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Formation of Rice Bran Glycosphingolipids Microemulsion Powders with Vitamin B1, B2, B12, and Folate as Additives for Elderly Food

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Abstract

This research investigated the extraction of glycosphingolipids from rice bran as an emulsifier to form microemulsions with vitamins B1, B2, B12 and folate being added to prepare the emulsion powder as the dietary supplement materials. Rice bran, a low-value agricultural material, was used as raw material for glycosphingolipids extraction using a solvent mixed between dichloromethane and methanol at a ratio of 2:1. The crude extract was observed in physical characteristics. The sphingosine in crude extract was analyzed by spectrophotometer technique. The emulsion was prepared using a mixture of crude extract, Polysorbate 80 and coconut oil. The average particle size of the emulsion was determined using Particle Size Analyzer. The emulsion was made into powder using various drying methods including hot air drying, spray drying and freeze-drying method. The mannan-oligosaccharides were used as an additive to replace the expensive mannitol or the energy-producing sucrose. The distribution property in distilled water of the emulsion powder was determined. The morphology and the surface of the powder emulsion were measured using SEM. The resistance to the imitation of the digestive system and encapsulation efficiency were determined. After extraction, the yield of crude glycosphingolipids extract from rice bran was 20.65%. The crude extract was a clear liquid, slightly yellow, insoluble in water and looks like oil. We found that the crude extract contained sphingosine 22.75 μg/g of crude extract from rice bran. When the emulsion was prepared, the characteristic of the emulsion showed the colloidal solution with a milky white color. The size of the emulsion without vitamins and with vitamins B1, B2, B12, and folate (total 100 ppm) were 70-75 nm and 74.4 -78.1 nm, respectively. This result illustrated that the emulsion was classified as glycosphingolipid microemulsion. The emulsion powder was prepared and we found that the hot air drying and spray drying methods showed a viscous liquid with an oily smell. Whereas the freeze-drying method created the form of a light yellow,

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odorless and fine emulsion powder. The average particle size of emulsion was 80-100 nm. The solubility test showed that the emulsion powder was able to dissolve up to 300 g/L and 450 g/L at 25°C and 70°C, respectively. The morphological analysis showed that the powder emulsion was quite spherical with a diameter of less than 100 micrometers. In a simulated digestive system test, it was found that glycosphingolipid emulsion releases all vitamins in gastric simulated fluids and simulated small intestinal fluid conditions with values greater than 80%.

Introduction

Thailand is an agricultural country with rice as the main food crop. Rice bran is the outer brown layer of paddy, and it is one of the major byproducts produced from the rice milling process. It can be used as livestock feed or extracted and produced as rice bran oil. Rice bran is a good source of crude fibers, proteins, lipids and carbohydrates (Subramanian et al., 2018). One of the compounds found in rice bran is glycosphingolipid, an important compound found in the plasma membrane of eukaryotic cells. The extraction and separation of glycolipids were subjected to a fractionation process. Glycolipid quantification does not require specialized techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), or mass spectroscopy (MS). It can be done in a normal laboratory (Saito & Hakomori, 1971). Glycosphingolipids from rice bran have been developed into various forms and applied in food supplements and cosmeceuticals. Similar to animal-derived glycosphingolipids, the main structure is ceramide with sphingoids connected to fatty acids by amide bonds and a hydroxyl group of sphingosines connected to glucose or galactose. Glycosphingolipids are amphipathic molecules with both polar regions that exhibit hydrophilic properties and non-polar regions that show hydrophobic properties within the same molecule. This allows glycosphingolipids to form a micelle structure that can contain substances and show emulsifying potential in emulsions.

Microemulsion is a thermodynamically stable oil-water structure. It is a transparent, isotropic colloid system with particle sizes less than 100 nm (Lawrence & Rees, 2000). It shows greater skin permeability than other emulsion types due to its smaller size. Moreover, it has advantages from its stability, easy invention and scale-up and became popular in dermatological cosmetics and food industries over the past several years (Flanagan & Singh, 2006; Talegaonkar et al., 2008).

