



Free aromatic amino acids in egg yolk show antioxidant properties

Chamila Nimalaratne, Daise Lopes-Lutz, Andreas Schieber, Jianping Wu*

Department of Agricultural, Food and Nutritional Science (AFNS), 4-10 Ag/For Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

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ABSTRACT

Laying hens are known to be able to “bio-convert” health-promoting components from the diet into eggs. Wheat and corn are important sources of antioxidant phenolics in the human diet. Given the fact that wheat and corn are major feed ingredients of laying hens in Canada, the objectives of this study were to characterise the presence of novel phenolic compounds in egg yolk and to determine the effect of cooking methods on their content and activity. The total phenolic content of yolk extracts was 3.83 and 3.49 μmol gallic acid equivalents/g dry yolk for wheat-based and corn-based yolks, respectively. Antioxidant properties of yolk extracts were measured by the ORAC, DPPH and ABTS assays. ORAC values were 151.1 ± 26.1 and 155.9 ± 9.5 $\mu\text{mol TE/g}$ for wheat-based and corn-based yolks, respectively. All cooking methods significantly reduced ($p < 0.05$) the antioxidant values. Ferulic acid was detected in trace amounts and no other phenolic compounds were found. However, tryptophan and tyrosine were found to be two main contributors to the antioxidant property of egg yolk. The contents of total free amino acids were 10081.0 and 10009.5 $\mu\text{g/g}$ in wheat-based and corn-based yolks, respectively; cooking methods were found to reduce significantly the amount of free amino acids.

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1. Introduction

Eggs are considered as one of nature's perfect foods that have been consumed for centuries all over the world. Although they contain all the necessary nutrients for a new life, consumption of eggs in many developed countries has declined due to the public perception on its high content of cholesterol. However, current evidence suggests that there is no direct link between egg consumption and blood cholesterol levels (Lee & Griffin, 2006; Qureshi et al., 2007). Egg yolk is a rich source of both nutritive and non-nutritive compounds important to human health. It is well known that hens' diet influences yolk composition. Through dietary manipulation, certain phytochemicals with important health benefits can be enriched in egg yolk (Surai & Sparks, 2001). Over the past few years, many research studies have demonstrated that bioactive feed compounds may be transferred from hens' feed into the yolk. Lutein and zeaxanthin are the most extensively studied phytochemicals in egg yolk. They can help prevent age-related macular degeneration (AMD) and eye cataract either as antioxidants and/or by filtering harmful blue light (Chung, Rasmussen, & Johnson, 2004; Moeller, Jacques, & Blumberg, 2000). Phytoestrogens with potential health benefits such as soy isoflavones have also been reported to be present in egg yolk (Kuhnle et al., 2008; Saitoh, Sato, Harada, & Matsuda, 2004).

Phenolic compounds commonly found in cereal grains, fruits and vegetables are important antioxidants that are suggested to play a preventive role in the development of many chronic human diseases such as cancer and cardiovascular diseases (Adom, Sorrells, & Liu, 2005; Li, Pickard, & Beta, 2007; Liu, 2007). Yet, limited information is available about the presence of phenolic compounds in egg yolk. In Canada, wheat and corn are cereal grains commonly used in poultry feed rations; both are considered as rich sources of various phenolic compounds such as ferulic, *p*-coumaric, vanillic acids, etc. with high antioxidant activities. It has been reported that dietary sinapic acid (4-hydroxy-3,5-dimethoxycinnamic acid) affect egg quality characteristics and low levels of sinapic acid was detected in egg yolk (Johnson, Dahiya, Olkowski, & Classen, 2008); it was also reported that the deposition of simple phenolic acids, unlike the isoflavonoids, in egg yolk is very low under natural conditions. However, it is not known if other phenolic compounds are present in egg yolk.

This study was designed to investigate the possibility of deposition of phenolic acids in egg yolk and the total antioxidant capacity of yolk extracts. It has been reported in the literature that cooking may change the chemical properties of these compounds and therefore affect their antioxidant activity (Li et al., 2007; Xu, Li, Lu, Beta, & Hydamaka, 2009). Thus, the effect of cooking methods on the composition and the antioxidant activities was studied. Interestingly, we identified two aromatic amino acids from yolk phenolic extracts with high antioxidant capacity; therefore, the composition of free amino acids of yolk and the effect of cooking methods on free amino acids were also determined.

* Corresponding author. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jwu3@ualberta.ca (J. Wu).

