JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Domestic Cooking Methods on Egg Yolk Xanthophylls

Chamila Nimalaratne,[†] Daise Lopes-Lutz,[†] Andreas Schieber,*[,†,‡] and Jianping Wu*[,†]

[†]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

[‡]Department of Nutritional and Food Sciences, University of Bonn, Römerstrasse 164, D-53117 Bonn, Germany

ABSTRACT: Xanthophylls are a class of bioactive compounds known to play an important role in preventing age-related macular degeneration. Egg yolk is a rich source of highly bioavailable xanthophylls including lutein and zeaxanthin. The effects of domestic cooking methods (boiling, frying, microwaving) on egg yolk xanthophyll content were investigated. A LC-(APCI)-MS/MS method was used to identify and quantify all-*E*- and *Z*-isomers of lutein, zeaxanthin, canthaxanthin, and *β*-apo-8′-carotenoic acid ethyl ester in fresh and cooked egg yolks. Both fresh and cooked yolks showed similar xanthophyll profiles but with higher contents of *Z*-isomers in cooked samples. All-*E*-lutein was the most affected, with 22.5%, 16.7%, and 19.3% reductions in boiled, microwaved, and fried yolk extracts, respectively. Total xanthophyll losses ranged from 6% to 18%. The results presented here could be useful in calculating the dietary intake of xanthophylls and also in assessing the xanthophyll profiles and contents of egg-containing products.

KEYWORDS: *egg yolk, xanthophylls, domestic cooking methods, LC-(APCI)-MS/MS*

INTRODUCTION

Xanthophylls, the yellow pigments of egg yolk, have long been associated with the quality of eggs by consumers.[1] Later, with more detailed understanding of the link between xanthophylls and human health, they became an important class of bioactive compounds.[2,3] Among many food types, egg yolk is considered one of the important sources of xanthophylls with higher bioavailability than other common sources such as dark-green leafy vegetables.[4,5] The xanthophyll carotenoids, especially lutein and zeaxanthin, are known to accumulate in the macular region of the human retina and play an important role in human health, especially in preventing age-related macular degeneration, the main reason for vision loss of the elderly in western countries.[4,6,7] Several studies reported the elevation of plasma lutein and zeaxanthin levels followed by diets containing egg yolks.[6,8]

An average egg yolk usually contains about 175−400 μg of lutein and about 200−300 μg of zeaxanthin, but this could be significantly affected by hens' feed composition.[6] In commercial poultry farming, synthetic xanthophylls are permitted to be used in poultry feed within the maximum allowable limits. In organic farming, as synthetic additives are not permitted, xanthophyll-rich plants are used to obtain the desired amounts in the eggs.[9] In designer eggs, which are enriched with desired compounds through modified poultry feed, lutein content was reported to be as high as 1.91 mg of per yolk.[8]

Xanthophylls are oxygenated carotenoids with long conjugated polyene chains which predominantly exist in nature in their all-*E*-isomeric form. They are susceptible to processing conditions such as heat, light, and oxygen and can be converted into their *cis*-isomeric forms, which may result in changes of their functionality and bioavailability.[10,11] The effects of thermal processing on carotenoids in various fruits and vegetables such as oranges, broccoli, corn, spinach, etc., have been studied in the past years.[12−15] However, very few studies report the effect of cooking on egg yolk xanthophyll composition. One study showed that boiling of eggs decreased the xanthophyll content of the yolk with 20% loss of lutein, whereas the content of other xanthophylls was reduced by 10% to 20%.[5] Pasteurization of liquid egg yolk has no influence on the xanthophyll content, while significant reductions were observed during the storage time regardless of the storage temperature (−18 or 20 °C).[16,17] Another study found that boiling of designer eggs did not change the lutein concentration in egg yolk.[8] There is limited information about the effects of different home cooking methods such as boiling, microwaving, frying, etc., on egg yolk xanthophylls. Therefore, the objective of this study was to investigate how different cooking methods affect the content and composition of egg yolk xanthophylls.

MATERIALS AND METHODS

Materials and Chemicals. Light petroleum ether, methanol, acetone, ethyl acetate, and TBME (*tert*-butyl methyl ether) were purchased from Fisher Scientific (Ottawa, ON, Canada) and of HPLC grade. Lutein (xanthophyll from marigold), zeaxanthin, canthaxanthin, *β*-apo-8′-carotenal, silica gel 60 (0.063−0.200 mm), and butylated hydroxytoluene (BHT) were purchased from Sigma (Oakville, ON, Canada); *β*-apo-8′-carotenoic acid ethyl ester was obtained from CaroteNature (Lupsingen, Switzerland). *Z*-Isomers were obtained by iodine-catalyzed photoisomerization of the all-*E*-carotenoids.[13]

