mung bean meal. All conditions of encapsulated WPH had higher values of $S_0$ than non-encapsulated WPH. It was the result of γ-CD. The $S_0$ of encapsulated WPH increased compared with non-encapsulated WPH because high hydrophobic groups inside the molecules of γ-CD. For the incubation time effect, the $S_0$ of E1 (3hr) and E2 (6hr) did not significantly different. But incubation time increased to 9 hr (E3 condition), the $S_0$ significantly increased compared to E1 and E2 conditions. For the ratio effect, the $S_0$ increased with γ-CD increased because γ-CD had a lot of hydrophobic groups in the molecules. For the temperature effect, the $S_0$ did not significantly affect. At long time, the hydrophobic groups of protein hydrolysate may be rearranged the position from inside the molecules to the surface. The high $S_0$ could occur in case of low water solubility of encapsulated WPH because the small peptides had lower $S_0$ than large peptides. Therefore, the increased solubility could be due to smaller molecular peptides (Wu et al., 1998)

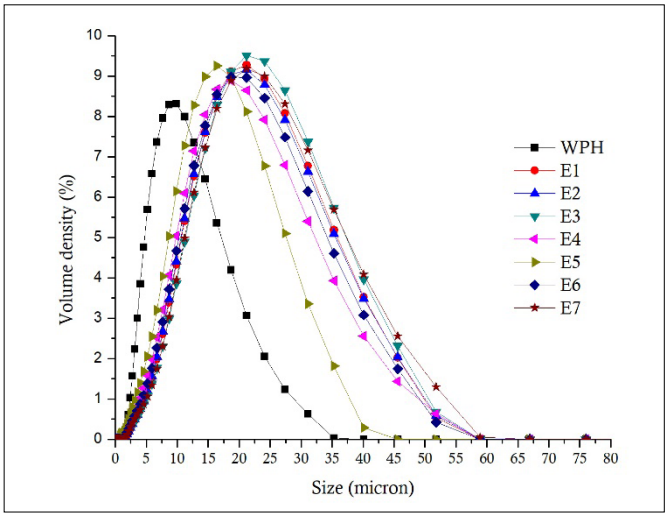


**Figure 1.** Particles distribution of Non-encapsulated WPH, Encapsulated WPH E1, E2, E3, E4, E5, E6 and E7 conditions.

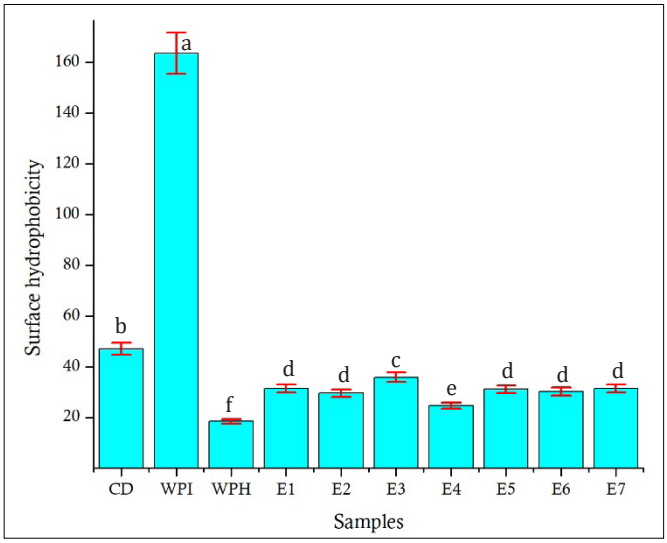


**Figure 2.** Surface hydrophobicity of non-encapsulated WPH, encapsulated WPH E1, E2, E3, E4, E5, E6 and E7 conditions.

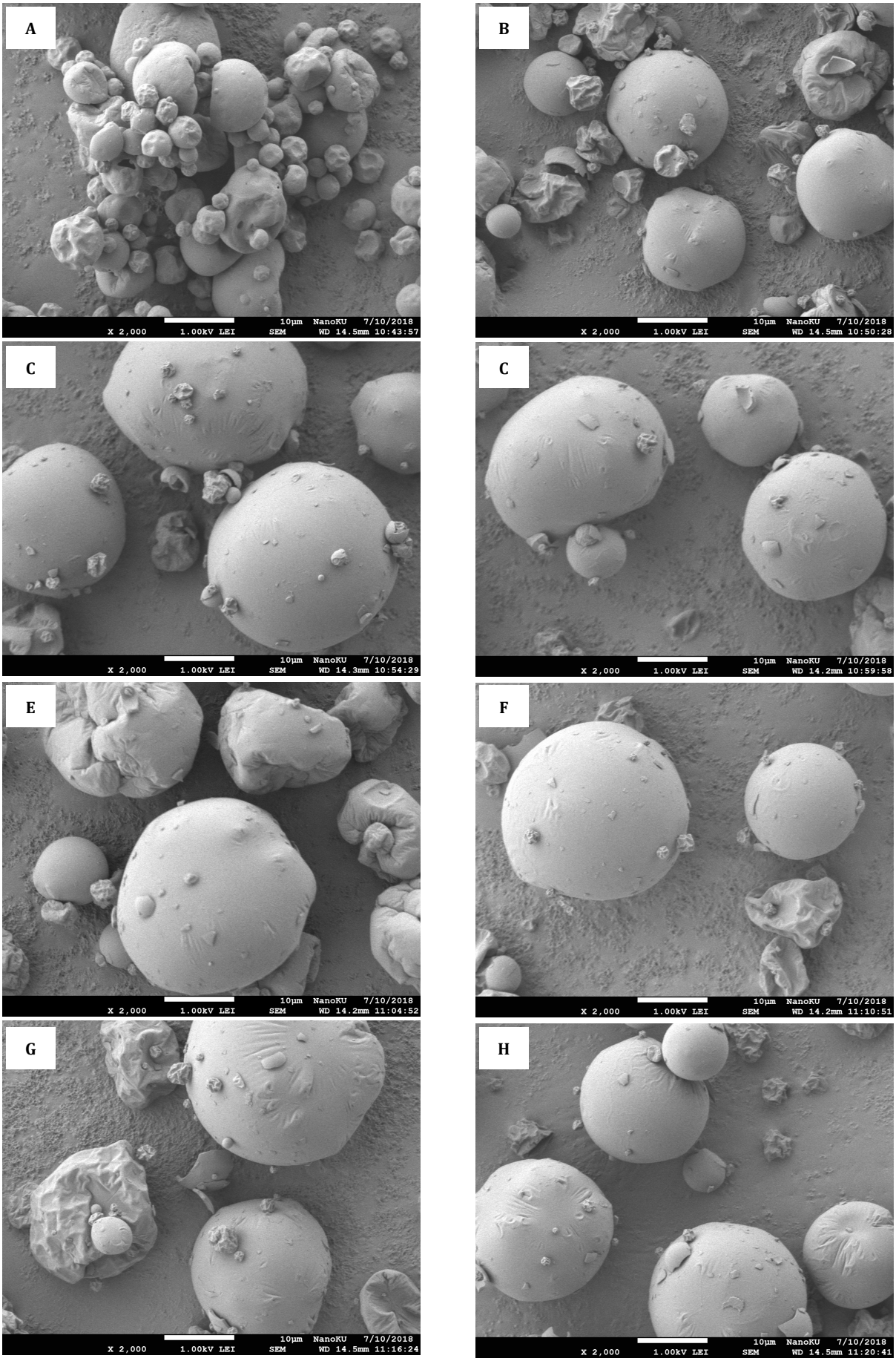


**Figure 3.** Morphological characterization (SEM micrographs) of non-encapsulated WPH and encapsulated WPH with magnification of x2000. **A** non-encapsulated whey protein hydrolysate (WPH); **B** encapsulated whey protein hydrolysate E1 condition; **C** E2 condition; **D** E3 condition; **E** E4 condition; **F** E5 condition; **G** E6 condition and **H** E7 condition.

