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Fatty Acid and Antioxidant Composition of Conventional Compared to Pastured Eggs: Characterization of Conjugated Linoleic Acid and Branched Chain Fatty Acid Isomers in Eggs

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ABSTRACT: The objective of this study was to compare the antioxidant and fatty acid composition of conventionally raised commercial, free-range commercial, and pasture-raised local eggs. Egg characteristics and antioxidants were assessed, and the fatty acid composition was determined by gas chromatography-mass spectrometry. Pasture-raised egg yolk contained more retinol and significantly higher levels of carotenoids and α-tocopherol ($p < 0.05$) with no significant differences in total phenolic content. The percents of total ω-3 fatty acids were higher and ω-6:ω-3 fatty acid ratios were lower in pasture-raised and free-range eggs ($p < 0.05$). Branched chain fatty acids (BCFAs) and conjugated linoleic acid (CLA) isomers were identified in egg yolk. Pasture-raised eggs had significantly higher levels of BCFAs ($p < 0.05$). However, no differences in CLA isomers were detected. These results indicate that a beneficial profile of antioxidants and fatty acids is found in egg yolks from hens with pasture access.

KEYWORDS: *poultry, yolk, free-range, odd chain, omega-3, GC-MS*

INTRODUCTION

Consumer demand for specialty eggs has increased due to interest in hen welfare and nutrient-dense products.[1] In particular, consumers are interested in free-range production systems for their reported improved animal welfare, increased product quality, and decreased environmental impact. U.S. production systems have no legal definitions for "free-range production", but this refers to a system in which poultry have outdoor access and may or may not be provided with a shelter and fresh pasture. "Pasture-raised" (PR) refers to a free-range system (FR) in which pasture for poultry ingestion must be provided. Some pasture systems utilize moveable housing structures that allow hens to be moved to fresh pasture regularly.[2] Other pasture systems provide hens unrestricted access to pasture in which the hen can choose from a variety of potentially phytochemically rich plant and insect species. Animals' "attuned palate", as described by Provenza et al.,[3] allows them to select foods that meet their nutrient needs and result in more nutrient-rich animal products. These systems are contrasted to conventional caged production systems in which hens are provided a standard layer hen diet high in corn and soy.[4]

Eggs are a nutrient-dense food rich in protein, lipids, and a variety of micronutrients.[5] Eggs are also an important food source of antioxidants for humans, providing retinol, tocopherols, and carotenoids among other beneficial bioactive compounds that may enhance human health.[6] A hen's diet significantly influences the nutrient density and sensory quality of the egg. For example, supplementation with linseed increased egg antioxidant content, including total phenolic content (TPC) and carotenoids.[7] In addition, supplementation with spirulina resulted in greater red yolk color and increased carotenoid content.[8] Providing hens with access to pasture results in a 2-fold increase in egg yolk carotenoid content[9] and a 30% increase in α-tocopherol content.[10] Therefore, hens who forage grasses and seeds may produce eggs that have more bioactive nutrients.

Egg yolk fatty acid (FA) profiles are also influenced by hen diet. Hen eggs are particularly responsive to dietary changes in linoleic and linolenic acids.[10] Access to forage material, high in α-linolenic acid (ALA), increases the level of egg yolk polyunsaturated fatty acids (PUFAs). In particular, forage increases egg yolk ω-3 fatty acid (n-3) content, resulting in a lower ω-6:ω-3 (n-6:n-3) fatty acid ratio around 5 compared to ratios of 11−19 observed in conventional eggs.[9] Similar findings related to antioxidant and FA profiles are observed in PR beef and dairy cattle.[11,12] The increased phytochemical richness of cattle diet is thought to influence the biochemical richness of meat and dairy products and, therefore, impact consumer health.[13] Likewise, an increased phytochemical richness of hen diets should result in more phytochemically rich eggs.

Conjugated linoleic acid (CLA) and branched chain fatty acids (BCFAs), levels of which are higher in PR beef[14] and dairy products,[11,15] are two bioactive FA groups explored due to their potential benefits for human health.[14,16] CLA consumption is associated with numerous health effects such as improved body composition and decreased risk of cancer and diabetes.[16] CLA

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isomers are synthesized by bacterial isomerization and/or biohydrogenation of PUFAs in ruminant animals and by endogenous synthesis in all animals. Monogastric animals such as poultry do not possess a rumen, so CLA is likely synthesized endogenously.[17] BCFAs are saturated fatty acids (SFAs) with at least one branching point on their carbon chain. While their function in humans is largely unknown, they may play a role in microbiota, enterocytes, and skin health.[14] Dairy and beef are two of the few major food sources of BCFAs in the human diet, though research is limited on BCFAs in foods.[14] Levels of BCFAs are reported to be below detection limits in eggs,[14] yet BCFAs are often obscured by other FA peaks during analysis.[18] A newer FA methodology developed to better characterize FA isomers in ruminant products[19] can be utilized in egg yolk FA analysis to achieve improved identification of BCFAs, along with some separation of CLA isomers. The CLA and BCFA content may be increased in PR eggs given the increased antioxidant and PUFA content of PR eggs.

The increased nutritional variety available to FR and PR hens may result in altered antioxidant and FA profiles of eggs. Therefore, the objective of this study was to compare antioxidant and FA profiles of conventionally raised commercial, free-range commercial, and pasture-raised local eggs. In this study, previously uncharacterized CLA and BCFA isomers in egg yolk were identified by utilizing updated fatty acid methodology and significant differences in egg yolk antioxidant and FA profiles were demonstrated among conventional and pastured eggs.

