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Determination of cholesterol in Italian chicken eggs

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ABSTRACT

Chicken eggs represent a relevant diet source of cholesterol. With the aim of evaluating the cholesterol content of the eggs from Campania Region (Italy) and to test a new analytical procedure, a sample of 100 chicken eggs from local market was analysed by gas chromatography using a polar capillary column. The total free cholesterol was extracted from the egg yolk with *n*-hexane and trichloroacetic acid, and analysed without the saponification step commonly used. This alternative procedure was compared with the official method. The results indicate that the total free cholesterol content is in the range of 120–193 mg/egg (average value 157 ± 3 mg/egg) lower than the previously reported value of 213 mg/egg. The total free cholesterol quantity is not related to the yolk weight. The bound cholesterol contribution to the total cholesterol was negligible. Dietary recommendations aimed at restricting the egg consumption should be taken with caution and should not include all individuals.

1. Introduction

As a whole food, eggs are an inexpensive and low calorie source of nutrients such as folate, riboflavin, selenium, choline, vitamin B-12 and vitamin A; eggs are also one of the few exogenous sources of vitamins K and D. Furthermore, eggs are a source of high quality protein, and the lipid matrix of the yolk serves to enhance the bioavailability of nutrients such as lutein and zeaxanthin (Herron & Fernandez, 2004; INRAN, 2000). Compared to other animal protein sources, eggs contain proportionately less saturated fat, which has generally been recognised as a strong dietary determinant of elevated low-density lipoprotein (LDL) levels and increased risk of coronary heart disease (CHD). The main components of an average egg of 65 g weight (edible part 58 g) are, water 45 g (69%), proteins 7.2 g (11%), carbohydrates 0.5 g (0.8%), lipids 5.3 g (8.1%), according to reported data (Herron & Fernandez, 2004). Eggs are also a major source of dietary cholesterol, totally contained in the yolk, and consumer concern about the association of cholesterol with coronary heart disease has lowered their consumption (USDA, 1975).

Cholesterol, namely (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H cyclopenta[a] phenanthren-3-ol, is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals; it is the principal sterol synthesized by animals. Cholesterol is important to good health at a reasonable level, because it is a component of the cell membranes and it is converted into hormones.

Cholesterol determination in foodstuff, particularly in eggs has been done using different techniques, including spectrophotometry (Bohac, Rhee, Cross, & Ono, 1988; Herron & Fernandez, 2004; USDA, 2000), gas chromatography (GC) (Guardiola, Codony, Rafecas, & Boatella, 1994), liquid chromatography (LC) (Fenton & Sim, 1991; Hwang, Wang, & Choong, 2003), high-performance liquid chromatography (HPLC) (Mazalli, Sawaya, Eberlin, & Bragagnolo, 2006) and capillary electrophoresis (Riekkola, Jussila, Porras, & Valkò, 2000; Xu et al., 2002). All these methods share the same procedure for sample preparation: a saponification extraction step of total lipids and a multistage solvent extraction followed by purification and concentration (Fenton & Sim, 1991; Naeemi, Ahmad, Al-Sharrah, & Behbahani, 1995; Van Elswyk, Schake, & Hargis, 1991). Natural variation between samples has also been reported: the cholesterol level in eggs in fact changes with species, breed, hen's age, egg and yolk weight, and diet (Maurice, Lightsey, Hsen, Gaylord, & Reddy, 1994; Pandey, Panda, Maitra, & Mahapatra, 1989; Riad, Kicka, Osman, & Kamar, 1981; Zemková, Simeonovová, Lichovníková, & Somerlíková, 2007). It is worth to note that there are many conflicting points of view and results in relation to the analytical methods for cholesterol determination and in relation to the results obtained.

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The cholesterol content in eggs, according to literature data (USDA, 1975), has been reported as 274 mg/egg as set by the Consumer and Food Economics Institute of the USDA (1976), and was used as reference value by the medical community to determine the recommended daily intake of cholesterol (Beyer & Jensen, 1989). Consequently, physicians recommended a consumption of two eggs per person and per week, considering that the maximum daily intake of cholesterol should be 100 mg per 1000 kcal energy diet assumption to avoid problems related to the accumulation of cholesterol in arteries and to prevent cardiovascular diseases. Data from recent studies show that the consumption of one or two eggs per day, when part of a low fat diet, does not adversely affect the lipid profile, in particular low density lipoprotein concentration (Harman, Leeds, & Griffin, 2008; Spence, Jenkins, & Davignon, 2010). Cholesterol is essential for the production of bile acids; without bile acids, fats cannot be digested. Bile acids are crucial for the assimilation of fat soluble vitamins, such as vitamins A, D, E and K. Current evidence indicates that dietary cholesterol has a modest effect on plasma cholesterol (1.9-mg change in LDL and 0.4-mg change in HDL per 100 mg/d of dietary cholesterol) in the general population; more importantly, dietary cholesterol does not appear to influence the ratio of LDL to HDL cholesterol (the most important predictor of CHD) in the general population (Baron & Hylemon, 1997; Katz et al., 2005). The importance of an accurate determination of the cholesterol content in eggs is vital for the control of cholesterol uptake; in 1976, due the high reported cholesterol content in eggs, the American Heart Association's Committee, advised a weekly consumption of two eggs at most. Later, the American Agricultural Department determined a cholesterol content in eggs of 213 mg per egg (USDA, 2000) and not of 274 mg, and consequently in 2002 the American Heart Association suggested four eggs as a maximum weekly intake. Nowadays this value of cholesterol content in eggs is considered acceptable according to recent literature data (Herron & Fernandez, 2004).

