Rice phytochemicals concentrated by molecular distillation process and their use as co-surfactant in water dispersion

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Abstract This study investigated the effects of evaporating temperature during molecular distillation (MD) process employed to deodorizer distillate (DD) on the retention of rice phytochemicals in the unevaporated fraction (UMDs), which were then further used as co-surfactants in the fabrication of water-dispersible vesicles. The pilot-scale MD unit was operated at 120, 140 or 160 °C and 0.1 Pa to concentrate rice phytosterols from 1540.8 mg in 100 g DD to 3990.2–4904.8 mg in 100 g UMDs by evaporating out free fatty acids. Although γ -oryzanol content was increased from 598.9 mg in 100 g DD to 870.0–1018.1 mg in 100 g UMDs when the temperature was raised to 160 °C, such high temperature decreased tocopherols from 2185.7 mg in 100 g DD to 850.5 mg in 100 g UMDs and antioxidant capacity of UMDs measured as 2,2-diphenyl-1-picrylhydrazyl scavenging capacity. The UMD obtained after distillation at 140 °C was used as

co-surfactant with soy lecithin, sucrose palmitate or polyoxyethylene sorbitan monooleate (Tween 80) to fabricate vesicles in pH 7.0 phosphate buffered saline (PBS). This study showed potential use of the UMD as a source of rice phytochemicals and a co-surfactant when used with Tween80 in small vesicle fabrication. The fabricated Tween 80/UMD vesicles in PBS had the size range of 200–300 nm and were stable within a temperature range of 4 to 37 °C for 96 h.

Keywords Distillation · γ -Oryzanol · Phytosterol · Rice · Surfactant · Tocol

Introduction

Rice bran oil (RBO) is well recognized as healthy oil that contains proper ratio of saturated, monounsaturated and polyunsaturated fatty acid. Not only suitable fatty acid profile, RBO is also known as the source of health-promoting phytochemicals, especially tocopherols, tocotrienols, phytosterols and γ -oryzanol (Van Hoed et al. 2006; Sengupta et al. 2014). However, some of these phytochemicals are reduced in refined RBO during refining process and become concentrated in the co-products, in particular gum, soapstock, deodorizer distillate (DD) and wax.

Our previous investigation revealed that the DD, a co-product obtained from steam deodorization process during physical refining of rice bran oil, could be used as a source for the production of γ -oryzanol, phytosterols, monoacylglycerol (MAG) and diacylglycerol (DAG) mixtures (Nukit et al. 2014; Sawadikiat and Hongsprabhas 2014) after free fatty acids (FFAs) were removed by molecular distillation (MD).

MD is a high-vacuum distillation process suitable for separation and purification of high-molecular-weight and thermally

Research highlights

- Deodorizer distillate from oil refinery was used as the source of rice phytochemicals
- Rice phytosterols, tocopherols and γ -oryzanols in DD was concentrated by MD process
- Reduction in tocopherols and antioxidant activity was due to evaporation during MD process
- The UMD obtained can be used as co-surfactant and water-dispersible vesicle stabilizer

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sensitive materials (Lei et al. 2005). The separation principle of MD is dependent on the difference in the molecular mean free path of materials (Lei et al. 2005). Generally, MD is characterized by low operating temperature (due to high vacuum), short exposure of the distilled liquid to elevated temperature, high vacuum, and a small distance between the evaporator and the condenser. Due to the high vacuum condition, oxidation that might occur in the presence of oxygen is reduced. The separation efficiency of MD is dependent on many operational factors, i.e. evaporation temperature, feed flow rate, and operating pressure (Batistella et al. 2002; Liu et al. 2008; Martins et al. 2006; Posada et al. 2007). Therefore, the influences of evaporation temperature during MD process on the retention of oil-soluble rice phytochemicals; namely tocopherols, phytosterols and γ -oryzanol, in the unevaporated fraction (UMDs) were further investigated in the current study.

However, insolubility in the aqueous phase of the concentrated oil-soluble rice phytochemicals obtained from MD could lead to their limited use in aqueous food systems. In the present study, we also proposed that the UMDs could be used in the fabrication of small vesicles that can disperse in water due to their indigenous phytosterols, MAG and DAG in the UMDs (Nukit et al. 2014; Sawadikiat and Hongprabhas 2014). A closed spherical structure of vesicle is usually fabricated by amphiphilic molecules in a bilayer membrane (Mollet and Grubenmann 2001). A well-known vesicle fabricated by phospholipids is called a liposome. Normally the fabrication of a vesicle also requires cholesterol to stabilize the vesicular structure (Vemuri and Rhodes 1995), which would be replaced by rice phytosterols in the current study.