MATERIALS AND METHODS

Chemicals. Dichloromethane was purchased from VWR Chemicals (Radnor, PA). A GC-MS reference standard curve was created by combining Supelco 37 Component FAME Mix (Sigma-Aldrich, St. Louis, MO) with mead acid, docosatetraenoic acid, n-3 docosapentaenoic acid (DPA), n-6 DPA, and palmitelaidic acid purchased from Cayman Chemical (Ann Arbor, MI). BCFAs were compared to Mixture BR 3 purchased from Larodan AB (Solna, Sweden). CLA reference standard UC-59M (Nu-Chek Prep, Elysian, MN) was used to identify CLA isomers. All other chemicals were purchased from Sigma-Aldrich unless otherwise noted.

Materials. One dozen eggs each were acquired from four different Midwest suppliers. Conventionally raised commercial eggs from a standard grocery store brand (COM) and free-range commercial eggs from a free-range, organic brand promising intensely colored yolks (CFR) were acquired from the supermarket. Pasture-raised eggs were acquired from both a small-scale local farm (SPR) and a large-scale local farm (LPR). SPR hens were provided unrestricted access to pasture, and LPR eggs were provided pasture access in a 20 acre rotated hoop house. All eggs were stored no longer than 7 days at 4 °C until they were analyzed.

Physical Characteristics of Eggs. Eggs were weighed to quantify the physical characteristics: albumen, yolk, and shell weights. Egg albumen was separated from the yolk by hand, and then the yolk was gently rolled down a paper towel to remove excess albumen. Egg yolk color was analyzed using a DSM Yolk Color Fan (DSM Nutritional Products, Basel, Switzerland) with values ranging from 1 to 14 (1 for pale yellow and 14 for dark orange).

Retinol and α-Tocopherol Analysis. Retinol and α-tocopherol were analyzed as described by Rettenmaier and Schuep[20] and Schmitz et al.[21] The portion of egg yolk for retinol analysis was mechanically homogenized in degassed methanol containing butylated hydroxytoluene (BHT) as an antioxidant. Potassium hydroxide (40%) was added, and the sample was heated to 75 °C in an atmosphere of nitrogen for 1 h. Retinyl esters were saponified by this procedure, resulting in free retinol. The portion of egg yolk for α-tocopherol analysis was mechanically homogenized in 2 mL of water and then frozen to lyse cells. After the yolk had thawed, ethanol was added to an aliquot of the solution to precipitate proteins.

Hexane was added to either sample to extract the retinol or α-tocopherol, and the solution was centrifuged to separate the hexane layer. A measured portion of the hexane was removed and evaporated under reduced pressure in a vortexing chamber (10 min, 35 °C, 300 mbar vacuum). The remaining matter was solubilized in a measured portion of the chromatographic mobile phase and placed in autosampler vials. Samples were analyzed chromatographically using a Waters Acquity system and Waters Empower Pro Chromatography Manager software (Waters Corp., Milford, MA). Elution was isocratic using an acetonitrile/methylene chloride/methanol mobile phase [70:20:10 (v/v/v)] and a Symmetry C18, 1.7 μm, 2.1 mm × 50 mm analytical column (Waters Corp.). The system also contained a Sentry C18, 3.5 μm guard column (Waters Corp.). The flow rate was 0.5 mL/min, and detection was achieved by UV absorption at 325 nm for retinol samples and UV absorption at 292 nm for α-tocopherol samples. To quantify, six-point calibration curves were prepared by serial dilution of a stock retinol solution (10 ppb to 10 ppm) and an α-tocopherol standard (0.2−50 ppm). Peak integration was achieved by the ApexTrack method of Empower Pro (Waters Corp.). All peaks were reviewed manually after initial autointegration. Peaks that were otherwise questionable were reviewed for purity using spectral data at the peak wavelength for that analyte. Results were reported as micrograms of retinol per gram of yolk and micrograms of α-tocopherol per gram of yolk.

Carotenoid Analysis. The total carotenoid content was determined using methods adapted from refs 22 and 23. Briefly, 0.5 g of egg yolk sample was combined with 5 mL of cold acetone (0.05% BHT) and homogenized until solid pieces of the sample were liquefied into solution. Samples were vortexed for 2 min and then placed in an ultrasound water bath for 5 min. Next, samples were centrifuged for 15 min (3000 rpm and 4 °C). The supernatant was recovered and evaluated in the spectrophotometer at 450 nm against an acetone blank. The total carotenoid content was calculated according to the method of Biehler et al.[24] using an ε of 140663 L/mol for β-carotene in acetone and was expressed as micrograms of β-carotene per gram of egg yolk.

Phenolic Analysis. A modified method based on that of Nimalaratne et al.[25] was used to extract phenolic compounds. Briefly, 2 g of the homogenized yolk sample was added to 20 mL of a methanol/distilled water/acetic acid solvent [80:18:2 (v/v/v)]. The tube was shaken for 30 min and then centrifuged for 20 min (2500 rpm and 4 °C). The supernatant was transferred to a new tube. A second solution of 20 mL of an acetone/distilled water/acetic acid solvent [80:18:2 (v/v/v)] was added to the original tube. The original tube was shaken again for 10 min and centrifuged for 15 min (2500 rpm and 4 °C). The supernatants were combined and stored at 4 °C until they were analyzed.

