Interfacial Behavior of Egg Yolk with Reduced Cholesterol Content

Adamantini Paraskevopoulou,[†] Vassilis Kiosseoglou,*[,†] and Sofia Pegiadou[‡]

Laboratories of Food Chemistry and Technology and of Organic Chemical Technology, Faculty of Chemistry, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece

The interfacial activity of low-cholesterol yolk protein concentrates, prepared by treating spray-dried yolk with various extraction media, was investigated using the Wilhelmy plate method. The rate of interfacial pressure increase and the equilibrium value reached after 4 h of adsorption were influenced by the method of extraction applied, indicating that, in addition to composition changes, structural alterations in yolk protein structure brought about by the extraction determine to some extent their adsorption behavior. The presence of surface-active constituents, i.e., monoglycerides, diglycerides, and phospholipids, in vegetable oil also affected the interfacial pressure value of adsorbed yolk films, possibly the result of interactions between the yolk and the oil surface-adsorbing molecules at the oil−water interface.

Keywords: *Egg yolk; cholesterol; interfacial pressure; adsorption*

INTRODUCTION

Many food products appear in the form of oil-in-water (o/w) emulsions where the oil droplets are usually stabilized by protein molecules which, either alone or in collaboration with other surface-active food components of low molecular weight, adsorb and form a strong and cohesive interfacial film that may resist tensile or shearing stresses and protect the oil droplets from coalescence (Dickinson and Stainsby, 1982). An important step in the formation and stabilization of protein-based emulsions is the adsorption and unfolding/spreading of protein molecules at the oil−water interface. The interfacial behavior of proteins is determined by their physicochemical and structural characteristics, i.e., molecular size; shape and flexibility; charge and surface hydrophobicity; secondary, tertiary, and quaternary structure; and interactions with other food constituents (Kinsella, 1976; Halling, 1981; Dickinson et al., 1988).

Egg yolk constitutes an important ingredient of products such as mayonnaise, salad dressings, cakes, and creams and is a very effective emulsifier owing its unique emulsification properties to the existence of a phospholipid−protein complex (Kiosseoglou, 1989). A problem, however, associated with the yolk is that its high cholesterol content has been connected in the consumers' mind as a causative agent for heart disease. Removal of cholesterol from yolk by extracting with organic solvents (Larsen and Froning, 1981; Paraskevopoulou and Kiosseoglou, 1994, 1995) or with supercritical CO_2 (Froning et al., 1990; Paraskevopoulou et al., 1997) could be a solution to the problem provided that the yolk's functional properties, especially its unique emulsifying activity, are retained. Paraskevopoulou and Kiosseoglou (1994) observed that extraction of yolk with petroleum ether resulted in a yolk protein concentrate with emulsifying properties comparable to those of yolk while a significant deterioration was observed when the yolk was extracted with a mixture of petroleum ether and ethanol. Extraction with supercritical CO_2 resulted in yolk protein concentrates with emulsification and emulsion stabilization properties compared to egg yolk (Paraskevopoulou et al., 1997). Froning et al. (1990) found some increase in functional properties and then a loss as the level of supercritical extraction increased. Considering that the above treatments may alter the structure of yolk lipoproteins, and since yolk's lipoprotein structure changes in turn influence their interfacial behavior (Kiosseoglou and Sherman, 1983a,b), this study was conducted to investigate the adsorption behavior of yolk extracted with various solvent media to determine how the yolk's emulsifying properties may be affected by the extraction systems.

MATERIALS AND METHODS

Materials. Spray-dried egg yolk was obtained from Sigma Chemical Co. (St. Louis, MO). Polyoxyethylene sorbitan monooleate (Polysorbate 80) was bought from Fluka AG (Buchs, Switzerland) while petroleum ether and absolute ethanol of analytical grade were provided by Riedel-de-Häen (Seelze, Germany). Cholesterol and 5α-cholestane standards for egg yolk cholesterol determination were obtained from Sigma Chemical Co. Corn oil used for the preparation of o/w emulsions was commercially available. It was used either as "natural corn oil" or as triglycerides following removal of the more polar surface-active components (sterols, free fatty acids, monoglycerides, diglycerides, phospholipids) by passing 250 g through a glass column (30 × 3 cm) packed with 100 g of silica gel, which was previously activated at 200 °C for 10 h (Min and Mistry, 1987). The lack of oil surface-active components (free fatty acids, mono- and diglycerides, phospholipids, etc.) was then checked with thin-layer chromatography (TLC) employing a hexane/diethyl ether/formic acid (80:20:2) mixture as the developing medium (Christie, 1982). The resulting oil was called "purified corn oil".

