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Fatty Acid Profiles of Processed Chicken Egg Yolks

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The fatty acid compositions of egg yolks subjected to industrial processing treatments, namely, homogenization, pasteurization, drying, and "omega-3-enrichment", were studied. In general, the total contents of C16:0, C18:0, C18:1 n-9, and C18:2 n-6 fatty acids accounted for close to 90% of the total fatty acids. Statistical analysis of the data revealed correlations among the fatty acids; significant differences existed depending on the egg source and type of processing. Yolk samples enriched with omega-3 fatty acids clustered together owing to their higher C16:0, C16:1 n-7, C18:3 n-6, and C24:0 contents. Nonpasteurized/non-heat-treated samples formed another cluster because of their higher C18:1 n-11 and C18:1 n-9 contents, and the remaining samples formed another group due to their higher proportions of C18:0, C18:2 n-6, and C20:4 n-6. The relative proportions of essential fatty acids were similar in the four types of samples examined.

KEYWORDS: Yolks; eggs; fatty acids; chemometric

INTRODUCTION

Chicken eggs are considered to be essential components of a balanced diet. Each European consumes some 252 eggs per year, and estimates for North Americans are 320 eggs per year. Their protein composition, including all essential amino acids, energy supply, and fatty acid, and vitamin and mineral contents make chicken eggs a staple high-protein food. Egg proteins with their balanced amino acids are of high biological value; thus, egg proteins are used as "reference proteins" for studies dealing with protein intake. Egg proteins also possess a series of interesting properties (solidification through heat, an ability for stabilization, emulsification, and foam formation, among others) that have led to their use in the industrial manufacturing of several food products (*1*).

Notwithstanding, since 1970 consumers in many countries have limited their egg intake because of adverse publicity about saturated fats and cholesterol. Today, we know more than ever before about the relationships among diet, lifestyle, and good health. There is a growing body of evidence that diet and health are inter-related, and these are a function of both what is in the diet and what is missing from it.

Several studies have shown that the chemical composition of eggs is variable and depends on the breeding system and the composition of the diet of the hens (*2*). Eggs contain 10.8% fat, almost entirely in the yolk; the egg white, or albumen, contains <0.05% fat. Stadelman and Pratt (*3*) reported that the lipid content of chicken eggs is linked to genetics, age, feeding program, and also to the level and type of dietary lipids. Several studies have also established a relationship between the polyunsaturated (n-3, n-6) fatty acid intake of hens and the fatty acid composition of their eggs (*4*−*6*). Moreover, it may even be possible to modify the cholesterol (*7*, *8*) and vitamin (*9*) composition of eggs.

Other compositional variations may also arise due to processing, as eggs are not always consumed fresh. Thus, eggs may be eaten pasteurized, frozen, or dried as well as in many manufactured products such as mayonnaise and enriched pasta, among others.

The aim of the present study was to examine the effect of several processing treatments on the fatty acid profiles of eggs from different farms and to identify possible significant differences in the final products that could have consequences for factors related to health.

MATERIALS AND METHODS

Reagents. A 150.0 mL chloroform/methanol (2:1, v/v) solution containing 0,1 g of the antioxidant 2,6-di-*tert*-butyl-4-methylphenol (BHT) was prepared using trichloromethane stabilized with ethanol (0.5%) (ACS-ISO) and methanol (HPLC grade). $MgCl_2 \cdot 6H_2O$ was used to prepare a 0.017% $MgCl_2$ solution. Boron trifluoride was obtained from Prolabo-Merck (Barcelona, Spain) (synthetic grade).

Equipment. An Agilent Technologies 6890N Network GC system chromatograph was used under the following conditions: a splitless mode automated injector, a 220 °C constant analysis temperature, and automatically controlled pressure. A capillary column Varian model CP8822, 260 °C maximum, 30 m × 250 μm × 0.25 μm nominal (Varian Ibérica, Madrid, Spain), with an internal linked-phase coating VF 23MS housed in an oven was programmed for the following cycle: 1 min at 50 °C, 5 °C/min ramp to 225 °C, held for 15 min. Gas carrier was N_2 at a flow rate of 11.25 mL min^{-1}. A flame ionization detector (FID) used the following flow rates: O_2, 450 mL min^{-1}; H_2, 40 mL min^{-1}. The software used was supplied by Agilent Technologies for Windows 2000 Professional.