With the aim of evaluating the cholesterol content of eggs from different provinces of the Campania Region, Italy, and to test the potential use of a new analytical method, one hundred chicken eggs from local markets were analysed by gas chromatography using a polar capillary column. The total free cholesterol was extracted from egg yolk with *n*-hexane and trichloroacetic acid (TCA), and analysed without the saponification step commonly used. This alternative procedure was compared with the extraction of total lipids by a chloroform/methanol solution followed by the saponification of fat (official method).

1.1. Some nutritional considerations

Cardiovascular disease is one of the major factors responsible for death in industrialised countries. Lifestyle and eating habits, in particular, are considered essential in any intervention aimed at reducing blood cholesterol. Hypercholesterolemia is usually caused by a problem of the regulation of cholesterol, that is the delicate balance between intake, endogenous biosynthesis and elimination trough bile and organic wastes. For this reason, for many years, great attention has been devoted to the identification of the main risk factors for cardiovascular disease, and in this context, several epidemiological studies have demonstrated the existence of a relationship between mortality from cardiovascular disease and cholesterol blood levels (LaRosa et al., 1990; Mancini & Stamler, 2004; Martin, Hulley, Browner, Kuller, & Wentworth, 1986; Verschuren & Jacobs, 1995). Other epidemiological studies have demonstrated the multifactorial aspect of cardiovascular risk and the relevance of metabolic factors, such as the levels of triglycerides and lipoprotein HDL (High Density Lipoprotein) (Assmann, Cullen, & Schulte, 1998; Evans et al., 2001; Hokanson & Austin, 1996); other controllable risk factors, like smoking, hypertension, type 2 diabetes, obesity, sedentary lifestyle as well as noncontrollable risk factors e.g. age, sex, family history can contribute to raising the cardiovascular risk. In obesity disease, adipose tissue synthesizes and secretes biologically active molecules believed to affect the metabolic syndrome and cardiovascular disease (Eckel & Krauss, 1998). Carbohydrate restricted diets (CRD) have been shown to reduce the weight and thereby reduce the cardiovascular health risk (Volek & Feinman, 2005; Wood et al., 2006).

In a recent study, eggs for their high protein and low in carbohydrates were used as part of a CRD in overweight men diet (Mutungi et al., 2008). The authors speculated that the eggs under the CRD would not alter the beneficial effects of CRD on plasma lipids and body composition. Another study showed that the consumption of 2 and 4 egg yolks/day for 5 weeks increased the concentration of macular pigment in older adults with low macular pigment allowing the statins to lower cholesterol (Vishwanathan, Goodrow-Kotyla, Wooten, Wilson, & Nicolosi, 2009).

To date, no research has clearly established a link between egg consumption and risk for coronary heart disease (Vislocky et al., 2009) and this should suggest the need of a more in depth re-evaluation and reconsideration of the association between the intake of cholesterol related foodstuff and human health (Lee & Griffin, 2006; McNamara, 2000). This lack of connection can be explained by two main reasons: first, the eggs are a good source of many nutrients including lutein and zeaxanthin, powerful antioxidants that may have a protective effect against oxidation of lipoproteins. Secondly, it has been well established that cholesterol in the diet increases the concentration of both circulating LDL and HDL cholesterol in those individuals who have a higher increase in plasma cholesterol after the consumption of eggs (hyper-responders). It is also important to note that 75% of the population experiences a slight increase or no change in the plasma cholesterol concentrations when challenged with high amounts of dietary cholesterol (normal responders and hypo-responders) (Fernandez, 2010). For these reasons, the dietary recommendations aimed at restricting egg consumption should be taken with caution and do not include all individuals. Different healthy populations experience no risk in the development of coronary heart disease by increasing their intake of cholesterol, but on the contrary, they may have multiple beneficial effects for the inclusion of eggs in the regular diet, due to high protein content (mainly ovalbumin) and antioxidants.

