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Effect of processing and storage on the chemical quality markers of spray-dried whole egg

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Abstract

Chromatographic techniques were used in order to determine the modifications induced in egg, by a spray-drying treatment, on the native constituents and on the formation of cholesterol oxidation products (COPs) and early Maillard reaction products. The same powdered egg was stored for 12 months at room temperature and at 4 °C, and sampled at 1, 3, 6 and 12 months, respectively. The spray-drying treatment did not affect tocopherol or retinol composition, but caused a severe Maillard reaction (furosine increased from 15 to 500 mg/100 g protein) and accelerated cholesterol oxidation (the sum of COPs increased from 24 to 55 μg/g fat). Only slight modifications were registered in powdered egg stored at 4 °C. During storage at room temperature, however, COPs increased significantly (up to 167 μg/g fat), tocopherols and retinols decreased, whereas furosine was not significantly modified. Thus, furosine can be considered as a suitable technology marker, whereas COPs are important markers of the storage conditions.

Keywords: Powdered eggs; Cholesterol oxidation products; Furosine; Tocopherols; Retinols; Storage conditions; Multivariate analysis

1. Introduction

Hen eggs are a primary source of high quality proteins of low cost and offer a balanced distribution of minerals and vitamins, particularly vitamins E, A, B_{12}, B_2 and folate (Surai & Sparks, 2001), as well as high amounts of lipids, such as triacylglycerols, phospholipids and cholesterol (Watkins, 1995).

Dried eggs are widely used in food preparations because of their microbiological safety and their reduced volume with respect to unshelled or liquid eggs (Bergquist, 1995). Moreover, the appeal of dried eggs is their conveniently and long shelf-life; in fact, this product is usually stored without particular care. However, the quality of the raw material, the processing and the storage conditions, strongly influence the quality and safety of egg powder (Galobart, Guardiola, Barroeta, López-Ferrer, & Baucells, 2002).

The safety and quality of powdered whole egg depend on two critical steps: the drying process and the storage. Chemical modifications, such as oxidation and non-enzymatic browning with formation of undesirable compounds (Bergquist, 1995; Galobart, Barroeta, Baucells, Cortinas, & Guardiola, 2001; Missler, Wasilchuk, & Merrit, 1985; Utzmann & Lederer, 2000; Wahle, Hoppe, & McIntosh, 1993), can occur during these processing steps.

Lipid oxidation in spray-dried eggs is currently monitored by determination of the peroxide value or

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thiobarbituric acid-reactive substances (TBARS) (Katušin-Ražem, Mihaljević, & Ražem, 1992). However, there is not a linear correlation between TBARS or peroxides and total oxidation products; the concentration of TBARS and peroxides can decrease after a maximum due to the formation of evolution products with a wide range of egg components. This can lead to underestimation of the real oxidative status of the egg product. On the other hand, the presence of cholesterol oxides (COPs) in dried egg products has been reported by several authors in the past two decades, (Guardiola, Codony, Miskin, Refecas, & Boatella, 1995; Lai, Gray, Buckley, & Kelly, 1995; Missler et al., 1985; Morgan & Armstrong, 1992; Nouroouz-Zadeh & Appelqvist, 1987; Sander, Addis, Park, & Smith, 1989; Tsai & Hudson, 1985; Zunin et al., 1995). Most COPs are readily absorbed and carried to tissues where they can exert their negative biological effects (Peng & Morin, 1992; Sevanian & MacLeod, 1987; Smith, 1981).

Other newly formed compounds can arise during the processing and storage of egg products due to the Maillard reaction (MR): the presence of proteins and reducing sugars is the essential condition for the non-enzymatic condensation of carbonyl and amino groups of sugars and protein (amino acids) (Belitz & Grosch, 1999; Eskin, 1990; Friedman, 1996), which leads to the browning of the egg-containing product. The MR is responsible for the main off-flavour and off-colour of powdered eggs (Bergquist, 1995). Thus, a desugarization treatment of pasteurised whole egg is sometimes carried out before spray-drying (Sebring, 1995) in order to protect the product during heating and storage against MR products.

The furosine assay, which is specific for ε-*N*-deoxyketosyl-lysine (Amadori compound), is considered the most sensitive and most accepted method for determining the extent of early MR in a wide range of thermally treated products (Acquistucci, Panfili, & Marconi, 1996; Erbersdobler, Dhen, Nangpal, & Reuter, 1987; Guerra-Hernandez, Corzo, & Garcia-Villanova, 1999; Hidalgo & Pompei, 2000; Pagani, Resmini, & Pellegrino, 1992; Pellegrino, Resmini, & Luf, 1995; Ramirez-Jimenez, Guerra-Hernandez, & Garcia-Villanova, 2000; Sanz, Castillo, Corzo, & Olano, 1999; Villamiel, del Castillo, Corzo, & Olano, 2001). The furosine assay can be used, not only for assessing the intensity of thermal treatment, but also to assess freshness and shelf-life, since the MR can also occur at room temperature. The determination of furosine has been used to assess the storage conditions, shelf life and freshness of milk powder, royal jelly, honey, infant foods, dehydrated foods, jams (Guerra-Hernandez, Leon, Garcia-Villanova, Corzo, & Romera, 2002; Hurrell, Finot, & Ford, 1983; Marconi, Caboni, Messia, & Panfili, 2002; Rada-Mendoza, Sanz, Olano, & Villamiel, 2004; Ramirez-Jimenez, Guerra-Hernandez, & Garcia-Villanova, 2003; Sanz, del Castillo, Corzo, & Olano, 2001, 2003) and shell eggs (Hidalgo, Lucisano, Comelli, & Pompei, 1996; Hidalgo, Rossi, & Pompei, 1995). Maillard products can also have interesting antioxidant activity which makes the product more stable during storage (Alaiz, Zamora, & Hidalgo, 1996; Alfawaz, Smith, & Jeon, 1994; Bayley & Um, 1992; Shizuchi & Hayase, 2003; Smith & Alfawaz, 1995).

