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Stability of wheat germ oil obtained by supercritical carbon dioxide associated with lipase ethanolysis

Jeong-Eun Sim, Joo-Hee Lee and Byung-Soo Chun*

Department of Food Science and Technology, Pukyong National University, 45 Yongso-ro, Nam-Gu, Busan 608-737, Republic of Korea.

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Wheat germ oil was extracted using an environmental friendly solvent, supercritical carbon dioxide (SC-CO_2) at a semi-batch flow extraction process. The supercritical carbon dioxide (SC-CO_2) extraction was carried out to extract oil at temperature of 40°C and pressure of 25 MPa. Ethanolysis was performed with 1,3-regiospecific lipase at different temperatures (40 to 70°C) to produce diglycerides and monoglycerides containing fatty acid ethyl esters. For determination of stability, wheat germ oil obtained by ethanolysis reactants was characterized by measuring the acid value, peroxide value, free fatty acid contents, thiocyanate method, DPPH radical scavenging effect and rancimat test. The optimized condition of 40°C shows the highest oil stability among the various conditions.

Key words: Supercritical carbon dioxide, wheat germ oil, ethanolysis, immobilized lipases, oil stability.

INTRODUCTION

Wheat germ is a by-product of the wheat milling industry. It is known that wheat germ may adversely affect the keeping and reprocessing quality of flour. The human consumption of wheat germ is very limited, meanwhile it is used for animal feeding and other purposes (Zhu et al., 2006). It is rich in unsaturated fatty acids, mainly oleic, linoleic and α-linoleic acids (Sjovall et al., 2000). Wheat germ containing as much as 10% oil used in products such as foods, biological insect control agents, medicals and cosmetic industries (Kahlon et al., 1989; Dunford et al., 2003). It is demanded in technology for functional food development due to its high content of polyunsaturated fatty acids and bioactive compounds (Shao et al., 2008).

Supercritical carbon dioxide (SC-CO_2) is an environment friendly solvent which is widely used to extract non polar lipids with lipid soluble bioactive compounds from different sources (Esquivel et al., 1997; Davarnejad et al., 2008; Rubio-Rodriguez et al., 2009). Further, SC-CO_2 extraction provides some advantages over conventional extraction processes because carbon dioxide (CO_2) is non-flammable, nontoxic, inert to most materials, inexpensive, and can be used under mild operational conditions (Ge et al., 2002; Lopez et al., 2004).

Currently, esterification reaction is a great interest because of its application in several branches of industries. Low value oils and fats can be converted to biodiesel, trans-free fats and medium chain-length triglyceride by several methods including alcoholysis, acidolysis and interesterfication. In conventional chemical processing, synthesis of esters by transesterification is achieved by either acid or alkaline esterification. This can lead to reduced selectivity and undesirable side reactions

*Corresponding author. E-mail: bschun@pknu.ac.kr. Tel: +82-51-629-5830. Fax: +82-51-629-5824.

(Soumanou and Bornscheuer, 2003; Meher et al., 2006). Lipase catalysts modify the properties of esterified lipids by altering the locations of the fatty acid in the glyceride and replacing one or more of the fatty acids with new ones. These exchange reactions usually proceed with high region- and/or enantio-selectivity, making lipases an important group of biocatalysts (Jaeger and Reetz, 1998). The by-products recovered from food processing can be turned into valuable products or at least converted into useful products. Manufacturing lipid products from natural resources normally yield significant amounts of low-value fats and oils next to the focused high valued processes. With better refining technologies, these resources can be turned into value added products. Fats are possible to be converted into glycerol and fatty acid esters by alcoholysis. Accordingly, wheat germ oils are industrially subjected to alcoholysis, to the triglyceride into di-, monoglycerides and fatty acid esters.