Iodine-Catalyzed Photoisomerization to Obtain *Z*-Isomers. Stock solutions of all-*E* compounds were prepared at a concentration of 500 μg/mL in TBME:methanol (3:1 v/v) containing 0.1% BHT and stored in amber glassware. To obtain *Z*-isomers of reference compounds, the following procedure adapted from Aman et al.[13] was used with minor modifications. For 200 μL of all-*E* standards, 150 μL of iodine in hexane (c = 40 umol/L) (to obtain a final iodine concentration of approximately 1−2% (w/w) of the carotenoid) was added. Solutions were exposed to fluorescent light (four fluorescent lamps: 54 W, T5, Cool white, Philips, Holland) for 30 min at room

Received: September 4, 2012
Revised: November 28, 2012
Accepted: December 3, 2012
Published: December 3, 2012

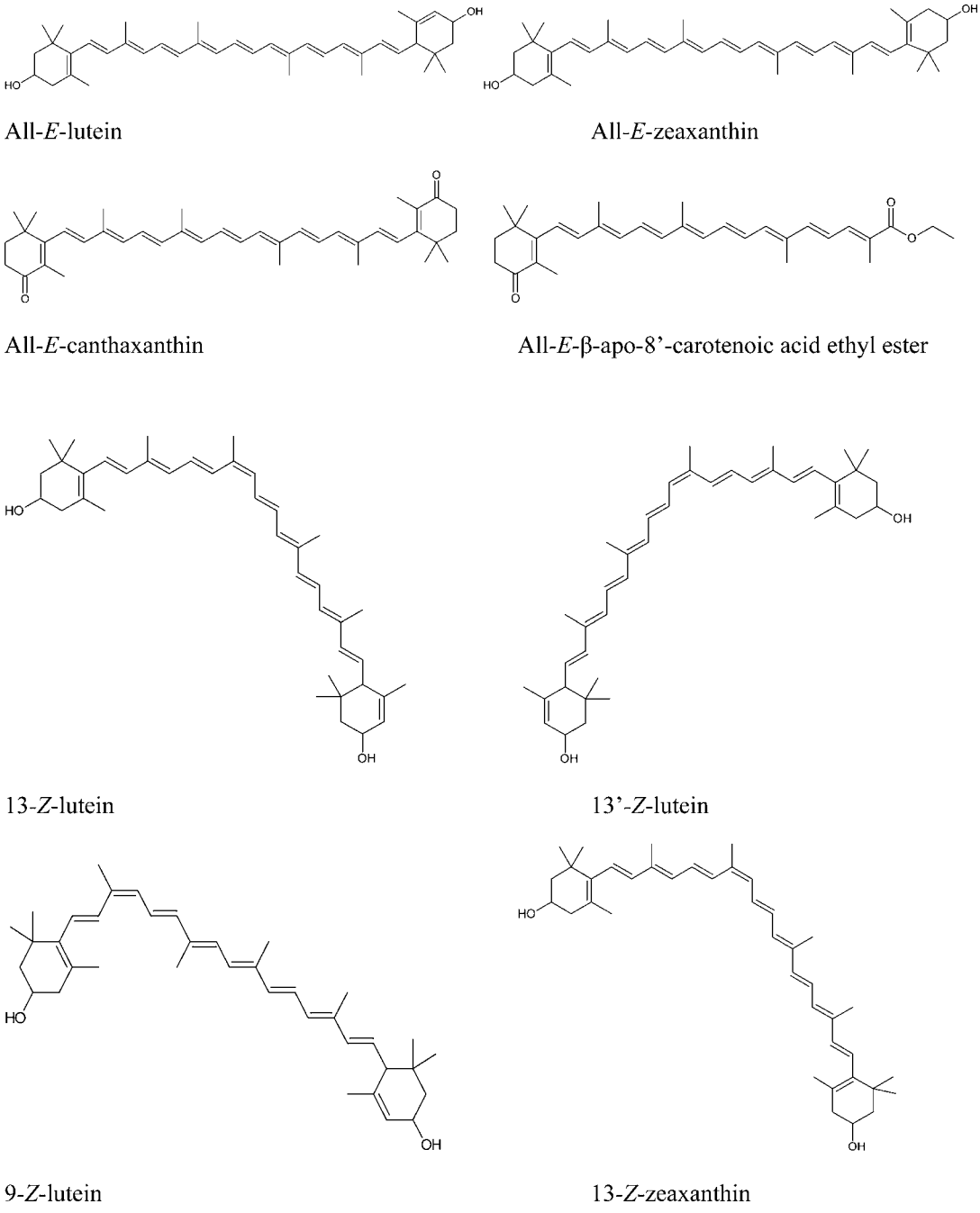

Figure 1. Chemical structure of xanthophylls found in egg yolk.

temperature. The illumination intensity was measured using a luxmeter (3900 lx) (LI-188 B, Li-Cor Inc., Lincoln, NE, USA). Solutions were then washed twice with $Na_2S_2O_3$ solution (1 mol/L) to remove excess iodine and evaporated under nitrogen gas. Residues were dissolved in a mixture of TBME:methanol (3:1, v/v) containing 0.1% BHT and made up to 1 mL (final concentration 100 μg/mL). These isomers were characterized by their UV spectra, retention times compared to the standards, and elution order of the compounds.