High temperature, high surface area (powder, thin film) and forced oxygen flow are factors promoting lipid autoxidation (Guardiola et al., 1995; Maerker, 1987) during spray-drying; thus the protective effect of antioxidants, phospholipids and lipoproteins against lipid oxidation is significantly reduced. Moreover, tocopherols and other natural antioxidants present in eggs are drastically degraded during thermal treatments and inadequate storage conditions (Galobart et al., 2001; Wahle et al., 1993).

From these premises, it is evident that the markers of a processing technology can be defined by two types of chemical reactions: (1) degradation, denaturation and inactivation of heat-labile components or (2) formation of new compounds which are not present, or only at trace level, in an unprocessed product (Pellegrino et al., 1995). Previous work dealt with the stability of some nutrients during spray-drying treatment and storage of egg powder (Brinkerhoff, Huber, Huber, & Pike, 2002; Guardiola et al., 1995; Zunin et al., 1995). However, a comprehensive and systematic study of the influence of processing and storage technology, on the markers of both types, contemporaneously, is missing to our knowledge.

Therefore, the goal of the present paper was to assess the quality of egg powder after heating and during storage using both types of indicators. The degradation of certain nutrients (glucose, reducing sugars, retinols, tocopherols, lysine) and the formation of non-natural products (Amadori compounds and COPs) were analyzed by chromatographic techniques in order to identify the most suitable markers of egg powder quality and safety. The role of these components, as thermal and storage markers, is considered using chemometric analysis.

2. Materials and methods

2.1. Sampling

The spray-dried whole egg was a commercial sample obtained from an Italian producer, as well as the corresponding pasteurized liquid egg sample.

The dried sample was divided into 18 aliquots (10 g for each sample); each aliquot was placed in a vial and sealed. Two aliquots were immediately analysed for dry matter, COPs, protein, furosine, lysine, reducing sugar, tocopherol and retinol contents and represented the control (time 0). The remaining aliquots were divided into two groups. The first group was stored at room

temperature in the dark for 1, 3, 6 and 12 months in two replications. The second group was stored at 4 °C for the same time intervals.

2.2. pH

The pH was measured in the pasteurized liquid egg using a Crison Basic20 pH meter (Crison Instruments, S.A., Barcelona, Spain).

2.3. Dry matter

Dry matter was determined by the AOAC method 925.30 (AOAC, 2000).

2.4. Water activity

Water activity, a_w, of the spray dried egg samples was measured at 20 °C as vapour pressure, through the dew point, using an Aqualab CX-2 instrument (Decagon Devices, Pullman, WA, USA).

2.5. Protein

Protein content (N × 6.25) was determined according to the Dumas combustion method, AOAC method 992.23 (AOAC, 2000), using a Leco nitrogen determiner, model FP 528 (Leco Corp., St. Joseph, MI, USA). Pasteurized liquid egg was weighed (250 mg) in a tin capsule (Leco tin capsule 502-040); the powdered egg samples were weighed (100 mg) in tin foil (Leco tinfoil cups 502-186), using a foil holder (Leco 604-493) and twisting the ends of the foil to form a teardrop-shaped pocket.

2.6. Reducing sugars (glucose and fructose)

The egg sample was dissolved (20 and 150 mg for powdered and liquid sample, respectively) in 25 ml of water and then filtered through Whatman paper no.1. The reducing sugar content was determined using the Dionex system (Dionex Corporation, Sunnyvale, CA, USA), composed of a gradient pump (mod GP50) with on-line degasser and an electrochemical detector (model ED40). The instrument control, data collection and total quantification were managed using Peak Net chromatography software (Dionex). The flow-through electrochemical cell (Dionex) consisted of a 1 mm diameter gold working electrode, a pH reference electrode, and a titanium body of the cell as the counter electrode. A controlled Rheodyne injector (Cotati, CA, USA) with 25 μl sample loop was used for sample injection. Separation was performed with an Aminopac PA10 analytical column 250 × 2 mm, with 8.5 μm particle size (Dionex). The quantitative determination was carried out at a flow rate of 0.25 ml/min using a mobile phase gradient of water, 250 mM sodium hydroxide and 1.0 M sodium acetate as shown in Table 1. The optimized time–potential waveform used is shown in Table 2.

