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# Effect of Storage on Cholesterol Oxide Formation and Fatty Acid Alterations in Egg Powder

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The effect of the storage of egg powder on cholesterol oxide formation and alterations in the fatty acid composition was studied. Two commercial brands, A and B, were studied at 0 time and then monthly for up to 6 and 12 months, respectively. Five cholesterol oxides were identified and quantified (7-ketocholesterol, 7$\beta$-hydroxycholesterol, 7$\alpha$-hydroxycholesterol, 5,6$\alpha$-epoxycholesterol, and 5,6$\beta$-epoxycholesterol), the amounts of which increased during storage, although the cholesterol contents remained constant. The polyunsaturated fatty acid contents reduced during storage, whereas the levels of saturated and monounsaturated fatty acids and total lipids remained constant. High cholesterol oxide and trans fatty acid contents were found, a fact of concern because these compounds are hazardous to health, and egg powder is used in various food products widely consumed by the population, principally by infants.

**KEYWORDS: Cholesterol oxides; cholesterol; fatty acids; HPLC-UV-RI; egg powder**

## INTRODUCTION

The great consumption of industrialized foods containing eggs, such as bakery products and pasta, imposed the need to obtain this raw material in a form with a longer shelf life, resulting in the use of egg powder. However, the egg is a cholesterol- and lipid-rich food, and the process used to obtain powdered egg by spray-drying uses high temperatures, accelerating reactions between the lipids and molecular oxygen, resulting in the formation of cholesterol oxides (*1*, *2*). In addition, the polyunsaturated fatty acids present in eggs are extremely susceptible to oxidation, producing principally free radicals that promote the formation of cholesterol oxides. Finally, the transport and storage conditions of egg powder also contribute to cholesterol and fatty acid degradation.

The cholesterol oxides normally found in egg powder samples are 7$\alpha$-hydroxycholesterol (7$\alpha$-OH), 7$\beta$-hydroxycholesterol (7$\beta$-OH), and its dehydrogenation product, 7-ketocholesterol (7-keto). In addition, 5,6$\alpha$-epoxycholesterol (5,6$\alpha$-epoxy) and 5,6$\beta$-epoxycholesterol (5,6$\beta$-epoxy) and their hydration product, cholestantriol, and also oxides formed by the oxidation of the cholesterol side chain, such as 20$\alpha$-hydroxycholesterol (20$\alpha$-OH) and 25-hydroxycholesterol (25-OH), have been found (*3*, *4*). Cholesterol oxides are related to a series of factors hazardous to human health, such as atherogenic, cytotoxic, mutagenic, and carcinogenic factors (*5*, *6*) and can also be related to degenerative diseases such as Parkinson's and Alzheimer's diseases and diabetes (*7*, *8*). Among these oxides, the 25-OH and the triol are described as being the most atherogenic (*9*).

Recent studies have shown that trans fatty acids are associated with the development of cardiovascular infirmities, due to increases in the blood LDL-cholesterol and Lp(a) levels, in a way similar to the consumption of saturated fatty acids (*10*, *11*). On the other hand, the trans fatty acids are considered to be of greater concern than the saturated fatty acids, because they also decrease the HDL-cholesterol level (*12*).

Although the prejudicial effects on health caused by the continuous consumption of foods containing significant levels of cholesterol oxides and trans fatty acids are still under study, improvements in production and distribution systems and in commercially used storage techniques, aimed at minimizing the production of these compounds in processed foods, have become necessary. Additionally, the development of research on alterations occurring in the levels of fatty acids during the processing and storage of these foods has become equally necessary. Thus, the objective of this study was to verify the formation of cholesterol oxides and alterations in the fatty acid composition in egg powder during the storage period.

## MATERIALS AND METHODS

**Material.** Cholesterol and COP standards, including 19-hydroxycholesterol (19-OH), 20$\alpha$-hydroxycholesterol (20$\alpha$-OH), 22*S*-hydroxycholesterol (22*S*-OH), 22*R*-hydroxycholesterol (22*R*-OH), 25-hydroxycholesterol (25-OH), 7-ketocholesterol (7-keto), 7$\beta$-hydroxycholesterol (7$\beta$-OH), 5,6$\alpha$-epoxycholesterol (5,6$\alpha$-Ep), 5,6$\beta$-epoxycholesterol (5,6$\beta$-Ep), and cholestan-3$\beta$,5$\alpha$,6$\beta$-triol (triol) were from Sigma (Milford, MA). The other COP standards, 24*S*-hydroxycholesterol (24*S*-OH) and 7$\alpha$-hydroxycholesterol (7$\alpha$-OH), were obtained from Steraloids (Newport, RI). A total of 37 saturated, monounsaturated, and polyunsaturated fatty acid standards (Supelco FAME Mix 18919, Bellefonte, PA) were used.

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Methyl esters (99% pure) of tricosanoic (23:0) and tridecanoic (13:0) acids (Sigma) were used as the internal standards for quantification. Reference standards of whole egg powder (SRM 1846 and SRM 8415) from NIST (Gaithersburg, MD) were used to verify the acurracy of the cholesterol and fatty acid analyses, respectively.

**Samples.** Two commercial brands (A and B) of egg powder were acquired directly from the factory on the day of production. Two batches of each brand, with different production dates, were analyzed. Each sample, consisting of 3 kg, was homogenized, divided into 100 g subsamples, packaged under vacuum in polyethylene bags, and stored in the dark at 25 ± 2 °C. The samples were analyzed at 0 time and then every month for 6 months up to the expiration date of brand A and for 12 months to the expiration date of brand B. All samples were analyzed in triplicate.

**Lipid Extraction.** The lipids were extracted according to the method of Folch et al. (*13*). Aliquots of 10 mL were taken and the total lipids determined gravimetrically.

