Solvent Extraction of Lipid Components From Egg Yolk Solids

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The effects of solvent systems on extraction of lipid components from commercially spray-dried egg yolks were investigated. Hexane, hexane:isopropanol (2:1), and chloroform:methanol (2:1) were studied, and the effects of temperature of extraction and ratio of solvent to egg were examined in a 3 × 2 × 2 factorial design. Hexane removed more fat and phospholipid and less cholesterol and pigment than the more polar solvents. Increased solvent ratio extracted more of the lipid components, although temperature of the extraction had little effect on removal of the lipid components.

Consumption of eggs and egg products has been affected by concern about dietary cholesterol. Several low-cholesterol egg products are being marketed which contain egg white, whey solids and nonfat dried milk but little or no egg yolk because cholesterol is found only in the yolk portion of the egg.

Research has investigated techniques for extraction of the egg oil from fresh yolks (1,2). These techniques involve mixing the fresh yolks with a solvent, adjusting the pH and then centrifuging them at very high speeds to achieve a clear separation.

The lipids of fresh yolk are intimately associated with the lipoprotein and are not easily extractable with nonpolar solvents such as hexane. However, when egg yolk is spray dried, free fat from the highly emulsified lipoproteins is released, and a large percentage of the fat can be extracted (3). Melnick (4) extracted egg yolk solids with hexane in a simple method of slurrying solvent with solids and then filtering the mixture. The remaining solids were then used in a scrambled-egg product. Melnick (4) suggested that disruption of the lipid protein bonds by a mixture of polar and nonpolar solvents would provide a more complete removal of the lipid constituents.

The objective of this study was to investigate the effect of solvent systems on the extraction of pigment, fat, cholesterol and phosphorus from commercially spray-dried egg yolks.

MATERIALS AND METHODS

Commercially standard egg yolk solids were obtained from Monark Egg Corp. (Kansas City, Missouri) and stored at -18 C under nitrogen. The solids were allowed to adjust to room temperature before the extractions.

The study was statistically designed in a 3 × 2 × 2 factorial (3=solvent, 2=temperature, 2=solvent:egg ratio) with two randomized incomplete blocks per each of three replications.

Extraction. For each extraction, 150 ml of solvent [hexane, or hexane (HEX):isopropanol (ISO) (2:1 ratio) or chloroform (CHO):methanol (METH) (2:1)] was slurried with 50 g of yolk solids in a beaker on a magnetic stir plate for 30 min at either 23 C or 55 C. The slurry was filtered through Whatman #1 filter paper in a Buchner funnel with suction. The residue was rinsed with 50 ml of the solvent once (4:1 solvent:egg ratio) or twice (5:1 solvent:egg ratio). The residue was allowed to air dry overnight in a laboratory hood.

The air dried, "defatted" yolk solids were placed in plastic bags and stored at -18 C under nitrogen until analyzed. Because the CHO:METH and HEX:ISO defatted yolks were not as free flowing as the hexane defatted yolks, these samples were crushed with a mortar and pestle for pigment and lipid analysis.

Analyses: pigment. The pigment of the full-fat yolks and defatted yolks was determined using AOAC method 17.004 (5). A Varian Model 634S spectrophotometer was used to determine the absorbance. Concentration was expressed as μg β-carotene/100 g yolk; because a constant weight of sample was used, the data were converted to a fat-free, "as is" basis using the following formula:

$$\frac{\mu g\ \beta\text{-carotene}}{g\ yolk} \rightarrow \frac{\mu g\ \beta\text{-carotene}}{1-\frac{g\ oil}{g\ yolk\ (\text{"as is" basis})}} \rightarrow \frac{\mu g\ \beta\text{-carotene}}{g\ fat\text{-free yolk}\ (\text{"as is" basis})}$$

Analyses: fat. To determine lipid components, full-fat and defatted yolks were dried in a vacuum oven according to AOAC method 17.007 (5), and fat was extracted using a Goldfisch apparatus according to AOCS method BC 3-49 (6).