Table 1
Gradient conditions for anion-exchange separation of carbohydrates

Time (min)	H_2O (%)	NaOH (%)	Sodium acetate (%)	Gradient curve[a]
0.0	80	20	0	
2.0	80	20	0	
12.0	80	20	0	
16.0	68	32	0	8
24.0	36	24	40	8
40.0	36	24	40	
40.1	20	80	0	5
42.1	20	80	0	
42.2	80	20	0	5
62	80	20	0	

[a] Shapes of gradient curves are defined in the GP50 Pump Manual (Dionex).

Table 2
Integrated amperometry waveform used to detect sugars and lysine

Time (s)	Potential (V)	Integration
0.00	+0.13	
0.04	+0.13	
0.05	+0.28	
0.11	+0.28	Begin
0.12	+0.60	
0.41	+0.60	
0.42	+0.28	
0.56	+0.28	End
0.57	−1.67	
0.58	−1.67	
0.59	+0.93	
0.60	+0.13	

The duplicate (two different aliquots of each sample) values differed by less than 10% from their mean values.

2.7. Lipid

Egg sample (2 g for egg powder and 5 g for pasteurised egg) was extracted according to the procedure described by Folch, Lees, and Stanley (1957), slightly modified as reported by Boselli, Velazco, Caboni, and Lercker (2001). The amount of total lipids was determined gravimetrically.

2.8. Cholesterol and its oxides

2.8.1. Preparation

Cholesterol and its oxides (COPs) were obtained by cold saponification (Sander et al., 1989), after addition of an exact volume (25 μl) of a 19-hydroxy-cholesterol solution (0.5 mg/ml) and 25 μl of betulin (2 mg/ml) to 250 mg lipids.

2.8.2. Cholesterol analysis

The unsaponifiable matter was divided into 2 aliquots: one aliquot was used for the GC analysis of

cholesterol and the remaining aliquot was used for the determination of COPs. The first aliquot was silylated (Sweeley, Bentley, Makita, & Wells, 1963) and injected in to the GC. The GC–FID apparatus consisted of a HRGC 5300 gas chromatograph (Carlo Erba Instruments, Rodano, MI, Italy) equipped with a split-splitless injector and a flame ionisation detector (FID). The capillary column (30 m × 0.32 mm i.d.) was coated with a film (0.25 μm film thickness) of 100% dimethylpolysiloxane (DB-1, J&W Scientific, Folsom, CA, USA). The samples were injected in the split mode (1 μl) with a split ratio of 1:15. Helium was the carrier gas (0.8 ml/min, gas flow). The oven temperature was increased from 250 to 325 °C at a rate of 3 °C/min. Both the injector and detector temperature were set at 325 °C.

2.8.3. COPs analysis

COPs were purified from the unsaponifiable matter, obtained as described above, by NH_2 solid-phase extraction according to Rose-Sallin, Hugget, Bosset, Tabacchi, and Fay (1995). Successively, the eluted fraction was silylated, and then analysed by capillary gas chromatography (GC–FID). The gas chromatographic apparatus and conditions were the same as described above. The identification of COPs was confirmed by gas chromatography–mass spectrometric (GC–MS) analysis. A gas chromatograph 3400 (Varian, Palo Alto, CA, USA) equipped with a ITS40 mass spectrometry detector (Finnigan, Manchester, UK) was used. The same gas chromatographic conditions as for GC–FID analysis were used and the transfer line was heated at 300 °C. The mass detector was used in the electronic impact (70 eV) mode; the emission current was 10 mA and the scan rate was 1 scan/s.

The standards used for the identification of COPs were supplied by Sigma Chemical Co. (St. Louis, MO, USA): 5-cholesten-3β-ol-7-one (7-k), 5-cholestan-5α, 6α-epoxy-3β-ol (α-epo), 5-cholestan-5β,6β-epoxy-3β-ol (β-epo), 5-cholesten-3β,20α-diol (20-OH), 5-cholesten-3β,5α,6β-triol (triol) and 5-cholesten-3β,19-diol (19-OH) were used as the internal standards. The standards 5-cholesten-3β,7α-diol (7α-OH), 5-cholesten-3β,7β-diol (7β-OH), 5-cholesten-3β,25-diol (25-OH) were obtained from Steraloids (Wilton, NH, USA).

The duplicate (two different aliquots of each sample) values differed by less than 10% and less than 20% from their mean values for 7α-OH, 7β-OH, β-epo and α-epo, and for 20-OH, triol and 7-k, respectively.