**Determination of Moisture Content.** Aliquots of 1 ± 0.5 g were dried to constant weight in an oven at 110 °C according to the AOAC methodology (*14*).

**Determination of Fatty Acids.** The lipid extract was dried in a rotary evaporator and 25.0 ± 0.1 mg of the oil obtained methylated according to the method of Joseph and Ackman (*15*). The fatty acid methyl esters were separated in a gas chromatograph (Varian 3400 CX, Walnut Creek, CA) equipped with a flame ionization detector, a split injector, a CP-SIL 88 column (100 m, 0.25 mm, 0.20 μm), and a workstation. The chromatographic conditions were according to those of Mazalli and Bragagnolo (*16*). The fatty acids were identified by comparing the retention times of the standards with those of the sample, and quantification was by internal standardization using the methyl esters of tricosanoic and tridecanoic acids as the internal standards. The results were calculated in milligrams per 100 g of sample according to the AOCS method (*17*). The method was validated using the certified reference egg powder standard (SRM 8415, NIST), the results being similar to those specified for the reference material.

**Simultaneous Determination of Cholesterol and Cholesterol Oxides by HPLC-UV-RI.** Cholesterol and the 19-OH, 20α-OH, 22*R*-OH, 24*S*-OH, 22*S*-OH, 25-OH, 7-keto, 7β-OH, 7α-OH, 5,6α-epoxy, 5,6β-epoxy, and triol cholesterol oxides were separated and quantified according to the method of Mazalli et al. (*18*). In summary, the sample was saponified with KOH in the dark for 18 h at room temperature and the nonsaponifiable matter extracted five times with ethyl ether. The ethyl ether fraction was concentrated, and the cholesterol and cholesterol oxides were determined by liquid chromatography. A Shimadzu liquid chromatograph (Kyoto, Japan), equipped with UV ($SPD\text{-}10AV_{vp}$) and refractive index (RID-10A) detectors, was used. A Nova Pak CN HP, 300 mm × 3.9 mm × 4 μm, column (Waters, Milford, MA) was used with a temperature of 32 °C. Quantification was by external standardization. To confirm the identity of cholesterol and cholesterol oxides liquid chromatography was interfaced with chemical ionization at atmospheric pressure (APCI) and mass detection (MS). HPLC-APCI-MS was carried out using a Waters Alliance 2695 pump and the same chromatographic conditions as above. The mass spectrometer used was a Qtrap (Applied Biosystems, Concord, ON, Canada) with a QqQ (linear ion trap) configuration. Injections of cholesterol and cholesterol oxide standards were used to optimize the conditions. The optimum conditions were as follows: positive mode APCI, scan range *m*/*z* 250−500, 375 °C, nitrogen as carrier gas (70 L/min), sheath gas (60 L/min) and curtain gas (30 L/min), nebulizer current set at 3000 V, declustering potential at 30 V, and entrance potential at 9 V. Chromatograms were obtained in the selective ion monitoring (SIM) mode for the ions *m*/*z* 367, 369, 385, 401, and 403 (*18*).

Cholesterol recovery varied from 92 to 102% and that of the cholesterol oxides from 93 to 96%. The limits of detection (LD) and quantification (LQ) were calculated according to the method of Long et al. (*19*). The LD encountered for the cholesterol oxides varied from 0.002 to 0.031 μg/g, and the LD for cholesterol was 0.026 μg/g. The LQ encountered for the cholesterol oxides varied from 0.002 to 0.079 μg/g, and the LQ for cholesterol was 0.088 μg/g. The cholesterol content obtained for the certified reference material (SRM 1846, NIST) was 19.0 ± 0.2 mg/g of yolk, equal to the value referred to for the certified reference material.

**Table 1.** Total Lipids and Moisture in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C Analyzed during 6 Months for Brand A and 12 Months for Brand B[a]

| | lipids (g/100 g, dwb) | | moisture (g/100 g) | |
|---|---|---|---|---|
| months | brand a | brand b | brand a | brand b |
| 0 | 35 ± 1 a | 35 ± 1 a | 2.8 ± 0.3 a | 3.1 ± 0.6 a |
| 1 | 35 ± 0 a | 35 ± 1 a | 3.1 ± 0.2 a | 2.8 ± 0.3 a |
| 2 | 35 ± 1 a | 35 ± 0 a | 2.9 ± 0.3 a | 3.1 ± 0.1 a |
| 3 | 35 ± 1 a | 35 ± 1 a | 2.7 ± 0.1 a | 3.1 ± 0.2 a |
| 4 | 36 ± 0 a | 35 ± 1 a | 3.0 ± 0.4 a | 3.1 ± 0.2 a |
| 5 | 35 ± 1 a | 34 ± 1 a | 2.9 ± 0.2 a | 2.8 ± 0.3 a |
| 6 | 34 ± 1 a | 34 ± 1 a | 3.1 ± 0.1 a | 3.3 ± 0.3 a |
| 7 | | 35 ± 0 a | | 3.2 ± 0.3 a |
| 8 | | 34 ± 0 a | | 2.8 ± 0.1 a |
| 9 | | 34 ± 1 a | | 2.8 ± 0.3 a |
| 10 | | 34 ± 1 a | | 2.8 ± 0.0 a |
| 11 | | 34 ± 1 a | | 3.1 ± 0.0 a |
| 12 | | 34 ± 1 a | | 2.8 ± 0.1 a |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

**Statistical Analysis.** The analysis of variance (ANOVA) was applied to the data obtained for total lipids, moisture, cholesterol, cholesterol oxides, and fatty acids, using the software Statistic 5.5 (Santa Clara, CA). The means were compared by the Tukey test at the 5% level of probability.