2. Materials and methods

2.1. Materials and chemicals

Fresh white shell eggs, corn-fed eggs from Ontario and wheat-fed eggs from Manitoba, both were supplied by Burnbrae Farms Limited, Canada. Poultry feed samples used by the above farms were also provided by Burnbrae Farms Limited, Canada. Fluorescein disodium and Trolox were obtained from Acros Organics (Morris Plains, NJ). Methanol (HPLC and analytical grades), trichloroacetic acid, ferulic acid, gallic acid, 2,2'-azobis (2-methylpropionamide) dihydrochloride (AAPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-di (4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH), amino acid standards, ethanolamine (EA), β -aminobutyric acid (BABA) and *o*-phthalaldehyde (OPA) were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.2. Cooking of eggs

Boiled eggs were prepared by placing whole shell eggs in single layer in a saucepan, with water up to 1–2 inches above the egg layer and boiled for 10 min. After boiling they were placed under running tap water for 5 min, peeled and egg yolks were separated from whites. For microwaving, whole eggs were placed in a microwavable glass bowl and cooked in a household microwave oven (model DMW 113w, Danby Products, Ontario, Canada) for 90 s at cooking level of 1100 W and 2450 MHz. Egg yolks were carefully separated from whites. Fried samples were prepared using a frying pan preheated to 205 °C (model SK200TY non-stick frying pan, Black & Decker Canada Inc., Brockville, ON). 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. Thirty eggs were used for preparing each sample, i.e. a total of 120 eggs were used for all four samples. Cooked and raw egg yolks were frozen immediately at –20 °C and then subjected to freeze drying in containers impermeable to light. Freeze-dried egg yolks were ground with a coffee blender to obtain a fine homogenised powder and sieved with a 0.425 mm sieve under dim light conditions. The processed samples were stored in the dark at –20 °C in airtight sealed plastic bags until further analysis. Feed samples were ground, sieved under the same conditions and stored at –20 °C.

2.3. Extraction of phenolic compounds

Approximately 1 g of freeze dried egg yolk sample was extracted with 10 mL of 80% methanol (80:20, vol/vol) adjusted to pH 1.5 with 1 M HCl. The sample was then mixed thoroughly using a vortex mixer (standard vortex mixer model 945404, Fisher Scientific, Edmonton, AB, Canada) for 2 min and centrifuged at 6000g for 10 min at 4 °C. The supernatant was evaporated under vacuum at 35 °C using a rotary evaporator and reconstituted with 1 mL of methanol. All samples were filtered with a 0.22 μ m Nylon syringe filter and subjected to further analyses. Extracts from feed samples were also prepared using the same procedure mentioned above. Extractions were performed in triplicate.

2.4. Determination of total phenolic content (TPC)

The Folin–Ciocalteu assay was used to determine the total phenolic content (Singleton & Rossi, 1965). For this purpose, 0.1 mL of sample was mixed with 0.5 mL of the Folin–Ciocalteu reagent and 1.5 mL of 20% sodium carbonate (Na_2CO_3) solution.

The mixture was made up to 10 mL and allowed to stand at room temperature for 2 h. The absorption was measured at 760 nm using a spectrophotometer (Jasco Corp., Tokyo, Japan). Gallic acid was used to develop standard calibration curves and the results were expressed as μ mol of gallic acid equivalents (GAE)/g dry weight. Determinations were performed in duplicate for each sample and the values represent the means.

2.5. Oxygen radical absorbance capacity–fluorescein (ORAC-FL) assay

The ORAC-FL assay was performed according to the method of Ou, Hampsch-Woodill, and Prior (2001) modified by Dávalos, Miguel, Bartolomé, and López-Fandiño (2004). The reaction was carried out in 96F black, untreated 96-well microplates (Nunc, Roskilde, Denmark) and Trolox (2–16 μ M) was used as standard. The final assay mixture containing fluorescein (200 nM), 75 mM phosphate buffer (pH 7.4) and the sample at different concentrations was incubated at 37 °C for 10 min. Then, 50 μ L of APPH solution (80 mM) were added to each well using a dispenser and the plate was automatically shaken. The fluorescence was recorded using a fluorometric microplate reader (Fluoroskan Ascent; Thermo Electron Corp., Vantaa, Finland) at 1 min intervals for 60 min at excitation and emission wavelengths of 485 and 538 nm, respectively. All assays were conducted in duplicates and at least three independent tests were done for each sample. A blank assay was carried out which did not contain antioxidants. The area under curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. Regression equations obtained from net value of Trolox was used to calculate the ORAC-FL value for each assay. Final ORAC-FL values were expressed as micromoles of Trolox equivalent (TE) per mg of dried yolk (μ mol TE/mg).

2.6. Free radical-scavenging activity on ABTS⁺

The free radical scavenging capacity using the ABTS radical cation (ABTS⁺) is based on the reduction of ABTS⁺ by antioxidants present in the sample tested. The assay was conducted according Madhujith, Izydorczyk, and Shahidi (2006) with slight modifications. To generate the ABTS⁺, the ABTS stock solution (7 mM) and potassium persulphate (2.45 mM) in phosphate buffer saline (PBS, pH 7.4) were allowed to stand for 16 h. For the reaction, 100 μ L of the samples was mixed with 100 μ L of diluted ABTS⁺ (absorbance 0.7 at 734 nm) in a 96-well microplate (Costar #9017, Corning, Inc., NY). The decline in absorbance was measured at 734 nm after 8 min incubation at 37 °C (Spectramax 190, Molecular Devices Corporation, CA). A standard curve was prepared using a series of concentrations of Trolox (0, 2, 4, 8, 12, 16 and 20 μ M) with 100 μ L of diluted ABTS⁺ solution. The radical scavenging capacity of the yolk samples was calculated based on the Trolox standard curve and was expressed as μ mol of TE/mg of dried yolk.