Preparation of Samples. Eggs (n = 48) were purchased from a local supermarket (Edmonton, AB, Canada), and all experiments were performed at least 2 weeks prior to the expiry date. Eggs were divided into four equal sets, and three sets were subjected to different cooking treatments, boiling, microwaving, and frying, and the fourth was used as the control.

Cooking of Eggs. One set of whole shell eggs was placed in a saucepan as a single layer, with water up to 1−2 in. above the eggs, and boiled for 10 min. After boiling, they were placed under running tap water for 5 min and peeled and yolks were separated from whites. For microwaving, whole egg without the shell was placed in a microwavable glass bowl and cooked in a household microwave oven (model DMW 113w, Danby Products, ON, Canada) for 90 s at cooking level (1100 W, 2450 MHz); egg yolks were carefully separated from whites. Another set of eggs was fried using a frying pan (model SK200TY nonstick frying pan, Black & and Decker Canada Inc., Brockville, ON, Canada) preheated to 205 °C. Whole eggs were fried for 6 min (3 min each side), and the yolks were separated from whites. Raw egg yolks were used as a control. To prepare raw yolk samples, egg yolks were manually separated from whites and wiped with a filter paper to remove adhered albumins. Cooked and raw egg yolks were pooled, homogenized, and placed immediately at −20 °C and then subjected to freeze drying in containers impermeable to light. Freeze-dried samples were ground to obtain a fine powder. Processed samples

