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Microencapsulation of Zn-chlorophyll pigment from Pandan leaf by spray drying and its characteristic

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Abstract: Microencapsulated compound of Zn-chlorophyll derivatives extracted from pandan leaf by using spray dried method can be utilized as a natural green colorant. Three different wall material types, gum arabic (GA), maltodextrin (MD) and osa-modified starch (MS), were studied based on their physicochemical properties and stabilities of the encapsulated powder. Results showed that MS powder was spherical and smooth, whereas GA and MD powders exhibited shrinkage on the surface. At 30% MS as wall material, Zn-chlorophyll powder obtained had the highest greenness value, total chlorophyll and antioxidant activity as -14.64, 187.34 mg/100gfw and 772.50 μMTEAC/g fresh mass, respectively. The MS powder provided a longer predicted half life (462 days) compared to the GA and the MD powders. This powder had green color, 2-acetyl 1- pyrroline as volatile compound with pandan leaf characteristic flavor and Zn content of 13.12 mg/kg which was safe to use as food additive.

Keywords: Zn-chlorophyll derivatives, microencapsulation, wall material, pandan leaf

Introduction

There is growing interest in food industry for natural colorant and flavor elicited characteristic of authentic food (Uhl, 1995). Pandan (Pandanus amaryllifolius) leaf can be found widely in tropical countries including Thailand because of its high chlorophylls content, and hence pandan is becoming popular to use as green colorant in food. Pandan leaves also contain the aromatic compound 2-acetyl-1-pyrroline which has a similar compound found in Basmati and Jasmine rice (Jiang, 1990; Laksanalamai and Ilangantileke, 1993). Furthermore, pandan leaf displays antioxidant properties due to the presence of quercetin (Miean and Mohamed, 2001), carotenoids, chlorophyll derivatives (Ferruzzi *et al*., 2002), tocopherols, tocotrienols (Lee *et al*., 2004), and polyphenols. Nor, *et al*. (2008) reported that polyphenols from the pandan leaf are excellent heat-stable antioxidants. From the information obtained, it indicates that pandan leaf is an interesting plant because of its various bioactive compounds and a rich source of natural extracts especially green colorants which are widely used in food industries (Wissgott and Bortlik, 1996).

However, chlorophyll in natural sources is in the unsuitable form for use as a colorant because of its rapid degradation. It is rapidly degraded by enzymatic reaction or other factors such as acid, oxygen, light and heat, resulting in chlorophyll derivatives such as pheophytin, pheophorbide, pyropheophytin and pyropheophorbide. The formation of a stable chlorophyll molecule can be managed by replacing the magnesium ion in the porphyrin ring with divalent cations such as zinc or copper to change the native form to a more stable molecular structure (Humphrey, 1980). These derivatives are green in color like native chlorophyll but more stable to acid and heat and behave more effectively as antioxidants (Tonucci and von Elbe, 1992). Senklang and Anprung (2010) reported that formation of Zn-chlorophyll derivative in pandan leaf can be done by reaction of fresh leaves with 300 ppm $ZnCl_2$ at pH 5 and 110 ^oC for 15 min. The derivatives obtained were Zn-pheophytin and Zn-pyropheophytin, then extraction of them with Pectinex®Ultra SP-L enzyme. The outcome was high concentration of chlorophyll in liquid form.

Spray drying is a useful technique for changing liquids into solid powder form which is not only easy to handle but also improves shelf life and stability of the product. The initial step in drying a colorant involves the selection of a suitable wall material as known as carrier or encapsulating agent. Gum arabic (GA), hydrolyzed starches, and modified starches are the three most important classes of wall materials that are widespread used in pigment microencapsulation such as betacyanin (Cai and Corke, 2000; Azeredo *et al*., 2007), anthocyanin (Ersus and Yurdagel, 2007),

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betalain (Obón *et al*., 2008) and carotenes (Wagner and Warthesen, 1995).

GA is the traditional standard used as the encapsulating agent because it shows good retention of active compounds (Thevenet, 1988). However, GA is an expensive and quality is dependent on the climate conditions. These disadvantages have prompted many manufactures to look for substitutes for GA. Starch and products derived from it, such as maltodextrin (MD) and *n*-octenyl succinic anhydride-treated starch have proved to be popular choices. MD is a good compromise between cost and effectiveness, as it is bland in flavor, has low viscosity at a high solid ratio and is available in a variety of molecular weights (Apintanapong and Noomhorm, 2003). Osa-starch contains both hydrophobic and hydrophilic groups. These starches exhibit excellent volatile retention during spray drying.

Collectively, the above information suggested that pandan leaf was suitable to be evaluated as a potential source for natural green colorant. However, the chlorophyll molecule would have to be changed to a stable derivative prior to enzymatic extraction. The solid Zn-chlorophyll derivative was substituted for the liquid form by using spray drying technique. The suitable wall materials were studied for the highest encapsulating efficiency. Then, the physicochemical properties and stability of Zn-chlorophyll powder was determined. Results of this research proved useful in development of Zn-chlorophyll derivatives powder to use as colorant in food processing.