2.9. Tocopherols and retinols

The unsaponifiable matter was obtained according to Panfili, Manzi, and Pizzoferrato (1998) with slight modifications. Egg powder (0.5 g) or liquid egg (2 g) were put under nitrogen in a screw-capped tube with 5 ml ethanolic pyrogallol (60 g/l), added as antioxidant, 2 ml of 95% ethanol, 1 ml of sodium chloride (10 g/l) and 3 ml of potassium hydroxide (600 g/l). Alkaline digestion was carried out in a water bath at 70 °C over 30 min. Successively, the tubes were rapidly cooled in an ice bath and 15 ml of NaCl (10 g/l) were added. The suspension was then extracted twice with 15 ml of *n*-hexane/ethyl acetate (9:1, v/v); the organic layers were collected and evaporated to dryness; the residue was dissolved in *n*-hexane containing 1% of 2-propanol. HPLC analysis was performed using a Waters (Milford, MA, USA) system consisting of two pumps (mod. 510), and a Rheodyne injector, mod. 7125. The system was equipped with a programmable spectrofluorimeter detector (Waters, mod. 470). The chromatographic analysis was achieved with a Kromasil Phenomenex Si column (250 × 4.6 mm, 5 μm particle size) (Torrance, CA, USA). The separation was performed at a flow rate of 1.5 ml/min with 2-propanol (1%) in *n*-hexane and *n*-hexane in a multi linear gradient as described by Panfili, Manzi, and Pizzoferrato (1994). Fluorimetric determination of tocopherols was performed at 280 and 325 nm as excitation and emission wavelenghts, respectively. Retinols were detected fluorimetrically at 325 nm excitation wavelength and 475 nm emission wavelength. Data were stored and processed using Millennium 32 chromatography software (Waters Corp.). The standards of retinols were purchased from Sigma Chemical Co. (St. Louis, MO, USA); tocopherol standards were from Merck (Darmstadt, Germany).

The duplicate (two different aliquots of each sample) values differed by less than 10% from their mean values.

2.10. Lysine

Protein hydrolysis. An egg sample, corresponding to 25 mg of protein, was placed in a flask with an acid-resistant rubber stopper. After the addition of 25 ml 6 N HCl, the flask was introduced under vacuum to an oven at 110 °C for 24 h. The flask was then cooled and the sample was evaporated to dryness under gentle vacuum and dissolved in 0.1 N HCl. Lysine was determined by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), using the same apparatus as described for reducing sugars analysis. Separation was performed with an AminoPac PA10 analytical column 250 × 2 mm, with 8.5 μm particle size (Dionex). The quantitative determination of lysine was carried out by isocratic elution, using 50 mM NaOH with a flow rate of 0.25 ml/min, as reported by Marconi et al. (2002). The time–potential waveform used was the same as described for reducing sugars (see Table 2).

The duplicate (two different aliquots of each sample) values differed by less than 3% from their mean values.

2.11. Furosine

The furosine content was determined by HPLC according to Resmini, Pellegrino, and Battelli (1990). A sample amount (0.2 and 0.5 g for powdered and liquid egg, respectively), corresponding to about 30–70 mg of protein, was hydrolysed in nitrogen with 8 ml of 8 N HCl at 110 °C for 23 h. Afterwards, the hydrolysate (0.5 ml) was purified on a Sep-Pak C18 cartridge (Waters), diluted and analysed by HPLC (Waters) equipped with an Alltech furosine-dedicated column (250 × 4.6 mm) (Alltech, Derfield, IL, USA). Furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France).

The duplicate (two different aliquots of each sample) values differed by less than 3% from their mean values.

2.12. Blocked lysine

Blocked lysine was indirectly measured from the furosine content by the following equations (O'Brien & Morissey, 1989; Finot & Mauron, 1972):

$$\%\text{Blocked lysine} = \text{blocked lysine} \times 100/(\text{blocked lysine} + \text{available lysine}),$$

where

$$\text{Blocked lysine} = (\text{furosine}/0.30) \times \text{MW}_{\text{lysine}}/\text{MW}_{\text{furosine}}.$$

$$\text{Available lysine} = \text{chromatographed lysine} - (0.45 \times \text{blocked lysine})$$

2.13. Statistical analysis

ANOVA was performed on two replications of each treatment and the Tukey comparison test was used to identify differences at $P < 0.05$. Data reported for all the considered parameters are the average values of two results obtained from analysis of two different aliquots of each sample. The significance of the variation is also reported ($P < 0.05$): groups of samples which are significantly different are indicated with different letters.

Principal component analysis (PCA) was performed in order to obtain differences or groupings among the samples of dried whole egg. The Unscrambler v 7.6 (Camo Inc, Corvallis, OR, USA) software for multivariate statistical analysis was used.

3. Results and discussion

3.1. Thermal treatment assessment

The drying (thermal) treatment caused substantial chemical modifications in processed egg, as shown in Table 3. In particular, the furosine amount increased significantly, varying from 15 to 500 mg/100 g protein after drying. The intense development of the MR is surely favoured by the alkaline pH of pasteurised whole egg (pH 7.7; Li-Chan, Powrie, & Nakay, 1995; Rossi, Pompei, & Hidalgo, 1996) and by adequate amounts of reactants, such as, reducing sugars (glucose = 1.22 g/100 g dry basis) and ε-aminogroups of proteins (lysine = 6.99 g/100 g protein). The furosine content of the liquid pasteurised sample (15.2 mg/100 g protein) is evidence of fresh raw material (shell egg). In fact, Rossi et al. (1996) and Hidalgo et al. (1995) found similar values in fresh and pasteurised eggs (one day after laying) since the pasteurisation process did not significantly influence the furosine level of whole egg (Hidalgo et al., 1996). This low glycation level of protein corresponds to about 1.2 g/100 g dry basis for free D-glucose and is in agreement with literature data for the native D-glucose content in fresh/pasteurised whole egg (Toney & Bergquist, 1983). In contrast, whole egg powder contains only 0.74 g/100 g (dry basis) of free D-glucose due to glycation reaction in the course of industrial spray-drying. The glucose involved in Amadori compounds (0.53 g/100 g, dry basis) calculated from blocked lysine fits very well with the difference between glucose found in pasteurized egg and in dried egg (1.22–0.74 g/100 g = 0.48 g/100 g). The blocked lysine in spray-dried and pasteurized eggs was 13.3% and 0.46% of total lysine, respectively (Table 4).