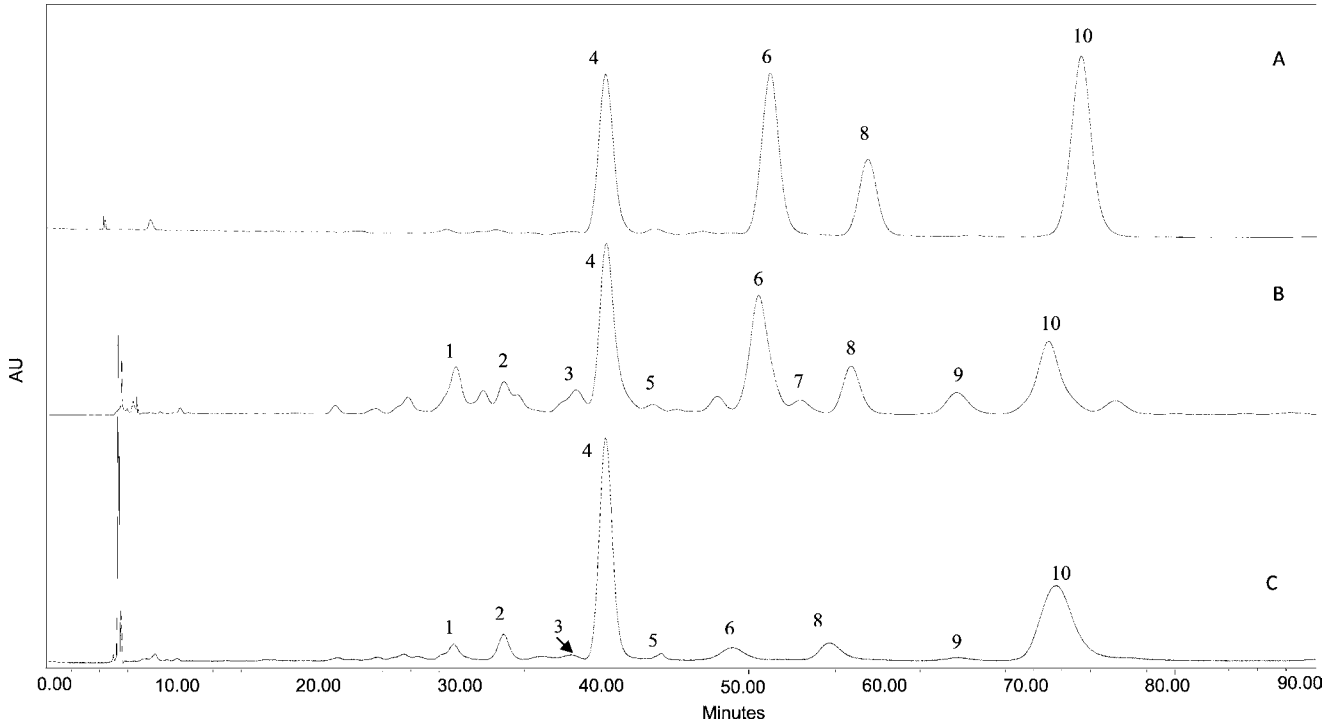

Figure 2. HPLC with photodiode-array UV−vis absorbance detection of a standard mixture of all-*E* xanthophylls (A), a standard mixture showing separation of isomers of xanthophylls (B), and an extract from egg yolk (raw) showing separation of xanthophylls (C). Peak identification: (1) 13-*Z*-lutein, (2) 13′-*Z*-lutein, (3) 13-*Z*-zeaxanthin, (4) all-*E*-lutein, (5) *Z*-isomer of canthaxanthin, (6) all-*E*-zeaxanthin, (7) 9-*Z*-lutein, (8) all-*E*-canthaxanthin, (9) 9′-*Z*-lutein, and (10) all-*E*-*β*-apo-8′-carotenoic acid ethyl ester.

were stored in the dark at −20 °C in airtight sealed plastic containers until analysis.

Extraction of Xanthophylls from Egg Yolks. Yolk samples were extracted according to Schlatterer et al.[5] with minor modifications. Approximately 5 g of freeze-dried egg yolk powder was weighed into a glass extraction tube covered with aluminum foil to protect xanthophylls from light. Samples were extracted three times (15 mL each) using a ternary solvent mixture (methanol:ethyl acetate:petroleum ether, 1:1:1, v/v/v) with 0.1% BHT. Supernatants were combined and evaporated under nitrogen gas. The oily residue was completely transferred to a 10 mL volumetric flask and made up to the volume with TBME:methanol (3:1 v/v) containing 0.1% BHT. These samples were filtered through a 0.45 μm nylon membrane filter and analyzed by HPLC. Dim light conditions and amber glassware were used throughout extraction and analysis to protect the xanthophylls from light-induced isomerization. Samples were prepared in triplicate.

Chromatography. *HPLC-DAD Analysis.* Samples were analyzed with a Waters 600 HPLC system (Waters, Millford, MA) equipped with a 2702 thermoautosampler, a binary gradient pump, and a 2998 photodiode array detector. Reversed-phase separation was performed on a C_{30} reversed-phase column (YMC 250 mm × 4.6 mm, i.d., 5 μm) protected with a C_{18} guard column (20 mm × 3 mm, i.d., 5 μm) at a flow rate of 1 mL/min. Compounds were separated with gradient elution as described by Aman et al.[13] Carotenoids were eluted with methanol/TBME/water (92:4:4 v/v/v with 0.1% BHT) as solvent A and methanol/TBME/water (6:90:4 v/v/v with 0.1% BHT) as solvent B using a linear gradient from 100% A to 6% B within 90 min. The injection volume was 20 μL. Monitoring was performed at 450 nm, and analyses were performed in duplicate.

Quantification. Seven-point standard calibration graphs were prepared for quantification purposes. Calibration graphs were recorded with sample concentrations ranging from 0.05 to 30.00 μg/mL by plotting the respective analyte peak area against concentration. Both *E*- and *Z*-isomer concentrations were calculated using the corresponding all-*E* standard calibration curves. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by injecting a series of dilute solutions with known concentrations. LOD and LOQ were defined as the signal-to-noise ratio equal to 3 and 10, respectively, according to the International Conference on Harmonization (ICH) Guideline.[18]

Purification of Yolk Samples for LC-MS/MS Analysis. Yolk extracts were evaporated under nitrogen and reconstituted in 10 mL of acetone and kept at −20 °C overnight. Subsequently, samples were vacuum filtered using a sintered glass funnel in a freezer compartment at −20 °C to remove the crystallized lipids.[19] The filtrate was evaporated under nitrogen and made up to a volume of 10 mL with TBME/methanol (3:1 v/v with 0.1% BHT). All samples were filtered through a 0.45 μm nylon filter before LC-MS/MS analysis.

LC-(APCI)-MS/MS Conditions. For separation and identification of xanthophylls, an Agilent 1200 HPLC system including degasser, binary pump, autosampler, thermostatted column compartment, and diode array detector (DAD) (Agilent Technologies, Palo Alto, CA) was connected to a 4000 QTrap mass spectrometer (AB Sciex, Concord, ON, Canada) fitted with an atmospheric pressure chemical ionization (APCI) source. Separation was performed on a C_{30} reversed-phase column (YMC 250 mm × 4.6 mm i.d., 5 μm) and a C_{18} guard column (20 mm × 3 mm, i.d., 5 μm) at a flow rate of 0.5 mL/min. The solvent system and gradient program were the same as described above. Mass spectrometric data were acquired and processed using Analyst software (version 1.5). The mass spectrometer was operated in positive ionization mode, and data were obtained using enhanced MS (EMS) and enhanced product ion (EPI) scans. Mass spectra were recorded over the m/z range from 300 to 600 Da at a scan rate of 1000 Da/s for EMS and 4000 Da/s for EPI scans. The instrument parameters for curtain gas, nebulizer gas (GS1), needle current, and ion source temperature were 10 psi, 25 psi, 5 μA, and 400 °C, respectively. Optimum values for other MS parameters were as follows: declustering potential (DP) at 36 V, entrance potential (EP) at 10 V, collision energy (CE) at 21 eV, and collision cell exit potential (CXP) at 12 V.