Percent fat on a fat-free, dry matter basis was calculated by using the following formula:

$$\frac{\text{wt fat extracted}}{\text{wt dry yolk-wt fat extracted}} \times 100 = \%\ \text{fat, fat-free, dry matter basis}$$

The amount of fat extracted by each solvent system was determined by the difference between percent fat, fat-free, dry matter basis in the full-fat yolks, and (residual) percent fat, fat-free, dry matter basis in the defatted yolks.

The extracted egg oil was quantitatively transferred and brought to volume in 100-ml volumetric flasks, with hexane. Samples of the extracted egg oil were removed for phosphorus and cholesterol determinations.

Analyses: phosphorus. Phospholipid-phosphorus was determined using a method developed by Bartlett (7), using KH_2PO_4 as the standard in concentrations of 1-10 μg phosphorus. The amount of phosphorus which was extracted by the solvent systems was determined by the difference between the phosphorus content in the full-fat yolks and the residual phosphorus in the defatted yolks, and was expressed as mg/100 g fat-free, dry matter yolk.

Analyses: cholesterol. Quantitation of cholesterol was achieved using a technique developed by Brown (8). A Beckman Model 322 liquid chromatograph with a 15-cm silica (Ultra sphere-Si, Beckman) column (5 μm diameter) was used. The mobile phase was 0.75% ISO/HEX with 2 ml/min flow rate. The detector was a variable wavelength spectrophotometer (Hitachi 155-00, 20 μl cell with one cm path length) at 210 nm. A

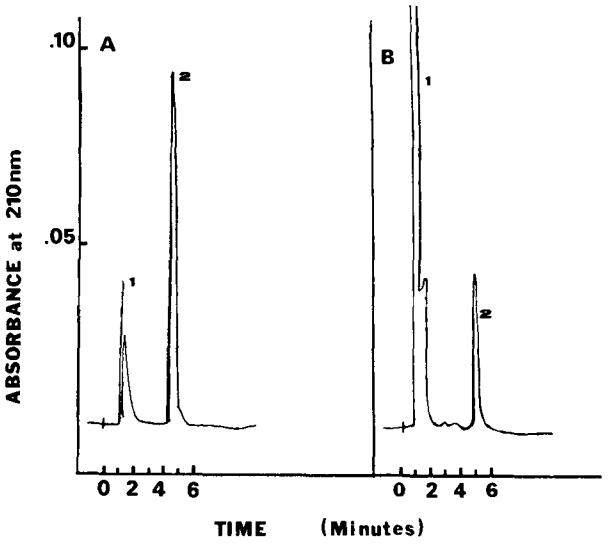

FIG. 1. HPLC chromatogram of 5 μg cholesterol (A) and egg oil containing 2.4 μg cholesterol (B). Column ultrasphere silica (15 cm × 4.6 nm); eluant: 0.75% ISO in HEX; flow rate 2 ml/min; peaks: (1) solvent and triglyceride; (2) cholesterol; μg cholesterol = area—under peak × (0.00002) + (0.16657).

20-μl sample injector loop was filled with the egg oil in hexane of known concentration. Area under chromatographic peaks was obtained using a CDS 111 electronic integrator. A standard curve of cholesterol (Nu-Chek Prep, Elysian, Minnesota) from 1-10 μg at the appropriate absorbance range was used for calculating concentration of cholesterol which was expressed as g/100 g yolk fat-free, dry matter basis. A typical chromatogram of the cholesterol and egg oil peak is found in Figure 1. Peak 2 is the cholesterol peak.

RESULTS AND DISCUSSION

The analysis of variance for the main and interactive effects is shown in Table 1. The solvent effect was significant for the extraction of fat, cholesterol, pigment ($P < 0.01$) and phosphorus ($P < 0.05$).