Vitamin B is a group of water-soluble vitamins. Each of them shows important roles in human metabolism and maintaining a healthy body. Normally, humans get vitamins from their daily diet, but some age groups, especially the elderly have problems with chewing food, deterioration of the digestive system, decreased sense of smell and taste and difficulty swallowing leading to nutrient deficiencies, especially micronutrients such as thiamine (vitamin B1), riboflavin (vitamin B2), folate (vitamin B9, also known as folic acid, cobalamin (vitamin B12), iron (Fe) and calcium (Ca) (Kennedy, 2016). Nowadays, Thai society is stepping into an ageing society like many other countries in the world. The elderly are classified as consumers with high purchasing power and a high demand for quality products and services. The problem found among the elderly in Thailand is malnutrition from macronutrients and micronutrients, especially vitamins and minerals (Baik & Russell, 1999).

Prior research have reported on the food additive for dietary supplements, such as the combination of antioxidants and calcium used as a functional food for the elderly to reduce the risk of osteoporosis (Arnold et al., 2021). Kim & Lee (2016) reported that vitamin C was used as a food additive to decrease the risk of osteoporosis in elderly Korean (aged over 50 years with low physical activity). Both research involved adding dietary supplements directly to food. However, some researchers use carriers to transport food additives or functional food, such as research by Darole et al. (2008). They reported on the formation of the microemulsion-based delivery system for the transport of antifungal antibiotic in the treatment of severe systemic fungal infections, Amphotericin B.

In this research, rice bran, a low-value by-product from the rice milling process, is used as raw material for glycosphingolipids extraction. The microemulsion is prepared using crude extract as an emulsifier. The rice bran-extracted glycosphingolipids were investigated as additives to prepare vitamin-containing microemulsion powder. It can be used as a dietary supplement material in healthy instant soups, functional foods for elderly food products, or used as a model for the preparation of emulsion powder to further transport other important substances into the human body.

Materials and methods

1. Rice bran glycosphingolipid preparation

The preparation of glycosphingolipid from rice bran was modified from Paosila et al. (2020). The rice bran was cleaned and dried in an oven (Eyela NDD-600 ND hot air oven, Tokyo Rikakikai Co., Ltd., Japan) at 60°C. Then, 20 g of rice bran was weighed into a 500 mL beaker. Next, dichloromethane:methanol solution at a ratio of 2:1 was added about ten times of the sample volume. The mixed solution was left to stand for 1 hr to ensure that the rice bran was completely soaked. The mixed solution was centrifuged (RS-SC plus, Sorvall Dupont Ltd., USA) at 3000 rpm for 20 min and the supernatant was collected. The pellet was re-extracted with 200 mL of dichloromethane:methanol (ratio 1:1) and left to stand overnight. The mixed solution was centrifuged at 3000 rpm for 20 min and the supernatant was collected. Combined the supernatant from step 2 and 3 and evaporated in a vacuum evaporator until the sample was dried. The dried sample was dissolved in 400 mL of dichloromethane:methanol solution at the ratio 2:1 and 0.1M KCl was added about two times the sample volume and left to stand overnight. Transfered the lower solution to the new beaker and the TUP solution (dichloromethane:methanol:0.1M KCl, ratio 3:48:47) was added about 0.2 times the volume. The solution was extracted and left aside to separate. Transfered the lower solution to the new beaker. The solution was evaporated with a rotary vacuum evaporator (EYELA 1300VF, Tokyo, Japan). Stored the solution in a desiccant before usage in next step.

2. Glycosphingolipid microemulsion preparation

The preparation of glycosphingolipid microemulsion was modified from Uchiyama et al. (2019). The crude glycosphingolipid from rice bran was mixed with Polysorbate 80 (TWEEN 80) and then coconut oil was added and mixed. The mixed solution was dissolved in 1,250 mL of the mixture of ethanol: acetone (ratio 1:1). The mixture of ethanol:acetone was removed by evaporation in a rotary evaporator with a pressure of 102 mbar at 50°C. The dried sample was dissolved in distilled water and sonicated for 10 min and the particle size was measured using Particle Size Analyzer. The vitamins-loaded glycosphingolipid microemulsion was prepared as above, but the sample was dissolved in a mixed vitamins solution with excess concentration of each vitamin (Vitamin B1 50 ppm, B2 50 ppm, B12 15 ppm, and folate 10 ppm).