Materials and Methods

Materials

Pandan leaves were purchased from a reputable local market in Bangkok, Thailand in May-July, 2007. Gum arabic was purchased from S.R. Lab, Ltd. (Thailand). Maltodextrin was obtained from Berli Jucker Specialties, Ltd. (Bangkok, Thailand). Osa-modified starch was a kindly provided by Sayuan Wong company, Ltd. (Nakhon Ratchasima, Thailand). Pectinex® Ultra SP-L enzyme was purchased from Novozyme (Denmark) manufactured from *Aspergillus aculeatus* which had an activity of 10292 PGU/mL. All chemicals used in this study were analytical grade.

Methods

The formation of metallochlorophyll complexes in pandan leaf extract was performed by method of Senklang and Anprung (2010) as follows: first, preparation of pandan leaf pulp by washing fresh pandan leaves, chopping into small pieces, homogenizing in the warning blender for 2 min, and mixing in the ratio 1:4 (pandan leaf : water), then this pulp was reacted with 300 ppm zinc chloride at pH 5 and 110 °C for 15 min in autoclave, finally, the Zn-chlorophyll derivatives were extracted by 2.5% (v/w) Pectinex® Ultra SP-L, 260 min incubation time, and extraction repeated twice. This Zn-chlorophyll obtained was used to find out the suitable wall material.

Preparation of microencapsulation by spray drying

Preparing 10, 20 and 30% solution of each wall material (GA, MD and MS) by dispersion in water and final volume was made to 100 mL. The 10% (v/w) of Zn-chlorophyll solution was added to the mixture and 1 mL of Tween 80 was added to be emulsifying agent. The mixture was homogenized for 3 min at 3000 rpm until complete dispersion. The resulting slurry was spray dried in a spray dryer Eyela equipped with 0.5 mm diameter nozzle. The atomizer pressure and blower controls were adjusted to 50 kPa and 0.70 m^3/min, respectively. The inlet and outlet temperatures were maintained at 150 ± 5 °C and 90 ± 5 °C, respectively, and the feed rate was set at 300 mL/h. The powder obtained was collected, filled in airtight, self-sealable polyethylene pouches, and stored in a dessicator until further studies.

Scanning electron microscope (SEM)

Scanning electron microscope (FE-SEM, FEI, Sirion, USA) was used to examine the morphology and surface appearance of microencapsulated powder. The samples were attached with a two-sided adhesive tape to specimen stubs and then Pt coated in a sputter coater (BAL-TEC, SCD 005, Germany) at 30 mA for 150 s. The coated microcapsules were examined in a Sirion SEM at 10 kV with 1.5 nm resolutions. Examinations were made at 500 x and 3000x magnifications.

Determine the particle size distribution

A laser diffraction-based Malvern particle size analyzer Mastersizer 2000 (Malvern Instruments Inc., UK) was used for determination of particle diameter. The Malvern Mastersizer 2000, which is considered a spatial sampling device, correlates with the phenomena that a powder particle fallen through the laser beam can cause laser light to scatter in many angles dependent on the diameter of the particle. Particle characteristics were computed automatically from a compressed range. Particle size measurement tests were replicated four times.

Determination of color values

Minolta colorimeter (Minolta Spectrophotometer CR300 and CT310 was used to determine *L*, a*, b**, chroma (*C*), and Hue (h^o) values in which *L** is the lightness of color (100 = white, 0 = black), *a** value (+*a** = red, -*a** = green), *b** value (+*b** = yellow, -*b** = blue) , C= $((a^*)^2 + (b^*)^2)^{1/2}$ and $h^o = (\tan^{-1}(b^*/a^*)$.

Determination of chlorophyll content

Measurement of chlorophyll contents was done by Vernon method (1960) as follows: 5 g of chlorophyll powders were accurately weighed, then extracted with 20 mL of 80% acetone, mixed by homogenizer, centrifuged at 6200 g for 15 min, filtered through Whatman no. 1 and 42 filter papers, adjusted to 25 mL in a volumetric flask, and measured the absorbance values at 663 and 645 nm. Total chlorophyll content (mg/g fw) = 20.2 A_{663} + 8.02 A_{645} × Dilution factor / 1000

Assessment of Trolox equivalent antioxidant capacity (TEAC)

Evaluation of scavenging properties using long-lived $ABTS^{\cdot+}$ radical was performed by using the method of Thaipong *et al.* (2006). A 7.4 mM of $ABTS^{\cdot+}$ solution was used to react with 2.6 mM of potassium persulfate solution in a ratio 1:1, then allowing the solution to react and form free radicals for 12 h at room temperature in the dark (the output chemical should be used within 4 h). Then 1 mL of $ABTS^{\cdot+}$ solution was mixed with 60 mL of methanol to obtain an absorbance of 1.1 ± 0.2 units at 734 nm ($ABTS^{\cdot+}$ should be prepared daily). Subsequently, pandan leaf extracts (150 µL) were allowed to react with 2850 µL of the $ABTS^{\cdot+}$ solution for 2 h in a dark condition and absorbance value was measured again at 734 nm. The standard curve was prepared by using Trolox as a standard substance in a concentration range of 25 – 600 µM and results were expressed in µM Trolox equivalent (TE)/g fresh mass.

Moisture content and water activity (a_w)

Moisture content of the powder was determined by using a moisture determination balance FR-600. Water activity was measured using Testo 650.