The Folin-Ciocalteu assay modified from that of Chen et al.[22] was used to quantify the total phenolic content. A 100 μL portion of the supernatant was combined with 100 μL of Folin-Ciocalteu reagent and 800 μL of 5% sodium bicarbonate and heated at 40 °C for 30 min. Cooled samples were plated in triplicate in a 96-well plate, scanned at 765 nm, compared against a gallic acid standard curve, and reported as milligrams of gallic acid equivalents per gram of yolk.

Fatty Acid Analysis. A modified version of the microwave-assisted extraction method described by Bronkema et al.[12] was used to extract FAs from egg samples using the CEM Mars 6 microwave digestion system, equipped with a 24-vessel rotor and GlassChem vessel set (CEM Corp., Matthews, NC). Briefly, 400 mg of the homogenized yolk sample was added to a microwave vessel with 8 mL of a 4:1 (v/v) ethyl acetate/methanol solution and 0.1% BHT as an antioxidant. FAs were extracted using the following microwave parameters: 55 °C for 15 min with an initial ramp of 2 min at a 400 W maximum power. The contents of the vessel were filtered using Whatman qualitative filter paper (grade 597) into a test tube containing 3.5 mL of HPLC water. Samples were centrifuged at 2500 rpm for 6 min, and the top organic layer was transferred to a new tube and dried under nitrogen. The extracted oil was resuspended in a 4:1 (v/v) dichloromethane/methanol solution with 0.1% BHT to bring each sample to 20 mg of oil/mL.

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Table 1. Physical Characteristics of the Eggs[a]

producer	egg weight (g)	yolk weight (g)	albumen weight (g)	shell weight (g)	DSM yolk color fan (range of 1−14)
small-scale local pasture-raised	59.4 ± 6.8 ab	17.2 ± 3.0	36.8 ± 9.0	5.4 ± 1.1	12.7 ± 0.6 a
large-scale local pasture-raised	61.5 ± 1.5 a	16.4 ± 1.2	38.9 ± 2.7	6.2 ± 0.3	9.7 ± 1.2 b
commercial free range	53.1 ± 0.2 b	14.7 ± 0.7	33.2 ± 0.6	5.3 ± 0.3	14.0 ± 0.0 a
commercial	66.3 ± 0.7 a	17.3 ± 0.6	43.2 ± 1.9	5.8 ± 0.7	10.3 ± 0.6 b
p value[b]	0.012	0.272	0.157	0.358	<0.001

[a]Data are reported as means ± the standard deviation of three replicates (n = 3) from each producer. [b]p values indicate results of one-way ANOVA. Means within a column that have different letters are significantly different according to Tukey's HSD test ($p < 0.05$).

Table 2. Antioxidant Profile of Egg Yolks[a]

producer	retinol (μg/g)	α-tocopherol (μg/g)	total carotenoids (μg/g)	total phenolic content (mg of GAE[b]/g)
small-scale local pasture-raised	43.55 ± 1.31	920.07 ± 198.10 a	57.21 ± 5.29 a	1.17 ± 0.09
large-scale local pasture-raised	35.20 ± 1.10	388.59 ± 147.80 b	28.31 ± 4.94 b	1.30 ± 0.17
commercial free range	36.18 ± 7.28	440.72 ± 27.18 b	34.78 ± 11.54 b	1.72 ± 0.44
commercial	37.49 ± 5.86	323.96 ± 56.19 b	27.99 ± 4.70 b	1.49 ± 0.14
p value[c]	0.213	0.002	0.003	0.112

[a]Data are reported as means ± the standard deviation of three replicates (n = 3) from each producer. [b]GAE, gallic acid equivalents. [c]p values indicate results of one-way ANOVA. Means within a column that have different letters are significantly different according to Tukey's HSD test ($p < 0.05$).

For the creation of fatty acid methyl esters (FAMEs), a modified methylation described by Jenkins[26] was conducted. Two milligrams of suspended oil (100 μL) was aliquoted from each sample, dried under nitrogen, and resuspended in toluene with 20 μg of an internal standard (methyl 12-tridecenoate, U-35M, Nu-Chek Prep). Two milliliters of 0.5 N anhydrous potassium methoxide was added, and samples were heated at 50 °C for 10 min. Once the mixture had cooled, 3 mL of 5% methanolic HCl was added, and samples were heated at 80 °C for 10 min. Once the mixture had cooled, 2 mL of water and 2 mL of hexane were added, and the upper organic phase was removed and dried to yield FAMEs. FAMEs were suspended in 1 mL of isooctane to reach a concentration of 2 mg/mL and transferred to gas chromatography-mass spectrometry (GC-MS) vials with glass inserts. Samples were stored at −20 °C until they were analyzed.

