DOI:10.1021/jf903488b

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Influence of Pasteurization, Spray- and Freeze-Drying, and Storage on the Carotenoid Content in Egg Yolk

Michael Wenzel,*,† Ingrid Seuss-Baum,† and Elmar Schlich‡

†Department of Food Technology, University of Applied Sciences Fulda, Marquardstrasse 35, 36039 Fulda, Germany and ‡Department of Process Engineering in Food and Servicing Business, Justus Liebig University, Giessen, Germany

A liquid chromatography−atmospheric pressure chemical ionization mass spectrometry [LC-(APCI)-MS] method was developed to identify and quantify the carotenoids present in fresh, pasteurized, and freeze- and spray-dried egg yolk in two independent batches. The egg yolk powders in each batch were stored in the dark for 6 months at −18 or 20 °C. Carotenoids were isolated by solvent extraction without saponification and analyzed by HPLC using a C_{30} column coupled to a photodiode array and mass detector. The most abundant carotenoids were *all-E*-canthaxanthin, *all-E*-lutein, *all-E*-zeaxanthin, *9-Z*-canthaxanthin, and β-apo-8′-carotenoic acid ethyl ester. Pasteurization of the egg yolk caused no critical changes in the carotenoid content. On the contrary, drying to a dry matter of 98−99% led to higher carotenoid contents, induced by a denaturation of binding proteins, and a destabilization of the cell matrix. After the 6 months of storage, the contents of all main carotenoids in the egg yolk powder were significantly lower. The synthetic carotenoids canthaxanthin and β-apo-8′-carotenoic acid ethyl ester showed a higher retention rate, and the greatest losses occurred within the first 8 weeks. Statistical tests (ANOVA, $P < 0.05$) also proved that after 26 weeks, the egg yolk powders stored at −18 °C showed only a slightly higher retention of carotenoids when compared to the powders stored at 20 °C.

KEYWORDS: Egg yolk; carotenoids; lutein; zeaxanthin; pasteurization; freeze-drying; spray-drying; storage

INTRODUCTION

Egg yolks contain substantial concentrations of highly bioavailable carotenoids, especially the xanthophylls lutein and zeaxanthin (*1*). Lutein and zeaxanthin are xanthophylls with antioxidant properties; they interest nutritionists and physicians because it is presumed that higher nutritional uptake of these substances may increase macular pigment (MP) density and thereby lower the risk for age-related macular degeneration (AMD). In Western countries, AMD is the main reason for loss of vision (*2*, *3*) and affects millions of people worldwide.Visual acuity is greatest at the macula lutea, the central region of the retina (*4*), whereby MP is considered to act as a free radical scavenger (*5*) and bluelight filter (*6*). Because consumers associate intense colors with high food quality, xanthophylls have been used to enrich poultry feed (*7*). In the European Union (EU), the law permits the addition of eight xanthophylls possessing various functional groups and carbon chain lengths (C_{30}−C_{40}) to the feed of poultry and laying hens in amounts of up to 80 mg/kg of feeding stuff (*8*). These substances are the natural xanthophylls capsanthin (C_{40}), β-cryptoxanthin (C_{40}), lutein (C_{40}), and zeaxanthin (C_{40}) and the synthetic xanthophylls β-apo-8′-carotenal (C_{30}), β-apo-8′-carotenoic acid ethyl ester (C_{30}), canthaxanthin (C_{40}), and citranaxanthin (C_{33}).

*Author to whom correspondence should be addressed (fax 49-661-9640445; e-mail michael.wenzel@hs-fulda.de).

The presence of these carotenoids in the egg yolk can differ enormously and depends on husbandry type and genetic variations (*9*). In the EU, commercial eggs are classified after rearing method, resulting in four different classes (0, ecological; 1, free range; 2, barn; 3, cage) as well as classifications for size (S, < 53 g; M, 53− < 63 g; L, 63− < 73 g; XL, ≥73 g) and grade (A extra; A; B) (*10*).

For microbiological and economic reasons, in the bakery and pasta industry eggs from class 3 (cage) are commonly processed to produce pasteurized liquid egg yolk or spray-dried egg yolk powder. As a result, the xanthophyll content of the final products depends on the amount of egg yolk and the processing.

There are various methods that describe the identification of carotenoids in fat-free foodstuffs using LC-MS analysis (see, e.g., refs *11*−*13*), but only a few studies concerning the determination of xanthophylls of foods rich in fat, for example eggs, have been conducted (*9*, *14*, *15*). The influence of processing and storage on foodstuffs, especially tomato products, juices, and carrots, is well-known, and several study results are available (*16*−*19*). Findings about the influence of freeze-drying on the functional properties of egg yolk (*20*) and the stability of polyunsaturated fatty acids (PUFAs) during processing and storage of egg yolk powder (*21*) have also been published. To the best of our knowledge, however, there is no study available considering the influence of pasteurization, drying, and storage on the content of xanthophylls in egg yolk.

 Published on Web 01/13/2010

The aim of the present study was to obtain extensive information about the xanthophyll concentrations to be found in fresh, pasteurized, spray- and freeze-dried egg yolks, and egg yolk powders with a high dry matter (98−99%) stored for up to 6 months. Accordingly, a fast, economical, and innovative HPLC method was developed whereby peak identification was supported by LC-(APCI)MS analysis.