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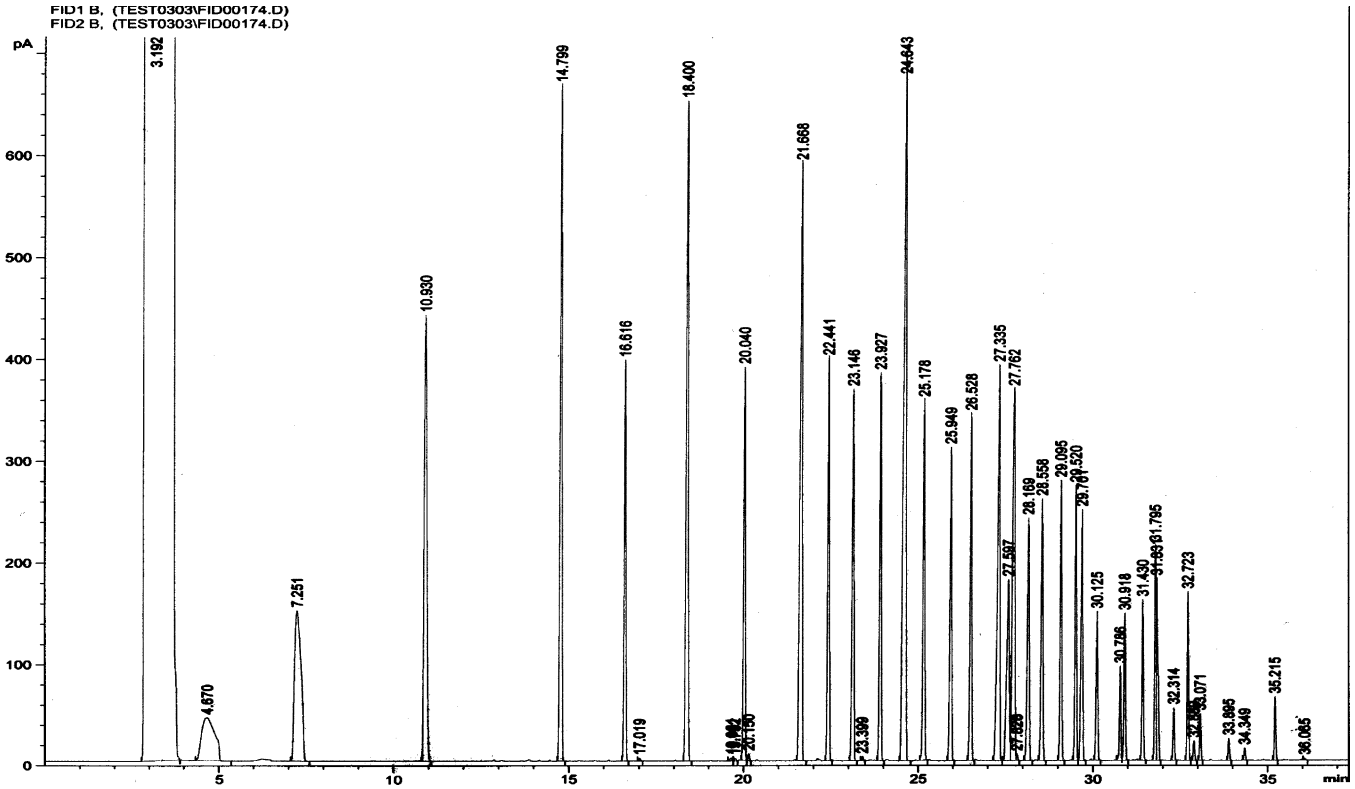

Figure 1. Chromatogram of the Supelco lipid standard 189-19 in the optimal conditions.

Table 1. Types of Egg Yolk Sample Used and Sample Numbers

group	type	heat treatment	sample no.
A	homogenized	no	A1
		yes	A2
	pasteurized	no	A3
		yes	A4
	powdered	no	A5
B	homogenized	no	B1
		yes	B2
	pasteurized	no	B3
		yes	B4
	powdered	no	B5
C	omega-3	no	C1
		yes	C2

Table 2. Information Supplied with the Omega-3 Eggs

nutritional information	mean for 100 g
fats (g)	9.9
saturated (g)	3.0
monounsatutated (g)	3.4
polyunsaturated (g)	1.6
omega-3 (g)	0.44
docosahexaenoic acid (g of omega-3)	0.28
omega-6	1.48
cholesterol (g)	0.34