Statistical Analysis. All data were processed by analysis of variance (ANOVA) followed by Tukey's multiple range test using Statistical Analysis System Software (SAS version 9.0, SAS Institute,

Table 1. UV−Vis and Mass Spectrometric Data of Xanthophylls in Egg Yolk Obtained by HPLC-DAD-APCI-MS

peak	name	λ_{max} (nm)	$[M + H]^+$ (m/z)	fragment ions (m/z)
1	13-*Z*-lutein	330, 415, 436, 463	569	477 [M + H − 92], 551 [M + H − 18]
2	13′-*Z*-lutein	330, 415, 437, 465	569	477 [M + H − 92], 551 [M + H − 18]
3	13-*Z*-zeaxanthin	337, 422, 442, 465	569	477 [M + H − 92], 551 [M + H − 18]
4	all-*E*-lutein	332, 421, 444, 472	569	477 [M + H − 92], 551 [M + H − 18], 459 [M + H − 18 − 92]
5	*Z*-isomer of canthaxanthin	364, 465	565	547 [M + H − 18], 473 [M + H − 92], 363 [M + H − 92 − 92 − 18]
6	all-*E*-zeaxanthin	348, 426, 450, 478	569	477 [M + H − 92], 551 [M + H − 18]
7	9-*Z*-lutein	327, 414, 439, 466	569	477 [M + H − 92], 551 [M + H - 18]
8	all-*E*-canthaxanthin	477	565	547 [M + H − 18], 473 [M + H − 92], 363 [M + H − 92 − 92 − 18]
9	9′-*Z*-lutein	416, 440, 468	569	477 [M + H − 92], 551 [M + H − 18]
10	all-*E*-β-apo-8′-carotenoic acid ethyl ester	445	461	443 [M + H − 18], 369 [M + H − 92]

Cary, NC). Significance of differences was defined at the 5% level ($p < 0.05$).

RESULTS AND DISCUSSION

Purification and Identification of Xanthophylls in Egg Yolk. Egg yolk extracts have very high lipid content and need to be purified before mass spectrometric analysis. Saponification of egg yolk to remove these coextracted lipids was not employed to avoid the possible degradation of alkali labile xanthophylls. Lipids of extracted yolk samples can be removed by fractionating on a silica gel column[5] or by filtration in a freezer compartment.[19] Open column chromatography with a silica gel column is time consuming and requires a high amount of organic solvents. Therefore, in this experiment, samples were subjected to filtration in a freezer compartment, which has been proven to remove about 90% of total lipids.

A C_{30} reversed-phase column was used to obtain the baseline separation of nine xanthophylls including the *Z*-isomers of lutein, zeaxanthin, and canthaxanthin that were found in egg yolk (Figures 1 and 2). Identification of xanthophylls was based on their UV spectra, retention times, mass spectra, fragmentation pattern, and elution order of the compounds as shown in Table 1. Because of their similar mass spectra, identification of *Z*-isomers of lutein and zeaxanthin was mainly based on the UV spectra, retention times, and elution order.[13] All compounds showed the $[M + H]^+$ ion as the base peak. Most abundant product ions were $[M + H - 18 - 92]^+$ (loss of water and toluene) and $[M + H - 18]^+$ (loss of water) (data not shown). UV−vis spectra of all-*E* xanthophylls were similar in shape to those of *Z*-isomers, but small hypsochromic shifts were observed. As reported previously, introduction of cis double bond shifts the maximum absorption to a shorter wavelength.[20] Compared to all-*E*-lutein, 9-*Z*-, 9′-*Z*-, 13-*Z*-, and 13′-*Z*-isomers showed hypsochromic shifts between 4 and 8 nm (Table 1). *cis*-Canthaxanthin showed a hypsochromic shift of 12 nm, and an additional "cis peak" at 364 nm was also present.[21] Figure 2 illustrates the HPLC separation of compounds for the standard mixture of all-*E* xanthophylls and their *Z*-isomers (obtained after iodine-catalyzed photoisomerization of all-*E* standards).