MATERIALS AND METHODS

Chemicals. Methanol (MEOH) and *tert*-butyl methyl ether (TBME) were purchased from LGC Promochem (Wesel, Germany); formic acid was purchased from Merck (Darmstadt, Germany). High-purity water was prepared with an Arium 611 VF water purification system (Sartorius, Goettingen, Germany). All solvents were degassed by ultrasonic treatment before use.

Reference Compounds. Lutein (purity = 98.8%) and zeaxanthin (purity = 93.2%) were purchased from LGC Promochem. Canthaxanthin (purity = 98.0%) and β-apo-8′-carotenoic acid ethyl ester (purity = 96.0%) were obtained from CaroteNature (Lupsingen, Switzerland).

Preparation of Samples. *Samples*. Two batches of eggs (n = 80 each) from one egg-producing factory from husbandry class 1 (size M, quality A) were purchased from a local supermarket. Analyses were always performed > 14 days before the date of expiry.

Homogenization, Drying, Storage. Eggs were cracked and the yolks manually separated from the egg white under yellow light. Eighty egg yolks of each batch were pooled and homogenized (60 s, speed level 1, KitchenAid Professional). Half of the homogenized masses were pasteurized immediately (61.5 °C, 3.5 min, Lab Reactor LR-A 1000, IKA, Germany), the other half of the sample masses were nitrogen-purged and temporarily stored in a cooling chamber at 2 °C. After pasteurization, all of the samples were immediately processed in the spray- and freeze-dryer.

For spray-drying, a mini-spray-dryer was used (B 190, Büchi, Switzerland). The temperature of the supply air was 72 °C, and flow was adjusted to 400 nL/h. Because of the high viscosity of the egg yolk, the mass had to be mixed with high-purity water (1:1, v/v). Before freeze-drying in the laboratory freeze-dryer (β 2-16, Christ, Germany), the pure egg yolk material had to be shock-frozen at −40 °C. During the drying procedures, all of the equipment was covered by foil impermeable to light to protect the xanthophylls in the egg yolk. Immediately after drying, aliquots were procured for LC-MS analysis. The main amounts of the sample materials were carefully placed in plastic bags (approximately 2 g of egg yolk powder per bag), nitrogen-purged, and vacuum-sealed. Half of these sample materials was stored at −18 °C in a freezer and the other half at 20 °C in an air-conditioned room. After 2, 4, 8, and 26 weeks of storage in the dark, the samples were analyzed again. From all of the samples the dry matter was determined in duplicate to attain correct results for quantifying. All of the LC-MS analyses were performed in triplicate with three injections each.

Extraction of Xanthophylls. Aliquots (0.5 g) from fresh and processed egg yolk were placed directly into glass centrifuge tubes (40 mL). After the addition of 13 mL of methanol, the tubes were covered with Parafilm to avoid solvent evaporation and immediately treated with ultrasound (60 s) to improve extraction. One-time ultrasound-assisted extraction (UAE) of xanthophylls from fresh and dried egg yolk with pure methanol revealed in several advance tests a significantly higher retention from the same source material than a two-time extraction with pure methanol without assistance of ultrasound, and in comparison with a two-time UAE with methanol/TBME (2:1 v/v), a not significantly differing retention. The residue after one-time UAE from fresh and dried egg yolk with methanol was colorless, so it was concluded that extraction was complete. Remarkable efficiency of ultrasound-assisted extraction of lutein from egg yolk had previously been described (*22*, *23*). Saponification was not applied because it could result in the degradation of xanthophylls (*24*). To assist extraction, the samples were homogenized using the Ultra-Turrax T25 (IKA, Staufen, Germany) at 9500 rpm (60 s). After homogenization, the stirring unit of the Ultra-Turrax was carefully washed twice with 1 mL of methanol each. Parafilm was used again to cover the glass tubes, and after an incubation time of 20 min, the samples were centrifuged (Labofuge 400R, Thermo Scientific, Karlsruhe, Germany) at 4500 rpm (5 min, 20 °C). Aliquots of the supernatants were directly placed into HPLC vials using a plastic syringe (10 mL) and a membrane filter (0.45 μm).

Table 1. Calibration Graphs and Limits of Quantitation (LOQ) and Determination (LOD) Used for Quantification of Xanthophylls in Fresh and Processed Egg Yolks by HPLC-DAD

	calibration range (μg/mL)	calibration graphs c (μg/mL)	LOQ (μg/L)	LOD (μg/L)
lutein[a]	0.125−0.75	[height (mAUs) − 321.88]/19368	46.4	13.9
zeaxanthin[a]	0.125−0.75	[height (mAUs) + 73.68]/14695	33.7	10.1
canthaxanthin[a]	0.10−0.80	[height (mAUs) + 120.63]/10777	44.7	13.4
β-apo-8′-carotenoic acid ethyl ester	0.25−1.00	[height (mAUs) − 26.50]/13743	44.3	13.3

[a] The same calibration graph was used for the calculation of dedicated isomers.