## RESULTS AND DISCUSSION

**Total Lipid and Moisture Contents in Spray-Dried Whole Egg Powder.** There was no variation in moisture content between the different egg powders either between batches, between brands, or during storage. Even so, the results for total lipids, fatty acids, cholesterol, and cholesterol oxides were expressed on a dry weight basis (dwb). Between batches the total lipids were similar and were expressed as the mean of the two batches, analyzed in triplicate (**Table 1**). For both brands, A and B, the total lipid contents remained fairly stable throughout storage. As compared to the values reported by the USDA (*20*), the total lipid contents for both brands of egg powder were lower and the moisture contents similar.

**Fatty Acids in Spray-Dried Whole Egg Powder.** There were no significant differences between the fatty acids of the batches for either brand, and they were therefore expressed as the mean of the two batches in both cases. Of a total of 29 fatty acids identified, the main ones determined in the egg powders of brands A and B were C14:0, C16:0, C18:0, C16:1 n7, C18:1 n9, C18:2 n6, C20:4 n6, C18:3 n3, and C18:1 n9 trans. The majority of these fatty acids were unsaturated. The following fatty acids were not found in egg powders: C4:0; C6:0; C8:0; C10:0; C11:0; C12:0, C13:0; and C23:0; and fatty acids C15:1 n9, C17:1 n7, C22:1 n9, and C22:2 n6 were found in only trace amounts (<1 mg/100 g).

The saturated (**Table 2**) and monounsaturated (**Table 3**) fatty acids presented no significant changes ($p > 0.05$) during storage. Only the polyunsaturated fatty acids C18:2 n6 and C20:4 n6 (**Table 4**) and polyunsaturated fatty acids C18:3 n3 and C22:6 n3 (**Table 5**) were reduced significantly for both brands during storage. For both brands A and B, C18:2 n6 and C20:4 n6 were reduced for up to 3 months and 6 months, respectively. For brand A, C18:3 n3 and C22:6 n3 were reduced only up to the end of storage, and for brand B, C18:3 n3 was reduced for up to 12 months and C22:6 n3 for up to 6 months.

**Table 2.** Saturated Fatty Acids in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C Analyzed at 0 Time and during 6 Months for Brand A and 12 Months for Brand B[a]

| | fatty acid (mg/100 g, dwb) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| months | C14:0 | C15:0 | C16:0 | C17:0 | C18:0 | C20:0 | C21:0 | C22:0 | C24:0 |
| | | | | | Brand A | | | | |
| 0 | 947 ± 8 a | 160 ± 3 a | 5906 ± 44 a | 414 ± 15 a | 4049 ± 139 a | 129 ± 4 a | 178 ± 4 a | 201 ± 3 a | 64 ± 2 a |
| 1 | 944 ± 6 a | 158 ± 4 a | 5935 ± 36 a | 399 ± 18 a | 3998 ± 115 a | 131 ± 3 a | 176 ± 2 a | 200 ± 4 a | 64 ± 3 a |
| 2 | 948 ± 5 a | 161 ± 3 a | 5888 ± 45 a | 421 ± 17 a | 4015 ± 108 a | 127 ± 4 a | 180 ± 4 a | 198 ± 4 a | 60 ± 3 a |
| 3 | 942 ± 5 a | 160 ± 4 a | 5889 ± 39 a | 414 ± 4 a | 4049 ± 100 a | 129 ± 4 a | 177 ± 4 a | 200 ± 2 a | 61 ± 2 a |
| 4 | 943 ± 6 a | 155 ± 5 a | 5905 ± 52 a | 425 ± 11 a | 4102 ± 130 a | 129 ± 4 a | 181 ± 4 a | 201 ± 3 a | 64 ± 2 a |
| 5 | 944 ± 4 a | 162 ± 4 a | 5913 ± 48 a | 415 ± 12 a | 4111 ± 132 a | 130 ± 2 a | 177 ± 2 a | 204 ± 2 a | 62 ± 3 a |
| 6 | 941 ± 8 a | 162 ± 2 a | 5891 ± 31 a | 409 ± 11 a | 4171 ± 71 a | 131 ± 4 a | 178 ± 3 a | 203 ± 3 a | 64 ± 3 a |
| | | | | | Brand B | | | | |
| 0 | 988 ± 6 a | 162 ± 2 a | 6150 ± 40 a | 472 ± 5 a | 4230 ± 70 a | 292 ± 3 a | 231 ± 4 a | 103 ± 4 a | 42 ± 1 a |
| 1 | 990 ± 4 a | 161 ± 2 a | 6155 ± 45 a | 469 ± 5 a | 4234 ± 74 a | 290 ± 3 a | 228 ± 5 a | 102 ± 5 a | 40 ± 2 a |
| 2 | 984 ± 6 a | 160 ± 3 a | 6152 ± 38 a | 475 ± 4 a | 4198 ± 75 a | 290 ± 2 a | 225 ± 6 a | 100 ± 4 a | 40 ± 2 a |
| 3 | 988 ± 5 a | 165 ± 3 a | 6148 ± 45 a | 471 ± 4 a | 4241 ± 55 a | 296 ± 4 a | 232 ± 4 a | 100 ± 4 a | 41 ± 2 a |
| 4 | 991 ± 6 a | 164 ± 3 a | 6170 ± 47 a | 464 ± 7 a | 4222 ± 78 a | 292 ± 3 a | 231 ± 4 a | 104 ± 5 a | 39 ± 2 a |
| 5 | 984 ± 4 a | 162 ± 3 a | 6169 ± 42 a | 470 ± 6 a | 4196 ± 81 a | 295 ± 3 a | 228 ± 5 a | 101 ± 4 a | 41 ± 2 a |
| 6 | 990 ± 4 a | 165 ± 4 a | 6160 ± 28 a | 465 ± 5 a | 4223 ± 62 a | 296 ± 4 a | 226 ± 5 a | 103 ± 2 a | 42 ± 2 a |
| 7 | 991 ± 6 a | 164 ± 3 a | 6145 ± 32 a | 467 ± 6 a | 4235 ± 65 a | 291 ± 4 a | 225 ± 5 a | 102 ± 3 a | 40 ± 2 a |
| 8 | 984 ± 7 a | 160 ± 3 a | 6148 ± 40 a | 464 ± 6 a | 4244 ± 68 a | 291 ± 3 a | 235 ± 3 a | 102 ± 3 a | 41 ± 1 a |
| 9 | 990 ± 4 a | 159 ± 4 a | 6161 ± 36 a | 463 ± 7 a | 4242 ± 74 a | 295 ± 4 a | 229 ± 4 a | 98 ± 3 a | 40 ± 1 a |
| 10 | 985 ± 6 a | 159 ± 4 a | 6154 ± 39 a | 470 ± 6 a | 4255 ± 55 a | 292 ± 3 a | 230 ± 4 a | 100 ± 3 a | 41 ± 2 a |
| 11 | 983 ± 6 a | 161 ± 3 a | 6172 ± 51 a | 472 ± 5 a | 4224 ± 60 a | 297 ± 5 a | 230 ± 5 a | 101 ± 4 a | 40 ± 1 a |
| 12 | 992 ± 4 a | 158 ± 3 a | 6161 ± 32 a | 463 ± 8 a | 4238 ± 58 a | 293 ± 3 a | 233 ± 8 a | 102 ± 4 a | 42 ± 2 a |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