The emulsifying properties of monoglycerides have long been exploited by food, pharmaceutical and lubricant manufactures. Recently, new applications, such as nutraceuticals and controlled-release medicinal tablets, also make use of food grade monoglycerides. The diglycerides have recently attracted attention as it helps to prevent obesity, while being beneficial to diabetics and helps in preventing atherosclerosis (Moquin et al., 2006). And they help in preventing weight and fat accumulation (Murase et al., 2001; Maki et al., 2002). The chemically catalyzed reaction of lipids with alcohols is simple to carry out, but can generate many side products like soaps and free fatty acids which can be tedious to remove from the reaction mixture. As an alternative, the lipase catalyzed reaction is more selective and ideally leads only to monoglycerides and fatty acid esters (Koichi et al., 2004). Wheat germ oils contain a high percentage of polyunsaturated fatty acid at the sn-2 position (Mazhidov et al., 1996). 1,3-regioselective lipase (lipozyme TL-IM) derived from *thermomuces lanuginosus* is used for enzymatic ethanolysis.

The aim of this study was to evaluate the stability of the ethanolysis of SC-CO_2 extracted wheat germ oil by lipase at different temperatures.

MATERIALS AND METHODS

The sample was kindly donated by Young-nam Flour Mills Company (Busan, Republic of Korea). Carbon dioxide (99.99% pure) was supplied by KOSEM (Yangsan, Republic of Korea). Immobilized commercial lipase was purchased by Novozymes (Bagsvaerd, Denmark). All other chemicals used in different analysis were analytical or HPLC grade.

Sample preparation

After drying in an oven at 60°C, wheat germ was crushed in a mechanical blender and sieved (500 µm) through a mesh. Then the samples were stored at 2°C and used for SC-CO_2 extraction.

Supercritical carbon dioxide (SC-CO_2) extraction

A laboratory scale supercritical fluid extraction unit was used for extracting oil from wheat germ. This apparatus can be operated at pressure up to 30 MPa. 50 g of wheat germ samples were filled into the stainless steel extraction vessel which was 200 mL in volume. A thin layer of cotton was placed at the bottom of the extraction vessel. Before plugging with cap another layer of cotton was used at the top of the sample. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump up to the desired pressure. A back pressure regulator was used to control the pressure of CO_2. The extraction temperature was maintained by connecting the extraction vessel with water bath. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter. Wheat germ oil was extracted by SC-CO_2 at a temperature of 40°C and pressure of 25 MPa. The flow rate of CO_2 was 26.81 g/min and it was constant for extractions at the entire extraction period of 2 h. The extracted oil was collected on the glass separation vessels and stored at -20°C until further analysis.

Ethanolysis reaction of wheat germ oil

Ethanolysis was performed with lipozyme TL-IM (Novozymes; Bagsvaerd, Denmark) 4 w% of SC-CO_2 extracted oil in the erlenmeyer flasks containing a mixture of wheat germ oil (5 g) and ethanol (1.0 mole ratio). The mixtures were incubated from 40 to 70°C for 2 h and shaken at 120 rpm.

Measurement of oil stability

Free fatty acid content of ethanolysis reactants

Free fatty acid (FFA) content of extracted oil from ethanolysis reactants were analyzed as described by Bernardez et al. (2005). Accurately, 50 mg of oil was placed into pyrex tubes with the addition of 3 mL of cyclohexane and then 1 mL of cupric acetate-pyridine reagent was added. Tubes were vortexed for 30 s. After centrifugation at 2000 *g* for 10 min, the upper layer was read at 710 nm. The FFA content of oil was measured on a calibration curve constructed from oleic acid standard. Copper reagent was prepared according to Lowry and Tinsley (1976). Briefly, 5% (w/v) aqueous solution of cupric acetate was prepared and filtered. Then, the pH of cupric acetate solution was adjusted to 6.1 using pyridine.

Acid value (AV)

The AV was assessed according to AOCS official method of Cd 3d-63 (AOCS, 2006). 1 g of sample was dissolved in 100 mL of ether: ethanol (1:1) and shaken. Then phenolphthalein as an indicator was added in 4 drops. The solution was titrated with 0.1 N KOH-ethanol until it became pink in color. Acid value was expressed as mg KOH per g of sample:

$$\text{Acid value (AV)} = \frac{56.11 \times A \times F}{S}$$

Where, A is the volume of KOH-ethanol solution of the titration (mL), F is concentration of the KOH-ethanol factor, S is mass of oil (g) and 56.11 is the molecular weight of KOH.