Bulk density

Bulk density of powder was measured by weighing 5 g of a sample into a 10 mL graduated cylinder. Cylinder was vibrated until nearly meet the optimum packing level, and when a steady volume was reached, the bulk density was calculated as g/mL.

Analysis of zinc content

Zinc content in the powder was determined by AOAC (2005) 999.10 using atomic absorption spectrometry (AAS) and inductively coupled plasma–optical emission spectrometry (ICP-OES) method.

Flavor analysis

Volatile flavors in headspace of sample were extracted using solid phase microextraction (SPME) fiber assembly (Supelco, Bellefonte,PA). SPME was performed with fibers of Divinyl Benzene-Caroson-Polydimethyl Silosane. The fibers were exposed the headspace of flavors for 20 min at ambient temperature. Subsequently, the fibers were withdrawn into the housing, the SPME device was removed from the sample vial, and the fibers were desorbed into the GC–MS injector.

Gas chromatography/ mass spectrometry condition.

An Agilent 6890 GC equipped with an Agilent 5973 mass-selective detector (Agilent Technologies) was used with the injector and detector maintained at 200 and 250 °C, respectively. The column (HP-Innowax) dimensions were 0.25 mm (i.d.) × 30 m × 0.25 µm (film thickness). The carrier gas (Helium) had a flow rate of 5.0 mL/min. The temperature program was isothermal at 50°C for 10 min, increased to 170°C at the rate of 15 °C/ min, and held for 10 min. The mass spectrometer was operated in the electron impact (EI) mode with an electron energy of 70 eV, ion source and quadrupole maintained at 230 and 150 °C, respectively, and mass range (m/z) of 10-350. Compounds were identified by matching mass spectra (quality match >80%) and retention indices with the Chemstation Wiley Spectral Library of standard compounds. Moreover, the standards Alkane C1-C19 were used for determination of the retention indices (R.I).

The decay of Zn-chlorophyll powder

Pigment retention (%) was calculated by the formula: (chlorophyll content at × storage time) ×100 / (chlorophyll content at zero storage time). Rate constant (*k*) and half-life time ($t_{½}$) were calculated by the method of Cai *et al.* (1998) using the regression analysis of *ln*(pigment retention) against storage time when plotted on a natural logarithmic scale.

Results and Discussion

SEM micrograph

Figure 1 represents the shape of spray dried particle as results of wall materials of GA, MD and MS. The GA and MD had the spherical particles with

dented surface, while MS had the spherical particle with smooth surface. These related to Rosenberg *et al.* (1990) and Kim and Morr (1996) who reported that GA encapsulated powder had high dense on surface and also high release rate of orange oil flavor which was a cause of short term storage. Drush and Schwarz (2006) found that *n*-OSA starch resulted in spherical particles. Furthermore, Desobry *et al*. (1997) suggested that the spray dried particle in spherical shape had high ratio of surface/volume showing the appropriate character of spray dried product. Reineccius (2004) recommended that particle in spherical shape can retain the highest amount of flavoring agent. In this study, it can be suggested from considering the shape of the powder that MS was the proper wall material.

Particle size distributions

Average particle sizes of GA, MD and MS were 34.46, 30.11 and 16.13 μM, respectively (Figure 2). The average particle sizes of GA and MD were ~ 2 times greater than that of MS. These large particle sizes of GA and MD caused high dense on surface when they were processed by spray drying as shown in SEM micrographs.

Bulk density

Bulk density value correlated with the particle size as founded that powders derived from GA and MS had large sizes, while that from MS had the smallest size and hence MS can be contained most tightly and represented the highest bulk density value (Table 1). Reineccius (2004) found that particle in spherical shape had the highest bulk density value, best packing and best flowing ability. Moreover, Buffo and Reineccius (2000) have concluded that powder in spherical shape can be compacted. There was an advantage of the high bulk density that was reduction of the oxygen permeability which can cause oxidation, so the shelf life of product can be extended. Therefore, the smallest particle with density of MS can elicit the suitable characteristic of wall material. Bulk density of powder was affected by chemical composition, particle size and moisture content as well as by processing and storage conditions (Beristain *et al*. 2001).

Chlorophyll content

Properties of the wall material depended on its chemical composition (Goubet *et al*., 1998). Results of Zn-chlorophyll derivatives content derived from different types of wall material as presented in Figure 3 showed that MS had the highest amount of chlorophylls derivatives, the second was GA and the lowest was from MD due to the difference in chemical structure as follows: GA comprised with subunits of oligosaccharides, polysaccharides, and especially glycoproteins which had property of emulsion, thus they can bind with chlorophyll molecules that had both hydrophobic part of phytol group and hydrophilic part of porphyrin ring.

For MD, it derived from the starch modified with acid or enzyme to get monosaccharide or short chain polymer which subsequently formed thin film to cover the flavoring agent and coloring agents and prevent the loss of them during drying. The involved chemical bonds were hydrophobic, van der Waals, hydrogen bond and electrostatic (Goubet *et al.*, 1998). Nevertheless, some disadvantages of MD were having no property of emulsification and lack of surface binding activities at oil-water interface, causing low greenness of Zn-chlorophyll derivatives content.

