Asian Journal of Chemistry Vol. 18, No. 1 (2006), 57–64

Solid-liquid Extraction of Fatty Acids of Some Variety of Iranian Rice in Closed Vessel in the Absence and Presence of Ultrasonic Waves

MOHAMMAD REZA BABAEI†, ALI JABBARI† and YADOLLAH YAMINI*
Department of Chemistry, Tarbiat Modarres University, Tehran, Iran
Fax: (98)(21)8006544; E-mail: yyamini@modares.ac.ir

Extraction of the free fatty acids of four varieties of Iranian rice (Neda, Neamat, Fajr and Sahel) was done in closed vessels both in the absence and presence of ultrasonic waves. Then, the free fatty acids were converted into their methyl esters by the MeOH/BF_3 reagent and were separated and determined by high-resolution gas chromatography equipped with flame ionization detector and a fused silica CBP-10 capillary column. The parameters influencing extraction efficiency, such as temperature, size of grains, volume ratio of methanol to chloroform as extracting solvent and extraction time, were optimized using a three level orthogonal array design with an OA_9 (3^4) matrix. Furthermore, extraction efficiencies both in the presence and absence of ultrasound wave were compared at optimum conditions. The optimum conditions of T = 80°C, mesh > 100, t = 35 min and volume ratio of methanol to chloroform, 2 : 1 were obtained in the absence of ultrasound wave. On the other hand, the time necessary for the quantitative extraction of free fatty acids in the presence of ultrasonic waves was reduced to 5 min. Based on the results, among the extraction parameters, temperature and size of grains had significant effects on the extraction efficiency and extraction yield was increased by an increase in temperature and a decrease in particle size. The amount of total fatty acids in these different rice varieties was in the range of 0.99–1.62% and Fajr variety had the greatest amount of free fatty acids.

Key Words: Rice grain, Free fatty acids, Gas chromatography, Extraction, Ultrasonic wave.

INTRODUCTION

Fatty acids are organic compounds that contain a carboxyl group at one end and a methyl group at the other. Based on the degree of saturation, fatty acids may be divided into saturated and unsaturated fatty acids[1]. The major contents of fatty acids in most vegetable oils[2] are linear saturated species with a carbon number ranging from 12 to 22 and linear unsaturated species with a carbon number of 18 and a double bond number ranging from 1 to 3. Essential fatty acids (EFA), such as *cis*-linoleic acid, arachidonic acid and linolenic acid, cannot be synthesized inside the human body and must be supplied externally from the diet. Non-essential fatty acids, like oleic acid, can be synthesized in the body[1]. Although the unsaturated fatty acids normally exist as a *cis*- configuration, the

†Department of Chemistry, K.N. Toosi University of Technology, Tehran, Iran.

trans-fatty acids that may cause health problems exist as a relatively small content[2]. Essential fatty acids are important for nutrition and are used in the biosynthesis of eicosanoid hormones[3]. In fact, dietary deficiency of EFA is manifested as poor growth, increased water content of the muscle, high liver lipid content, poor feed efficiency, shock syndrome, fin erosion, mitochondrial swelling and a decrease in hemoglobin[4]. Fatty acids play an important role in biological tissues. For example, fatty acids as constituents of lipid, influence membrane properties, such as fluidity, integrity, permeability and the activities of membrane bond enzymes and they are a source of energy for man and animal[5]. Linoleic acid is one of the essential fatty acids and is found at a high concentration in vegetable oil[6]. In comparison to other vegetable oils, rice oil tends to contain higher levels of free fatty acids[7]. Most of the oil in the grain is located in the bran and germ, which usually contain about 15–22 weight per cent[8]. Most fatty acids of rice grain are located[8–10] as C16:0, C18:0, C18:1, C18:2, C18:3. The most common procedure of fat removal from solid matrix is the conventional Soxhlet extraction; nevertheless, new extraction methods, such as microwave-assisted extraction (MAE)[11, 12], solid-phase micro extraction (SPME)[13, 14], supercritical fluid extraction (SFE)[8, 15, 16], ultrasonic[17] and so on are displacing the conventional Soxhlet extraction method because of its drawbacks. These drawbacks mostly include the strong dependence of the lipid's extraction on the solvent used, the necessity of a hydrolysis step before extraction and the large volume of solvents released into the atmosphere, in addition to the slowness of the procedure[11]. The alternative methods are not only faster and sophisticated but also reduce the organic solvent consumption[11]. The conventional methods for the determination of fatty acids are GC, HPLC[18] and CE[19]. In order to determine fatty acids by GC, these acids have to be converted into derivatives with a lower boiling point[5, 20], such as alcoholic esters. The *trans*-estrification of fatty acids was performed with different reagents, such as borontrifloride-methanol reagent (that is most often used for *trans*-estrification of all type of lipids) or with methanolic acids or sodium methoxide and potassium hydroxide in methanol or diazomethane[5]. In separation and determination of a fatty acid by HPLC, these acids are usually converted into diazoalkanes or into 9-bromoacridine derivatives. In fact, the quantification of fatty acids as their 9-bromoacridin derivatives seems to be more advantageous than that based on the use of very hazardous diazoalkenes[8].

