Journal of Engineering Science and Technology
Special Issue on SOMCHE 2014 & RSCE 2014 Conference, January (2015) 41 - 49

SEPARATING POLY-UNSATURATED FATTY ACIDS FROM VEGETABLE OIL USING UREA COMPLEXATION : THE CRYSTALLISATION TEMPERATURE EFFECTS

DWI ARDIANA SETYAWARDHANI[1,2,*], HARY SULISTYO[2], WAHYUDI BUDI SEDIAWAN[2], MOHAMMAD FAHRURROZI[2]

[1] Department of Chemical Engineering, Faculty of Engineering, Sebelas Maret University, Jalan Ir. Sutami No.36 A, Surakarta 57126, Indonesia
[2]Department of Chemical Engineering, Faculty of Engineering, Gadjah Mada University, Jalan Grafika No.2, Kampus UGM, Yogyakarta 55281, Indonesia
*Corresponding Author: dwi_ardiana@yahoo.com

Abstract

Omega-6 fatty acids, such as Linoleic Acid (LA) are essential Poly-Unsaturated Fatty Acids (PUFAs) which are very important on human health. The omega fatty acids intake as concentrate is preferred than consumes the whole oil. One of the methods used for separating PUFAs from the oil is urea complexation. This separation method is based on the capability of saturated fatty acids (SFAs) to form complexes compound with urea. It was observed that temperature affected the PUFAs composition and yield of the concentrate (non-urea complexes fraction/NUCF). Corn oil, which is rich of PUFAs, was saponified and purified to produce fatty acids. Urea was dissolved in ethanol then was added to the fatty acids to obtain crystal compound complexes. In this research, crystallisation was studied at different temperatures, i.e., ambient temperature (30 °C), cooled temperature (5 °C) and frozen temperature (-15 °C). The filtrate consisted of NUCF, which was rich of PUFAs. Fatty acid composition in filtrate was analysed using gas chromatography. The NUCF yields were measured by gravimetric method. The results showed that urea complexation needed crystallisation temperature below the ambient, to obtain significant yield of urea adduct. Crystallisation in lower temperature produced higher concentration of PUFAs. However, this condition obtained lesser yield.

Keywords: Fatty acids, Crystallisation, PUFA, Temperature, Urea complexation.

1. Introduction

Poly-unsaturated Fatty Acids (PUFAs), such as Linoleic Acid (LA), Alpha-Linoleic Acid (ALA), Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid

Nomenclatures

FA	Fatty acids composition, %
T	Crystallisation temperature, °C

Abbreviations

CO	Corn oil
FA	Fatty acid
JCSO	Jatropha curcas seed oil
MUFAs	Mono-unsaturated fatty acids
NUCF	Non-urea complexed fraction
PUFAs	Poly-unsaturated fatty acids
SBO	Seal blubber oil
SFAs	Saturated fatty acids
UCF	Urea complexed fraction

(DHA) are important for human health. PUFAs are needed for brain development and neuro system for infants. They also prevent degenerative diseases such as cancer, atherosclerosis and cardiovascular diseases [1-5]. PUFAs are essential fatty acids which must be obtained through food. LA, known as omega-6 fatty acid, is a major fatty acid in plant lipids. Whereas EPA and DHA as omega-3 are mainly consist in fish oil. The PUFAs intake as concentrate is preferred than consumes the whole oil [6]. This form provides a useful alternative for consuming required amount of fatty acids without any saturated one (SFAs), to keep the daily intake of lipids as low as possible. As the public awareness of the nutritional benefits of consuming PUFAs-concentrates is growing, the market demand is expected to rise.

Recently, fish oil is the most potential feedstock for concentrating PUFAs. But, some fishes may contain methyl mercury (MeHg) which harms the developing fetus [7]. As the fatty acids consist in fish oil is similar with vegetable oil, the substitution of fish oil is greatly possible to avoid the poisonous effects. Indonesia is one of the largest edible-oil producers in Asia, and is very potential in non-edible oil resources. Developing the oleo-chemical industries here, such as PUFAs separation, is very promising.