For the isolation of FAMEs, the Perkin Elmer (Waltham, MA) 680/600S GC-MS instrument in the electron impact mode was equipped with the Agilent Technologies (Santa Clara, CA) HP-88 column (100 m, 0.25 mm inner diameter, 0.2 μm film thickness). The column temperature parameters were as follows: initial temperature of 80 °C for 4 min, ramp at a rate of 13.0 °C/min to 175 °C, held for 27 min, ramp at a rate of 4.0 °C/min to 215 °C, and held for 35 min (modified from ref 19 previously used for improved separation of FA isomers in beef and dairy products). Helium was used as the carrier gas at a flow rate of 1 mL/min. Both a 30:1 split and a splitless injection (0.75 min splitless hold time, 40 mL/min flow exiting the vent) were conducted for each sample at an injection temperature of 250 °C with a 1 μL sample volume. Samples were injected using two different injections to capture both lower-concentration analytes and higher-concentration analytes too concentrated on the splitless injection. The electron energy was 70 eV, and the MS data were recorded in full scan mode (mass range of m/z 70−400). The MS transfer line and ion source temperature were set to 180 °C.

For identification of FAMEs, data analysis was conducted using MassLynx version 4.1 SCN 714 (Waters Corp.). FAs were identified by retention time and EI mass fragmentation in comparison to those of our reference standard (described above). FAs were analyzed using extracted ion chromatograms of the respective quantitative ions. FAs not included in our reference standard were identified according to the elution order reported in the literature[19] and confirmed by EI mass fragmentation. Identification of split or splitless injection used, retention times, mass fragmentation, and quantitative ions used are outlined in Supplementary Table 1. For the quantification of FAMEs, a standard curve constructed from our reference and internal standard was utilized. The internal standard peak area and analyte peak area in the egg sample relative to those of the standard curve were used to calculate each FAME concentration. Fatty acids were reported as the percent of total fatty acids quantified.

Statistical Analysis. Data from the egg characteristic, retinol, α-tocopherol, carotenoid, and phenolic analyses were analyzed using Prism version 7.0d for Mac OS X (GraphPad Software, La Jolla, CA). Data from the fatty acid analysis were analyzed using R version 3.6.1, and figures were created using Prism version 7.0d. Group comparisons in all analyses were performed using one-way analysis of variance (ANOVA) and Tukey's HSD, correcting for multiple comparisons. Values below the lower limit of detection in fatty acid analyses were treated as zeroes in analysis. Statistical significance for all analyses was set at the $p < 0.05$ level.

RESULTS AND DISCUSSION

The following findings support the hypothesis that yolk color, antioxidant, and FA profiles were influenced by production system. The levels of antioxidants and n-3 FAs were higher in pasture-fed eggs. Previously unknown BCFAs were detected in FR and PR eggs. Surprisingly, there were no significant differences in phenolic concentration or CLA isomers among the egg production systems tested in this study.

Egg Physical Characteristics and Antioxidant Profiles. Yolk weight, albumen weight, and shell weight were not significantly different among producers. Egg weight was significantly higher in COM and LPR eggs than in CFR eggs. Egg yolk color was significantly darker orange in SPR and CFR eggs than in COM and LPR eggs (Table 1). Egg yolk color is an important factor for consumers and is largely influenced by carotenoid in hen diets.[6] Eggs are regarded as an excellent source of carotenoids for humans given the increased bioavailability of egg carotenoids compared to green vegetables due to yolk lipids.[6] The darker orange color observed in SPR and CFR eggs is congruent with their higher carotenoid content (Table 2). Carotenoids may be used as poultry feed additives to improve egg color in commercial systems, so it is unclear if the increased pigmentation observed in CFR eggs is due to pasture access or supplementation.[6]

The antioxidant profile of small-scale pasture-raised eggs was distinct from those of other production systems in this study, especially with regard to carotenoids and α-tocopherol (Table 2). Complex grass mixtures in a pasture have more tocopherol,

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Table 3. Fatty Acid Profile of Egg Yolks (percent of total fatty acids)[a]