Peroxide value (POV)

The POV was determined by the AOCS method Cd 8-53 (AOCS,

2006) with modified amount of sample taken. 1 g sample was dissolved in 6 mL of chloroform : acetic acid (2:3) solution. Then 0.1 mL of saturated KI solution was added to the mixtures and the solution was allowed to stand with occasional shaking for 1 min. Distilled water (6 mL) was immediately added to the solution. The solution was titrated with 0.1 N of sodium thiosulfate until the yellow iodine color had almost disappeared. Then 0.4 mL of starch indicator solution was added and again titrated until the blue color disappeared. A blank determination was conducted with the same procedure. Peroxide value was expressed as milliequivalents peroxide/1000 g sample:

$$\text{Peroxide value (POV)} = \frac{(S-B)\times N\times 1000}{M}$$

Where, S is volume of titrant of sample (mL), B is volume of titrant blank (mL), N is normality of sodium thiosulfate solution and M is mass of sample (g).

Thiocyanate method

To measure the oxidative stability, emulsion of ethanolysis reactant in water were oxidized at 37°C. Three emulsions of ethanolysis reactant in water (w/w) (ethanolysis reactant 5%, water 95%; astaxanthin 2%, ethanolysis reactant 4%, water 94%; linoleic acid 10%, water 90%) were prepared. The deionized and degassed water was used for emulsion preparation. Linoleic acid and standard astaxanthin were used to measure the oxidative stability of wheat germ oil ethanolysis reactant. The mixture was properly homogenized by a homogenizer. Oxidative stability was checked by thiocyanate method (Mitsuda et al., 1996). The peroxide formed by lipid peroxidation reacts with ferrous chloride and form ferric ions. Ferric ions then unite with ammonium thiocyanate and produce ferric thiocyanate. Briefly, 0.1 mL of emulsion solution was added to 4.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. Precisely, 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm (UVIKON 933, Kontron Instrument, Milano, Italy). A blank determination was conducted with the same procedure. The absorbance was recorded at 24 h intervals during the incubation.

DPPH radical scavenging method

The scavenging of DPPH radical was carried out according to the method described by Hsu et al. (2003). Aliquots of 1 mL of methanolic samples and 5 mL of freshly prepared 0.1 mM DPPH methanolic solutions were thoroughly mixed, and kept for 50 min in the dark. The absorbance of the reaction mixture at 517 nm was read with a spectrophotometer (UVIKON 933, Kontron Instrument). Methanol (1 mL), replacing the extract, was used as the blank. The percentage of free radical scavenging effect was calculated as follows:

Scavenging effect (%) = [1- ($A_{517\ nm,\ sample}/A_{517\ nm,\ blank}$)] x 100

Rancimat test

Three grams of oil was accurately weighed into the reaction vessels and the following procedure was carried out according to the method described by Hasen Huettl and Wan (1992). A Metrohm Rancimat model 743 (Methrom Instruments, Herusau, Swizerland) was utilized. A steam of filtered, cleaned and dried air at flow rate 20 L/h is bubbled into samples contained in reaction vessel. These vessels were placed in an electric heating block which is set at 110°C. Effluent air containing volatile organic acids from the oil samples are collected in a measuring vessel with 60 mL of distilled water. The conductivity of the water is continuously recorded and the OSIs of the oil samples were automatically recorded at 120°C. The oil samples for all determinations were randomized to determine their position in the heating block.

