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Bioaccessibility and Digestive Stability of Carotenoids in Cooked Eggs Studied Using a Dynamic in Vitro Gastrointestinal Model

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ABSTRACT: Among dietary carotenoids, lutein and zeaxanthin are known to protect against age-related macular degeneration, a leading cause of irreversible vision loss in the elderly. Egg yolk is rich in lutein and zeaxanthin, however, the effect of cooking and gastrointestinal digestion on yolk carotenoids is poorly understood. An in vitro dynamic gastrointestinal model (TIM-1) was used to investigate the digestive stability and bioaccessibility of carotenoids from boiled, fried, and scrambled eggs. Bioaccessibility but not digestive stability was significantly affected by the method of cooking. The main egg carotenoids, all-*E*-lutein and all-*E*-zeaxanthin, were stable during the digestion with average recoveries of 90 and 88%, respectively. No *trans*−*cis* isomerization of carotenoids was observed during digestion. Both all-*E*-lutein and all-*E*-zeaxanthin from scrambled eggs showed significantly lower bioaccessibility compared to boiled eggs. The results indicate that the bioaccessibility of egg carotenoids can be affected by different food preparation methods.

KEYWORDS: *egg carotenoids, cooking, bioaccessibility, digestive stability, TIM-1 gastrointestinal model*

INTRODUCTION

Dietary carotenoids, especially lutein and zeaxanthin, have been reported to reduce the risk of age-related macular degeneration (AMD), a leading cause of irreversible vision loss in individuals over the age of 55.[1] The disease develops slowly over a number of years, characterized by the formation of extracellular deposits called drusen concentrated in and around the macula and degenerative changes in retinal pigment epithelium.[44,45]

In addition to various plant-derived dietary sources such as fruits, vegetables, and green leafy vegetables, egg yolk serves as an important animal-derived source of lutein and zeaxanthin. Because carotenoids are lipophilic compounds, the digestible lipid matrix of the egg yolk makes it an ideal carrier to deliver highly bioavailable carotenoids; indeed, bioavailability of lutein from lutein-enriched egg yolk was found to be greater than from lutein supplements or spinach.[2,3] Through manipulation of hens' feed, egg yolk can be enriched with nutrients such as carotenoids, vitamin E, selenium, and omega-3 fatty acids.[4]

To be bioavailable, a compound first needs to be released from its food matrix and micellarized into an absorbable (bioaccessible) form, which can then be taken up by the intestinal cells and metabolized.[5] Human studies would be the ideal approach to obtain the most accurate information on nutrient bioavailability; however, high cost, technical difficulties, and ethical constraints involved with human trials have increased the need for alternative methods.[6,7] As a result, in vitro digestion methodologies have been developed to mimic in vivo human digestion and to assess the bioaccessibility of bioactive compounds, which is usually followed by dialysis to determine the absorbable portion.[8,9] Bioaccessibility varies depending on the type of carotenoids in a given food and across different foods for a particular carotenoid.[29] Several studies using in vitro digestion models[9−12] as well as human studies[13−15] indicate that xanthophylls are more bioaccessible compared to carotenes. Furthermore, various food-related factors such as matrix, food composition, and cooking and processing conditions may change the micellarization efficiency.[16−19] Ryan et al. showed that retention and transfer of carotenoids to the micelles may depend on the method of cooking and the type of carotenoids present.[47] For example, grilling and microwave cooking of vegetable were detrimental on micellarization of β-cryptoxanthin, whereas others (boiling, grilling, microwave cooking, and steaming) enhanced β-carotene transfer to micelles.[47]

The TIM (TNO's Intestinal Model) system is considered the most advanced in vitro digestive model developed to date,[40] where TIM-1 simulates the digestive processes of the stomach and small intestine and TIM-2 represents the colon and includes microbial gut-derived flora, validated on the basis of the studies performed with healthy individuals.[8] The main advantages of TIM-1 are its accuracy, reproducibility, and the possibility of collecting samples at any level of the gastrointestinal (GI) tract at any time during the digestion. There are some studies using TIM-1 to assess the bioaccessibility and digestive stability of carotenoids from various food products such as different tomato varieties and mixed diets with fruits and vegetables.[20−23]