2.7. Free radical-scavenging activity on DPPH

The DPPH radical scavenging capacity (RSA) of each extract was determined according to the method of Madhujith et al. (2006) with slight modifications. All assays were duplicated for three independent determinations. The DPPH \cdot (0.2 mM) solution in 95% ethanol was mixed with the same volume of sample solution in a 96-well flat bottom microplate (Costar #9017, Corning, Inc., NY) and incubated at 37 °C for 60 min using a shaking incubator. The absorbance was measured at 517 nm using a microplate reader (Spectramax 190, Molecular Devices Corporation, CA). A standard radical scavenging activity curve was prepared by reacting 100 μ L of a series of concentrations of Trolox (0, 2, 4, 8, 12, 16 and 20 μ M) with 100 μ L of diluted DPPH solution. RSA of yolk

samples was calculated on the basis of the Trolox standard curve and expressed as μmol of TE/ mg of dried yolk.

2.8. HPLC (high-performance liquid chromatography) analysis of phenolic acids

A Waters 600 HPLC system (Waters, Millford, MA) equipped with a 2702 thermoautosampler, a binary gradient pump and a 2998 photodiode array detector was used. All samples were analysed using a C_{18} reversed-phase column (Waters XBridge 250 mm \times 3 mm, i.d., 5 μM) protected with a C_{18} guard column (20 mm \times 3 mm, i.d., 5 μM). Mobile phases were 2% acetic acid in water (A) and acetonitrile (B). The flow rate was 0.5 mL/min and the total run time was 60 min with the following linear gradient according to Kim, Tsao, Yang, and Cui (2006): From 0% to 15% B in 30 min, from 15% to 50% B in 20 min, from 50% to 100% B in 5 min and from 100% to 0% B in 5 min. Between the injections there was a 20 min equilibration time for reconditioning. The analyses were detected at 280 nm and 320 nm. The injection volume was 20 μL . HPLC analyses were performed in duplicates.

2.9. HPLC-DAD-ESI-MS/MS analysis of phenolic acids

An Agilent Technologies 1200 series HPLC equipment including degasser, binary pump, autosampler, thermostated column compartment and diode array detector (DAD) (Agilent Technologies, Palo Alto, CA) was connected to an Applied Biosystem MDS SCIEX 4000 Q TRAP LC/MS/MS System (AB Sciex, Concord, ON, Canada) for separation and identification of phenolic compounds. Analyst (version 1.5) software was used for data processing. The separation was performed on a C_{18} reversed phase column (Waters XBridge 250 mm \times 3 mm i.d., 5 μM) and a C_{18} guard column (20 mm \times 3 mm, i.d., 5 μM) at a flow rate of 0.5 mL/min. The solvent system and the gradient program were similar as described above. Mass spectrometric data was acquired using an API 4000 QTRAP[®] hybrid QqQ-linear ion-trap mass spectrometer operating on a TurbolonSpray[®] source in negative ionisation mode. Major parameters were set as follows: scan speed of 1000 Da/s; 138 kPa for curtain gas; 600 °C for source temperature; 345 kPa for nebulizing gas; 138 kPa for heating gas (GS2) and -3.500 V for spray voltage. An information-dependent acquisition (IDA) method was used to profile the phenolic compounds. Both Q1 and Q3 were operated unit mass resolution. The spectra were obtained over a scan range from m/z 50 to 1300 in 2 s. The IDA threshold was set at 100 cps, above which enhanced product ion (EPI) spectra were collected from the eight most intense peaks. The EPI scan rate was 4000 amu s^{-1} . Collision-induced dissociation spectra were acquired using high purity Nitrogen as the collision gas under collision energy (CE) of -20 eV. The other MS parameters used were: declustering potential -70 V, entrance potential -10 V and -7 V for collision exit potential. Identification of compounds was achieved based on the retention time, UV spectrum and the fragmentation pattern compared with the authentic standards. The identification of ferulic and isoferulic acids in egg yolk was achieved using multiple reaction monitoring (MRM) scan experiments that included three transitions: 193.0 \rightarrow 133.8 (CE -24 eV); 193.0 \rightarrow 133.2 (CE -40 eV) and 193.0 \rightarrow 148.8 (CE -16 eV).

2.10. Preparative HPLC analysis and collection of fractions

Major components identified from egg yolk were isolated using a C_{18} preparative column (150 mm \times 10 mm i.d., 5 μM) with C_{18} guard column. The same HPLC system and HPLC conditions similar to those described above were used with a flow rate of 3 mL/min and an injection volume of 477 μL . The two major compounds

(P1 and P2 in Fig. 1) were collected separately, freeze-dried and used for antioxidant assays.

