J. Trop. Agric. and Fd. Sc. 42(2)(2014): 149 – 155

Preparation of concentrated polyunsaturated fatty acids (PUFA) from soybean oil

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Abstract
The study was aimed to prepare concentrated polyunsaturated fatty acids (PUFA) from soybean oil by hydrolysis and urea complexation to obtain the maximum concentration of PUFA. Linoleic acid (LA) and α-linolenic acid (ALA) derived from chemically hydrolysed soybean oil were concentrated by urea complexation. The chemical hydrolysis was carried out in 1M ethanolic alkaline solution by refluxing at 60 ºC for 30 min. The production of LA and ALA from chemical hydrolysis were 53.4% and 6.4% respectively. Concentration of LA and ALA were affected by urea/fatty acid ratio, crystallisation temperature and time. After complexation of saturated and less unsaturated fatty acids, the maximum concentration of LA (84.0%) and ALA (15.3%) was obtained using an ethanolic solution with urea:fatty acid ratio (3:1) at 4 ºC for 24 h crystallisation. A combination of chemical hydrolysis and urea complexation is a promising method to obtain highly concentrated omega-3 and omega-6 PUFA from soybean oil.

Keywords: urea/fatty acid ratio, urea complexation, linoleic acid, α-linolenic acid, crystallisation time and temperature

Introduction

The omega-3 and omega-6 polyunsaturated fatty acids (PUFA) have biochemical effects in the prevention or treatment of several human diseases (Gamez-Meza et al. 2003). Linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) are essential omega-6 and omega-3 PUFA and must be provided from food because they cannot be synthesised by human body. LA is important for a healthy skin, helping to keep it smooth and supple, to protect it from injury and infections and to regulate body temperature and water loss (Senanayake and Shahidi 2002; Chin et al. 2010). Individuals with atopic eczema (a skin disorder) are thought to have a deficiency that interferes with the production of other omega-6 PUFA from linolenic acid. LA applied to skin or taken orally, has often helped to relieve symptoms of this disorder.

The beneficial effects of omega-3 PUFA have been ascribed to their ability to lower serum triacylglycerol and cholesterol levels; also considered essential for normal growth and development (Simopoulos et al. 2000) and may play important role in the prevention treatment of cardiovascular diseases (Senanayake and Shahidi 2002; Ruxton et al. 2004). Therefore, consumption

Article history
Received: 1.11.2012
Accepted: 19.6.2014

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of appropriate amounts and proportions of omega-6 and omega-3 PUFA needs to be considered.

The market of PUFA concentrates is expected to grow since it has been suggested that consuming PUFA concentrates devoid of more saturated fatty acids are much better than consuming marine oils (high in omega-3 fatty acids). Overconsumption of marine oils to obtain omega-3 PUFA may increase the intake of cholesterol and other saturated fatty acids. Therefore, concentration or enrichment of omega-3 PUFA from marine oils or other sources could help avoid such a concern.

Urea complexation is the most efficient and well established technique for obtaining omega-6 and omega-3 PUFA concentrates in free fatty acid form (Wanasundara and Shahidi 1999; Mendes et al. 2007). The complexed crystals in urea complexation are extremely stable and filtration does not necessarily has to be carried out at the very low temperatures which solvent crystallisation of fatty acids would require. This method is also favoured by many researchers because complexation depends upon the configuration of the fatty acid moieties due to the presence of multiple double bonds, rather than pure physical properties such as melting point or solubility (Shahidi and Wanasundara 1998; Chin et al. 2010).

Refined, bleached and deodorised (RBD) soybean oil was commonly used as raw material to prepare PUFA concentrates. It is a widely used edible oil in the world. Soybean oil is the largest oil crop in the world where 13 million tonnes of oil is produced each year (O'Brien 1998). The oil is in high demand in the market due to the high oil and essential fatty acid content. Soybean oil contained low saturated fat (15%) and high unsaturated fat (61% polyunsaturated and 24% monounsaturated). Two of polyunsaturated fatty acids in this oil are known as essential fatty acids (EFA) of linoleic (omega-6) and α-linolenic (omega-3) fatty acids that are not produced by the human body but must be taken from food sources (Lai 2002).

The study was aimed to prepare concentrated PUFA from soybean oil by chemical hydrolysis and urea complexation process. Factors (variables) such as urea to fatty acid ratio (w/w), crystallisation time (h) and temperature (ºC) were evaluated to obtain a maximum concentration of omega-6 and omega-3 PUFA.

Materials and methods

Refined, bleached and deodorised soybean oil (RBD-SBO) was purchased from supermarket (commercialised cooking oil product). All chemicals and reagents used in this study were analytical grade.