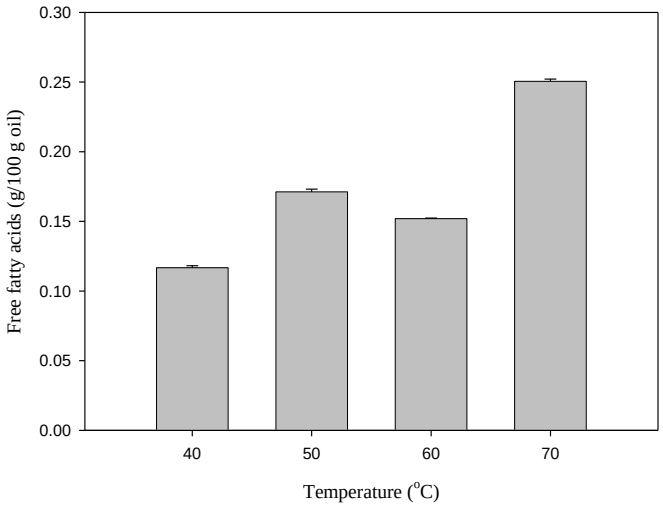


Figure 1. Free fatty acid content of wheat germ oil by ethanolysis reactants.

RESULTS AND DISCUSSION

Free fatty acid (FFA) content

The quality of oil deteriorated at production and storage conditions. Lipid hydrolysis, with consequent free fatty acid (FFA) generation occurred by chemical or enzymic action. In this study, FFA content of enzymatic ethanolysis reactants were measured at different temperatures and are given in Figure 1. It was found that as the temperature increase, the amounts of FFA content significantly increased. Temperature influenced lipid hydrolysis in enzymatic ethanolysis reaction. Similar observation was found in the hake byproduct oil (Rubio-Rodriquez et al., 2008).

Acid and peroxide values

In the present study, storage properties obtained by enzymatic ethanolysis reactant were compared with different temperatures (Figures 2 and 3). AV was used to measure the acidity of oil. In contrast, POV was carried out to measure the quality of the oil and oxidation state of lipid. It is used to measure the rancidity which occurs by autoxidation state of lipid.

It was also found that at temperature of 40°C both AV and PV was reduced and it increased as the temperature increases. This result is associated with FFAs contents.

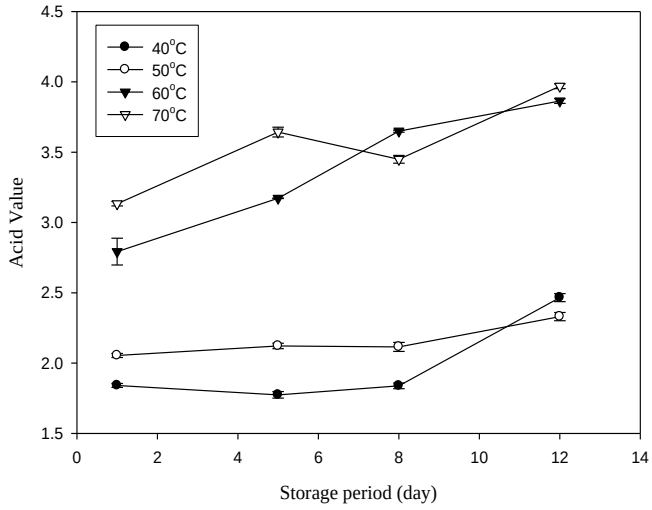


Figure 2. Comparison of the acid value of enzymatic ethanolysis reactants.

