Oxysterol Formation in Spray-Dried Egg Processed and Stored under Various Conditions: Prevention and Relationship with Other Quality Parameters

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A factorial arrangement was planned to study the influence of various factors (spray-drying temperature, antioxidant type, antioxidant concentration, packing conditions, and storage time) on various responses (oxysterol formation, fat UV absorptions, polyunsaturated fatty acid loss, color loss due to oxidation of carotenoids, and Maillard browning intensity) in egg powder. Positive correlations were found between oxysterol formation and the other responses. Use of low spray-drying temperatures prevented oxidation during processing and storage of egg powder. Vacuum packing and dark conditions were highly effective in preventing oxidation during storage. Propyl gallate seemed to be slightly effective in preventing polyunsaturated fatty acid loss, oxysterol formation, and color loss during processing and storage, whereas synergistic combination of ascorbyl palmitate plus *dl*-α-tocopherol seemed to show a slight prooxidant effect in terms of fatty acid and cholesterol oxidation. Propyl gallate concentrations of 100 and 200 ppm seemed to be optimal to prevent, respectively, polyunsaturated fatty acid loss and oxysterol formation and color loss. Propyl gallate seemed to be more effective under highly oxidative conditions.

Keywords: *Egg powder; spray-drying temperature; antioxidants; storage conditions; oxysterols; fatty acid oxidation; polyunsaturated fatty acid loss; Maillard browning*

INTRODUCTION

The method used in obtaining dried egg products, be it pan-drying, foam-drying, freeze-drying, or spray-drying, determines their quality (Bergquist, 1964, 1977). Freeze-drying has been used only in research due to its high cost. It is, however, the system that supplies the best quality because it is easy to reconstitute the dried egg and deterioration during the deshydration process is negligible. Thus, oxysterols (OS) are not detected in freeze-dried egg (Fontana et al., 1992; Tsai and Hudson, 1984), or their presence is minimal (Morgan and Armstrong, 1989; Nourooz-Zadeh and Appelqvist, 1987). Spray-drying is the most frequently used method in preparing powdered eggs. OS in spray-dried egg have been widely reported (Emanuel et al., 1991; Fontana et al., 1992, 1993; Guardiola et al., 1995b; Lai et al., 1995a,b; Nourooz-Zadeh, 1990; Sugino et al., 1986), and their formation is much greater in direct fired dryers than in indirect ones (Lai et al., 1995b; Missler et al., 1985; Morgan and Armstrong, 1987, 1992; Nourooz-Zadeh and Appelqvist, 1987; Tsai and Hudson, 1985). This has been attributed to the formation of nitrogen oxides (NO, NO_2) in air heated directly by a natural gas flame (Lai et al., 1995b; Missler et al., 1985; Morgan and Armstrong, 1987, 1992; Tsai and Hudson, 1985). Morgan and Arsmtrong (1987) showed that addition of N_2O to an indirect heating atomizer increased OS content in dried egg yolk, since N_2O decomposes to NO and NO_2 at high temperatures. In addition, Lai et al. (1995b) observed that OS formation during the storage of egg powder was greater in samples dried by direct heating than in samples dried by inderect heating. Other factors that affect OS formation are inlet and outlet temperatures (Morgan and Armstrong, 1987, 1992; Tsai and Hudson, 1985; Guardiola et al., 1995b) as well, it would seem, as the type of atomizer (box- or cyclone-type) and the product residence time inside the spray-drying chamber (Tsai and Hudson, 1985).

Fatty acids (FA) also undergo considerable oxidation during spray-drying of egg (Guardiola et al., 1995a); therefore, the nutritive value of egg powder decreases owing to oxidation of essential fatty acids. In addition, for oxidized FA (Chow, 1992; Kubow, 1990) and OS (Guardiola et al., 1996; Smith and Johnson, 1989) several biological effects have been reported: cytotoxicity, atherogenesis, mutagenesis, carcinogenesis, changes in cellular membrane properties, inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, etc.

This paper examines the influence of spray-drying temperature, type and concentration of antioxidant, and duration and conditions of storage on OS formation, FA oxidation, and color change due to oxidation of carotenoids and Maillard browning intensity (MBI) in egg powder. FA oxidation was studied by examining fat UV absorptions at the characteristic maxima of FA oxidation products (232, 270, and 303 nm) and polyunsaturated fatty acid (PUFA) loss. Possible correlations between OS formation and the other quality parameters were studied.

Two types of antioxidant were studied: one was the synergistic combination of ascorbyl palmitate (AP) and *dl*-α-tocopherol (α-T), and the other was propyl gallate (PG). AP and α-T are, respectively, an oxygen scavenger and a radical interceptor, and both are without toxicity problems and permitted in egg products by the European Union (European Parliament, 1995). Tocopherols are highly effective in preventing carotenoid oxidation but they show moderate thermostability (Dall'Aglio and Nicoli, 1992; Madhavi et al., 1996). However, Park and

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Table 1. Determinations and Definitions of Responses Studied

determinations (units of measure)	frozen egg ($n = 1$)	freshly produced egg powder ($n = 24$)	5-month egg powder ($n = 48$)	10-month egg powder ($n = 48$)	definition of responses
moisture[a] (% water)	D[b]	D	D	D	moisture of egg powder
a_W	ND[b]	D	D	D	a_W of egg powder
fat UV absorption at 232, 270, and 303 nm (specific absorbances: K_{232}, K_{270}, and K_{303})[a,c]	D	D	D	D	$\Delta K_\lambda = K_\lambda$ of egg powder $- K_\lambda$ of frozen egg[c]
fatty acid composition (compensated area normalization in parts per thousand)[d]	D	ND	ND	D	PUFA loss (C20:4n−6 and C22:6n−3) = PUFA in frozen egg − PUFA in egg powder[b]
OS content (ppm in solids)[b,e]	D	D	D	D	ΔOS (Δα-CE, Δ7β-HC, ΔCT, Δ7-KC, and Δ25-HC) = OS in egg powder − OS in frozen egg[b]
color (ppm of β-carotene in solids)[a]	D	D	D	D	color loss = color of frozen egg − color of egg powder
Maillard browning intensity (absorbance at 420 nm/g of solids)[a]	ND	D	D	D	Maillard browning intensity (MBI) of egg powder

[a] Determined as described by Guardiola et al. (1995b). [b] D, determined; ND, not determined; PUFA, polyunsaturated fatty acids; OS, oxysterols; α-CE, cholesterol 5α,6α-epoxide; 7β-HC, 7β-hydroxycholesterol; CT, cholestanetriol; 7-KC, 7-ketocholesterol; 25-HC, 25-hydroxycholesterol. [c] Specific absorbances were calculated by applying the formula $K_\lambda = E^{1\%}{}_{1\text{cm}\ \lambda} = A_\lambda/(CW)$, where A_λ is the absorbance at λ, C is the concentration of the cyclohexanic solution expressed as grams of fat/100 mL, and W is the width of the spectrophotometer cell in cm. [d] Determined as described by Guardiola et al. (1994b). [e] Determined as described by Guardiola et al. (1995c).

Addis (1986) showed that combination of AP (500 ppm) and α-T (100 ppm) prevented the formation of OS in tallow which had been heated at 135 °C for 70 h. In addition, AP and synergistic mixtures with tocopherols are highly effective in protecting deep-frying fats and oils (Gordon and Kouřimská, 1995a,b; Madhavi et al., 1996). Among synthetic radical terminators, gallates are less toxic and thermostable. However, PG is stable up to 190 °C (Dziezak, 1986; Madhavi et al., 1996), which is more than enough considering that the maximun temperature reached by egg powder particles inside the spray-dryer chamber was 140 °C (maximum outlet temperature).

MATERIALS AND METHODS

Reagents and Standards. AP (>99%) was obtained from Fluka Chemie AG (Buchs, Switzerland). α-T (95%) and PG (>99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Glyceryl monostearate was supplied by Henkel KGaA (Düsseldorf, Germany). Standards used for OS determination were as follows: cholesterol 5α,6α-epoxide (α-CE), 7β-hydroxycholesterol (7β-HC), cholestanetriol (CT), 7-ketocholesterol (7-KC), 25-hydroxycholesterol (25-HC), and 19-hydroxycholesterol from Sigma. All standards were weighed, to an accuracy of 0.01 mg, and were made up as ethyl acetate solutions. The remaining reagents and standards have been described previously by Guardiola et al. (1995b).

Experimental Design. A 2 × 2 × 3 × 2 × 3 factorial arrangement was planned to study the influence of the following factors, spray-drying temperature (2), antioxidant type (2), antioxidant concentration (3), packing conditions (2), and storage time (3), on the following responses, moisture, water activity (a_W), increase in fat UV absorptions, PUFA loss, OS formation, color loss due to oxidation of carotenoids, and MBI in egg powder (Table 1). This arrangement was conducted twice.

Sample Preparation. Frozen pasteurized egg stored at −20 °C for 1 month was spray-dried to obtain egg powder samples. A 12 kg container of frozen egg was thawed and homogenized for 2 min, at 20 000 rpm, with an Ystral electric drive 10/20 3000 homogenizer (Liverpool, U.K.), and then an aliquot was taken to determine the parameters defined in Table 1 and cholesterol content, as reference values for frozen egg. The rest of the thawed egg was divided into 12 parts, in which the antioxidant was added at the corresponding concentration. Each part was assigned an order number at random and then stored at −20 °C until spray-drying.

For the first replicate of the factorial design, half of each part (500 g) was spray-dried in accordance with the assigned order number. Then, for the second replicate, the 500 g remaining was spray-dried following the same order.

Just before spray-drying, the egg was homogenized again for 30 s at 20 000 rpm and diluted by adding 25% of distilled water to facilitate the spray-dryer feeding. A diagram of this sample preparation is shown in Figure 1.

Thus, two batches were obtained for each of the 12 spray-drying conditions assayed in the factorial arrangement (Figure 1). Each batch was divided into five parts: one was used to determine responses in freshly produced egg powder; two were non-vacuum-packed and stored at room temperature for 5 and 10 months, respectively; and the remaining two were vacuum-packed, wrapped in aluminum foil, and stored at room temperature for 5 and 10 months, respectively.