**Table 3.** Monounsaturated Fatty Acids in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C Analyzed at 0 Time and Then Monthly to the End of the Shelf Life of 6 Months for Brand A and 12 Months for Brand B[a]

| | fatty acid (mg/100 g, dwb) | | | | |
|---|---|---|---|---|---|
| months | C14:1 n9 | C16:1 n7 | C18:1 n9 | C20:1 n9 | C24:1 n9 |
| | | | Brand A | | |
| 0 | 130 ± 2 a | 1153 ± 53 a | 4804 ± 111 a | 38 ± 2 a | 59 ± 4 a |
| 1 | 130 ± 3 a | 1139 ± 50 a | 4789 ± 95 a | 36 ± 2 a | 56 ± 2 a |
| 2 | 128 ± 3 a | 1168 ± 45 a | 4825 ± 115 a | 35 ± 1 a | 56 ± 3 a |
| 3 | 132 ± 1 a | 1160 ± 39 a | 4789 ± 126 a | 36 ± 2 a | 57 ± 1 a |
| 4 | 130 ± 2 a | 1142 ± 41 a | 4832 ± 115 a | 35 ± 2 a | 55 ± 2 a |
| 5 | 130 ± 2 a | 1159 ± 52 a | 4792 ± 98 a | 33 ± 2 a | 54 ± 2 a |
| 6 | 128 ± 2 a | 1150 ± 60 a | 4815 ± 105 a | 35 ± 2 a | 55 ± 2 a |
| | | | Brand B | | |
| 0 | 121 ± 5 a | 1368 ± 65 a | 8977 ± 454 a | 81 ± 3 a | 43 ± 2 a |
| 1 | 117 ± 4 a | 1422 ± 58 a | 8679 ± 423 a | 79 ± 2 a | 43 ± 2 a |
| 2 | 126 ± 4 a | 1378 ± 46 a | 8704 ± 434 a | 80 ± 3 a | 42 ± 1 a |
| 3 | 118 ± 5 a | 1391 ± 70 a | 8529 ± 421 a | 82 ± 2 a | 44 ± 2 a |
| 4 | 123 ± 3 a | 1402 ± 54 a | 8666 ± 359 a | 80 ± 3 a | 40 ± 2 a |
| 5 | 121 ± 4 a | 1359 ± 48 a | 8821 ± 418 a | 79 ± 2 a | 44 ± 2 a |
| 6 | 119 ± 4 a | 1374 ± 85 a | 8784 ± 443 a | 79 ± 3 a | 40 ± 1 a |
| 7 | 125 ± 5 a | 1415 ± 49 a | 9201 ± 417 a | 83 ± 3 a | 41 ± 2 a |
| 8 | 118 ± 4 a | 1343 ± 41 a | 8915 ± 392 a | 83 ± 3 a | 44 ± 2 a |
| 9 | 123 ± 5 a | 1395 ± 54 a | 9222 ± 389 a | 81 ± 3 a | 41 ± 2 a |
| 10 | 125 ± 4 a | 1411 ± 62 a | 8724 ± 367 a | 79 ± 2 a | 41 ± 2 a |
| 11 | 119 ± 5 a | 1409 ± 63 a | 8888 ± 412 a | 80 ± 3 a | 43 ± 2 a |
| 12 | 124 ± 1 a | 1347 ± 52 a | 8909 ± 479 a | 78 ± 3 a | 42 ± 1 a |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

