Determination of Egg Content in Pasta

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Abstract

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The recent Czech Food Law (Decree No. 264/2003 Col., 93/2000 Col. and 57/2003 Col. of the law No 110/1997 Col. as amended) specifies the requirements for the presence or minimum concentration of egg or egg yolk contents in relevant food products (mayonnaises, egg pastas, egg liqueurs). However, the methods for the determination of egg and/or egg yolk contents are not sufficiently specified. Three methods based on the determination of cholesterol, lysozyme, fatty acids and lipid contents were experimentally validated to evaluate the egg content in egg pasta. The concentration of egg solid in the real egg pasta samples was calculated according to (1) average cholesterol content in the raw material analysed, (2) average lysozyme content in the raw material analysed, and (3) multiple regression equation for 21 model samples with the known egg contents. The comparison of the obtained data with the requirements of Czech legislation revealed that only 12 of 23 analysed samples (52%) and 3 of 13 samples of Czech origin (27%), declared as egg pasta, contained two or more eggs per 1 kg of flour.

Keywords: egg; estimation of egg content; egg pasta; adulteration

Pasta made of the flour produced from common wheat (*Triticum aestivum)* is often fortified with a number of ingredients. Eggs are the most common and important ingredient, either as whole eggs or egg yolk. The eggs in pasta improve its nutritional value as well as physical and organoleptic properties which results in a higher price of egg pasta on the market compared to pasta without eggs. Moreover, while wheat flour contains no cholesterol, eggs have a high cholesterol content which is of concern to consumers interested in maintaining a low cholesterol intake. The Czech Food Law No. 110/1997 Col. as amended and subsequent decrees that deal with the egg products require that these products contain certain amounts of eggs or egg yolks (Table 1).

There are numerous published methods for the determination (or estimation) of the egg content in pasta. As the basic marker of the egg content, cholesterol concentration is mentioned both in literature and legislative requirements of several countries (Beyer & Jensen 1989; Kovacs 1990; Agulló & Gélos 1996). An important disadvantage of cholesterol-based calculations of the egg contents resides in the high variability of the raw material (from 840 to 1970 and from 170 to 550 mg per 100 g of yolk and edible part of egg, respectively) (Maurice *et al.* 1994; Simeonovová *et al.* 2001). The egg products

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Table 1. Czech legislative requirement for the egg products

Product	Minimal content			Decree No.
	sucrose (g/l)	yolks	egg/kg flour	
Egg liqueur	150	140 g/l	*	57/2003 Col.
Egg cream	250	140 g/l	*	57/2003 Col.
Liqueur with eggs	100	70 g/l	*	57/2003 Col.
Egg pasta	*	*	2	93/2000 Col.
Pasta, home made	*	*	6	93/2000 Col.
Egg bakery products	*	180 g/kg of flour	64 g	93/2000 Col.
Mayonnaise	*	2 %	*	264/2003 Col.

*not relevant

should contain: mayonnaise 168 mg/kg, egg pasta 197 mg/kg, home made egg pasta 591 mg/kg, egg liqueur 1069 mg/kg and liqueurs with added eggs 534 mg/kg of cholesterol to fulfill the law requirements. The specifications of egg pasta abroad are different, instead of the required number of eggs per kg of flour, the weight of egg solids or directly the minimal content of cholesterol is given. In USA, Food Standard Regulations (Kovacs 1990) set a minimum of 5.5% by weight of egg solids or egg yolk solids in the total solids of egg noodle products. A minimum cholesterol content of 198 mg/100 g has been reported for noodles containing 5.56% of yolk solids. Argentine Food Code (Agulló & Gélos 1996) requires egg pasta to contain two egg yolks per kg of flour, semolina, or their mixture, and minimally 0.4 g cholesterol per 100 g of dry substance. EU regulations do not specify the minimum amounts of eggs in the egg products, but the national legislations do, e.g. in Italy (Pressi *et al.* 1994) a minimum of 4 eggs (200 g) is set per kg of flour.

Based on the specific composition of the egg, it is possible to choose some other characteristic chemical markers of egg solids (Burini *et al.* 1978; Bostel 1981; Pressi *et al.* 1994):

- Total content and composition of fatty acids
- Total lipids content
- Specific egg proteins to prove the presence of egg white (ovoalbumin, lysozyme, avidin) and yolk (lipovitelins, phosphatidin).