Native starch and starch hydrolysis products were hydrophilic and had no affinity for hydrophobic substances such as oils. However, modifying the starch with fatty acids can introduce hydrophobic groups. For *osa*-MS, it has been modified by octenyl succinic anhydride to add hydrophobic groups to the starch molecules that comprised with amphiphilic character (Viswanathan, 1999; Kshirsagar and Singhal, 2007). Subsequently, it can bind with chlorophyll molecule or this meant MS had emulsifying property. Furthermore, it had character of strong film at oil-water interface area, so this can be resistant to re-agglomeration (Bhosale and Singhal, 2006) and hence MS was the suitable wall material for Zn-chlorophyll derivatives encapsulation.

Greenness of the powder

The greenness of the Zn-chlorophyll derivatives powder derived from different wall materials can be compared by considering at the same amount of total solid of wall material (Table1). It was found that using MS as wall material gave the highest greenness, the second was GA and MD had the lowest one. The greenness decreased when total solid content increased and it was the same trend with the result of Zn-chlorophyll derivatives content.

Antioxidant activity

The result of antioxidant activity value is shown in Figure 4. The result showed that the highest value derived from the MS powder and the lower levels from GA, and MD, respectively. The antioxidant value was related with Zn-chlorophyll derivative content, thus the higher Zn-chlorophyll derivative content affected to higher in antioxidant value (Endo *et al.*,

Figure 1. SEM micrographs of spray dried Zn-chlorophylls encapsulated powders. Wall material: (A) Gum arabic, (B) Maltodextrin, and(C) Modified starch.

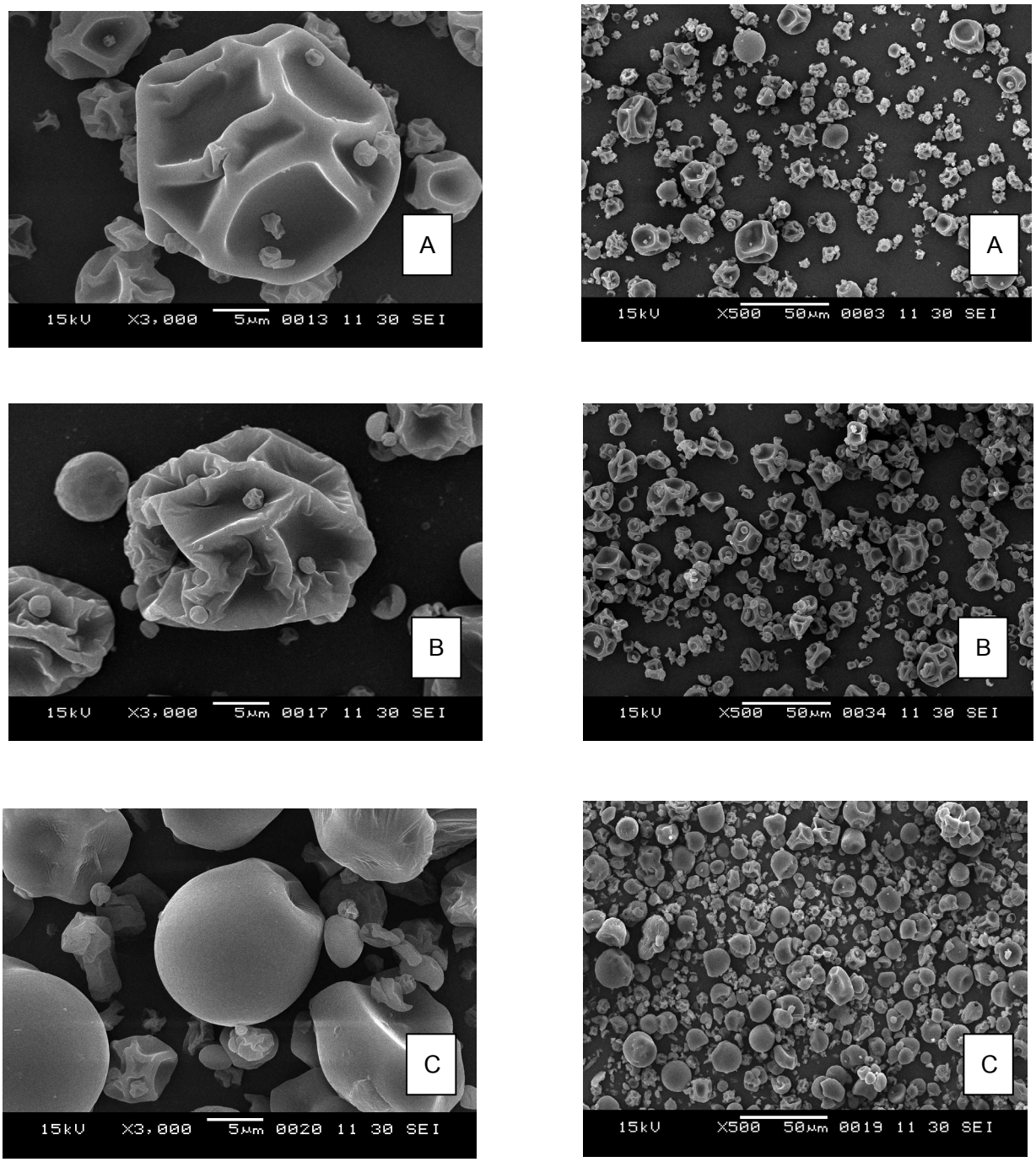


Figure 2. Particle size distribution of Zn-chlorophyll powder with different wall material: (A) Gum arabic (.....), (B) Maltodextrin (-------), and (C) Modified starch (_____) wall material.