Table 3. FAMEs Contained in the Standard That Have Been Quantified in the Samples

denomination	name of corresponding fatty acid	retention time (min)
C14:0	myristic acid	21.7
C16:0	palmitic acid	24.6
C16:1 n-9	palmitoleic acid	25.2
C17:0	heptadecanoic acid	25.9
C18:0	stearic acid	27.3
C18:1 n-9	oleic acid	27.6
C18:1 n-11	vaccenic acid	27.9
C18:2 n-6	linoleic acid	28.2
C18:3 n-6	linolenic acid	29.5
C20:4 n-6	arachidonic acid	31.8
C24:0	lignoceric acid	35.2

Samples. Three types of yolk sample were examined (see **Table 1**): (A) those obtained from eggs from the same chicken farm; (B) samples obtained from eggs obtained from different suppliers; and (C) samples obtained by homogenizing the yolks of commercial "omega-3-enriched" eggs. According to the marketing firm, these last eggs are obtained by supplementing the feed of the hens with foods rich in these components, and they are sold with the "nutritional information" shown in **Table 2**.

The group A samples were obtained from 300 boxes containing 360 eggs each supplied by the same farm. After the shells had been removed and discarded, the whites were separated and the remaining yolks were homogenized in an industrial tank. Portions of the homogenized yolks were obtained to provide two sets of samples, one directly processed for lipid extraction (A1) and a second set dried in an oven for 4.5 h at 104 ± 2 °C to remove all water (A2). The remaining yolks in the tank were pasteurized at 140 °C for 2 min, and a further two portions were subjected to treatment as above, one directly processed for lipid extraction (A3) and the other dried for 4.5 h at 104 ± 2 °C (A4). The yolk mixture left in the tank was hot-air-dried to provide a powdered sample (A5).

The samples for group B were obtained in the same way as described above starting with 300 boxes of eggs from three different farms. The group C samples were obtained by homogenizing the yolks of 36 eggs from a single lot sold as "omega-3-enriched", one portion being directly processed for lipid extraction (C1) and a second portion dried in an oven for 4.5 h at 104 ± 2 °C to remove all water (C2).

Procedures. Fats were extracted using the Folch procedure according to the Spanish Official Method (*10*). This involves treating the samples with chloroform, methanol, and deionized water to obtain a lipid extract free of amino acids, non-lipid products, or water-soluble carbohydrates. Three analyses were performed on each sample. To this end, 10.0 mL of chloroform/methanol (2:1, v/v) was added to a 0.5 g aliquot of sample

Table 4. Relative Contents (Percent) of Different Fatty Acids in Egg Yolk Samples

	fatty acid										
sample	C14:0	C16:0	C16:1 n-9	C17:0	C18:0	C18:1 n-9	C18:1 n-11	C18:2 n-6	C18:3 n-6	C20:4 n-6	C24:0
A1	0.50	29.93	3.38	0.24	6.64	43.56	2.14	11.55	0.28	1.09	0.16
A2	0.00	30.27	3.41	0.00	6.30	46.96	2.12	11.61	0.00	0.00	0.00
A3	0.56	30.79	3.41	0.36	7.44	40.98	2.03	12.42	0.36	1.25	0.07
A4	0.57	31.01	3.43	0.35	7.29	41.29	2.01	12.43	0.24	1.17	0.00
A5	0.53	29.89	3.29	0.35	7.25	41.11	1.96	12.43	0.37	1.21	0.20
B1	0.61	30.21	3.95	0.42	7.09	41.32	2.04	11.46	0.41	1.02	0.21
B2	0.00	29.15	3.76	0.00	5.98	47.19	2.28	11.60	0.00	0.00	0.00
B3	0.61	31.35	3.55	0.31	7.84	41.24	2.09	11.02	0.33	1.24	0.13
B4	0.54	30.52	3.38	0.35	7.32	41.52	2.02	12.47	0.35	1.18	0.00
B5	0.75	29.80	3.34	0.38	7.48	38.11	1.91	13.78	0.62	1.09	0.21
C1	0.65	31.51	4.04	0.39	6.55	40.94	2.18	10.81	0.92	0.57	0.71
C2	0.65	31.51	4.08	0.39	6.50	40.81	2.16	10.91	0.94	0.57	0.70