The calculation of the egg content in the final product using the selected chemical markers can be, similarly as for cholesterol, rather inaccurate and vary from 50% to 150% of the real value.

The purpose of this investigation was to develop a reliable method for the evaluation of the egg content in pasta based on the determination of the characteristic chemical markers of the egg solids.

MATERIALS AND METHODS

The following chemical markers of the egg content were chosen: cholesterol content, the composition and total content of fatty acids, lysozyme content, fat content. The set of eggs and flours used was analysed for the evaluation of the raw material composition. Twenty-three model samples with known egg contents were prepared and analysed to evaluate the relation between the egg content and chemical composition. Finally, 25 samples of pasta obtained from the local market were analysed and the egg content was estimated.

Material. Samples of raw materials obtained from the local markets: 4 trademarks of eggs (grade A), 3 trademarks of wheat flour (from *Triticum aestivum).* Twenty-five real samples of pasta obtained from the local markets (of these 22 declared as egg pasta). A set of model samples of pastas, with the egg content 0 (four samples), 1 (five samples), 2 (five samples), 3 (one sample), 4 (four samples), and 6 (two samples) pieces per kg of flour was prepared under laboratory conditions. Three ways of drying were used: 1st set (15 samples) was dried at laboratory temperature (25°C) for 48 h, 2nd set (content 1, 2 and 4 egg/kg flour) was dried in thermostat 10 min at 70°C and than 12 h at 35°C, 3rd set (content 1, 2 and 6 egg/kg flour, recalculated according to the melange dry mater) was prepared from industrially produced dried melange and

dried at laboratory temperature (25°C) for 48 h. All samples were analysed in duplicates.

Analytical methods. Cholesterol (Kovacs 1990) – 10 ml of KOH (50%) and ethanol mixture (1:9) and an internal standard (5-α-cholestane) were added to 2.5 g of sample and heated at 60°C for 60 min in a shaking water bath to saponify cholesteryl esters. Cholesterol was extracted by 3 × 10 ml of hexane and filtred through anhydrous Na_2SO_4. Hexane phases were evaporated to dryness, diluted with 0.5 ml of ethanol and injected into the gas chromatograph. GC conditions: column DB-5 (30 m × 0.32 mm × 0.25 μm), carrier gas: nitrogen, constant flow 0.45 ml/min, temperature program: 260°C, 6°C/min, 290°C (8 min), injector: 300°C, split 1:1, detector (FID): 300°C.

Fatty acids. Amount of 0.5 g of sample was added to 100 ml flask together with 50 ml of 2% solution of H_2SO_4 in methanol and internal standard solution (heptadecanoic acid). The mixture was boiled for two hours under reflux. Further, 5 ml of heptane was added and the heating was finished. The flask was cooled under running water and saturated solution of NaCl was then added to separate the phases. The upper (hexane) layer was transferred with a syringe into a vial and injected into the gas chromatograph. GC conditions: column DB-wax (30 m × 0.32 mm × 0.25 μm), carrier gas: nitrogen, constant flow rate 1.7 ml/min, temperature program: 60°C (1 min), 10°C/min, 250°C (10 min), injector: 230°C, split 1:1, detector (FID): 280°C.

Fat content. The samples were extracted according to Boselli *et al*. (2001). The sample (10 g) was homogenised in a flask with 10 ml of chloroform/methanol mixture (1:1, v/v) for 3 min. Subsequently, 50 ml of chloroform was added and after 3 min of homogenisation the content was filtered through filter paper. The filtrate was mixed thoroughly with 1M KCl solution and left overnight. The lower phase (organic) was collected and dried in vacuum evaporator.

Lysozyme. Sample (2.5 g) with 30 ml 1M acetic acid was extracted in a shaking water bath at 40°C for 1 h (Kvasnička 2003). The extract was made up to 50 ml with water. The extract was diluted 10 times and filtered before injecting. CITP-CZE (on-line coupled capillary isotachophoresis with capillary zone electrophoresis) conditions: electronic integrator CSW 1,7 (DataApex, CR), UV detector LCD 2084 (ECOM s. r. o.) at 280 nm and electrophoretic analyser EA 101 (Villa-Labeco, SK), capillaries 110 × 0.8 mm and 140 × 0.3 mm, electrolytic system: TE (5mM HAc + 5mM ε-aminocaproic acid), LE (20mM HAc + 10mM NH_4OH), BGE (40mM EACA + 20mM HAc + 0.1% HEC).