Previously, we showed that cooking affects the carotenoid profile of egg yolks.[24] Nevertheless, the effect of cooking on the

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Table 1. Parameters of the TIM-1 System When Simulating Digestive Conditions of a Healthy Adult after Intake of a High-Fat Semisolid Meal ("Fed State")[a]

compartment	starting content/secretions/filtration fluid	volume (mL)	pH (min/pH)	$t_{1/2}/\beta$
stomach	starting content:	310	0/5.5	70/2
	• 10 g of gastric juice (pH 2), 10400 U of pepsin, and 400 U of lipase in GES		10/5.0	
			20/4.2	
	secretions:		40/2.8	
	• 1040 U/mL of pepsin and 40 U/mL of lipase in GES circulating at 0.5 mL/min		60/2.1	
			90/1.8	
	• water or HCl (1 M) circulating at 0.5 mL/min		120/1.7	
			300/1.7	
duodenum	starting content:	60	6.3	160/1.6
	• 15 g of a pancreatin solution (21%), 30 g of fresh bile, 1 mL of trypsin solution (2 mg/mL), and 15 g of SIES (pH 7)			
	secretions:			
	• pancreatin solution (21%) circulating at 0.25 mL/min			
	• fresh bile circulating at 0.50 mL/min			
	• SIES[b] or $NaHCO_3$ (1 M) circulating at 0.25 mL/min			
	• SIES circulating at 3.2 mL/min			
jejunum	starting content:	160	6.5	160/1.6
	• 40 g of SIES, 80 g of fresh bile, and 40 g of pancreatin solution (21%)			
	secretions:			
	• $NaHCO_3$ (1 M) if necessary			
	• SIES containing 10% of fresh bile circulating at 3.2 mL/min			
	filtration fluid:			
	• SIES circulating at 4.5 mL/min			
ileum	starting content:	160	7.4	160/1.6
	• 160 g of SIES			
	secretions:			
	• $NaHCO_3$ (1 M) if necessary			
	• SIES circulating at 3.0 mL/min			
	filtration fluid:			
	• SIES circulating at 4.5 mL/min			

[a]Gastric and ileal deliveries are modeled with a power exponential formula: $f = 1 - 2^{-(t/t1/2)\beta}$, where f represents the fraction of meal delivered; t, the time of delivery; $t^{1/2}$, the half-time of delivery; and β, the coefficient describing the shape of the curve. [b]SIES, small intestine electrolyte solution.

digestive stability and bioaccessibility of egg carotenoids is poorly understood. Therefore, the objectives of this study were to determine the effect of cooking and digestion on the stability and bioaccessibility of carotenoids and to characterize the carotenoid profile in digested eggs using TIM-1.

MATERIALS AND METHODS

Chemicals. Pepsin (3200 U/mg, P6887, from porcine gastric mucosa), trypsin (7500 U/mg, T9201, from bovine pancreas), and pancreatin (4xUSP, P1750, from porcine pancreas), lutein (xanthophyll from marigold), zeaxanthin, canthaxanthin, and butylated hydroxytoluene (BHT) were obtained from Sigma-Aldrich (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.[24] Lipase (150 units/mg, from *Rhizopus oryzae* DF 15) was obtained from Amano Enzyme USA Co. The electrolyte solutions used for the digestion of proteins were gastric electrolyte solution (GES) containing NaCl (4.8 g/L), KCl (2.2 g/L), $CaCl_2$ (0.3 g/L), and $NaHCO_3$ (1.25 g/L) and small intestinal electrolyte solution (SIES) containing NaCl (5.0 g/L), KCl (0.6 g/L), and $CaCl_2$ (0.3 g/L). Fresh pig bile was collected from Olymel slaughterhouse (Vallée-Jonction, QC, Canada), aliquoted for individual TIM experiments and stored at −20 °C until use. Bile bladder had a minimal size of 8 cm in length. HPLC grade solvents (methanol, ethyl acetate, petroleum ether, and *tert*-butyl methyl ether) were purchased from Fisher Scientific (Ottawa, ON, Canada).