Eggs have been perceived as unhealthy food for many years because of their high cholesterol content, but in our work it was demonstrated that the cholesterol average content is 157 mg/egg in Campania. People have assumed that egg consumption would lead to increased risk of heart disease, disregarding that the slight adverse effect of an egg's cholesterol content is balanced by the beneficial contents of its other nutrients. Elevated low-density lipoprotein (LDL) cholesterol is a major risk factor for coronary heart disease (CHD): dietary cholesterol raises LDL cholesterol levels and causes atherosclerosis in numerous animal models. In controlled metabolic studies conducted in humans, the dietary cholesterol raises levels of total and LDL cholesterol in blood, but the effects are relatively small if compared with saturated and trans fatty acids. Studies have found that individuals vary widely in their responses to dietary cholesterol based on monitoring their plasma levels (Hu et al., 1999). Recent studies, however, indicated that egg consumption is dangerous for people with diabetes, possibly because of their altered ability to metabolize cholesterol (Barman, Leeds, & Griffin, 2008; Njike, Faridi, Dutta, Gonzalez-Simon, & Katz, 2010; Spence et al., 2010). Moderate egg consumption led to a 40–50% increased risk of heart disease for diabetics. Data from recent studies show that the consumption of one or two eggs per day, when part of a low fat diet, does not adversely affect the lipid profile; in fact, the preclusion of eggs from the diet may represent a

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potential reduction in the overall dietary quality (Djoussé et al., 2010).

2. Methods and materials

2.1. Sampling

Twenty "A category" chicken eggs were purchased on the local markets of each of the five provinces constituting the Campania Region. The total sample, constituted by 100 eggs with a weight in the range 55–79 g, was divided in 5 sub samples (each containing 20 eggs), one for each province, for the subsequent analyses and identified as Avellino (AV) samples from 1 to 20, Benevento (BN) samples 21 to 40, Caserta (CE) samples from 41 to 60, Napoli (NA), samples from 61 to 80, and Salerno (SA) samples from 81 to 100. The production day, whole weight, albumen weight, yolk weight, and shell weight of each egg were recorded.

2.2. Materials

A gas chromatograph autosystem XL (Perkin Elmer, Norwalk, CT, USA) equipped with a programmed split splitless (PSS) injector and flame ionisation detector (FID), connected to a Turbochrom version 4.1 data acquisition system, was used for the cholesterol analysis. Diethyl ether (Fluka, Buchs, Switzerland), chloroform (Fluka, Buchs, Switzerland), methanol (Carlo Erba, Milan, Italy), trichloroacetic acid (TCA), potassium hydroxide (Carlo Erba, Milan, Italy), *n*-hexane (Fluka, Buchs, Switzerland), cholesterol (Fluka, Buchs, Switzerland), squalene, cholestane, *n*-octacosane (Fluka, Buchs, Switzerland), nitrogen gas (SOL, Naples, Italy), all pure at analytical grade, were used. For the separation steps, a centrifuge PK 131 (ALC International, Milan, Italy) and a rotating evaporator (Heidolph, Laborota 4000) were used.

2.3. Gas chromatographic conditions

Gas chromatographic conditions were: capillary column with a stationary phase 65% diphenyl/35% dimethyl polysiloxane (RTX 65-TG HT); l = 30 m; i.d. 0.25 mm; f.t. = 0.10 μ (Restek, Bellefonte, CA, USA) was used. The injector program was set as follow: temperature 50 °C for 15 s, then a temperature increase of 999 °C/min to 370 °C then hold for 3 min. The initial low temperature of the injector contributes to eliminate the solvent before the vaporisation step of the sample. The oven program was set as: temperature kept at 250 °C for 2 min, then increased at 5 °C/min rate up to 360 °C, then hold for 3 min. The detector temperature was set at 370 °C, the carrier hydrogen gas flow was set at 2 ml/min and the split ratio 1:80 was used. All the experimental measurements were repeated three times and the average values reported. The precision of the method was less then 2%.

2.4. Total lipid extraction from yolk

Eggs belonging to each subsample were weighted using a technical balance and the weight (P) was recorded; the yolk was carefully separated from albumen and weighted, and the weight recorded (T). The yolk was transferred into a 50 ml centrifuge tube and 18 ml of a chloroform/methanol 2:1 solution were added; after shaking vigorously, the mixture was centrifuged at 5263×*g* for 5 min at 20 °C. The bottom fraction was separated with a Pasteur glass pipette and transferred into a 100 ml flask. This procedure was repeated using other two aliquots of 18 and 10 ml of a chloroform/methanol 2:1 solution. After centrifugation, the bottom liquid fraction was recovered and added to the same flask. The solvent was vacuum removed from the 100 ml flask using a rotating evaporator at a temperature of 40 °C and nitrogen gas was used to completely remove all traces of solvent. The total extracted fat (G) weight was obtained by the difference from the final weight and the previously determined weight of the empty flask. The fat percentage in the yolk was calculated using the following relationships:

$$\text{Fat in egg } (\%) = G \cdot 100/P$$

$$\text{Fat in yolk } (\%) = G \cdot 100/T$$

where G = weight of extracted fat (g); T = weight of yolk (g); P = weight of whole egg (g).