fatty acids	small-scale local pasture-raised	large-scale local pasture-raised	commercial free range	commercial	p value[b]
10:0	0.005 ± 0.002	0.006 ± 0.002	0.005 ± 0.001	0.006 ± 0.001	0.764
12:0	0.01 ± 0.002	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.001	0.963
14:0	0.33 ± 0.04	0.32 ± 0.04	0.30 ± 0.03	0.33 ± 0.02	0.803
14:1	0.10 ± 0.04	0.07 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.410
16:0	22.31 ± 0.80 ab	21.21 ± 1.03 b	21.54 ± 0.67 ab	23.40 ± 0.83 a	0.019
16:1 n-7	3.13 ± 0.73	2.63 ± 0.75	2.22 ± 0.29	2.58 ± 0.31	0.418
16:1 n-9	0.57 ± 0.04 ab	0.64 ± 0.12 a	0.56 ± 0.07 ab	0.44 ± 0.07 b	0.050
18:0	6.57 ± 0.73 ab	6.19 ± 0.43 b	6.72 ± 0.60 ab	7.71 ± 1.14 a	0.026
18:1 n-7	1.86 ± 0.21	1.78 ± 0.24	1.51 ± 0.16	1.40 ± 0.18	0.045
18:1 n-9	43.21 ± 0.86 a	42.70 ± 1.73 a	36.78 ± 1.21 b	40.83 ± 2.81 ab	0.001
18:1 n-9 t	0.27 ± 0.03	0.26 ± 0.07	0.21 ± 0.004	0.24 ± 0.01	0.577
18:2 n-6	13.68 ± 2.05 b	16.22 ± 3.66 ab	21.64 ± 1.81 a	15.78 ± 2.50 ab	0.039
18:3 n-3 (ALA[c])	0.62 ± 0.15 b	0.70 ± 0.13 b	0.97 ± 0.19 a	0.31 ± 0.04 c	<0.001
18:3 n-6	0.12 ± 0.02	0.13 ± 0.03	0.17 ± 0.03	0.18 ± 0.04	0.027
20:0	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.01	0.13 ± 0.06	0.081
20:1 n-9	0.40 ± 0.04	0.48 ± 0.11	0.38 ± 0.02	0.42 ± 0.02	0.351
20:1 n-11	0.07 ± 0.06	0.05 ± 0.06	0.04 ± 0.07	0.12 ± 0.02	0.296
20:2 n-6	0.33 ± 0.04	0.37 ± 0.10	0.37 ± 0.02	0.35 ± 0.02	0.835
20:3 n-3	0.03 ± 0.02 b	0.03 ± 0.01 b	0.06 ± 0.01 a	0.01 ± 0.02 b	0.004
20:3 n-6	0.24 ± 0.05 b	0.24 ± 0.04 b	0.28 ± 0.01 ab	0.37 ± 0.06 a	0.002
20:3 n-9	0.13 ± 0.02	0.13 ± 0.04	0.08 ± 0.01	0.17 ± 0.01	0.065
20:4 n-6	1.63 ± 0.08	1.49 ± 0.20	1.80 ± 0.12	1.56 ± 0.05	0.075
20:5 n-3 (EPA[d])	0.05 ± 0.04 ab	0.07 ± 0.03 a	0.02 ± 0.03 ab	<LLOD[e] b	0.018
22:0	0.08 ± 0.02	0.07 ± 0.04	0.10 ± 0.01	0.11 ± 0.02	0.335
22:4 n-6	0.57 ± 0.06 a	0.46 ± 0.08 a	0.45 ± 0.01 ab	0.33 ± 0.03 b	0.005
22:5 n-3	0.61 ± 0.15 a	0.48 ± 0.11 a	0.46 ± 0.14 ab	0.23 ± 0.02 b	0.006
22:5 n-6	1.33 ± 0.24 ab	1.21 ± 0.22 b	1.24 ± 0.37 ab	1.77 ± 0.06 a	0.023
22:6 n-3 (DHA[f])	1.04 ± 0.13 ab	1.31 ± 0.30 a	1.32 ± 0.01 a	0.65 ± 0.06 b	0.005

[a]Data are reported as means ± the standard deviation of three replicates (n = 3) from each producer, excluding large-scale local pasture-raised with nine replicates (n = 9). [b]p values indicate the results of one-way ANOVA. Means within a row that have different letters are significantly different according to Tukey's HSD test (p < 0.05). [c]ALA, α-linolenic acid. [d]EPA, eicosapentaenoic acid. [e]<LLOD, below the lower limit of detection. [f]DHA, docosahexaenoic acid.

carotenoids, polyphenols, and other bioactive compounds compared to those of commercial feeds.[5,27] In this study, the SPR system, with the most unrestricted access to biodiverse pasture, provided hens with a diet high in such antioxidants. These results are consistent with other studies reporting the high capacity of hens to transfer fat-soluble vitamins such as carotenoids and α-tocopherol into yolks.[5,28] Similarly, access to grass, legume, and herb-rich pasture increased both egg carotenoid and tocopherol content compared to those of caged or conventionally fed hens.[5,27] In a pasture system including either grass, clover, or alfalfa, Karsten et al.[10] observed twice as much vitamin E in pastured compared to commercially fed eggs, consistent with the high tocopherol content of SPR eggs in this study. The level of retinol was higher on average in SPR eggs, though not statistically significantly (Table 2). This is consistent with the unlimited access to pasture in SPR eggs given that β-carotene is the most abundant carotenoid in plants and is efficiently converted to vitamin A by hens.[29] Karsten et al.[10] observed a 38% higher vitamin A concentration in egg yolks from hens provided grass, clover, or alfalfa compared to egg yolks of commercially fed hens. Anderson[1] observed no difference in the vitamin A content of caged and free-range eggs yet observed more β-carotene in free-range eggs. The similar vitamin A contents observed by Anderson[1] and in this study may reflect vitamin A or β-carotene added to a standard layer hen diet, presumably provided to both caged and free-range hens.[30] Hens with access to a phytochemically rich complex pasture produce more antioxidant-rich egg yolks, which may provide benefits for consumer health.[3]

In this study, TPC in egg yolk did not differ by production system. TPC was relatively low, ranging from 1.17 to 1.72 mg of GAE/g of yolk (Table 2). In a similar study of supermarket eggs, including both FR and caged eggs, the phenolic content of egg yolks was also low, between 1.1 and 1.7 mg of GAE/g of yolk, and was similar across all groups.[31] Likewise, hen diet supplementation containing phenolic compounds did not increase the phenolic content of the yolk.[7] However, in another study, levels of egg yolk flavonoids were significantly higher in pastured than in caged hens.[5] Nimalaratne et al.[25] found trace amounts of phenolic compounds in egg yolks and suggested that the hydrophilic nature of some phenolic classes may limit their deposition in egg yolks. The similarity in phenolic content observed in this study may be due to the assessment of TPC rather than specific phenolic classes or compounds.