3. Glycosphingolipid and vitamins-loaded glycosphingolipid microemulsion powder preparation

The preparation of glycosphingolipid and vitamins-loaded glycosphingolipid microemulsion powder were prepared with mannitol, sucrose or mannan-oligosaccharide using the freeze drying technique (Uchiyama et al., 2019). It began by adding at 10, 20 and 30% of mannitol, sucrose, or mannan-oligosaccharide and then frozen at -80°C for 30 min. The evaporation drying process was carried out using a freeze dryer (FDU-2100 freeze dryer, Tokyo Rikakikai Co., Ltd., Japan) for 24 hr. Re-dispersibility of freeze-dried emulsion powder was determined.

4. Dissolution test

The dissolution test was modified from Uchiyama et al. (2019). The freeze-dried emulsion powder was re-dispersed in 10 mL of distilled water to achieve the maximum concentration. The particle size of re-dispersed glycosphingolipid microemulsion powder and vitamins-loaded glycosphingolipid microemulsion were measured. The maximum solubility was studied at 25 and 70°C. The weight of the emulsion powder was measured.

5. Solubility test

The solubility test was modified from Galia et al. (1998). The emulsion powder was carried out in simulated gastric fluid (0.1 M HCl) or simulated intestinal fluid (6 mM sodium taurocholate, 1.5 mM lecithin, 8.9 mM KH_2PO_4 and 0.21 M of KCl pH 6.5) at 37°C, 100 rpm using water bath shaker (Shaker, WNB 29 model, Memmert, Germany). Freeze-dried powder prepared with mannan-oligosaccharide were added to 10 mL of dissolution media. The 1.0 mL of samples was removed after 24 hr and filtered through a 0.45 μm PTFE-filter. The concentration of each vitamin was determined by the HPLC (HPLC, CBM-20A model, Shimadzu, Japan).

Results and discussion

1. Rice bran glycosphingolipid preparation

From the extraction of glycosphingolipid from rice bran, which is a low-value agricultural material, using dichloromethane:methanol (2:1), we found the yield of crude glycosphingolipids extract was 20.65%. It showed the same trend of the yield of lipid extraction of glycosphingolipid from rice bran using methyl tert-butyl ether/methanol as the solvent extraction was 19.3% (Guazzotti et al., 2023). Physical characteristics were observed: it was a clear, light-yellow solution, insoluble in water, like oil. Sphingosine content was analyzed by spectrophotometer (UV-visible spectrophotometer Shimadzu Model UV-160, Japan) (A_{510}, λ_{max} of sphingosine). We found that the obtained crude extract illustrated the peak which is related to sphingosine as shown in Fig. 1. After comparing with standard sphingosine, the crude extract showed that it contained 22.75 µg sphingosine/g of crude extract. When the solution was tested by the Molisch method (Foulger, 1931), a purple ring was found at the interface between the layers of the solution. From these results, we suggested that the rice bran extracted was probably glycosphingolipids because the sphingosine and carbohydrates were found in its structure from the results of Spectrophotometry and Molisch method.