2.5. Extraction of the yolk non-polar lipid fraction

Eggs from each subsample weight (P) and yolk weight (T) were recorded. The yolk was transferred into a 50 ml centrifuge tube and 15 ml of 12% (w/v) trichloroacetic acid (TCA) were added; after vigorously shaking for two minutes, 15 ml of *n*-hexane were added; the mixture was vigorously shaken for three minutes and centrifuged at 10,526×*g* for 5 min at 20 °C. The upper part was removed from the tube and transferred it into a 50 ml separating funnel. The extraction was repeated using other two aliquots of 15 ml of *n*-hexane and the upper part, after centrifugation, was also transferred into the same separating funnel. The *n*-hexane fraction was washed three times with 10 ml of deionised water and then transferred into a 100 ml flask. The organic solvent was removed using a rotating evaporator, and traces of solvent were eliminated using nitrogen gas flow. The flask was weighted and the amount of extracted fat (L) was calculated from the difference in the weight of the empty flask previously recorded. The non-polar lipid fraction (NPLF) was reported by percentage in yolk and in eggs using the following formulas:

$$\text{NPLF in yolk } (\%) = L \cdot 100/T$$

$$\text{NPLF in eggs } (\%) = L \cdot 100/P$$

where L = weight of extracted non-polar fat (g); T = weight of yolk (g); P = weight of whole egg (g).

2.6. Calibration line for free cholesterol

To evaluate the best internal standard (IS) for the free and bounded cholesterol GC analysis, three IS were tested, namely squalene, cholestane and *n*-octacosane. The best separation was obtained using squalene as IS (see Fig. 1); this compound was then used for the calibration line built as described in the following. Three hundred mg of squalene were weighted in a 100 ml flask, and the minimum amount of diethyl ether was used to dissolve the squalene before adding to the mark (C_{IS} = 3000 mg/l). About 45 mg of cholesterol were accurately weighted and transferred in a 10 ml flask; then, also, 45 mg of cholesterol were accurately weighted and transferred in a 15 ml flask; whereas, another 45 mg of cholesterol were accurately weighted and then transferred in a 30 ml flask. Diethyl ether solution containing the IS was used to dissolve the cholesterol and was added to the mark for all the flasks.

The three prepared solutions contained 4500, 3000 and 1500 mg/l of cholesterol, respectively, and 3000 mg/l of squalene (IS); for the construction of the calibration curve 0.5 μl of each solution was injected into the gas-chromatograph using the gas chromatographic conditions reported in Section 2.3. The cholesterol/squalene ratio for each standard solution (Ri) was represented as a function of the cholesterol concentration (mg/l) as a plot; the experimental points obtained in our analytical conditions were interpolated by the function: $R = 0.000242 \cdot C$, where *R* is the

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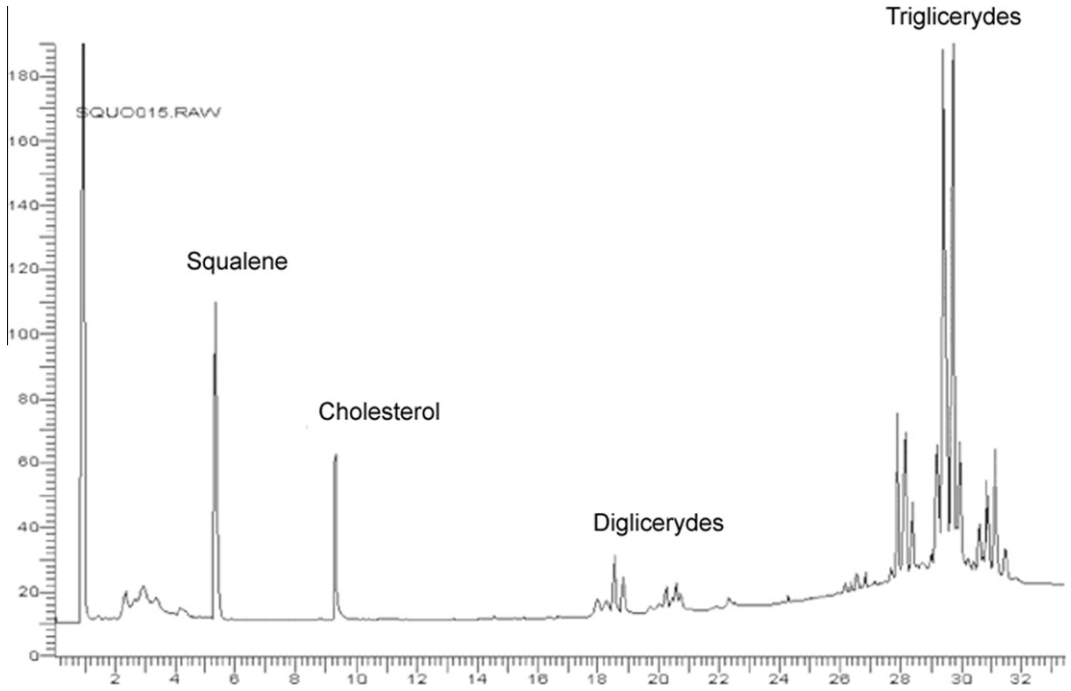