Egg Yolk Fatty Acid Profiles. The egg production system also influences yolk FA profiles (Table 3). In this study, the total levels of SFAs were highest in COM eggs, but generally, there were only small differences in total levels of SFAs by production system. Total levels of monounsaturated fatty acids (MUFAs) were higher in both PR eggs than in CFR eggs, and total levels of PUFAs were highest in CFR eggs. Total levels of n-6 PUFAs were lower in PR eggs than in FR eggs, and total levels of n-3 PUFAs were higher in all eggs with some pasture access, demonstrating that hens with pasture access produce eggs with

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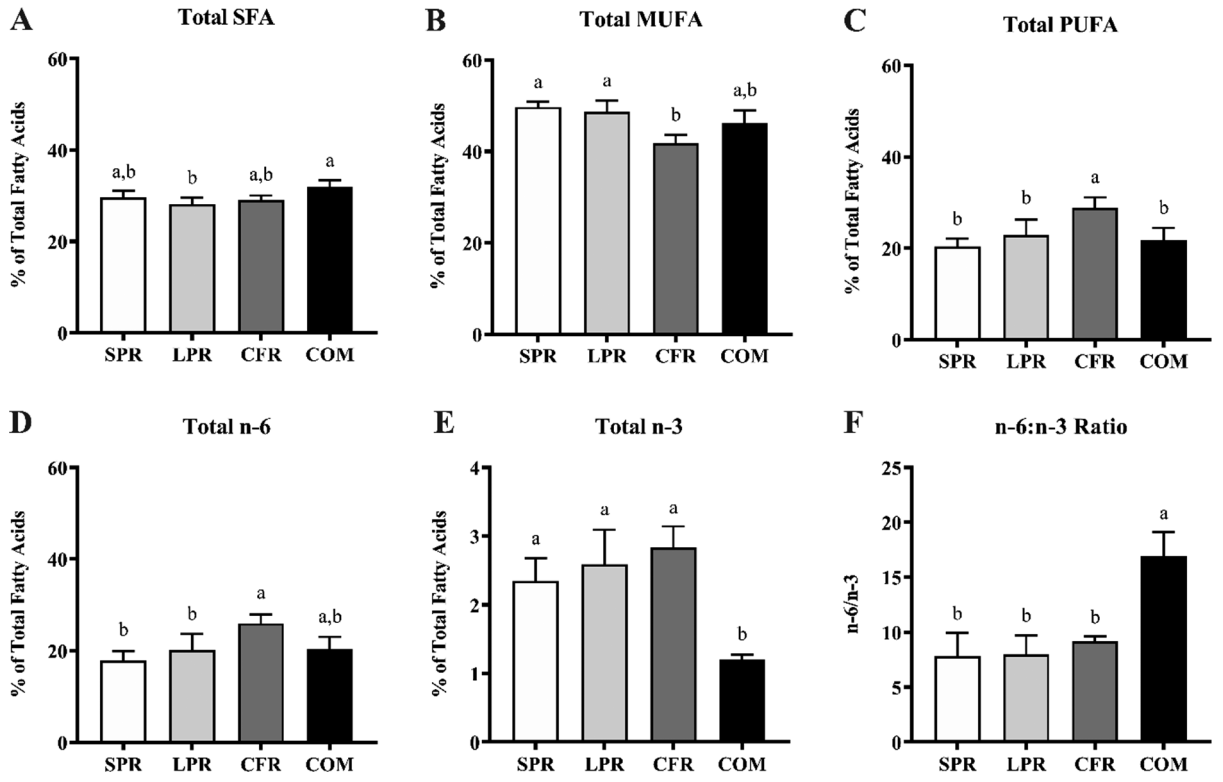

Figure 1. Major fatty acid totals by producer reported as means ± the standard deviation of three replicates ($n = 3$) from each producer, excluding LPR with nine replicates ($n = 9$). Different letters denote statistically significant differences according to Tukey's HSD test ($p < 0.05$). Individual values are listed in Supplementary Table 2.

Table 4. Conjugated Linoleic Acids, Odd Chain Fatty Acids, and Branched Chain Fatty Acids in Egg Yolks (percent of total fatty acids)[a]

fatty acids	small-scale local pasture-raised	large-scale local pasture-raised	commercial free range	commercial	*p* value[b]
conjugated linoleic acid					
9*c*, 11*t* 18:2	0.11 ± 0.01	0.09 ± 0.03	0.10 ± 0.01	0.08 ± 0.02	0.603
10*t*, 12*c* 18:2	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.792
t,*t* 18:2	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.525
odd chain fatty acids					
15:0	0.06 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.109
17:0	0.20 ± 0.02	0.24 ± 0.05	0.22 ± 0.01	0.19 ± 0.01	0.212
17:1	0.12 ± 0.03	0.12 ± 0.02	0.10 ± 0.02	0.06 ± 0.06	0.060
branched chain fatty acids					
15:0-*iso*	0.006 ± 0.002 a	0.003 ± 0.002 b	0.001 ± 0.001 bc	<LLOD[c] c	<0.001
15:0-*anteiso*	0.001 ± 0.001	0.001 ± 0.001	0.000 ± 0.001	<LLOD	0.386
17:0-*iso*	0.01 ± 0.01 a	0.01 ± 0.00 a	<LLOD b	<LLOD b	<0.001
17:0-*anteiso*	0.03 ± 0.01 a	0.02 ± 0.01 a	<LLOD b	<LLOD b	<0.001