We hypothesized that the rice phytochemicals concentrated in the UMDs could act as co-surfactant and vesicle stabilizer. To our knowledge, the use of the unevaporated fraction (UMDs) – obtained after molecular distillation of DD from the physical refining process of rice bran oil – which contains a mixture of rice phytochemicals and surfactants, has received little attention and thus merits further investigation. The objectives of the present study were thus to explore the influences of evaporating temperature during the MD process to evaporate out the FFAs from DD, and to investigate the effects of UMDs in the fabrication of vesicles that were stable in the aqueous phase. The insights obtained could be used to enhance the utilization of co-products from rice bran oil refinery in the production of water-dispersible rice nutraceuticals.

Materials and methods

Materials

Deodorizer distillate (DD) from commercial production of physically refined rice bran oil was used as the raw material for the production of rice phytochemicals using a pilot-scale

molecular distillation (MD) unit at Surin Bran Oil Co. (Surin, Thailand). De-oiled soy lecithin (Solae, St. Louis, MO, USA) and sucrose palmitate (HLB 16) (P1670; Mitsubishi-Kagaku Foods Corporation, Tokyo, Japan) were kindly provided by Rama Production Co., Ltd., Thailand, and Caltech Corp., Ltd., Thailand, respectively. Polyoxyethylene sorbitan monooleate (Tween 80) was purchased from Sigma-Aldrich. Other chemical reagents were of analytical grade.

Characteristics of rice bran oil deodorizer distillate (DD)

Thermo-oxidative stability of DD

The thermo-oxidative stability of DD was characterized by a Mettler Toledo DSC821e differential scanning calorimeter (Schwerzenbach, Switzerland). Four to five mg of DD were weighed into a 40 μ L aluminum sample pan, closed with a lid having a hole of 1 mm internal diameter drilled in the center. This hole allowed the sample to be in contact with an oxygen (O_2) or nitrogen (N_2) stream. A sealed aluminum empty pan was used as a reference. The sample and reference pans were heated at heating rates (β) of 2, 5, 10, 16 and 20 $^{\circ}C/min$. For thermal stability evaluation, experiments were performed under a N_2 stream at 100 mL/min flow rate. The thermo-oxidative stability of DD was determined under an O_2 stream at a flow rate of 100 mL/min. When the run was completed, the onset temperature (T_o) of oxidation was determined as the intersection of the extrapolated baseline and the tangent line (leading edge) of the exothermic peak (Ostrowska-Ligeza et al. 2010). The characterization was performed in duplicate.

The Ozawa–Flynn–Wall method (OFW) was used to determine activation energy (E_a) (Ostrowska-Ligeza et al. 2010). Linear regression of $\log \beta$ versus $1/T_o$ was plotted using Eq. (1) to determine the slope A:

$$\log \beta = A(T_o) + B \quad (1)$$

where β is the heating rate ($^{\circ}C/min$) and T_o is the onset temperature of oxidation in Kelvin (K). The activation energy (E_a) was calculated using Eq. (2), where R is a gas constant:

$$E_a = -2.19R \frac{\partial \log \beta}{\partial (1/T_o)} \quad (2)$$

Acid value of DD

The acid value of DD was determined according to the AOCS Official Method Cd 3d-63 (AOCS 1997a). Briefly, 2 mL of 1 % phenolphthalein in isopropyl alcohol was added to 125 mL of solvent mixture (isopropyl alcohol:toluene at the ratio of 1:1 v/v) and neutralized to a faint pink using 0.1 N KOH. Samples were weighed into an Erlenmeyer flask; 125 mL of solvent mixture containing phenolphthalein was

added, followed by titration with 0.1 M KOH. The acid value was calculated using Eq. (3):

$$\text{Acid value (mg KOH/g sample)} = \frac{(A-B) * \text{Normality of KOH} * 56.1}{\text{g sample}} \quad (3)$$

where A is mL of 0.1 N KOH titrated with sample, and B is mL of 0.1 N KOH used in titrating a blank (solvent mixture).