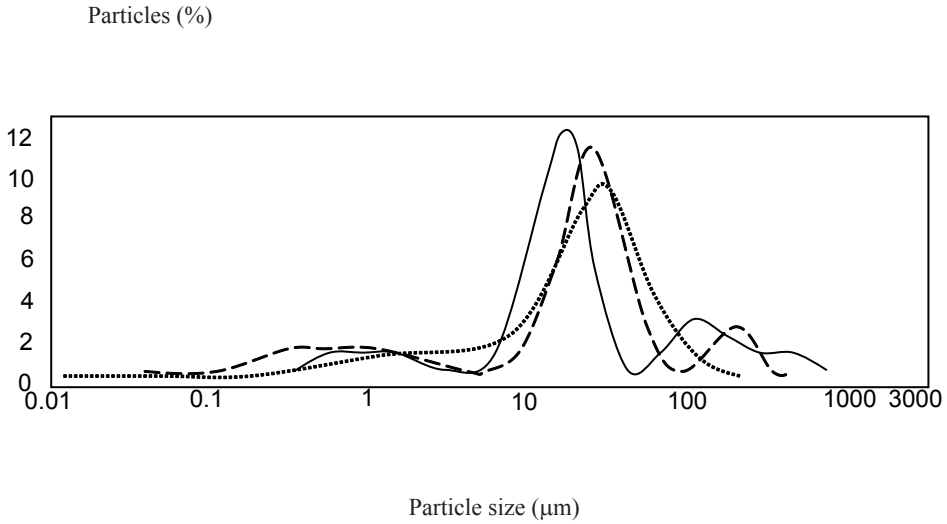


Figure 3. The chlorophyll contents of the powder derived from GA, MD, and MS powders at total solid content in range of 10-30% (v/w)

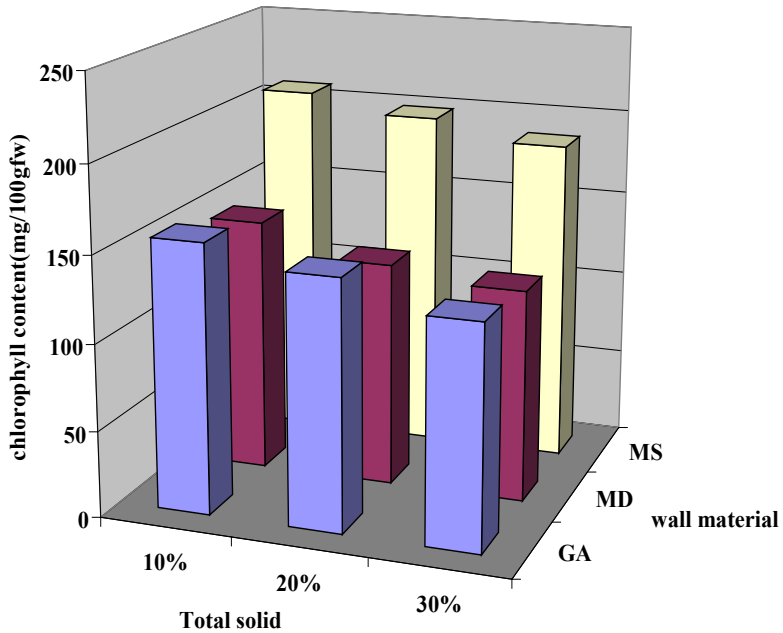


Figure 4. The antioxidant activity of Zn-chlorophyll powder with different wall material: GA, MD and MS powder at total solid range 10-30% (v/w)

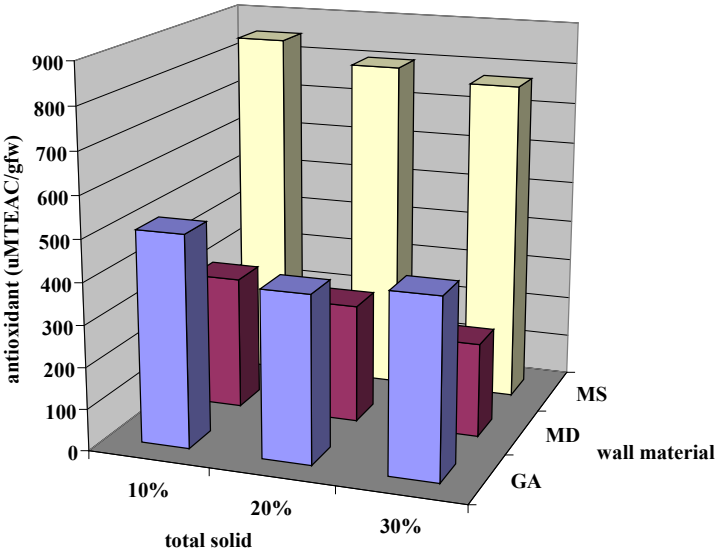


Figure 5. Degradation of Zn-chlorophyll derivatives in spray-dried powders with (a) GA, (b) MD, and (c) MS.