Table 5. Relative Proportions According to the Degree of Unsaturation

	sample											
type of FA	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2
saturated	37.54	36.58	39.22	39.22	38.50	38.54	35.13	40.24	38.73	38.84	39.93	40.05
monounsaturated	49.49	52.82	46.75	46.95	47.03	48.32	53.23	47.16	47.25	43.97	47.69	47.63
polyunsaturated	12.99	11.61	14.03	13.84	14.34	13.15	11.60	12.59	14.00	15.90	12.30	12.42

in a test tube. The tubes were then sealed and shaken to give a single phase and placed in a refrigerator at 4−5 °C for 20 min. After cold storage, the samples were filtered using degreased paper into tubes of similar size to the starting test tubes, to which 2 mL of 0.017% $MgCl_2$ solution was added. The samples were stirred by bubbling N_2 gas and centrifuged at 4500 rpm for 5 min. This treatment produced two phases for each sample: a top layer containing non-lipid substances and a lower layer containing different lipids. The top layer was discarded, and the resultant lower phase was washed in 10.0 mL of chloroform/methanol/water (5:48:47, v/v/v). This operation was conducted in duplicate. The resultant lower phases were mixed and transferred to 250 mL round-bottom flasks. Finally, the samples were dried in a Rotavapor at 40 °C under vacuum.

The residue from previous operations was dissolved in 5.0 mL of chloroform. Next, 1.0 mL of the liquid was transferred to a test tube with a Teflon stopper to which 2.0 mL of boron trifluoride/methanol and 1.0 mL dichloromethane were added. The tube was then hermetically sealed and left still for 1 h in an oven at 100 °C to obtain methyl esters of the fatty acids present.

Once the tubes had cooled, 1.5 mL of deionized water and 3.0 mL of hexane were added to give two phases. The upper phase was cooled and the lower phase treated as before. The two upper phases were then combined, and the sample was then ready for injection to the chromatograph.

RESULTS AND DISCUSSION

To establish the most appropriate conditions for fatty acid determination, we tested several method designs (factorial designs) using a series of standards: Supelco lipid standard 189-19 fatty acid methyl ester mix; Supelco 46900-U cis-7 octadecenoic methyl ester (C18: n-7); Supelco 46904 cis-11 vaccenic methyl ester (C18:1 n-11c); Supelco 46905-U vaccenic methyl ester (C18:1 n-11t) ; Supelco 46906 cis-12 octadecenoic methyl ester (C18:1 n-12); Supelco 46907-U trans-12 octadecenoic methyl ester (C18:1 n-12t); Supelco 47198 cis-6 petroselinic methyl ester (C18: n-6c); and Supelco 47199 trans-6 petroselinic methyl ester (C18:1 n-6t). **Figure 1** shows the chromatogram obtained for standard 189-19. This figure shows that when the conditions described under Equipment are used,the 37 fatty acids present can be suitably differentiated and quantified and that the data coincide with those specified by the manufacturer. Standards and samples were analyzed three times, and standard deviations of the different fatty acids varied between 0.01 and 0.34%. **Table 3** shows the retention times of those fatty acids methyl esters (FAME) quantified in samples and the denomination of the corresponding fatty acids used throughout this paper.

We then determined the fatty acid contents of different samples under the proposed conditions to provide the results shown in **Table 4**. These results indicate that the sum of C16:0 and C18:1 n-9 fatty acids accounts for practically 70% of the total fatty acids detected in every sample. The C18:2 appeared in relative proportions above 10%, C18:0 from 6 to 8%, C16:1 from 3 to 4%, and C18:1 n-11 around 2%. The fatty acids C14:0, C17:0, C18:3, and C24:0 occurred in relative proportions below 1%. In non-heat-treated/nonpasteurized (A1 and B1) and omega-3-enriched yolk samples (C1), C20:4 was found in proportions under 1%.

In **Table 5**, the fatty acids are assembled according to their degree of unsaturation, indicating that approximately 12−16% of fatty acids were polyunsaturated, 43−53% monounsaturated, and 37−40% saturated. In general, these proportions are consistent with those provided in the literature (*1*, *5*). Focusing on the essential fatty acids, it may be seen that C18:3 appears in greater proportions in the omega-3-enriched samples, which in contrast contain a lower proportion of C18:2 and C20:4, also essential for human health. Overall, it can be stated that the relative proportions of essential fatty acids were similar in the four types of samples tested.