**Table 4.** Polyunsaturated n6 Fatty Acids in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C and Analyzed at 0 Time and during 6 Months for Brand A and 12 Months for Brand B[a]

| | fatty acid (mg/100 g, dwb) | | | | |
|---|---|---|---|---|---|
| months | C18:2 n6 | C18:3 n6 | C20:2 n6 | C20:3 n6 | C20:4 n6 |
| | | | Brand A | | |
| 0 | 3986 ± 166 a | 34 ± 1 a | 38 ± 1 a | 33 ± 1 a | 319 ± 15 a |
| 1 | 3929 ± 131 a | 33 ± 1 a | 39 ± 2 a | 32 ± 1 a | 323 ± 15 a |
| 2 | 3955 ± 158 a | 34 ± 1 a | 37 ± 2 a | 33 ± 1 a | 340 ± 18 a |
| 3 | 3300 ± 60 b | 33 ± 1 a | 37 ± 2 a | 32 ± 1 a | 206 ± 8 b |
| 4 | 3318 ± 74 b | 34 ± 1 a | 37 ± 2 a | 32 ± 0 a | 195 ± 11 b |
| 5 | 3329 ± 52 b | 33 ± 1 a | 38 ± 2 a | 33 ± 2 a | 201 ± 15 b |
| 6 | 3301 ± 62 b | 34 ± 1 a | 39 ± 1 a | 32 ± 1 a | 194 ± 17 b |
| | | | Brand B | | |
| 0 | 3777 ± 226 a | 26 ± 1 a | 21 ± 1 a | 22 ± 1 a | 262 ± 7 a |
| 1 | 3789 ± 211 a | 26 ± 2 a | 21 ± 0 a | 20 ± 2 a | 264 ± 9 a |
| 2 | 3868 ± 187 a | 25 ± 1 a | 20 ± 1 a | 20 ± 1 a | 265 ± 9 a |
| 3 | 3855 ± 225 a | 25 ± 1 a | 20 ± 1 a | 21 ± 1 a | 263 ± 9 a |
| 4 | 3799 ± 242 a | 24 ± 1 a | 21 ± 0 a | 21 ± 1 a | 264 ± 8 a |
| 5 | 3844 ± 121 a | 25 ± 1 a | 21 ± 1 a | 20 ± 1 a | 262 ± 8 a |
| 6 | 3440 ± 75 ab | 25 ± 1 a | 20 ± 1 a | 21 ± 1 a | 234 ± 4 b |
| 7 | 3432 ± 54 b | 26 ± 2 a | 20 ± 1 a | 22 ± 1 a | 232 ± 4 b |
| 8 | 3411 ± 72 b | 26 ± 1 a | 20 ± 1 a | 20 ± 1 a | 236 ± 5 b |
| 9 | 3369 ± 85 b | 25 ± 1 a | 21 ± 1 a | 22 ± 1 a | 235 ± 4 b |
| 10 | 3401 ± 78 b | 25 ± 1 a | 20 ± 1 a | 20 ± 1 a | 237 ± 8 b |
| 11 | 3396 ± 90 b | 25 ± 1 a | 20 ± 1 a | 20 ± 1 a | 233 ± 8 b |
| 12 | 2845 ± 74 b | 25 ± 2 a | 20 ± 1 a | 20 ± 1 a | 214 ± 6 b |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

For brand A at 0 time, the mean concentrations of saturated, monounsaturated, and polyunsaturated fatty acids were 12.0, 6.2, and 5.7 g/100 g of egg powder, respectively. At the end of the 6 month storage period, the n6 polyunsaturated FA had reduced by 18% and the n3 polyunsaturated FA by 32%. For brand B, the mean concentrations of saturated and monounsaturated FA were higher and similar, respectively, to the polyunsaturated FA content of brand A. At the end of the 12 month storage period, the amount of n6 polyunsaturated FA was reduced by 24% and the n3 polyunsaturated FA by 28%.

In the present study, the samples were vacuum packed and stored in the dark at 25 °C, but even so significant reductions in the polyunsaturated fatty acids occurred. Nevertheless, Guardiola et al. (*21*) observed that vacuum packing egg powder

**Table 5.** Polyunsaturated n6 Fatty Acids in Whole Spray-dried Egg Powder Stored in the Dark at a Temperature of 25 ± 2 °C and Analyzed at Zero Time and during Six Months for Brand A and Twelve Months for Brand B[a]

| | fatty acid (mg/100 g, dwb) | | | | | |
|---|---|---|---|---|---|---|
| months | C18:3 n3 | C20:3 n3 | C20:5 n3 | C22:6 n3 | C18:1 n9*t* | C18:2 n6*t* |
| | | | Brand A | | | |
| 0 | 455 ± 11 a | 30 ± 1 a | 41 ± 1 a | 774 ± 33 a | 1777 ± 177 a | 31 ± 3 a |
| 1 | 458 ± 10 a | 32 ± 2 a | 40 ± 2 a | 800 ± 41 a | 1645 ± 159 a | 29 ± 2 a |
| 2 | 460 ± 9 a | 31 ± 1 a | 41 ± 2 a | 805 ± 39 a | 1802 ± 132 a | 30 ± 2 a |
| 3 | 390 ± 9 b | 24 ± 1 b | 39 ± 1 a | 775 ± 12 a | 1735 ± 155 a | 32 ± 2 a |
| 4 | 394 ± 6 b | 23 ± 1 b | 36 ± 1 a | 778 ± 18 a | 1836 ± 160 a | 28 ± 2 a |
| 5 | 386 ± 5 b | 23 ± 1 b | 36 ± 1 a | 764 ± 14 a | 1713 ± 129 a | 30 ± 2 a |
| 6 | 321 ± 6 c | 18 ± 1 b | 37 ± 1 a | 505 ± 10 b | 1702 ± 134 a | 30 ± 2 a |
| | | | brand B | | | |
| 0 | 672 ± 14 a | 26 ± 1 a | 32 ± 1 a | 817 ± 46 a | 2407 ± 80 a | 44 ± 2 a |
| 1 | 662 ± 16 a | 26 ± 1 a | 32 ± 1 a | 815 ± 44 a | 2330 ± 92 a | 43 ± 2 a |
| 2 | 675 ± 12 a | 25 ± 2 a | 31 ± 1 a | 825 ± 46 a | 2458 ± 85 a | 44 ± 1 a |
| 3 | 677 ± 15 a | 26 ± 1 a | 31 ± 1 a | 829 ± 39 a | 2401 ± 91 a | 42 ± 2 a |
| 4 | 649 ± 18 a | 25 ± 1 a | 31 ± 1 a | 831 ± 43 a | 2333 ± 75 a | 42 ± 1 a |
| 5 | 655 ± 17 a | 25 ± 1 a | 31 ± 1 a | 818 ± 52 a | 2356 ± 70 a | 41 ± 2 a |
| 6 | 607 ± 10 a | 26 ± 1 a | 31 ± 1 a | 543 ± 32 b | 2330 ± 76 a | 43 ± 1 a |
| 7 | 607 ± 11 a | 26 ± 1 a | 31 ± 1 a | 532 ± 39 b | 2410 ± 81 a | 41 ± 2 a |
| 8 | 600 ± 15 a | 26 ± 1 a | 31 ± 1 a | 555 ± 44 b | 2345 ± 73 a | 44 ± 2 a |
| 9 | 611 ± 11 a | 25 ± 1 a | 31 ± 1 a | 561 ± 36 b | 2444 ± 70 a | 42 ± 2 a |
| 10 | 615 ± 12 a | 25 ± 1 a | 31 ± 1 a | 533 ± 48 b | 2423 ± 68 a | 41 ± 2 a |
| 11 | 600 ± 16 a | 26 ± 1 a | 32 ± 1 a | 496 ± 50 b | 2331 ± 79 a | 43 ± 2 a |
| 12 | 507 ± 10 b | 25 ± 1 a | 31 ± 1 a | 544 ± 45 b | 2260 ± 79 a | 43 ± 1 a |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