Nine xanthophylls were identified in raw egg yolk. Surprisingly, all cooked yolk samples showed xanthophyll profiles similar to that obtained for raw egg yolks, indicating that there was no formation of cis isomers after cooking. HPLC chromatograms were very similar except that the peaks were of different intensities. This agrees with a previous study on boiled egg yolks,[5] according to which no additional peaks appeared in HPLC chromatograms of boiled yolk extracts compared to that of raw yolk.

Quantification of Xanthophylls in Yolk Samples. Quantitation was based on the standard calibration obtained from UV detection. The sensitivity of the method was assessed by analyzing the limits of detection and quantitation (LOD and LOQ) for each compound. Values obtained and calibration ranges are presented in Table 2. Correlation coefficients (R^2) of calibration graphs were >0.999 for all compounds. Extracts obtained from egg yolks subjected to different types of domestic cooking, i.e., boiling, frying, and microwave cooking, were analyzed, and quantities were determined for all nine xanthophylls identified. The extracts of raw and cooked egg yolks were compared for their total xanthophyll content and degree of isomerization.

Table 2. Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Xanthophyll Analysis by HPLC-DAD[a]

analyte	calibration range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
lutein[a]	0.10−30.0	0.0094	0.0314
zeaxanthin[a]	0.20−30.0	0.0095	0.0316
canthaxanthin[a]	0.05−30.0	0.0166	0.0555
all-*E*-β-apo-8′-carotenoic acid ethyl ester	0.05−20.0	0.0133	0.0442

[a]The quantities of the isomers of each xanthophyll in the egg yolk samples were calculated based on the same calibration graph.

Effects of Cooking on Xanthophylls in Egg Yolk. All-*E*-lutein was the predominant xanthophyll present in egg yolk, followed by zeaxanthin, β-apo-8′-carotenoic acid ethyl ester, and canthaxanthin. It should be noted that the amount of canthaxanthin in egg yolk (193.43 ± 17.1 μg/100 g) found in this study is considerably lower than those reported previously.[5,16] This can be attributed to the different poultry feed. As mentioned earlier, the composition and content of xanthophylls in egg yolk depend greatly on the hen's feed. Depending on the regulation on animal feed, the permitted levels and types of xanthophylls may vary. In the European Union, eight xanthophylls are allowed to be added to the poultry feeding, including lutein, capsanthin, zeaxanthin, β-apo-8′-carotenal, canthaxanthin, β-apo-8′-carotenoic acid ethyl ester, β-cryptoxanthin, and citranaxanthin.[9] Currently, in Canada only three xanthophylls are permitted to be added to poultry feeds: lutein (as marigold oil extracts), β-apo-8′-carotenoic acid ethyl ester (crystalline), and canthaxanthin (crystalline).[22]

Table 3. Quantities of Each Compound in Raw and Processed Samples[a]

	raw egg yolk, μg/100 g of egg yolk	boiled egg yolk, μg/100 g of egg yolk	% change	microwaved egg yolk, μg/100 g of egg yolk	% change	fried egg yolk, μg/100 g of egg yolk	% change
13-*Z*-lutein	67.5 ± 12.9	74.7 ± 10.4	10.8	71.1 ± 8.5	5.4	74.3 ± 7.8	10.1
13′-*Z*-lutein	85.6 ± 2.7	93.5 ± 12.5	9.2	94.1 ± 19.4	10.0	92.5 ± 16.6	8.1
all-*E*-lutein	1102.7[a] ± 159.9	854.7[b] ± 67.6	−22.5	918.5[b] ± 69.5	−16.7	890.4[b] ± 44.7	−19.3
9′-*Z*-lutein	26.6 ± 6.6	28.2 ± 3.0	6.1	27.4 ± 2.0	3.2	30.1 ± 2.0	13.3
total lutein	1282.3 ± 182.1	1051.1 ± 93.5	−18.0	1111.1 ± 99.4	−13.3	1087.3 ± 71.1	−15.2
13-*Z*-zeaxanthin	79.1 ± 8.5	89.7 ± 9.9	13.4	86.8 ± 22.1	9.7	89.5 ± 12.2	13.1
all-*E*-zeaxanthin	560.7[a] ± 131.4	475.7[b] ± 34.6	−15.2	515.6[ab] ± 58.6	−8.0	511.8[ab] ± 50.1	−8.7
total zeaxanthin	639.8 ± 139.9	565.4 ± 44.5	−11.6	602.4 ± 80.7	−5.9	601.3 ± 62.3	−6.0
Z-canthaxanthin	18.6 [a] ± 5.2	28.2 [b] ± 5.2	51.8	27.3 [b] ± 3.1	46.8	27.8 [b] ± 4.4	49.7
all-*E*-canthaxanthin	174.8 ± 11.9	152.5 ± 5.0	−12.8	155.1 ± 10.9	−11.3	154.5 ± 14.9	−11.6
total canthaxanthin	193.4 ± 17.1	180.8 ± 10.2	−6.6	182.4 ± 14.0	−5.7	182.3 ± 19.3	−5.7
all-*E*- β-apo-8′-carotenoic acid ethyl ester	432.5[a] ± 18.4	393.5[b] ± 17.9	−9.0	402.9[ab] ± 36.6	−6.9	397.0[ab] ± 17.5	−8.2

[a] $n = 6$. [a-b]different letters in same row denote significant difference ($p < 0.05$).