Quantification of Xanthophylls. *Calibration*. Stock solutions of the xanthophylls *all-E*-lutein, *all-E*-zeaxanthin, canthaxanthin, and β-apo-8′-carotenoic acid ethyl ester were prepared under yellow light using ultrasonic treatment. Calibration was performed using dilutions in the range of 0.125−1.00 μg of xanthophyll/mL. Calibration graphs were recorded by plotting the appropriate peak heights (450 nm, AUs) against the concentrations (μg/mL) due to slightly overlapping peaks. Coefficients of determination were always > 0.996. Limits of quantitation (LOQ) and determination (LOD) were calculated from the respective calibration graphs using signal/noise ratios of 10:1 and 3:1 (*25*) (**Table 1**).

Validation of the Extraction Method. To investigate the method's reproducibility, homogenized fresh egg yolk samples poor in synthetic xanthophylls (class 2, grade A) were individually spiked with aliquots (500 μL each) of stock solutions of lutein (c = 0.5 μg/mL methanol), zeaxanthin (c = 2.6 μg/mL methanol), canthaxanthin (c = 1.7 μg/mL methanol), and β-apo-8′-carotenoic acid ethyl ester (c = 2.4 μg/mL methanol). After spiking, the samples were extracted and analyzed by HPLC-DAD. Recovery rates of pure xanthophylls were calculated (*26*) with the following results (n = 9 each): 95.3% (lutein), 95.0% (zeaxanthin and canthaxanthin), and 95.4% (β-apo-8′-carotenoic acid ethyl ester).

LC-(APCI)MS: Apparatus and Conditions. To analyze the xanthophylls, a system consisting of a Waters Alliance 2695 (Eschborn, Germany) and a photodiode array detector 996 set to 450 nm and coupled to a micromass Quattro LC mass spectrometer (Manchester, U.K.) were used. For separation, a Stability 100 C30 (250 × 4.6 mm i.d.) end-capped analytical column (Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany) filled with 5 μm phase material, including a precolumn of the same material (10 × 4.0 mm i.d.), was used and kept at 30 °C. The mobile phase consisted of methanol/purified water/formic acid [994:5:1 v/v/v (A)] and TBME/methanol [93:7 v/v (B)] using isocratic elution (93:7 A/B, 20 min, 1.1 mL/min, 40 μL). (APCI)MS operated in the positive mode, the APCI source was heated to 150 °C, and the APCI probe was kept at 450 °C. Corona voltage was set to 3.1 kV, the cone to 35.0 V, the extractor to 3.0 V, and the Rangefinder (RF) lens to 0.1 V. Nitrogen was used as a nebulizer and desolvation gas at 150 and 800 L/h, respectively. Within a scan range of m/z 400−600 mass spectra were acquired (scan time = 1.0 s, interscan delay = 0.1 s). UV−vis spectra were recorded from 220 to 600 nm with a resolution of 1.2 nm. Data were processed with MassLynx 4.0 software.

Statistical Analysis. Quantitative data are presented as mean ± SD. One-sided analysis of variance (ANOVA) was performed on the obtained data using Microsoft Excel XP software. The significant statistical level was set to $P < 0.05$.

RESULTS AND DISCUSSION

Identification of Xanthophylls in the Standard Solutions and Egg Yolk Samples by LC-(APCI)MS. Besides unambiguous retention times of the xanthophylls and their UV−vis, maxima obtained by DAD LC-MS were used to support peak assignment. In particular, (*Z*)-isomers not found in the standard solutions could be assigned because of their mass. The mass traces were extracted

Table 2. Spectroscopic and LC-(APCI)MS Data Used for Identification of Xanthophylls in Fresh and Processed Egg Yolks

	VIS maxima[a] (nm)				
xanthophyll[b]	I	II	III	*Q* ratio	main ions (*m*/*z*; intensity)
all-E-lutein (**1**)	420	443	474	III/II 0.61	569 (78%) $[M + H]^+$, 551 (100%) $[M + H - H_20]^+$
all-E-zeaxanthin (**2**)	420	446	475	III/II 0.23	569 (100%)
13-Z-lutein (**3**)	417	441	472	III/II 0.30	569 (100%)
13-Z-zeaxanthin (**4**)	416	442	473	III/II 0.11	569 (100%)
9-Z-canthaxanthin (**5**)	363	478		I/II 0.23	565 (100%)
all-E-canthaxanthin (**6**)		478			565 (100%)
Z-isomer canthaxanthin (**7**)		478[c]			565 (100%)[c]
β-apo-8′-carotenoic acid ethyl ester (**8**)		445			461 (100%)

[a] Determined in the HPLC eluents. [b] Shown in elution order. [c] Weak signal.