Addition of Antioxidants. Two types of antioxidant were added: one was the synergistic combination of AP and α-T, and the other was PG. The antioxidants were added at three concentrations: 0, 100, and 200 ppm in liquid egg (for AP + α-T: 50 + 50 and 100 + 100). PG and α-T were added from solutions in ethanol. Solutions were prepared in appropriate concentrations so that 1 mL of solution added to 100 mL of liquid egg produced the desired final concentration. AP was added in the same way from a glyceryl monostearate emulsion.

Spray-Drying Conditions. All egg samples were processed in a cyclone-type spray-dryer (Spray-Drying Unit Type Minor 53, Niro Atomizer, Copenhagen, Denmark) equipped with an electric heater, with a feed rate of 10 mL/min, an air pressure of 6 kg/cm^2, and an egg powder residence time in the spray-dryer < 2.5 min. Inlet and outlet temperatures were fixed simultaneously by controlling the air flow through the system. The two following temperature conditions were assayed: TA, inlet = 170 °C and outlet = 117 °C; and TB, inlet = 225 °C and outlet = 140 °C. These two conditions are within the range of temperatures usually applied in experimental studies of egg spray-drying (Morgan and Armstrong, 1987, 1992; Lai et al., 1995b). However, commercially, the current tendency is to use the lowest temperature possible, and so outlet temperatures between 60 and 70 °C are usual (Bergquist, 1964, 1977; Tsai and Hudson, 1985).

Packing Procedure. Samples were vacuum- or non-vacuum-packed in 20 × 20 cm polypropylene five-layer film barrier bags, using a multivac machine (Wolfertschwenden, Germany). The vacuum-packed samples were wrapped in aluminum foil. Vacuum-packed and non-vacuum-packed samples were then set in groups of five and again, respectively, vacuum-packed or simply sealed in 32 × 28 cm polypropylene five-layer film barrier bags.

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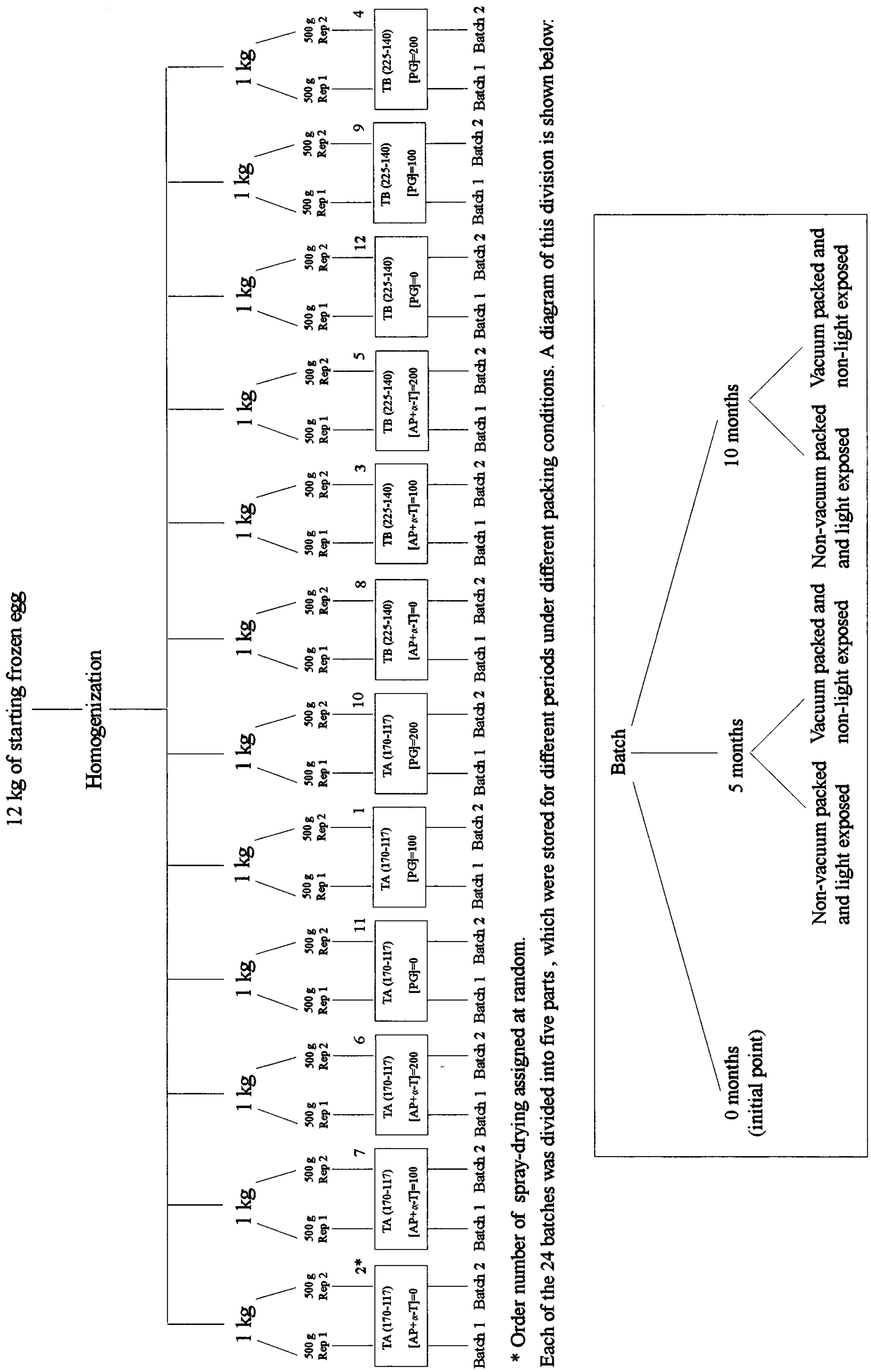

Figure 1. Sample preparation diagram.

Table 2. Pearson Correlation Coefficients and Significance Levels ($n = 120$)[a]

moisture	a_W	ΔK_{232}	ΔK_{270}	ΔK_{303}	C20:4n−6 loss[b]	C22:6n−3 loss[b]	Δα-CE	Δ7β-HC	ΔCT	Δ7-KC	Δ25-HC	color loss	MBI	
1.0000[c]	0.9895	0.6505	0.1715	0.1350	0.3412	0.3653	0.6990	0.7215	0.7803	0.6681	0.6738	0.7040	−0.0630	moisture
	P = 0.0000[d]	P = 0.0000	NS[e]	NS	P = 0.0176	P = 0.0107	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	NS	
	1.0000	0.6651	0.1897	0.1452	0.4148	0.4241	0.7145	0.7268	0.7915	0.6979	0.6789	0.7348	−0.0915	a_W
		P = 0.0000	P = 0.0380	NS	P = 0.0034	P = 0.0027	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	NS	
		1.0000	0.7606	0.7090	0.6999	0.7557	0.8238	0.7835	0.7859	0.7696	0.6754	0.7072	0.1566	ΔK_{232}
			P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	NS	
			1.0000	0.9871	0.6622	0.7319	0.4928	0.4180	0.3901	0.4330	0.3397	0.2634	0.2533	ΔK_{270}
				P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0001	P = 0.0037	P = 0.0053	
				1.0000	0.6054	0.6816	0.4397	0.3730	0.3229	0.3646	0.3068	0.1967	0.2847	ΔK_{303}
					P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0002	P = 0.0000	P = 0.0007	P = 0.0313	P = 0.0016	
					1.0000	0.9097	0.7253	0.7470	0.6110	0.5880	0.5506	0.5579	0.0470	C20:4n−6 loss
						P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0001	P = 0.0000	NS	
						1.0000	0.7711	0.7645	0.6466	0.6127	0.5911	0.5781	0.0671	C22:6n−3 loss
							P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	NS	
							1.0000	0.9550	0.9279	0.9254	0.8820	0.8031	0.3077	Δα-CE
								P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0006	
								1.0000	0.9135	0.8899	0.9033	0.8067	0.3303	Δ7β-HC
									P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0002	
									1.0000	0.9219	0.8959	0.8030	0.1849	ΔCT
										P = 0.0000	P = 0.0000	P = 0.0000	P = 0.0432	
										1.0000	0.8455	0.7999	0.2216	Δ7-KC
											P = 0.0000	P = 0.0000	P = 0.0150	
											1.0000	0.7356	0.3052	Δ25-HC
												P = 0.0000	P = 0.0007	
												1.0000	0.0920	color loss
													NS	
													1.0000	MBI

[a] See Table 1 for abbreviations. [b] $n = 48$. [c] Pearson correlation coefficient. [d] Significance level (P value). [e] Nonsignificant.

Methods. Moisture, fat UV absorptions, color, and MBI were determined as previously described by Guardiola et al. (1995b). FA composition was determined as previously described by Guardiola et al. (1994b, 1995a). OS determination was carried out following the method proposed by Guardiola et al. (1995c). Cholesterol was determined as previously described by Guardiola et al. (1994a). a_W was determined using a Novasina Thermoconstanter Humidat T-2 (Pfäffikon, Switzerland).

Statistics. Pearson coefficients were used to examine possible linear correlations between responses. P values $\leq$ 0.05 were considered significant.

To determine whether any significant effects were produced by the studied factors (spray-drying temperature, antioxidant type, antioxidant concentration, storage time, and packing conditions) on the responses, three multifactor ANOVA (MANOVA) were performed. The first MANOVA (n = 120) was performed considering storage time and packing conditions as a single factor. The second MANOVA (n = 96, results from initial point were excluded) was perfomed considering storage time and packing conditions as separate factors. The third MANOVA (n = 48) was applied to examine the influence of spray-drying temperature, antioxidant type, antioxidant concentration, and packing conditions on PUFA losses in samples stored for 10 months. In all cases, interactions higher than order 2 were ignored and P values $\leq$ 0.05 were considered significant.