Cholesterol Extraction. Extraction of cholesterol from spray-dried yolk (50 g) was effected by suspending the yolk in 500 mL of petroleum ether (PE) or a mixture of petroleum ether/ethanol (PE/E; 35:65 v/v) and stirring continuously for 3 h with the aid of a propeller-type mechanical stirrer. Following filtration of the yolk−solvent mixture, the yolk residue was washed with 200 mL of solvent and dried for 6 h at 50−55 °C in a vacuum oven (Paraskevopoulou and Kiosseoglou, 1994).

Yolk protein concentrate with reduced cholesterol content was also prepared by mixing the yolk with an ethanol/water mixture (E/W; 20:80 v/v) containing 1.5% (w/v) polysorbate 80 and homogenizing with an Ultra-Turrax T25 homogenizer (IKA Instruments, Staufen in Breigau, Germany), equipped with a S25KG-25F dispersing tool at 8500 rpm for 10 min. The homogenized yolk dispersion was centrifuged at 3000*g* for

[†] Laboratory of Food Chemistry and Technology.
[‡] Laboratory of Organic Chemical Technology.

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1 h, and the yolk precipitate was washed twice with ethanol/water (20:80, v/v), collected, and freeze-dried (Paraskevopoulou and Kiosseoglou, 1995).

Supercritical CO_2 (SC) extraction was effected using a Gilson (Gilson Medical Electronics Inc., Middleton, WJ) SF3 supercritical fluid chromatography apparatus, especially modified by adjusting a 10 cm long × 14 mm i.d. extraction tube (Keystone Scientific Inc., Bellefonte, PA) for use in the supercritical fluid extraction mode. The supercritical CO_2 was passed through the yolk (15 g) at 35 °C and 310 atm, and the flow was continued until 50 g of CO_2/g of sample had been passed through the extractor.

Chemical Analysis. Total solids content was determined by the vacuum oven method at 98−100 °C (AOAC, 1975). Total lipid content was determined by extracting with chloroform/methanol (AOAC, 1975) and protein content by applying the Kjeldahl method (Pearson, 1976). Cholesterol was determined by applying gas−liquid chromatography (Beyer et al., 1989) on the total lipid extract obtained by chloroform/methanol extraction (Paraskevopoulou and Kiosseoglou, 1995). Lipid phosphorus was quantified by applying the IUPAC method (1987) on the total lipid extracted with chloroform/methanol.

Determination of Adsorbed and Precipitated Protein in Emulsions. In order to determine the amount of protein adsorbed per unit oil droplet surface area, o/w emulsions were prepared by dispersing 30 mL of corn oil into 70 mL of 10% (w/v) yolk or yolk protein concentrate suspension in a citrate buffer (0.02 N disodium hydrogen citrate, 0.2 M NaCl) at pH 3.8 with the aid of a mechanical stirrer. The crude emulsions were then homogenized for 1 min using the Ultra-Turrax homogenizer equipped with a S25KG-25F dispersing tool and operating at 9500 rpm. Following storage at 5 °C for 24 h, the emulsions were centrifuged for 10 min at 2000*g* in a Heraeus Model VJ2 centrifuge (Heraeus Christ, Instruments, Hanau, Germany). The cream was collected, washed twice with 150 mL of the buffer, and finally collected by centrifugation and frozen at −15 °C for 24 h. The precipitated yolk fraction of the emulsion was also collected. The frozen cream was then thawed at 50 °C and centrifuged for 60 min at 3000 rpm to break the emulsion. The separated oil was discarded and the water phase collected. This process was repeated three more times, and the four water phases were combined together, lyophilized, and used for protein determination. The precipitated yolk fractions of the emulsions were also combined, lyophilized, and analyzed for protein.