Sample Preparation. Fresh eggs (n = 180) were obtained from the Poultry Research Centre of University of Alberta (Edmonton, AB, Canada). Eggs were divided into three groups of 60 each and subjected to boiling, frying, and scrambling. Boiled and fried eggs were prepared using the same cooking conditions that were reported previously.[24] Briefly, shell eggs were boiled in a boiling water bath for 10 min, cooled under running tap water, and peeled, and the yolks were separated from the whites. A nonstick frying pan (model SK200TY nonstick frying pan, Black & and Decker Canada Inc., Brockville, ON, Canada) preheated to 205 °C was used to prepare fried eggs. Whole eggs were fried for 6 min (3 min each side), and the yolks were separated from the whites. To prepare scrambled eggs, yolks were separated and combined and stirred on a preheated (205 °C) model SK200TY nonstick frying pan (Black & Decker Canada Inc.) by stirring yolks for 1 min. Each sample of cooked egg yolks was divided into two subgroups. Samples were vacuum packed, covered with aluminum foil to protect carotenoids from light exposure, and frozen immediately at −20 °C. The next day, all samples were shipped in a Styrofoam box along with dry ice to the Institute of Nutrition and Functional Foods (INAF) at Laval University (Quebec City, QC, Canada), where they were stored at −80 °C until subjected to digestion.

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TIM-1 Dynamic Gastrointestinal Model. TIM-1, which has been previously described in detail[8] and commercialized by TNO Nutrition and Food Research (Zeist, The Netherlands), was used for this study. In brief, the computer-controlled system consists of four compartments made of a glass outer wall and a flexible inner wall to represent the stomach, duodenum, jejunum, and ileum. These compartments are connected by peristaltic valve pumps, which facilitate the passage of a constant volume of chyme during each open-and-close cycle. The space between the two walls is filled with water at 37 °C to mimic body temperature. Changing the water pressure enables peristaltic movements and churning of chyme by alternated compression and relaxation of the inner walls. Previously formulated solutions to mimic salivary, gastric, biliary, and pancreatic secretions are introduced into the system via a computer-controlled pumping system. Two hollow-fiber membranes are connected to the jejunum and ileum to simulate the absorption of nutrients/drug and water from chyme. The volume in each compartment and pH, which is regulated using hydrochloric acid and/or sodium bicarbonate, are continuously monitored and controlled.

Digestion of Samples with TIM-1. Approximately 200 g of cooked egg sample was homogenized with 200 g of distilled water to prepare the "meal" using a hand-held blender model CSB-79C (Cuisinart, Woodbridge, ON, Canadae) for 12 s. A sample of 100 g from the meal was taken out as a control, and the rest (300 g) was immediately fed into the gastric compartment of the TIM-1 system. The digestion was carried out using predetermined parameters of TIM-1 modified from Speranza et al.[25] and adapted to reproduce the digestion of a semisolid meal in a healthy human adult (Table 1). The jejunal and ileal compartments were connected to specific hollow-filter membranes (MiniKros module M80S-300-01P, Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) that separate the micellar phase from the fat phase to determine bioaccessibility of lipophilic products.[26,27] The mixed micelles that contain the bioaccessible lipophilic fraction are able to pass through these membranes, whereas the undigested fat is retained. These bioaccessible fractions are collected in separate pouches kept on ice. After the passage of the chyme throughout the system, the residual digestion mixture is discharged as the effluent, which was kept on ice during the experiment. The digestion was carried out under subdued light to prevent photodegradation of carotenoids. All solutions/secretions used during the digestion were purged with nitrogen, and continuous nitrogen flow to the jejunal, ileal, and effluent compartments was maintained to minimize carotenoid oxidation and to reproduce the in vivo luminal conditions. Duplicated digestions were carried out for each cooked egg sample. A blank digestion was also performed using the same parameters with 200 g of distilled water in place of egg sample to estimate the carotenoids from digestive secretions and bile found in each sample.