Determination of tocopherols and tocotrienols in DD

The AOCS Recommended Practice Ce 7-87 (AOCS 1997b) was used to quantify tocopherol (α -T and γ -T) and tocotrienol (α -T3, β -T3, γ -T3 and δ -T3) contents. Briefly, 20 to 30 mg of samples were silylated by sylon BFT, i.e. 1 mL of pyridine and 2 mL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) mixture (99:1). Samples were heated at 50 °C for 10 min. The internal standard (heptadecanyl stearate) was added to silylated samples and mixed thoroughly. An aliquot (1 μ L) of each sample was injected into a gas chromatographic apparatus and quantified for tocopherol and tocotrienol contents using the response factor (FC) equation shown in Eq. (4):

$$FC = \frac{A_{IS} \times C_{\text{Standard}}}{A_{\text{Standard}} \times C_{IS}} \quad (4)$$

where A_{IS} is the area of internal standard (heptadecanyl stearate), A_{Standard} is the area of tocol standard, C_{IS} is mg of internal standard and C_{Standard} is mg of tocol standard.

Capillary gas chromatography was performed by a gas chromatograph equipped with a flame ionization detector (HP 6890 Series GC system; Agilent Technologies, Santa Clara, CA, USA) and HP-5 capillary column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m; Agilent Technologies). Helium was used as a carrier gas at a flow rate of 2 mL/min. Oven temperature was programmed to increase from 140 to 300 °C at a rate of 10 °C/min, with a 6 min hold at 300 °C; the temperature was then increased from 300 to 320 °C at a rate of 5 °C/min and held for 10 min. The injector and detector were maintained at 240 and 345 °C, respectively.

Determination of γ -oryzanol in DD

The γ -oryzanol content in each sample was determined spectrophotometrically using the method described by Khatoun and Gopala Krishna (2004). Briefly, 10 mg of sample was weighed into a 10 mL volumetric flask, dissolved in hexane and determined for γ -oryzanol content by a UV spectrophotometer (Genesys 10S UV-Vis; Thermo Fisher Scientific,

Waltham, MA, USA) at 314 nm using 1 cm cell length. The γ -oryzanol content was calculated as shown in Eq. (5):

$$\gamma\text{-oryzanol (mg/100g)} = \frac{\text{Absorbance at 314 nm in hexane solution} * 10000}{\text{g of sample} * 358.9} \quad (5)$$

Determination of phytosterols in DD

Rice phytosterol contents were determined according to the method described by Schwartz et al. (2008), using a flame ionization detector (HP 6890 Series GC system) and HP-5 capillary column (30 m $\times$ 0.32 mm $\times$ 0.25 μ m; Agilent Technologies) as previously described by Sawadikiat and Hongsprabhas (2014).

Antioxidant capacity of DD

The total free radical scavenging capacity of DD, determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, was evaluated using a modified method described by Rossi et al. (2007) and Ghafoorunissa (2007). DPPH was dissolved in ethyl acetate at a concentration of 126.8 μ M and the dilution adjusted to obtain an absorbance at 515 nm of 0.6237 absorbance units (AU) (Infinite® M200 PRO microplate reader; Tecan Group Ltd., Männedorf, Switzerland). UMD samples were diluted with ethyl acetate to obtain approximately 200–3000 μ g/mL of UMD. A mixture of 100 μ L of DPPH solution and 100 μ L of diluted UMD sample, having a final DPPH concentration of 63.4 μ M, was incubated in the dark at 25 ± 0.1 °C for 30 min. The absorbance (Abs) of the mixture was measured at 515 nm. The % scavenging of sample was determined as shown in Eq. (6):

$$\% \text{ scavenging} = \frac{Abs_{\text{control}} - Abs_{\text{sample}}}{Abs_{\text{control}}} * 100 \quad (6)$$

where Abs_{control} is the absorbance at 515 nm of DPPH solution with ethyl acetate (instead of sample), and Abs_{sample} is the absorbance at 515 nm of DPPH solution containing sample. The 50 % inhibition concentration (IC_{50} , μ g of sample/mL) was determined graphically by plotting a graph between % scavenging and sample concentration, and calculated as μ g of sample per mL of solution required to obtain 50 % of the maximum scavenging capacity. For comparison, α -tocopherol was evaluated under the same conditions.

