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RESEARCH NOTES

Fatty Acid Determination in Chicken Egg Yolk: A Comparison of Different Methods

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ABSTRACT Four different methods (direct-methylation, saponification, chloroform-methanol extraction, and postextraction saponification) were compared to determine the fatty acids in egg yolk. About 50 mg of pooled egg yolk samples, with C23:0 as an internal standard, was used for all assays. No difference ($P > 0.05$) was observed among the four methods for C17:0, C18:0, C18:1, C20:1, C18:2n-6, and C22:6n-3 content of egg yolk. Direct saponification resulted in a lower ($P < 0.05$) content of C14:0, C16:1, C18:3n-3, and C20:4n-6. Fatty acids at less than 0.5%, such as C15:0 and C14:1, were not detectable in the direct saponification method. The total saturated, monounsaturated, or polyunsaturated fatty acids did not differ ($P > 0.05$) among the four methods. Direct methylation of egg yolk resulted in lower variability than other methods and is fast and economic for determining egg fatty acid composition.

(*Key words*: fatty acid assay, egg yolk)

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INTRODUCTION

Claims of beneficial health effects of certain dietary fatty acids have led to extensive research on manipulating the fatty acid composition of food-animal products (Caston and Leeson, 1990; Ajuyah et al., 1993). The poultry industry responded to such needs by increasing the n-3, n-6, and n-9 fatty acid content of chicken eggs (Cherian et al., 1996; Farrell, 1998; Van Elswyk, 1998). Accordingly, many name brands of fatty acid-modified eggs with varying levels of n-3 fatty acids are now available in the market.

Several methods are currently available for the determination of fatty acids in egg. The most commonly used procedure is extraction of lipids using chloroform:methanol (2:1, vol/vol) (Folch et al., 1957) and esterification to fatty acid methyl esters. Other reported methods include direct saponification and postextraction saponification. However, chloroform-methanol extraction and saponification are time consuming (Lepage and Roy, 1986), hazardous to health, costly, and may lead to errors because of the multiple steps involved. Drucker (1972) reported an alternative procedure for determining the bacterial lipids by direct methylation, which has been reported for other biological samples to have greater accuracy and recovery (Sattler et al., 1991; Rodriguez-Palmero et al., 1997).

Considering the increased public awareness (Marshall et al., 1994) and market availability of health-promoting fatty acid-modified eggs, there is a need for fast, less expensive, accurate, and environmentally-friendly methods for determining the fatty acid content in food products. Consumer acceptance of eggs also depends on quality parameters and proper nutritional information. Therefore, a fast and accurate method for determining egg fatty acid content may help egg consumers and the egg industry. In this context, the objective of the present study was to compare four different methods for determining the fatty acid in egg yolk.

MATERIALS AND METHODS

Egg Collection

Extra large (n = 12) commercial eggs were collected from a supermarket in Edmonton, Canada. The yolks were separated from albumen and weighed. The yolk samples were pooled and were kept frozen at –20 C until analysis. For each method tested, approximately 50 mg of yolk samples was taken in eight replicates.

Direct Methylation

Pooled egg yolk was weighed into a 50-mL, teflon-lined, screw-capped tube. Tricosanoic acid (C23:0) (2.0 mg)[3] dissolved in 1 mL hexane was added as an internal

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standard, followed by 1 mL of methanol and 3 mL of 3*N* methanolic-HCl. The tubes were capped tightly and refluxed in a water bath at 95 C for 1 h. After cooling to the room temperature, 8 mL of 0.88% (wt/vol) NaCl solution and 3 mL of hexane were added to the tube, and the contents were mixed well. After centrifugation, an aliquot of the top layer was taken into a 1.5-mL vial and adjusted so that the final concentration of total lipids was 0.2 to 0.3 mg/mL hexane. The fatty acid methyl esters were separated and quantified using an automated gas chromatograph equipped with an on-column injector.[4]

Saponification

About 50 mg of pooled egg yolk was weighed into a 50-mL, teflon-lined, screw-capped tube. Tricosanoic acid (C23:0) (2.0 mg) dissolved in 1 mL hexane was added as an internal standard. The egg samples were saponified with 10 mL of ethanolic-potassium hydroxide (5 mL of 45% KOH and 95 ml of 95% ethanol) at 60 C in a water bath for 1 h with occasional shaking. After cooling to room temperature, 5 mL of water and 4 mL hexane were added, shaken well, and left to separate into phases. The top layer containing nonsaponifiable lipids was discarded. An additional 4 mL hexane was added, and the samples were again allowed to separate. The nonsaponifiable lipids from the top layer were discarded. The bottom layer containing saponifiables (fatty acids) was acidified with HCl solution (HCl:water, 1:1, vol/vol) to pH 2 to 2.5, followed by the addition of 4 mL hexane. The samples were shaken vigorously and left for complete separation. About 2 mL of the top layer was moved into a 15-mL, teflon-lined, screw-capped tube and dried under an atmosphere of nitrogen. About 3 mL of methanolic-HCl (3*N*) was added, and the tube was kept in a water bath at 95 C for 1 h. After cooling, 5 mL of 0.88% NaCl (wt/vol) and 3 mL hexane were added to the tube. The solution was then mixed by shaking vigorously to extract fatty acid methyl esters. After separation, the top layer was pipetted off into a 1.5-mL glass vial and adjusted to contain 0.2 to 0.3 mg lipids/mL hexane. The fatty acid methyl esters were separated and quantified using an automated gas chromatograph.[4]