Sampling Location and Time. During the total digestion time of 300 min, samples were obtained from different compartments as follows: 8 g of stomach chyme at 120 min and 8 g samples from duodenal, jejunal, and ileal chyme at 150 min were taken out. Jejunal filtrate, ileal filtrate, and effluent were collected from 0 to 150 min and from 150 to 300 min into separate pouches kept on ice. The residual chyme samples in duodenal, jejunal, and ileal compartments at the end of the digestion were also collected. Samples were weighed, aliquoted, and immediately transferred to a −80 °C freezer in airtight containers. All samples were freeze-dried and transported to the University of Alberta (Canada) with ice packs, where they were stored at −20 °C until analysis.

Analysis of Carotenoids in Digested Samples. Carotenoids in each sample were extracted as described previously with modifications.[28] Briefly, 500 mg of sample was accurately weighed into a microcentrifuge tube and extracted twice with 0.5 mL of a ternary solvent mixture consisting of methanol/ethyl acetate/petroleum ether (1:1:1 v/v/v) containing 0.1% BHT. The supernatants were combined in microcentrifuge tubes, evaporated under nitrogen, and reconstituted in 0.5 mL of acetone. The acetone extracts were kept at −80 °C for ∼1 h, and then the samples were vacuum filtered using a sintered glass funnel in a freezer compartment at −20 °C to remove the crystallized lipids.[24,46] The crystallized white color lipids were retained in the funnel; the filtrates containing carotenoids were centrifuged at 16500*g* for 30 s, and the supernatants were immediately transferred to a separate microcentrifuge tube, evaporated under nitrogen, and reconstituted in 200 μL of methanol. The extracts were filtered through a 0.45 μm nylon syringe filter and analyzed by HPLC using a Waters 600 HPLC system (Waters, Millford, MA, USA) equipped with a 2702 thermoautosampler, a binary gradient pump, and a 2998 photodiode array detector. The separation of carotenoids was performed on a C_{30} reversed-phase column (YMC 250 mm × 4.6 mm, i.d. = 5 μm) operated at room temperature (22 °C) using the conditions reported before.[24] Duplicate analyses were performed. To protect carotenoids from light- and oxygen-induced degradation, dim light conditions were used during sample handling, extraction, and analysis, and solvents used were purged with nitrogen. Identification of *trans* and *cis* isomers of lutein and zeaxanthin standards were performed on the basis of our previous study.[24] Carotenoids in the samples were identified on the basis of their UV spectra, retention time, and order of elution compared to the standard compounds and quantified using seven-point standard calibration curves.

Calculations and Statistical Analysis. The recovery of carotenoid compounds (separately for lutein and zeaxanthin) in each cooked egg sample was calculated as a percentage of the total amount of carotenoid compounds in the initial meal fed into the system using following equation:

$$\begin{aligned}&\text{recovery of carotenoids (\%)}\\&= (\text{total amount in jejunal filtrate} + \text{ileal filtrate} + \text{effluent}\\&\quad + \text{chyme residue at 300 min})/(\text{total amount fed into the system}\\&\quad - \text{amount detected during blank digestion}) \times 100\end{aligned}$$

All data were analyzed by analysis of variance (ANOVA) followed by Tukey's multiple-range test using Statistical Analysis System software (SAS version 9.3, SAS Institute Inc., Cary, NC, USA). Significance of differences was defined at the 5% level ($p < 0.05$).