2.11. Extraction of free amino acids in egg yolk

Free amino acids were extracted using trichloroacetic acid (Ohkubo, Sawaguchi, Hamatsu, & Matsubara, 2006). Approximately 1 g of the freeze-dried egg yolk was extracted with 5 mL of 6% trichloroacetic acid by vortex mixing for 2 min. The mixture was centrifuged at 6000g for 20 min at 4 °C and the supernatant was filtered with 0.22 μm Nylon syringe filter (Mandel Scientific Corp., Guelph, ON, Canada) prior injecting to the HPLC analysis.

2.12. HPLC analysis of free amino acids

HPLC analysis of free amino acids was carried out according to the method described by Sedgwick, Fenton, and Thompson (1991). Ethanolamine (EA) and β -amino-*n*-butyric acid (BABA) were used as internal standards. Quantification was carried out using standard calibration curves.

2.13. Statistical analysis

All data were analysed by analysis of variance (ANOVA) followed by Duncan's multiple range test using Statistical Analysis System Software (SAS version 9.0, SAS Institute, Cary, NC). Significance of differences was defined at the 5% level ($p < 0.05$).

3. Results and discussion

3.1. Total phenolic content in egg yolk

In this study, four extraction methods, petroleum ether, ethyl acetate, 50% methanol, 100% methanol and 80% acidified methanol, were used to determine the optimum solvent for the extraction of phenolic compounds from egg yolk. The best extraction conditions were established by analysing the TPC of the sample and by performing a recovery test with the addition of a ferulic acid standard. Acidified 80% methanol gave the highest TPC and the best recovery (*data not shown*) and was thus used for further studies. The total phenolic contents of the yolk samples, expressed as mg of GAE/100 g yolk are shown in Table 1. Raw egg yolks showed a TPC content of 72.5 ± 1.1 mg of GAE/100 g yolk and 66.1 ± 0.5 mg of GAE/100 g yolk in corn-fed and wheat-fed yolks, respectively. Cooking of eggs caused a reduction of the TPC by 5% to 18.3% in both yolk types. The decrease in TPC in fried corn-fed egg yolks was higher than those observed for other cooking methods, whereas in wheat-fed yolks there were no differences among the methods of cooking. It was reported that the degree of polyphenol degradation depends very much on the nature of the food and condition of processing (Sikora, Cieřlik, Leszczyńska, Filipiak-Florkiewicz, & Pisulewski, 2008). There were no reports in the literature on the TPC of chicken eggs; however, some studies showed reduced TPC with thermal processing of selected vegetables (Faller & Fialho, 2009; Zhang & Hamazu, 2004). Boiling was found to be the most damaging cooking method for total phenolics in coloured peppers, whereas stir-frying and microwaving did not cause a significant change (Chuah et al., 2008).

3.2. Antioxidant properties of raw and cooked yolk samples

The ORAC-FL assay has been widely applied to measure total antioxidant capacity of many compounds, food and other biological samples. It is the only method that considers both the degree of inhibition and inhibition time in one assay (Prior et al., 2003).