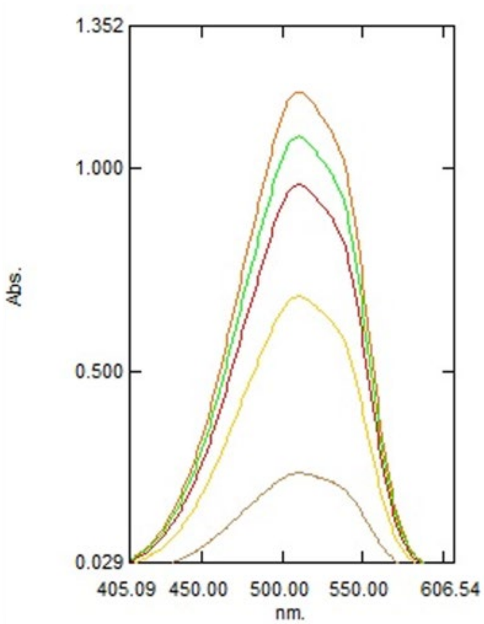


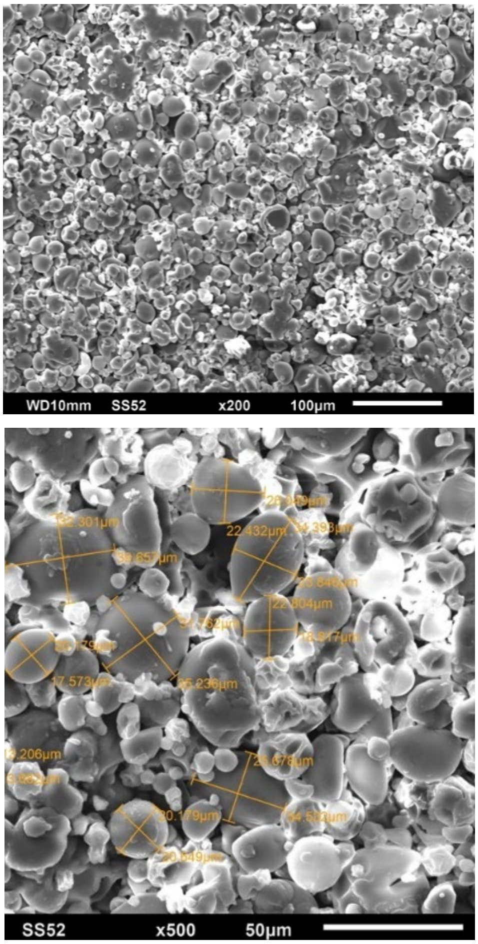
Fig. 1 A510 of the crude extract of glycosphingolipid from rice bran

2. Glycosphingolipid and vitamins-loaded glycosphingolipid microemulsion powder preparation

After that, the crude rice bran extract was then used to prepare a microemulsion with Polysorbate 80 and coconut oil in the ratio of 1:4:2 using the sonication technique, the result showed that the emulsion was a

Fig. 2 The emulsion particles were analyzed under a 100x magnification microscope

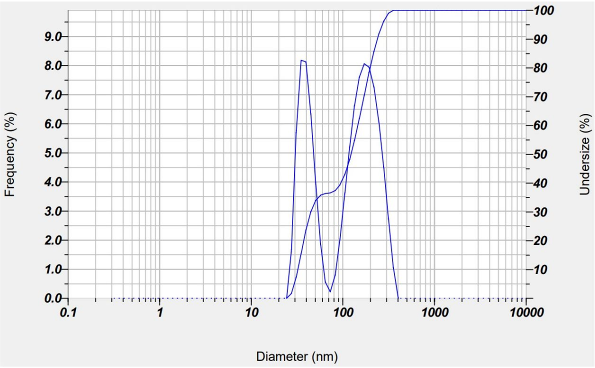
(A)



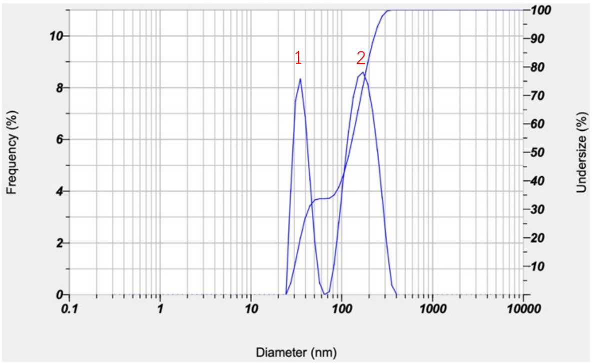
(B)

Fig. 3 The emulsion particles were analyzed using Scanning Electron Microscopy (SEM) clear white solution. The emulsion was analyzed under a 100x magnification microscope, we found the small spherical particles were evenly distributed as shown in Fig. 2. By studying emulsion powder morphology using Scanning Electron Microscopy (SEM) (FEI QUANTA 450, USA), we found the morphology of the vitamin-added powder emulsion using the freeze-drying method was quite round with a diameter of less than 100 micrometers (Fig. 3).