Although these observations are of interest, through multivariate factor and cluster analysis we were able to further analyze the information obtained (*11*−*13*)

Correlation of Variables. Data were normalized to zero mean and unit variance to avoid misclassifications arising from the different orders of magnitude of both the numerical values and the percentage variance of the different fatty acids determined. The correlation matrix of the variables (**Table 6**) was calculated from the normalized data (critical correlation coefficient for 10 degrees of freedom and $P = 0.05$ was 0.576). High correlation was shown among different fatty acids. Thus, C14:0, C17:0, C18:0, C18:1 n-9, C18:3 n-6, and C20:4 appeared

Table 6. Correlation Matrix of the 11 Fatty Acids Analyzed

	C14:0	C16:0	C16:1 n-7	C17:0	C18:0	C18:1 n-9	C18:1 n-11	C18:2 n-6	C18:3 n-6	C20:4 n-6	C24:0
C14:0	1.000										
C16:0	0.506	1.000									
C16:1	0.104	0.401	1.000								
C17:0	0.964	0.508	0.158	1.000							
C18:0	0.671	0.271	–0.449	0.648	1.000						
C18:1 n-9	–0.963	–0.422	0.003	–0.935	–0.730	1.000					
C18:1 n-11	–0.554	0.037	0.592	–0.572	–0.792	0.687	1.000				
C18:2 cc	0.181	–0.458	–0.710	0.161	0.456	–0.372	–0.771	1.000			
C18:3 n-6	0.744	0.598	0.559	0.717	0.101	–0.704	–0.074	–0.178	1.000		
C20:4	0.771	0.210	–0.414	0.766	0.889	–0.752	–0.735	0.408	0.185	1.000	
C24:0	0.475	0.551	0.730	0.454	–0.228	–0.393	0.249	–0.476	0.914	–0.125	1.000

Table 7. Eigen Analysis of the Correlation Matrix Loadings of the First Three Factors

variable	PC1	PC2	PC3
C14:0	0.412	0.093	–0.035
C16:0	0.202	0.301	0.559
C16:1 n-7	–0.031	0.467	–0.073
C17:0	0.406	0.104	–0.022
C18:0	0.339	–0.227	0.359
C18:1 n-9	–0.418	–0.026	0.161
C18:1 n-11	–0.307	0.307	0.155
C18:2 n-6	0.143	–0.398	–0.501
C18:3 n-6	0.271	0.356	–0.313
C20:4 n-6	0.357	–0.190	0.270
C24:0	0.138	0.452	–0.282
eigenvalue	5.517	3.821	0.858
proportion	0.502	0.347	0.078
cumulative (%)	50.2	84.9	92.7

as a group. C16:1 correlated with C18:1 n-11, C18:2 cc, and C24:0, and high correlation was also shown between C16:0 and C18:3 n-6.

Factor Analysis. Factor analysis allows a clustering of variables on the basis of mutual correlations and a grouping of objects based on their similarities. For this analysis, the correlation matrix was diagonalized, and the new factors or principal components were obtained as weighted linear combinations of the original variables. By extracting the eigenvalues and eigenvectors of the correlation matrix, we know the number of significant factors, the percentage of variance explained by each of them, and the participation of the old variables (fatty acids) in the new "latent" ones (see ref *14* for more details). **Table 7** shows the main significant components (eigenvalues greater than or close to unity) and their loadings (coefficients expressing the contribution of the original variable to the main component). It may be noted that the first three factors account for 92.7% of the variance or information contained in **Table 4**. The fatty acids that contribute to factor 1 (representing 55% of the total variance) are those that were previously found to cluster with C14:0 with the exception of C18:3 n-6. Factor 2, to which the fatty acids C16:1 and C24:0 contribute, accounts for 38.0% of the variance. Fatty acids C16:0 and C18:2 cc contribute to factor 3, accounting for 7.8% of the variance. All of the fatty acids determined contributed to one of the three factors.