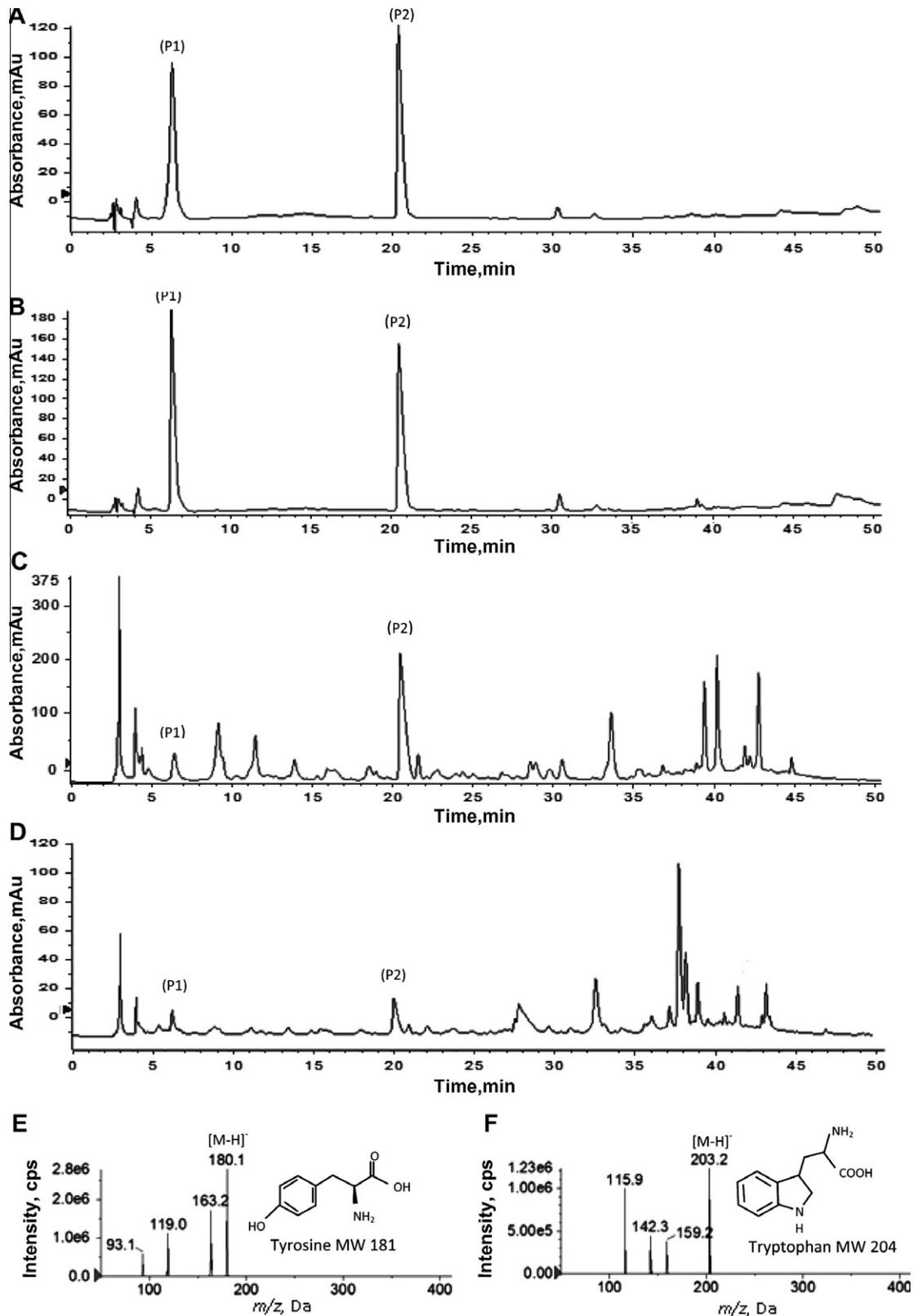


Fig. 1. Wheat fed egg yolk extract (A), Corn fed egg yolk extract (B), wheat based poultry feed extract (C) and corn based poultry feed extract (D) showing the common peaks identified as tyrosine (P1) and tryptophan (P2). Product ion spectra of tyrosine m/z 181 (E) and tryptophan m/z 204 (F). HPLC-MS/MS conditions are as described in the text.

The ORAC-FL, DPPH and ABTS values obtained in this study are shown in Table 2. ORAC-FL values were 151.1 $\mu\text{mol TE/g}$ dry yolk and 155.9 $\mu\text{mol TE/g}$ dry yolk in wheat-fed and corn-fed raw yolk samples (Table 2), which is comparable to the ORAC values of most

fruits and vegetables listed in the most recently published USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2 (Nutrient Data Laboratory, 2010). Our results showed that cooked eggs have significantly lower ORAC-FL

Table 1
Effect of different cooking methods on total phenolic content (TPC) of wheat-fed yolk samples and corn-fed yolk samples.*

Cooking condition	Wheat-based yolk		Corn-based yolk	
	TPC (mg GAE/100 g dried yolk)	Loss%	TPC (mg GAE/100 g dried yolk)	Loss%
Raw yolk	66.1 ^{ab} ± 0.5		72.5 ^a ± 1.1	
Micro-waved	62.8 ^{bc} ± 0.9	5.0	66.9 ^{ab} ± 7.1	7.7
Boiled	58.1 ^c ± 1.8	12.1	67.5 ^{ab} ± 2.4	6.9
Fried	56.2 ^c ± 0.2	15.0	59.2 ^c ± 1.0	18.3

* Mean ± SD values in each column with no common superscripts differ significantly ($P < 0.05$).

Table 2
Antioxidant activities of the yolk extracts as affected by the method of cooking*.

	ABTS (μmol TE/g dry yolk)	DPPH (μmol TE/g dry yolk)	ORAC (μmol TE/g dry yolk)
<i>Wheat-fed yolk</i>			
Micro-waved	45.0 ^e ± 1.6	6.3 ^e ± 0.7	64.5 ^b ± 0.3
Boiled	45.4 ^e ± 0.5	7.9 ^{de} ± 0.1	77.3 ^b ± 4.5
Fried	40.6 ^e ± 1.2	12.0 ^c ± 0.3	66.3 ^b ± 2.2
Raw	54.0 ^d ± 0.6	13.2 ^c ± 0.2	151.1 ^a ± 26.1
<i>Corn-fed yolk</i>			
Micro-waved	55.4 ^c ± 0.6	8.6 ^d ± 0.9	68.9 ^b ± 4.4
Boiled	58.7 ^b ± 0.2	13.7 ^c ± 0.9	77.9 ^b ± 10.7
Fried	64.7 ^a ± 0.4	21.3 ^b ± 1.9	90.7 ^b ± 11.7
Raw	66.0 ^a ± 0.9	26.8 ^a ± 0.9	155.9 ^a ± 9.5

* Mean ± SD values in each column with no common superscripts differ significantly ($P < 0.05$).

values than those of raw samples. However, there was no significant difference among the boiled, fried and microwaved samples.