Several statistical techniques, such as simplex optimization and factorial design, were administered for the optimization of analytical methods. Factorial design has some advantages over simplex optimization in that a global optimum can be provided, large amounts of quantitative information can be extracted and both discrete and continuous factors can be estimated. One obvious disadvantage of the factorial design is the large number of experiments required when several variables are examined. However, the number of experiments can be considerably reduced by the use of an orthogonal array design[13].

The aim of the present study was the optimization of the different parameters, such as temperature, size of rice grain, extraction time and the ratio of methanol to chloroform (as the extraction solvent) on the extraction of free fatty acids of four varieties of Iranian rice grain in closed vessels both in the absence and presence of ultrasonic waves. In addition, the free fatty acid contents of different Iranian rice varieties were compared.

EXPERIMENTAL

Fatty acid methyl esters were analyzed using a Shimadzu GC-14B gas chromatograph equipped with a FID and a CBP-10 fused silica capillary column (25 m × 0.22 mm i.d., film thickness 0.25 μm). Oven temperature was held at 200°C for 2 min and then programmed up to 230°C at a rate of 5°C/min and held at this temperature for 3 min. Detector and injector port temperatures were 250°C. Helium was used as the carrier gas with a linear velocity of 35 cm/s. The samples and standards (1 μL) were injected into the GC using the split mode with a split ratio of 1 : 40.

Extraction of fatty acids was performed in an ultrasound bath of model Starsonic (Italy) at frequencies of 28–34 KHz.

HPLC grade solvents of chloroform, methanol, *n*-hexane and ethyl acetate were purchased from Merck. The boron trifloride reagent in ethyl ether and standards of fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and tridecanoic acid as internal standard) were obtained from Aldrich and used without any further purification.

Four varieties of Iranian rice (Neda, Neamat, Sahel, and Fajr) were kindly offered as a gift by the Agriculture Center of Mazandaran (province of Mazandaran, Iran) and Sadri rice was purchased from the market.

Extraction of fatty acids: The rice grain was extracted from its bran and pulverized. Particles with average meshes of 16–40, 40–100 and 100–200 were obtained by sieving. 0.25 g of each variety was extracted with 6 mL of methanol : chloroform (2 : 1) mixture at 85°C for 35 min in a 15 mL closed vessel. The desired solution was separated from the rice grain and then evaporated. The residue in the vessel was converted into methyl ester according to the following method:

Esterification of fatty acids: 130 μg $C_{13:0}$ as an internal standard and 0.5 mL of boron triflouride reagent (10% BF_3 in methanol) were added to each vessel that contained extracted fatty acids and the vessels were left for 15 min at 60°C. Methyl esters of fatty acids were extracted twice with *n*-hexane. The *n*-hexane layer was washed with water and dried in the presence of $CaCl_2$. Hexane was evaporated and the final volume of the extract was adjusted to 1 mL with ethyl acetate. 1 μL of the resulting sample was injected to the GC-FID using split injection.

Calibration: Five methanolic solutions containing $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ with concentrations of 50, 100, 500, 1000 and 2000 μg/mL and 130 μg/mL $C_{13:0}$ as an internal standard were sterified as described above and 1 μL of each sample was injected into the GC and proper calibration curves (based on the relative peak area *vs.* concentration) were obtained for each fatty acid with correlation coefficients higher than 0.99 (Table-1).