Cooking of eggs did not generate new xanthophyll isomers. All nine xanthophylls detected in cooked yolk extracts were present in raw yolks, but the amounts varied. Table 3 summarizes the amounts of xanthophylls present in yolk extracts, the total amount of individual xanthophylls (both *E*- and *Z*-isomers), and the percentage change of a compound in cooked yolk extracts relative to the raw yolk extracts. After cooking, all-*E* xanthophyll contents decreased while their *Z*-isomers increased. All-*E*-lutein seems to be affected most among the xanthophylls, showing 22.5%, 16.7%, and 19.3% reduction in boiled, microwaved, and fried yolk extracts, respectively. Cooking resulted in a 8−15.2% decrease in all-*E*-zeaxanthin, 11.3−12.8% in all-*E*-canthaxanthin, and 6.9−9% decrease in all-*E*-β-apo-8′-carotenoic acid ethyl ester. Similar results were observed in a study on boiled eggs.[5] Total xanthophyll content decreased in all cooked yolk extracts. These losses might reflect a possible degradation of xanthophylls owing to the time−temperature regime. Degradation causes irreversible loss of the compounds due to oxidation and subsequent formation of volatile compounds. The effects and products of thermal degradation were studied only recently and to a limited extent.[23−25]

Statistical analysis revealed that the effects of the three cooking methods, boiling, frying, and microwaving, on yolk xanthophylls are not significantly different among each other. Yet, all three cooking methods cause significant losses of all-*E*-lutein, while boiling causes considerable losses of all-*E*-zeaxanthin and all-*E*-β-apo-8′-carotenoic acid ethyl ester. Except for decreased all-*E*-lutein and increased *Z*-canthaxanthin contents, microwave cooking and frying have no significant effect on yolk xanthophylls. With respect to the types of stereoisomers, both 13-*Z*- and 9-*Z*-isomers increased after thermal processing. 13-*Z*-Isomers were the predominant form of *Z*-isomers of lutein and zeaxanthin found in egg yolk, and the amounts increased with heating. It has been observed in several studies that heating causes formation of 13-*Z*-isomers of xanthophylls.[26,27]

Cooking reduced the quantity of all-*E* xanthophylls and increased *Z*-isomers. Boiling significantly reduced the amounts of all-*E*-lutein, all-*E*-zeaxanthin, and all-*E*-canthaxanthin, whereas microwave cooking and frying only showed significant losses for all-*E*-lutein. The total xanthophyll losses ranged from 6% to 18%. These results will help to improve the current knowledge about the effects of different cooking methods on yolk xanthophylls and thus may be useful in assessing the dietary intake of xanthophylls. Furthermore, the analytical method presented here could be useful in determining the xanthophyll profiles and contents of egg-containing products. Currently, we are working on developing improved methods with shorter analysis time and higher sensitivity using fast liquid chromatography coupled with tandem mass spectrometry.

AUTHOR INFORMATION

Corresponding Author

*Phone: (780) 492-6885 (J.W.); +49(0)228 73-4452 (A.S.). Fax: (780) 492-4265 (J.W.); +49(0)228 73-4429 (A.S.). E-mail: jwu3@ualberta.ca (J.W.); schieber@uni-bonn.de (A.S.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was funded by grants from Egg Farmers of Canada (EFC), Alberta Egg Producers (AEP), the Agriculture and Food Council, which is responsible for delivering Agriculture and Agri-Food Canada's Advancing Canadian Agriculture and Agri-Food (ACAAF) Program in Alberta, Food for Health Initiative (Vitamin Fund) of the Faculty of Agricultural, Life & Environmental Science of the University of Alberta, Burnbrae Farms Limited, Poultry Industry Council and Natural Sciences, and Engineering Research Council (NSERC) of Canada to J.W.

REFERENCES

(1) Gordon, H. T.; Bauernfeind, J. C.; Furia, T. E. Carotenoids as food colorants. *Crit. Rev. Food Sci. Nutr.* **1982**, *18*, 59−97.

(2) Stahl, W.; Sies, H. Bioactivity and protective effects of natural carotenoids. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2005**, *1740*, 101−107.

(3) Voutilainen, S.; Nurmi, T.; Mursu, J.; Rissanen, T. H. Carotenoids and cardiovascular health. *Am. J. Clin. Nutr.* **2006**, *83*, 1265.