Table 3
Chemical composition of pasteurised and dried eggs (means ± SD)

	Pasteurised egg	Spray-dried egg
Dry matter (g/100 g)	25.1 ± 0.21	95.3 ± 0.15
Reducing sugars[a] (g/100g db[b])	1.27 ± 0.02	0.84 ± 0.00
Protein (g/100 g db)	47.3 ± 0.58	47.5 ± 0.69
Total lipid (g/100 g db)	45.2 ± 0.00	45.8 ± 1.56
Furosine (mg/100 g protein)	15.2 ± 0.64	501 ± 12.8
Lysine (g/100 g protein)	6.99 ± 0.29	6.73 ± 0.09
Total tocopherols (mg/100 g db)	22.9 ± 0.03	23.0 ± 1.00
α-Tocopherol (mg/100 g db)	20.4 ± 0.03	20.3 ± 0.00
γ-Tocopherol (mg/100 g db)	2.53 ± 0.01	2.68 ± 0.09
Total retinols (mg/100 g db)	0.51 ± 0.00	0.51 ± 0.00
13-*cis* retinol (mg/100 g db)	0.07 ± 0.00	0.09 ± 0.00
All-*trans* retinol (mg/100 g db)	0.44 ± 0.01	0.42 ± 0.01
Total COPs (μg/g fat)	24.5 ± 3.90	54.7 ± 0.28
7α-OH (μg/g fat)	7.3 ± 1.77	14.3 ± 0.07
7β-OH (μg/g fat)	5.2 ± 0.21	11.0 ± 1.84
α-Epo (μg/g fat)	0.7 ± 0.85	4.9 ± 1.27
β-Epo (μg/g fat)	4.6 ± 0.57	10.0 ± 1.34
Triol (μg/g fat)	2.1 ± 0.19	2.0 ± 0.55
7-k (μg/g fat)	4.6 ± 1.56	9.0 ± 0.78

[a] Glucose + fructose.
[b] Dry basis.

The COPs in liquid pasteurised egg were about 24.5 μg/g fat. The spray-drying process doubled the COPs content, reaching values of 54.7 μg/g fat. Only α-epo showed a different trend, because its amount increased more than 7 times, as reported in Table 3 (from 0.7 to 4.9 μg/g fat).

Table 4
Furosine content and lysine fractions of pasteurised and spray-dried eggs

Sample	Furosine		Lysine fractions					
			Chromatographed		Available[a]	Blocked		Total[d]
	g/100 g db	g/100 g protein	g/100 g db	g/100 g protein	g/100 g db	g/100 g db[b]	% Total lysine[c]	g/100 db
Pasteurised egg	0.008	0.015	3.31	6.99	3.30	0.015	0.46	3.32
Spray dried egg	0.237	0.500	3.18	6.73	2.97	0.456	13.31	3.43

db, dry basis.
[a] Chromatographed lysine – (0.45 × blocked lysine).
[b] (1/0.30 × furosine)(MW lys/MW fur).
[c] (Blocked lysine × 100)/(blocked lysine + available lysine).
[d] Available lysine + blocked lysine.

There was no significant variation of tocols in pasteurised and spray-dried egg, whereas retinols showed a *trans–cis* isomerization, in accordance with data reported for dairy products by Panfili et al. (1998). The unusual presence of *cis*-isomers in raw eggs is probably due to their presence in feeds or to a metabolism of these isomers in hens different from other species. In fact, many animal products, such as unprocessed meat and milk, do not show detectable levels of *cis*-isomers.

These results showed that neoformation compounds, such as Amadori compound determined through furosine, COPs, and *cis*-isomers of retinols, are effective markers of the whole-egg drying treatment.

3.2. Storage assessment

The analytical results obtained during the storage of egg powder at 4 and 20 °C for 1, 3, 6 and 12 months and the control (the sample just spray-dried) are shown in Tables 5–8. Table 5 lists the reducing sugar and furosine evolutions during storage of powdered egg. The storage at 4 °C did not significantly affect the glucose and furosine amounts. In samples stored at 20 °C, glucose decreased markedly, reaching 1/5 of the initial value after a 12-month storage (from 0.74 to 0.15 g/100 g dry basis). However, a proportional increase of furosine in samples stored at the same temperature was not observed. A 20% increase of furosine was observed only during the first 3 months, then the value slightly decreased.