Vegetable oils, such as soybean, corn and palm oil contain of long-chain fatty acids, including saturated (palmitic, stearic), monosaturated/monoenes (oleic) and poly-unsaturated/polyenes (linoleic, linolenic) fatty acids [8]. It is important to eliminate the SFAs for consumption. They might be separated from the PUFAs, based on their solubility, melting point, and degree of unsaturation or their dimensional characteristics. There are several methods for separating PUFAs from the saturated and monosaturated fatty acids, but only few which can used for cost effectiveness and large-scale production, to fit the growing demand. One of the suitable methods for concentrating PUFAs is urea complexation. This method is favoured because the complexation depends upon the configuration due to presence of multiple bonds rather than the physical properties of the molecules, such as melting point or solubility. Pure urea crystallises in a tightly packed tetragonal structure with channels of 5.67 Å in diameter. SFAs, whose straight chain molecules, form hexagonal crystal with urea in 8-12 Å channel diameter. However,

monoenes are more readily complexed as compared to dienes, which in turn, are more readily complexed than trienes. Urea complexes formation depends on the degree of unsaturation of the fatty acids [9].

2. Materials and Methods

Vegetable oil used for the raw material for urea inclusion is RBD (Refined, Bleached, and Deodorised) corn oil, which is rich of Linoleic Acid (LA). Analytical grade urea was purchased from E-Merck, and 96% aqueous ethanol employed for wetting agent. The saponification equipment used for obtaining fatty acids shown on Fig. 1.

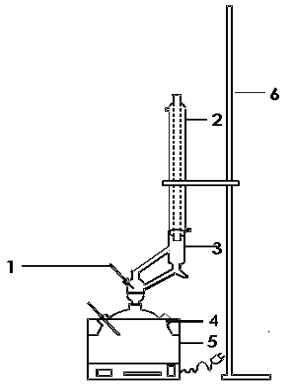

Fig. 1. Saponification apparatus for producing fatty acids mixtures.

Fatty acids preparation developed according to Wanasundara and Shahidi methods [10]. Corn oil was saponified with the mixture of KOH, water and aqueous ethanol at the boiling temperature of the mixture for 1 hour. Distilled water then added to the mixture and the unsaponifiable matters were extracted using n-hexane. Aqueous layer was acidified with HCl solution to release the fatty acids, which then extracted by n-hexane. Na_2SO_4 used for adsorbing the remaining water before evaporating n-hexane to produce fatty acids mixtures.

For the urea complexation process, 10 g FFA were mixed with urea and 96% aqueous ethanol, and then heated to 60 °C with stirring until the mixture formed a clear homogeneous solution. The urea-FFA adduct was allowed to crystallise at room temperature. For colder temperature, the mixtures were then cooled and frozen in the refrigerator. The crystal formed was urea complexed fraction (UCF), and the remaining solution was the non-urea complexed fraction (NUCF), which rich of PUFAs. The crystal was separated from the liquid using centrifugation and filtrated under suction using a Buchner funnel. The filtrate (NUCF) was diluted with water in an equal volume, and acidified with HCl to reach pH 4-5. Hexane in an equal volume then added to the mixture with stirring for 1 hour to extract the liberated fatty acids. The mixture separated in a separation funnel until formed two layers. The top layer (hexane fraction) contained fatty acids, and the discharging lower layer contained urea. The hexane layer was washed with distilled water to remove any

remaining urea, and then dried with anhydrous sodium sulphate. Rotary evaporator then needed to evaporate solvent from the fatty acids concentrate at the room temperature. The fatty acids composition was determined using gas chromatography.

3. Results and Discussion

In this research crystallisations were studied at different temperatures, i.e., ambient temperature (30 °C), cooled temperature (5 °C) and frozen temperature (-15 °C). Table 1 listed the process condition we used in this experiment.

Table 1. Process conditions designed for urea inclusion.

Variables	Quantity	Unity
FFA weight	10	grams
Crystallisation time	68	hours
Ethanolic urea : FFA ratio	40/10	mL/g
Ethanolic urea : FFA concentration	Saturated solution (10 g/100 mL ethanol 96%)	
Mixing time	1/60	hours
Crystallisation temperature	Ambient (30) Cooled (5) Frozen (-15)	°C

Crystallisation which developed in ambient temperature was not satisfied for obtaining the UCF. The amount of the crystal was so few that it was not an effective separation (Table 2). It was possibly due to 1) the amount of urea added, 2) the crystallisation time was too short, or 3) the crystallisation temperature was not low enough to obtain the supersaturated condition. But comparing with other crystallisation temperatures, it was shown that lower temperature providing more crystal, which means higher urea-SFAs adduct yielded. As the experiment developed in the same quantity of urea and crystallisation time (shown on the Table 1), it was clearly stated that the crystallisation temperature played the most important role to the forming of urea adducts.

