EFFECT OF STORAGE DURATION ON THE ANTIOXIDANT ACTIVITY OF THE HEN AND QUAIL EGGS USING ABTS METHOD

MARTINA VRSANSKA1, STANISLAVA VOBERKOVA1, VOJTECH KUMBAR2
1Department of Chemistry and Biochemistry
2Department of Technology and Automobile Transport
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC
martina.vrsanska@mendelu.cz

Abstract: Eggs are a source of proteins and bioactive molecules, including antioxidants. The effect of storage time on the antioxidant activity in hen and quail egg components was chosen as an important parameter in this work. For the determination of antioxidant activity ABTS method was used. Our results showed higher antioxidant activity in yolk in both kinds of eggs and quail eggs showed higher activity in comparison with hen eggs. The obtained results also suggested that the total antioxidant capacity was decreased during storage, which was probably caused by the loss of naturally occurring antioxidants or formation of new compounds with pro-oxidant activity during storage.

Key Words: hen egg, Japanese quail egg, antioxidant activity, ABTS

INTRODUCTION
The chemical and nutrient composition of egg is well known (Kovacs-Nolan et al. 2005, Seuss-Baum 2007, Li-Chan and Kim 2008). The nutritional composition of quail and hen eggs is similar, although quail eggs are highly prized for their composition, because they contain around 2.5 times more vitamins A, B1 and B2 (Baumgartner and Hetenyi 2001). Concurrently, literature sources suggest that quail eggs have a slightly higher proportion of protein, carbohydrates, minerals and water (Shanaway 1994, Oliveira et al. 2009).

The eggs are perceived as important food product due to multifunctional properties and their nutritional, biological and technological potential. In addition to the nutritional value, biological molecules in eggs are a source of proteins and bioactive molecules, including antioxidants (Yu et al. 2011), which have different activities and they can render important health benefits (Miranda et al. 2015), but they are not generally considered as antioxidant diets. They are subjected to various processing and storage conditions before consumption, which may influence the antioxidant capacity of egg components. The effect of egg processing and storage conditions on the overall antioxidant activity play important role.

An antioxidant is defined as any substance that slows down, changes or removes oxidative damage to a target molecule (Halliwell 2007), directly acts as reactive oxygen species (ROS) (Ngo et al. 2011) or indirectly acts to regulate antioxidant barricade or inhibit ROS production (Khlebnikov et al. 2007). Consumption of antioxidants through diet is thought to be important in reducing oxidative damage (Valko et al. 2007, Halliwell 2012). These antioxidants play a critical role in protecting cellular components from potentially damaging ROS and maintaining homeostasis and cellular functions.

The ability of an antioxidant to inhibit the oxidative degradation of various compounds is defined as antioxidant activity (to prevent lipid peroxidation etc.) (Surai 2007). Egg storage is associated with lipid peroxidation within egg membranes, particularly those containing high levels of polyunsaturated fatty acids (Kodovska 2013).

In recent years many various methods for determination of antioxidant activity have been developed in chemical analysis and biological evaluation of antioxidant characteristics. The ABTS method is one of the most used methods for determining of total antioxidant activity. The sample is tested for the ability to extinguish a radical cation (ABTS$^{+}$) + ABTS (2,2'-azinobis (3-
ethylbenzothiazoline-6-sulfonic acid), which is generated by oxidation of ABTS with potassium persulfate and it is reduced in the presence of hydrogen-donating antioxidants (Figure 1A–C). Radical ABTS\(^{\cdot+}\) antioxidants that act as hydrogen donors, measured spectrophotometrically at a wavelength length of 734 nm on the basis of changes in the absorption spectrum. This method is very fast, simple and allows a various evaluation of the antioxidant activity of many substances and mixed samples (Paulova et al. 2004).

The main aim of this work is to study the effect of storage duration on the antioxidant activity of the egg components of hen and quail eggs.

**Figure 1 A Molecular formula of ABTS, B Molecular formula of ABTS\(^{\cdot+}\), C Formula of ABTS**

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**MATERIAL AND METHODS**

**Hen and Japanese quail eggs**

A total of 90 eggs obtained from hens and 180 Japanese quail eggs were used to investigate the effect of storage time on quality of eggs. One sample mixture obtained 10 pieces of eggs, which were analysed in triplicate. Therefore total was examined 81 analyses. Hens (ISA BROWN) and Japanese quails (Coturnix coturnix japonica) were kept in cage technology at a commercial breeding farm in the South Moravia region (Czech Republic).

The eggs were tested fresh and after storage for 1, 2, 8, 14, 21, 28, 42 and 56 days. Eggs were stored using refrigeration at 4 °C. All chemicals were purchased from Sigma-Aldrich (USA).

**Sample preparation**

The albumen and yolk were separated, after they were blended for 10 minutes to get homogeneous mixtures and after that they were used for determination of antioxidant activity. The melange was prepared by mixing whole eggs samples and blended for 10 minutes.