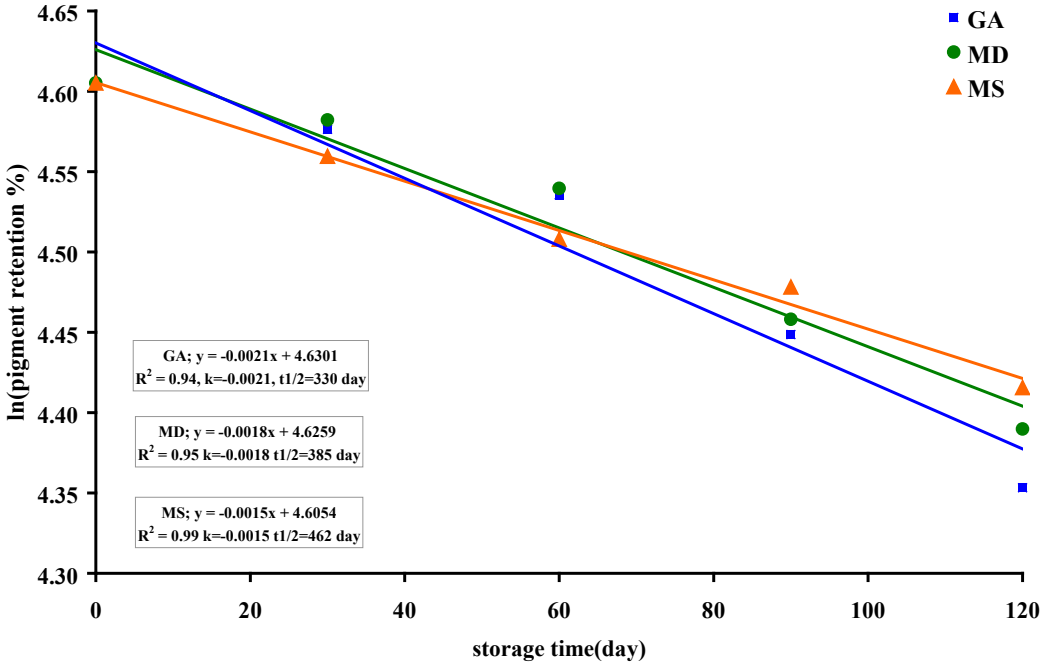
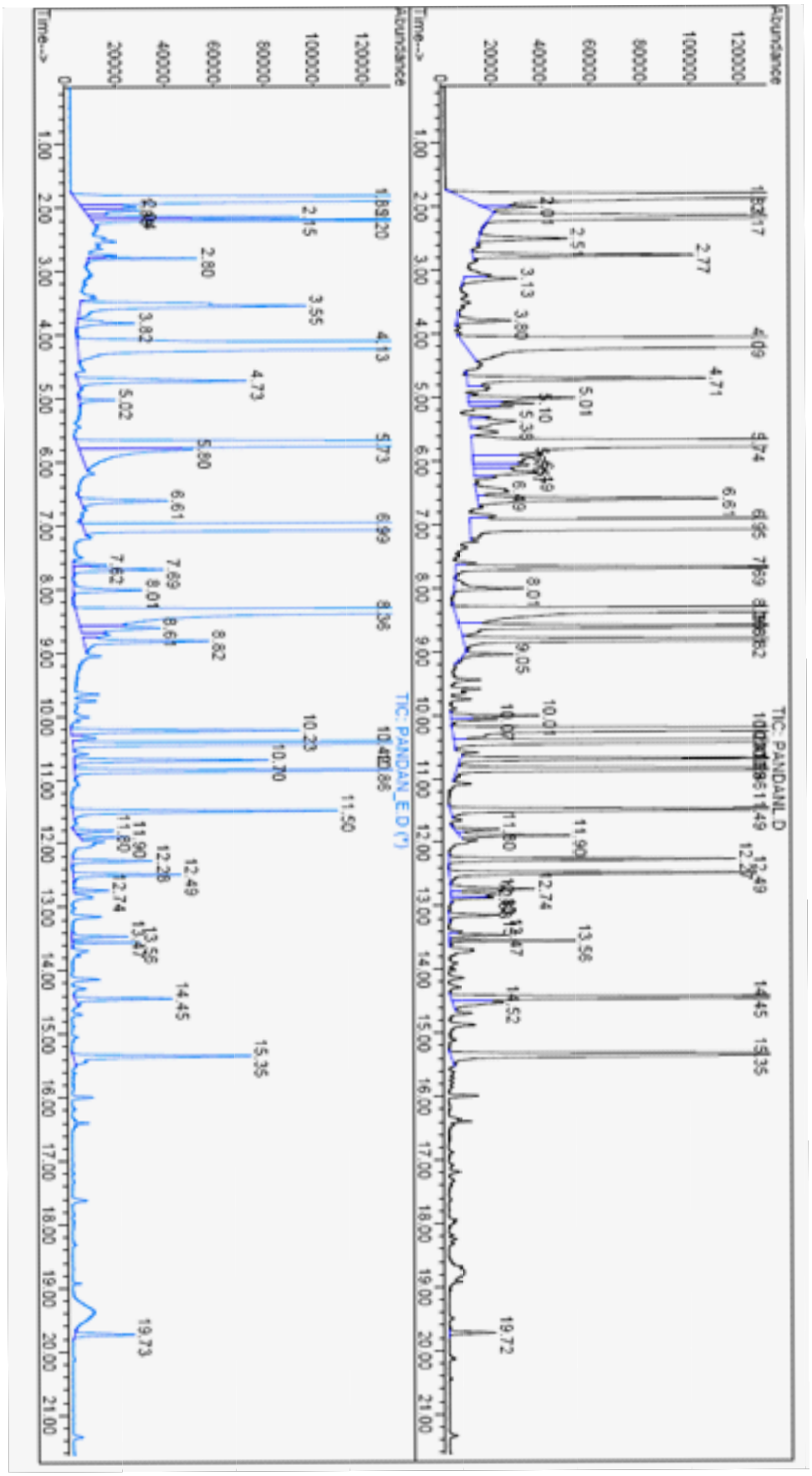