Multiple-regression equations were calculated to describe the influence of quantitative factors (spray-drying temperature, antioxidant concentration, and storage time) on the responses. A stepwise variable selection procedure was used to select the factors and their interactions that significantly influenced ($P \leq 0.10$) each response. Regression equations present the following quadratic polynomial equation:

$$Y = B_0 + \sum_{i=1}^{i=3} B_i X_i + \sum_{i=1}^{i=3} \sum_{j=1}^{j=3} B_{ij} X_i X_j \qquad (1)$$

Y is the response studied; B_0, B_i, and B_{ij} are the regression coefficients; and X_i and X_j are the quantitative factors. Prior to calculation, orthogonalization of quantitative factors was carried out by reducing and centering their values to estimate linear and quadratic effects independently (Peng, 1967). Reduced and centered values for factors were as follows: outlet temperature (X_1), 117 °C = −1 and 140 °C = 1; antioxidant concentration (X_2), 0 ppm = −1, 100 ppm = 0, and 200 ppm = 1; and storage time (X_3), 0 months = −1, 5 months = 0, and 10 months = 1.

As our design, in addition to the quantitative factors, incorporates two qualitative factors at two levels (antioxidant type and packing conditions), four regression equations were calculated for each response corresponding to the following combinations: (1) AP and α-T with no vacuum packing and light exposure; (2) PG with no vacuum packing and light exposure; (3) AP and α-T with vacuum packing and without light exposure; (4) PG with vacuum packing and without light exposure. As only those samples stored for 10 months were analyzed for PUFA loss, storage time could not be used to fit the equations.

Outlet temperature was used to calculate regression equations and to plot graphs, since this temperature is the maximum temperature reached by egg powder particles.

Influence of Antioxidant Addition on Spectrophotometric Readings. PG shows absorption maxima at 231 and 273 nm and the combination of AP and α-T at 209, 223, 245, and 285 nm. Methanolic solutions containing 100 and 200 ppm of antioxidants were prepared. Each methanolic solution (3.5 mL) was submitted to the entire analytical process for specific absorbance determination (K_{232}, K_{270}, and K_{303}). Final extracts of PG solutions showed very slight absorption at 232 nm > 270 nm > 303 nm. Final extracts of AP and α-T solutions showed slightly higher absorption at 232, 270, and 303 nm. These results may explain the effect of antioxidant concentration on ΔK_{232}, ΔK_{270}, and ΔK_{303}.

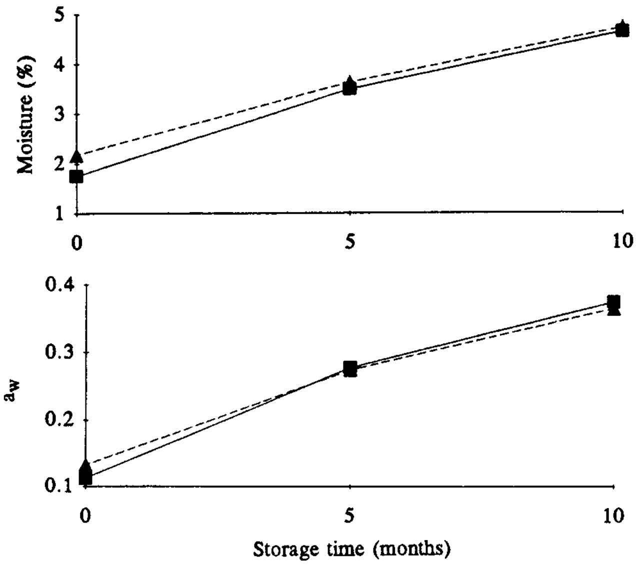

Figure 2. Influence of spray-drying temperature and storage time on moisture and a_W: (- -▲- -) TA (170−117 °C); (−■−) TB (225−140 °C).

RESULTS AND DISCUSSION

Results of moisture, UV fat absorptions, fatty acid composition, cholesterol and oxysterol content, and color from starting frozen egg did not differ from other frozen egg samples that were anlyzed (data not shown).

Table 2 shows a high correlation between several of the studied responses. Particularly, note the very high correlation between formation of different OS determined and the other studied responses, with the exceptions of MBI, ΔK_{270}, and ΔK_{303}. Color loss and ΔK_{232} were the oxidative parameters that correlated most highly with OS formation. Correlation between formation of different OS was very strong, which indicates a similar pattern of accumulation. As expected, correlation was high between moisture and a_W, between ΔK_{270} and ΔK_{303}, and between and C20:4n−6 and C22:6n−3 loss, which allowed these responses to be grouped when influence of factors were studied. Highest correlations of ΔK_{270} and ΔK_{303} were with C20:4n−6 and C22:6n−3 loss. Only correlations between ΔK_{270} and moisture; between ΔK_{303} and moisture and a_W; and between MBI moisture, a_W, ΔK_{232}, C20:4n−6, C22:6n−3, and color loss were not significant.

Table 3 shows least-squares means for responses as influenced by factors and P values for factors and their interactions that have a significant effect on responses. Results of this table were obtained from MANOVA (n = 120), considering storage time and packing conditions as a single factor. Least-squares means as influenced by interactions between factors are not shown in Table 3 to facilitate the understanding of results, since there are so many and a lot of them are without relevance. Table 4 shows P values for factors and their interactions that have a significant effect on responses. These P values were obtained from MANOVA (n = 96, results from initial point were excluded), considering storage time and packing conditions as separate factors. Least-squares means from this MANOVA are not shown.

Moisture and a_W. Storage time, packing conditions, and the interaction of the two influenced moisture and a_W (Tables 3 and 4). Increase in these responses over time was clearly greater in non-vacuum-packed samples (Table 3). As Figure 2 shows (data not shown in Table 3), spray-drying temperature and its interaction with

Table 3. Least-Squares Grand Mean (Global Mean) and Least-Squares Means for Responses As Influenced by Factors [*P* Values for Factors and Their Interactions That Have a Significant Effect on Responses Were Obtained from MANOVA (*n* = 120), Considering Storage Time and Packing Conditions as a Single Factor]

	egg powder												
	global mean	spray-drying temp (*n* = 60)		antioxidant type (*n* = 60)		antioxidant concn (ppm) (*n* = 40)			storage time and packing conditions (*n* = 24)				
responses[a]	(*n* = 120)	(170−117 °C)[b]	(225−140 °C)	AP + α-T[c]	PG	0	100	200	A[d]	B	C	D	E
moisture[f,g]	3.69	3.78	3.61****	3.72	3.67	3.55	3.74	3.79****	1.96	4.67	2.46	6.09	3.29****
a_W[g,j]	0.283	0.283	0.284	0.285	0.281	0.276	0.285	0.289***	0.123	0.378	0.173	0.496	0.247****
ΔK_{232}[f−h]	7.17	6.72	7.63****	7.13	7.22	6.89	7.20	7.42****	6.39	7.41	6.90	8.07	7.10****
ΔK_{270}[f−h]	1.45	1.21	1.70****	1.43	1.47	1.24	1.50	1.61****	1.37	1.47	1.45	1.51	1.45**
ΔK_{303}[f−h]	0.89	0.69	1.10****	0.89	0.90	0.69	0.94	1.05****	0.85	0.89	0.88	0.92	0.91
Δα-CE[f,g,i,j]	12.40	9.12	15.67****	12.99	11.81**	11.95	12.25	12.99	4.67	10.63	8.19	24.33	14.18****
Δ7β-HC[f−h]	30.80	24.50	37.10****	32.56	29.03***	31.63	30.16	30.60	12.77	27.44	18.94	55.86	38.97****
ΔCT[f,g,j]	6.14	4.87	7.42****	6.52	5.76**	6.07	5.81	6.54	2.06	6.63	3.49	11.99	6.55****
Δ7-KC[e−g,j]	23.31	18.54	28.08****	24.09	22.53	22.73	24.11	23.09	10.35	24.31	18.30	38.27	25.33****
Δ25-HC[f,g,j]	1.74	1.54	1.93****	1.82	1.66**	1.74	1.69	1.78	1.12	1.55	1.30	2.67	2.05****
color loss[e,g,h]	15.72	14.84	16.60****	15.77	15.67	16.29	15.61	15.26**	12.02	16.37	14.83	19.51	15.87****
MBI[f−h]	0.052	0.050	0.054**	0.053	0.051	0.050	0.053	0.052*	0.043	0.048	0.053	0.053	0.061****

[a] See abbreviations and units of measure for responses in Table 1. [b] (Inlet temperature − outlet temperature, °C). [c] AP + α-T, ascorbyl palmitate + *dl*-α-tocopherol; PG, propyl gallate. [d] A, 0 months; B (5 months) and D (10 months), non-vacuum-packed and light-exposed; C (5 months) and E (10 months), vacuum-packed and non-light-exposed. [e] Interaction of spray-drying temperature × antioxidant type significant at $P \leq 0.05$. [f] Interaction of spray-drying temperature × antioxidant concentration significant at $P \leq 0.05$. [g] Interaction of spray-drying temperature × storage time and packing conditions significant at $P \leq 0.05$. [h] Interaction of antioxidant type × antioxidant concentration significant at $P \leq 0.05$. [i] Interaction of antioxidant type × storage time and packing conditions significant at $P \leq 0.05$. [j] Interaction of antioxidant concentration × storage time and packing conditions significant at $P \leq 0.05$. *, Significant factor at $P \leq 0.05$ (**, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$).