TABLE-1
REGRESSION EQUATIONS FOR FREE FATTY ACID DETERMINATIONS IN RICE GRAINS

Fatty acid	Regression equation	Correlation coefficient (R_2)
Palmitic acid	Y[a] = 0.0089 C (μg/mL) + 0.165	0.9997
Stearic acid	Y = 0.0068 C (μg/mL) + 0.072	0.9995
Oleic acid	Y = 0.0069 C (μg/mL) + 0.255	0.9989
Linoleic acid	Y = 0.0068 C (μg/mL) + 0.1101	0.9992
Linolenic acid	Y = 0.0034 C (μg/mL) + 0.0079	0.9997

[a]Relative GC peak area of solute to internal standard.

RESULTS AND DISCUSSION

The aim of this study was to find out the conditions providing the highest extraction efficiency of fatty acids from rice grain by solid-liquid extraction (SLE) in closed vessel inside the experimental domain explored and the results were compared both in the absence and presence of ultrasonic waves.

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step. The optimization of this method can be carried out, step-by-step, or by using an experimental design. Table-2 shows different conditions of experiments carried out in the absence of ultrasonic waves for extraction of fatty acids from rice grain according to the Taguchi experimental design. All the selected factors were examined by using a three-level orthogonal array design with an OA_9 (3^4) matrix. In this study, interactions among variables were not incorporated in the matrix and emphasis was laid on the main effects of the four most important factors. The Sadri rice was used to achieve optimum conditions of extraction. The total extracted fatty acid from Sadri rice has been considered as the response of the method. The parameters that were optimized were temperature (65, 75 and 85°C), size of particles of rice grain based on the mesh (16–40, 40–100 and 100–200), extraction time (15, 25 and 35 min) and ratio of methanol to chloroform as extraction solvent (2 : 1, 1 : 1 and 1 : 2). 6 mL of the extracting solvent were used in all extractions. To determine the exact level of each factor, various preliminary experiments at different conditions were performed. Then the total impact of all factors can be obtained. For the next experiments, we selected the level of each factor through the obtained results.

The conditions of each experiment and results of the experiments, based on the extraction yields, are given in Table-1. The mean values of the extraction yields for the corresponding factors at each level were calculated according to the assignment of the experiment. For example, the extraction yields of the three trials at 85 : C were evaluated as a mean value of the corresponding three runs (trials 1–3). The mean values of the three levels of each factor (*e.g.*, temperature) reveal how the extraction yield will change when the level of that factor is

changed. Fig. 1 shows the variations in extraction yield as a function of change in different levels of the factors studied. According to Fig. 1, the optimum conditions for extraction in closed vessels in the absence of ultrasonic waves are: T = 85°C, mesh > 100, extraction time = 35 min and ratio of methanol to chloroform 2 : 1.

TABLE-2
EXPERIMENTAL CONDITIONS OF LIQUID-SOLID EXTRACTIONS AND EXTRACTION YIELD OF FREE FATTY ACIDS FROM RICE GRAINS

Trial No.[a]	T (°C)	T (min)	dp (mesh)	MeOH : $CHCl_3$ volume ratio	Yield (%)
1	65	15	16–40	2 : 1	1.52
2	65	25	40–100	1 : 1	1.80
3	65	35	100–200	1 : 2	2.60
4	75	15	40–100	1 : 2	2.38
5	75	25	100–200	2 : 1	3.87
6	75	35	16–40	1 : 1	2.76
7	85	15	100–200	1 : 1	4.01
8	85	25	16–40	1 : 2	2.90
9	85	35	40–100	2 : 1	3.44

[a]All experiments were performed using Sadri rice grains.

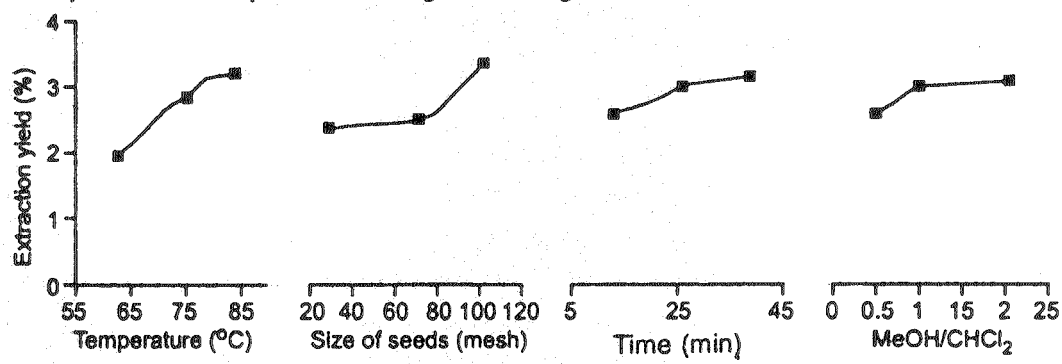

Fig. 1. Effects of temperature, size of particles, volume ratio of methanol : chloroform and extraction time on the extraction yield. In all of the extractions 0.25 g of the sample and 6 mL of the solvent were used.