Fig. 1. Gas chromatogram of the free non-polar lipid fraction using squalene as internal standard.

value of cholesterol/squalene area ratio and C is the cholesterol concentration (mg/l).

2.7. Calibration line for bound cholesterol

Aliquots of 1 ml of the standard solutions of cholesterol prepared as described in Section 2.6 were dried under nitrogen flow and then 1 ml of *n*-hexane was added; the obtained solutions were transesterified using 200 μl of potassium hydroxide 2 N in methanol using a Vortex stirring device for 1 min. After the separation of the two phases, 0.5 μl of the upper phase were injected in the gas-chromatograph using the gas chromatographic conditions reported in Section 2.3. The cholesterol/squalene ratio was plotted for each standard solution versus the cholesterol concentration. The experimental points obtained were interpolated, in our experimental conditions, by the function: $R = 0.000254 \cdot C$.

2.8. Determination of the free and esterified cholesterol in the non-polar lipid extracted with trichloroacetic acid (TCA) and n-hexane

Fifty milligrams (l) of the non-polar lipid fraction from the yolk were accurately weighted and 1.00 ml of the 3000 mg/l IS solution was added; 0.5 μl of the obtained solution was injected in the gas chromatograph using the gas chromatographic conditions reported in Section 2.3. The cholesterol/squalene area ratio was recorded and the amount of cholesterol was calculated using the calibration plot previously described; finally the free cholesterol content in egg was calculated by applying the following relation:

$$\text{Cholesterol (mg/egg)} = C \cdot V \cdot L/l$$

where C = concentration of cholesterol in the fat solution (mg/l); V = internal standard volume solution (ml); L = weight of total non-polar fraction from yolk (g); l = weight of lipid used for the gas-chromatographic analysis (mg).

To determine the concentration of esterified cholesterol, 50 mg (l) of the non-polar lipid fraction from the yolk were accurately weighted and 1.00 ml of the 3000 mg/l IS solution was added; solvent was dried under nitrogen flow and then 1.00 ml of *n*-hexane was added. The obtained solution was transesterified using 200 μl of potassium hydroxide 2 N in methanol under vigorous stirring for 1 min. After the separation of the two phases, 0.5 μl of the upper phase was injected in the gas-chromatograph using the gas chromatographic conditions reported in Section 2.3.

The cholesterol/squalene area ratio was recorded, and the amount of esterified cholesterol was calculated using the calibration plot previously described; finally, the esterified cholesterol content in egg was calculated by applying the following relation:

$$\text{Cholesterol (mg/egg)} = C \cdot V \cdot L/l$$

where C = concentration of cholesterol in the fat solution (mg/l); V = internal standard volume solution (ml); L = weight of total non-polar fraction from yolk (g); l = weight of lipid used for the gas-chromatographic analysis (mg).

2.9. Determination of the free and esterified cholesterol in the lipid extracted with chloroform and methanol

To compare the findings using the previously described procedures with the official AOAC suggested method (AOAC Method, 1990) the determination of the cholesterol was also performed using lipid extraction with a chloroform/methanol mixture. In the flask containing the total lipid fraction of weight G obtained with a 2:1 chloroform–methanol ratio extraction, an ether solution containing 3000 mg/l of squalene was added as the internal standard in order to obtain a 5.0% w/v solution; 0.5 μl of the obtained solution was injected in the gas chromatograph and the cholesterol/squalene area ratio recorded. The amount of free cholesterol in the lipid solution was calculated using the calibration line and the relation:

$$\text{Cholesterol (mg/egg)} = C \cdot V$$

where C = concentration of cholesterol in the fat solution (mg/l); V = final volume of 5.0% w/v solution (l).

To determine the concentration of esterified cholesterol, 1.00 ml of the 5.0% (w/v) solution was added with 1.00 ml of IS solution and the solution was dried under nitrogen flow; finally

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1.00 ml of *n*-hexane was added. The obtained solution was transesterified using 200 μl of 2 N potassium hydroxide in methanol under vigorous stirring for 1 min. After the separation of two phases, 0.5 μl of the upper phase was injected in the gas-chromatograph using the gas chromatographic conditions reported in Section 2.3. The cholesterol/squalene area ratio was recorded, and the amount of esterified cholesterol was calculated using the calibration plot previously described. The esterified cholesterol content in egg was calculated by applying the following relation:

$$\text{Cholesterol (mg/egg)} = C \cdot V$$

where C = concentration of cholesterol in the fat solution (mg/l); V = final volume of 5.0% w/v solution (l).