RESULTS AND DISCUSSION

Carotenoid Profile of Eggs. The main carotenoid isomers found in undigested egg yolk were all-*E*-lutein, all-*E*-zeaxanthin, 13′-*Z*-lutein, and 13-*Z*-zeaxanthin. The other carotenoids usually found in eggs, such as all-*E*-canthaxanthin and all-*E*-β-apo-8′ carotenoic acid ethyl ester, were found in only trace amounts (Figure 1). The eggs used in this study did not use synthetic carotenoids as poultry feed additives. This might explain their absence in eggs as the egg carotenoid profile is

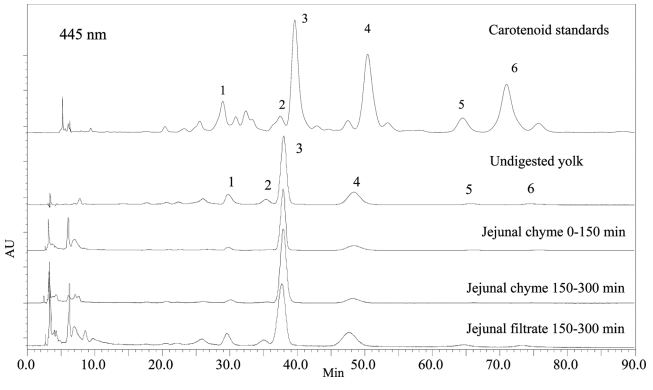

Figure 1. Representative HPLC chromatograms of carotenoid standards, egg yolk carotenoids from meal, and different intestinal compartments during digestion. Peaks: (1) 13′-*Z*-lutein; (2) 13-*Z*-zeaxanthin; (3) all-*E*-lutein; (4) all-*E*-zeaxanthin; (5) all-*E*-cantha-xanthin; (6) all-*E*-β-apo-8′-carotenoic acid ethyl ester.

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highly dependent on hens' diet.[28] The qualitative HPLC chromatographic profile of the egg carotenoid isomers remained unchanged during the digestion as no new isomer formation was observed (Figure 1). 13′-*Z*-Lutein and 13-*Z*-zeaxanthin were found in most of the samples, but were below the limit of quantitation in the duodenal, jejunal, and ileal chyme. Thus, the total recovery of these *cis* isomers could not be calculated. Coinciding with previous results,[29,30] no significant *trans*−*cis* isomerization of carotenoids was observed during the digestion due to the high recovery of *trans*-lutein and -zeaxanthin as well as the total quantity of *cis* isomers in filtrates and effluent being much lower than the amounts in the meal. Therefore, in this study we mainly focused on all-*E*-lutein and all-*E*-zeaxanthin levels in the gut during the digestion; unless mentioned otherwise, lutein and zeaxanthin represent the *trans* form of the respective carotenoid.