Effect of distillation temperature on chemical characteristics of the unevaporated fraction (UMD)

To evaporate out the FFAs, a pilot-scale MD unit modified at Surin Bran Oil Co. (Surin, Thailand) was operated at distillation temperatures of 120, 140 and 160 °C and a pressure of

0.1 Pa. The unevaporated fraction, designated as UMD, was analyzed for acid value, γ -oryzanol, tocotrienols, tocopherols and phytosterols, as well as DPPH antioxidant capacity, using the methods described above. All samples were kept at 4 °C in amber glass bottles before analyses.

Effect of UMD and commercial surfactants on vesicle fabrication

Vesicle preparation

Vesicles were prepared using the Bangham method, described by Takahashi et al. (2007). Commercial surfactants – soy lecithin, Tween 80, or sucrose palmitate (0.05 to 0.16 g) – and UMD samples (0.04–0.15 g) obtained from MD operated at 140 °C were dissolved in 8 mL chloroform in a screw-cap test tube and mixed thoroughly. The solvent was evaporated to dryness under a N₂ stream. The residual solvent was further dried overnight in a hood. Then 8 mL of phosphate buffered saline (PBS) pH 7.3 was added to the thin film of surfactant/UMD and heated at 55–60 °C for 10 min. The test tube was then shaken vigorously using a vortex mixer for 5 min. The solid concentration of surfactant/UMD vesicles, having different ratios of surfactant to UMD of 1:0, 1:0.25, 1:1, 1:2, 1:3, 1:4 and 1:5 in the suspension, was 2.5 % (w/v) in PBS.

Determination of vesicle size distribution in PBS

The size distribution of surfactant vesicles and surfactant/UMD vesicles in PBS was analyzed by a Zetasizer Nano-ZS (Zen 3600; Malvern Instruments Ltd., Worcestershire, UK). Only treatments capable of UMD holding capacity (no observable phase separation between aqueous phase and oil phase) were used.

Storage stability of vesicles in PBS

A 0.22 μ m nitrocellulose membrane (MF-Millipore™ membrane filter; Millipore, Billerica, MA, USA) was sprayed with 70 % ethyl alcohol and equipped with sterile housing and test tube before filtration. Surfactant/UMD vesicle suspensions in PBS were aseptically filtered to sterile the suspensions. The sterile suspensions were kept at 4–5 °C or 37 °C for 0, 24, 48, 72 and 96 h before determination of size distribution using a Zeta Nano-ZS.

Statistical analysis

One batch of DD was distilled at different temperatures using a pilot-scale MD unit in two separate trials. Results were subjected to analysis of variance (ANOVA) with confidence interval set at 95 % ($P < 0.05$) using the statistical software program SPSS for Windows version 12 (SPSS Inc., Chicago,

IL, USA). Differences among means were differentiated using Duncan's multiple range test at $P < 0.05$.

Results and discussion

Characteristics of unevaporated fractions (UMDs) after molecular distillation of deodorizer distillate (DD)

The DSC thermogram in Fig. 1 illustrates that in the absence of oxygen (under a N₂ stream), DD obtained from the physical refining process of rice bran oil was thermostable between 25 and 300 °C. However, when O₂ was present, the oxidation of DD started at 121.2 °C at a heating rate of 2 °C/min. Table 1 indicates that raising the heating rate increased the onset temperature (T_o) of oxidation. The calculated activation energy (E_a) of DD was 111.3 kJ/mol, which was higher than those of sunflower oil, soybean oil and corn oil (Adhvaryu et al. 2000). The E_a of rice bran oil DD reported in the present study was within the same range as olive oil (Ostrowska-Ligeza et al. 2010). The reason is probably because both oils contain around 2 % polyunsaturated fatty acids and around 85 % monounsaturated fatty acids; while sunflower oil, soybean oil and corn oil contain around 46–61 % polyunsaturated fatty acids (Adhvaryu et al. 2000; Ostrowska-Ligeza et al. 2010).

Table 2 indicates that DD was a rich source of oil-soluble rice phytochemicals. The γ -oryzanol content in DD was 598.9 mg/100 g. The DD also contained high contents of tocotrienols, tocopherols and phytosterols. The antioxidant capacity, expressed as the IC₅₀, was 2301.6 μ g/mL. However, the acid value was very high, i.e. 125.4 mg KOH/g (Table 2), which is unsuitable for food use.