Table 4. *P* Values Obtained from MANOVA (*n* = 96, Results from Initial Point Were Excluded) for Factors and Interactions That Have a Significant Effect on Responses

	responses[a]											
factor	moisture	a_W	ΔK_{232}	ΔK_{270}	ΔK_{303}	Δα-CE	Δ7β-HC	ΔCT	Δ7-KC	Δ25-HC	color loss	MBI
spray-drying temp (A)	0.0123[b]	0.0386	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0006
antioxidant type (B)	NS[c]	NS	AS[d]	0.0414	NS	0.0308	0.0004	0.0104	NS	0.0050	NS	0.0369
antioxidant concn (C)	0.0000	0.0000	0.0000	0.0000	0.0000	NS	NS	NS	NS	NS	0.0056	0.0074
storage time (D)	0.0000	0.0000	0.0000	NS	NS	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
packing conditions (E)	0.0000	0.0000	0.0000	NS	NS	0.0000	0.0000	0.0000	0.0000	0.0000	0.0120	0.0000
interactions												
A × B	0.0435	NS	NS	NS	NS	NS	NS	NS	AS	NS	0.0120	NS
A × C	0.0000	AS	0.0001	0.0000	0.0000	0.0000	0.0000	0.0034	0.0414	AS	NS	0.0016
A × D	NS	NS	NS	0.0017	0.0015	NS	NS	NS	NS	NS	0.0000	NS
A × E	NS	NS	0.0053	NS	NS	0.0028	0.0118	0.0012	0.0324	NS	AS	0.0004
B × C	NS	NS	0.0030	0.0006	0.0044	NS	0.0274	NS	NS	NS	NS	0.0040
B × D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B × E	NS	NS	NS	NS	NS	0.0112	NS	NS	NS	NS	NS	NS
C × D	NS	AS	NS	NS	NS	0.0003	NS	AS	NS	0.0257	NS	NS
C × E	0.0131	0.0205	NS	NS	NS	0.0398	AS	0.0415	NS	AS	NS	NS
D × E	0.0000	0.0000	0.0038	NS	NS	0.0000	0.0002	0.0011	0.0064	0.0048	0.0004	NS

[a] See Table 1 for abbreviations. [b] Significance level (*P* value). [c] Nonsignificant. [d] Almost significant ($P \leq 0.1$).

storage time influenced these responses (influence of interaction was not significant; Table 4). Samples obtained at high spray-drying temperatures initially showed lower moisture and a_W. During storage, these samples sorbed more water than samples produced at low temperatures and consequently a_W increased more, since sorbed water is freer.

ΔK_{232}, ΔK_{270}, and ΔK_{303}. Factors that significantly influenced these responses were spray-drying temperature, antioxidant concentration, and storage time and packing conditions (Tables 3 and 4). Increase of K_λ mostly occurred during spray-drying operation and was greater for samples produced at high spray-drying temperatures (Table 3). However, spray-drying temperature did not differentiate the evolution of ΔK_λ during storage (data not shown in Table 3). These responses (ΔK_λ) increased during storage, and they were greater for non-vacuum-packed and light-exposed samples (Table 3; nonsignificant for ΔK_{303}). Thus, oxidative degradation of egg powder was facilitated by high processing temperatures, long periods of storage, exposure to light, and non-vacuum-packing. Increase of these responses with antioxidant concentration was due, at least in part, to absorbance of these antioxidants in the UV region.

OS Formation. Since formation of determined OS was strongly correlated, factors that influenced their formation were the same. Spray-drying temperature, storage time, and packing conditions influenced OS formation (Tables 3 and 4). OS formation increased with spray-drying temperature and during storage (Table 3). OS formation during storage was greater for samples obtained at higher processing temperatures (data not shown in Table 3). Interaction between storage time and packing conditions significantly influenced OS formation, since OS formation during storage was clearly greater for non-vacuum-packed and light-exposed samples (Table 3). This confirms that packing methods which reduce oxygen availability (Chan et al.,

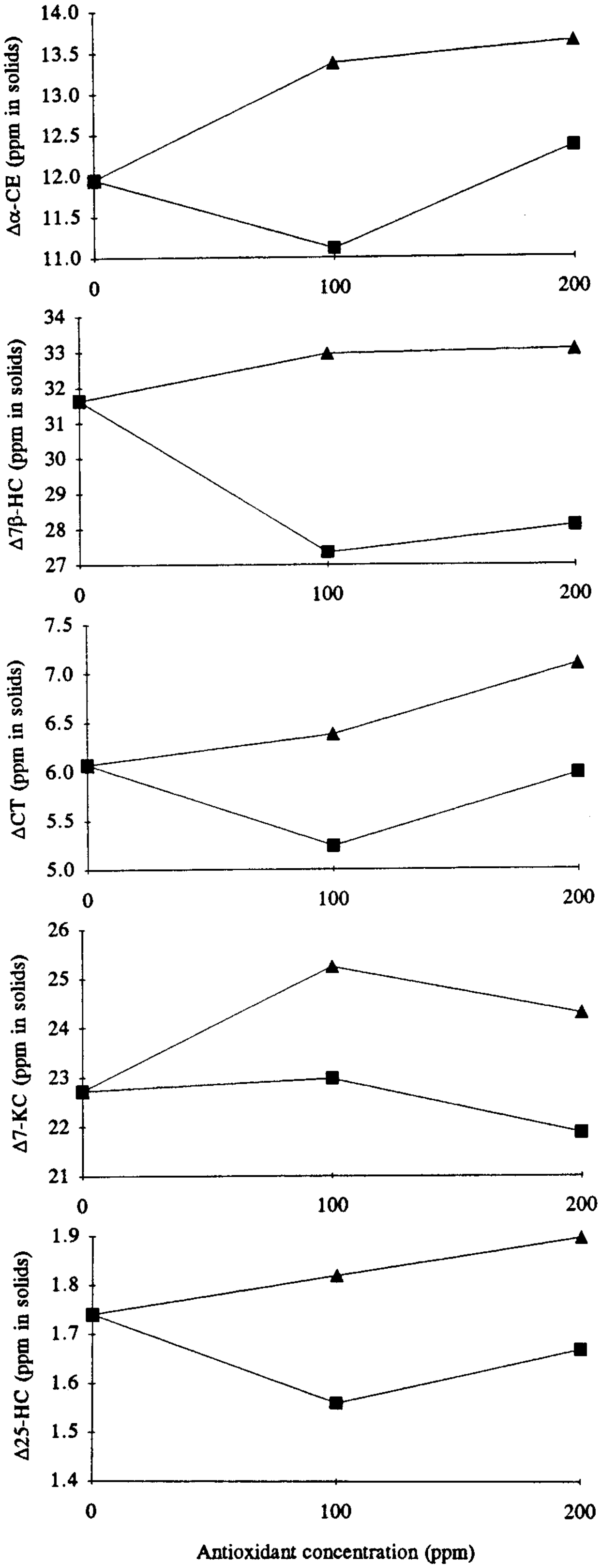

Figure 3. Influence of type and concentration of antioxidant on OS formation: (▲) AP + α-T; (■) PG.

1993) and darkness (Addis et al., 1996; Fontana et al., 1993) prevent cholesterol oxidation in foods.

PG prevented OS formation (Tables 3 and 4; nonsignificant for 7-KC). Although interaction between type and concentration of antioxidant was only significant for 7*β*-HC formation (Tables 3 and 4), Figure 3 (data not shown in Table 3) shows the effect of this interaction on formation of different OS determined. PG may have a slight effect in preventing OS formation, whereas synergistic combination of AP plus α-T seemed to show a slight prooxidant effect at 100 and 200 ppm (parts per million in liquid egg). PG concentration of 100 ppm seemed to be optimal in preventing OS formation. This agrees with the fact that gallates show optimum concentrations and may act as prooxidants at high levels (Madhavi et al., 1996). In addition, Morgan and Armstrong (1987) showed effectiveness of PG, BHA, and BHT in preventing hydrogen peroxide-induced cholesterol 5,6-epoxide formation during spray-drying of egg and PG was slightly more effective at 67 ppm than at 200 ppm (parts per million in yolk solids). However, high intrinsic vitamin E concentrations of egg (up to 500 ppm in solids), modified by dietary supplementation, prevented cholesterol oxidation during egg powder storage (Wahle et al., 1993). In addition, Huber et al. (1995) showed that AP (230 ppm in lipids), BHA (100 ppm), and a tocopherol blend (230 ppm) were effective

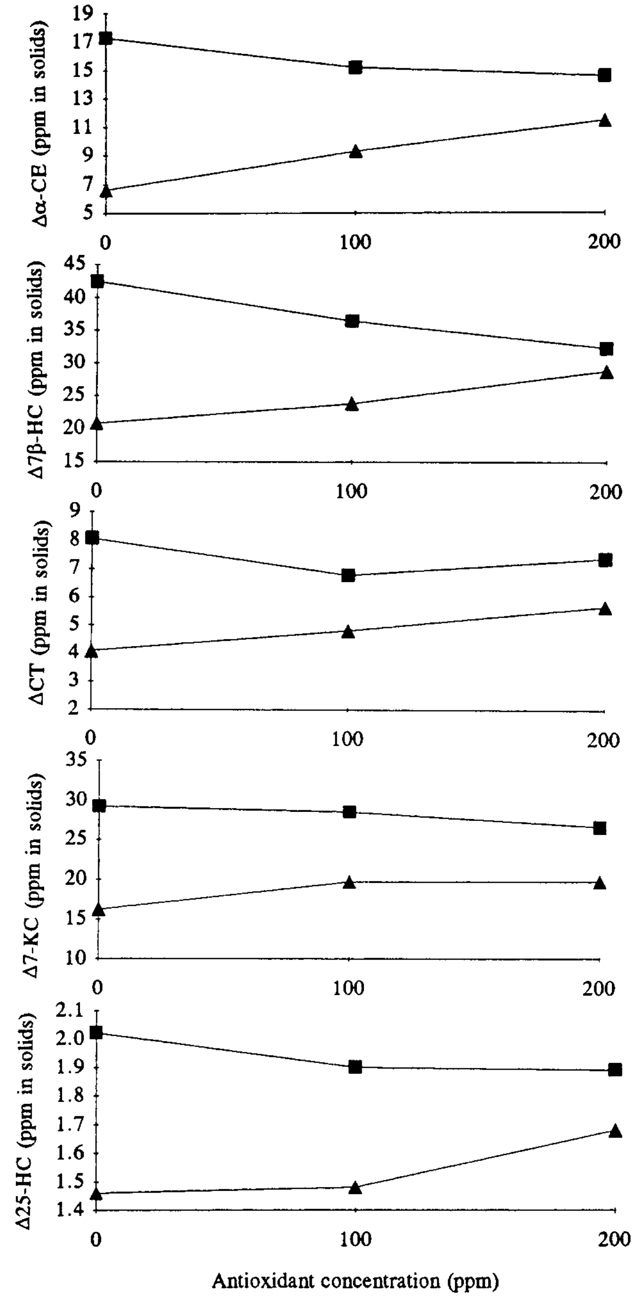

Figure 4. Influence of spray-drying temperature and antioxidant concentration on OS formation: (▲) TA (170−117 °C); (■) TB (225−140 °C).

