Stability of Polyunsaturated Fatty Acids in Egg Powder Processed and Stored under Various Conditions

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A 2 × 2 × 3 × 2 factorial arrangement was planned to study the influence of different factors (spray-drying temperature, kind of antioxidant, antioxidant concentration, and packing conditions) on polyunsaturated fatty acid (PUFA) losses in egg powder stored for 10 months. Higher spray-drying temperatures led to a higher loss of many PUFA, especially of C20:4$n-6$ ($P \leq 0.0001$) and C22:6$n-3$ ($P \leq 0.0001$). Storage of egg powder packed under vacuum and not exposed to light effectively prevents the loss of many PUFA, especially of C20:4$n-6$ ($P \leq 0.001$) and C22:6$n-3$ ($P \leq 0.001$). Antioxidants may be slightly effective in preventing PUFA losses under highly oxidative conditions. Spray-dried egg is a highly oxidized product, and, in conclusion, spray-dried egg yolk should be avoided as a long-chain PUFA source for enriching weaning foods, since oxidized fatty acids lose their essentiality and present several harmful biological effects.

Keywords: *Egg powder; spray-drying temperature; antioxidants; storage conditions; polyunsaturated fatty acid losses; infant feeding*

INTRODUCTION

Polyunsaturated fatty acids (PUFA) are important from a nutritional point of view. $n-3$ PUFA have been associated with cardiovascular disease, hypertension, and arthritis (Leaf and Weber, 1988; Lee et al., 1985; Phillipson et al., 1985). C22:6$n-3$ has a especial interest, since humans appear to have a limited ability for Δ^4-desaturation, the final step in the formation of C22:6$n-3$ from C18:3$n-3$ (Crawford, 1976). C22:6$n-3$ is essential in infant diets to avoid retinal function anomaly in neonates with deficient stores of this FA (Liu et al., 1987; Uauy et al., 1990). Moreover, in relation to C20:4$n-6$, Clandinin et al. (1981) have raised the question of the ability of preterm infants to convert C18:2$n-6$ to C20:4$n-6$. Therefore, the considerable amounts of C22:6$n-3$ and C20:4$n-6$ in eggs, which in the case of C22:6$n-3$ (1.2%) (Guardiola et al., 1994) are higher than those found in maternal milk (0.1–0.9%) (Boersma et al., 1991; Harris et al., 1984; Spear et al., 1992), have attracted considerable attention, since some authors have proposed egg yolk as a complement for enriching weaning foods (Simopoulos and Salem, 1992). In addition, eggs are a source of C20:4$n-6$ (2.0%) and could prevent the reduction of this acid, observed by Carlson et al. (1991), in red blood cells and plasma phospholipids of preterm infants fed formulas supplemented with marine oils. This possibility is supported by studies showing that the levels of C20:4$n-6$ in plasma phospholipids of children are related to egg consumption (Carlson et al., 1991). In addition, Oh et al. (1991) compared eggs enriched in $n-3$ PUFA from hens fed fish oil with control eggs and concluded that $n-3$ eggs were more healthy, since they lowered plasma cholesterol and triglyceride concentration and blood pressure.

In contrast, a high PUFA presence decreases the oxidative stability of eggs. Some authors observed that monitoring PUFA losses in phospholipid fractions could be a useful technique to evaluate oxidation (Dearden et al., 1985; Gokalp et al., 1983; Moerck and Ball, 1974). However, other reports showed that monitoring PUFA changes was not a good indicator of autoxidation in poultry meat (Kunsman et al., 1978). Moreover, for this technique to be sensitive, its application to foods requires the separation of lipid fractions to evaluate the fatty acid losses in phospholipids, which have higher PUFA content. In the case of egg powder obtained by spray-drying, the separation of lipid classes is unnecessary because the PUFA content is high and the heat treatment is severe. In addition, this treatment leads to protein denaturation, which modifies lipoprotein structure and, consequently, decreases oxidative stability of egg lipids interwoven in this structure (Pike and Peng, 1985, 1988). So, egg powder may not be a source of PUFA for enriching weaning foods, since oxidized fatty acids lose their essentiality and have several biological effects (atherogenicity, cytotoxicity, carcinogenicity, mutagenicity, etc.) (Chow, 1992; Kubow, 1990).