Chloroform-Methanol Extraction

About 50 mg of pooled egg yolk was weighed into a 50-mL, teflon-lined, screw-capped tube. Tricosanoic acid (C23:0) (2.0 mg), dissolved in 1 mL hexane, was added as an internal standard. A 10-mL, Folch-I solution (chloroform:methanol 2:1) was added. The samples were homogenized using a polytron[5] at high speed. The polytron head was rinsed with another 5 mL Folch-I solution that was added to the homogenate. After 3 h, 4 mL of 0.88% NaCl (wt/vol) was added to the egg homogenate and mixed. After phase separation, the top layer was carefully siphoned off. The bottom layer (3 mL) was taken into a 15-mL glass tube and dried in a block heater under a stream of nitrogen. The dried lipids were resolubilized in 3 mL of methanolic-HCl (3*N*), capped tightly, and incubated in a water bath at 95 C for 1 h. After cooling to room temperature, 5 mL of water and 3 mL hexane were added to the tube. The solution was mixed by shaking vigorously and allowed to separate. After complete separation, an aliquot of the top (hexane) layer was taken into a 1.5-mL vial, and the lipid concentration was adjusted to 0.2 to 0.3 mg/mL hexane. The fatty acid methyl esters were separated and quantified by gas chromatograph.

Postextraction Saponification

About 50 mg of pooled egg yolk was weighed into a 50-mL, teflon-lined, screw-capped tube. Tricosanoic acid (C23:0) (2.0 mg) dissolved in 1 mL hexane was added as an internal standard. A 10-mL Folch-I solution (chloroform:methanol, 2:1, vol/vol) was added. The samples were homogenized using the polytron at high speed. The polytron head was rinsed with another 5 ml Folch-I solution, which was added to the homogenate. After 3 h, 4 mL of 0.88% NaCl (wt/vol) was added to the egg homogenate and mixed by tilting the tubes. After phase separation, the top layer was carefully siphoned off. The bottom (3 mL) was taken into a 15-mL glass tube and dried in a block heater under a stream of nitrogen. The dried lipid samples were incubated with 10 mL of ethanolic-potassium hydroxide (5 mL of 45% KOH and 95 ml of 95% ethanol) at 60 C in a water bath for 1 h with occasional shaking. After cooling to room temperature, 5 mL of water and 3 mL hexane were added, shaken well, and left for the phases to separate. The top layer containing nonsaponifiable lipids was discarded. An additional 3 mL hexane was added, and the samples were again allowed to phase separate. The nonsaponifiable lipids (top layer) were discarded. The bottom layer containing saponifiables (fatty acids) was acidified with HCl solution (HCl:water, 1:1, vol/vol) to pH 2 to 2.5, followed by the addition of 4 mL hexane. The samples were shaken vigorously and left for complete separation. About 2 mL of the top layer was taken into a 15-mL, teflon-lined, screw-capped tube and dried under an atmosphere of nitrogen. About 3 mL methanolic-HCl (3*N*) was added, and the tubes were kept in a water bath at 95 C for 1 h. After cooling, 5 mL of 0.88% NaCl (wt/vol) and 3 mL hexane were added to the tube, mixed by shaking vigorously to extract fatty acid methyl esters, and after separation, the top layer was aliquoted into a 1.5-mL glass vial and adjusted to contain 0.2 to 0.3 mg lipids in 1 mL hexane. The fatty acid methyl esters were separated and quantified using an automated gas chromatograph.

Gas Chromatography

A fused silica capillary column (30 m × .25 mm i.d) on a Varian 3600 gas chromatograph, equipped with an auto-

[4]Varian, 3600 GLC, Varian Associates, Inc., Palo Alto, CA 94304.
[5]The Virtis Company, Inc., Gardiner, NY 12525.