The average surface area S, expressed in square meters per milliliter of oil, was derived by using the equation (Walstra, 1983)

$$S = 6/d_{vs} \quad (1)$$

where d_{vs} is the mean volume-surface diameter given by the equation

$$d_{vs} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (2)$$

where n_i is the number of droplets with diameter d_i determined by applying the microscope method (Mita et al., 1974) on an emulsion sample diluted with a glycerine solution (50% v/v).

Interfacial Pressure Determination. Interfacial pressure changes with time at the o/w interfaces were calculated from interfacial tension measurements conducted by applying the Wilhelmy plate method using a Sigma 70 tensiometer (KSV Instruments Ltd., Helsinki, Finland) connected to an Altec 386 SXL computer which aided in the continuous recording of the interfacial tension with time. The tensiometer was operated in the "manual run" mode. The platinum ring used in the experiments and the sample vessel were very carefully cleaned at the end of each run with perchromic acid and flamed in a bunsen burner to remove organic impurities. Dispersions of 1 or 0.1% (w/v) yolk or yolk protein concentrate were prepared by adding the dehydrated samples in a citrate buffer (0.02 N disodium hydrogen citrate, 0.2 M NaCl) at pH 3.8 while mixing with a mechanical stirrer. A lower level of yolk or yolk protein concentrate content was used in this experiment compared to that used in the case of emulsions (10%) since the total o/w intefacial area of the emulsions is considerably higher and more emulsifier was required for complete surface coverage. Following zero adjustment of the balance while the plate was completely immersed in oil (lighter phase), the sample vessel was raised until the plate touched the water surface and the position (target position) noted. The plate was then immersed about 2 mm in the water phase, the oil was carefully poured on the surface, and the plate was brought again to target position. The first measurement was taken 30 s after the layering of the oil, and the tension was monitored until it reached a near-steady value. All the surface tension measurements were conducted three or four times, and the widest range between the replicates found was not greater than 0.4%. The experiments were conducted at 25 °C.

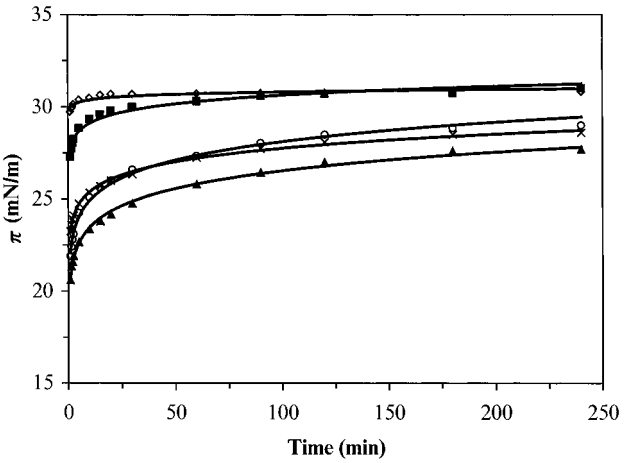

Figure 1. Rate of development of interfacial pressure of egg yolk and yolk protein concentrate films at the natural corn oil−water interface. Yolk solids content is 1% (w/v). Key: ◇, spray-dried yolk; ■, yolk extracted with petroleum ether; ▲, yolk extracted with 35:65 (v/v) petroleum ether/ethanol; ×, yolk extracted with 20:80 (v/v) ethanol/water containing 1.5% (w/v) polysorbate 80; ○, yolk extracted with supercritical CO_2.

The interfacial pressure (π) was calculated from $\pi = \gamma_0 - \gamma_t$, where γ_0 and γ_t are the interfacial tensions of the buffer (72.0 mN/m) and the protein dispersion at time t at the o/w interface, respectively.