3. Results and discussion

3.1. Analytical determination of cholesterol

The cholesterol content in eggs, sample preparation and quantification has been recently reported (Daneshfar, Khezeli, & Lotfi, 2009) and often leads to controversial results: in this paper, two different methods have been used for the cholesterol determination from Italian eggs, the official AOAC method (AOAC Method, 1990) as reference, and a modified extraction of the cholesterol, both followed by gas chromatographic analysis. The official method suggests to extract fat from the yolk with a chloroform/methanol mixture and then analyse the total recovered fat using cholestane as internal standard. This method, even though is simple and rapid, requires the analysis of all extracted fat. The alternative method that we propose here allows to evaluate the non-polar free lipid part by extraction in *n*-hexane and trichloroacetic acid (TCA) followed by gas chromatographic analysis performed using a capillary polar column with a high temperature stationary phase 65% diphenyl/35% dimethyl polysiloxane (RTX 65-TG HT). This column has never been used before for the determination of cholesterol from eggs. However, its use has been reported for the analysis of sterols from the transesterified fat from caprine milk (Fraga, Fontecha, Lozada, Martinez-Castro, & Juarez, 2000) and for the analysis of animal fats (Russo, De Leonardis, & Macciola, 2005).

Squalene revealed to be the optimum IS and the results are reported in Fig. 1; that clearly indicates no overlapping between the peaks. Cholestane, a commonly used internal standard for non-polar GC columns, was not appropriate, as shown in Fig. 2, because it overlaps with the cholesterol peak, due to its low polarity. If *n*-octacosane is used as IS, the corresponding peak is not sharp, overlapping with minor compounds, as shown in Fig. 3. In this case, the corresponding peak is not sharp due to the strong polarity of the stationary phase, and to the low polarity of *n*-octacosane.

Advantages of the proposed method lay in the possibility to analyse only a small portion of the total extracted lipid fraction instead of analysing the total extracted lipid fraction as suggested by the official method for cholesterol analysis. Table 1 shows the results obtained with the method alternative to the official one. The data obtained analysing the same mixtures of yolks for the cholesterol content with the two methods, are within the experimental error. The standard deviation between two data set was less than 3%, and the recovery was not less then 97%.

3.2. Triglycerides of egg's fat

The proposed method allows the simultaneous analysis of triglycerides, and highlighted the presence of high molecular weight compounds similar to the ones observed in vegetable oils. Table 2 shows the composition of each family of triglycerides. It can be observed that the standard deviation is quite wide, suggesting that the triglyceride composition, represented by the C48, C50, C52, C54 families, has a wide range of values depending on the eggs analysed. This observation could suggest that the determination of the triglycerides from egg's fat could be a useful information to asses the natural origin of the eggs used in egg-containing derivatives.

3.3. Variability among parts of the egg

Table 1 reports the non-polar fraction weight, the cholesterol content per egg, the cholesterol per mg of fat and the cholesterol