TABLE 1. The fatty acid content of the egg yolk analyzed by different methods[1]

Fatty acid[3]	Method[2] I	II	III	IV
C14:0	1.29 ± 0.06[ab]	1.23 ± 0.08[b]	1.39 ± 0.06[a]	1.29 ± 0.09[ab]
C15:0	0.21 ± 0.1	ND[4]	0.30 ± 0.04	0.26 ± 0.03
C16:0	100.64 ± 2.53	100.05 ± 2.45	103.33 ± 2.68	101.75 ± 3.77
C17:0	0.73 ± 0.08	0.67 ± 0.11	0.75 ± 0.05	0.72 ± 0.08
C18:0	29.52 ± 0.79	30.74 ± 0.75	30.19 ± 0.99	29.42 ± 1.15
C14:1	0.28 ± 0.02	ND	0.29 ± 0.02	0.32 ± 0.05
C16:1	17.98 ± 0.46[a]	16.84 ± 0.38[b]	18.62 ± 0.49[a]	18.35 ± 0.63[a]
C18:1	159.22 ± 4.40	160.89 ± 3.97	161.81 ± 4.92	158.22 ± 5.82
C20:1	1.21 ± 0.17	1.23 ± 0.34	1.10 ± 0.01	1.08 ± 0.17
$C18:2_{n-6}$	30.43 ± 0.79	29.53 ± 0.80	30.83 ± 0.80	30.52 ± 1.11
$C18:3_{n-3}$	1.11 ± 0.04[ab]	0.98 ± 0.14[b]	1.20 ± 0.16[a]	1.11 ± 0.19[ab]
$C20:4_{n-6}$	5.03 ± 0.16[ab]	4.75 ± 0.18[b]	4.98 ± 0.31[ab]	5.17 ± 0.29[a]
$C22:6_{n-3}$	1.63 ± 0.06	1.54 ± 0.12	1.43 ± 0.09	1.46 ± 0.08
SAFA	132.38 ± 3.40	132.51 ± 3.12	135.92 ± 3.58	133.44 ± 5.05
MUFA	178.69 ± 4.99	178.57 ± 4.27	181.67 ± 5.31	178.47 ± 6.58
PUFA	38.20 ± 1.00	36.80 ± 1.09	38.43 ± 1.03	38.26 ± 1.49

[a–d]Values with common superscripts are not significantly different ($P > 0.05$).

[1]Values are mean ± SD (mg/g).

[2]I, II, III, and IV are direct methylation, saponification followed by methylation, chloroform-methanol extraction followed by methylation, and postextraction saponification followed by methylation, respectively.

[3]SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.

[4]Not detectable.

sampler and flame ionization detector,[4] was used to separate and quantify the fatty acid methyl esters. The initial column temperature was set at 70 C for 3 min, increased to 180 C by 30 C/min, and held for 10 min. The column temperature was elevated to 230 C at 5 C/min and held at the final temperature for 3 min. Helium was used as the carrier gas at a flow rate of 3.0 mL/min. The detector was set at 240 C. Fatty acid methyl esters were identified by comparison with retention times of standard.[6] A Shimadzu EZChrom[7] laboratory data integration system was used to integrate peak areas. The fatty acid content of egg yolk was calculated as concentration (mg/g) = peak area of a given fatty acid × concentration of internal standard (mg/mL)/peak area of internal standard/sample weight (g).

Statistical Analysis

Data were analyzed using one-way analyses of variance. Significant differences among the four method means were tested using Tukey's studentized range test at a 5% probability level (Myers and Well, 1995). Computations were done using the general linear model procedure of SAS Institute (1990).

RESULTS AND DISCUSSION

Among the different fatty acids tested, no difference ($P > 0.05$) was observed among the four methods for C16:0, C17:0, C18:0, C18:1, C20:1, C18:2n-6, and C22:6n-3 (Table 1). However, direct saponification resulted in a lower ($P < 0.05$) content of C14:0, C16:1, C18:3n-3, and C20:4n-6. Similarly, fatty acids under 0.5%, such as C15:0 and C14:1, were not detectable in the direct saponification method. The fatty acid results from direct methylation were found to be similar to those after chloroform:methanol extraction and methylation. Better accuracy from direct methylation has been documented in fatty acid analyses of feed, digesta, feces (Outen et al., 1975), plasma, and erythrocytes (Rodriguez-Palmero et al., 1997). The total saturated, monounsaturated, or polyunsaturated fatty acids did not differ ($P > 0.05$) among the four methods.