(4) McNamara, D. J. Egg xanthophylls and health implications. In *The Amazing Egg: Nature's Perfect Functional Food for Health Promotion*; Sim, J. S., Sunwoo, H. H., Eds.; Department of Agricultural, Food and Nutritional Science, University of Alberta: Edmonton, Alberta, Canada, 2006; pp 95−110.

(5) 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.

(6) 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.
(7) Ribaya-Mercado, J. D.; Blumberg, J. B. Lutein and zeaxanthin and their potential roles in disease prevention. *J. Am. Coll. Nutr.* **2004**, *23*, 567S−587S.
(8) Surai, P. F.; MacPherson, A.; Speake, B. K.; Sparks, N. H. C. Designer egg evaluation in a controlled trial. *Eur. J. Clin. Nutr.* **2000**, *54*, 298.
(9) Breithaupt, D. R. Xanthophylls in Poultry Feeding. In *Carotenoids: Natural Functions*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 2008; Vol. *4*, pp 255−264.
(10) Schieber, A.; Carle, R. Occurrence of carotenoid *cis*-isomers in food: Technological, analytical, and nutritional implications. *Trends Food Sci. Technol.* **2005**, *16*, 416−422.
(11) Dugave, C.; Demange, L. *Cis-trans* isomerization of organic molecules and biomolecules: implications and applications. *Chem. Rev.* **2003**, *103*, 2475−2532.
(12) 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.
(13) Aman, R.; Biehl, J.; Carle, R.; Conrad, J.; Beifuss, U.; Schieber, A. Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. *Food Chem.* **2005**, *92*, 753−763.
(14) Gama, J. J. T.; de Sylos, C. M. Effect of thermal pasteurization and concentration on carotenoid composition of Brazilian Valencia orange juice. *Food Chem.* **2007**, *100*, 1686−1690.
(15) Updike, A. A.; Schwartz, S. J. Thermal processing of vegetables increases *cis* isomers of lutein and zeaxanthin. *J. Agric. Food Chem.* **2003**, *51*, 6184−6190.
(16) Wenzel, M.; Seuss-Baum, I.; Schlich, E. Influence of pasteurization, spray- and freeze-drying, and storage on the carotenoid content in egg yolk. *J. Agric. Food Chem.* **2010**, *58*, 1726−1731.
(17) Wenzel, M.; Seuss-Baum, I.; Schlich, E. Influences of storage time and temperature on the xanthophyll content of freeze-dried egg yolk. *Food Chem.* **2011**, *124*, 1343−1348.
(18) ICH guidelines International Conference on Harmonization (ICH), Q2b: Validation of Analytical Procedures: Methodology. *US FDA Fed. Reg.* **1997**, *62* (May), 27463.
(19) Rodriguez-Amaya, D. B. Food carotenoids: analysis, composition and alterations during storage and processing of foods. *Forum Nutr.* **2003**, *56*, 35−37.
(20) Dugo, P.; Skeriková, V.; Kumm, T.; Trozzi, A.; Jandera, P.; Mondello, L. Elucidation of carotenoid patterns in citrus products by means of comprehensive normal-phase x reversed-phase liquid chromatography. *Anal. Chem.* **2006**, *78*, 7743−7750.
(21) Nelis, H.; Lavens, P.; Moens, L.; Sorgeloos, P.; Jonckheere, J. A.; Criel, G.; De Leenheer, A. cis-Canthaxanthins. Unusual carotenoids in the eggs and the reproductive system of female brine shrimp artemia. *J. Biol. Chem.* **1984**, *259*, 6063.
(22) Feeds Regulations (SOR/83-593). 1983, Ministry of Justice, Canada. http://laws-lois.justice.gc.ca/eng/regulations/SOR-83-593/page-1.html (accessed on March 08, 2011)
(23) Fratianni, A.; Cinquanta, L.; Panfili, G. Degradation of carotenoids in orange juice during microwave heating. *LWT-Food Sci. Technol.* **2010**, *43*, 867−871.
(24) Zepka, L. Q.; Mercadante, A. Z. Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. *Food Chem.* **2009**, *117*, 28−34.
(25) Rios, J. J.; Fernández-García, E.; Mínguez-Mosquera, M. I.; Pérez-Gálvez, A. Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins. *Food Chem.* **2008**, *106*, 1145−1153.
(26) Chen, B.; Peng, H.; Chen, H. Changes of carotenoids, color, and vitamin A contents during processing of carrot juice. *J. Agric. Food Chem.* **1995**, *43*, 1912−1918.
(27) Marx, M.; Stuparic, M.; Schieber, A.; Carle, R. Effects of thermal processing on *trans-cis*-isomerization of *β*-carotene in carrot juices and carotene-containing preparations. *Food Chem.* **2003**, *83*, 609−617.