Free radical scavenging properties were determined using the ABTS⁺ and DPPH assays (Table 2). Corn-fed yolks showed significantly higher ABTS and DPPH values than wheat-fed yolk samples. All cooked samples had lower RSA values than the raw sample with the exception of the fried corn-fed yolk. The decreasing trend was different from the TPCs, indicating that there are some other compounds contributing to the antioxidant activity. In ABTS and DPPH assays for the corn-fed egg yolks, microwaving appears to be the most deleterious method to the antioxidant activity compared to boiling and frying. This could be ascribed to the degradation of antioxidative compounds due to high and uniform heat generation during microwave cooking. A study conducted by Faller and Fialho (2009) on the DPPH RSA of several vegetables (carrot, onion, potato, broccoli, white cabbage) also showed that both boiling and microwaving reduced the RSA. Zhang and Hamazu (2004) also reported that microwave and conventional cooking reduced the antioxidant activity of broccoli; they stated that the decrease might be due partially to leaching or attributable to loss of the primary structure of the antioxidative compounds.

3.3. Phenolic acids in egg yolk

Egg yolk extracts were analysed by HPLC-DAD-MS/MS to characterise the phenolic compounds. HPLC-DAD chromatograms obtained for yolk samples did not show the presence of any phenolic acids. Ferulic and isoferulic acids were detected in both types of yolk extracts in multiple reaction monitoring (MRM) mode (*data not shown*). Since these compounds were found only in trace quantities, no attempts were made for quantification. It appears that, under natural conditions, deposition of simple phenolic acids into the chicken egg yolk is very limited and this is in agreement with previous observations (Johnson et al., 2008). This could be due to the more hydrophilic nature of the simple phenolic acids compared to other polyphenols such as, isoflavones, lignans, coumestrol, etc., which have been reported to occur in chicken eggs (Kuhnle et al., 2008).

Interestingly, while phenolic acids could be detected only in trace amounts, two unknown compounds of aromatic nature were found in considerable quantities in all yolk samples and in poultry feed samples by HPLC-DAD. LC/MS-MS analyses revealed that these are tryptophan and tyrosine (Fig. 1), two aromatic amino acids with reported antioxidant activities (Xu et al., 2009; Yan et al., 1999). Therefore, it was assumed that these compounds contribute to the antioxidant capacity of egg yolk. To confirm this, tryptophan and tyrosine were isolated using preparative HPLC and subjected to antioxidant assays.

3.4. Antioxidant activity of tryptophan (Trp) and tyrosine (Tyr) from egg yolk

Yolk Tyr and Trp showed high ABTS⁺ and ORAC values (Table 3). The activities of yolk samples were compared with that of Trp and Tyr standards. ORAC values of Trp and Tyr from yolk were 25.74 ± 1.76 μmol TE/mg and 24.20 ± 3.94 μmol TE/mg, respectively and these values are comparable to values reported earlier (Huang, Majumder, & Wu, 2010). ABTS⁺ values of yolk Tyr and Trp were 20.58 ± 1.27 μmol TE/mg and 19.23 ± 1.43 μmol TE/mg, respectively, indicating the high radical scavenging ability. Tyr is an aromatic amino acid (see Fig. 1) and structurally related to *p*-coumaric acid, a phenolic acid with high antioxidant activity, except that Tyr contains an amino group. The aromatic nature of its structure is considered to be the major factor contributing to

Table 3
ORAC and ABTS of tryptophan and tyrosine.^a

Sample	ORAC value (μmol TE/mg)	ABTS value (μmol TE/mg)
Tryptophan	32.14 ± 0.56	16.02 ± 1.29
Tryptophan from egg yolk	25.74 ± 1.76	19.23 ± 1.43
Tyrosine	24.91 ± 0.24	11.49 ± 0.87
Tyrosine from egg yolk	24.20 ± 3.94	20.58 ± 1.27

^a All data were expressed as mean ± standard deviation.

its antioxidant activity. It was proposed that the antioxidant activity of Trp is mainly due to its indole group (Galisteo & Herraiz, 2004). Trp has been studied as an antioxidant in different food materials such as yacon (Yan et al., 1999) and potato (Xu et al., 2009). A recent study showed that Trp in mother's milk has very high antioxidant activity, particularly a high free radical scavenging ability and it is used to increase the antioxidant properties of infant formula (Tsopmo et al., 2009). Apart from being an essential amino acid and a precursor of neuromediators such as serotonin, Trp may exert some important biological functions through its high antioxidant activity. To the best of our knowledge, this is the first study to report the antioxidant activities of free tryptophan and tyrosine in egg yolk.