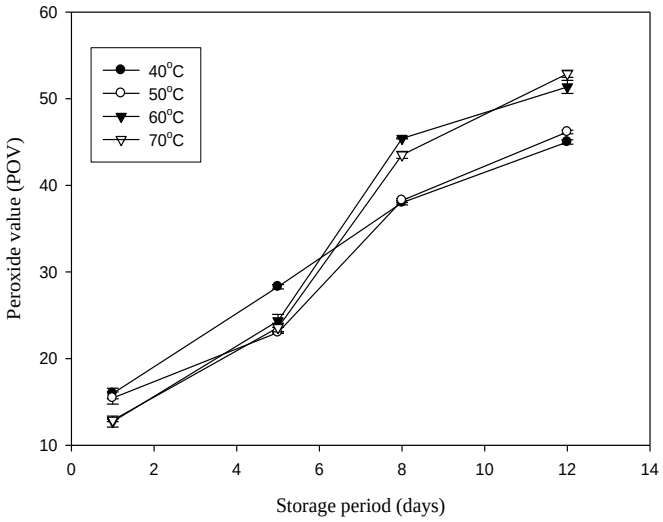


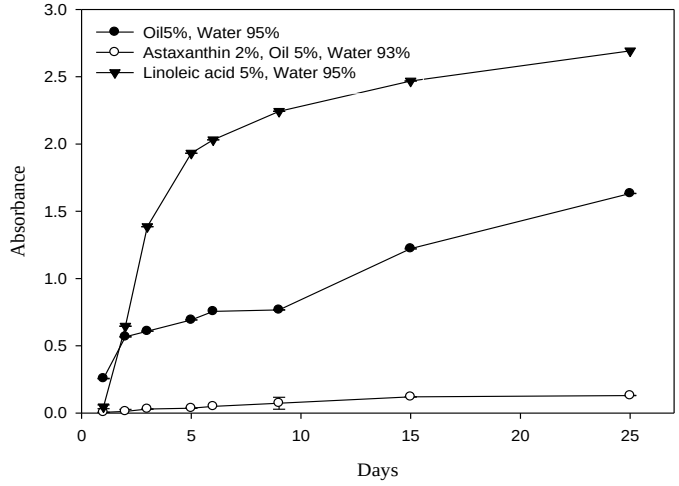
Figure 3. Comparison of the peroxide value of enzymatic ethanolysis reactants.

Meshref et al. (2008) have reported comparable results with milk and some dairy products on lipid oxidation.

Thiocyanate method

The oxidative stabilities of ethanolysis reactants are shown in Figures 4a and b. In this study, oxidation trend was evaluated by determining the state of oxidation of incubated sample. The increase in absorbance value was an indicator of autoxidation by formation of peroxides during incubation. Astaxanthin as antioxidant inhibited the peroxide formation from lipid. And linoleic acid emulsion indicated a low oxidative stability. Figure 4a indicated that

a)



b)

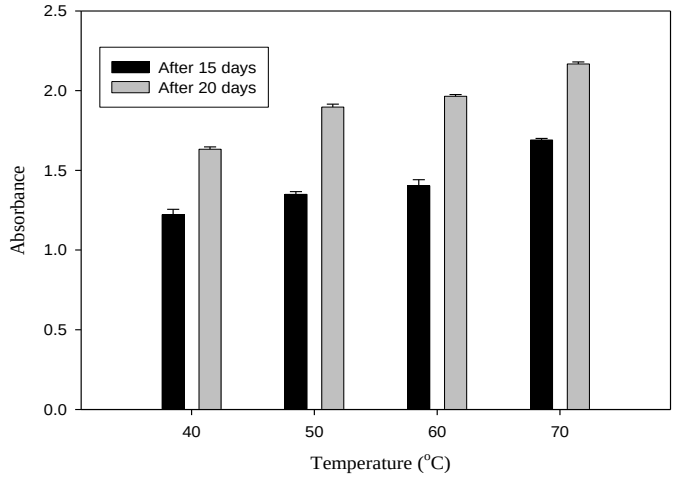


Figure 4. (a-b): Oxidative stability of ethanolysis reactants according to a) storage period and b) reactant temperature

the ethanolysis reactants increased oxidation during storage period. The ethanolysis reactants showed reduced oxidation up to 10 days. Initially, ethanolysis reactant emulsion showed slightly high absorbance as compared to lionoleic acid emulsion. This might be due to the presence of peroxide from the oxidation of neutral lipids of ethanolysis reaction of wheat germ oil. And Figure 4b shows that the higher reaction temperature increased oxidation.