Thus, our study focuses on fatty acid losses in egg powder stored under different conditions for 10 months, following production at different spray-drying temperatures, using two kinds of antioxidant and three concentrations.

MATERIALS AND METHODS

Reagents. Ascorbyl palmitate (AP) (>99%) was obtained from Fluka Chemie AG (Buchs, Switzerland). *dl*-α-Tocopherol (*dl*-α-T) (95%) and propyl gallate (PG) (>99%) were purchased from Sigma Chemical Co. (St. Louis, MO). Glyceryl monostearate was supplied by Henkel KGaA (Düsseldorf, Germany).

Experimental Design. A 2 × 2 × 3 × 2 factorial arrangement was planned to study the influence of the following

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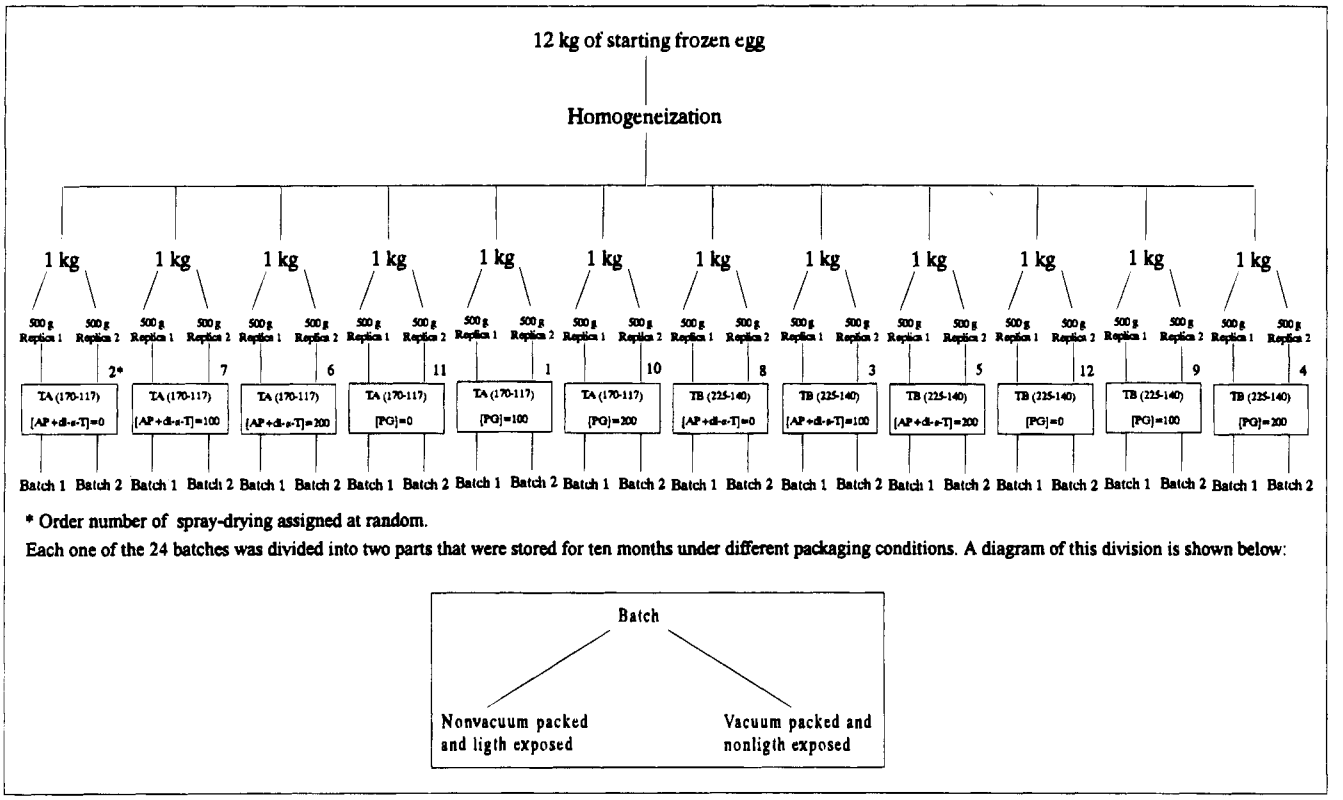