3.5. Free amino acids in egg yolk

The composition of free amino acids in egg yolk was analysed after in-column derivatization with *o*-phthalaldehyde (OPA). The contents of free amino acids in raw and cooked samples in both wheat-fed and corn-fed yolks are shown in Table 4. The contents of total free amino acids were 10081.0 and 10009.5 µg/g of dry yolk in wheat-based and corn-based yolks, respectively. No difference in the composition of amino acids between corn-fed and wheat-fed eggs was observed. Essential amino acids account for ~46–47% while aromatic amino acids account for ~11% of the total amino acids; arginine was the most abundant amino acid (~1500 µg/g of dry yolk) in all samples followed by glutamic acid (~1200 µg/g of dry yolk) and lysine (~900 µg/g of dry yolk). Although the presence of free amino acids in eggs was observed as early as in 1927, there is scarce information available in the literature on the quantity of individual free amino acids in egg yolk (Ducay, Kline, & Mandeles, 1960). The total free amino acid in egg yolk was reported to be ~40 µM/mL of fresh yolk (Ducay et al., 1960; Fitzsimmons & Waibel, 1968).

The total amino acid content of yolk samples is significantly reduced during all types of cooking treatments except for the boiled egg yolk of corn-fed eggs. There is a trend that microwave caused greater reduction of the total amino acid content than other methods. A significant decrease in amino acids during steaming of faba beans (Ziena, Youssef, & El-Mahdy, 1991) and "ginseng" herb (Cho et al., 2008) was attributed to Maillard reactions. Most of the

amino acids, including aromatic amino acids, were significantly reduced in quantity during cooking; microwave cooking caused the highest losses in both type of samples (Table 4). A previous study on conventional and microwave cooking of pork, beef and lamb showed that most essential amino acids including leucine, isoleucine, phenylalanine and methionine were affected during cooking (Baldwin, Korschgen, Russel, & Mabesa, 1976).

4. Conclusions

In the present study, egg yolk was initially screened to determine phenolic compounds by reversed-phase HPLC-DAD-MS/MS. Transfer of simple phenolic acids to egg yolk appeared to be minimal under natural conditions and only ferulic acid was detected in trace amounts in the MRM scan mode. Two unknown compounds of aromatic nature found in considerable amounts in all yolk samples were identified as tryptophan and tyrosine. These compounds are the major contributors to the total antioxidant capacity of egg yolk extracts. The total phenolic content and total antioxidant capacity were affected significantly during cooking; microwave cooking caused the highest reduction in antioxidant capacity while frying reduced the total phenolic content to the highest extent. For the first time, our results showed that free amino acids contribute to the total antioxidant capacity of egg yolk and domestic cooking methods significantly reduced the quantity of free amino acids in egg yolk, with microwaving caused the highest losses. The onset and progression of many forms of chronic diseases including cardiovascular diseases are highly related to oxidative stress. The results of our studies suggest that eggs contain significant quantities of antioxidants in addition to its well-known nutrients. Consumption of cholesterol-rich foods such as eggs was once indicated as a possible causing factor for cardiovascular diseases; findings from the present study might even give rise to a paradigm change in the nutritional evaluation of eggs.

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Table 4
Free amino acid contents of the raw and cooked yolk samples (µg/g of dry weight).^a