RESULTS AND DISCUSSION

The change in interfacial pressure during the adsorption from a 1% (w/v) yolk (control) or yolk protein concentrate dispersion at a natural corn oil−water interface is shown in Figure 1. All the points represent mean values of at least three experiments with a standard deviation not exceeding ±0.2. It is apparent that, in all the samples, with the exception of the control, near-steady-state values were reached after an adsorption period of 4 h. Both the rate of increase of interfacial pressure and its steady-state value were higher for the yolk and the PE yolk protein concentrate compared to the rest of the concentrates with PE/E concentrate having the lowest steady-state value. When the yolk or yolk protein concentrate content of the water phase was decreased to 0.1% (w/v), a lower rate of interfacial pressure development was observed (Figure 2) in the cases of SD yolk and PE protein concentrate while the steady-state pressure values for all the samples were similar to those of Figure 1. Following purification of corn oil by column chromatography, a marked reduction in both the rate of interfacial pressure development and the steady-state value was noticed for all the samples studied (Figure 3).

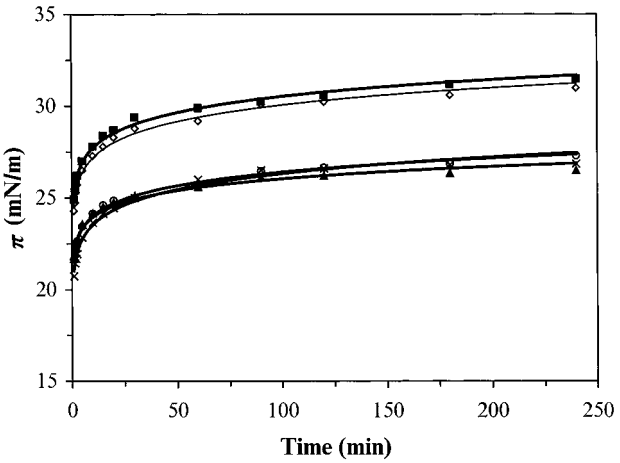

Figure 2. Rate of development of interfacial pressure of egg yolk and yolk protein concentrate films at the natural corn oil−water interface. Yolk solids content is 0.1% (w/v). Key: ◇, spray-dried yolk; ■, yolk extracted with petroleum ether; ▲, yolk extracted with 35:65 (v/v) petroleum ether/ethanol; ×, yolk extracted with 20:80 (v/v) ethanol/water containing 1.5% (w/v) polysorbate 80; ○ yolk extracted with supercritical CO_2.

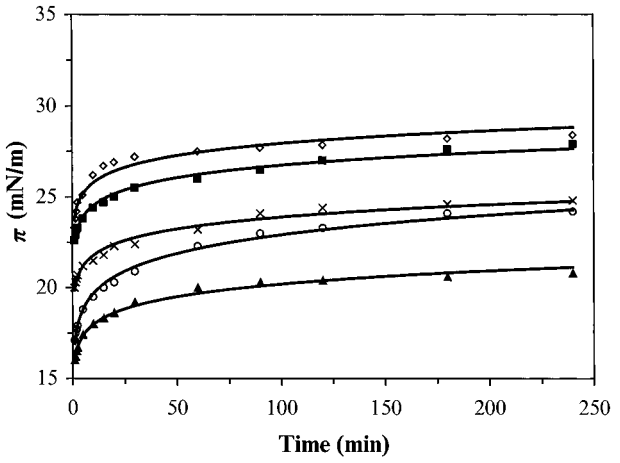

Figure 3. Rate of development of interfacial pressure of egg yolk and yolk protein concentrate films at the purified corn oil−water interface. Yolk solids content is 1% (w/v). Key: ◇, spray-dried yolk; ■, yolk extracted with petroleum ether; ▲, yolk extracted with 35:65 (v/v) petroleum ether/ethanol; × yolk extracted with 20:80 (v/v) ethanol/water containing 1.5% (w/v) polysorbate 80; ○, yolk extracted with supercritical CO_2.