DPPH radical scavenging effect

The DPPH radical scavenging effect of reaction temperature to ethanolysis reactants are shown in Figure 5. According to Figure 5, the condition of 40°C shows the highest scavenging activity among the various conditions,

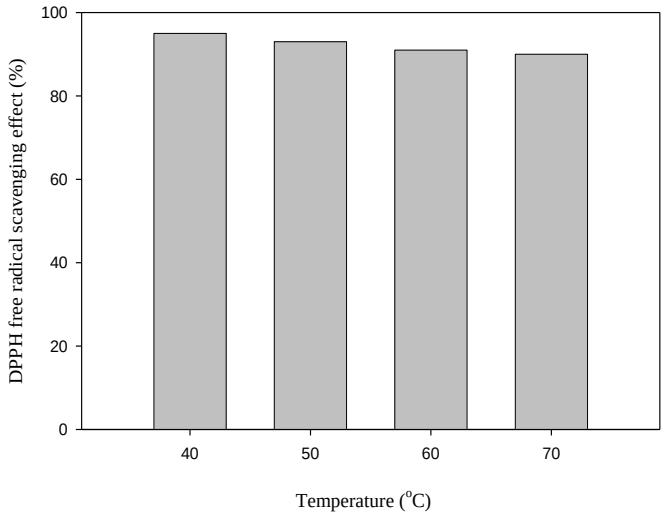


Figure 5. DPPH free radical scavenging effect of reaction temperature on ethanolysis reactants.

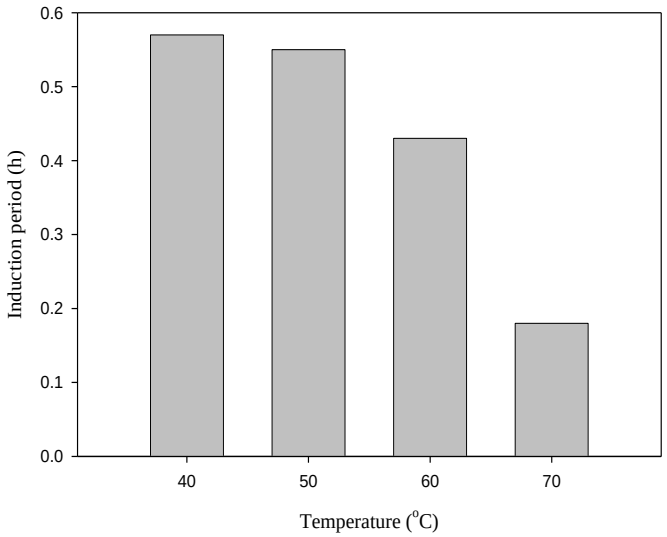


Figure 6. Induction period of ethanolysis reactants at different reaction temperatures.

but there was no significant difference of the DPPH radical scavenging activity of wheat germ oil obtained by ethanolysis reactants in other conditions. Related results was created with effect of temperature on the free radical scavenging capacity of extracts from red and white grape pomace peels (Jose et al., 1998).

Rancimat test effect

The Rancimat is used to determine the oxidative stability of fatty acid methyl ester. Tests of the induction period were performed. The instrument measures the ability of a sample to resist oxidation under conditions of heat and continuous air flow. The instrument is used extensively in determining the detrimental effects of metallic contaminants and the ameliorating effects of chelators and antioxidants. Figure 6 shows the induction time of the ethanolysis reactants with reaction temperatures. At 40°C, the induction period of ethanolysis reactants was higher. When comparing reactants, reaction temperature is lower, the induction period is higher. As a result, at the process of ethanolysis from the high temperature, the oxidation was already in progress. This result approved kinetic parameter determination of vegetable oil oxidation under rancimat test conditions (Reza et al., 2008).

Conclusions

Monoglycerides and diglycerides have great demand as emulsifier in food industries and yield higher market prices than oils. For quality containing mono and diglyceride separation, ethanolysis reaction is very important. Temperature may affect quality degradation of oil in reaction period, for the enzymatic ethanolysis of wheat germ oil was characterized by measuring stability at different temperatures. With the higher temperature, the amounts of FFA, AV and POV significantly increased. With the result of thiocyanate method, ethanolysis reactants showed significantly increased oxidation after 10 days and at 40°C, it showed reduced oxidation than other temperatures. Peroxide formation was inhibited in ethanolysis reactants using astaxanthin. DPPH radical scavenging effect (%) and induction period of rancimat test was higher at 40°C. Among the various conditions, 40°C shows the highest oxidative stability.

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