**Sensory evaluation**

The score of bitterness taste are shown in Figure 3. All conditions of encapsulated WPH demonstrated the lower bitterness compared with non-encapsulated WPH. The score of E5 showed maximum reduction of bitterness at 89.78% while E4 showed minimum reduction of bitterness at 51.61%. Because E5 conditions consisted of highest content of wall material and lowest content of wall material was E4 condition. The score of bitterness mainly depended on wall material. The γ-CD formed the complexed with hydrophobic amino acid or small peptide fragments that hydrophobic groups of peptides are wrapped into the hydrophobic cavity of γ-CD. So the bitterness decreased with an increase of γ-CD. In addition, E5 condition led to lowest water solubility. While the sensory test the E5 condition may be partially dissolved so, the bitterness score was lowest. For the time effect, the bitterness scores were 69.35 to 76.34% reduction compared to non-encapsulated WPH. The bitterness of E1 and E2 were not significantly different. But the bitterness of E3 was significantly higher compared to E1 and E2 conditions. For the long time of incubation, the buried hydrophobic groups inside the molecules may be rearranged the position to the surface. This result was in the same trend of surface hydrophobicity (Figure 2) that E3 had surface hydrophobicity more than E1 and E2 thus contributing to the higher bitterness. The result was similar to the report of Lixia *et al*. (2013). For the temperature effect, the bitterness score decreased in the range of 70.50 to 74.19% compared with non-encapsulation WPH. The bitterness was not significantly affected by incubation temperature.

From these results, γ-CD can be used to mask the bitterness of hydrophobic peptides by forming complexes which similar to the results of Shu *et al*. (2012), Yulin *et al*. (2015), Andre *et al*. (2017), Steffi *et al*. (2017) and Jiaqi *et al*. (2018). The cyclodextrin demonstrated that formed stable inclusion complexes with hydrophobic amino acid. The aromatic rings of amino acid were trapped into the cavity of cyclodextrin. Therefore, inclusion of bioactive peptides with CD is supposed to effectively remove their own bitterness (Jiaqi *et al*., 2018).

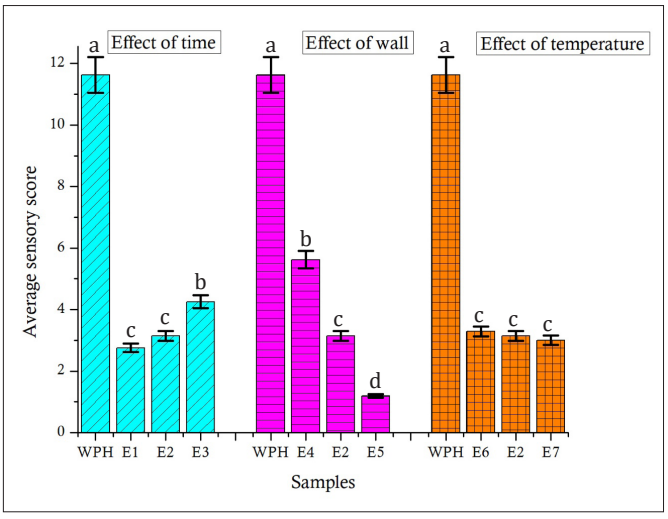


**Figure 4.** Bitterness taste by sensory evaluation of Non-encapsulated WPH, Encapsulated WPH E1, E2, E3, E4, E5, E6 and E7 conditions.

## CONCLUSIONS

Encapsulation by inclusion complexes method with γ-CD as wall material and spray drying can improve the characteristics of protein hydrolysate as well as masking the bitter taste of hydrophobic amino acid in WPH. The condition to obtain the whey protein hydrolysate with the minimum bitterness and lowest hygroscopicity was E5, which were incubation temperature of 35 °C, incubation time for 6 hr and the ratio of γ-CD/WPH of 60:40. The minimum bitterness score was 1.18 but water solubility was lowest value at 86.28 g/100 g.

The second best condition was E1 that the bitterness score was 2.75. It also had some properties that were better than E5 such, moisture content and water solubility. If the amount of protein intake was also mainly considered, E1 condition was an interesting condition because it consisted of high protein content with low bitterness.

## ACKNOWLEDGEMENTS

All authors express their sincere appreciation to the Kasetsart University Research and Development Institute (KURDI) for supporting the study financially. Author Rukluarh expresses her sincere appreciation to the Graduate School of Kasetsart University for financial support to participate in the conference.

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