Figure 1. Sample preparation diagram.

factors on fatty acid losses in egg powder: spray-drying temperature (2), kind of antioxidant (2), antioxidant concentration (3), and packing conditions (2) (two replicates of this arrangement were carried out).

Sample Preparation. Frozen egg 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, in an Ystral electric drive 10/20 3000 homogenizer (Liverpool, U.K.), and then an aliquot was taken to determine FA composition in quadruplicate, as reference value. 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 they were stored at −20 °C until spray-drying.

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

Just before spray-drying, 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.

As a result of our experimental design, two batches were obtained for each one of the 12 spray-drying conditions assayed in the factorial arrangement (Figure 1). Each batch was divided into two parts, one packed under vacuum and wrapped in aluminum foil, and the other packed at atmospheric pressure. Samples ($n = 48$) were stored at room temperature (23 °C) for 10 months, and fatty acid composition was determined. In addition, six of these samples were analyzed after 8.5 months of storage.

Spray-Drying Conditions. All egg samples were processed in a Niro Atomizer A/S (Copenhagen, Denmark) equipped with an electric heater, with a feed rate of 10 mL/min, an air pressure of 6 kg/cm^2, and a powder egg residence time in the spray-dryer of 25 min. Inlet and outlet temperatures were fixed simultaneously by controlling the air flow through the system. The two following temperature conditions were assayed: T_A, inlet = 170 °C and outlet = 117 °C; and T_B, inlet = 225 °C and outlet = 140 °C. These two conditions are within the range of temperatures usually applied in experimental studies on egg spray-drying (Morgan and Armstrong, 1987). However, the current tendency is to use the lowest temperature possible (Bergquist, 1964, 1977; Tsai and Hudson, 1985). Conditions A fall in the higher range of temperatures used commercially, and conditions B are outside this range.

Addition of Antioxidants. Two kinds of antioxidant were added: one was the synergistic combination of AP and *dl*-α-T, and the other was PG. The antioxidants were added at three concentrations: 0, 100, and 200 μg/L in liquid egg (for AP + *dl*-α-T, 50 + 50 and 100 + 100).

PG and *dl*-α-T were added from solutions in ethanol. Solutions were prepared in appropriate concentration 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.

Packing. Samples were vacuum or nonvacuum 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. All samples were then set in groups of five and respectively packed under vacuum or sealed in 32 × 28 cm polypropylene five-layer film barrier bags.

Methods. Extraction of lipids, preparation of fatty acid methyl esters, and gas chromatographic determination were carried out as described by Guardiola et al. (1994).

Statistics. A Student−Fisher t test was used to examine the significance of storing time ($n = 6$). P values ≤ 0.05 were considered significant.

To check whether there were any significant effects of factors studied (spray-drying temperature, kind of antioxidant, antioxidant concentration, and packing conditions), a multifactor ANOVA was performed ($n = 48$). Interactions higher than order 2 were ignored. P values ≤ 0.05 were considered significant.