Free fatty acids from RBD-SBO was prepared according to the method of Gamez-Meza et al. (2003). A sample of 20 g RBD-SBO was saponified by refluxing for 30 min at boiling temperature of the mixture (60 ± 2 ºC) under a blanket of nitrogen using 100 ml 1M KOH in 95% ethanol. Distilled water (50 ml) was added to the saponified mixture and the unsaponifiable matter was extracted into hexane (2 x 70 ml) and discarded. The aqueous layer (saponified matter) was then acidified (pH = 1.0) with 3N HCl. The mixture was transferred into a separatory funnel and the liberated fatty acids were then extracted into 50 ml of hexane. The hexane layer (contain free fatty acids) was dried over anhydrous sodium sulphate and the solvent removed at 40 °C to recover free fatty acids which were then stored at –20 ºC until used in the urea complexation.

The PUFA concentrates were prepared by mixing 10 g free fatty acids with 30 g urea in 150 ml aqueous ethanol (95%) in a capped bottle and heated at 60 ºC with stirring until the whole mixture turned into a clear homogeneous solution. The ratio of urea to fatty acids was changed by using different amounts of urea. This solution was then allowed to crystallise at room temperature and then kept at cold temperatures (–24, –15, –5, 0, 4 and

8 ºC) for different periods for further crystallisation. All reactions were performed in triplicates. To study the effect of urea to fatty acid ratio, the clear homogeneous solution was then allowed to crystallise at room temperature and then kept at 4 ºC for 24 h.

To study the effect of crystallisation temperature, the clear homogeneous solution was then allowed to crystallise at room temperature and then kept at cold temperatures (–24, –15, –5, 0, 4 and 8 ºC) for 24 h. For the effect of crystallisation time, the clear homogeneous solution was allowed to crystallise at room temperature and then kept at 4 ºC for 12 – 60 h.

The formed crystals (urea complexing fraction; UCF) were then separated from the liquid (non urea complexing fraction; NUCF) by vacuum filtration. The filtrate (NUCF) containing PUFA was diluted with an equal volume of distilled water and acidified (pH 4 – 5) with 6N HCl. The PUFA was then extracted by hexane according to the method of Wanasundara and Shahidi (1999). An equal volume of hexane was added and the mixture was stirred for an hour. The mixture was then transferred into the separatory funnel and the hexane layer containing PUFA was washed with distilled water to remove any remaining urea and then dried over anhydrous sodium sulphate. The solvent was removed at 40 ºC using a vacuum rotary evaporator to recover PUFA which was then stored at –20 ºC until further analysis.

For gas chromatography (GC) analysis, samples were dissolved in 1 ml of hexane in a sample tube. Then 1 ml of sodium methoxide in methanol was added for fatty acid methylation process. The mixture was allowed to stand for 15 min before GC analysis, using Shimadzu GC 17A gas chromatography equipped with a flame ionisation detector (FID). Nitrogen was used as the carrier gas and the total gas flow rate 0.3 ml/min. BPX 70 (30 m x 0.25 mm x 0.25 μm) was used as the GC column. The injector and detector temperatures were set at 250 ºC and 280 ºC. Fatty acid compositions calculated were based on the percentage peak area of the GC chromatogram. All analyses were performed in triplicates and average values were reported.

Statistical analysis

All determinations were carried out in triplicates and the mean data ± standard deviation (SD) was reported. Experimental data were statistically analysed by analysis of variance (ANOVA) and the significant differences among means were determined by Duncan multiple range test (DMRT) at a level of $p < 0.05$.

Results and discussion

Effects of urea to fatty acids ratio

The ratio of urea to fatty acid increased from 1:1 to 3:1, thus percentage of polyunsaturated fatty acids (PUFA) increased significantly ($p < 0.05$). However, the PUFA content decreased significantly ($p < 0.05$) when more than 3:1 urea to fatty acid ratio was employed (*Table 1*). There was no significant difference of PUFA percentage when the ratio of urea to fatty acid was between 4:1 and 5:1. This means 3:1 urea to fatty acid ratio (w/w) is needed to complex the saturated (SFA) and monounsaturated fatty acids (MUFA) with urea.