The process of adsorption of protein molecules at an interface and the associated pressure increase can be divided into three likely stages: (a) diffusion of native protein molecules to the interface, (b) penetration of protein molecules into the interface and their unfolding, and (c) rearrangement of the adsorbed, denatured molecules to a state of minimum free energy (Graham and Phillips, 1979). Egg yolk, however, has a very complex composition. It consists of flexible lipoproteins, globular proteins called livetins, phospholipids, cholesterol, and neutral lipids. While livetins are water-soluble and found in yolk serum, the rest of the components are organized in the form of high-density (HD) granules and low-density (LD) micelles (Kiosseoglou, 1989). Thus, a variety of molecules can be found in yolk that differ in structure, physical properties, and surface activity. Additionally, commercial spray-dried yolk may be contaminated by up to 20% egg white (Powrie and Nakai, 1985). Despite the complexity in composition and structure, the adsorption process of yolk can be analyzed in terms of the previous theory if one takes into account that its globular proteins (livetins and accompanying egg white proteins) exhibit a very limited adsorption ability in the presence of the more readily adsorbing lipoproteins (Camejo et al., 1968). The later have a more flexible structure compared to the water-soluble proteins due to the absence of disulfide bridges. Shenton (1979) reported that only 20% of the livetins and no egg white proteins contributed to the interfacial membrane of artificial creams stabilized by egg and milk proteins. The interfacial film, therefore, in the present experiments can be visualized as a mixed film made up of low molecular weight surface-active yolk components (phospholipids and cholesterol) and low- and high-density lipoprotein molecules which can penetrate and rearrange at the interface even under high surface pressure conditions due to their structure flexibility. Additionally, surface-active components from oil will also be found in the film since the use of purified corn oil in the place of natural resulted in a marked decrease in the steady-state values of interfacial pressure (Figures 1 and 3). The presence of such surface-active oil components was verified by tension experiments of clean oil−water interfaces. The interfacial tension values at the natural corn oil−water and purified corn oil−water interfaces were 32 and 25 mN/m, respectively. According to Gaonkar (1989), the surface activity of commercial oil−water interfaces is due to the adsorption of the more surface-active monoglycerides and to a lesser extent to other oil constituents such as diglycerides and free fatty acids. The low molecular weight surface-active components of yolk and oil adsorb faster than yolk proteins which when they arrive at the interface must, in order to adsorb, penetrate and rearrange in the film against an already developed interfacial pressure. When the yolk content of the water phase is very low, the initial stages of yolk protein adsorption are diffusion controlled (Kiosseoglou and Sherman, 1983a). At relatively higher yolk contents, however, penetration, unfolding, and rearrangement of the adsorbed protein molecules determine the development of interfacial pressure with time. The adsorption behavior of proteins is greatly influenced, among other parameters, by their structural and conformational characteristics. The interfacial activity of yolk is influenced by factors that affect the structural organization of high- and low-density lipoprotein structures (Kiosseoglou and Sherman, 1983a). Yolk treatment, therefore, by various lipid-extracting media will result in compositional changes can affect the structural properties of the yolk concentrate. Table 1 presents the composition of yolk protein concentrates prepared as described above. All the concentrates had a higher protein content compared to the control yolk. Their lipid content, however, was markedly reduced while differences may be observed in their cholesterol and phospholipids (as expressed by lipid phosphorus) content.

Graham and Phillips (1979) stressed that the primary layer of molecules is to a great extent responsible for determining the interfacial pressure, and therefore, the formation of multilayers during adsorption should only have a negligible effect on pressure. The apparent steady-state values reached, following adsorption from a 0.1% (w/v) yolk dispersion (Figure 2), were not markedly lower than those reached in the case of 1.0% yolk dispersion (Figure 1), indicating that an adsorbed monolayer may form even when the yolk content was as low as 0.1%. Differences in interfacial tension between the samples cannot, therefore, be attributed to differences in yolk solubility since there is enough