RESULTS

This study on fatty acid losses is part of a study of egg powder storage, for which different oxidation parameters were determined at 0, 5, and 10 months (UV absorptions at 232, 270, and 303 nm; oxidation of

Table 1. Fatty Acid Composition, Expressed as Compensated Area Normalization (‰), of Starting Frozen Egg and Six Egg Powder Samples Stored for 8.5 and 10 Months

fatty acid	starting frozen egg[a]	egg powder (n = 6) storage time	
		8.5 months	10 months
total SFA[b]	305.6 (3.3)	315.4	307.4
total *trans*-MUFA[b]	4.9 (1.1)	5.4	5.2
total *cis*-MUFA	468.6 (1.2)	467.6	473.8
$n-6$ PUFA[b]			
C18:2$n-6$	168.6 (1.8)	163.7	165.2
C18:3$n-6$	1.0 (2.9)	1.0	1.0
C20:2$n-6$	2.1 (13.6)	2.8	3.0
C20:3$n-6$	1.4 (9.7)	1.7	1.5
C20:4$n-6$	18.4 (1.1)	15.8	16.0
C22:4$n-6$	2.1 (8.3)	1.8	1.9
C22:5$n-6$	2.5 (3.8)	2.3	2.4
total	195.4 (1.9)	189.2	190.1
$n-3$ PUFA			
C18:3$n-3$	6.7 (3.2)	6.8	7.0
C22:5$n-3$	1.9 (5.9)	1.7	1.8
C22:6$n-3$	16.3 (2.6)	13.5	13.3
total	25.0 (3.0)	21.9	22.1
$n-6/n-3$	7.83 (1.4)	8.65	8.64
PUFA/SFA	0.72 (5.3)	0.67	0.69

[a] Determination in quadruplicate. Coefficient of variation in parentheses. [b] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

carotenoids; and content of oxysterols). To check the reliability of fatty acid losses as an oxidation parameter, it was first determined in only six samples at 8.5 months. Table 1 shows a significant loss for different PUFA at 8.5 months, which led to the determination of this parameter in all samples stored for 10 months (n = 48).

Table 1 shows a summarized fatty acid composition of starting frozen egg and six egg powder samples stored for 8.5 and 10 months, detailing only $n-6$ and $n-3$ PUFA composition. FA composition is expressed as compensated area normalization (parts per thousand). The coefficients of variation for quadruplicate analysis of frozen egg show the precision of the method. All samples analyzed were processed at high spray-drying temperature and were the most oxidized.

Although C20:5$n-3$ is normally present in eggs at low levels (Guardiola et al., 1994), starting frozen egg presented only traces of this fatty acid.

Table 2 shows fatty acid composition of starting frozen egg and all egg powder samples stored for 10 months. Least-squares grand mean (global mean) and least-squares means as influenced by factors are provided in this table. In addition, this table presents significance levels obtained from multifactor ANOVA for factors and interactions that have a significant effect on fatty acid losses.

DISCUSSION

Table 1 shows that starting frozen egg has a higher proportion of PUFA than egg powder samples. Total PUFA $n-6$ and especially C20:4$n-6$ are higher in frozen egg. The C20:4$n-6$ loss in egg powder, expressed as percentage referred to the initial proportion of this fatty acid in frozen egg, is 13.0–14.1%. Total PUFA $n-3$ (17.2–18.4%) and C22:6$n-3$ (11.6–12.4%) losses are highly relevant. In addition, from a nutritional point of view, PUFA losses in egg powder led to less favorable $n-6/n-3$ and PUFA/SFA ratios, especially if it is taken into account that the $n-6/n-3$ increase is mostly due to the high loss in C22:6$n-3$.