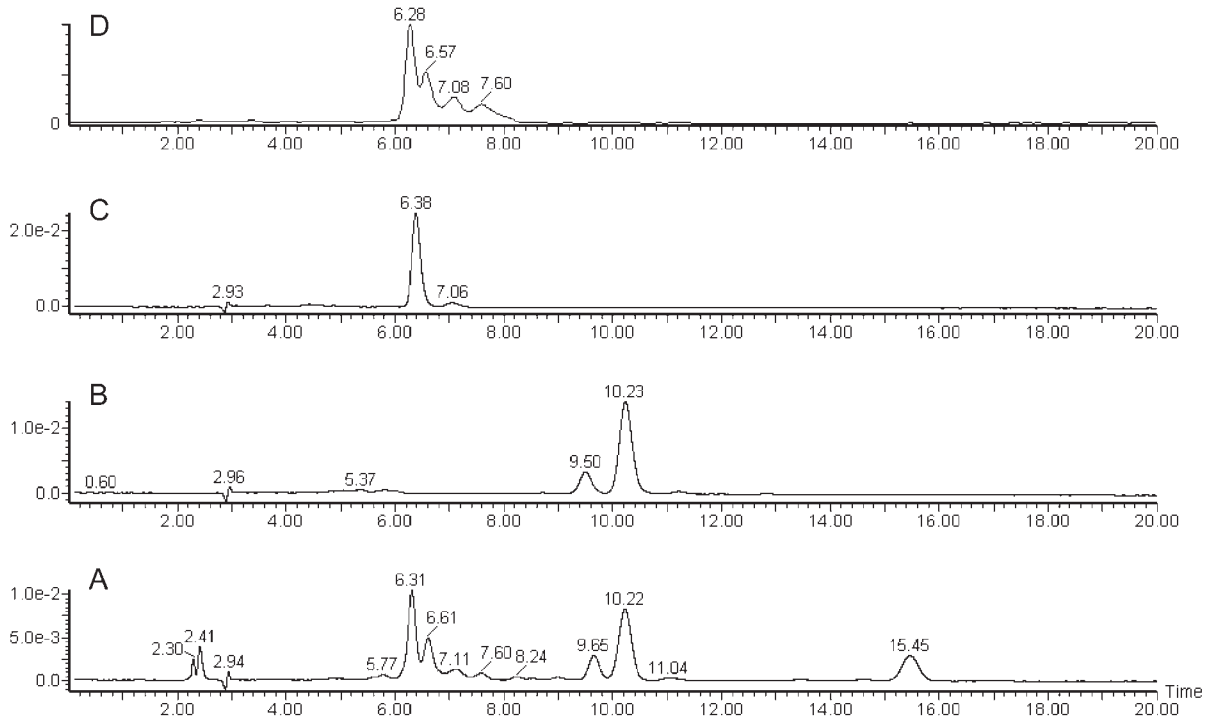

Figure 1. LC-(APCI)MS analysis of a freeze-dried egg yolk extract. The bottom trace (**A**) corresponds to detection at 450 nm (DAD). The upper trace (**D**) represents the appropriate mass trace for lutein and zeaxanthin (*m*/*z* 568) extracted from the TIC. Traces **B** and **C** correspond to DAD signals (450 nm) from calibration solutions of canthaxanthin and lutein. Peak numbers comply with the assignments given in **Table 2**.

from the total ion chromatogram (TIC) and were considered to be sufficient, although no cleanup of the egg yolk samples was performed beyond the xanthophyll extraction process. The data set used for identification of the xanthophylls present in standard solutions and egg yolk samples is shown in **Table 2**.

Eight substances of relevant quantity in the sample material were obtained. A representative HPLC chromatogram is depicted in **Figure 1**. *all-E*-Lutein (**1**), *13-Z*-lutein (**2**), *all-E*-zeaxanthin (**3**), *13-Z*-zeaxanthin (**4**), *9-Z*-canthaxanthin (**5**), *all-E*-canthaxanthin (**6**), and β-apo-8′-carotenoic acid ethyl ester (**8**) were clearly identified by their characteristic retention times, masses, and, in addition, their spectral fine structures (% III/II, I/II). Traces of *13-Z*-lutein and *13-Z*-zeaxanthin and mentionable contents of *9-Z*-canthaxanthin could already be detected in the stock solutions besides their dedicated *all-E*-compounds (see **Figure 1**). Masses used for identification were 569 Da (**1**−**4**), 565 Da (**5**−**7**), and 461 Da (**8**). Besides these masses of quasimolecular ions, strong mass signals of dedicated molecular ions $[M^+]$ could also be observed as well (see **Figure 1**). Because spectral data and masses were almost equal to **6**, it was concluded that substance **7** might represent a (*Z*)-isomer of canthaxanthin. Furthermore, in every HPLC chromatogram about half a dozen peaks indicating minor components were observed (see **Figure 1**), but identification and quantification of these substances failed because standard solutions gave no hint and UV−vis and mass signals were not distinctive.