Comparison of fatty acids among the four different methods was made more accurate using an internal standard. Tricosanoic acid (C23:0) is not detectable in chicken egg yolk and was added at the beginning of sample preparation in all procedures to quantitatively determine the fatty acid content in egg yolk without significant losses being incurred during the preparative process. About 50 mg of egg yolk was used in each method (n = 8), resulting in 15 mg of lipids (about 30% of lipids in fresh egg yolk) undergoing the lipid extraction and methylation processes. Lower concentrations of lipids usually give higher yields of fatty acids when using the method of direct methylation (Lepage and Roy, 1986; Sattler et al., 1991). A large amount of fatty acids in the samples could reduce the relative influence of error and the proportion of possible losses of fatty acids during the sample preparation. The recovery of fatty acids or fatty acid esters may also depend on the type of samples. The reason for the lower level concentration of fatty acids in direct saponification is not known. Further improvement to optimize the method is warranted.

Application of any method is subject to its precision. In the present study, direct methylation had less variation than the chloroform extraction or postextraction saponi-

[6]Matreya, Inc., 500 Tressle Street, Pleasant Gap, PA 16823.

[7]Shimadzu Scientific Instruments, Inc., 7120 Riverwood Drive, Columbia, MD 21046.

fication methods. Except for C14:0, C17:0, C20:1, and C22:6n-3, the saponification method had less variation in most fatty acids than the chloroform extraction or postextraction saponification methods. The chloroform extraction and postextraction saponification methods produced the greatest variation for most fatty acids, suggesting a greater chance of sample loss and error as the number of preparative steps are increased (Lepage and Roy, 1986). Direct methylation has been reported to produce less variation when compared with Folch-extraction and methylation (Outen et al., 1975), which again may be due to the fewer number of steps involved in the extraction and preparative process, affecting recovery.

With the availability of different brands of fatty acid-modified egg and other food products on market, a fast and accurate method to measure fatty acid content of egg yolk is needed. In the current study, fatty acids analyzed by the saponification method resulted in lower ($P < 0.05$) values than the other methods. The direct methylation showed comparable results in fatty acid composition and fatty acid content with the conventional method; chloroform-methanol extraction followed by methylation or followed by saponification and methylation. Direct methylation circumvents most of the preparative steps, is faster, uses less chemicals, and is more economical. Thus, this method is simpler and more environmentally friendly than the conventional methods and has less variation.

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REFERENCES

Ajuyah, A. O., R. T. Hardin, and J. S. Sim, 1993. Effect of dietary full-fat flaxseed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats. Poultry Sci. 72:125–136.

Caston, L., and S. Leeson, 1990. Dietary flax and egg composition. Poultry Sci. 69:1617–1620.

Cherian, G., R. T. Wolfe, and J. S. Sim, 1996. Dietary oils with added tocopherols: Effects on egg or tissue tocopherols, fatty acids and oxidative stability. Poultry Sci. 75:423–431.

Drucker, D. B., 1972. The identification of streptococci by gas liquid chromatography. Microbios 5:109–112.

Farrell, D., 1998. Enrichment of chicken eggs with n-3 polyunsaturated fatty acids and evaluation of enriched eggs in humans. Am. J. Clin. Nutr. 68:538–544.

Folch, J., M. Lees, and G. H. Sloane-Stanely, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–507.

Lepage, G., and C. C. Roy, 1986. Direct transesterification of all classes of lipids in a one step reaction. J. Lipids Res. 27:114–120.

Marshall, A. C., K. S. Kubena, K. R. Hinton, P. S. Hargis, and M. E. Van Elswyk, 1994. N-3 fatty acid enriched table eggs: a survey of consumer acceptability. Poultry Sci 73:1334–1340.

Myers, J. L., and A. D. Well, 1995. Research design and statistical analysis. Lawrence Erlbaum Associates, Hillsdale, NJ.

Outen, G. E., D. E. Beever, and J. S. Fenlon, 1975. Direct methylation of long chain fatty acid in feeds, digesta and feces without prior extraction. J. Sci. Feed Agric. 27:419–425.

Rodriguez-Palmero, M., M. C. Lopez-Sabater, A. I. Castellote-Bargallo, M. C. De La Torre-Boronat, and M. Rivero-Urgell, 1997. Comparison of two methods for the determination of fatty acid profile in plasma and erythrocytes. J. Chromatogr. A. 778:435–439.

SAS, 1990. User's Guide. Version 6.06 ed. SAS Institute Inc., Cary, NC.

Sattler, W, H. Puhl, M. Hayn, G. M. Kostner, and H. Esterbauer, 1991. Determination of fatty acids in the main lipoprotein classes by capillary gas chromatography: BF_3/methanol transesterification of lyophilized samples instead of Folch extraction gives higher yields. Anal. Biochem. 198:184–190.

Van Elswyk, M. E., 1998. Egg as a functional food alternative to fish and supplements for the consumption of docosahexaenoic acid. Page 20 *in*: Second International Symposium on Egg Nutrition and Newly Emerging Ovo Biotechnologies. Banff, AB, Canada. (Abstr.).