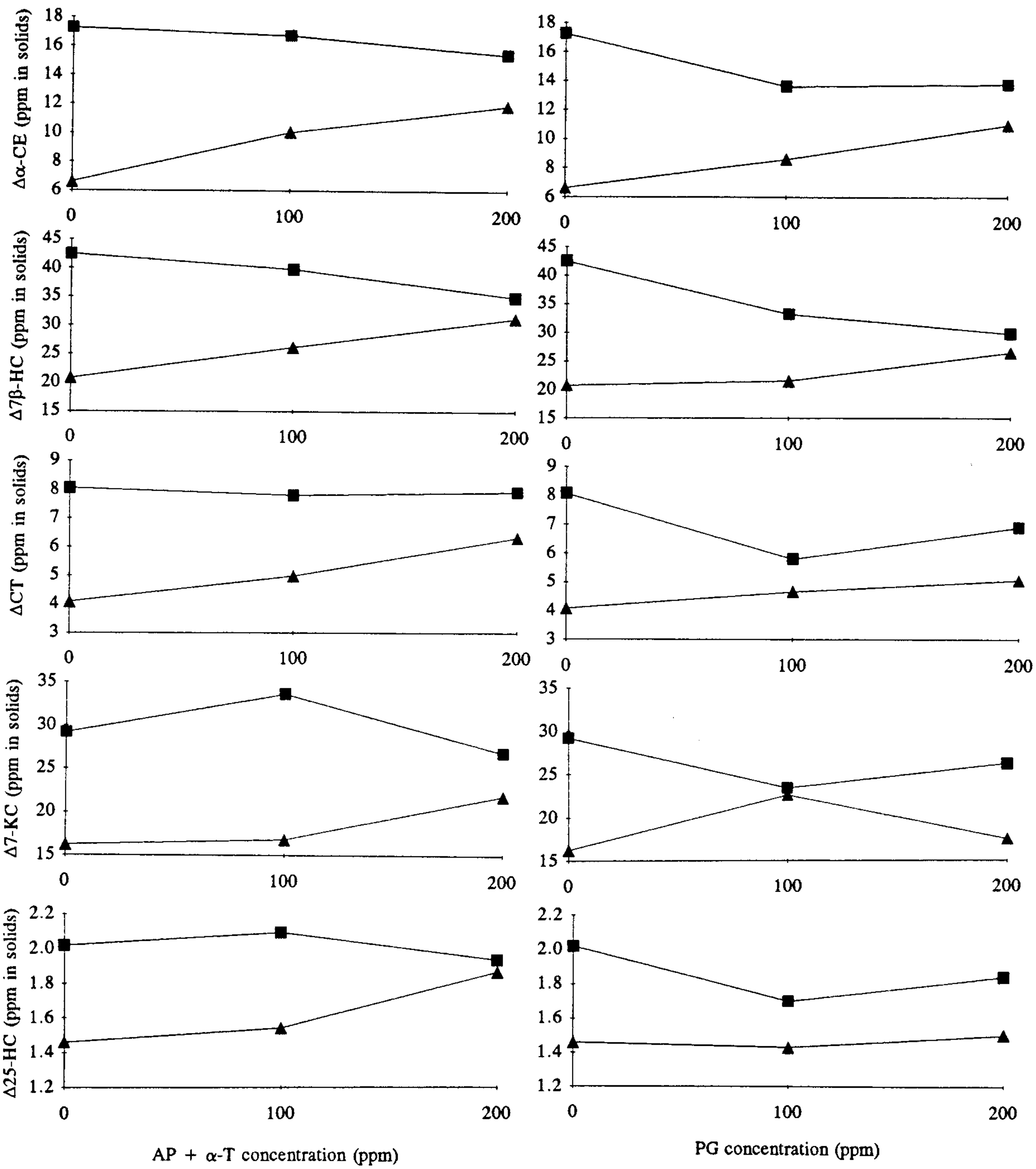

Figure 5. Influence of spray-drying temperature and type and concentration of antioxidant on OS formation: (▲) TA (170−117 °C); (■) TB (225−140 °C).

in preventing OS formation during accelerated storage of spray-dried egg yolk (Cu^{2+} catalyzed, 60 °C). Lai et al. (1995b) showed that rosemary oleoresin (500 ppm in lipids) is effective in preventing nitrogen oxide-induced OS formation during spray-drying and storage of egg powder. Interaction between spray-drying temperature and antioxidant concentration affected OS formation (Tables 3 and 4), which indicates that antioxidants were only effective at high temperatures (Figure 4, data not shown in Table 3). Although interactions higher than order 2 were ignored in the statistical treatment, Figure 5 (data not shown in Table 3) shows the influence of an order 3 interaction (spray-drying × antioxidant type × concentration of antioxidant) on OS formation. PG was the only antioxidant clearly effective at high spray-drying temperatures, whereas at low temperatures PG seemed to show a prooxidant effect to a lesser extent than AP + α-T.

Interaction between spray-drying temperature and packing conditions influenced OS formation, so that the higher the spray-drying temperature, the greater OS formation was, especially when samples were non-vacuum-packed (Figure 6; data not shown in Table 3). Effect of this interaction on 25-HC was not significant (Table 4).

Color Loss. This response increased with spray-drying temperature, storage time, non-vacuum packing, and light exposure and decreased with antioxidant concentration (Table 3). Interaction between antioxidant type and spray-drying temperature had a significant influence on this response (Figure 7; data not shown in Table 3), since PG prevented color loss more effectively through spray-drying and storage at high spray-drying temperatures, whereas a synergistic combination of AP and α-T prevented this loss more effectively at low spray-drying temperatures. As Figure

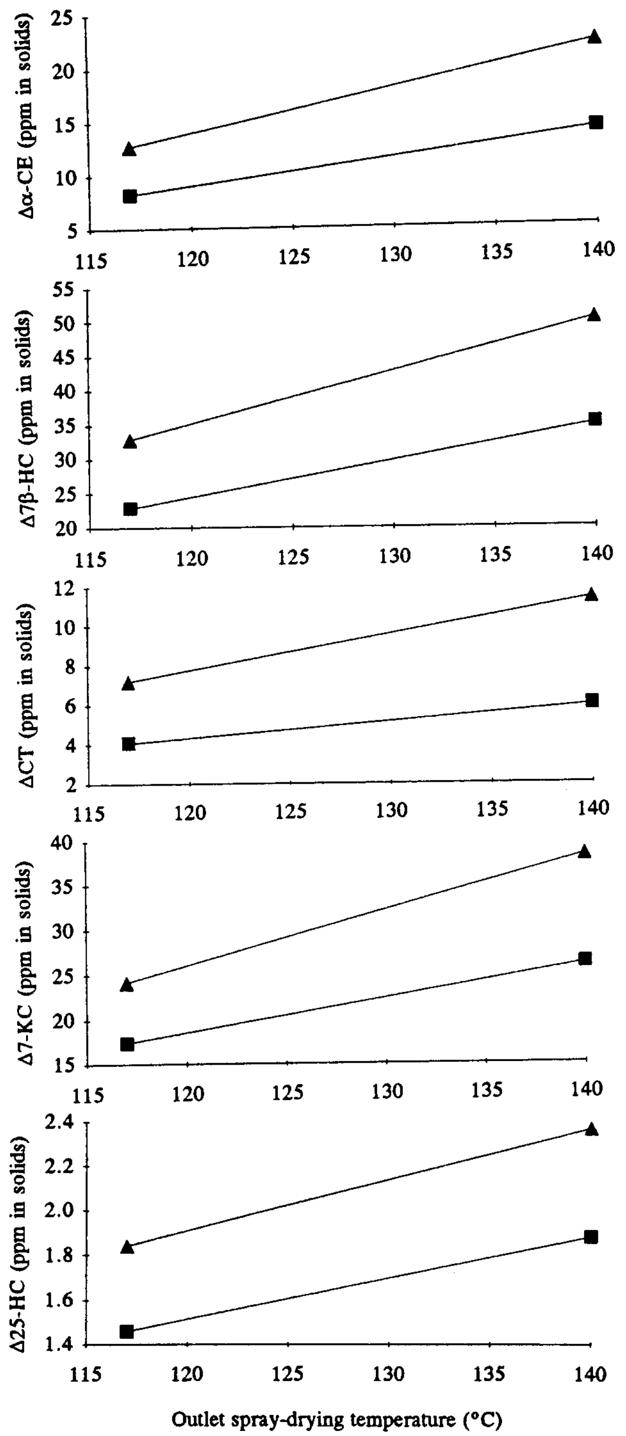

Figure 6. Influence of spray-drying temperature and packing conditions on OS formation: (▲) nonvacuum and light; (■) vacuum and nonlight.

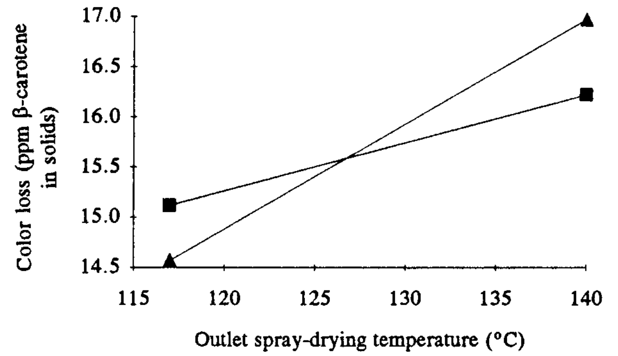

Figure 7. Infuence of spray-drying temperature and antioxidant type on color loss: (▲) AP + α-T; (■) PG.