Quantification of Xanthophylls in Fresh and Processed Egg Yolk Samples. In this study, the extraction of xanthophylls was accomplished by using pure (slightly nonpolar) methanol supported by ultrasound treatment. The use of ultrasound treatment in facilitating the extraction of carotenoids from foods is widely acknowledged (*22*, *27*) but has to be applied carefully because effects on degradation and isomerization are possible (*28*). Calibration graphs, LOQs, and LODs of substances determined in this study are shown in **Table 1**.

all-E-Canthaxanthin was the predominant xanthophyll present in two batches of fresh commercial egg yolk from husbandry class 1, followed by *all-E*-lutein, *all-E*-zeaxanthin, *9-Z*-canthaxanthin, and β-apo-8′-carotenoic acid ethyl ester. Xanthophyll contents of batch 1 were slightly higher than those of batch 2, but

Table 3. Concentrations of Xanthophylls in Fresh and Spray-Dried Egg Yolks (Stored Powders: Upper Values Correspond to Storage at −18 °C and Lower Values to Storage at 20 °C) from Husbandry Class 1 from Batch 2 Presented as Mean Value ± Standard Deviation (Micrograms per 100 g of Egg Yolk, $n = 9$ Each)[a]

	μg/100 g of egg yolk								
sample	1	2	3	4	5	6	7	8	total
fresh	549.0 ± 17.1	350.7 ± 19.8	14.9 ± 4.6	69.7 ± 4.7	322.4 ± 11.3	887.5 ± 37.0	67.4 ± 3.9	211.4 ± 12.7	2473.1 ± 99.2
pasteurized	549.7 ± 10.8 a	341.4 ± 6.2 a	10.2 ± 5.4 a	72.9 ± 3.5 a	331.3 ± 7.8 a	893.4 ± 19.2 a	68.3 ± 3.6 a	215.6 ± 12.9 a	2482.9 ± 40.5
spray-dried	701.2 ± 27.2 b	429.6 ± 22.3 b	38.0 ± 2.9 b	94.7 ± 5.2 b	356.5 ± 12.7 b	920.5 ± 38.0 ab	57.8 ± 3.2 bdfg	225.0 ± 13.0 ab	2823.2 ± 114.0
2 weeks	434.2 ± 20.4 c	256.9 ± 11.4 c	48.9 ± 2.4 c	51.2 ± 4.5 c	357.2 ± 2.6 b	941.6 ± 18.9 b	63.4 ± 3.0 ceg	234.3 ± 7.4 b	2387.6 ± 42.2
	384.1 ± 10.5 f	210.7 ± 6.5 e	46.7 ± 2.2 c	51.0 ± 2.4 c	315.5 ± 9.7 e	831.1 ± 30.4 e	62.0 ± 2.4 ceg	201.8 ± 6.1 d	2102.9 ± 60.0
4 weeks	274.9 ± 17.1 d	223.0 ± 7.8 d	29.8 ± 3.0 dg	32.2 ± 2.0 d	385.0 ± 9.8 c	830.4 ± 12.4 ce	55.9 ± 3.7 bf	217.8 ± 3.5 a	2049.2 ± 26.4
	199.7 ± 11.4 g	190.8 ± 4.1 f	20.9 ± 2.6 f	31.4 ± 2.1 d	340.2 ± 16.3 a	793.2 ± 12.7 f	55.1 ± 3.6 f	202.7 ± 6.1 d	1834.0 ± 35.9
8 weeks	263.1 ± 5.4 d	218.5 ± 4.8 d	36.4 ± 3.2 b	48.5 ± 4.2 c	374.9 ± 11.1 cd	810.2 ± 10.9 de	59.3 ± 3.7 bdeg	193.1 ± 7.7 c	2003.9 ± 34.7
	179.6 ± 3.7 h	186.1 ± 4.2 g	29.3 ± 2.2 d	38.9 ± 1.7 f	334.0 ± 8.7 a	792.2 ± 13.6 df	60.6 ± 4.7 g	174.7 ± 3.3 e	1800.3 ± 25.1
26 weeks	251.4 ± 4.6 e	211.6 ± 3.4 e	43.3 ± 2.2 e	56.9 ± 2.6 e	368.9 ± 9.2 d	804.5 ± 9.2 d	60.4 ± 2.2 deg	190.7 ± 2.0 c	1987.8 ± 15.4
	177.7 ± 3.2 h	184.9 ± 2.7 g	32.2 ± 2.7 g	45.1 ± 1.9 g	323.0 ± 4.9 e	804.0 ± 6.8 d	72.4 ± 3.4 h	175.1 ± 4.5 e	1814.4 ± 4.7

[a] Peak numbers comply with the assignments given in **Table 2**. Means within columns with different letters are significantly different ($P < 0.05$).

Table 4. Concentrations of Xanthophylls in Fresh and Freeze-Dried Egg Yolks (Stored Powders: Upper Values Correspond to Storage at −18 °C and Lower Values to Storage at 20 °C) from Husbandry Class 1 from Batch 2 Presented as Mean Value ± Standard Deviation (Micrograms per 100 g of Egg Yolk, $n = 9$ Each)[a]