Digestive Stability of Carotenoids and Distribution in TIM-1 Compartments during Digestion. Only trace amounts of endogenous lutein and zeaxanthin from bile and/or other intestinal secretions were observed in the samples from the blank digestion, which were below the quantitative limits of HPLC. Hence, the distribution and recovery of carotenoids were calculated as a percentage of carotenoid content present in eggs fed into the TIM-1. Overall, both lutein and zeaxanthin were stable at 300 min of the digestion, with average recoveries of 90 and 88% calculated for all-*E*-lutein and all-*E*-zeaxanthin, respectively. At half time of the digestion (150 min), approximately 37% of lutein, irrespective of the type of sample, exited the small intestine (indicated as "effluent") without absorption (Figure 2A). Around 16 and 8% of lutein were passed through the hollow fiber membrane as bioaccessible lutein into the jejunal and ileal filtrates, respectively. Most of the remaining lutein was still in the jejunal and ileal compartments, whereas only about 2% was present in the duodenum. No lutein was detected in the stomach samples even at 120 min through digestion, and by 150 min the stomach was completely free from meal. All-*E*-zeaxanthin had distribution profiles similar to those of lutein at 150 min; however, a comparatively higher proportion (∼50%) in scrambled eggs was found in effluent as nonbioaccessible zeaxanthin (Figure 2). The average recovery of zeaxanthin from fried eggs at 150 min was 69.7%, much lower than those of boiled and scrambled samples (Figure 2B). This is because the zeaxanthin contents in duodenal, jejunal, and ileal chyme samples at 150 min from one of the two fried egg digestions were below the quantifiable limits of HPLC. Consequently, the calculated recovery at 150 min was lower than the true recovery of zeaxanthin. However, this does not influence the digestive stability calculations at 300 min, as we have collected all of the chyme remaining in the intestinal compartments and had enough samples for quantification (see the equation under Materials and Methods). At the end of the digestion (at 300 min), almost 50% of the total lutein was found in effluent followed by 30% in jejunal filtrates. Around 10% of lutein was in ileal filtrate, whereas only a small amount remained in duodenal, jejunal, and ileal compartments. A similar distribution pattern was observed with zeaxanthin except that a higher amount (∼63%) was found in the effluent in scrambled eggs (Figure 2B), apparently the reason for comparatively lower bioaccessibility (Figure 3), which is discussed below. Even

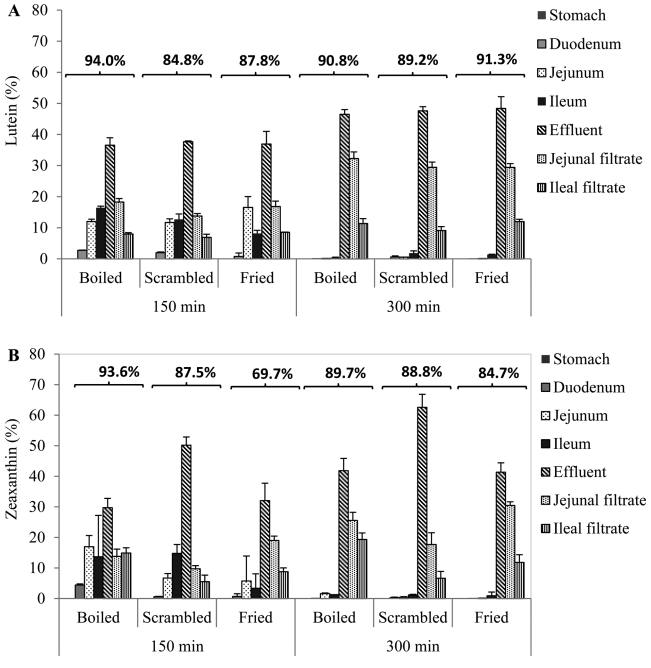

Figure 2. Total recovery (indicated on top of the bars) and distribution of all-*E*-lutein (A) and all-*E*-zeaxanthin (B) in the gut during digestion (as a % of the feeding). Data are the average of two measurements.

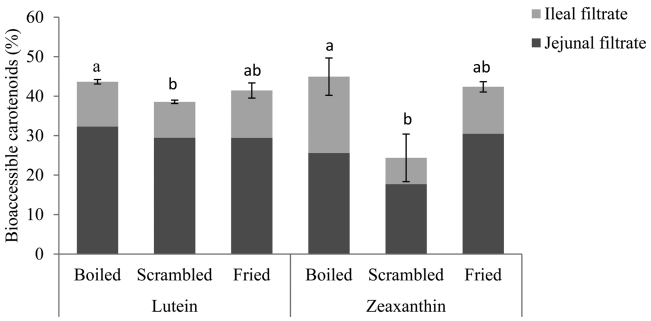

Figure 3. Cumulative carotenoids in jejunal and ileal filtrates (bioaccessible carotenoids) as a percentage of the feeding at the end of the digestion (n = 2; different letters (a, b) denote significant difference (p < 0.05)).