RESULTS AND DISCUSSION

The majority of the recent works dealing with the estimation of the egg content in pasta were based on the determination of a single analytical parameter, mainly cholesterol or specific egg white proteins (lysozyme, ovalbumin etc.). Cholesterol is a parameter used by official authorities for the evaluation of the genuinness of egg pasta. Its content in eggs, however, is highly variable and as a marker it provides information only about the egg yolk content in the product. The content of particular egg white proteins in eggs is less variable compared to the cholesterol content (Sajdok *et al*. 1990). However, the methods for its determination (enzymatic, immunochemical, electrophoretical) are not widely used in the control laboratories; the concentrations and activities of specific proteins are reduced by higher temperatures used for the drying of egg melange and pasta, and some methods are not suitable for the thermally treated products in general (Pressi *et al.* 1994). To eliminate the disadvantages caused by a single chemical marker, several analytical parameters (cholesterol, fatty acids, lipids and lysozyme) were chosen to statistically evaluate their correlations and suggest the methods for the calculation of the content of egg matter in real samples of egg pasta obtained from the local market.

The analysis of raw material, which is generally used in the production of egg pasta, was focused on the determination of the chemical markers chosen (Table 2). The obtained results confirmed the tabulated compositions of wheat flour (no content of cholesterol and lysozyme, low contents of lipids and fatty acids) (Vojtašáková *et al*. 1999) and whole egg (high variability of all parameters) (Simeonovová *et al.* 2001).

The concentrations of chemical markers in model samples are also given in Table 2. The values determined for the samples (of the total of 23 samples, 2 were removed as outliers) with the known egg content were used for the construction of multiple regression equation for the estimation of the egg content in real samples of egg pasta. The accuracy of estimation was acceptable. Correlation coefficients (R) were 0.98, 0.98, 0.96, 0.97, 0.98 for cholesterol, fatty acids, lipids and lysozyme, respectively, the

Table 2. Real content of eggs and concentrations of chemical markers in model samples of pasta and raw materials

Content (egg/kg flour)	Cholesterol		Fatty acids		Lipids		Lysozyme	
	(mg/kg)	S.D.	(g/kg)	S.D	(%)	S.D	(mg/kg)	S.D
No eggs (*n** = 4)	3	4	7.7	1.0	0.9	0.2	0	0
One egg (*n* = 5)	419	135	14.6	3.9	1.5	0.4	114	17
Two eggs (*n* = 5)	771	296	16.4	7.3	1.9	0.5	198	37
Three eggs (*n* = 1)	388	0	24.4	0.0	3.0	0.0	210	0
Four eggs (*n* = 4)	1599	67	25.6	7.5	3.3	0.1	275	13
Six eggs (*n* = 2)	2466	416	34.6	1.5	4.2	0.4	439	47
Fresh whole egg (*n* = 4)	5391	191	77.1	10.5	10.6	0.7	2350	253
White flour (*n* = 3)	0	0	8.6	0.4	1.2	0.2	0	0

*number of analysed samples

correlation of the predicted and the observed values for the individual model samples (on 95% confidence level) is shown in Figure 1. Cluster analyses distribution of the model samples according to their egg contents and the concentrations of the chemical markers measured is given in Figure 2.

The influence of temperature and drying conditions on the selected chemical markers was monitored. It was observed that: (1) there is no significant influence of higher temperatures on the degradation of chemical markers; the influence of the drying conditions during the production is generally low where calculation on the dry matter content is necessary (Table 2; with regard to the generally low standard deviation, the samples are not distributed in relation to the way of drying), (2) industrially dried melange contained slightly higher contents of cholesterol and the lipid fraction that resulted in the outlay of all 3 samples prepared of it.