The bell-shaped concentration of furosine could be attributed to the degradation of the Amadori compound at advanced stages of MR since the Amadori compound is only an intermediate product; at this stage the degradation of Amadori compound was faster than its formation, as already reported in previous studies (Ferrer

Table 5
Reducing sugar and furosine contents of spray-dried egg during storage

	Glucose (g/100 g db)		Fructose (g/100 g db)		Furosine (mg/100 g protein)	
Time 0	0.74[a]		0.10[a]		501[d,c]	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
1 month	0.79[a]	0.43[c]	0.11[a]	0.10[a,b]	512[c]	585[a]
3 month	0.76[a]	0.27[d]	0.09[a,b]	0.09[a,b]	484[d]	598[a]
6 month	0.75[a]	0.17[e]	0.09[a,b]	0.07[a,b]	501[c,d]	579[a]
12 month	0.66[b]	0.15[e]	0.11[a]	0.06[b]	510[c]	556[b]

For each parameter, different letters indicate statistically significant differences at $P \leqslant 0.05$.
db, dry basis.

Table 6
COPs content (μg/g fat) of spray-dried egg during storage

	7α-OH		7β-OH		α-epo		β-epo		Triol		7-k		Total COPs	
Time 0	14.3[c]		11.0[e]		4.9[b]		10.0[c]		2.0[b]		9.0[b,c]		54.7[c,d]	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
1 month	11.7[c]	17.4[c]	11.6[e]	16.3[d]	2.2[b,c]	4.1[b,c]	11.4[c]	14.0 [b,c]	2.1[b]	1.8[b]	5.5[c]	9.8[b,c]	47.8[c,d]	63.8[c,d]
3 month	12.1[c]	24.8[b]	12.3[d,e]	28.9[c]	2.7[b,c]	9.8[a]	11.1[c]	25.9[b]	2.0[b]	2.4[b]	6.3[b,c]	14.8[a,b]	46.9[c,d]	109[b]
6 month	12.3[c]	33.4[a]	9.3[e]	39.8[b]	2.7[b,c]	11.7[a]	8.5[c]	40.9[a]	1.0[b]	1.6[b]	5.6[b,c]	16.2[a]	40.8[d]	145[a]
12 month	16.4[c]	39.4[a]	14.6[d,e]	47.7[a]	5.7[b]	11.0[a]	13.2[c]	42.2[a]	4.7[a]	4.8[a]	10.8[b]	16.5[a]	68.5[c]	167[a]

For each parameter, different letters indicate statistically significant differences at $P \leqslant 0.05$.

Table 7
Tocopherol content (mg/100 g db) of spray-dried egg during storage

	α-Tocopherol		γ-Tocopherol		Total tocopherols	
Time 0	20.3[a]		2.68[a]		23.0[a]	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
1 month	19.5[a]	17.8[b]	2.27[b]	1.96[c]	21.8[b]	19.8[c]
3 month	19.5[a]	18.2[b]	2.29[b]	1.83[c,d]	21.8[b]	20[c]
6 month	19.8[a]	17.5[b]	2.24[b]	1.75[c,d]	22[b]	19.3[c]
12 month	20.0[a]	17.6[b]	2.08[c]	1.82[c,d]	22.1[b]	19.4[c]

For each parameter, different letters indicate statistically significant differences at $P \leqslant 0.05$.
db, dry basis.

Table 8
Retinol content (mg/100g db) of spray-dried egg during storage

	13-*cis* retinol		all-*trans* retinol		Total retinols	
Time 0	0.09[a]		0.42[a]		0.51[a,b]	
	4 °C	20 °C	4 °C	20 °C	4 °C	20 °C
1 month	0.09[a]	0.08[b]	0.44[a]	0.36[b]	0.53[a]	0.44[c]
3 month	0.09[a]	0.06[c]	0.43[a]	0.26[c]	0.52[a]	0.32[d]
6 month	0.08[b]	0.04[d]	0.42[a]	0.20[d]	0.50[a,b]	0.24[e]
12 month	0.08[b]	0.04[d]	0.38[b]	0.17[d]	0.46[b]	0.21[f]

For each parameter, different letters indicate statistically significant differences at $P \leqslant 0.05$.
db, dry basis.

et al., 2003; Guerra-Hernandez et al., 2002; Kramholler, Pischetsrieder, & Severin, 1993; Pellegrino et al., 1995; Ramirez-Jimenez et al., 2003; Resmini & Pellegrino, 1994; Sanz, del Castillo, Corzo, & Olano, 2003).

The limited increase in furosine during the storage may also be due to the low a_w of egg powder (0.32–0.35) since the optimal range of a_w for MR is 0.52–0.75 (O'Brien & Morissey, 1989).

In addition, some reports indicate that aminophospholipids, which represent a relevant amount of egg yolk (about 12%) (Li-Chan et al., 1995; Watkins, 1995) may likewise be targets for non-enzymatic browning, originating aminophospholipid-linked MR products (Utzmann & Lederer, 2000; Whitfield, 1992). The determination of these compounds could represent a further marker to evaluate the quality of dried eggs during storage (Bergquist, 1995; Utzmann & Lederer, 2000).