Table 1. Composition of Spray-Dried Yolk and of Yolk Protein Concentrates

yolk sample	total lipids[a] (%)	cholesterol (mg/g)	lipid phosphorus (mg/g)	protein (%)
spray-dried yolk	60.0 ± 0.5[b]	18.8 ± 0.4	4.9 ± 0.2	38.2 ± 0.1
extracted with petroleum ether	24.5 ± 0.7	5.0 ± 0.2	4.1 ± 0.2	72.0 ± 0.6
extracted with petroleum ether/ethanol (35:65)	6.9 ± 0.4	<1.0	1.1 ± 0.1	88.5 ± 0.6
extracted with 20:80 ethanol/water (1.5% w/v polysorbate 80)	32.2 ± 0.9	7.1 ± 0.3	3.2 ± 0.1	63.2 ± 0.8
extracted with supercritical CO_2	33.9 ± 1.0	4.3 ± 0.2	5.9 ± 0.1	64.0 ± 0.6

[a] Based on dry matter. [b] Mean values ± standard deviation of three determinations.

Table 2. Amount of Protein Adsorbed per Unit Surface Area (Γ_α) and Percentage of Protein Precipitated (Γ_p) in o/w Emulsions Stabilized by Yolk and Yolk Protein Concentrate

yolk sample	D_{vs} (μm)	Γ_α (mg/m^2)	Γ_p (%)
spray-dried yolk	25.0 ± 0.5[a]	7.5 ± 0.30	27.1 ± 1.0
extracted with petroleum ether	26.1 ± 0.3	10.2 ± 0.40	34.6 ± 2.1
extracted with petroleum ether/ethanol (35:65)	24.4 ± 0.4	7.2 ± 0.35	43.6 ± 1.5
extracted with 20:80 ethanol/water (1.5% w/v polysorbate 80)	23.7 ± 0.3	7.0 ± 0.50	39.4 ± 1.7
extracted with supercritical CO_2	25.9 ± 0.2	9.9 ± 0.15	30.5 ± 1.3

[a] Mean values ± standard deviation of three experiments.

soluble yolk material in all the cases for the formation of at least a monomolecular film. As can be concluded from the results of Table 2, the percentage of yolk protein precipitated in emulsions differed among the samples but enough protein remained soluble to cover the oil droplets and give surface coverages ranging between 7.0 and 10.2 mg of protein/m^2 of the interface. Differences in phospholipid content may be partly responsible for the different interfacial tension values reached by the various yolk samples, and in fact, PE/E yolk protein concentrate gave the lowest steady-state pressure values, possibly as a result of its very low phospholipids content. This parameter, however, cannot be the sole determinant of interfacial pressure since the concentrate prepared by extracting the yolk with supercritical CO_2 (SC), in spite of its high phospholipid content, resulted in lower pressure values than SD yolk, PE, and E/W protein concentrates. In order to investigate the influence of yolk protein concentrate structure, the results were analyzed by applying the first-order equation (Graham and Phillips, 1979)

$$\ln\left(\frac{\pi_{ss} - \pi_t}{\pi_{ss} - \pi_0}\right) = -\frac{t}{\tau} \qquad (3)$$

where π_{ss}, π_0, and π_t are the interfacial pressure values at steady-state, at time $t = 0$ and at time t, respectively, and τ is the relaxation time ($\tau = K^{-1}$, rate constant).

An example of the application of eq 3 to the results of Figure 1 is given in Figure 4. All the yolk samples exhibited curvilinear first-order plots. Waniska and Kinsella (1985) and Song and Damodaran (1987) also reported curvilinear plots for β-lactoglobulin and structurally modified bovine serum albumin, respectively, following adsorption at air−water surface. These curves were resolved into two first-order kinetic phases. The first one, having rate constant K_1 and occurring in the first few minutes, corresponds to initial adsorption, penetration, and unfolding of protein molecules, while the second kinetic phase, having rate constant K_2, is related to rearrangement of the adsorbed molecules to a state of minimum free energy. The first-order rate