Table 2. The temperature effect to UCF yields.

Temperature (°C)	Ethanolic urea : FFA ratio	UCF yield (g)
30	40 : 10	N.A.
5	40 : 10	0.52
-15	40 : 10	2.61

Vegetable oils contain long chain fatty acids, mostly in 16 (palmitic) and 18 (stearic, oleic and linoleic) carbon atoms. Figure 2 shows that the shorter fatty acids molecules (palmitic curve) formed complex more readily than the longer ones. The curve exhibits the steepest minus slope, which means the lower temperature gives the fewer palmitic acids in the NUCF. This result was in accordance with the experiment developed by Hassan [11], which reported that on

the separation of long chain n-paraffins from isooctane solution using urea complexation, the uptake of shorter chain n-paraffin was greater. Due to the high restriction in the urea crystal, the long chain molecules cannot move freely in the tunnel. The loss of translational and rotational energies of the molecules may result in a less favourable packing in urea, thereby resulting in a smaller uptake capacity. However, it conversely with his previous experiment for the medium chain n-paraffins [12].

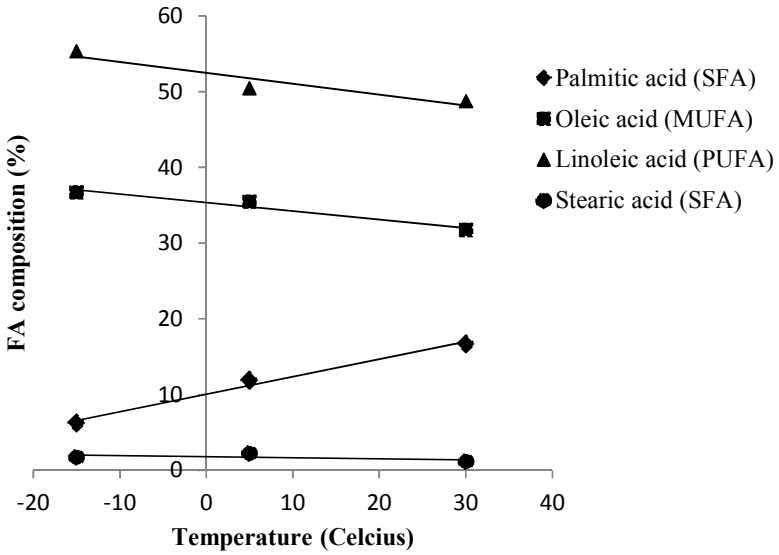

Fig. 2. The relationships between each fatty acids composition in the NUCF and crystallisation temperature.

Most saturated fatty acids are straight hydrocarbon chain with even number of carbon molecules (usually 12-24 carbon atoms). Mono-unsaturated fatty acids have one carbon-carbon double bond, which can occur in different positions. The most common monoenes have a cis-configuration, which gives a kink in the molecular shapes and make it bigger in diameter. Poly-unsaturated fatty acids have two or more double bonds between their carbon-carbon atoms in some positions. Therefore, PUFAs molecules have bigger diameter, which make them impossible to form adduct with urea crystal. MUFAs, whose only one kink sometimes can occlude to the urea channel, compete with the SFAs.

In the NUCF, MUFAs composition only slightly changed, but SFAs was greater (as shown on Fig. 3). The relationships between fatty acids composition (FA) and the crystallisation temperature (T) was described in the linear curve, which estimated by the least square methods. The SFAs curve showed positive slope, means that lower temperature resulted fewer SFAs in the concentrate. The solubility of urea in ethanol is depending on temperature. Whereas we used saturated urea solution, the condition below ambient temperature encouraged the solution to the supersaturated condition. The supersaturation formed rapidly, especially with direct

cooling in the refrigeration systems. Therefore, SFAs molecules complexed with urea readily as UCF and obtaining less concentration in NUCF.