	μg/100 g of egg yolk								
sample	1	2	3	4	5	6	7	8	total
fresh	549.0 ± 17.1	350.7 ± 19.8	14.9 ± 4.6	69.7 ± 4.7	322.4 ± 11.3	887.5 ± 37.0	67.4 ± 3.9	211.4 ± 12.7	2473.1 ± 99.2
pasteurized	549.7 ± 10.8 a	341.4 ± 6.2 a	10.2 ± 5.4 a	72.9 ± 3.5 a	331.3 ± 7.8 ae	893.4 ± 19.2 ae	68.3 ± 3.6 a	215.6 ± 12.9 a	2482.9 ± 40.5
freeze-dried	692.3 ± 28.6 b	428.8 ± 23.3 b	67.5 ± 3.2 b	77.0 ± 2.4 b	395.2 ± 24.9 b	1053.1 ± 41.4 b	58.9 ± 2.9 b	270.8 ± 8.9 b	3043.6 ± 112
2 weeks	470.3 ± 32.7 c	250.8 ± 24.5 cg	50.2 ± 1.9 c	55.0 ± 3.3 c	328.8 ± 21.2 ade	900.1 ± 46.1 ace	55.6 ± 3.3 c	235.6 ± 15.3 c	2346.5 ± 139.6
	486.5 ± 12.0 c	265.9 ± 7.4 c	55.6 ± 2.0 g	52.8 ± 1.9 c	339.4 ± 9.9 e	922.4 ± 38.1 ef	56.8 ± 4.7 bc	233.2 ± 10.9 c	2412.7 ± 65.3
4 weeks	261.2 ± 17.5 d	233.3 ± 1.8 d	22.4 ± 2.4 d	27.1 ± 2.0 d	349.8 ± 16.8 ce	876.7 ± 28.4 acd	46.9 ± 2.3 d	239.2 ± 7.3 c	2056.6 ± 51.8
	375.3 ± 20.3 g	248.2 ± 6.3 g	31.8 ± 3.8 ef	32.1 ± 2.7 g	422.9 ± 18.3 f	943.1 ± 34.0 f	57.0 ± 3.7 bc	256.8 ± 8.7 f	2367.2 ± 80.3
8 weeks	241.8 ± 4.7 e	218.4 ± 4.4 e	29.7 ± 1.1 e	38.9 ± 1.4 e	329.2 ± 6.7 a	872.1 ± 16.0 cd	51.6 ± 2.2 e	192.8 ± 5.0 d	1974.5 ± 27.3
	327.7 ± 13.0 h	233.8 ± 7.0 cdh	39.0 ± 1.6 h	49.6 ± 4.1 h	359.0 ± 9.5 c	833.4 ± 17.2 g	50.2 ± 1.4 e	183.7 ± 4.6 e	2076.2 ± 53.0
26 weeks	233.7 ± 2.4 f	213.0 ± 4.2 f	30.9 ± 1.1 f	42.7 ± 2.4 f	322.6 ± 3.8 d	862.8 ± 4.8 d	57.1 ± 1.7 bc	187.1 ± 1.6 e	1950.0 ± 9.2
	328.2 ± 2.9 h	228.3 ± 3.9 h	40.6 ± 0.9 i	53.4 ± 1.6 c	353.2 ± 6.5 c	828.8 ± 13.6 g	62.4 ± 1.1 f	178.0 ± 2.9 g	2072.9 ± 16.6

[a] Peak numbers comply with the assignments given in **Table 2**. Means within columns with different letters are significantly different ($P < 0.05$).

both showed a very similar distribution pattern indicating that the hens' feed was of the same quality. The observed distribution and amounts of xanthophylls detected in eggs from husbandry class 1 are in line with a recent study (*9*) and in accordance with legal regimentations (*8*).

Statistical data (mean value ± standard deviation; $n = 9$ each) of xanthophylls from fresh egg yolks, processed (spray-dried, freeze-dried) egg yolks, and egg yolk powders stored at −18 and 20 °C for up to 6 months from one egg batch are presented in **Tables 3** and **4**. Because of different dry matters, all values were standardized on the basis of 100 g of fresh egg yolk.

Pasteurization. As expected, pasteurization of fresh (liquid) egg yolk caused no significant changes in the xanthophyll content. A heating temperature of 61.5 °C, an exposure time of 3.5 min, and exposure to oxygen during pasteurization had no obviously significant effects.

Drying. Spray- and freeze-drying of the same basic raw material to dry matters to 97.9 and 98.9%, respectively, predominantly led to a significantly higher retention of xanthophylls (see **Tables 3** and **4**). A reasonable explanation for this effect could be a destabilization of the egg yolk granule fraction caused by a high dry matter, leading to an irreversible denaturation of egg yolk (lipo)proteins and followed by a release of associated xanthophylls that had not been previously extractable. A recent study (*20*) accentuated that, on the one hand, lowering the water content of freeze-dried egg yolk powders significantly correlates with a higher ability of egg yolk proteins to produce stable three-dimensional networks if rehydrated. On the other hand, a prior pasteurization at inappropriate temperatures and times leads to a remarkable degree of denaturation of egg yolk proteins. Other authors (*17*, *29*, *30*) dealing with the effects of thermal processing such as spray-drying on carotenoid-rich foodstuffs such as tomatoes and potatoes emphasized that heat-induced matrix softening causes relative changes in the carotenoid content by up to 50%.

Relative changes in *13-Z*-lutein and *13-Z*-zeaxanthin were noticeably greater, indicating an isomerization that could have

been caused by the parameters of temperature, time, and exposure to oxygen during the drying processes. This effect is well-known and has been observed in several carotenoid-containing model systems of foods (*30*−*32*).