Nonetheless, most FFAs were evaporated after the MD process, resulting in UMDs having low acid values (Table 3). The acid values of UMDs were reduced dramatically at distillation temperatures at and above 140 °C, and reached the minimum values of less than 2.1 mg KOH/g. After FFAs were removed, some oil-soluble rice phytochemicals were concentrated in the UMD fractions. Table 3

Fig. 1 DSC thermograms of deodorizer distillate (DD) at a heating rate of 10 °C/min under oxygen (solid line) and nitrogen (dotted line) streams at a flow rate of 100 mL/min

Table 1 Effect of heating rate on onset temperature of oxidation (T_o) and activation energy (E_a) of deodorizer distillate (DD)

Heating rate (°C/min)	T_o (°C)
2	121.2 ^c ± 0.2
5	128.8 ^d ± 0.4
10	137.1 ^c ± 0.4
16	142.2 ^b ± 0.8
20	149.0 ^a ± 0.5
E_a (kJ/mol), calculated from Arrhenius equation	111.2 ± 2.1

Means ± s.d. followed by different superscripts are significantly different ($P < 0.05$)

indicates that γ -oryzanol content was the highest in UMD obtained at a distillation temperature of 140 °C ($P < 0.05$).

Tocotrienols were the main tocols in DD and all UMD samples. Tables 2 and 3 indicate that β -tocotrienol was the most abundant isomer, followed by δ -tocotrienol, γ -tocotrienol and α -tocotrienol, respectively. Both tocopherols and tocotrienols were effectively concentrated at a low distillation temperature of 120 °C. As distillation temperature increased to 160 °C, all tocol concentrations in UMDs were dramatically decreased ($P < 0.05$). The chromatogram in Fig. 2 indicates that a high distillation temperature of 160 °C reduced some unsaponifiable matter, i.e. tocols having a retention time between 12 and 20 min, shown as a lower number of peaks in UMD obtained after distillation at 160 °C.

Among all four isomers of tocotrienol, more of the δ isomer was lost during MD. More α isomer, however, was retained at high distillation temperature than the others. The variation in the reduction of different isomers may be due to the differences in thermal stability and boiling point of each isomer.

Table 2 Chemical characteristics of rice bran oil deodorizer distillate (DD) obtained from physical refining process

Chemical constituents	Mean ± s.d.
Acid value (mg KOH/g)	125.4 ± 1.0
γ -Oryzanol (mg/100 g)	598.9 ± 0.5
Tocotrienol contents (mg/100 g)	
α -Tocotrienol	77.7 ± 1.4
β -Tocotrienol	1132.6 ± 16.1
γ -Tocotrienol	139.2 ± 3.2
δ -Tocotrienol	612.9 ± 15.5
Tocopherol contents (mg/100 g)	
α -Tocopherol	166.1 ± 1.2
γ -Tocopherol	57.2 ± 0.4
Phytosterol contents (mg/100 g)	
β -Sitosterol	970.8 ± 77.9
Campesterol	259.2 ± 24.3
Stigmasterol	310.8 ± 17.4
DPPH radical scavenging capacity (IC ₅₀ ; μ g/mL)	2301.6 ± 45.3

The boiling point of each isomer at 760 mmHg was predicted using ACD/PhysChem Suite software from ACD/Labs (Toronto, Canada); the α -tocotrienol isomer had a boiling point of 542 °C, while δ -tocotrienol had a lower boiling point of 517 °C (The Royal Society of Chemistry 2013). The difference in the boiling point could be due to the different structure of the chromanol head group, of which α -tocotrienol has three methyl-substituted groups, whereas δ -tocotrienol has only one methyl-substituted group. The increase in methyl-substituted groups on the chromanol head group likely resulted in an increase in intermolecular forces. Consequently, the α -tocotrienol isomer has a higher boiling point compared with the δ -tocotrienol isomer, and was retained to a high extent when MD was operated at 140 and 160 °C.