3.3. COPs and total cholesterol

Cholesterol in egg samples was 2.6 g/100 g fat; after 12 months of storage at 20 °C the oxidation affected about 0.6% of cholesterol since COPs concentration reached 167 μg/g fat.

Table 6 shows the evolution of each COP and total COPs during storage. The storage at 4 °C did not cause significant variation of COPs concentration. The storage at 20 °C caused a significant increase of total COPs in the first 6 months. Successively, during the following six months, the COPs increase appeared to be statistically insignificant. Some pathways of degradation or secondary reactions of COPs probably reduced their concentration, as already observed in other matrices (Boselli, Caboni, Frega, & Lercker, 2004; Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997). The main COPs detected were 7α-OH, 7β-OH, α-epo, β-epo, 7-k and triol, as shown in Fig. 1; their identification was confirmed by gas chromatography, coupled with mass spectrometric detection. The trend of COPs was peculiar and different with respect to results observed in other experiments. 7-k represented about 17% of total COPs, both in pasteurised egg and immediately after spray-drying. However, although 7-k increased during 12 months of storage at 20 °C, at the end of the storage it represented only 10% of total COPs. Model systems, meat, bakery products and milk products usually show predominant concentration of 7-k over all the other COPs (Caboni & Lercker, 1993; Gallina Toschi et al., 1995; Zunin, Evangelisti, Calcagno, & Tiscornia, 1996). In fresh and preserved meat, the 7-k amount was 30% of total COPs, whereas, in food containing eggs, the percentage decreased, such as in egg noodle, where 7-k was not the main COP (Boselli et al., 2004). The fact that 7-k was not the most represented COP, although the sample preparation causes the formation of additional amounts of 7-k from hydroperoxide degradation (Caboni & Costa, 1996; Rodriguez-Estrada, Costa, Pelillo, Caboni, & Lercker, 2004), suggests that the ketonic function has a selective reactivity, leading to formation of still unidentified compounds. The sum of

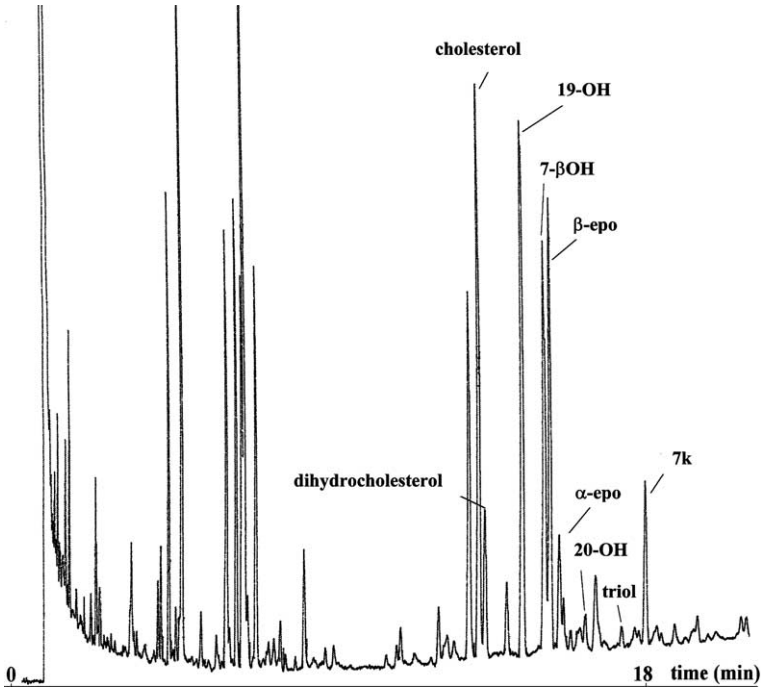

Fig. 1. Analysis of COPs in an egg sample stored at room temperature for 12 months.

7α-OH and 7β-OH represented 52% of total COPs at the end of the storage at room temperature. At the same stage, the sum of α-epo, β-epo and triol (which is formed by hydrolysis of the oxyranic ring) is about 35% of total COPs. The COPs amount, registered after spray-drying, was comparable to that reported in the literature (Guardiola et al., 1995). However, this author determined only α-epo and 7β-OH. 20-OH was also identified, but its amount reached 3.5 μg/g fat and no significant variation was observed during storage. Only samples stored for 12 months at 20 °C contained detectable amounts of 25-OH (2.0 μg/g fat).

It is remarkable that the shelf life of powdered eggs is currently fixed at 12 months. Moreover, egg powder is commonly used as an ingredient for different food preparations, sometimes needing further heat treatments, such as cooking or baking and further storage time; thus, the oxidation process can proceed further (Zunin et al., 1995).