Storage. Whereas the storage of spray-dried egg yolk at room temperature resulted in a slightly lower xanthophyll retention, the storage of freeze-dried egg yolk at 20 °C implicated weakly higher xanthophyll contents by tendency compared to freeze-dried egg yolk stored at −18 °C (see **Tables 3** and **4**). The results of this study support the conclusion that egg yolk powders do not have to be stored at low temperatures because a protective influence on xanthophyll retention could be refuted.

Contents of *all-E*-lutein, *all-E*-zeaxanthin, *all-E*-canthaxanthin, and *β*-apo-8′-carotenoic acid ethyl ester in spray- and freeze-dried egg yolk fell continuously during a storage time of 6 months, following an exponential function whereby the greatest losses occurred during the first 4 weeks of storage time. This progress is consistent with results of other xanthophyll storage studies (*33*, *34*). In contrast, *13-Z*-lutein, *13-Z*-zeaxanthin, *9-Z*-canthaxanthin, and *Z*-isomer canthaxanthin (**7**) contents proved to be quite stable. The results of this study also suggest that natural pigments such as *all-E*-lutein and *all-E*-zeaxanthin are more liable to an intense degradation in the egg matrix than synthetic xanthophylls *all-E*-canthaxanthin and *β*-apo-8′-carotenoic acid ethyl ester. In this context, the findings of prior studies, which emphasized the relatively high stability of *all-E*-canthaxanthin during a longer storage period in a fatty matrix (*35*) and a low degradation rate of *β*-apo-8′-carotenoic acid ethyl ester during boiling of eggs (*9*), could be confirmed. The low degradation of the quantitatively dominating synthetic xanthophylls in the chosen egg material can be underscored as the main reason for relatively low xanthophyll loss (17−27%) during 26 weeks of storage. On the basis of the outcome of this study, the retention of total xanthophyll content during 6 months of lightless storage depends mostly on the distribution of natural and synthetic pigments in the egg yolk and on storage temperature only to a lesser degree.

It must be emphasized that stability data based on model systems may not be transferred to complex food matrices containing inevitably volatile amounts of bioactive compounds such as antioxidants without intensive investigation. Furthermore, knowledge about xanthophyll concentrations in dried egg yolks at all stages of storage will help to support estimations of the intake of xanthophylls from foods partly or completely made of processed egg yolk.

LITERATURE CITED

(1) Handelman, G. J.; Nightingale, Z. D.; Lichtenstein, A. H.; Schaefer, E. J.; Blumberg, J. B. Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. *Am. J. Clin. Nutr*. **1999**, *70*, 247–251.

(2) Gale, C. R.; Hall, N. F.; Phillips, D. I. W.; Martyn, C. N. Lutein and zeaxanthin status and risk of age-related macular degeneration. *Investigative Ophthalmology & Visual Science* **2003**, *44*, 2461–2465.

(3) Guymer, R. H.; Chong, E. W.-T. Modifiable risk factors for age-related macular degeneration. *The Medical Journal of Australia* **2006**, *184*, 455–458.

(4) Broekmans, W. M. R.; Berendschot, T. T. J. M.; Klöpping-Ketelaars, I. A. A.; de Vries, A. J.; Goldbohm, R. A.; Tijburg, L. B. M.; Kardinaal, A. F. M.; van Poppel, G. Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. *Am. J. Clin. Nutr*. **2002**, *76*, 595–603.

(5) Khachik, F.; Bernstein, P. S.; Garland, D. L. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Investigative Ophthalmology & Visual Science* **1997**, *38*, 1802–1811.

(6) Landrum, J. T.; Bone, R. A.; Chen, Y.; Herrero, C.; Llerena, C. M.; Twarowska, E. Carotenoids in the human retina. *Pure Appl. Chem*. **1999**, *71*, 2237–2244.

(7) Christensen, C. M. Effects of color on aroma, flavor and texture judgments of foods. *J. Food Sci*. **1983**, *48*, 787–790.

(8) Community register of feed additives pursuant to Regulation (EC) 1831/2003, released 17 November 2009 (http://ec.europa.eu/comm/food/food/animalnutrition/feedadditives/registeradditives_en.htm); Regulation (EC) 1831/2003 of the European Parliament and of the council of 22 September 2003 on additives for use in animal nutrition.

(9) Schlatterer, J.; Breithaupt, D. E. Xanthophylls in commercial egg yolks: quantification and identification by HPLC and LC-(APCI)MS using a C30 phase. *J. Agric. Food Chem*. **2006**, *54*, 2267–2273.

(10) Commission Regulation (EC) 2295/2003 of 23 December 2003 introducing detailed rules for implementing Council Regulation (EEC) 1907/90 on certain marketing standards for eggs. Art. 5-7, Art. 13, Annex II.

(11) Breithaupt, D. E.; Schwack, W. Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. *Eur. Food Res. Technol*. **2000**, *211*, 52–55.

(12) De Jesus Ornelas-Paz, J.; Yahia, E. M.; Gardea-Bejar, A. Identification and quantification of xanthophyll esters, carotenes, and tocopherols in the fruit of seven Mexican mango cultivars by liquid chromatography-atmospheric pressure chemical ionization-time-of-flight mass spectrometry [LC-(APCI+)-MS]. *J. Agric. Food Chem*. **2007**, *55*, 6628–6635.