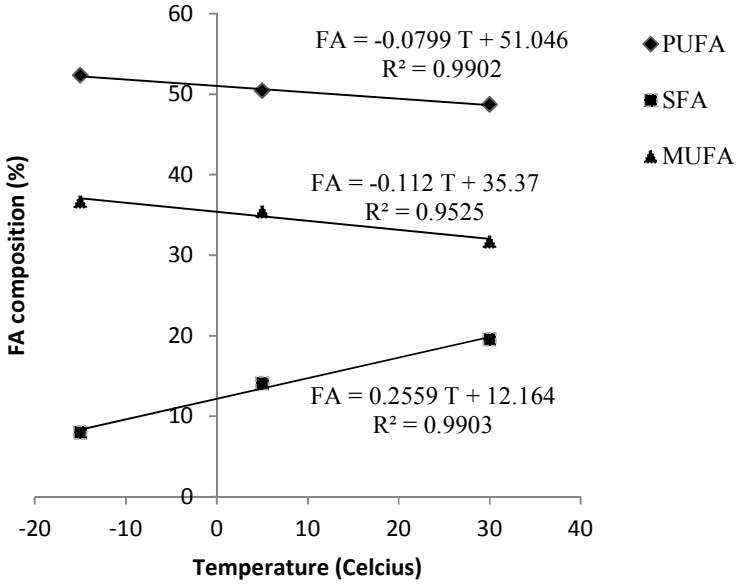

Fig. 3. The relationships between PUFA, MUFA, SFA compositions in NUCF (liquid fraction) and crystallisation temperature.

Table 3 and Fig. 4 show that the yield of fatty acids concentrate (NUCF) decreased significantly with decreasing temperature. Lower crystallisation temperature gives lower urea solubility in aqueous ethanol, which in turn, more SFAs form adduct with urea. The UCF or NUCF yield was estimated by gravimetric method (the weight of UCF or NUCF obtained from the complexation of 10 g FFA). Conversely, the higher yield solidified in UCF gives the lower yield in NUCF. The relationships between NUCF (concentrates) yield and crystallisation temperature also described in linear curve by the least square methods.

Table 3. The temperature effect to NUCF yields.

Temperature (°C)	Ethanolic urea : FFA ratio	UCF yield (g)
30	40 : 10	4.847
5	40 : 10	2.730
-15	40 : 10	1.410

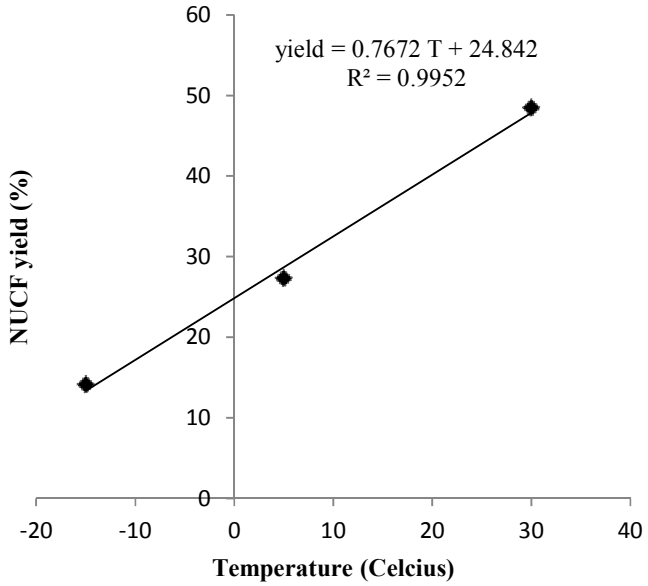

Fig. 4. The relationships between PUFA yields in NUCF and crystallisation temperature.

This result was in accordance with the experiment developed by Salimon et al. [13] which studied crystallisation temperature effects for jatropha curcas seed oil (JCSO). The temperature was observed between -10 °C and 10 °C. Their result showed that lower temperature gives higher PUFA concentration, whereas the NUCF yield was lower. It was also compatible with the result by Wanasundara and Shahidi [10], for the temperature between 6 °C and -18 °C. The comparison of these researches showed in Table 4.

Table 4. PUFAs concentrations and yields on the NUCF (compared with previous research [10, 13]).