The egg content of real samples was calculated according to the following methods:

(1) Average cholesterol content in the analysed raw material (Hurst *et al.* 1985; Kovacs 1990; Agulló & Gélos 1996)

(2) Average lysozyme content in the analysed raw material (Kvasnička 2003)

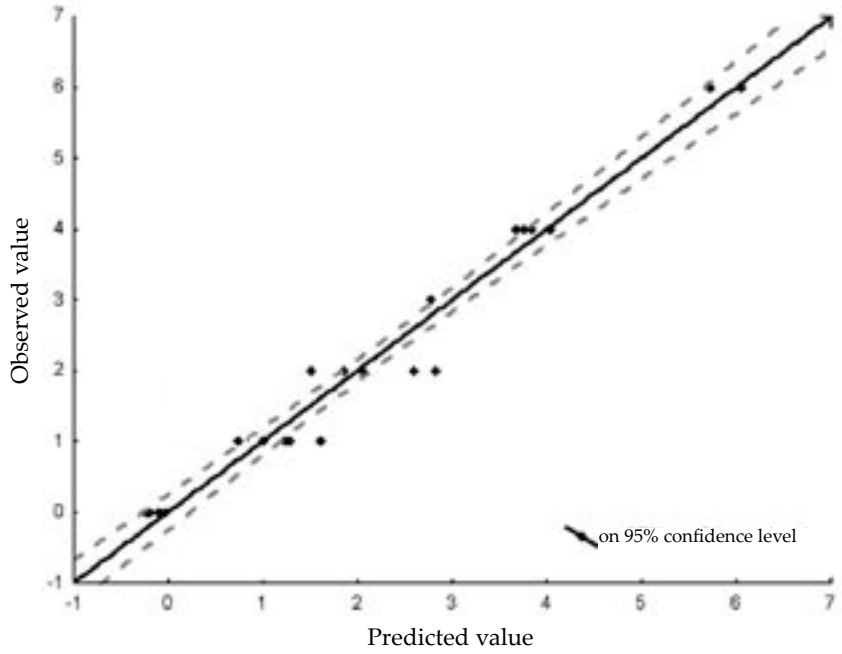

Figure 1. Correlation of predicted and observed values for individual model samples (*n* = 21)

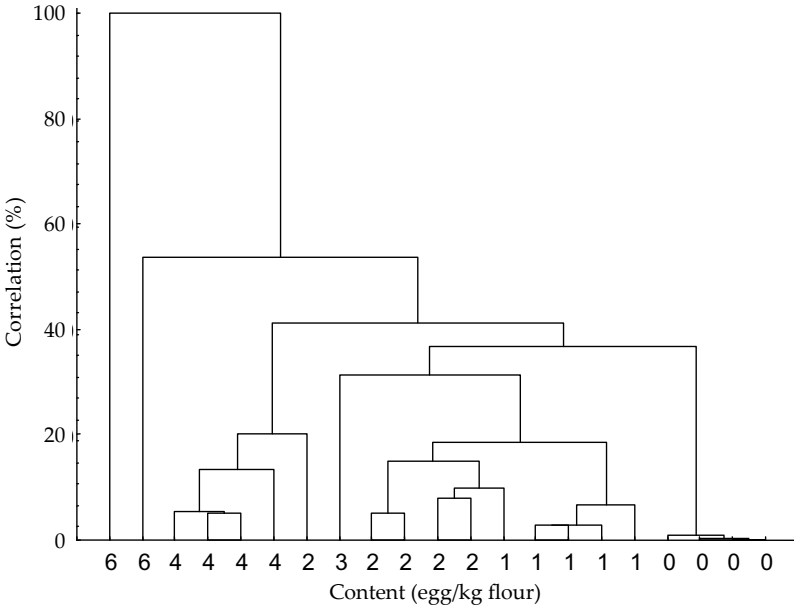

Figure 2. Cluster analyses of model samples of pasta based on the concentration of measured chemical markers (n = 21)

(3) Multiple regression equation for 21 model samples with the known egg content (Burini *et al.* 1978; Čížková *et al*. 2004)

egg content (number/kg of flour) = –0.47 + 0.00084 × cholesterol (mg/kg) + 0.0096 × fatty acid (g/kg) + 0.27 × lipids (%) + 0.0064 × lysozyme (mg/kg)

The estimated and the declared contents of eggs in the pasta samples are presented in Figure 3. The differences between the estimations obtained by different markers vary from 0 to 10% (for 16 samples) and from 11 to 100% (for 9 samples). The reason for the high variation in the second group of samples can be explained by (a) great differences observed