TABLE-3
ANOVA TABLE OF THE EXPERIMENTS (AT 90% CONFIDENCE)

Source of variance	Sum of squares	Degree of freedom	Mean square	F[a] value
Temperature	3.44	2	1.72	25.57
Size of grains	2.15	2	1.07	15.29
Solvent	0.16	2	0.08	1.14
Extraction time	0.15	2	0.07	1
Pooled error	0.15	2	0.07	1
Total	5.89	8		

[a]$F_{critical} = 9.00$

The analysis of variance (ANOVA) of the results is shown in Table-3. The results indicate that, with a confidence level of 90%, the extraction time and the chloroform : methanol ratio had no significant effects on the recovery percentage of fatty acids.

The ANOVA results of this experiment indicate that the temperature and size of grains play an important role in the extraction of fatty acids. Temperature is an important factor contributing to all extraction techniques. When extraction is conducted in the closed vessel (high pressure), temperature may reach well above the boiling point of the solvent. Such high temperatures result in improved extraction efficiencies since desorption of analytes from active sites will increase. Additionally, solvents have higher capacity to solubilize analytes at higher temperatures while surface tension and solvent viscosity decrease with temperature, which will improve sample wetting and matrix penetration, respectively[21]. Therefore, in classical methods better extraction was achieved at a higher temperature[22]. The critical point of these experiments was the reduction of extraction time in comparison to conventional Soxhlet extraction that refers to an increase in the pressure of the extraction and an increase in the temperature to higher than boiling point of the extracting solvent in the closed vessel[21]. Moreover, in this method, the volume of extraction solvent was decreased as compared to the conventional extraction methods, such as Soxhlet (6 mL in comparison with at least 250 mL for Soxhlet extraction).

Under optimum conditions, in the presence of ultrasonic waves with frequency range of 28–34 KHz, the extraction of fatty acids was done to determine the effect of ultrasonic waves on the rate of extraction. Extraction of fatty acids in the presence of ultrasonic waves was completed in 5 min. The reduced time of extraction depends on a complex system of physical and chemical reactions called acoustic cavitations, which produce a high energy and a high contact between solvent and solute[17]. In addition, the rate of extraction of fatty acids increases since ultrasonic waves increase the rate of stirring of solution. It is clear that the extraction rate will be higher if the mixture is shaken as compared to a motionless mixture. The reason for the low extraction rate in the motionless method can be due to the formation of a saturated layer of extracted materials around the material. This phenomenon can slow down the extraction rate of materials. The mechanical or ultrasonic stirring removes this layer from the surroundings of the particles of rice grains and thus the particles in contact with fresh solvent will result in a higher extraction rate. Moreover, the LSE is based on the diffusion process and increasing the temperature will increase this physical property.

The results of extraction of fatty acids of four types of Iranian rice under the optimum conditions are tabulated in Table-4. For determination of fatty acid each experiment was repeated 3 times at optimum conditions and the value of per cent relative standard deviation (% RSD) for total free acids that is an indicator for reproducibility was reported. The process of extraction was repeated and we did not observe any fatty acid in extracted sample. This indicate all of the fatty acids were extracted and there is no residue of fatty acids in our samples.

TABLE-4
FREE FATTY ACID COMPOSITION OF SOME VARIETIES OF IRANIAN RICE

Fatty acid / Rice variety	Concentration of free fatty acids in rice grain (mg/g)					Total amount of fatty acid (%)	RSD (%)[a]
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$		
Fajr	2.44	0.31	6.46	6.27	0.60	1.61	9.17
Neda	1.77	0.19	3.30	4.18	0.43	0.99	3.14
Sahel	2.24	0.47	4.41	5.27	0.66	1.31	4.96
Neamat	2.00	0.31	3.91	4.37	0.68	1.13	16.83

[a]Relative standard deviation based on three replicate measurements.