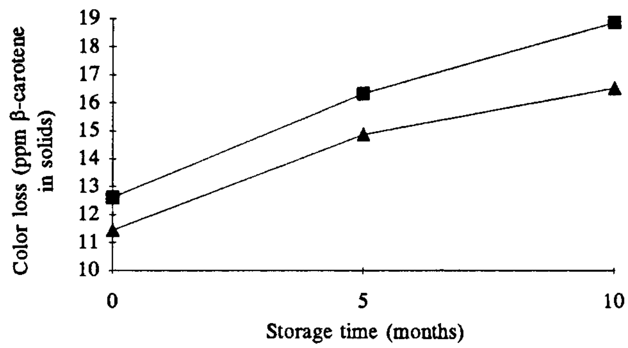

Figure 8. Influence of spray-drying temperature and storage time on color loss: (▲) TA (170−117 °C); (■) TB (225−140 °C).

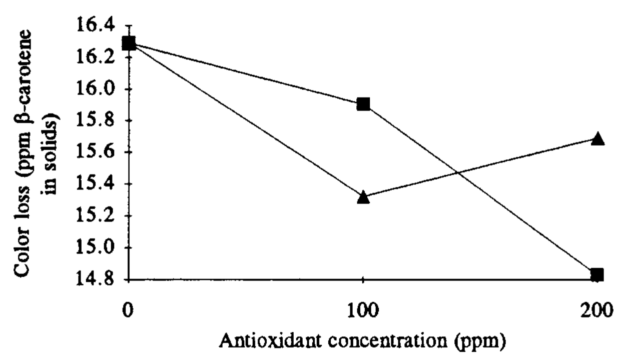

Figure 9. Influence of type and concentration of antioxidant on color loss: (▲) AP + α-T; (■) PG.

8 shows (data not shown in Table 3), interaction between spray-drying temperature and storage time had a significant influence on color loss. Egg powder produced at high temperatures lost color more easily through storage. Figure 9 (data not shown in Table 3) shows the effect of the interaction between type and concentration of antioxidant. PG was more effective in preventing color loss at 200 ppm, whereas combination of AP plus α-T was more effective at 100 ppm. This could be related to the prooxidant effect of α-T at high concentrations (Madhavi et al., 1996).

MBI. MBI was dependent on spray-drying temperature, storage time, and packing conditions (Tables 3 and 4). This response increased with spray-drying temperature and storage time and was higher when samples were vacuum-packed and not light-exposed (Table 3), which disagrees with the fact that the presence of oxygen does not modify or exceptionally increase Maillard browning and that Maillard browning increases with a_W up to values of 0.5−0.8 (Adrian, 1986; Ames, 1990). Thus, explanation of these results should be related to light, but a recent study conducted by Solomon et al. (1995) showed no effect of light on Maillard browning in orange juice.

C20:4*n*−6 and C22:6*n*−3 Loss. These responses were only studied in samples stored for 10 months, and they were dependent on spray-drying temperature, packing conditions, and antioxidant type (Table 5). PUFA loss during spray-drying and storage was greater when powdered egg was produced at high temperatures. It seems that PUFA loss occurs mostly during spray-drying (Guardiola et al., 1995a). These responses were very highly correlated with fat UV absorptions, which increased markedly during spray-drying and to a lesser extent during storage for 5 and 10 months (Table 3), which supports the fact that PUFA loss occurs mostly during spray-drying. PUFA loss was effectively pre-

Table 5. Least-Squares Grand Mean (Global Mean) and Least-Squares Means As Influenced by Factors for Polyunsaturated Fatty Acid (PUFA) Losses in Egg Powder Samples Stored for 10 Months [*P* Values for Factors and Their Interactions That Have a Significant Effect on PUFA Losses Were Obtained from MANOVA ($n = 48$)]

	egg powder									
	global mean	spray-drying temp ($n = 24$)		antioxidant type ($n = 24$)		antioxidant concn (ppm) ($n = 16$)			packing conditions ($n = 24$)	
response[a]	($n = 48$)	(170−117 °C)[b]	(225−140 °C)	AP + α-T[c]	PG	0	100	200	A[d]	B
C20:4*n*−6 loss	1.58	1.01	2.14****	1.76	1.39*	1.46	1.44	1.83	1.89	1.26***
C22:6*n*−3 loss[e]	1.99	1.29	2.70****	2.15	1.84	1.77	1.95	2.26	2.37	1.61***

[a] See units of measure for responses in Table 1. [b] (Inlet temperature − outlet temperature, °C). [c] AP + α-T, ascorbyl palmitate + *dl*-α-tocopherol; PG, propyl gallate. [d] A, non-vacuum-packed and light-exposed; B, vacuum-packed and non-light-exposed. [e] Interaction of spray-drying temperature × antioxidant concentration significant at $P \leq 0.05$. *, Significant factor at $P \leq 0.05$ (**, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$).

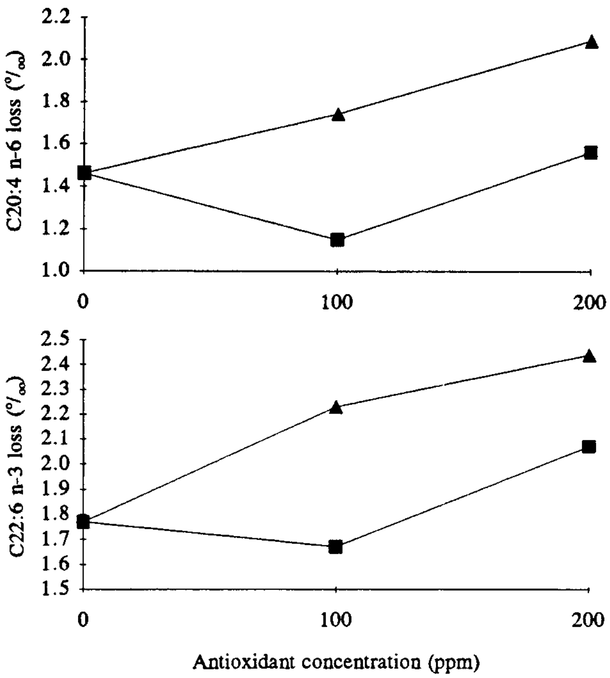

Figure 10. Influence of type and concentration of antioxidant on PUFA loss: (▲) AP + α-T; (■) PG.

vented by vacuum packing and darkness. PG prevented PUFA loss (Table 5; nonsignificant for C22:6*n*−3). Although interaction between type and concentration of antioxidant was not significant, Figure 10 (data not shown in Table 5) shows the effect of this interaction on PUFA loss. PG seemed to be slightly effective in preventing PUFA loss at 100 ppm, whereas synergistic combination of AP plus α-T seemed to show a slight prooxidant effect at 100 and 200 ppm. Prooxidant effect of tocopherols at high concentrations in terms of FA oxidation has been previously reported (Huang et al., 1994, 1995; Mukai et al., 1993; Mukai and Okouchi, 1989; Satué et al., 1995; Jung and Min, 1990). α-Tocopherol showed maximum antioxidant activity in various oils at 100 ppm (Huang et al., 1994; Jung and Min, 1990). Husain et al. (1987) showed prooxidant effect at 54 ppm *in an aqueous model with linolenic and α-tocopherol*. Prooxidant effect of AP in phospholipid vesicles has been reported (Fukazawa et al., 1993; Yin, et al., 1993). In addition, in terms of TBA value, ascorbic acid at different concentrations (30, 200, and 2000 ppm) showed prooxidant or antioxidant activity in oyster homogenate and ground fish depending on processing and storage conditions (Hatate and Kochi, 1992; Ramanathan and Das, 1992). However, a prooxidant effect of AP plus α-T has not been reported before, and this raises the question of how sample matrix and processing and storage conditions could influence antioxidant activity of this combination. Figure 11 (data not shown in Table 5) shows the influence of an order 3 interaction (spray-drying × antioxidant type × concentration of antioxidant) on PUFA loss. This figure shows that PG seemed to be effective in preventing PUFA loss at high spray-drying temperatures, whereas at low temperatures PG showed lower prooxidant effect than AP + α-T.

In conclusion, low spray-drying temperatures prevented oxidation during processing and storage of egg powder. Vacuum packing and darkness were very effective in protecting egg powder against oxidation during storage. PG seemed to be slightly effective in preventing PUFA loss, OS formation, and color loss during processing and storage, whereas synergistic combination of AP plus α-T seemed to show a slight prooxidant effect in terms of FA and cholesterol oxidation. PG concentrations of 100 and 200 ppm seemed to be optimal to prevent, respectively, PUFA loss and OS formation and color loss. PG seemed to be more effective under highly oxidative conditions (high spray-drying temperatures). Extrapolation of these conclusions to commercial practice must take into consideration that this study was conducted starting with frozen egg and stressing oxidative conditions during spray-drying (high outlet spray-drying temperatures). In addition, the effect of egg variability was not considered in this study since only one starting frozen egg sample was used.

From multiple-regression equations and their regression coefficients (Tables 6−8) the influence of quantitative factors on responses is described and response surfaces can be drawn to optimize experimental conditions for egg powder production and storage. As Tables 6 and 7 show, most equations presented high multiple-determination coefficients (r^2) and F ratio, which means that fitted equations accounted for a significant proportion of total variation in response. Fitted equations for MBI explained a low proportion of response variation. Fitted equations for a_W and moisture when samples were non-vacuum-packed and light-exposed showed much higher r^2 and F ratio than the other responses. In relation to PUFA loss, fitted equations showed lower r^2 and F ratio than for the other responses (Table 8). This was due to the fact that storage time was not studied and that a smaller sample size was used ($n = 12$, only samples stored for 10 months).

For example, to show the meaning of the regression coefficients, fitted equations for moisture when samples were non-vacuum-packed and light-exposed will be used (Table 6). From these regression coefficients it could be concluded that most of the variation for this response under these conditions was explained by storage time.