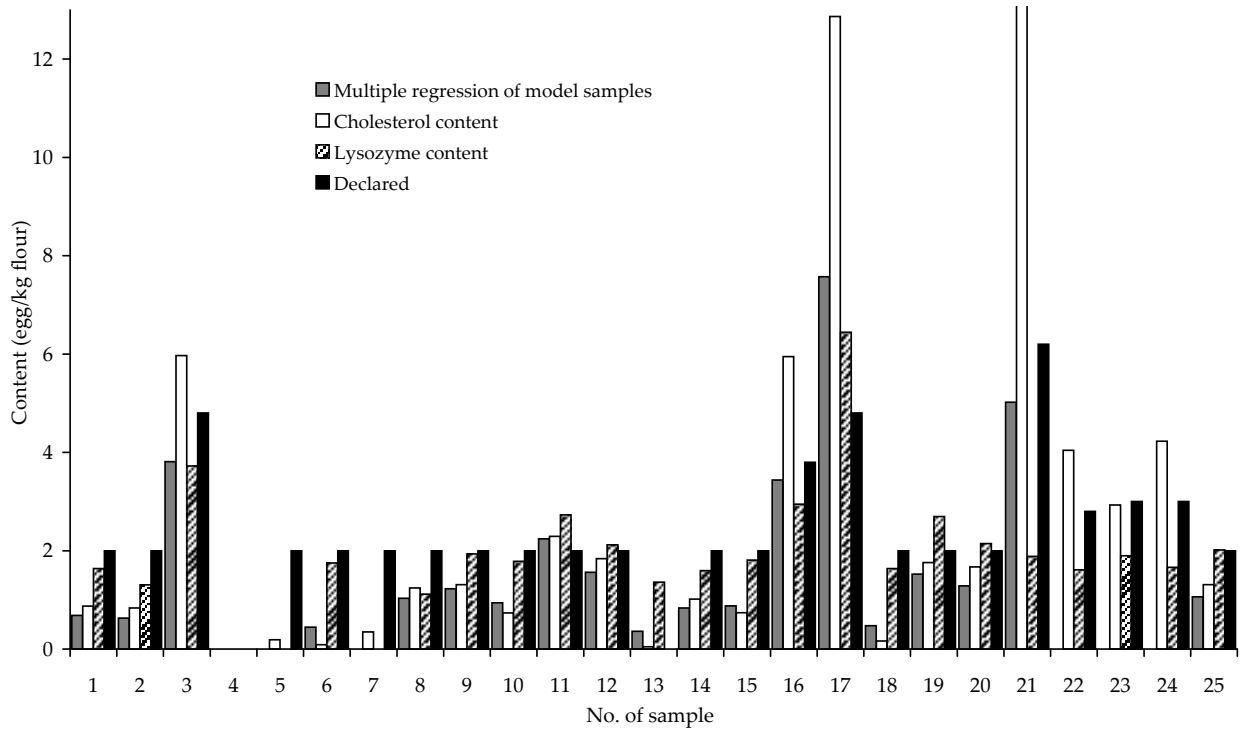

Figure 3. Estimated (according to multiple regression of model samples, cholesterol content and lysozyme content) and declared contents of eggs in real pasta samples (n = 25)