The amount of fat in the full-fat yolks and the residual after the solvent extraction are represented in Table 2. Full-fat yolks contained 94.0 g fat/100 g (±1.4 g) on a fat-free, dry matter basis. Hexane extracted 77.8 g/100 g of this fat (on a fat-free, dry matter basis). It is important to express the fat-soluble components being removed by the solvent treatments in this manner because fat comprised so much (47% "as is" basis) of the total weight of the full-fat yolks. Hexane removed more fat than CHO:METH (69.9 g) and HEX:ISO (69.8 g). Temperature did not affect the extraction of components. However, the solvent-temperature interaction was significant for the extraction of fat ($P < 0.01$). HEX:ISO extracted more fat than CHO:METH at the unheated temperature, while CHO:METH extracted more fat than HEX:ISO at the heated temperature, and there was no difference due to temperature for hexane.

Using HPLC to quantitate cholesterol, the full-fat yolks contained 5.93 g cholesterol (±0.25 g) per 100 g, fat-free, dry matter basis (Table 2). This corresponds to 2.95 g/100 g spray-dried egg yolk on an "as is" basis. Posati and Orr (9) reported that the average cholesterol content for dried yolks was 2.928 g/100 g "as is". Hexane extracted 3.9 g cholesterol from the yolks while CHO:METH extracted 4.4 g and HEX:ISO extracted 4.3 g. Although the hexane extracted more fat, CHO:METH and HEX:ISO extracted more cholesterol from the egg yolks.

Yolk lipid is made up of triglycerides, phospholipids and cholesterol in a lipoprotein matrix. The full-fat dry yolks contained 370 mg phosphorus, i.e., phospholipid phosphorus, per 100 g yolk (±15 mg) (Table 2). The extraction of phosphorus paralleled the extraction of fat. Hexane extracted more phosphorus than CHO:METH and HEX:ISO (347 mg vs 332 mg and 338 mg, respectively). There was no difference between the more polar solvents (LSD = 9.0). Some of the functional properties of yolks may be due to phospholipid. However, it is possible that phospholipid is not the factor controlling functional dissolution properties. We observed that hexane-extracted yolks were more easily dissolved in water than those defatted

TABLE 1

Analysis of Variance: Significance of Independent Variables

Independent variables		Dependent variables			
Source	DF	Fat	Cholesterol	Phosphorus	Pigment
Solvent	2	**	**	*	**
Rinse	1	**	*	**	**
Temperature	1	—	—	—	—
Solvent-temp interaction	2	**	—	—	—
Solvent-rinse interaction	2	—	—	—	—
Temp-rinse interaction	1	—	—	—	—
Solvent-temp-rinse interaction	2	—	—	—	—
Error	19				

*, $p < 0.05$.

**, $p < 0.01$.

—, not significant.

TABLE 2

Lipid Composition of Yolks

	Full fat yolks	Solvent defatted yolks		
		Hexane	CHO:METH	HEX:ISO
Fat (g/100g)[a]	94.0	16.2	24.1	24.2
Cholesterol (g/100 g)[a]	5.93	2.0	1.5	1.6
Phosphorus (mg/100 g)[a]	370.0	23.0	38.0	32.0
β-Carotene (μg/100 g)[b]	510.0	285.0	164.0	160.0

[a]Data expressed on a fat-free, dry matter basis.
[b]Data expressed on an "as is" basis.