though no studies are available regarding the gut distribution and/or stability of lutein and zeaxanthin from egg yolk, these carotenoids have been reported as highly stable during gastrointestinal digestion regardless of the food source. Blanquet et al.[20] used TIM-1 to compare the digestive stability of xanthophylls and carotenes and found that lutein and zeaxanthin were stable throughout the digestion and the digestive stability of xanthophylls is higher than that of carotenes. Studies that used other in vitro digestion methods also showed a higher digestive stability (>80%) of lutein and zeaxanthin derived from different food sources.[9,10,19,29]

Bioaccessibility of Carotenoids from Cooked Eggs. Bioaccessibility of carotenoids was the total amount of ileal and jejunal filtrates expressed as a percentage of the total carotenoids present in starting meal. According to Etcheverry et al.,[31] there are principally four in vitro methods for measuring bioaccessibility and/or bioavailability: solubility, dialyzability, or a gastrointestinal model (e.g.,TIM) for bioaccessibility and cell culture for bioavailability. There exist no in vitro digestion models that have the capability to mimic

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perfectly the complex environment of the GI tract. However, TIM-1 is the only in vitro digestion model with the capacity to simulate the dynamic conditions of the stomach and small intestine during digestion.[32] As shown in Figure 1, the isomeric profiles of carotenoids were similar in both nonbioaccessible (jejunal chyme, 0−150 and 150−300 min) and bioaccessible samples (jejunal filtrate, 150−300 min), indicating both *trans* and *cis* isomers of carotenoids passed through the hollow fiber membranes into the bioaccessible filtrates. In boiled, fried, and scrambled eggs, the bioaccessibility of lutein was 44, 41, and 39%, whereas that of zeaxanthin was 45, 42, and 24%, respectively (Figure 3). Total bioaccessible carotenoids were higher in the jejunal filtrate than in the ileal filtrate. A similar trend was observed in recent studies using TIM-1 to study the effect of fat on blueberry anthocyanins[33] and β-carotene bioaccessibility.[21] Although we did not find any study on lutein bioaccessibility using TIM-1, there are many other papers on lutein bioaccessibility using other in vitro digestion models. Garrett et al.[9] developed an in vitro digestion model and found the lutein bioavailability (solubility assay + cells culture) in a standard baby food to be 25−40%, which is consistent with our results. Using the same model, O'Connell et al. demonstrated that lutein from fruits including orange, kiwi, grapefruit, and honeydew melon (100−109%) is more bioaccessible compared to that from dark green vegetables, spinach (19%), and broccoli (38%).[11] However, Chitchumroonchokchai et al.[10] found a much higher lutein micellarization (53%) in microwave-cooked spinach using solubility assay coupling with Caco-2 cells, presumably due to the disruption of cell walls. Bioaccessibility of lutein from pure durum wheat pasta amounted to 71%, whereas from egg pasta it was 57%,[34] which seems contradictory to the fact of high bioavailability of egg lutein. It was speculated that egg lutein was incorporated into the complex mixture of proteins and starch during pasta processing, leading to low bioaccessibility. Lutein-fortified whole, semiskimmed, and skimmed milk showed bioaccessible levels of 46.5, 45.8, and 19.7%, whereas in whole, semiskimmed, and skimmed yogurts, it was 47.5, 38.3, and 17.8%, respectively;[35] these data are comparable to bioaccessibility levels observed in our study.