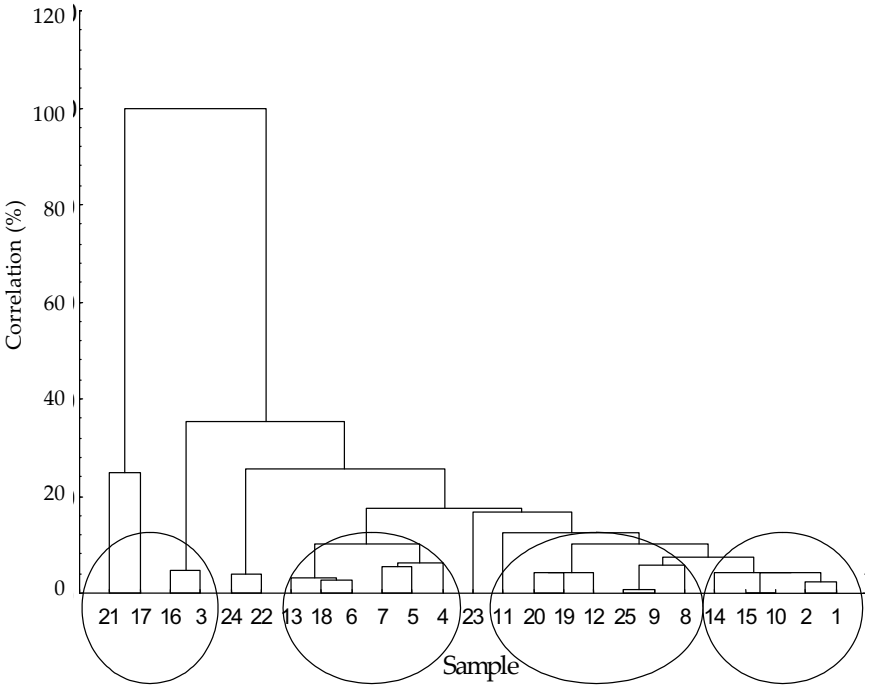

Figure 4. Cluster analyses of real samples of pasta based on the concentration of measured chemical markers (n = 25), division into four groups according to egg content is described in the text

in the cases of very low or no content of eggs in sample, (b) variability of individual markers, (c) the differences of yolk:white ratio in the used egg melange caused by conscious or unconscious extra additions of yolk or white instead of neat melange (e.g. samples No. 21 and 22 with the declared extra addition of 2 yolks). The most acceptable results were obtained using multiple regression equation which involves all the measured markers in the calculation.

According to cluster analysis of the samples based on the concentrations of the chemical markers measured (Figure 4), the samples can be divided into 4 groups according to the egg content per kg of flour:

(1) Samples with significantly higher contents than 2 (samples No. 21, 17, 16, 3)
(2) Samples with the egg content in the range from 1.5 to 2 (samples No. 11, 20, 19, 12, 25, 9, 8)
(3) Samples with the egg content about 1 (samples No. 14, 15, 10, 2, 1)
(4) Samples with no or very low egg content (samples No. 13, 18, 6, 7, 5, 4).

The samples No. 24, 22 and 23 are out of the suggested range because they are labelled as the egg pasta with various fillings and the fillings themselves cause the outlay of selected markers.

Three methods based on the determination of cholesterol, lysozyme, fatty acids and lipids for the evaluation of the egg content in egg pasta were experimentally validated. Comparing the data obtained with the requirements of Czech legislation only 12 from 23 samples (52%) and 3 from 13 of Czech origin (27%) declared as egg pasta were found to contain two or more eggs per one kg of flour. Samples were marked as satisfactory if at least one calculation method gave the egg content of 2 or higher. Natural variability was found as expected. Czech legislative similarly as the legislative of most of the EU member states requires egg pasta to contain certain amount of eggs, nevertheless, the official methods for the determination of egg solids are poor or absent in many cases; for that reason, the obtained and presented data and the suggested methods may be useful in evaluating the genuinness of the egg pasta samples.

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Souhrn

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Česká legislativa (vyhlášky č. 264/2003 Sb., 93/2000 Sb. a 57/2003 Sb. Zákona o potravinách č. 110/1997 Sb. v původním znění) specifikuje požadavky na přítomnost nebo obsah vajec nebo vaječných žloutků ve vaječných výrobcích (majonézy, vaječné těstoviny, vaječné likéry). Vhodné analytické metody ani způsoby stanovení však nejsou určeny. V prezentované práci byly navrženy a experimentálně ověřeny tři metody stanovení vaječného podílu v těstovinách označovaných jako vaječné. Obsah vajec ve výrobcích byl vypočítán na základě: 1. průměrného obsahu cholesterolu v surovině, 2. průměrného obsahu lysozymu v surovině a 3. multiregresní rovnice vytvořené na základě rozboru složení (obsah cholesterolu, mastných kyselin, tuku a lysozymu) 21 modelových vzorků těstovin se známým obsahem vajec. Z 23 analyzovaných vaječných těstovin pocházejících z tržní sítě pouze 12, tj. 52 % (ze 13 vzorků českého původu pouze 3, tj. 27 %) splnilo limit 2 ks vajec na kg mouky požadovaný českou legislativou.

Klíčová slova: vejce; stanovení vaječného podílu; vaječné těstoviny; falšování

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