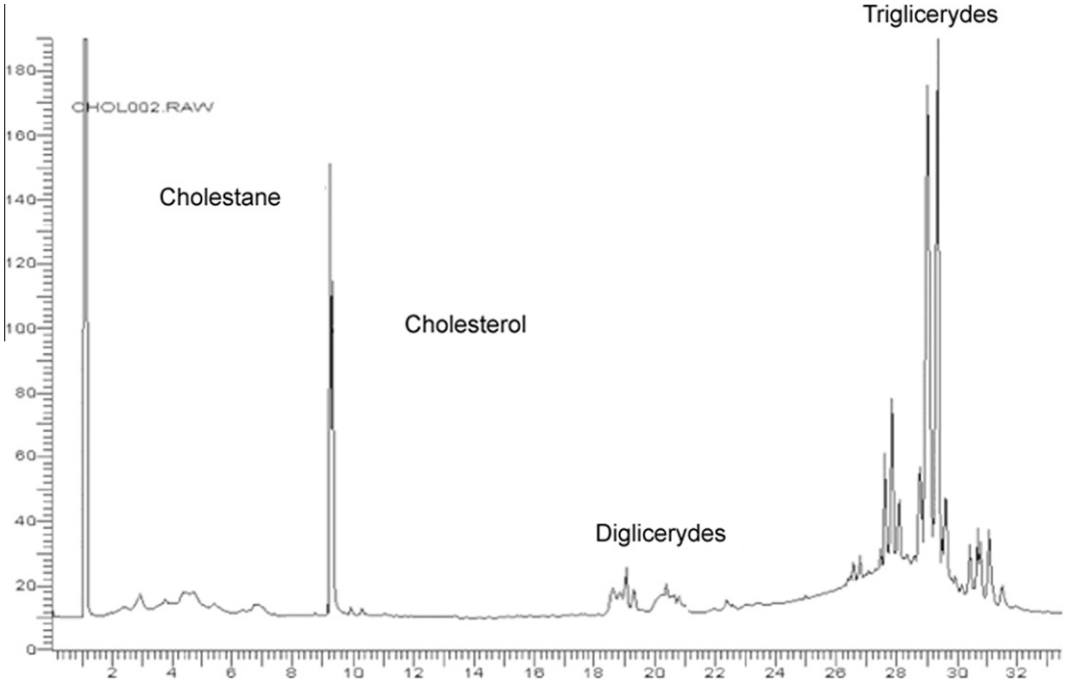

Fig. 2. Gas chromatogram of the free non-polar lipid fraction using as cholestane as internal standard.

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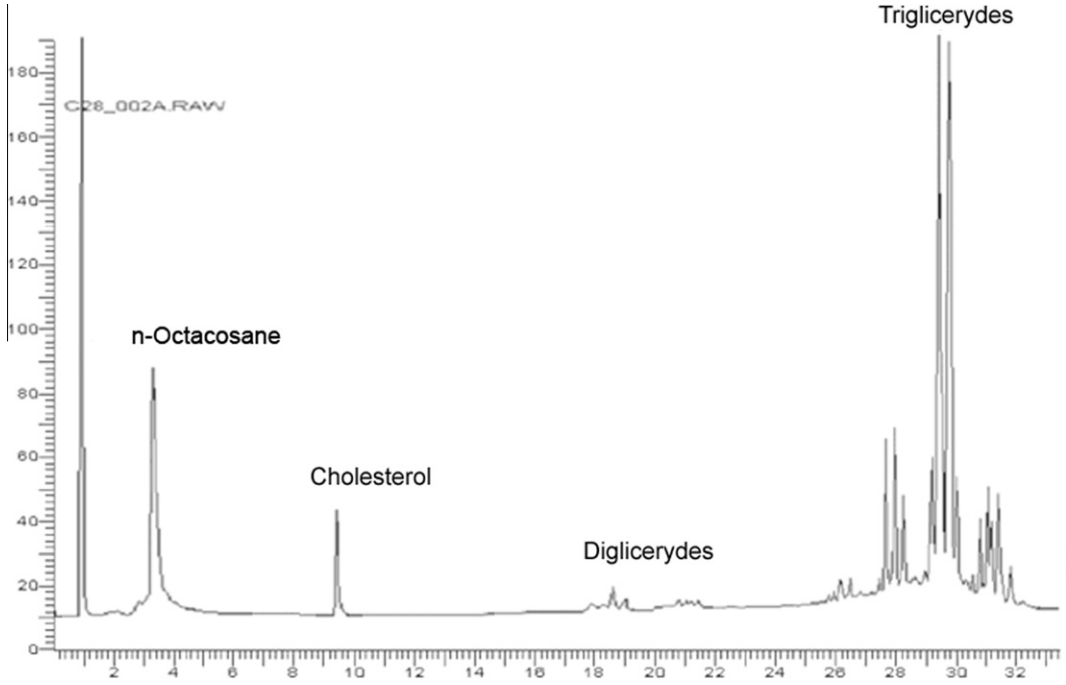

Fig. 3. Gas chromatogram of the free non-polar lipid fraction using *n*-octacosane as internal standard.

Table 1
Cholesterol content in a sample of 100 eggs analysed from the five Provinces of Campania Region (Italy), and on the basis of the non-polar free lipid fraction and weight of yolk.