Figure 6. The GC-MS chromatograms of (A) fresh pandan leaf and(B) Zn-chlorophyll derivative powder.



1985; Cahyana, *et al*., 1993; Hoshina *et al*., 1998; Ferruzzi *et al*., 2002). Furthermore, higher amount of chlorophyll increased antioxidant activity because Zn-chlorophyll derivative was able to break the radical chains due to being an electron donor (Endo *et al*., 1985). Hoshina *et al*. (1998) found that the porphyrin's structure in chlorophyll molecules was capable of inhibiting the formation of lipid hydroperoxide from thiocyanate and ferric nitrolotriacetate. Consequently, the formation of metallochlorophyll complexes by changing chlorophyll to Zn-chlorophyll derivative not only made the color of chlorophyll stable due to the porphyrin's stability, but also brought about more antioxidant activity than native chlorophyll (Ferruzzi *et al*., 2002). According to the abilities of MS and GA in binding with both hydrophilic and hydrophobic groups of chlorophyll molecules, their antioxidant values were higher than that of the MD which had no emulsion property and then can bind only with low amount of chlorophyll, leading to low antioxidant value.

Water activity (a_w)

The a_w values resulted from MS, GA and MD were in range of 0.28-0.30. At the higher total solid contents there were more rapid drying and lower a_w value of the encapsulated powder. Fenema (1985) suggested that the suitable a_w values were between 0.2 and 0.3 because many chemical reactions such as oxidation, Maillard reaction and chlorophyll degradation can occur slowly, and no microbial proliferation in this condition. Moreover, the a_w value at less than 0.300 can be considered as ensure of product stability (Drusch and Schwarz, 2006).

Decay of Zn-chlorphyll powder

Stability of Zn-chlorophyll derivative powders derived from different wall materials and kept in clear glass bottles at room temperature for 120 days showed the results in Figure 5. Regression equation of *ln*(pigment retention %) against storage time showed the linear relation with negative slope when plotted on a natural logarithmic scale. This decrease of the Zn-chlorophyll derivative can be concluded as first-order kinetics with rate constant (k) of 2.1×10^{-3}, 1.8×10^{-3} and 1.5×10^{-3} day^{-1} for the GA, MD and MS, respectively and the half-life value of 330, 385 and 462 days for GA, MD and MS, respectively. From this experiment results, it was clear that MS was the appropriate wall material which can retain most of Zn-chlorophyll derivative as shown the longest shelf life.

Flavor analysis in modified starch pandan leaf powder

Zn-chlorophyll derivatives powder comprised 21 types of flavoring agents as shown in Figure 6 and Table 2 which were 2-Ethyl furan and 2-Pentyl furan of furan group; 1-Penten-3-one, 2-heptanone and 4-(2,6,6-trimethyl-2-cyclohexan-1-ol)-3buten-2-one

Table1. Physicochemical properties of Zn-chlorophyll derivatives at 30%total solid of wall material

Physicochemical properties	GA	MD	MS
Color value			
Lightness(*L**value)	64.34 ± 3.29a	66.71 ± 4.84a	52.90 ± 4.13b
Greenness(-*a**value)	-8.75 ± 3.20c	-8.37 ± 1.31b	-14.64 ± 1.54a
Yellowness (*b**value)	14.71 ± 2.12b	15.73 ± 1.34b	20.16 ± 2.16a
Chroma(C)	14.78 ± 1.25c	16.22 ± 1.71b	23.50 ± 1.11a
Hue (^{o}h)	123.40 ± 2.29b	120.86 ± 1.98b	130.17 ± 1.94a
Chlorophyll content(mg/100gfw)	128.65 ± 1.45b	122.79 ± 1.03b	187.34 ± 2.19a
pH	6.55 ± 0.34c	7.35 ± 0.63a	7.04 ± 0.03b
a_w	0.30 ± 0.01a	0.28 ± 0.01b	0.28 ± 0.02b
Moisture content	9.43 ± 0.95a	8.45 ± 0.81b	9.27 ± 0.74a
Bulk density	0.48 ± 0.03b	0.50 ± 0.01b	0.55 ± 0.01a
Antioxidant activity(μMTE/gfw)	428.02 ± 3.12b	225.05 ± 5.63c	772.50 ± 4.32a
Particle size distribution, D(0.5)	34.46 ± 3.23a	30.11 ± 1.59b	16.13 ± 1.36c
Zinc content(mg/kg)	14.45 ± 0.44ns	13.79 ± 0.60ns	13.12 ± 0.49ns

Reported means (± standard deviations) derived from 3 replications with 3 samples per replication. Means within a same row followed by the same letter were not significantly different ($P<0.05$).