Unsaturated fatty acid losses have been widely reported as an indicator of lipid oxidation. Some papers described monounsaturated fatty acid and PUFA losses (Dearden et al., 1985; Gokalp et al., 1983; Yan and White, 1991), but others only referred to PUFA losses (Moerck and Ball, 1974). In addition, some studies have been focused only on $n-3$ PUFA losses because of their high nutritional relevance (Ajuyah et al., 1993). However, Kunsman et al. (1978) reported that PUFA losses were not a sensitive indicator of lipid oxidation in deboned red meat. As a rule, in foods, susceptibility to oxidation of phospholipids increases with the unsaturation (Pikul and Kummerov, 1991). However, Bruna et al. (1989) showed that susceptibility to peroxidation of C20:5$n-3$ and C22:6$n-3$ after photoirradiaton in aqueous solution, evaluated by measuring fatty acid losses, was surprisingly lower than susceptibility of C18:3$n-3$, C18:3$n-6$, C20:3$n-6$, and C20:4$n-6$. Terao et al. (1987) evaluated peroxidation in spray-dried egg and muscle foods, measuring fatty acid losses in phospholipids and other oxidation parameters, and concluded that spray-dried egg is highly oxidized and very susceptible to oxidation in comparison with raw egg (Marshall et al., 1994). This fact is related to the structure of phospholipids in the raw yolk that protect against oxidation. Phospholipids are interwoven in the exterior structure of low-density lipoprotein, and this compact surface prevents the contact of oxygen with the lipid core of the particle (Burley and Vadehra, 1989). Spray-drying leads to protein disruption, which modifies lipoprotein structure and, consequently, susceptibility of egg lipid to oxidation (Pike and Peng, 1985, 1988).

For several years, it has been known that weaning foods should be enriched in $n-3$ and $n-6$ long-chain PUFA (LCPUFA, PUFA with carbon chain length greater than 18). Jackson and Gibson (1989) examined LCPUFA in egg yolk, brains, and fresh and canned baby foods and concluded that infants would have to eat unphysiologic amounts of food to reach the intake of LCPUFA provided by lactation. Thus, Simopoulos and Salem (1992) proposed yolks of LCPUFA-enriched eggs as a source of this fatty acid in infant feeding. Moreover, Marshall et al. (1994) concluded that the oxidative stability of $n-3$ PUFA enriched shell eggs from hens fed 1.5% dietary menhaden oil for 4 weeks is comparable to that from control shell egg. However, the use of these spray-dried $n-3$ PUFA egg yolks should be avoided, since oxidized fatty acids lose their essentiality and present several biological effects, although they constitute the easier way to incorporate yolk in powdered infant formulas. For these formulas egg yolk oil could be used. A Student–Fisher t test was used to check whether there were any significant differences between fatty acid composition of egg powder samples stored for 8.5 and 10 months (Table 1). This test showed that differences were not significant. Thereby, it could be concluded that lipid oxidation mostly occurs during spray-drying but care should be taken in considering this conclusion because only 1.5 months elapsed between analysis and, moreover, the samples analyzed were all processed at high spray-drying temperature and highly oxidized. Terao et al. (1987) observed, as is widely known, that egg lipids underwent high oxidation during spray-drying; moreover, they observed that this oxidation significantly increases during storage (1 and 3 months). In addition, in our study, C20:4$n-6$ and

Table 2. Fatty Acid Composition, Expressed as Compensated Area Normalization (‰), of Starting Frozen Egg and All Egg Powder Samples Stored for 10 Months [Least-Squares Grand Mean (Global Mean) and Least-Squares Means As Influenced by Factors Are Presented for Egg Powder Samples. *P* Values Were Obtained from Multifactor ANOVA for Factors and Interactions That Have Significant Effect on Fatty Acid Losses]