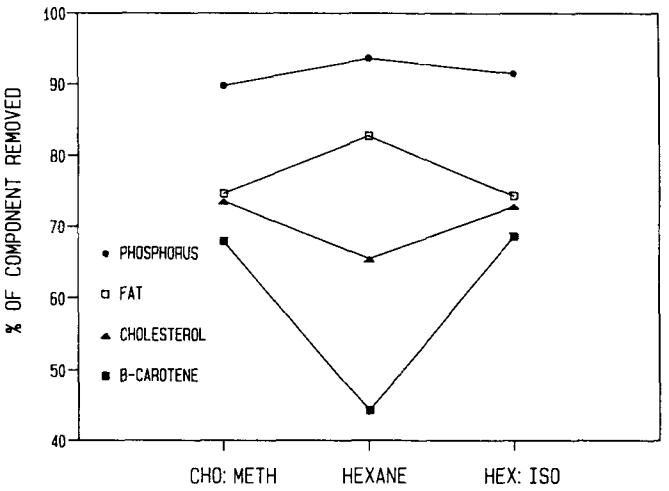

FIG. 2. Effect of solvent on the extraction of lipid components.

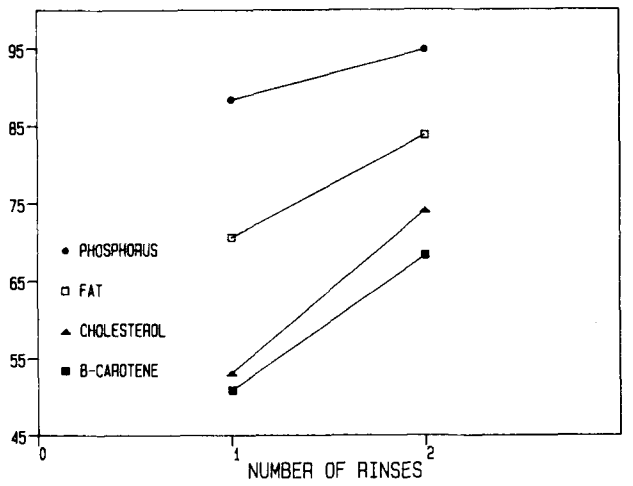

FIG. 3. Effect of solvent to egg ratio on the extraction of lipid components.

with the more polar solvents (which had more phospholipid).

The pigment content and residual pigment content of the yolks are represented in Table 2. The full-fat yolks contained 510 μg β-carotene/100 g. The extraction of pigment paralleled the extraction of cholesterol. CHO:METH and HEX:ISO extracted more pigment than hexane (346 μg and 350 μg vs 225 μg, respectively). Again there was no difference between the amount of pigment extracted by CHO:METH and HEX:ISO.

It is generally thought that cholesterol is extracted with the fat because both are nonpolar lipid components and the pigment is also associated with the nonpolar lipids. Phospholipids are polar in nature and should be extracted to a greater extent with more polar solvents, i.e., hexane with isopropanol and chloroform with methanol. However, these data show that nonpolar lipids and phospholipid phosphorus were extracted in a similar pattern, and cholesterol and pigment were extracted in a similar manner (Fig. 2).

There was a significant effect on the extraction of the fat, phosphorus and pigment at $P<0.01$ and cholesterol at $P<0.05$ due to the number of rinses. However, the interactive effect of rinse-solvent was not significant. An increase of solvent to yolk ratio, by doubling the number of times the residue was rinsed with the solvent, extracted more of the lipid components from the yolks (Fig. 3). A second rinse removed 13.3% more fat, 21.1% more cholesterol, 6.5% more phosphorus and 17.4% more pigment. Because a yellow layer was visible on top of the residue during filtration, perhaps the second rinse did not "extract" more, but "rinsed" the extracted components from the surface of the yolks.

It is desirable to extract cholesterol and fat with minimal changes in the color and functional properties of the yolks. Although the HEX:ISO and CHO:METH extracted more of the cholesterol, the hexane-extracted yolks retained more pigment, were easier to dissolve in water, and were more freeflowing. A study was undertaken concurrently to see how the hexane-extracted yolks functioned in cakes (11). Using yolk solids defatted with hexane in place of full fat yolks resulted in no difference in tenderness, flavor and volume of cakes and only a small color difference. Yet, practical nutritional benefit would result because one egg made with these hexane-extracted yolk solids would contain only 51 mg of cholesterol while a fresh egg contains 245 mg of cholesterol.

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