As indicated in Table-4, most of the fatty acids that are observed in Iranian rice include $C_{16:0}$, $C_{18:1}$, $C_{18:2}$. Since the most important fatty acids in human body are $C_{18:1}$, $C_{18:2}$, then the consumption of rice could compensate the poverty of the fatty acid in the human body. Fajr rice is the richest rice among the 4 types of Iranian rice. If we suppose the amount of fatty acids in rice is an indicator for the quality of rice then Fajr rice is the best.

Conclusions

In the present study, the effects of four experimental parameters (temperature, size of grains, extraction time and ratio of methanol to chloroform) on the extraction recovery of fatty acids from four varieties of Iranian rice were studied. The use of an experimental design approach allowed the selection of the best experimental conditions for the SLE of fatty acids in closed vessel from rice grains inside the experimental domain considered.

The ANOVA results identified temperature as a significant factor. In particular, it was found that an increase in the extraction temperature from 65 to 85°C enhances the extractability of fatty acids from rice grains.

In the presence of ultrasonic waves, extraction time was reduced to 5 min. Also, among the four varieties of Iranian rice, Neda has the best quality due to its free fatty acid contents.

REFERENCES

1. B.S.R. Badal, Supercritical Carbon Dioxide Extraction of Lipid from Raw and Bioconverted Rice Bran, Regional Engineering College, Jalandhar (1998).
2. S.H. Chen, K.C. Chen and H.M. Lien, *J. Chromatogr. A*, **849**, 357 (1999).
3. V.K. Svetlana, *Phytochemistry*, **49**, 2363 (1998).
4. D.M. Smith, B.J. Hunter, G.L. Allan, D.C.K. Roberts, M.A. Booth and B.D. Glencross, *Aquaculture*, **236**, 377 (2004).
5. K. Eder, *J. Chromatogr. B*, **671**, 113 (1995).
6. F.M. Ramezanzadeh, R.M. Rao, W. Prinyawiwatkul, W.E. Marshal and M. Windhauser, *J. Agric. Food Chem.*, **48**, 464 (2000).
7. C.E.C. Rodrigues, R. Antoniassi and A.J.A. Meirelles, *J. Chem. Eng. Data*, **48**, 367 (2003),
8. H.J. Kim, S.B. Lee, K.A. Park and I.K. Hong, *Sep. Purif. Technol.*, **15**, 1 (1999).

9. H. Taira. M. Nakagahra and T. Nagamine, *J. Agric. Food Chem.*, **36**, 45 (1988).
10. H. Taira and T. Itani, *J. Agric. Food Chem.*, **36**, 3460 (1988).
11. L.E. Garcia-Ayuso, J. Velasco, M.C. Dobarganes and M.D. Luque de Castro, *J. Agric. Food Chem.*, **47**, 2308 (1999); J.L. Luque-Garcia, J. Velasco, M.C. Dobarganes and M.D. Luque de Castro, *Food Chem.*, **76**, 241 (2002).
12. E. De Pedro, M. Casillas and C.M. Miranda, *Meat Sci.*, **45**, 45 (1997).
13. W. Lan, K.K. Chee, M.K. Wong, H.K. Lee and Y.M. Sin, *Analyst*, **120**, 281 (1995).
14. O. Pinho, I.M.P.L.V.O. Ferreira and M.A. Ferreira, *Anal. Chem.*, **74**, 5199 (2002).
15. A.A. Gharaibeh and K.J. Voorhees, *Anal. Chem.*, **68**, 2805 (1996).
16. M. Fischer and T.M. Jefferies, *J. Agric. Food Chem.*, **43**, 1259 (1995).
17. M. Mecozzi, M. Amici, G. Romanelli E. Pietrantonio and A. Deluc, *J. Chromatogr. A*, **963**, 363 (2002).
18. I. Molnar-Perl, *J. Chromatogr. A*, **891**, 132 (2000).
19. D.L. Gallaher (Jr.) and M.E. Johnson, *Anal. Chem.*, **72**, 2080 (2000).
20. M. Rodriguez-Palmero, M.C. Lopez-Sabater, A.I. Castellote-Bargallo, M.C. De La Torre-Boronat and M. Rivero-Urgell, *J. Chromatogr. A*, **849**, 357 (1999).
21. C.S. Eskilsson and E. Bjorkland, *J. Chromatogr. A*, **902**, 227 (2000).
22. M.H. Entezari, S. Hagh Nazary and M.H. Haddad Khodaparast, *Ultrasonic Sonochemistry*, **11**, 379 (2004).

(*Received*: 1 September 2004; *Accepted*: 22 August 2005) AJC-4364