The MD process in the present study was performed under high vacuum pressure (i.e. 0.1 Pa). As a result, oxidation of tocols was likely minimized due to low O₂ content. Verleyen et al. (2001) reported that the headspace pressure and O₂ concentration above the α -tocopherol in triolein hardly influenced the degradation of α -tocopherol at a high temperature of around 180–260 °C and reduced pressure of 400–4000 Pa. Therefore, it is most likely that the loss of tocols (Table 3) was mainly due to evaporation rather than thermo-oxidative degradation since the high vacuum pressure was used in the pilot-scale MD unit investigated in this study. This was due to the thermal stability of DD against the high temperature and very low pressure (0.1 Pa) used during the MD process investigated in the current study. Phytosterols and γ -oryzanol, however, had higher boiling points than the tocols, and thus more were concentrated at higher distillation temperature.

Nonetheless, the reduction in tocol contents at high distillation temperature at 160 °C, as well as the low content of γ -oryzanol at 120 °C, influenced the DPPH radical scavenging capacity, shown as a value of IC₅₀ of UMD obtained after distillation at 160 and 140 °C, respectively (Table 3). A distillation temperature of 140 °C was then used in further investigation as the source of phytosterols, tocols and γ -oryzanols to be encapsulated due to high content of γ -oryzanol and reasonably high tocols.

Effect of UMD and commercial surfactants on vesicle fabrication

Vesicles containing UMD in the presence of a commercial surfactant, i.e. soy lecithin, Tween 80 and sucrose palmitate, were fabricated. In the absence of UMD, soy lecithin vesicles showed a bimodal size distribution, with a high % intensity of the particles having sizes around 170 nm and 970 nm (Fig. 3a). Incorporation of UMD into soy lecithin/UMD vesicles using a lecithin to UMD ratio of 1:0.25 (w/w) resulted in a bimodal size distribution with particle sizes of 60 and 380 nm. Further increasing the UMD ratios in lecithin/UMD vesicles to 1:1, 1:2 and 1:3 resulted in polydispersed colloidal suspensions.

Table 3 Effect of distillation temperature during molecular distillation on chemical contents of unevaporated fraction after molecular distillation (UMD)

Chemical constituents	Distillation temperature		
	120 °C	140 °C	160 °C
Acid value (mg KOH/g)	42.9 ^a ± 2.6	2.1 ^b ± 0.5	1.5 ^b ± 0.1
γ-Oryzanol (mg/100 g)	870.0 ^b ± 25.1	1070.3 ^a ± 4.1	1018.1 ^a ± 28.1
Tocotrienol contents (mg/100 g)			
α-Tocotrienol	150.5 ^a ± 5.2	151.6 ^a ± 6.7	81.8 ^b ± 6.4
β-Tocotrienol	1804.0 ^a ± 42.2	1206.4 ^b ± 179.0	471.6 ^c ± 6.3
γ-Tocotrienol	205.7 ^b ± 14.9	257.7 ^a ± 18.3	83.4 ^c ± 7.4
δ-Tocotrienol	955.1 ^a ± 20.3	429.8 ^b ± 72.8	152.7 ^c ± 21.2
Tocopherol contents (mg/100 g)			
α-Tocopherol	265.5 ^b ± 11.9	295.8 ^a ± 19.4	61.0 ^c ± 3.9
γ-Tocopherol	108.9 ^a ± 0.4	76.0 ^b ± 5.8	not detected
Phytosterol contents (mg/100 g)			
β-Sitosterol	2635.0 ^b ± 134.6	2778.7 ^b ± 190.4	3160.7 ^a ± 37.5
Campesterol	763.4 ^b ± 48.5	748.0 ^b ± 54.5	860.8 ^a ± 17.6
Stigmasterol	591.8 ^b ± 44.1	816.2 ^a ± 60.1	883.3 ^a ± 23.5
DPPH radical scavenging capacity (IC ₅₀ ; µg/mL)	1053.9 ^b ± 61.7	899.0 ^c ± 30.7	1385.8 ^a ± 1.1

Means in the same row followed by different superscripts are significantly different ($P < 0.05$)

This indicated instability of soy lecithin/UMD vesicles in PBS.