If we represent the values of each component that each item takes for each new variable as scores, two-dimensional graphs are obtained that clearly illustrate these contributions. **Figure 2A** shows the representation of score 2 versus score 1 of the values obtained for the whole table. This figure shows groupings related to the origin and different treatments of the samples. It may be observed that the samples enriched with omega-3, pasteurized samples, and nonpasteurized samples form separate

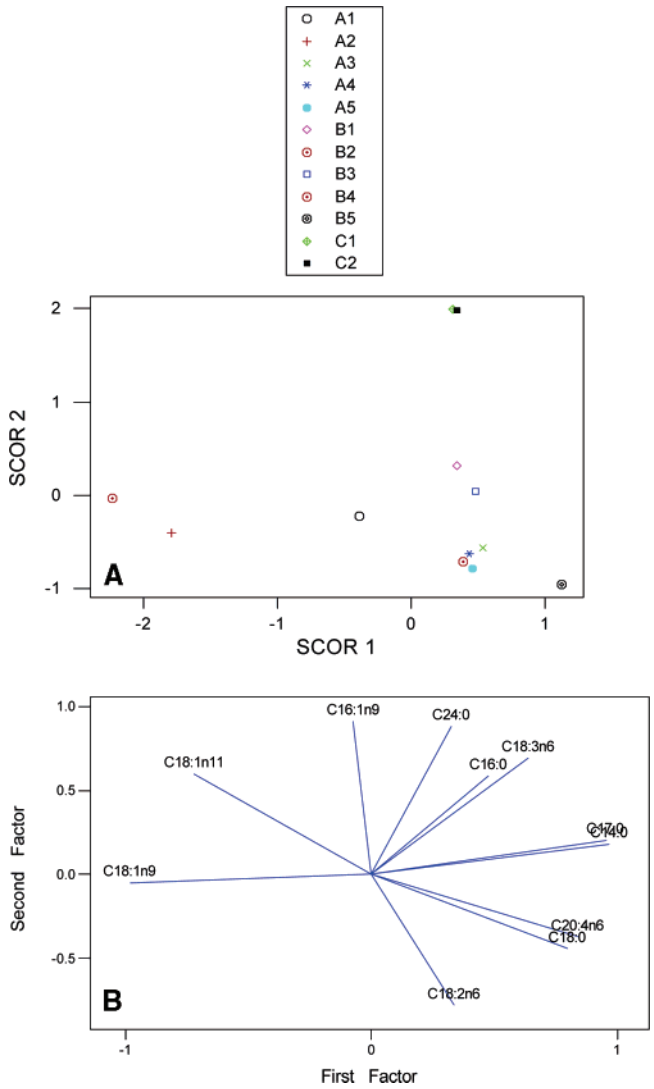

Figure 2. Factor analysis of the whole data: (**A**) representation of score 2 versus score 1 shows groupings related to the origin and different treatment of the samples; (**B**) representation of the loading of the two first factors, showing the contribution to the fatty acids to the scores.

groupings. This finding has significant implications in the design of equilibrated diets.

Figure 2B was drawn to explain these results. This figure indicates the loadings of the first two factors. If we compare the two graphs, it becomes clear that the omega-3-rich yolk samples group together because of their higher C16:0, C16:1, C18:3 n-6, and C24:0 contents, in agreement with **Table 3**. Fatty acids C18:1 n-11 and C18:1 n-9 mainly contribute to the nonpasteurized/non-heat-treated sample grouping. The group