Amino acids	Corn fed eggs				Wheat fed eggs			
	Raw	Boiled	Fried	Microwaved	Raw	Boiled	Fried	Microwaved
Asp	499.5 ± 4.6 ^{ab}	489.1 ± 24.9 ^{ab}	492.2 ± 25.2 ^{ab}	448.2 ± 5.2 ^{cd}	520.4 ± 6.5 ^a	447.1 ± 3.8 ^{cd}	476.1 ± 3.3 ^{bc}	435.4 ± 4.5 ^d
Glu	1171.9 ± 10.7 ^{bc}	1214.8 ± 54.9 ^b	1158.4 ± 1.8 ^c	1153.5 ± 4.8 ^c	1280.2 ± 11.6 ^a	1153.0 ± 2.1 ^c	1175.5 ± 0.6 ^{bc}	1151.6 ± 15.5 ^c
Asn	290.6 ± 2.5 ^{ab}	286.9 ± 15.5 ^{bc}	280.3 ± 13.9 ^{bcd}	266.3 ± 3.7 ^d	306.5 ± 3.2 ^a	266.8 ± 2.7 ^d	282.9 ± 4.3 ^{bcd}	267.7 ± 0.4 ^{cd}
Ser	603.4 ± 6.4 ^{ab}	595.1 ± 37.2 ^{ab}	583.9 ± 19.6 ^{abc}	562.8 ± 7.2 ^{bcd}	610.4 ± 9.5 ^a	534.6 ± 2.8 ^d	574.4 ± 12.8 ^{abcd}	551.6 ± 3.8 ^{cd}
Gln	345.6 ± 3.7 ^b	285.6 ± 15.0 ^c	318.1 ± 12.6 ^{cd}	299.4 ± 4.4 ^{de}	385.3 ± 8.2 ^a	305.3 ± 2.2 ^d	335.7 ± 1.6 ^{bc}	336.6 ± 2.8 ^{bc}
His	145.4 ± 0.1 ^a	137.0 ± 8.0 ^b	128.5 ± 4.3 ^{bc}	123.0 ± 2.2 ^c	149.8 ± 3.3 ^a	121.3 ± 0.1 ^c	134.0 ± 1.2 ^b	122.5 ± 0.5 ^c
Gly	251.8 ± 10.0 ^a	225.6 ± 12.6 ^{bc}	236.0 ± 18.7 ^{ab}	213.7 ± 6.9 ^c	206.3 ± 1.7 ^{cd}	179.0 ± 1.3 ^c	186.4 ± 3.3 ^{de}	180.0 ± 0.5 ^e
Thr	573.4 ± 4.9 ^a	542.9 ± 27.8 ^{bc}	531.1 ± 18.5 ^{cd}	502.3 ± 6.7 ^d	564.2 ± 6.0 ^{ab}	503.0 ± 2.0 ^d	515.9 ± 5.4 ^{cd}	503.2 ± 1.7 ^d
Arg	1584.3 ± 9.1 ^a	1602.4 ± 97.9 ^a	1494.3 ± 36.4 ^b	1439.7 ± 12.2 ^{bc}	1390.7 ± 1.4 ^c	1228.6 ± 7.7 ^{de}	1271.6 ± 13.1 ^d	1172.8 ± 7.2 ^e
Ala	625.5 ± 5.6 ^a	616.0 ± 32.1 ^{ab}	603.3 ± 23.2 ^{abc}	584.4 ± 8.0 ^{bc}	615.7 ± 1.2 ^{ab}	541.5 ± 2.0 ^d	575.9 ± 9.1 ^{cd}	547.7 ± 2.6 ^d
Tyr	688.6 ± 2.0 ^a	656.5 ± 33.3 ^a	613.1 ± 22.9 ^b	560.7 ± 5.8 ^c	690.6 ± 3.0 ^a	600.0 ± 5.7 ^b	599.6 ± 10.3 ^{bc}	540.1 ± 3.2 ^c
Trp	44.1 ± 0.2 ^a	40.1 ± 2.5 ^b	36.0 ± 2.3 ^c	32.7 ± 0.4 ^d	44.5 ± 0.1 ^a	36.1 ± 0.2 ^c	35.6 ± 0.9 ^c	31.1 ± 0.5 ^d
Met	160.3 ± 1.2 ^{ab}	152.2 ± 8.7 ^{bc}	145.0 ± 5.3 ^{cde}	138.88 ± 1.8 ^{de}	166.5 ± 1.7 ^a	140.9 ± 0.1 ^{de}	147.9 ± 2.2 ^{cd}	135.7 ± 0.2 ^e
Val	659.2 ± 3.8 ^{ab}	632.0 ± 34.5 ^{bc}	609.2 ± 25.9 ^{cd}	580.83 ± 5.3 ^d	679.4 ± 6.5 ^a	590.6 ± 1.7 ^d	616.6 ± 6.7 ^{cd}	589.2 ± 1.5 ^d
Phe	338.6 ± 0.7 ^a	316.8 ± 16.5 ^b	297.3 ± 11.4 ^c	271.15 ± 2.9 ^{de}	352.3 ± 0.2 ^a	286.4 ± 1.4 ^{cd}	292.9 ± 4.6 ^c	263.4 ± 0.4 ^e
Ile	388.3 ± 0.2 ^b	369.8 ± 19.1 ^{bc}	358.7 ± 15.9 ^{cd}	332.45 ± 4.7 ^e	418.5 ± 5.2 ^a	345.6 ± 2.7 ^{de}	365.7 ± 5.8 ^{bcd}	334.2 ± 0.8 ^e
Leu	744.0 ± 3.2 ^{ab}	706.4 ± 35.0 ^c	550.0 ± 16.9 ^{bc}	629.40 ± 9.8 ^e	769.7 ± 6.7 ^a	654.2 ± 3.2 ^{de}	669.5 ± 9.7 ^d	619.3 ± 0.6 ^e
Orn	19.87 ± 0.4 ^b	17.50 ± 1.6 ^{bc}	16.89 ± 0.4 ^{cd}	14.03 ± 0.6 ^d	23.49 ± 2.8 ^a	16.51 ± 0.1 ^{cd}	20.16 ± 0.5 ^b	16.70 ± 0.4 ^{cd}
Lys	874.2 ± 1.7 ^{ab}	876.5 ± 39.9 ^{ab}	832.2 ± 28.6 ^{bc}	814.44 ± 13.0 ^{cd}	905.7 ± 2.1 ^a	785.7 ± 7.9 ^{cd}	829.0 ± 19.5 ^{bc}	774.3 ± 2.1 ^d
Total	10009.52 ± 70.6 ^a	9764.20 ± 515.4 ^{ab}	9285.22 ± 303.4 ^{bc}	8968.44 ± 105 ^{cd}	10081.0 ± 78.1 ^a	8737.09 ± 49.6 ^d	9106.10 ± 114.4 ^{cd}	8573.86 ± 48.8 ^d

^a Results are means ± Standard deviation (*n* = 6), a–e values with different superscripts within a row indicate significant differences at *P* < 0.05 separated by Duncan's multiple range test.

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