Table 2. Flavor compounds detected in the fresh pandan leaf powder and Zn-chlorophyll derivative powder by SPME-GC-MS

No	Structure	Group	RI	CAS	Flavor	Description	Retention time		Zn-chl.2(%)
1	2-ethyl furan	furan	133.41	3208-16-0		sweet, burnt, earthy, malts	3.81	-	0.52
2	1-penten-3-one	ketone	245.20	1629-58-9		fish, pungent	4.73	1.26	1.37
3	hexanal	aldehyde	1083.65	66-25-1		grass, green, woody, green	5.73	41.39	48.27
4	(*E*)-2-pentenal	aldehyde	1131.82	1576-87-0		strawberry, fruit, tomato	6.61	1.39	0.74
5	2-heptanone	ketone	1184.34	110-43-0		soap	7.62	-	0.24
6	heptanal	aldehyde	1187.98	111-71-7		green, fresh green, sweet, herbal, rancid	7.69	2.37	0.69
7	2-hexenal	aldehyde	1221.54	6728-26-3		fresh green, fruity , apple	8.01	19.10	34.46
8	2-penty-furan	furan	1234.60	3777-69-3		green bean, butter	8.61	2.78	0.94
9	4-heptenal	aldehyde	1245.15	6728-31-0		biscuit, cream	8.82	3.71	1.09
10	2-penten-1-ol	alcohols	131.32	1576-95-0		green, plastic, rubber	10.23	10.15	1.62
11	2-acetyl-1-pyrrolline	Nitrogeneou compounds	1339.93	85213-22-5		nut, roast, pandan	10.42	1.64	1.33
12	1-hexanol	alcohols	1348.66	111-27-3		resin,flower,green	10.86	4.32	2.52
13	3-hexen-1-ol	alcohols	1381.51	928-96-1		fresh green, cut grass	11.50	2.38	1.84
14	nonanal	aldehyde	1396.92	124-19-6		fat, citrus, green	11.80	-	0.19
15	2-hexenol	alcohols	1402.14	928-95-0		green, leaf, walnut	11.90	-	0.28
16	2-octenal	aldehyde	1433.69	2363-89-5		Green	12.49	1.49	0.74
17	1-octen-3-ol	alcohols	1447.06	53907-72-5		mushroom, green coffee	12.74	-	0.20
18	2-ethyl hexanol	alcohols	1486.09	104-76-7		rose, green	13.47	-	0.36
19	2-nonenal	aldehyde	1540.38	18829-56-6		cucumber, fat, green	14.45	3.49	0.75
20	(E,Z) 2,6-nonadienal	aldehyde	1590.85	557-48-2		cucumber, wax, green	15.35	4.47	1.36
21	4-(2,6,6-trimethyl-2-cyclohexan-1-ol)-3buten-2-one	ketone	1840.22	127-41-3		wood, violet	19.73	-	0.43

Note; 1 Flavor compounds found in Fresh pandan leaf powder; 2Flavor compounds found in Zn-chlorophyll derivative powder

of ketone group; hexanal, (E)-2-pentenal, Heptanal, 2-Hexenal ,4-Heptenal, nonanal ,2-octenal,2-Nonenal and (E,Z) 2,6-nonadienal of aldehyde group; 2-Penten-1-ol, 1-Hexanol, 3-Hexen-1-ol ,2-hexenol, 1-octen-3-ol, and 2-ethyl hexanol of alcohol group; including 2-Acetyl-1-pyrroline of nitrogeneous compounds group.

Comparison between fresh pandan leaf (Table 2) and Zn-chlorophyll derivative powder made from fresh pandan leaf showed that Zn-chlorophyll powder had seven flavoring agents more than the fresh pandan leaf. These compounds were (1) 2-Ethyl furan (sweet and burnt flavor), (2) 2-heptanone (soap flavor), (3) nonanal (fat and green flavor), (4) 2-hexenol (green leaf flavor), (5) 1-octen-3-ol (green flavor), (6) 2-ethyl hexanol (green flavor) and (7) 4-(2,6,6-trimethyl-2-cyclohexan-1-ol)-3buten-2-one (wood flavor). These flavoring agents may derive from heat process like metallo-chlorophyll formation and spray drying, leading to some flavors like burnt, fat, soap and wood. When considered flavor of 2-AP, it was found that chlorophyll powder from pandan leaf had low amount of this flavor than fresh leaf because 2-AP compound was not stable and can be degraded rapidly, subsequently its concentration decreased after process.

Conclusions

Comparisons of the encapsulation properties among GA, MD and MS in Zn-chlorophyll derivative powders produced from pandan leaf can be concluded that using MS as wall material brought about the highest greenness, chlorophyll derivative content and antioxidant property. The MS powder can find the 2-AP compound which was the characteristic flavor of pandan leaf, and zinc content of 13.12 mg/kg which was not excess the limit of FDA (75 mg/kg). The deduction of Zn-chlorophyll derivative in MS powder showed the first-order kinetic with rate constant (k) 1.5×10^{-3} day^{-1} and half-life 462 days.

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