This finding was similar with Shahidi and Wanasundara (1998) and Ratnayake et al. (2006) who reported that the application of more than 3:1 urea to fatty acid ratio will trap the PUFA and PUFA may be detected in urea complexed fraction (UCF). The high content of PUFA will lower down the potential of urea complexation. Large molecules and bent fatty acids such as PUFA cannot enter the urea structure and will cause them suspended in the solvent (Ganga et al. 1998; Chin et al. 2010). Thus, PUFA can be easily separated from the SFA and MUFA. The stability of fatty acid-urea complex is consistent with the geometry of the molecules involved. Any deviation from

the straight chain fatty acid arrangement will undermine the stability of the complex. This indicates that the formation of urea depends on the degree of unsaturated fatty acid (Medina et al. 1995; Chin et al. 2010).

Urea complexation of soybean oil increased PUFA content from 60.6% to 99.3% in the concentrate. The minimum percentage of MUFA (0.7%) was obtained at urea to fatty acid ratio of 3. It is not easy to remove all saturated and monounsaturated fatty acids to obtain 100% PUFA in the concentrate. Liu et al. (2006) and Wanasundara and Shahidi (1999) have also reported that complete removal of saturated fatty acids by urea complexation may be impossible since some of the saturated fatty acids do not complex with urea during crystallisation.

Effects of temperature on crystallisation of urea complex

The PUFA enrichment in the concentrate was slightly decreased as the crystallisation temperature decreased from 4 ºC to –24 ºC (*Table 1*). The PUFA percentage decreased from 99.3% to 97.8, 97.1, 96.7 and 95.9%; consistent with the decrease of crystallisation temperature. The results showed that there were significant differences of PUFA percentage (p <0.05) due to the decrease of crystallisation temperature from 8 ºC to –15 ºC. However, the percentage of PUFA in NUCF fraction increased with the decreasing temperature of 8 – 4 ºC (p <0.05). The PUFA reached a maximum (99.3%) at 4 ºC.

Shahidi and Wanasundara (1998) and Liu et al. (2006) reported that the absence of urea, fatty acids cannot crystallise even at 0 ºC. The fatty acids can crystallise well

Table 1. The effects of urea/fatty acid ratio, crystallisation temperature and crystallisation time on total MUFA and PUFA content in the concentrate

	MUFA (%)	PUFA (%)
Urea to fatty acid ratio (w/w)		
1:1	20.86 ± 0.11a	79.19 ± 0.04d
2:1	5.04 ± 0.22b	94.96 ± 0.14c
3:1	0.68 ± 0.11d	99.32 ± 0.11a
4:1	3.56 ± 0.41c	96.44 ± 0.07b
5:1	3.68 ± 0.02c	96.32 ± 0.10b
Crystallisation temperature (°C)		
8	7.47 ± 0.12a	92.53 ± 0.82e
4	0.68 ± 0.11d	99.32 ± 0.11a
0	2.15 ± 0.42c	97.85 ± 0.08b
–5	2.94 ± 0.74bc	97.06 ± 1.16bc
–15	3.29 ± 1.21bc	96.71 ± 0.39cd
–24	4.13 ± 0.67b	95.87 ± 0.13d
Crystallisation time (h)		
12	14.04 ± 0.49a	85.96 ± 0.86d
24	0.68 ± 0.11d	99.32 ± 0.11a
36	1.14 ± 0.15d	98.85 ± 0.64a
48	2.80 ± 0.56c	97.19 ± 0.68b
60	4.79 ± 0.83b	95.31 ± 0.65c

Means with the same letter in the same column are not significantly different (p <0.05)

at temperature of 0 ºC in the presence of high concentrations of urea. Crystallisation of fatty acids in the presence of urea occurs at lower temperatures depending on the degree of the unsaturated fatty acids. The complexation limit depends on the concentration of urea and crystallisation temperature. It can be concluded that lower crystallisation temperature derived low percentage of PUFA. This is related to the crystallisation of fatty acids at a suitable temperature depending on the required degree of complex concentration.

Optimum temperature showed 99.3% of PUFA obtained after urea complexation process at 4 ºC. The results are in agreement with Gunstone (1998) who has reported that the optimum crystallisation temperatures for urea complexation are between 0 – 4 ºC.

Effects of time on crystallisation of urea complex

The total PUFA reached the maximum at 24 h of crystallisation and started declining slightly as the time was further increased (*Table 1*). The percentage of PUFA was significantly increased (p <0.05) from 12 h (85.9%) to 24 h (99.3%). However, after 24 h, the percentage of PUFA began to decrease with increasing crystallisation time (p <0.05). One possible explaination is that the PUFA was trapped in the urea crystals due to the further additional time. Klinkersorn et al. (2004) also reported that the temperature of 4 ºC and 24 h of crystallisation time were the optimum conditions to enrich PUFA in the concentrate by urea complexation.