Urea/FA	Time	Temperature	Yield	PUFAs	Vegetable
2	12	-18	28.5	69.2	SBO [13]
2	12	0	34.6	57.5	SBO [13]
2	24	-18	27.2	68.8	SBO [13]
2	24	0	35.2	59.6	SBO [13]
5	24	-18	20.0	89.5	SBO [13]
5	24	0	20.3	87.4	SBO [13]
3.5	18	-9	19.3	88.4	SBO [13]
3.5	18	6	24.6	80.8	SBO [13]
1	8	0	31.3	54.63	JCSO [10]

Urea/FA	Time	Temperature	Yield	PUFAs	Vegetable
1	8	10	45.6	52.53	JCSO [10]
5	24	-10	7.8	92.81	JCSO [10]
5	24	10	7.7	88.6	JCSO [10]
1	24	-10	34.1	61.46	JCSO [10]
1	24	10	50.6	54.91	JCSO [10]
5	8	-10	6.6	88.92	JCSO [10]
5	8	10	8.8	87.82	JCSO [10]
1	16	-10	48.1	59.85	JCSO [10]
1	16	10	49.7	54.87	JCSO [10]
0.4	68	-15	14.1	52.36	CO*
0.4	68	5	27.3	50.44	CO*
0.4	68	30	48.47	48.74	CO*

4. Conclusions

According to these research results, we concluded that:

- The crystallisation temperature played an important role to optimise the condition of urea complexation.
- Urea complexation needed crystallisation temperature below the ambient, to obtain significant yield of urea adduct.
- The PUFAs composition in the NUCF (FA) increased with the decreasing of the crystallisation temperature (T), as FA = -0.0799 T + 51.046.
- The NUCF yield reduced as the crystallisation temperature decreased, as yield = 0.7672 T + 24.842.

Acknowledgement

The authors would like to acknowledge The Directorate General of Higher Education Indonesia of financial supports for this work through scholarship of doctorate program (BPPS) at Gadjah Mada University to D.A. Setyawardhani, and the research grant Hibah Unggulan Fakultas PNBP 2014 Sebelas Maret University.

References

1. Dyerberg, J. (1986). Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. *Nutrition Review*, 44(4), 125-134.
2. Harris, W.S.; Mozaffarian, D.; Rimm, E.; Etherton, P.K.; Rudel, L.L.; Appel, L.J.; Engler, M.M.; Engler, M.B.; and Sacks, F. (2009). Omega-6 fatty acids and risk for cardiovascular disease. *American Heart* Association, 119, 902-907.

3. Kinsella, J.E. (1986). Food components with potential therapeutic benefits: the n-3 polyunsaturated fatty acids of fish oil. *Food Technology*, 40(2), 89-97.
4. Mehta, L.; Lopez, L.M.; Lowton, D.; and Wargovich, T. (1988). Dietary supplementation with omega-3 polyunsaturated fatty acids in patients with stable coronary diseases: effects on indices of platelet and neutrophil function and exercise performance. *American Journal of Medicine*, 84(1), 45-52.
5. Poudyal,H.; Panchal, S.K.; Diwan, V.; and Brown, L. (2011). Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. *Progress in Lipid Research*, 50(4), 372-387.
6. Bronsgeest-Schoute, H.C.; Van Gent, C.M; Luten, J.B.; and Ruiter, A. (1981). The effect ofvarious intakes of ω-3 fatty acids on the lipid composition in healthy human subjects. *Americal Journal of Clinical Nutrition*, 34(9), 1752-1757.
7. Bouzan, C.; Cohen, J.T.; Connor, W.E.; Kris-Etherton, P.M.; Gray, G.M.; Konig, A.; Lawrence, R.S.; Savitz, D.A.; and Teutsch, S.M. (2005). A quantitative analysis of fish consumption and stroke risk. *American Journal of Preentive Meicine.*, 29 (4), 347-352.
8. Swern, D. (1964). *Bailey's Industrial Oil and Fat Products* (3rd ed.). John Wiley and Sons, New York.
9. Hayes, D.G. (2002). Urea inclusion compound formation. *INFORM*, 13, 781-783.
10. Wanasundara, U.N.; and Sahidi, F. (1999). Concentration of Omega-3 polyunsaturated fatty acids of sea blubber oil by urea complexation : optimization of reaction condition. *Food Chemistry*, 64,41-49.
11. Hassan, N.M. (1994). The adsorptionof long-chain n-paraffin from isooctane solution on crystalline urea. *Separations Technology*, 4(1), 62-64.
12. Al-Ameeri,R.S.; and Hassan, N.M. (1990). Adsorption of normal alkanes from isooctane solution onto crystalline urea. *Journal of Chemical and Engineering Data*, 35(2), 110-114.
13. Salimon, J.; Abdullah, B.M.; and Salih, N. (2012). Selectively increasing of polyunsaturated (18:2) and monounsaturated (18:1) fatty acids in Jatropha curcas seed oil by crystallization using d-optimal design, *Chemistry Central Journal*, 6(65), 1-15.