(13) De Rosso, V. V.; Mercadante, A. Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. *J. Agric. Food Chem*. **2007**, *55*, 5062–5072.

(14) Kang, D. K.; Kim, S. I.; Cho, C. H.; Yim, Y. H.; Kim, H. S. Use of lycopene, an antioxidant carotenoid, in laying hens for egg yolk pigmentation. *Asian*−*Aust. J. Anim. Sci*. **2003**, *16*, 1799–1803.

(15) Liu, L.; Zhang, M.; Peng, G.; Wang, H.; Zhang, S. Separation and identification of red pigments in natural red yolk of duck's eggs by HPLC-MS-MS. *Chinese J. Chrom*. **2004**, *22*, 197–201.

(16) Baloch, A. K.; Buckle, K. A.; Edwards, R. A. Effect of processing variables on the quality of dehydrated carrot. *J. Food Technol*. **1977**, *12*, 285–293.

(17) Chang, C.-H.; Liu, Y.-C. Study on lycopene and antioxidant contents variations in tomatoes under air-drying process. *J. Food Sci*. **2007**, *72*, E532–E540.

(18) Cortés, C.; Esteve, M. J.; Rodrigo, D.; Torregrosa, F.; Frígola, A. Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices. *Food Chem. Toxicol*. **2006**, *44*, 1932–1939.

(19) Gahler, S.; Otto, K.; Böhm, V. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *J. Agric. Food Chem*. **2003**, *51*, 7962–7968.

(20) Jaekel, T.; Dautel, K.; Ternes, W. Preserving functional properties of hen's egg yolk during freeze-drying. *J. Food Eng*. **2008**, *87*, 522–526.

(21) Guardiola, F.; Codony, R.; Manich, A.; Rafecas, M.; Boatella, J. Stability of polyunsatured fatty acids in egg powder processed and stored under various conditions. *J. Agric. Food Chem*. **1995**, *43*, 2254–2259.

(22) Mason, T. J.; Paniwnyk, L.; Lorimer, J. P. The uses of ultrasound in food technology. *Ultrason. Sonochem*. **1996**, *3*, S253–S260.

(23) Yue, X.; Xu, Z.; Prinyawiwatkul, W.; King, J. M. Improving extraction of lutein from egg yolk using an ultrasound-assisted solvent method. *J. Food Sci*. **2006**, *71*, C239–C241.

(24) Larsen, E.; Christensen, L. P. Simple saponification method for the quantitative determination of carotenoids in green vegetables. *J. Agric. Food Chem*. **2005**, *53*, 6598–6602.

(25) Kromidas, S., Kuss, H.-J., Eds. *Chromatogramme richtig integrieren und bewerten - Ein Praxishandbuch für die HPLC und GC*; Wiley-VCH: Weinheim, Germany, 2008.

(26) Kromidas, S., Ed. *Validierung in der Analytik*; Wiley-VCH: Weinheim, Germany, 2000.

(27) Sun, T.; Xu, Z.; Godber, J. S. Ultrasound assisted extraction in quantifying lutein from chicken liver using high-performance liquid chromatography. *J. Chromatogr., B* **2006**, *830*, 158–160.
(28) Zhao, L.; Zhao, G.; Chen, F.; Wang, Z.; Wu, J.; Hu, X. Different effects of microwave and ultrasound on the stability of (*all-E*)-astaxanthin. *J. Agric. Food Chem.* **2006**, *54*, 8346–8351.
(29) Dewanto, V.; Wu, X.; Adom, K. K.; Liu, R. H. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.* **2002**, *50*, 3010–3014.
(30) Chandler, L. A.; Schwartz, S. J. Isomerization and losses of *trans*-β-carotene in sweet potatoes as affected by processing treatments. *J. Agric. Food Chem.* **1988**, *36*, 129–133.
(31) Aman, R.; Schieber, A.; Carle, R. Effects of heating and illumination on trans−cis isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *J. Agric. Food Chem.* **2005**, *53*, 9512–9518.
(32) Milanowska, J.; Gruszecki, W. I. Heat-induced and light-induced isomerization of the xanthophyll pigment zeaxanthin. *J. Photochem. Photobiol. B: Biol.* **2005**, *80*, 178–186.
(33) Lin, C. H.; Chen, B. H. Stability of carotenoids in tomato juice during storage. *Food Chem.* **2005**, *90*, 837–846.
(34) Shi, X.-M.; Chen, F. Stability of lutein under various storage conditions. *Nahrung* **1997**, *41*, 38–41.
(35) Sheehan, E. M.; O'Connor, T. P.; Sheehy, P. J. A.; Buckley, D. J.; FitzGerald, R. Stability of astaxanthin and canthaxanthin in raw and smoked Atlantic salmon (*Salmo salar*) during frozen storage. *Food Chem.* **1998**, *63*, 313–317.

Received for review October 6, 2009. Revised manuscript received December 21, 2009. Accepted December 26, 2009.