Percentage of recovery

The optimum conditions of urea complexation showed linoleic and α-linolenic acids content in the non-urea complexing fraction (NUCF) increased significantly. The fatty acids content in soybean oil and NUCF fraction were shown in *Figure 1*. After urea complexation process, only 1.5 g of PUFA derived (14.5%) from the total of 10 g of the fatty acids mixture used. A total of 100% and 97% of SFA and MUFA were successfully removed by this process.

From *Figure 1*, α-linolenic acid in NUCF fraction increased higher than linoleic acid, respectively 58% and 36%. This is due to urea complexation that is highly depended on the degree of unsaturated fatty acid. Shahidi and Wanasundara (1998) reported

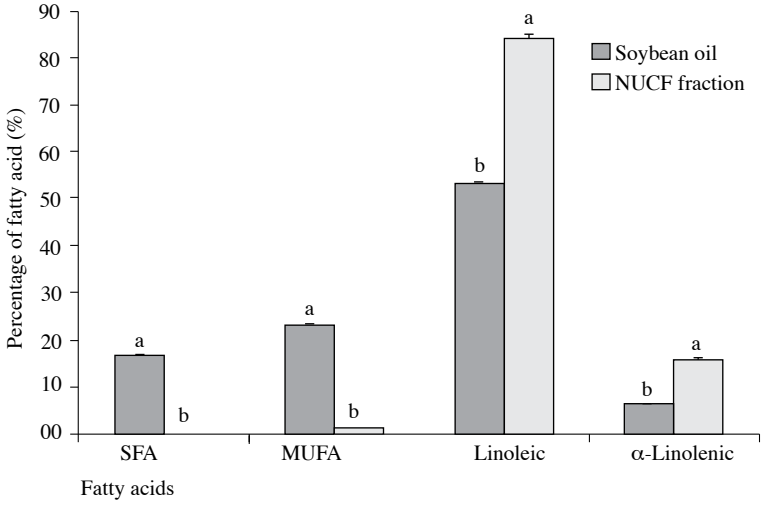

Figure 1. Fatty acids content in soybean oil and non-urea complexing fraction (NUCF). Means with the same letter, for each column are not significantly different (SFA = Saturated fatty acids, MUFA = Monounsaturated fatty acid)

that the existence of double bonds in the carbon chain fatty acids increased the size of the molecules themselves, thus reducing the possibility of these fatty acids to complex with urea. The fatty acids with two double bonds (dienes) are more easily to complex than trienes. Thus, α-linolenic acid is not easily complexed with urea compared to linoleic acid. The α-linolenic acid is more easily separated into NUCF fractions than linoleic acid.

Conclusion

A combination of chemical hydrolysis and urea complexation is a promising method to obtain highly concentrated omega-3 and omega-6 PUFA with a maximum recovery from soybean oil. Urea complexation process by using urea/fatty acid ratio of 3, crystallisation temperature of 4 ºC and crystallisation time of 24 h, can increase the amount of total PUFA up to 99.3% with a recovery (yield) of 14.5% of the weight of fatty acid mixture.

Acknowledgement

Tha authors are grateful to Universiti Kebangsaan Malaysia and Ministry of Science Technology and Innovation for the funding research grant IRPA 09-0202-0115EA277.

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Abstrak

Matlamat kajian ini adalah untuk menyediakan pekatan asid lemak politaktepu (PUFA) daripada minyak kacang soya melalui hidrolisis dan pengkompleksan urea bagi mendapatkan pekatan PUFA maksimum. Asid linoleik (LA) dan asid α-linolenik (ALA) yang diperoleh daripada hidrolisis kimia minyak kacang soya dipekatkan melalui pengkompleksan urea. Hidrolisis kimia dijalankan menggunakan larutan alkali beretanol 1M melalui refluks pada suhu 60 ºC selama 30 minit. Penghasilan LA dan ALA daripada hidrolisis kimia masing-masing sebanyak 53.4% dan 6.4%. Pemekatan LA dan ALA dipengaruhi oleh nisbah urea/asid lemak, suhu penghabluran dan tempoh penghabluran. Selepas pengkompleksan asid lemak tepu dan sedikit asid lemak tak tepu, pekatan maksimum LA (84.0%) dan ALA (15.3%) diperoleh menggunakan larutan beretanol dengan nisbah urea:asid lemak (3:1) pada suhu 4 ºC selama 24 jam tempoh penghabluran. Gabungan hidrolisis kimia dengan pengkompleksan urea adalah satu kaedah yang berpotensi untuk mendapatkan pekatan omega-3 dan omega-6 PUFA yang tinggi daripada minyak kacang soya.