Sample[a]	Weight (g)	Non-polar fraction (g)	Non-polar fraction (%)	Cholesterol per egg (mg)	Cholesterol/fat (mg/g)	Cholesterol/yolk (mg/g)
AV (1–20)	66.28 ± 0.01	4.79 ± 0.05	28.4 ± 0.3	157 ± 3	32.8 ± 0.4	9.3 ± 0.1
Min–max	55.25–78.92	3.77–5.38	26.9–31.6	126–193	28.5–37.1	8.2–10.4
RSD (%)	0.01	1.0	1.1	1.9	1.2	1.1
BN (21–40)	63.88 ± 0.01	4.68 ± 0.05	28.8 ± 0.3	150 ± 3	32.1 ± 0.4	9.4 ± 0.1
Min–max	55.25–79.26	4.08–5.96	25.0–31.8	120–173	26.1–38.6	8.5–10.3
RSD (%)	0.02	1.1	1.0	2.0	1.2	1.1
NA (41–60)	65.58 ± 0.01	4.72 ± 0.05	29.8 ± 0.3	165 ± 3	35.2 ± 0.4	9.4 ± 0.1
Min–max	55.33–79.01	3.93–5.40	24.4–38.6	133–191	28.7–42.8	8.7–10.3
RSD (%)	0.02	1.1	1.0	1,8	1.1	1.1
CE (61–80)	65.24 ± 0.01	4.80 ± 0.05	30.3 ± 0.3	156 ± 3	36.0 ± 0.4	9.5 ± 0.1
Min–max	55.17–78.64	4.04–5.67	25.9–38.9	135–179	29.6–40.9	8.7–10.3
RSD (%)	0.02	1.0	1.0	1,9	1.1	1.1
SA (81–100)	66.72 ± 0.01	4.76 ± 0.05	30.3 ± 0.3	159 ± 3	35.8 ± 0.4	9.6 ± 0.1
Min–max	56.42–75.38	3.83–5.85	25.4–37.6	131–185	29.5–41.5	8.9–10.3
RSD (%)	0.01	1.1	1.0	1.9	1.1	1.0

[a] Explicatory note: Samples from local market of the five Campania Region Provinces, namely samples 1–20 are from Avellino (AV), 21–40 are from Benevento (BN), 41–60 are from Napoli (NA), 51–80 are from Caserta (CE), 81–100 are from Salerno (SA).

Table 2
Composition of free non-polar lipidic fraction: diglycerides, triglycerides, and cholesterol in analysed eggs.

Non-polar component	Area % range	Area % average	Percentage deviation (%)
Diglycerides	7.7–12	9.9	22
Triglycerides (C48)	0.7–2.1	1.4	50
Triglycerides (C50)	9.0–17	13	31
Triglycerides (C52)	56–61	59	5.1
Triglycerides (C54)	9.4–12	11	15
Cholesterol	5.3–6.3	5.8	9.0

per mg of yolk. It can be observed that there is no correlation between the weight of the egg and the corresponding content of cholesterol. Table 1 reports also the ratio between the cholesterol content (mg) and the non-polar fat fraction (mg). These data suggest that a possible tentative of normalisation does not lead to any linear relationship between cholesterol content and the egg weight. As a result, it can be observed also that there is no correlation between the dimensions and the weight of the whole egg and its cholesterol content, as previously reported in the literature (Pandey et al., 1989; Riad et al., 1981; Zemková et al., 2007). This observation supports the fact that smaller eggs from the market should not cost more than bigger ones claiming their low cholesterol content.

3.4. Cholesterol content and eggs storage time

A further observation to correlate if there is a qualitative variability between the storage time and cholesterol content has been done. Additionally, five eggs of different size randomly selected and purchased on the local market from the five different provinces as previously described, were stored at a temperature of 4 °C until a week after their expiry date. The cholesterol content was determined, and results are reported in Table 3. The average cholesterol content in the yolk was 156 ± 14 mg. This value is

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Table 3
Cholesterol content in sample eggs analysed a week after the expiration date.

Sample	Cholesterol/egg (mg)
1	147 ± 3
2	158 ± 3
3	175 ± 3
4	163 ± 3
5	137 ± 3

similar to that observed for fresh eggs. Another measurement was done on the yolks from another five different eggs, which were sampled and purchased as described before. Yolks were mixed and mixture stored for one month at a temperature of 4 °C. Every week, the cholesterol content was determined in the mixture. In this case, the initial cholesterol content, determined in 183 ± 3 mg, did not vary during the storage time. Minor weight changes observed were always within the experimental error.

4. Conclusions

Many different methods have been proposed and used for the cholesterol determination in eggs, and in particular the spectrophotometric determination has been questioned because interfering compounds could lead to significant overestimation. Based on the many reported data suggesting gas chromatography as a very useful method for cholesterol determination and for the complete separation of the coexisting lipids, this paper presents results of cholesterol analysis on Italian chicken eggs from the five different provinces of the Campania Region (Italy). A polar GC column, and the use of squalene as internal standard allowed us to accurately determine in the above described simple and fast procedure, the cholesterol content of egg yolk without the lipid saponification reaction, as suggested by the official method for the cholesterol analysis. The proposed method used the recovery of apolar lipids fraction and the results obtained with both methods have been compared.

The cholesterol content in analysed eggs was on average 157 ± 3 mg/egg, a value lower than the ones reported in the literature. Considering the reported values, the new data suggest that a more in depth re-evaluation and reconsideration of the association between the intake of cholesterol related foodstuff and human health should take place. Moreover, the obtained results seemed to suggest that there is no correlation between the egg weight or its dimensions, and its cholesterol content. The attempt to normalize the cholesterol content with respect to the non-polar fraction and the yolk weight did not show any correlation. Finally, the cholesterol content did not vary after storage, after the expiration date suggested for consumption.

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