in aluminum foil without exposure to light avoided the loss of a lot of polyunsaturated fatty acids, especially C20:4 n6 and C22:6 n3.

Polyunsaturated fatty acids show low oxidative stability (*22*). In addition, all material of biological origin contains small amounts of transition metals, such as iron and copper. These metals present two or more valency states, making them susceptible to oxidation−reduction, and they can even abstract hydrogen from the fatty acids, as well as controlling the velocity of autoxidation. However, for these metals to have such activity, they must be in the lower valency state, which can be maintained by electron donors such as the cysteine present in eggs (*23*). In the present study, oxidation of the polyunsaturated fatty acids was probably favored by the presence of metal ions, which are potent pro-oxidants, associated with the low water activity of the egg powder.

High concentrations of trans fatty acids were observed in both brands of egg powder (**Table 6**) produced by a spray-drying process by the direct heating (180 °C) of the air by gas combustion. When unsaturated cis fatty acids are heated at high temperature in the presence of catalyzers, such as nitrogen oxides, these fatty acids are transformed into their trans stereoisomers. Besides, nitrous oxide is considered to initiate the oxidation of unsaturated lipids in model systems because it is a free radical (*24*−*26*).

**Cholesterol and Cholesterol Oxides in Spray-Dried Whole Egg Powder.** There was no significant difference ($p > 0.05$) in the cholesterol contents during storage for either brand A or B, but brand A showed higher amounts than brand B (**Table 7**). The mean values for cholesterol content were 1716 and 1570 mg/100 g of egg powder for brands A and B, respectively, values respectively similar to and lower than those cited by the USDA (*20*).

The following five oxides were identified and quantified in both brands of egg powder: 7-keto, 7$\beta$-OH, 7$\alpha$-OH, 5,6$\beta$-epoxy,

**Table 6.** Saturated, Monounsaturated, Polyunsaturated, and Trans Fatty Acids in Whole Spray-Dried Egg Powder Analyzed at 0 Time and after 6 Months for Brand A and 12 Months for Brand B[a]

| | | mg/100 g | |
|---|---|---|---|
| | | brand A | brand B |
| saturated (SAT) | T0 | 12048 | 12670 |
| | Tf | 12150 | 12682 |
| monounsaturated (MONO) | T0 | 6184 | 10590 |
| | Tf | 6183 | 10500 |
| n6 PUFA | T0 | 4410 | 4108 |
| | Tf | 3600 | 3124 |
| n3 PUFA | T0 | 1300 | 1547 |
| | Tf | 881 | 1107 |
| polyunsaturated (PUFA) | T0 | 5710 | 5655 |
| | Tf | 4481 | 4231 |
| unsaturated | T0 | 11894 | 16245 |
| | Tf | 10664 | 14731 |
| trans fatty acids | T0 | 1808 | 2451 |
| | Tf | 1774 | 2303 |
| ratio | | | |
| PUFA/SAT | T0 | 0.47 | 0.45 |
| | Tf | 0.37 | 0.33 |
| n6 PUFA/n3 PUFA | T0 | 3.39 | 2.71 |
| | Tf | 4.08 | 2.82 |

**Table 7.** Cholesterol (Milligrams per 100 g, dwb) in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C and Analyzed at 0 Time and during 6 Months for Brand A and 12 Months for Brand B[a]

| months | brand A | brand B |
|---|---|---|
| 0 | 1717 ± 3 a | 1566 ± 111 a |
| 1 | 1713 ± 3 a | 1580 ± 111 a |
| 2 | 1719 ± 6 a | 1558 ± 88 a |
| 3 | 1712 ± 11 a | 1562 ± 103 a |
| 4 | 1720 ± 2 a | 1581 ± 100 a |
| 5 | 1720 ± 7 a | 1562 ± 123 a |
| 6 | 1710 ± 13 a | 1583 ± 109 a |
| 7 | | 1575 ± 84 a |
| 8 | | 1570 ± 109 a |
| 9 | | 1571 ± 119 a |
| 10 | | 1563 ± 84 a |
| 11 | | 1576 ± 92 a |
| 12 | | 1564 ± 78 a |

[a] Mean and relative standard deviation (RSD) of the six analyses (two batches in triplicate). Means in the same column with the same letter do not differ significantly ($p > 0.05$).

and 5,6$\alpha$-epoxy. The different batches presented significantly different results for both brands A and B and were therefore expressed with respect to each batch (**Tables 8** and **9**). At 0 time, the cholesterol oxide contents were different between batches as well as between different brands, probably due to differences in the temperatures used during processing and principally due to differences in the concentrations of the nitrogen oxides produced by gas combustion during the spray-drying process. According to Morgan and Armstrong (*2*), the amount of cholesterol oxides in egg powder increases proportionally with the concentration of nitric oxide produced by gas combustion during the spray-drying process.