[a]Data are reported as means ± the standard deviation of three replicates ($n = 3$) from each producer, excluding large-scale local pasture-raised with nine replicates ($n = 9$). [b]*p* values indicate results of one-way ANOVA. Means within a row that have different letters are significantly different according to Tukey's HSD test ($p < 0.05$). [c]<LLOD, below the lower limit of detection.

lower n-6:n-3 ratios (Figure 1 and Supplementary Table 2). Likewise, Samman et al.[32] observed slightly lower total levels of SFAs in caged eggs compared to organic FR eggs but no difference in total MUFAs or PUFAs. They concluded that FA differences among organic and caged eggs were unlikely to impact consumer health. In contrast, numerous other studies have observed increased levels of MUFAs and n-3 PUFAs when comparing caged eggs to production systems with pasture access. Eggs yolks from hens with pasture access have higher levels of ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) than caged eggs,[1,5,9,10] consistent with the results in this study. Similarly, the nearly 2-fold decrease in egg yolk n-6:n-3 ratios from ∼17 in caged eggs to between 7.8 and 9.2 in hens provided pasture observed in this study (Figure 1 and Supplementary Table 2) is similar to the findings of a review of pastured and conventional eggs that observed reductions in egg yolk n-6:n-3 ratios from 11−19 to ∼5.[9] Generally, hens raised on a biodiverse diet in PR systems produce egg yolks with FA profiles more consistent with dietary recommendations, particularly regarding increased levels of n-3 PUFAs.[33]

Conjugated Linoleic Acid in Egg Yolks. Three CLA isomers not previously characterized in egg yolk were identified,

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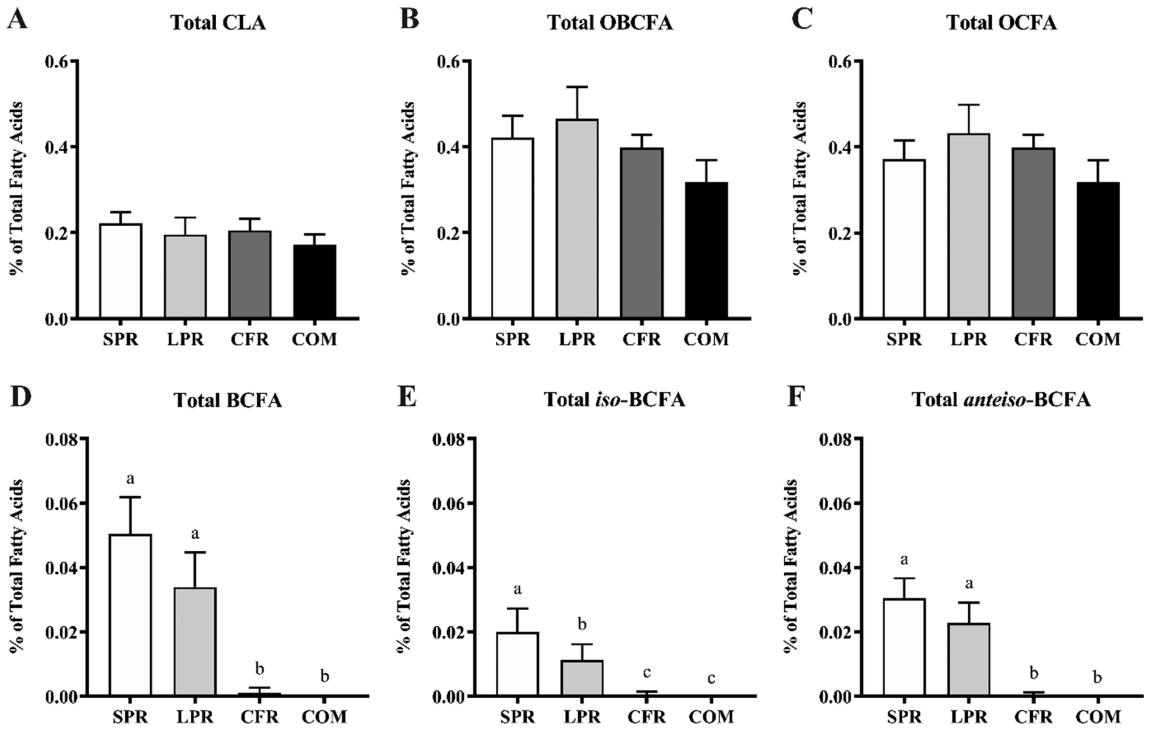

Figure 2. Conjugated linoleic acid, odd chain fatty acid, and branched chain fatty acid totals by producer reported as means ± the standard deviation of three replicates (n = 3) from each producer, excluding LPR with nine replicates (n = 9). Different letters denote statistically significant differences according to Tukey's HSD test (p < 0.05). Individual values are listed in Supplementary Table 2.

including *cis*-9, *trans*-11; *trans*-10, *cis*-12; and *trans*−*trans* CLA (Table 4). It was hypothesized that the level of CLA would be higher in PR than in caged eggs. Though SPR eggs contained the most CLA on average, there was no significant difference among the eggs studied. CLA isomers were detected in all production systems, but total CLA contributed only ∼0.2% of total FAs for all groups (Figure 2 and Supplementary Table 2). These results do not indicate any difference in endogenous CLA production by egg production system. In addition, the trace amounts of CLA detected in egg yolks may not make a significant contribution to dietary CLA in humans. Ruminant products, including dairy and beef, are significant food sources of CLA and contain larger amounts than monogastric products such as eggs,[16] though CLA can be enriched in eggs by adding it directly to hen diets[34] or with bacterial supplementation.[35] Eggs enriched with CLA contained 2.02% total CLA[34] compared to 0.22% in SPR eggs in this study, 1.6% in conventional milk,[36] and between 0.58% and 0.87% in either grass- or grain-fed beef.[17] Further investigation of CLA in eggs is needed to understand whether it makes an important contribution to CLA consumption and its health benefits and whether differences in production systems can increase CLA content.