**Antioxidant activity determination**

ABTS 2,2’-Azinobis-(3-ethylbenzthiazoline-6-sulfonate acid) assay was based on the method of Re et al. (1999) with a slight modification. ABTS radical cation (ABTS\(^{\cdot+}\)) was produced by the reaction between 3.5 mmol/l ABTS solution and 0.06 mmol/l potassium persulfate (K\(_2\)S\(_2\)O\(_8\)). The solutions were mixed in a ratio 50 : 1 (ABTS : K\(_2\)S\(_2\)O\(_8\)) and the mixture was allowed to stand in the dark at room temperature for 16 h before use. After this time, the mixture was mixed with freshly prepared acetate buffer (pH 4.3) in a ratio 39 : 1 (buffer : ABTS). 2 ml of the prepared mixture and 25 ml of tested sample (albumen/melange/yolk) were pipetted into the tubes. The acetate buffer was used as a blank.

The absorbance of the mixture was measured at 734 nm after 30 minutes of incubation at room temperature. All determinations were carried out in triplicate.

**Statistical analyses**

Statistical analyses of antioxidant activity in hen and quail eggs were made using one-way analyses of variance (ANOVA) and statistical significance was declared when p value was equal to or less than 0.05.
RESULTS AND DISCUSSION

Calibration curve of gallic acid

The absorbance was compared with a standard curve of prepared gallic acid solutions (0.01, 0.02, 0.03, 0.04, 0.05, 0.06 μmol/l) and expressed as % of gallic acid.

An absorbance was calculated by the formula:

\[ A(\%) = A_0 - \left( \frac{A_1}{A_0} \right) \cdot 100 \]

where \( A_0 \) is the absorbance of the prepared mixture, and \( A_1 \) is the absorbance of the extract measured after 30 minutes.

The resulting dependence of the loss of absorbance \( A_0 \) versus concentration of gallic acid is shown in Figure 2 using a calibration curve of gallic acid.

Figure 2 Calibration curve of gallic acid

Antioxidant activity in hen and quail eggs

Figures 3 and 4 show antioxidant activity of hen and quail eggs during 56 days of storage. The higher antioxidant activity was observed in yolk compared with albumen \((p < 0.05)\) in both kinds of eggs, which could be caused by oxidation of egg lipids, located in yolk. Approximately 65% of yolk lipids are triglycerides, while phospholipids, cholesterol and carotenoids create 30% and 4%.

The aromatic amino acids and carotenoids contained in egg yolk are the major contributors to the antioxidant properties and probably due to these components the highest antioxidant activity in hen and quail yolk compared with albumen \((p < 0.05)\) is shown in the figures 3 and 4. Our observation agrees with results of Remanan and Wu (2014), which suggest that higher antioxidant activity was determined for fresh egg yolk in comparison with fresh egg albumen and whole eggs.

The present study demonstrated that egg yolk antioxidant activity is stable during storage for all experiments. The results of Nimalaratne et al. (2016) showed that the antioxidant activity is stable during six weeks of simulated retail storage. In contrast, in the study of Barbosa et al. (2011), the total antioxidant activity of egg yolk decreased significantly after 14 days of storage at refrigeration temperature and after 7 days at room temperature.

It is known that the egg albumen protein hydrolysate has good antioxidant activity (Chen and Chi 2011).

Obtained results suggest that storage can influence antioxidant activity, which decreased during storage after 28 days of experiment \((p > 0.05)\), mainly for albumen components in both kinds of eggs. Similar results were observed in work of Xu et al. (2007), who tested antioxidant activity of hen egg ovalbumin in albumen.

Figure 3 Antioxidant activity of hen egg components (albumen, melange, yolk) during 56 days of storage
Figure 4 Antioxidant activity of quail egg components (albumen, melange, yolk) during 56 days of storage

The bioactivity of egg antioxidants can be affected by food processing and storage. However, the antioxidant properties of egg may vary depending on several factors, for instance egg types, processing and storage conditions (Nimalaratne et al. 2016).

CONCLUSION

This study was focused on the effect of storage duration of the hen and quail eggs on the antioxidant activity using ABTS method. The antioxidant activity of egg components was measured and results of this study showed that higher antioxidant activity was observed in yolk compared with albumen \((p < 0.05)\) in both kinds of eggs. The comparison of hen and quail eggs showed that higher activity was observed in quail eggs \((p > 0.05)\). The antioxidant activity was decreased during storage \((p > 0.05)\), which was probably caused the loss of naturally occurring antioxidants or formation of new compounds with pro-oxidant activity during storage experiment.

ACKNOWLEDGEMENTS

This research was supported by project TP 6/2015 “Impact loading of agricultural products and foodstuffs” financed by Internal Grand Agency FA MENDELU.

This research has been financially supported by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).
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