For both brands, batch 1 showed a higher cholesterol oxide content than batch 2, and the values increased significantly with storage. For batch 1 of brand A (**Table 8**) the amount of 7-keto increased up to 3 months, and the contents of 7$\alpha$-OH and 5,6$\beta$-epoxy increased up to the fourth and fifth months, respectively.For batch 2, 7-keto increased for up to 5 months and 7$\alpha$-OH and 5,6$\beta$-epoxy up to the end of the storage period. For both

**Table 8.** Cholesterol Oxides in Whole Spray-dried Egg Powder Stored in the Dark at a Temperature of 25 ± 2 °C and Analyzed at Zero Time and during Six Months for Brand A[a]

| | cholesterol oxides (μg/g, dwb) | | | | |
|---|---|---|---|---|---|
| months | 7-keto | 7β-OH | 7α-OH | 5,6α-epoxy | 5,6β-epoxy |
| | | | Batch 1 | | |
| 0 | 3 ± 0 c | 6 ± 1 d | 5 ± 1 d | 10 ± 1 c | 18 ± 2 e |
| 1 | 4 ± 1 bc | 8 ± 1 d | 7 ± 1 cd | 10 ± 1 c | 20 ± 1 e |
| 2 | 8 ± 1 b | 14 ± 2 c | 10 ± 1 c | 11 ± 2 c | 29 ± 2 d |
| 3 | 17 ± 2 a | 21 ± 2 b | 17 ± 2 b | 12 ± 1 c | 31 ± 3 d |
| 4 | 20 ± 2 a | 24 ± 3 b | 25 ± 2 a | 16 ± 1 b | 33 ± 3 cd |
| 5 | 20 ± 3 a | 30 ± 2 a | 28 ± 2 a | 18 ± 2 ab | 38 ± 3 bc |
| 6 | 21 ± 3 a | 32 ± 3 a | 28 ± 2 a | 20 ± 2 a | 43 ± 5 ab |
| mean | 13 ± 8 a | 19 ± 10 a | 17 ± 10 a | 14 ± 4 a | 30 ± 9 a |
| | | | Batch 2 | | |
| 0 | 8 ± 1 d | 3 ± 0 e | 6 ± 1 d | 5 ± 1 c | 6 ± 1 g |
| 1 | 10 ± 1 cd | 3 ± 0 e | 6 ± 1 d | 8 ± 1 c | 9 ± 1 fg |
| 2 | 10 ± 1 cd | 4 ± 1 de | 7 ± 1 cd | 8 ± 0 c | 10 ± 1 ef |
| 3 | 13 ± 1 bc | 6 ± 1 d | 10 ± 1 c | 11 ± 1 b | 13 ± 1 de |
| 4 | 13 ± 2 bc | 10 ± 1 b | 11 ± 1 c | 11 ± 2 b | 23 ± 2 c |
| 5 | 16 ± 2 ab | 15 ± 2 a | 17 ± 2 b | 14 ± 1 ab | 29 ± 3 b |
| 6 | 19 ± 2 a | 16 ± 1 a | 21 ± 3 a | 16 ± 1 a | 34 ± 3 a |
| mean | 13 ± 4 a | 8 ± 6 a | 11 ± 6 b | 10 ± 4 b | 18 ± 11 b |

[a] Mean and relative standard deviation (RSD) of the triplicate analyses. Means in the same column with the same letter do not differ significantly ($p > 0.05$).

**Table 9.** Cholesterol Oxides in Whole Spray-Dried Egg Powder Stored in the Dark at 25 ± 2 °C and Analyzed at 0 Time and during 12 Months for Brand B[a]