Table 6. Regression Coefficients (B) with Their Significance Level, Multiple Determination Coefficients (r^2), and F Ratio for Multiple Regression Equations Calculated Using Data from Samples Non-Vacuum-Packed and Light-Exposed[a]

coeff	moisture	a_W	ΔK_{232}	ΔK_{270}	ΔK_{303}	Δα-CE	Δ7β-HC	ΔCT	Δ7-KC	Δ25-HC	color loss	MBI
						Ascorbyl Palmitate + *dl*-α-Tocopherol (n = 36)						
B_0	4.6783****	0.3807****	7.3016****	1.5105****	0.9578****	11.1682****	29.5045****	7.3954****	25.5888****	1.6297****	15.9826****	0.0512****
B_1	−0.1339***	*b*	0.4930****	0.2303****	0.1841****	3.7722****	6.8321****	1.6344****	6.1737****	0.1859***	1.5879****	0.0039***
B_2	0.0870*	0.0052**	0.2196***	0.1426****	0.1552****	1.6329***	*b*	0.8708**	*b*	0.1500**	*b*	*b*
B_3	2.0400****	0.1860****	0.7848****	0.0694**	*b*	10.7378****	22.7913****	5.2944****	15.1014****	0.8113****	3.8272****	*b*
B_{22}	*b*	*b*	*b*	−0.1095	−0.1068**	*b*	*b*	*b*	*b*	*b*	*b*	−0.0085***
B_{33}	−0.6225****	−0.0675****	*b*	*b*	*b*	4.6481****	6.9963***	*b*	*b*	0.3266***	*b*	0.0086***
B_{12}	−0.1996****	−0.0046**	−0.3504****	−0.1020****	−0.0911****	−2.1401****	−5.6286****	*b*	*b*	−0.1867***	*b*	*b*
B_{13}	*b*	0.0044**	0.1723***	*b*	*b*	2.2625****	4.3425****	0.7310*	3.3109**	*b*	0.8659***	0.0035**
B_{23}	*b*	*b*	*b*	*b*	*b*	1.6925**	*b*	0.9088*	*b*	*b*	*b*	*b*
r^2	0.9868	0.9970	0.9160	0.8423	0.8303	0.9469	0.9426	0.8716	0.8424	0.8585	0.8729	0.5156
F ratio	403.42****	1810.13****	65.42****	32.06****	37.93****	71.27****	98.44****	40.74****	56.99****	36.40****	73.27****	8.25****
						Propyl Gallate (n = 36)						
B_0	4.6617****	0.3762****	7.2779****	1.4632****	0.8874****	8.6922****	25.3684****	5.6154****	23.0305****	1.4699****	15.9497****	0.0406****
B_1	−0.0954****	*b*	0.5372****	0.2672****	0.2193****	3.2551****	6.0868****	1.3429****	3.8383***	0.1855***	0.9859****	0.0025*
B_2	0.0491*	*b*	0.3005****	0.2164****	0.1969****	*b*	−2.8431**	*b*	*b*	*b*	*b*	*b*
B_3	2.0797****	0.1844****	0.8911****	0.0727***	0.0497**	8.9157****	20.3027****	4.6355****	12.8119****	0.7380****	3.6697****	*b*
B_{22}	*b*	*b*	*b*	*b*	*b*	2.0885*	*b*	1.1601*	*b*	*b*	*b*	*b*
B_{33}	−0.6622****	−0.0684****	*b*	*b*	*b*	3.0956***	6.7623***	*b*	*b*	0.3572***	*b*	0.0121****
B_{12}	−0.2376****	−0.0048**	−0.3172****	−0.0970****	−0.0989****	−2.2678****	−5.6100****	*b*	*b*	*b*	−0.7730**	−0.0038**
B_{13}	0.1022****	0.0077****	*b*	*b*	*b*	2.5683****	4.2120***	1.1084***	3.4623**	0.1780***	0.6100*	*b*
B_{23}	b	b	*b*	*b*	*b*	1.4204*	*b*	*b*	*b*	*b*	*b*	*b*
r^2	0.9960	0.9968	0.8798	0.8950	0.9093	0.9165	0.9252	0.8473	0.7860	0.8451	0.8280	0.4524
F ratio	1115.33****	2242.25****	56.73****	66.05****	77.65****	43.91****	59.77****	43.00***	39.18****	42.27****	37.30****	8.81****

[a] See Table 1 for abbreviations. [b] Nonsignificant effect. *, Significant effect at $P \leq 0.10$ (**, $P \leq 0.05$; ***, $P \leq 0.01$; ****, $P \leq 0.001$).

Table 7. Regression Coefficients (*B*) with Their Significance Level, Multiple Determination Coefficients (r^2), and *F* Ratio for Multiple Regression Equations Calculated Using Data from Samples Vacuum-Packed and Non-Light- Exposed[a]

coeff	moisture	a_W	ΔK_{232}	ΔK_{270}	ΔK_{303}	Δα-CE	Δ7β-HC	ΔCT	Δ7-KC	Δ25-HC	color loss	MBI
Ascorbyl Palmitate + *dl*-α-Tocopherol (n = 36)												
B_0	2.5988****	0.1741****	6.9603****	1.5292****	0.9961****	9.0702****	19.7669****	3.6250****	18.0624***	1.3513****	14.3364****	0.0512****
B_1	−0.1035***	*b*	0.3567****	0.2128****	0.1835****	2.4974****	4.7403****	0.8015****	4.0501****	0.1587***	0.6600***	*b*
B_2	0.2123****	0.0128**	0.1579***	0.1401****	0.1501****	*b*	*b*	*b*	*b*	*b*	−0.4858**	*b*
B_3	0.6148****	0.0600****	0.2234****	*b*	*b*	4.3404****	13.4053****	2.3107****	7.4404****	0.5220****	2.0101****	0.0044***
B_{22}	−0.1419*	*b*	−0.3416***	−0.1919****	−0.1773****	*b*	*b*	*b*	*b*	*b*	0.8500**	−0.0038*
B_{33}	0.1894**	0.0145**	*b*	*b*	*b*	*b*	7.3478****	0.8991***	*b*	0.3158***	−0.9346**	0.0085****
B_{12}	−0.0877*	*b*	−0.1043*	−0.0538**	−0.0575***	−0.8889**	−1.9248**	−0.4037**	*b*	*b*	0.5449****	−0.0022*
B_{13}	*b*	*b*	*b*	*b*	*b*	*b*	0.3818**	*b*	*b*	*b*	*b*	
B_{23}	*b*	0.0119**	−0.1236*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
r^2	0.9030	0.9176	0.7819	0.8732	0.8783	0.8515	0.9225	0.8992	0.7962	0.7341	0.7979	0.5177
F ratio	40.34****	77.91****	17.33****	53.35****	55.95****	61.16****	92.29****	53.50****	64.44****	29.45****	19.09****	8.32****
Propyl Gallate (n = 36)												
B_0	2.4108****	0.1728****	6.9641****	1.4962****	0.8824****	7.9930****	18.1161****	3.3477****	17.9212****	1.2553****	14.7558****	0.0477****
B_1	*b*	*b*	0.3848****	0.2561****	0.2290****	2.1500****	4.5472****	0.6573****	2.3755**	0.1393****	*b*	*b*
B_2	0.1825****	0.0077**	0.4100****	0.2487****	0.2140****	*b*	*b*	*b*	*b*	*b*	−0.9326****	*b*
B_3	0.6358****	0.0605****	0.4846****	0.0552**	0.0465**	5.1673****	12.7937****	2.1759****	7.5320****	0.4072****	1.8430****	0.0029**
B_{22}	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B_{33}	0.2192***	0.0114*	−0.1605*	−0.0710*	*b*	1.4387*	6.5058****	0.7815***	*b*	0.2410***	−0.8374**	0.0091****
B_{12}	−0.0742*	*b*	−0.0944*	−0.0678***	−0.0693****	−1.1927**	−2.2875***	−0.4515***	*b*	*b*	*b*	−0.0037***
B_{13}	0.1225***	0.0091**	−0.1280**	*b*	*b*	*b*	2.1203***	0.3918**	*b*	0.1453***	*b*	*b*
B_{23}	*b*	0.0099**	0.1238**	*b*	*b*	*b*	*b*	*b*	*b*	−0.0963*	*b*	*b*
r^2	0.9211	0.9160	0.9115	0.9256	0.9269	0.8592	0.9367	0.9044	0.6336	0.8208	0.7327	0.5231
F ratio	65.36****	61.03****	41.18****	74.61****	98.22****	47.29****	88.83****	56.79****	28.53****	27.49****	29.24****	11.70****

[a] See Table 1 for abbreviations. [b] Nonsignificant effect. *, Significant effect at $P \leq 0.10$ (**, $P \leq 0.05$; ***, $P \leq 0.01$; ****, $P \leq 0.001$).

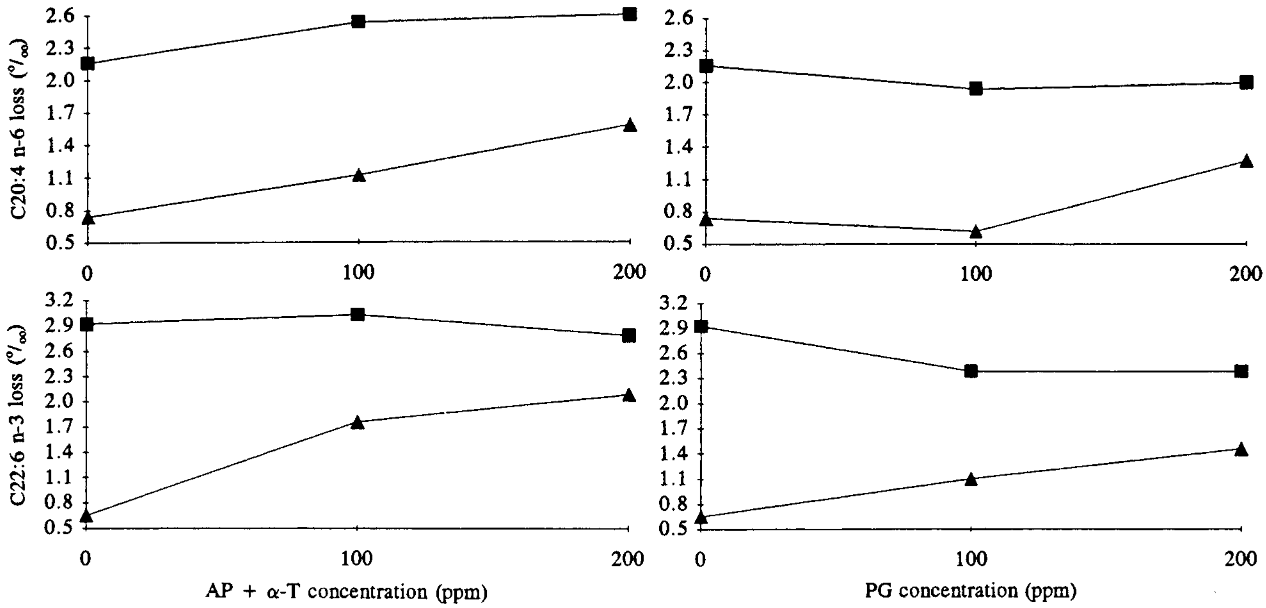

Figure 11. Influence of spray-drying temperature and type and concentration of antioxidant on PUFA loss: (▲) TA (170−117 °C); (■) TB (225−140 °C).