3.4. Tocopherols

Table 7 lists the α and γ-tocopherol amounts during storage. The most abundant was α-tocopherol, but the evolution of the two tocopherols was not the same. Storage at 4 °C maintained the native α-tocopherol level for 12 months without a significant loss; however, at 20 °C, a 14% reduction was registered after 12-months storage. γ-tocopherol decreased significantly at 4 °C after 3 months. A loss of about 35% was registered at the end of the storage at 20 °C. Although the percentage reduction of γ-tocopherol was higher than that of α-tocopherol, the absolute decrease of α-tocopherol (−2.71 mg) was higher than γ-tocopherol (−0.86 mg). The results found in our system are in accordance with the chemical structures of the tocopherols and tocotrienols that support a hydrogen-donating power in the order α > β > γ > δ. However, many side reactions, affected by tocopherol concentrations, by temperature and light, type of substrate and solvent, enzymes and by other chemical species acting as prooxidants and synergists in the system, may change the relative in vitro activity (Bramley et al., 2000; Kamal Eldin & Appelqvist, 1996).

3.5. Retinols

Table 8 shows an insignificant 10% decrease for 13-*cis* and all-*trans* retinols in samples stored at 4 °C. However, about 60% of the initial contents of 13-*cis* and all-*trans* retinol were found after 12-months of storage at 20 °C. In dependence on several physical and chemical factors, such as light, temperature, pH, O_2, food composition and presence of antioxidants, retinols may undergo to reactions at the unsaturated isoprenoid side chain, by either oxidation or geometric isomerization. Literature reports (Jung, Lee, & Kim, 1998) have demonstrated that the presence of antioxidants, such as ascorbic acid, could inhibit the oxidation of 13-*cis*-retinol to other compounds not analytically quantified. The reactions of isomerization should lead to increase

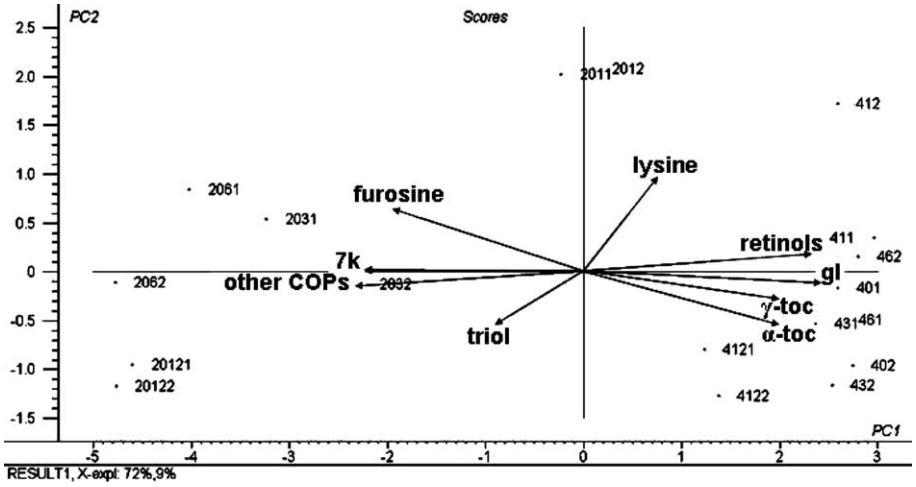

Fig. 2. Principal component analysis of stored egg samples. The samples are reported according to storage temperature, time and replication; for example 4121 is the sample stored at 4 °C for 12 months, first replicate. (gl = glucose)

of the 13-*cis* retinol levels (Panfili et al., 1998); however, the concomitant oxidative reactions of different forms of retinols and further isomerizations, lead, under our experimental conditions, to decreases of both 13-*cis*-retinol and all-*trans*-retinol.

The different decreases of tocopherol and retinol levels observed in this work may account for the synergism between the two cited antioxidants, as reported by Tesoriere et al. (1996).

3.6. Principal components analysis

The results of PCA of the storage of powdered egg are shown in Fig. 2 , which shows both the score plot of the samples and the loading of the variables. Therefore, this figure gives an overview of the results, which are analytically described in the previous sections. The first two principal components accounted for 72% and 9% of the variance; thus the plot describes 81% of the total variance. Two groups of variables were highly loaded on the first principal component: non-natural products, such as furosine and COPs, were negatively loaded. The native components, such as glucose (gl), tocopherols (α-toc and γ-toc) and retinols were positively loaded. Thus, the modifications of these two groups of chemical markers were inversely correlated. Samples with high contents of retinols, tocopherols and glucose are all those stored at 4 °C and are grouped in the same area on the right side of the score plot. These samples are clearly distinguished from the ones stored at 20 °C, which are rich in new formation products and are dispersed in the remaining part of the plot. The storage time has a dramatic influence on the formation of COPs, especially in samples stored at 20 °C: the longer the egg powder was stored at 20 °C, the greater was the formation of new products and thus the samples stored for longer time are gradually located at the left-bottom quadrant of the plot.

4. Conclusions

These results show that the storage conditions of powdered eggs affected their minor native constituents having high biological, vitamin and antioxidant activity.

The storage at room temperature caused noticeable losses of tocopherols and retinols. Moreover, their monitoring could be a useful tool to find the best technological and storage conditions.

Although furosine is a good marker for the technological treatment (pasteurisation and spray-drying), it is not appropriate for assessing the storage conditions of powdered whole egg. On the other hand, COPs represent an important marker of the storage conditions, since their concentration increases significantly during the shelf life of the product. The combination of these indicators is a significant tool for controlling the quality and safety of dried egg products.

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