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LETTER TO THE EDITOR

Rapid Lipid Extraction from Egg Yolks

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Sir,

Total or crude fat determination is commonly measured in lipid chemistry by traditional methods such as Soxhlet or Goldfisch extraction. These methods require hours of solvent reflux extraction, which may be necessary when lipids are well embedded into tissues and the surface area to weight ratio is low. However, many food materials may have oil that can be readily extracted without such exhaustive solvent extraction. Simple, practical, rapid extraction techniques are more cost effective when dealing with large number of samples if the lipids can be readily extracted from the food matrix.

Clark and Snyder [1] developed a rapid, 1-min equilibrium extraction method of a 1–2-g soy flour sample with hexane at ambient temperature that produced results similar to those obtained by Goldfisch extraction to allow rapid screening of soybean cultivars. Subsequently, we developed a rapid, 1-min method for total lipid extraction with hexane and isopropanol of 2 g of rice bran samples for free fatty acid determination that produced the same results as the Goldfisch extraction [2]. Similarly, the oil contents of milled rice and potato chips were determined by rapid solvent extraction [3, 4].

However, we have not developed any rapid animal lipid extraction methods. We anticipate incorporating CLA-rich oil [5] in eggs through poultry feed. Therefore, a rapid egg yolk lipid extraction technique would be invaluable prior to egg fatty acid analysis by FAMES GC-FID analysis. The Folch method [6] is a commonly used procedure for total lipid extraction from eggs, but uses chloroform–methanol (2:1, v/v) to extract the lipids, followed by a water wash using 0.2 times the volume of sample. This method is both time consuming and constitutes a significant health hazard because of the use of chloroform. The development of a practical, simple, rapid extraction technique would be more cost effective, particularly when dealing with a large number of samples. Hara and Radin [7] described an efficient 1-min extraction procedure that is particularly adapted to nervous tissues using hexane/isopropanol (3:2, v/v). Using this method, the whole liquid phase is evaporated, eliminating a phase separation step. The objective of this study was to determine if a rapid hexane/isopropanol extraction would extract the same amount of lipid and have the same fatty acid profile as lipids obtained by the Folch method.

Six eggs were collected from the University of Arkansas Poultry Science Department. Egg yolks were separated, combined and well mixed in a beaker with a stir-bar. Yolk samples were then diluted with distilled water to obtain 100, 75, 50 and 25 % dilutions of the original yolk mixture. Duplicate extractions of each dilution were made by the Folch method [6] and the following rapid extraction method. Duplicate 4-g samples were accurately weighed and vortexed with ten times the volume of hexane/isopropanol (1:1, v/v) for 5 min at room temperature. Homogenate was filtered using a funnel with Whatman no. 4 110-mm filter paper to recover only the liquid phase. Samples from both extraction methods were evaporated under vacuum in a rotary evaporator and weighed. A calibration curve was prepared comparing total lipid extraction by both methods. One-way ANOVA was determined by JMP 9.0.2 software to observe significant differences in the extraction methods.

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Table 1 Oil extracted from duplicate 4-g yolk dilutions by a rapid hexane/isopropanol extraction, relative to a control Folch extraction [6]

Yolk concentration (%)	Mean oil extracted (g) Folch method	Mean oil extracted (g) hexane/isopropanol method	Prob > F
25	7.65 ± 0.15[a]	8.35 ± 0.35	0.29
50	16.2 ± 1.0	16.75 ± 0.35	0.40
75	22.95 ± 0.15	21.9 ± 0.41	0.13
100	37.35 ± 0.05	36.5 ± 0.32	0.21

Means are expressed with standard error of mean

[a] Extracted oil amounts in the same row are not statistically different, shown by the large *P* values

Table 2 Fatty acid composition of whole egg yolk and 50 % egg yolk dilution obtained by Folch and rapid hexane/isopropanol extraction methods

Extraction method	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4
Undiluted yolk								
Folch	0.37 ± 0.003a	25.5 ± 0.20b	3.0 ± 0.08c	8.9 ± 0.21d	40.6 ± 0.20e	16.5 ± 0.01f	0.33 ± 0.03g	2.3 ± 0.13h
Hexane/IPA	0.38 ± 0.003a	25.6 ± 0.20b	3.0 ± 0.08c	8.5 ± 0.21d	40.5 ± 0.20e	16.6 ± 0.01f	0.33 ± 0.03g	2.3 ± 0.13h
50 % Yolk dilution								
Folch	0.37 ± 0.003a	25.5 ± 0.20b	3.0 ± 0.08c	8.6 ± 0.16d	40.7 ± 0.06e	16.5 ± 0.01f	0.35 ± 0.02g	2.4 ± 0.23h
Hexane/IPA	0.38 ± 0.003a	25.6 ± 0.20b	3.0 ± 0.08c	8.5 ± 0.16d	40.5 ± 0.06e	16.6 ± 0.01f	0.34 ± 0.03g	2.3 ± 0.23h

Fatty acid percentages with the same letter in the same column are not statistically different

Means are expressed with the standard error of mean

Each duplicate extraction from the whole yolk and 50 % dilution was subject to duplicate GC-FID FAMEs analysis. The FAMEs were prepared using a rapid, micro-FAMEs method [8], and FAMEs were analyzed by GC-FID by the method of Christie et al. [9]. One-way ANOVA was determined by JMP 9.0.2 software to observe any significant differences in the fatty acid content obtained by Folch and rapid hexane/isopropanol extraction methods.

Table 1 shows the comparison of total oil obtained by each extraction method from each yolk dilution. The data show that there is no significant difference between the extraction methods at any specific dilution. Furthermore, the correlation between the two extraction methods shows a correlation coefficient (R^2) of 0.997 with an intercept of 1.08. The fact that the regression line does not go through the origin may mean that hexane-isopropanol extracted more non-lipids than the Folch solvent. This could be due not including a water wash in the hexane–isopropanol extraction. However, this method does not claim to exclusively extract lipids, but that non-lipid extracted do not affect the data relative to lipid determination by the Folch method.

Table 2 shows fatty acid composition of lipid extractions from both the Folch method and rapid hexane/isopropanol extraction methods. No statistically significant differences were found in the levels of each fatty acid present in yolk lipids, which reflects the egg lipid profile previously reported [10].

In summary, we developed a rapid extraction method for egg lipids that is an effective alternative to the Folch method. This method also provides accurate fatty acid profiles when compared to those obtained using a Folch extraction and subsequent GC-FID FAMEs analysis.

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