| | cholesterol oxides (μg/g, dwb) | | | | |
|---|---|---|---|---|---|
| months | 7-keto | 7β-OH | 7α-OH | 5,6α-epoxy | 5,6β-epoxy |
| | | | Batch 1 | | |
| 0 | 1 ± 0 e | 6 ± 1 g | 9 ± 1 g | 7 ± 1 g | 16 ± 2 d |
| 1 | 2 ± 0 e | 10 ± 1 fg | 15 ± 2 f | 11 ± 1 efg | 17 ± 1 d |
| 2 | 3 ± 0 e | 17 ± 2 ef | 16 ± 1 ef | 10 ± 1 fg | 18 ± 3 d |
| 3 | 3 ± 0 e | 18 ± 1 e | 16 ± 1 ef | 12 ± 1 defg | 22 ± 2 cd |
| 4 | 5 ± 0 e | 20 ± 2 cde | 18 ± 1 def | 15 ± 1 def | 28 ± 2 cd |
| 5 | 11 ± 2 d | 20 ± 2 cde | 19 ± 2 def | 16 ± 1 de | 29 ± 2 cd |
| 6 | 16 ± 1 c | 26 ± 4 cde | 19 ± 1 cdef | 16 ± 1 cd | 32 ± 3 c |
| 7 | 21 ± 2 b | 27 ± 4 cd | 21 ± 2 cde | 21 ± 2 c | 36 ± 5 c |
| 8 | 24 ± 3 b | 29 ± 3 c | 23 ± 3 cd | 27 ± 4 b | 53 ± 6 b |
| 9 | 28 ± 2 ab | 33 ± 3 bc | 24 ± 3 bc | 29 ± 1 ab | 60 ± 10 b |
| 10 | 28 ± 1 a | 43 ± 3 a | 30 ± 2 ab | 33 ± 3 a | 70 ± 7 ab |
| 11 | 31 ± 2 a | 45 ± 4 a | 33 ± 3 a | 34 ± 3 a | 70 ± 11 ab |
| 12 | 30 ± 3 a | 51 ± 8 a | 35 ± 4 a | 34 ± 3 a | 77 ± 6 a |
| mean | 15 ± 12 a | 26 ± 13 a | 21 ± 8 a | 20 ± 10 a | 41 ± 22 a |
| | | | Batch 2 | | |
| 0 | 2 ± 0 g | 5 ± 1 h | 4 ± 1 g | 3 ± 0 e | 9 ± 1 g |
| 1 | 2 ± 0 g | 5 ± 1 gh | 5 ± 0 g | 4 ± 1 e | 12 ± 2 g |
| 2 | 2 ± 0 g | 7 ± 1 fg | 6 ± 1 g | 4 ± 1 e | 13 ± 2 fg |
| 3 | 3 ± 0 g | 10 ± 1 e | 7 ± 1 g | 5 ± 1 e | 13 ± 2 fg |
| 4 | 3 ± 0 g | 10 ± 1 e | 12 ± 2 f | 11 ± 2 d | 17 ± 3 ef |
| 5 | 6 ± 1 f | 10 ± 1 e | 14 ± 1 ef | 14 ± 2 cd | 23 ± 2 de |
| 6 | 7 ± 1 ef | 17 ± 2 d | 15 ± 1 def | 14 ± 1 c | 23 ± 2 de |
| 7 | 10 ± 1 d | 18 ± 2 d | 16 ± 2 de | 15 ± 1 c | 23 ± 2 de |
| 8 | 13 ± 1 c | 18 ± 1 d | 18 ± 1 cd | 15 ± 1 c | 29 ± 2 cd |
| 9 | 13 ± 1 c | 24 ± 3 c | 20 ± 2 c | 21 ± 2 b | 31 ± 4 c |
| 10 | 15 ± 1 b | 34 ± 3 b | 20 ± 1 c | 22 ± 2 b | 33 ± 3 c |
| 11 | 18 ± 1 a | 41 ± 4 a | 26 ± 1 b | 23 ± 3 ab | 40 ± 3 b |
| 12 | 18 ± 1 a | 40 ± 2 a | 33 ± 4 a | 27 ± 2 a | 48 ± 6 a |
| mean | 9 ± 6 b | 18 ± 13 b | 15 ± 9 b | 14 ± 8 b | 24 ± 12 b |

[a] Mean and relative standard deviation (RSD) of the triplicate analyses. Means in the same column with the same letter do not differ significantly ($p > 0.05$).

batches, the oxides 7β-OH and 5,6α-epoxy increased from 0 time to 5 months.

For brand B (**Table 9**), batch 1, the 7-keto and 5,6α-epoxy oxides increased from 0 time to the 9th month of storage and 7β-OH, 7α-OH, and 5,6β-epoxy up to the 10th month. For batch 2, the 7-keto, 7β-OH, and 5,6α-epoxy oxide contents increased from 0 time up to the 11th month and 7α-OH and 5,6β-epoxy up to the end of the storage period.

Other research with egg powder also showed the presence of the following oxides: 7-keto, 7α-OH, 7β-OH, 5,6β-epoxy, and 5,6α-epoxy (*26*−*28*). In addition, traces of 19-OH, 20-OH, and 25-OH have been determined in egg powder (*3*), although these oxides were not confirmed by mass spectroscopy. On the other hand, 25-OH and the triol were observed in egg powder samples that had undergone heating (*26*).

The results of this study showed that all of the oxides in both batches of both brands increased during storage, which can be attributed to the storage temperature and the low water activity, which favor lipid oxidation. In addition, the concentrations of all the polyunsaturated fatty acids decreased during storage and could therefore have suffered oxidation with the possible formation of free radicals and peroxides, which could have contributed to the increase in cholesterol oxides.

Various researchers have noted an increase in cholesterol oxides during the storage of egg powder (*2*, *3*, *27*, *28*−*30*). Obara et al. (*3*) verified an accumulation of cholesterol oxides in samples of egg powder stored for 3 months at room temperature, due to the low water activity.

Few studies can be found in the literature relating the formation of cholesterol oxides with the oxidation of polyunsaturated fatty acids in eggs. Wahle et al. (*31*) showed that marked changes occurred in the concentration of lipid peroxidation products (TBARS), free fatty acids, fatty acid oxidation, and the formation of cholesterol oxides such as 25-OH, 7-keto, 7β-OH, epoxides, and triol in spray-dried egg powder stored at room temperature and exposed to air and light for 18 months.

From the present results, it can be concluded that no oxides were formed during the storage of the egg powder samples, apart from those already present at 0 time. On the other hand, the contents of the oxides 7-keto, 7β-OH, 7α-OH, 5,6α-epoxy, and 5,6β-epoxy increased during the storage period, with a simultaneous decrease in the polyunsaturated fatty acids. High trans fatty acid contents were found in both brands of egg powder. The presence of high levels of cholesterol oxides and trans fatty acids is of concern because they could be hazardous to the health, especially because these eggs are used in various food products widely consumed by the population, principally infants. It must nevertheless be remembered that all of the results of this study were expressed on a dry weight basis and that the amounts used in the food products would depend on the product composition.

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