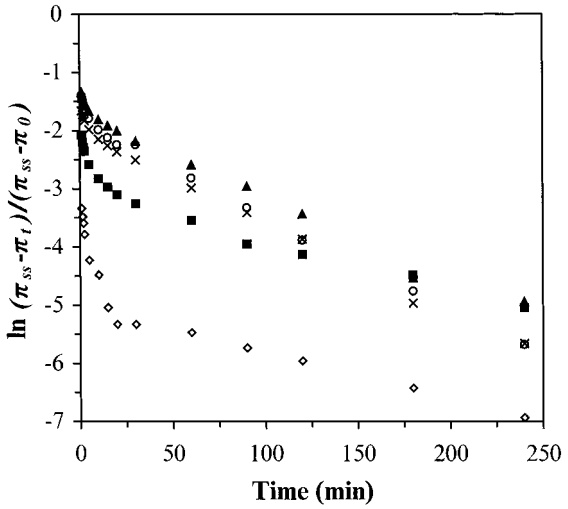

Figure 4. Rate of change of interfacial pressure ln [$\pi_{ss} - \pi_t$/$\pi_{ss} - \pi_0$] with time of egg yolk and yolk protein concentrate films at the natural corn oil−water interface. Yolk solids content of water phase is 1% (w/v). Key: ◇, spray-dried yolk; ■, yolk extracted with petroleum ether; ▲, yolk extracted with 35:65 (v/v) petroleum ether/ethanol; ×, yolk extracted with 20:80 (v/v) ethanol/water containing 1.5% (w/v) polysorbate 80; ○ yolk extracted with supercritical CO_2.

constant, K_1, values in the present experiments were not calculated since more points at shorter adsorption times would have been needed for that. It can, however, be inferred from the shapes of the plots that initial penetration and protein unfolding rates were higher in the cases of SD yolk and PE yolk protein concentrate. The same was observed when purified corn oil was used in place of natural oil (Figure 3). The rate constant, K_2, values, corresponding to molecular rearrangements and the associated relaxation times, τ_2, were calculated from the slopes of the linear parts of the graphs (80−240 min) and are presented in Table 3. In contrast to penetration behavior, lower first-order rate constant values and higher relaxation times can now be observed in the cases of SD and PE protein concentrate. According to Graham and Phillips (1979), τ_2 should depend on the extent of molecular interactions in the interfacial film and its value will be correlated with the incompressibility of the film, with higher τ_2 values occurring in highly incompressible films. The faster initial adsorption and unfolding of the protein molecules of the PE yolk protein concentrate, compared to the other concentrates, is an indication that spray-dried yolk structure is altered to a lesser degree than when yolk is treated with the tested extraction media. Such treatments may lead to molecular disorganization and aggregation, which in turn will influence their adsorption properties. Film incompressibility, then, will be higher in the cases of faster adsorbing SD yolk and PE yolk protein concentrate, as can be concluded from the longer relaxation times observed during molecular rearrangement to a state of minimum free energy.

Table 3. Rate Constants (K_2), Relaxation Times (τ_2), and Interfacial Area Cleared (ΔA_2) during Yolk Protein Adsorption (ΔA_1) and Rearrangement (ΔA_2) at the Corn Oil−Water Interface

	natural corn oil				purified corn oil			
yolk sample	$K_2 \times 10^3$ (min^{-1})	τ_2 (min)	ΔA_1 ($Å^2$)	ΔA_2 ($Å^2$)	$K_2 \times 10^3$ (min^{-1})	τ_2 (min)	ΔA_1 ($Å^2$)	ΔA_2 (Å)
spray-dried yolk	7.5	134	447.3	1227.6	7.3	137	95.9	374.8
extracted with petroleum ether	7.9	127	230.5	1139.8	8.4	119	103.6	312.7
extracted with petroleum ether/ethanol (35:65)	12.5	80	87.1	475.1	11.0	90	107.7	332.8
extracted with 20:80 ethanol/water (1.5% w/v polysorbate 80)	14.0	71	82.1	555.8	15.0	67	112.4	456.2
extracted with supercritical CO_2	15.0	67	111.2	307.9	15.1	66	76.5	418.4

