Scientific Works. Series C. Veterinary Medicine. Vol. LIX (3)
ISSN 2065-1295, ISSN Online 2067-3663, ISSN-L 2065-1295

THE EFFECT OF REFRIGERATION ON CAROTENOIDS AND LIPIDS IN EGG YOLK

Nicoleta Corina PREDESCU, Camelia Puia PAPUC, Valentin Răzvan NICORESCU

Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independentei, 050097, Bucharest, Romania,
Phone: +40213180469, Fax: +40213180498,
Email: durduncorina@yahoo.com, cami_papuc@yahoo.com, valinicorescu@yahoo.com

Corresponding author email: durduncorina@yahoo.com

Abstract

Hens' eggs represent a rich source of important nutrients, including lipids and carotenoids. Lipid composition of hens' eggs is influenced by genetic factors, age, and diet. Lipids of egg yolk represent an important source of animal fat for humans. Carotenoids are the pigments of egg yolk, and their concentration is an important attribute, since the consumers associate an intense colour with eggs that are both healthier and of higher quality. Since carotenoids are not produced by animals, their level in animal products, including egg yolk, is related strictly to diet. They play numerous physiological roles in both the laying hen and developing embryo. Analyzed eggs, both fresh and stored for 30 days at temperatures below 12°C, were obtained from hens fed with the same type of forage. The study was conducted in January and February, and the parameters analyzed were egg lycopene and β-carotene, conjugated dienes and trienes, lipid hydroperoxides and TBARS (thiobarbituric acid reactive substances). The amounts of β-carotene and lycopene found in refrigerated and fresh eggs were very low. Conjugated dienes concentration was higher then conjugated trienes concentration in refrigerated eggs yolk. TBARS concentration was higher for refrigerated eggs yolk samples compared to fresh eggs yolk samples. In the present research, we found that the refrigeration period has no significant effects on carotenoids concentration and also on lipids quality reflected by primary (conjugated dienes and trienes and peroxides) and secondary lipids peroxidation products (TBARS). Peroxidation products appeared during refrigeration period affect egg macromolecules' quality, probably due to damage of egg's antioxidants.

Key words: *β-carotene, conjugated dienes and trienes, hens' eggs, lycopene, thiobarbituric acid reactive substances.*

INTRODUCTION

Bird eggs are a common food and one of the most versatile ingredients used in many branches of the modern food industry. Bird egg is one of the natures' perfect and complete food material. The most commonly used bird eggs are those from chicken and represent a rich source of important nutrients, including carotenoids and lipids.

Carotenoids are the pigments of egg yolk, and their concentration is an important attribute, since the consumers associate an intense colour with eggs that are both healthier and of higher quality. Since carotenoids are not produced by animals, their level in animal products, including egg yolk, is related strictly to diet.

Lipids of egg yolk represent an important source of animal fat for humans. Hen eggs lipids, have already been studied but there is still not enough information on the profile of fatty acids in eggs from domestic birds. Triacylglycerols (63.1%) and phospholipids (26.9%) are the dominant lipids of hen eggs. The content of fatty acids is about 26.6 g/ 100 g yolk. Monoenic (MUFA) acids – 46.9% and polyenic (PUFA) acids – 22.4% are the dominant ones, whereas saturated acids (SFA) constitute the remaining 30.7% (Kaźmierska et al., 2005). PUFAs are not synthesized in human organism and have to be delivered with food.

Lipid oxidation during eggs storage is of major importance. As the PUFAs oxidize, they form hydro peroxides, which are susceptible to further oxidation or decomposition to secondary peroxidation products such as aldehydes, ketones and other compounds that may affect the quality of eggs, including flavour, taste, nutritional value and toxic compounds (Radwan N. et al., 2008).

Eggs seemed to have built-in antioxidant characteristics that maintain their quality during the storage period.

Constituents such as β-carotene and lycopene appear to be very effective in preventing oxidation of yolk lipids (Predescu et al., 2013).

MATERIALS AND METHODS

Sample preparation

In this experiment we used eggs from hens (*Gallus gallus*) reared extensively in the same conditions. For the assay were taken samples of fresh eggs and eggs held for a period of 30 days at refrigeration temperature. The two categories of eggs were divided in whole egg samples (yolk and albumen), yolk samples and albumen samples. For samples of whole egg, their shells were broken and the content (white and yolk) was homogenized using a laboratory blender. For samples of white and yolk, the two components were separated by hand, and then were homogenized using a laboratory blender.