The size distribution of vesicles fabricated using Tween 80 in PBS showed bimodal distribution (Fig. 3b). Considering % intensity, most Tween 80 vesicles had a size around 20 nm;

whereas a much lower population had a diameter around 1000 nm. The incorporation of UMD into Tween 80/UMD vesicles at a Tween 80 to UMD ratio of 1:0.25 (w/w) resulted in two major groups of vesicles. The first group with high % intensity had a size of about 20 nm. The second group of

Fig. 2 Effect of distillation temperature on phytochemical profiles of **a** deodorizer distillate, and unevaporated fractions obtained after molecular distillation at **b** 120 °C, **c** 140 °C and **d** 160 °C

Fig. 3 Particle size distributions of surfactant/UMD vesicles prepared using different ratios of surfactant to UMD: **a** soy lecithin/UMD vesicles, **b** Tween 80/UMD vesicles, and **c** sucrose palmitate (SP)/UMD vesicles

vesicles, with lower % intensity, had a size range between 200 to 300 nm. When the UMD ratio was increased to 1:1, 1:2 and 1:3 (*w/w*), the Tween 80/UMD vesicles showed monomodal size distribution, with the majority having sizes of around 200 to 300 nm, and no lipid separation was observed. This suggested that the fabricated Tween 80/UMD vesicles could hold a high content of UMD, indicating the potential for encapsulating rice phytochemicals concentrated from DD by MD process. Increasing the ratio of UMD to 1:4 and 1:5, however, resulted in the aggregation of Tween 80/UMD vesicles at a size of around 3000–4000 nm (results not shown),

which separated from the aqueous phase after 3 h of preparation. In this study, Tween 80/UMD vesicles had an average diameter greater than that of micellar Tween 80 of 35 Å (Amani et al. 2011).

Sucrose palmitate (SP), however, gelled in PBS at the concentration used in the current study. Therefore, the size distribution of vesicles in PBS could not be determined. Nonetheless, incorporation of UMD into SP/UMD vesicles resulted in suspensions instead of gel. However, the SP/UMD vesicles showed polydisperse distribution, ranging from submicron to micrometer size (Fig. 3c).

Fig. 4 Effect of storage time and temperature on particle size distribution of Tween 80/UMD vesicles stored at different temperatures

The greater ability of Tween 80, compared with soy lecithin and sucrose palmitate, in aiding the formation of surfactant/UMD vesicles may be due to the structure of the surfactant and the composition of the UMD itself. The UMD was composed of triacyl glycerol, DAG and MAG, with an oleyl chain as the major esterified fatty acid (Nukit et al. 2014). Therefore, the hydrophobic tail of the oleyl chain in Tween 80 molecules and the oleyl chain in the UMD reported in the current study may self-assemble into stable vesicles.

Despite the highly negatively charged groups of phosphatidyl choline and phosphatidyl ethanolamine, which generally favor liposome or vesicle formation by soy lecithin, the major acyl groups of commercial soy lecithin are usually linoleyl (65.9 %) and oleyl (10.6 %) chains (Magil et al. 1981). The conjugated double bond of linoleyl and oleyl chains may not favor the formation of thermodynamically stable bilayer of vesicles. Similar results were observed in SP/UMD vesicles, of which the esterified palmitate was the major acyl chain.

After filtered sterilization, the size distribution of Tween 80/UMD vesicles in PBS showed monomodal distribution of around 200 nm, with a narrow size range. The sterile suspensions were quite stable at low temperature of 4–5 °C and at 37 °C for 0, 24, 48, 72 and 96 h (Fig. 4). After storage, the average vesicle diameter remained around 200 nm (Fig. 4). The Tween 80/UMD vesicles, however, had a wider size range compared to that of 0 h. This was possibly due to vesicle flocculation. Nevertheless, Tween 80/UMD vesicles in PBS showed potential for use in the formulation of vesicle suspensions that were stable over a temperature range from chilled storage to body temperature.

Conclusions

This study indicated that deodorizer distillate (DD) from the physical refining process of rice bran oil was a rich source of tocotrienols, tocopherols, phytosterols and γ -oryzanol. DD was quite thermo-oxidatively stable for the MD process under the operating conditions used in the current study. Nevertheless, the distillation temperature during the MD process was a crucial processing parameter influencing the retention of rice phytochemicals in the unevaporated fraction in UMDs. The UMD containing high contents of γ -oryzanol, tocopherols and phytosterols could further be encapsulated in vesicles in the presence of Tween 80. Moreover, the Tween 80/UMD vesicles in the aqueous phase were quite stable and could retain an average size of 200 nm during storage at 4 and 37 °C.

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