		egg powder									
			spray-drying temp (°C) (n = 24)		kind of antioxidant (n = 24)		antioxidant concn (μg/L) (n = 16)			packing conditions (n = 24)	
fatty acid	starting frozen egg[a]	global mean (n = 48)	170–117[b]	225–140[b]	AP + *dl*-α-T[c]	PG[c]	0	100	200	A[d]	B[d]
total SFA[e,g]	305.6	309.3	307.9	310.8	308.4	310.3	309.6	310.0	308.4	309.5	309.2
total *trans*-MUFA[e,f,h]	4.9	5.1	5.0	5.1	5.0	5.1	5.1	5.0	5.0	5.2	4.9***
total *cis*-MUFA[g,k]	468.6	468.9	468.6	469.2	469.2	468.6	469.2	467.9	469.6	470.6	467.3*
$n-6$ PUFA[e]											
C18:2$n-6$	168.6	166.5	166.9	166.2	166.8	166.2	165.7	166.8	167.2	165.0	168.1****
C18:3$n-6$[i,k]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
C20:2$n-6$	2.1	2.6	2.5	2.7	2.6	2.6	2.5	2.6	2.7	3.0	2.2****
C20:3$n-6$	1.4	1.5	1.5	1.4	1.4	1.5	1.4	1.5	1.5	1.5	1.4***
C20:4$n-6$	18.4	16.8	17.4	16.2****	17.0	16.6*	16.9	16.9	16.6	16.5	17.1***
C22:4$n-6$	2.1	1.9	2.0	1.9****	1.9	1.9	2.0	1.9	1.9	1.9	1.9
C22:5$n-6$	2.5	2.5	2.6	2.4****	2.5	2.5	2.5	2.5	2.5	2.5	2.5
total	195.4	192.8	193.9	191.8*	193.3	192.3	192.0	193.3	193.2	191.5	194.2**
$n-3$ PUFA											
C18:3$n-3$[j,l]	6.7	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.0	7.2****
C22:5$n-3$	1.9	1.9	2.0	1.9*	1.9	1.9	2.0	1.9	2.0	1.9	2.0***
C22:6$n-3$[g]	16.3	14.3	15.0	13.6****	14.5	14.2	14.5	14.4	14.0	13.9	14.7***
total[g]	25.0	23.4	24.1	22.6****	23.5	23.2	23.5	23.4	23.1	22.8	23.9****
$n-6/n-3$[h]	7.83	8.28	8.05	8.50****	8.24	8.31	8.19	8.27	8.37	8.41	8.13****
PUFA/SFA	0.72	0.70	0.71	0.69*	0.70	0.70	0.70	0.70	0.70	0.69	0.71

[a] Determination in quadruplicate. [b] Inlet temperature–outlet temperature. [c] AP + *dl*-α-T, ascorbyl palmitate + *dl*-α-tocopherol; PG, propyl gallate. [d] A, nonvacuum packed and light exposed; B, vacuum packed and nonlight exposed. [e] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [f] Interaction of spray-drying temperature × kind of antioxidant significant at $P \leq 0.05$. [g] Interaction of spray-drying temperature × antioxidant concentration significant at $P \leq 0.05$. [h] Interaction of spray-drying temperature × antioxidant concentration significant at $P \leq 0.01$. [i] Interaction of spray-drying temperature × packing conditions significant at $P \leq 0.05$. [j] Interaction of spray-drying temperature × packing conditions significant at $P \leq 0.01$. [k] Interaction of spray-drying temperature × packing conditions significant at $P \leq 0.05$. [l] Interaction of antioxidant concentration × packing conditions significant at $P \leq 0.01$. * Significant factor $P \leq 0.05$ (** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$).

C22:6$n-3$ loss measured at 10 months of storage was well correlated with the other quality parameters studied, especially with increase in UV absorptions (unpublished data). UV absorptions increased markedly during spray-drying, and they also underwent a moderate, but statistically significant, increase during storage for 5 and 10 months.

Table 2 shows the influence of various factors studied on fatty acid losses. A higher spray-drying temperature led to higher $n-6$ and $n-3$ PUFA losses and to less favorable $n-6/n-3$ and PUFA/SFA ratios. Vacuum packing and nonlight storing prevent $n-3$ and $n-6$ PUFA losses and, consequently, the $n-6/n-3$ ratio for egg powder stored under these conditions is more favorable. The significance of these differences is shown in Table 2. Several authors, in accordance with our results, report the effect of temperature (Ajuyah et al., 1993) and vacuum packing (Gokalp et al., 1983) on PUFA losses. However, Deslypere et al. (1993) conclude that storage at 20 or −80 °C for 7 months yielded no perceptible changes in $n-3$ PUFA of fat tissue aspirates. AP + *dl*-α-T is significantly more effective than PG in preventing C20:4$n-6$ loss. The effectiveness of tocopherols in preventing $n-3$ PUFA losses was reported by Ajuyah et al. (1993). However, several papers reporting prooxidant effect of vitamin E derivatives at high concentrations should be taken into account (Husain et al., 1987; Mukai et al., 1993). In spite of that, neither concentration nor interaction between kind and concentration of antioxidant showed influence on PUFA losses (Table 2).