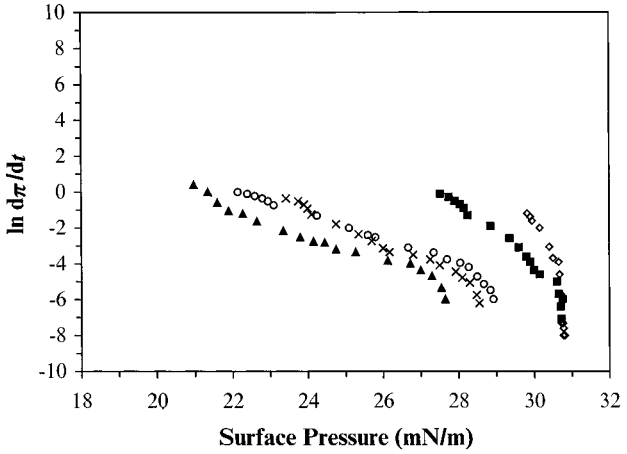

Figure 5. Ln(dπ/dt) as a function of π for yolk and yolk protein concentrates adsorbing at the natural corn oil−water interface. Yolk solids content of water phase is 1% (w/v). Key: ◇, spray-dried yolk; ■, yolk extracted with petroleum ether; ▲, yolk extracted with 35:65 (v/v) petroleum ether/ethanol; × yolk extracted with 20:80 (v/v) ethanol/water containing 1.5% (w/v) polysorbate 80; ○, yolk extracted with supercritical CO_2.

The areas cleared during molecular penetration and rearrangement were derived by applying the equation (Song and Damodaran, 1987):

$$\ln \frac{d\pi}{dt} = \ln \frac{KC_0}{d\Gamma/d\pi} - \frac{\pi \Delta A}{kT} \qquad (4)$$

where K is the rate constant of adsorption, C_0 is the subphase concentration, k is the Boltzmann constant, Γ is the amount of protein at the interface, and T is the absolute temperature. By plotting ln(dπ/dt) against π, the area cleared during protein adsorption can be calculated from the slope of the linear parts of the plots.

The ln(dπ/dt) against π plots, derived by analyzing the results of Figure 1 (natural corn oil) by linear regression analysis, were nonlinear for all samples but the curves could be divided into two linear parts (Figure 5). The correlation coefficients for the linear parts varied from 0.93 to 0.99. Similar graphs were obtained by other investigators for various proteins (Waniska and Kinsella, 1985; Song and Damodaran, 1987; Tornberg, 1978; Tornberg et al., 1982). According to Song and Damodaran (1987) the first linear region should basically correspond to the first kinetic phase of adsorption while the second should correspond to the kinetic phase of molecular rearrangement at the interface. By analyzing the plots, two ΔA values, i.e., ΔA_1 and ΔA_2, were obtained which are presented in Table 3 for both the natural and the purified corn oils. In general, higher ΔA values were observed compared to those reported by other investigators for various proteins studied (Waniska and Kinsella, 1985; Tornberg, 1978; Tornberg et al., 1982), probably as a result of the higher surface penetration power of egg yolk lipoproteins. In the case of natural oil, higher ΔA_1 and ΔA_2 values were obtained for the SD yolk and the PE yolk protein concentrate compared to the rest of the concentrates. This could be attributed to the presence at the interface of surface-active constituents from oil, resulting in more condensed films where they interact with the adsorbed yolk protein molecules, which may clear a greater surface area during penetration and molecular rearrangement. Proteins from the rest of the concentrates, on the other hand, exhibit a lower ability to interact with corn oil constituents at the interface due to structural alterations as a result of the cholesterol extraction process and, therefore, clear a smaller area when adsorbed and rearranged at the interface. In the case of purified corn oil, no significant differences between the samples could be observed in the ΔA_1 or the ΔA_2 values. The presence of the surface-active oil contituent is, therefore, very important in determining the behavior of yolk proteins during adsorption at oil−water interfaces. Kiosseoglou (1992) observed that minor surface-active lipids of olive oil can interact with yolk proteins at oil−water interfaces and confer viscoelasticity to the adsorbed film.

These data indicate that the adsorption behavior of low-cholesterol yolk is markedly affected by the method of lipid extraction applied, reflecting the significance of yolk protein interactions for the formation of the interfacial film network structure and the stabilization of the resulting o/w emulsions.

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