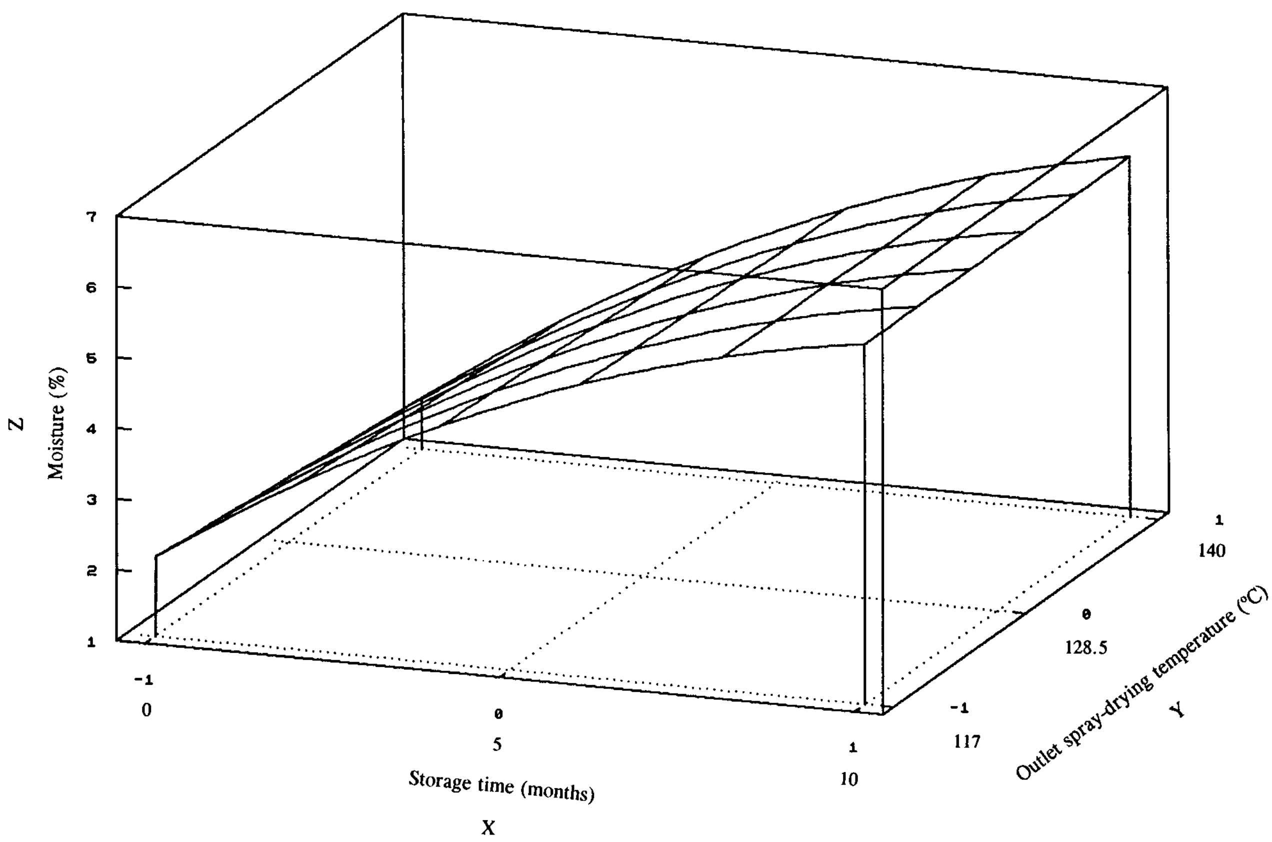

Figure 12. Moisture as a function of storage time and outlet spray-drying temperature when propyl gallate was used (100 ppm) and samples were non-vacuum-packed and exposed to light.

For instance, the fitted equation when PG was used is

$$Y = 4.6617 - 0.0954X_1 + 0.0491X_2 + 2.0797X_3 - 0.6622X_3^2 - 0.2376X_1X_2 + 0.1022X_1X_3 \quad (2)$$

This equation shows that the linear effect of storage time (B_3) on moisture was approximately 22 times greater than the linear effect of spray-drying temperature (B_1) and 42 times greater than the linear effect of antioxidant concentration (B_2). The positive sign of B_3 means that moisture increased during storage time, and the negative sign of B_1 means that egg powder obtained had less moisture as higher was spray-drying temperature. The constant term (B_0) for this equation was highly significant (Table 6). In addition to the linear term of storage time (B_3), the quadratic term (B_{33}) was also highly significant, which means that the increase of moisture during storage followed a curve instead of a straight line; the negative sign of B_{33} means that the curve was convex. Interaction terms B_{12} and B_{13} were also significant. The negative sign of B_{12} means that at a low level of spray-drying temperature

Table 8. Regression Coefficients (*B*) with Their Significance Level, Multiple Determination Coefficients (r^2), and *F* Ratio for Multiple Regression Equations Corresponding to C20:4*n*−6 and C22:6*n*−3 Loss

	non-vacuum- packed and light-exposed				vacuum-packed and non-light-exposed			
	AP + α-T[a] (n = 12)		PG (n = 12)		AP + α-T (n = 12)		PG (n = 12)	
coeff	C20:4*n*−6 loss	C22:6*n*−3 loss	C20:4*n*−6 loss	C22:6*n*−3 loss	C20:4*n*−6 loss	C22:6*n*−3 loss	C20:4*n*−6 loss	C22:6*n*−3 loss
B_0	2.1131****	2.6228****	1.6725****	2.1216****	1.4079****	1.6725****	1.1057****	1.5511****
B_1	0.8378***	0.8058**	0.5978***	0.8278***	0.3907**	0.4857**	0.4330***	0.7033***
B_2	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B_{22}	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
B_{12}	*b*	*b*	*b*	−0.4777*	*b*	*b*	*b*	*b*
r^2	0.5585	0.4975	0.5305	0.7247	0.4672	0.4851	0.5350	0.5736
F ratio	12.65***	9.90**	12.30***	11.85***	8.77**	9.42**	11.51***	13.45***

[a] AP + α-T, ascorbyl palmitate + *dl*-α-tocopherol; PG, propyl gallate. [b] Nonsignificant effect. *, Significant effect at $P \le 0.10$ (**, $P \le 0.05$; ***, $P \le 0.01$; ****, $P \le 0.001$).

(X_1) moisture increased with antioxidant concentration (X_2) and the contrary at a high level of spray-drying temperature. The positive sign of B_{13} means that at low spray-drying temperatures (X_1) moisture increased less during storage (X_3) than at high spray-drying temperatures. High values of r^2 (0.9960) and *F* ratio (111.33) showed the robustness of fitting.

From fitted eq 2 response surfaces can be drawn. Since response surface plots a response versus two factors, showing linear and quadratic effect of factors and their interaction, and three quantitative factors were studied, the two factors (X_1 and X_3) that more influenced moisture were chosen and three response surfaces were drawn corresponding to the different levels of the less influential factor (X_2). Equations of these response surfaces are as follows:

when $X_2 = -1$ (0 ppm)

$$Y = 4.6126 + 0.1422X_1 + 2.0797X_3 - 0.6622{X_3}^2 + 0.1022X_1X_3 \quad \text{or}$$

$$Z = 4.6126 + 2.0797X + 0.1422Y - 0.6622X^2 + 0.1022XY \quad (3)$$

when $X_2 = 0$ (100 ppm)

$$Y = 4.6617 - 0.0954X_1 + 2.0797X_3 - 0.6622{X_3}^2 + 0.1022X_1X_3 \quad \text{or}$$

$$Z = 4.6617 + 2.0797X - 0.0954Y - 0.6622X^2 + 0.1022XY \quad (4)$$

when $X_2 = +1$ (200 ppm)

$$Y = 4.7108 - 0.3330X_1 + 2.0797X_3 - 0.6622{X_3}^2 + 0.1022X_1X_3 \quad \text{or}$$

$$Z = 4.7108 + 2.0797X - 0.3330Y - 0.6622X^2 + 0.1022XY \quad (5)$$

By way of example, from eq 4 the response surface for moisture when PG was used (100 ppm) and samples were non-vacuum-packed and light-exposed can be drawn. Figure 12 shows that moisture slightly decreased with spray-drying temperature (B_1 negative and significant) and markedly increased during storage following a convex curve (B_3 positive and B_{33} negative, both significant). Also, the figure shows that moisture of freshly produced egg powder was greater when spray-drying temperature was low and that during storage the moisture became similar for both spray-drying temperatures (B_{13} positive and significant).

NOMENCLATURE

Cholestanetriol (CT), 5α-cholestane-3β,5,6β-triol; cholesterol, cholest-5-en-3β-ol; cholesterol 5α,6α-epoxide (α-CE), 5,6α-epoxy-5α-cholestan-3β-ol; 7β-hydroxycholesterol (7β-HC), cholest-5-ene-3β,7β-diol; 19-hydroxycholesterol, cholest-5-en-3β,19-diol; 25-hydroxycholesterol (25-HC), cholest-5-ene-3β,25-diol; 7-ketocholesterol (7-KC), 3β-hydroxycholest-5-en-7-one.

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