Determination of β-carotene and lycopene

One g of egg sample was extracted with 10 mL of 80% methanol (80:20, v:v) and adjusted to pH 1.5 with 1 M HCl. The sample was then mixed thoroughly using a vortex mixer for 2 min and centrifuged at 6000g for 10 min at 4°C. β-carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone-hexane mixture (4:6; v:v) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to equations (1) and (2):

$lycopene\ (\mu g/100\ mL) = -0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$ (1)

$\beta\text{-carotene}\ (\mu g/100\ mL) = 0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$ (2)

The assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as µg of carotenoid/100 mL of extract.

Determination of primary oxidation products

Determination of lipid hydroperoxides

Spectrophotometric ferric thiocyanate method was ussed for lipid hydroperoxides determination. The method is based on the ability of hydroperoxides to oxidize ferrous ions (Fe^{2+}) to ferric ions (Fe^{3+}) in an acidic solution. The ferric ions form chromophores when complexed to thiocyanate, which can be measured by spectrophotometry. Ferric thiocyanate is a red-violet complex with absorption spectra at 501 nm (Eymard and Genot, 2003). Blank samples were prepared using 5mL of ethanol:chloroform (v:v, 30:70), 100µL methanol:chloroform (v:v, 1:2), 100µL 30% ammoniumthiocyanate solution and 100µL Fe^{2+} in 3.7 % HCl solution. Exactly three minutes after addition of iron, absorbance was measured at 501nm against pure ethanol. Samples were made by the same procedure as blank samples, except that 100µL of methanol:chloroform (v:v, 1:2) was replaced by 100µL of sample. A standard curve was made based on 0.1 mg/mL Fe^{3+} standard work solution. PV was expressed as mEq peroxide kg^{-1}.

Determination of conjugated dienes and trienes

Hydroperoxides from PUFAs form conjugated dienes and trienes that can be measured quantitatively by spectrophotometric UV measurement at wavelength 233 nm and 268 nm. The method is considered very simple. The sample is diluted in isooctane and measured directly in a quartz cuvette placed in a spectrophotometer. The method does not depend on any chemical reaction or color development and requires relatively small amounts of sample (0.1g) (Frankel, 2005). The conjugated diene value and the conjugated trienes value are expressed as absorbance units at 233nm for conjugated diene and 268 nm conjugated trienes.

Determination of secondary oxidation products

Determination of thiobarbituric acid reactive substances (TBARS)

The method is based on the formation of a pink complex with strong absorbance at 532 nm when thiobarbituric acid (TBA) and oxidation products from unsaturated fatty acids react (Shahidi and Wanasundara, 2002). For calculations a standard curve based on known concentrations of 0.1 mM TEP (1.1.3.3 tetraethoxypropane) working solution was constructed. TBARS value was expressed as µmol /100 g.

RESULTS AND DISCUSSIONS

Determination of β-carotene and lycopene

Concentrations of carotenoids in egg yolks are strongly associated with diet and many factors influence the ability of hens to absorb carotenoids from their feed. Table 1 and table 2

show the concentration for β-carotene and lycopene in the whole eggs and yolk eggs extracts. β-carotene and lycopene were found in small amounts.

Table 1. Contents of lycopene in the whole egg and yolk methanolic extracts

Sample	Lycopene (μg /100 mL)	
	Fresh eggs	Refrigerated eggs
Whole egg	15.61±1.44	13.72±1.41
Yolk	19.55±2.09	18.94±1.74

β-carotene and lycopene concentrations found in fresh extracts were slightly and insignificant higher than the content found in chilled eggs extracts (Tables 1 and 2).

Table 2. Contents of β-carotene in the whole egg and yolk methanolic extracts

Sample	β-carotene (μg /100 mL)	
	Fresh eggs	Refrigerated eggs
Whole egg	23.54±2.47	20.79± 1.76
Yolk	29.12± 1.78	27.67 ±1.90

Determination of lipid hydroperoxides

From the analysis of Table 3, we can conclude that the lipid hydroperoxide value of refrigerated eggs methanolic extracts increase during refrigeration period when compared with fresh eggs.

The same situation was found for yolk egg. Lipid hydroperoxides were found in small concentration. For the egg yolks, the formation of lipid hydroperoxides may be prevented because of the antioxidant system presented in yolk like β-carotene and lycopene.

Table 3. Determination of lipid hydroperoxide value in fresh and refrigerated eggs

Sample	Lipid hydroperoxide value (mEq peroxide kg^{-1})	
	Fresh eggs	Refrigerated eggs
Whole egg	56.21±1.12	60.72±2.64
Yolk	54.45±2.43	56.94±1.98

Determination of conjugated dienes and trienes

During the formation of hydroperoxides from unsaturated fatty acids conjugated dienes are typically produced, due to the rearrangement of the double bonds. An increase in UV absorption theoretically reflects the formation of primary oxidation products in sample. It was found that for refrigerated eggs, the amount of conjugated dienes and trienes gradually increased.