Branched Chain Fatty Acids in Egg Yolks. Four BCFA isomers were detected, including 15:0-*iso*, 15:0-*anteiso*, 17:0-*iso*, and 17:0-*anteiso* in eggs from production systems with pasture access (Table 4). In addition, levels of BCFAs were significantly higher in PR eggs than in commercial FR eggs (Figure 2 and Supplementary Table 2). This suggests that increasing access to pasture for hens results in increased deposition of BCFAs in egg yolks. Chickens are monogastric animals that perform hindgut fermentation and therefore possess a complex microbial community in their gut.[37] BCFAs are major components of bacterial membranes[14] and are produced by bacteria in the hen hindgut.[38] Though the hen embryo is an isolated unit, Ding et al.[39] demonstrated the presence of microbes in hen embryos and suggested their deposition during egg formation. In addition, hen diet alters hen gut microbiota.[40] Bacteria residing on plants and soil may contribute to hen microbiomes, especially given that poultry litter strengthens soil bacterial communities.[41] Hens raised on pasture may possess a more diverse microbiome and/or have an improved ability to deposit microbes into embryos. Information about the gut microbiome of pasture-raised hens is limited, but diet alters microbial communities in hens provided pasture.[42] Therefore, differences in feed and pasture access may result in different deposition of microbes into eggs and explain the increased levels of BCFAs in FR and PR egg production systems in this study.

Beef and dairy products contain 1.89% and 2.05% BCFAs, respectively,[14] compared to 0.05% in SPR eggs in this study. However, information about the health effects of dietary BCFAs is limited, and this is the first report of BCFA in eggs. Due to branching in BCFA structure, they function like *cis*-unsaturated FAs that interfere with the tight packing of SFAs, implying their ability to function as anti-inflammatory compounds.[18] Accordingly, BCFAs increased the level of expression of anti-inflammatory cytokines in a rat model.[43] Decreased BCFA consumption was recently observed to be related to hypoxia and obesity in mammals.[44] The BCFAs 15:0-*iso* and 17:0-*iso* found in beef and dairy and PR eggs in this study have anticancer properties in vitro.[45] Frequent PR egg consumption could contribute to dietary BCFA intake and its potential health benefits.

Because the exact composition of the hens' diets is not known, some differences and anomalies in nutrient profiles cannot be explained. For one, CLA and BCFAs may have originated from small amounts of ruminant products in hen diets. In addition, increased levels of α-tocopherol, carotenoids, and n-3 FAs in FR and PR eggs are presumed to be a result of increased pasture

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access, but these differences could also be attributed to different hen feeds. These observations suggest future studies examine how specific differences in the botanical composition of the diets of hens affect the nutritional profiles examined in this study.

Another limitation is the small number of eggs analyzed, which may have limited the ability to detect some nutrient differences. However, the GC-MS method utilized in the study is a major advantage. The identification of three CLA isomers and four BCFA isomers (Table 4) not previously reported in eggs was achieved utilizing a 100 m, highly polar GC column and a GC temperature program optimal for separating the complex FA mixture in animal products.[19]

In conclusion, the results of this study demonstrate important differences in egg yolk antioxidant and FA composition by production system. Eggs from hens with access to pasture, labeled as "free-range" or "pasture-raised", possess nutrient profiles that are more likely to be health-promoting compared to those of conventionally raised eggs with regard to antioxidants and n-3 FAs. Improved identification of egg FAs was accomplished with the FA analysis methods utilized in this study, including not previously reported trace amounts of CLA and BCFAs in egg yolks. The greater BCFA content was found in eggs from PR hens, and a potential explanation for increased BCFA content in PR eggs was suggested. This study provides support for the increased interest in incorporating pasture-raised systems into animal product production and provides more detailed characterization of fatty acid differences between egg production systems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.0c00093.

GC-MS Supporting Information (Supplementary Table 1) (PDF)

Fatty acid values from Figures 1 and 2 (Supplementary Table 2) (PDF)

Fatty acid profile of egg yolks (grams per 100 g of egg yolk) (Supplementary Table 3) (PDF)

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■ ABBREVIATIONS USED

ALA, α-linolenic acid; BCFA, branched chain fatty acid; BHT, butylated hydroxytoluene; CFR, commercial free range; CLA, conjugated linoleic acid; COM, commercial; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; FAME, fatty acid methyl ester; FR, free-range; GAE, gallic acid equivalents; GC-MS, gas chromatography-mass spectrometry; LPR, large-scale local pasture-raised; OBCFA, odd and branched chain fatty acid; OCFA, odd chain fatty acid; PR, pasture-raised; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SPR, small-scale local pasture-raised; TPC, total phenolic content

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