The bioaccessibility of both lutein and zeaxanthin was affected by the type of cooking (Figure 3). Lutein and zeaxanthin from scrambled eggs yielded significantly lower bioaccessibility compared to boiled eggs, but were not different from fried eggs. The exact reason for this difference is not clear, but presumably the chemical and structural changes of proteins and lipoproteins in egg yolk occurring during different cooking conditions may have influenced the micellarization efficiency, that is, the transfer of carotenoids to the filtrates. Textural differences between the cooked eggs can be a considerable factor on micellarization as boiled eggs are easily blended with water compared to fried and scrambled eggs. Particle size, food processing, and food preparation[16−19] have been reported as influencing factors on carotenoid bioaccessibility. Panozzo et al.[18] showed bioaccessibility of carotenoids from tomato pulp was affected by homogenization in two main ways: (a) decreased particle size, which enables release of carotenoids; and (b) increased consistency due to the formation of a fiber network, which entraps the carotenoids. Similarly, the industrially extracted orange juice resulted in smaller particle sizes and increased the relative bioaccessibility compared to hand extraction.[16] The composition and degree of saturation of fatty acids are also considered important factors on carotenoid bioaccessibility and bioavailability.[36−38] Gleize et al.[38] observed that saturated and monounsaturated short-chain fatty acids (MUFA) lead to the formation of small micelles and higher bioaccessibility of lutein and zeaxanthin compared to the long-chain polyunsaturated fatty acids (PUFA). The significant inverse relationship between micelle size and bioaccessibility was attributed to increased specific surface area with smaller micelles. They hypothesized that due to the polar nature, xanthophylls located at the micelle surface and, therefore, increased surface area increases amount of xanthophylls that can be incorporated into the micelles. Different cooking methods resulted in diverse fatty acid profiles in egg yolk.[39,43] The amount of PUFA reported in scrambled eggs is significantly higher compared to boiled eggs, which may lead to the formation of larger micelles and lower bioaccessibility. This can also explain our observations on bioaccessibility of carotenoid from eggs after different cooking methods. Lutein and zeaxanthin differ only by the position of one double bond. This minor structural difference may influence how these two compounds incorporated into the micelles and their respective bioaccessibility. It has been shown that the orientations of lutein and zeaxanthin in a phospholipid bilayer are different. Zeaxanthin adopts an orientation spanning the membranes from outer polar headgroup layer to the other polar headgroup layer, whereas lutein can have two different orientations; one way of orientation is similar to that of zeaxanthin, whereas the other is arranged horizontally to the membrane.[48] In a similar way, lutein and zeaxanthin may have different orientation patterns in mixed micelles during digestion, which might contribute to their differences in bioaccessibility.

TIM-1 is so far considered the digestive model simulating the in vivo conditions of human gut and very useful in predicting the fate of nutrients and pharmaceuticals, optimizing the oral formulations for clinical studies and also allowing the simulation of the digestion of infants, young adults, seniors, and patients with impaired gastrointestinal conditions.[40] However, the data obtained through in vitro digestion models on the bioaccessibility of carotenoids may not directly be applied as bioavailability in humans and should be interpreted with caution. On the basis of previously published studies, Reboul et al.[19] made a comparison between the bioaccessibilities of carotenoids obtained using in vitro digestive models and bioavailability values measured in healthy subjects. They found that there is a significant relationship ($r = 0.98$, $p < 0.0001$) between bioaccessibility ratios measured in vitro and the mean bioavailability ratios. In contrast, Granado et al.[41] showed that the bioaccessibility of carotenoids and vitamin E from broccoli under in vitro conditions does not fully explain the changes they observed in healthy individuals. Another paper stated that, although the micellarization efficiencies of lutein from human milk and infant formula were similar, accumulation efficiency of human milk lutein was 4.5 times higher by Caco-2 human intestinal cells.[42]

In summary, we studied the effect of different domestic cooking methods on the stability and bioaccessibility of carotenoids from egg yolk. In alignment with the previous findings, lutein and zeaxanthin were stable during the in vitro digestion. Digestive stability was not affected by the different types of cooking, whereas scrambling resulted in significantly lower bioaccessibility compared to boiled eggs in both lutein and zeaxanthin. To the best of our knowledge, this is the first study to investigate the bioaccessibility of egg yolk carotenoids affected by the cooking methods. The results presented here provide detailed information on digestive stability and

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bioaccessibility of egg carotenoids under various thermal processing conditions and may be useful in estimating the bioavailable carotenoids from eggs and egg-containing foods.

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