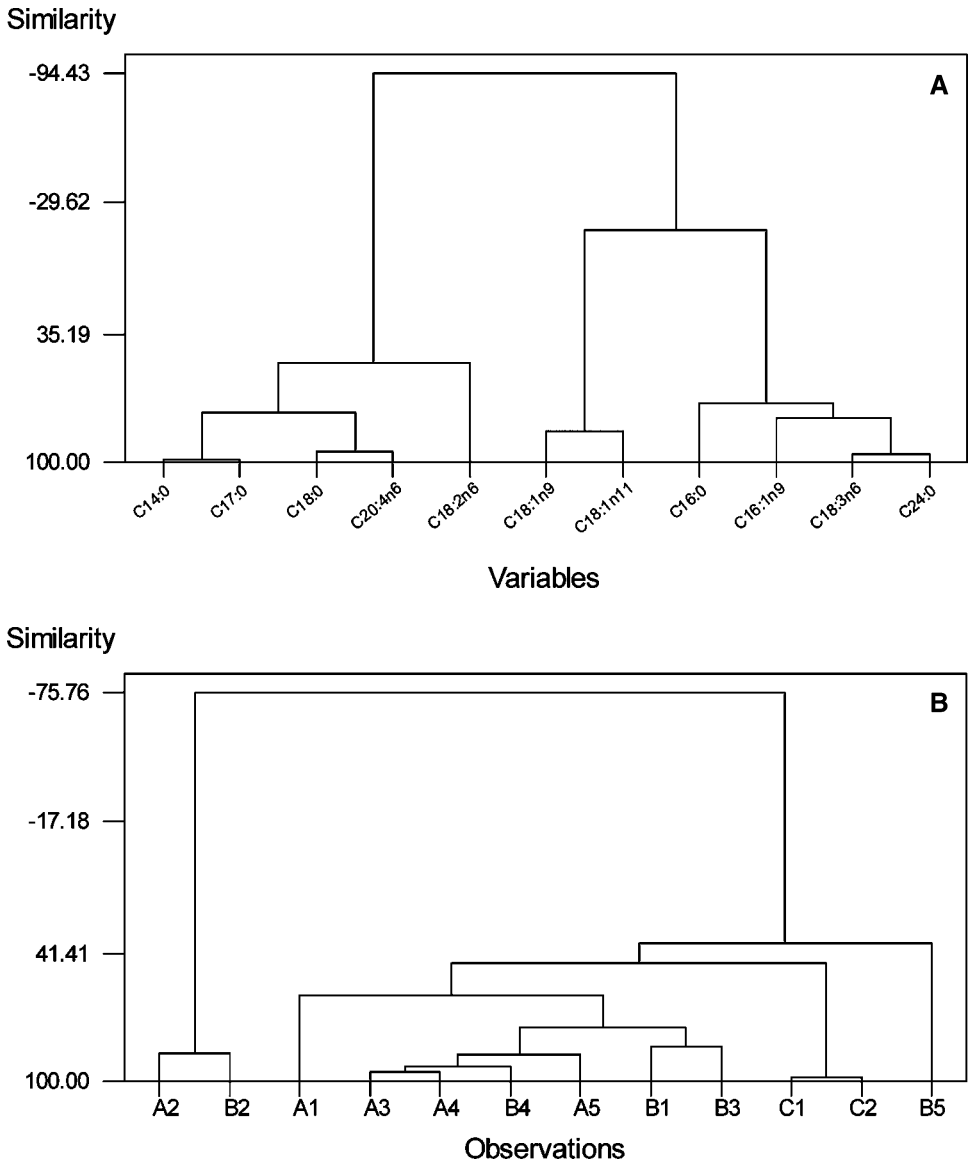

Figure 3. Cluster analysis: dendrograms based on cumulative hierarchical clustering by complete linkage (Ward distances) of (**A**) variables (fatty acids) and (**B**) samples analyzed (see **Table 1**).

gathering the rest of the samples shows relatively high levels of C18:0, C18:2, and C20:4. It may thus be concluded that fatty acid composition also serves to distinguish samples according to their different origins or treatments.

Cluster Analysis. Cluster analysis is used to classify objects, characterized by the values of set variables, into groups. Through cluster analysis, a dendrogram was produced grouping the variables (fatty acids) (**Figure 3A**). The set of clusters obtained reflects the derived data from the correlation matrix but further clarifies the correlations observed. Hence, for fatty acids, four clusters may clearly be discerned: one composed by C14:0 and C17:0; another with C18:0 and C20:4 n-6; a third composed by C18:1 n-9 and C18:1 n-11; and finally another with C24:0 and C18:3 n-6, leaving aside C18:2 n-6 as uncorrelated.

Cluster analysis applied to samples also revealed different groups (**Figure 3B**). One cluster corresponds to the nonpasteurized/non-heat-treated samples (samples A2 and B2), another to the omega-3-enriched samples (C1 and C2), and a final cluster grouping the remaining samples. In a separate group, although not isolated, appear the powdered yolk samples obtained from eggs from different farms and the nonpasteurized/heat-treated sample containing yolks from a single farm. These results are similar to those obtained in the principal component analysis.

Conclusions. The fatty acid profile of processed eggs depends on their origin and the treatment received before they reach the market. The results of this generic assessment revealed a group containing higher proportions of C16:0, C16:1, C18:3, and C24:0 fatty acids corresponding to eggs sold as "omega-3-enriched". Similarities were also noted among nonpasteurized yolks on the basis of their higher proportions of C18:1 n-11 and C18:1 n-9. The remaining samples contained greater proportions of C18:0, C18:2 cc, and C20:4.

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