Table 4 and table 5 shows the conjugated dienes and conjugated trienes absorbance in the whole egg and yolk egg extracts. Whereas conjugated dienes presented the major absorbance, conjugated trienes were found in small amounts ($0.11*10^2$ –$0.38*10^2$).

The absorbance at 232 nm, due to the formation of conjugated dienes, was a good index for measuring the degradation of whole eggs and yolk eggs samples. Good correlations between conjugated dienes and peroxide value have been found.

Table 4. UV Spectrophotometric determination of the formed conjugated dienes in fresh and refrigerated eggs

Sample	Conjugated dienes (A233 nm *10)	
	Fresh eggs	Refrigerated eggs
Whole egg	0,33±0.044	0,38±0.051
Yolk	0,11±0.04	0,22±0.04

The conjugated dienes were formed at higher levels than the conjugated trienes which corresponded with results published by Dostálová (2005).

Table 5. UV Spectrophotometric determination of the formed conjugated trienes in fresh and refrigerated eggs

Sample	Conjugates trienes (268 nm*10^2)	
	Fresh eggs	Refrigerated eggs
Whole egg	0.36± 0.05	0.38± 0.06
Yolk	0.11 ±0.02	0.14 ±0.02

Determination of thiobarbituric acid reactive substances (TBARS)

The primary oxidation products are unstable and susceptible to decomposistion. A complex mixture of volatile, nonvolatile, and polymeric secondary oxidation products is formed through decomposition reactions, providing various indices of lipid oxidation. Secondary oxidation products include aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds, among others.

Egg yolk TBARS value in fresh yolk eggs of chicken was less than refrigerated yolk eggs (15.55±0.49 vs. 22.94±1.59 μmol /100 g); it decreased linearly as lycopene and β-carotene concentration decreased ($p < 0.05$) (Table 6). Although the TBARS values obtained were very low and confirm the oxidative stability of fresh egg, as has been previously described by some authors (Galobart et al., 2001).

Table 6. Determination of TBARS value measured in fresh and refrigerated eggs

Sample	TBARS value (μmol /100 g)	
	Fresh eggs	Refrigerated eggs
Whole egg	40.71±1.21	65.22±2.81
Yolk	15.55±0.49	22.94±1.59

CONCLUSIONS

Yolk eggs presented important lycopene and β-carotene concentration, and during preservation by refrigeration their concentrations decreased. During storage by refrigeration, eggs suffered oxidative processes of the lipids, as reflected by increasing primary lipid peroxidation products (conjugated dienes and trienes, lipid hydroperoxides) and secondary lipid peroxidation products.

REFERENCES

Dostálová J., Hanzlík P., Réblová Z., Pokorný J. (2005): Oxidative changes of vegetable oils during microwave heating. Czech Journal of Food Sciences, 23, 230–239.

Eymard, S., Genot, C., 2003. A modified xylenol orange method to evaluate formation of lipid hydroperoxides during storage and processing of small pelagic fish. European Journal of Lipid Science and Technology, 105, 497-501.

Frankel, E. N., 2005. Lipid Oxidation, Bridgwater, England, The Oily Press.

Galobart, J., A. C. Barroeta, M. D. Baucells, and F. Guardiola, 2001. Lipid oxidation in fresh and spray-dried eggs enriched with ω3 and ω6 polyunsaturated fatty acids during storage as affected by vitamin E and canthaxanthin supplementation. Poultry Science, 80, 327–337.

Kamal-Eldin A., M., 2003. Makinen, and A. M. Lampi, in A. Kamal-Eldin, ed., Lipid Oxidation Pathways, AOCS Press, Champaign, Illinois, 1–36.

Kaźmierska M., Jarosz B., Korzeniowska M., Trziszka T., Dobrzański Z., 2005. Comparative analysis of fatty acid profile and cholesterol content of egg yolks of different bird species. Polish journal of food and nutrition sciences. 14(55), 69-73.

Nagata, M., I. Yamashita., 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 39(10), 925-928.

Predescu N. C., C. Papuc, V. Nicorescu, 2013. The effect of refrigeration on some antioxidants and soluble proteins in eggs. Bulletin of UASVM Cluj-Napoca - Veterinary Medicine, 70(1), 128-133.

Radwan N., L., Hassan R.A., Qota E.M., Fayek H.M., 2008. Effect of natural antioxidant on oxidative stability of eggs and productive and reproductive performance of laying hens. International Journal of Poultry Science, 7 (2): 134-150.

Shahidi, F., Wanasundara, U., 2002. Methods for measuring oxidative rancidity in fats and oils. Food science and technology-New York-Marcel Dekker, 465-488.