P values for interactions that have a significant effect on fatty acid losses are shown in Table 2. An interesting interaction is that between spray-drying temperature and packing conditions, which significantly influences

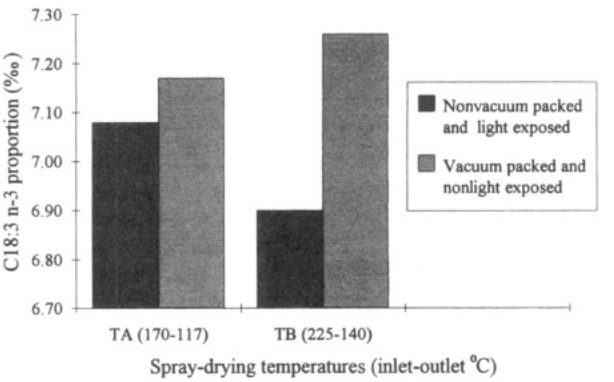

Figure 2. Influence of the interaction between spray-drying temperature and packing conditions on C18:3$n-3$ proportion in egg powder stored for 10 months.

C18:3$n-6$ and C18:3$n-3$ loss. Thus, when spray-drying temperature is high, vacuum packing is more effective in avoiding the loss of these fatty acids during storage (Figure 2). The interaction between spray-drying temperature and antioxidant concentration affects significantly total $n-3$ PUFA and C22:6$n-3$ loss, which means that high concentrations of antioxidants protect more effectively against oxidation of $n-3$ PUFA and, particularly, of C22:6$n-3$, when egg powder is produced at high spray-drying temperatures. The interaction between concentration of antioxidant and packing conditions shows a significant effect on C18:3$n-6$ and C18:3$n-3$ loss, which means that high antioxidant concentrations are more effective when egg powder is nonvacuum packed (Figure 3). In conclusion, antioxidants seem to be slightly effective when the product is processed and stored under highly oxidative conditions. Figures 2 and 3 are based on least-squares means as influenced by

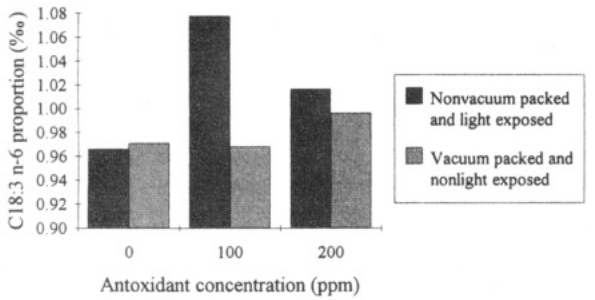

Figure 3. Influence of the interaction between antioxidant concentrations and packing conditions on C18:3*n*−6 proportion in egg powder stored for 10 months.

interactions, which are not shown to facilitate the understanding of the results.

ABBREVIATIONS USED

FA, fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; LCPUFA, long-chain polyunsaturated fatty acids; AP, ascorbyl palmitate; *dl*-α-T, *dl*-α-tocopherol; PG, propyl gallate.

ACKNOWLEDGMENT

A. Grau-Erra provided technical assistance.

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Received for review January 9, 1995. Accepted May 24, 1995.[⊗] This work was supported in part by a research grant from Comissió Interdepartamental de Recerca i Innovació Tecnològica (CIRIT).

JF950015U

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1995.