They were thought to be glycosphingolipid microemulsion particles. The particle size was analyzed. The average particle size of the emulsion was about 70-75 nm (Fig. 4A). The vitamins-loaded glycosphingolipid microemulsion was determined as above. The result illustrated that after vitamin B1, B2, B12 and folate were added, the average particle size of the vitamin-added emulsion was between 74.4-78.1 nm (Fig. 4B). We found that the particle size spectra of glycosphingolipid emulsion without vitamins and vitamins-loaded emulsion illustrated the same trend. There were two main peaks, Peak 1 and Peak 2, in a ratio of about 1:2. Peak 1 represented particle size approximately 34.5 ± 6.3 to 37.7 ± 7.7 nm. Peak 2 represents approximately 164.8 ± 54.6 to 175.0 ± 44.8 nm. The average particle size of the vitamins-loaded emulsion ranged from 74.4 – 78.1 nm. It was found that the average particle size before and after vitamin addition was less than 100 nm, consistent with the research of Uchiyama et al. (2019. Glycosphingolipids from rice were used to create microemulsions to transport CoQ10. (Uchiyama et al., 2019), where glycosphingolipids were found to act as emulsifiers when added to surfactant mixtures, polysorbate 80 and coconut oil making the particle size of the emulsion smaller. It has been shown that the presence of glycosphingolipids between the water and oil phases reduces the tension between the two phases resulting in smaller oil droplets. For the comparison of emulsions, it was found that the resulting emulsion was probably a glycosphingolipid microemulsion because it was transparent. It has an average particle size of less than 100 nm and is stable to temperature (Vittal & Aswathanarayan, 2019). It can be used in a variety of applications, for example, as an encapsulation vehicle for insoluble bioactive compounds to enhance transport stability and control. It can be used as an encapsulation vehicle for oxidation and photodegradation sensitive substances to enhance their stability and shelf life (Goindi et al., 2016).

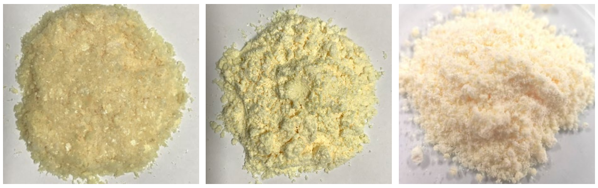


(A)



(B)

Fig. 4 Emulsion particle size spectra. (A) Emulsion without vitamin. (B) Vitamin-loaded emulsion



(A) (B) (C)

Fig. 5 Emulsion powder using 3 types of cryoprotectants. (A) Sucrose (B) Mannitol (C) Mannan-oligosaccharide

The vitamins-loaded glycosphingolipid microemulsion was made into powder using the freeze-drying method. Three types of cryoprotectants were compared: sucrose, mannitol and mannan-oligosaccharides. The results showed that emulsion can be changed into powder form with similar physical characteristics: light yellow, odorless and fine powder (Fig. 5). This is consistent with the study of Tozuka et al., using sucrose and mannitol as cryoprotectants in freeze drying (Uchiyama et al., 2019). Prebiotic oligosaccharides structure is a non-biodegradable short sugar that can be used as a cryoprotectant compared to sucrose and mannitol. It has been reported that mannan-oligosaccharide promotes intestinal microbial balance, effectively increasing the number of beneficial bacteria within the large intestine.

inhibits pathogens in animals and improves growth performance in experimental animals (Ghasemian & Jahanian, 2016). The inability of humans to break down mannan-oligosaccharides prevents the energy from the powder emulsion, while the high energy content of sucrose may have health implications. In addition, mannan-oligosaccharide is cheaper than mannitol, which can be considered a cost reduction in the powder emulsification process.

3. Solubility test

When testing the dispersion in distilled water, it was found that it could disperse well. The average particle size of the newly dispersed microemulsion was about 80-100 nm. The solubility showed that the powder emulsion in all conditions could dissolve up to 300 g/L at 25°C and up to 450 g/L at 70°C. The encapsulation efficiency of vitamins B1, B2, B12 and folate in glycosphingolipid microemulsions were 98, 55, 50 and 96%, respectively. The glycosphingolipid microemulsion was found to release vitamins in gastric-simulated fluid and small intestinal-simulated fluid. It is expected that when used as a food additive for dietary supplement, it can release the vitamins contained within the structure. It was also reported that rice glycosphingolipid microemulsion significantly increased the bioavailability of major metabolites through the oral mucosa (Uchiyama et al., 2019) and emulsion powder is a product that can be absorbed through the cells within the oral mucosa as well.

Conclusion

Glycosphingolipid from rice bran can assist in emulsifying the formulation consisting of polysorbate 80 and coconut oil. It can exist at the interface between the water and oil phases and decrease the interfacial tension. The vitamin-loaded microemulsion illustrated the high encapsulation efficiency of vitamins. Moreover, the vitamin-loaded microemulsion powder prepared by freeze-drying with a mannan-oligosaccharide as the cryoprotectant can be used as an ingredient in healthy instant soups, functional foods for elderly food products, or as a model for the preparation of emulsion powder to transport other important substances into the human body.

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