Determination of Egg Yolk Content in Egg Liqueurs

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Abstract

Čížková H., Voldřich M., Prokorátová V., Kvasnička F. (2004): **Determination of egg yolk content in egg liqueurs**. Czech J. Food Sci., **22**: 9–15.

The recent Czech Food law (Decree No. 264/2003, 93/2000 and 57/2003 of the law No. 110/1997 as amended) requires the minimal concentration of egg or egg yolk content in relevant food products (mayonnaises, egg pastas, egg liqueurs), however, the methods for the determination of egg and/or egg yolk content are not sufficiently specified. The presented study deals with the development and evaluation of the analytical methods for the determination of egg yolk content in egg liqueurs. Due to the high variability of the egg composition and a possible effect of processing on the composition of the product, several chemical markers were taken into the consideration: dry matter, phosphorus, fat, cholesterol, fatty acids, and lysozyme concentrations. The egg yolk content was estimated by means of multiple regression analyses of the calibration set (model samples) and the data obtained for raw materials and described in literature. According to the egg yolk content determined, only 6 from 10 analysed samples of egg liqueurs obtained from the local market met the limit of 140 g/l (calculated with the 10% standard deviation error of estimation) required by the recent Czech legislation.

Keywords: egg yolk; estimation of egg content; egg liqueur; adulteration; authenticity

Egg liqueurs (eggnogs, advocaats) are alcoholic beverages whose main natural components are egg yolks. This kind of beverages is prepared by the blanding of principal constituents (ethanol, milk, egg yolk solids and sugar) without any fermentation process. These beverages have alcoholic grade of about 20° and a high sugar content, i.e. more than 150 g of sugar (honey, glucose or sucrose sirup) per litre of product. They are usually coloured and other additives (stabilisers, emulgators) are added occasionally (Gutierrez *et al*. 1995).

Relating to the name, eggs are the most common and important ingredient of egg liqueurs, and improve their organoleptic, physical and nutritional properties. The Czech Food code No. 110/1197 Col. as amended and subsequent decreee No. 57/2003 Col. that deals with the properties of alcoholic beveradges requires these products to contain certain amount of egg yolks (Table 1).

Assuming strict enforcement of the standards for egg liqueurs raises an important question whether or not a simple or official test exists for the determination of egg yolk solids in the products. The methods generally used for assessing egg solids in foods are based on the determination of some characteristic egg yolk constituents such as phospholipids, phosphorus, cholesterol, fatty acids, or characteristic egg white constituents such as specific egg proteins (ovoalbumin, lysozyme, avidin etc.) (Nielsen 1968; Burini *et al*. 1978; Bostel 1981; Germs 1989; Sajdok *et al*. 1990). There are numerous published methods for the determination (or estimation) of the egg content of egg pasta according to the cholesterol concentration (Beyer

Supported by the Grant Agency of the Czech Republic (Grant No. 525/02/P118) and the National Ministry of Agriculture of the Czech Republic (Grant No. GC 1111).

Table 1. Czech legislative requirements for the egg liqueurs

Product	Minimal content of sucrose (g/l)	Minimal content of yolks (g/l)
Egg liqueur	150	140
Egg cream	250	140
Liqueur with eggs	150	70

& Jensen 1989; Agullo & Gelós 1996). An important disadvantage of cholesterol-based calculations of the egg contents resides in the high variability of the raw material (cholesterol content varies from 840 to 1970 and from 170 to 550 mg per 100 g in yolk and the edible part of egg, respectively) (Maurice *et al.* 1994; Simeonovová *et al.* 1999). The egg liqueurs should thus contain more than 1069 mg of cholesterol per liter, and liqueurs with added eggs 534 mg of cholesterol per liter to fulfill the law requirements. The methods mentioned are quite reliable for the food products in which all the selected markers of egg solid come from the egg, or in the products where the amount of these constituents coming from other sources are low, known and relatively uniform, so that reasonable assumptions regarding them can be made. The calculation of the egg content in the final product according to selected individual chemical markers can be, similarly as for cholesterol, rather inaccurate and vary from 50% to 150% of real value. Main factors affecting the estimation are (Kaffka & Kulscar 1982; Kovacs 1990; Simeonovová *et al.* 2001; Nogueira & Bragagnolo 2002): (1) High variability of the chemical markers in the raw egg yolk according to breed and age of laying hen, the composition of feed, laying period, and other factors; (2) Presence of the markers in the other raw materials and in the additives that are not commonly used or not declared; (3) Chemical and physical changes of the markers during the production and storage (thermal degradation, complex formation, autooxidation, evaporation, etc.); (4) Different technology and recipes; (5) Different properties and composition of the so called "egg with lower cholesterol content", which is popular nowadays; (6) Different methods of isolation and analyses may give different results.

MATERIAL AND METHODS

According to the literature, the following chemical markers of the egg yolk content were chosen: phosphorus, cholesterol, composition and total content of fatty acids, lysozyme and fat contents. The set of egg yolks were analysed for the evaluation of the raw material composition and quality. Seven model samples with the known egg yolk content were prepared and analysed to evaluate the relation between the egg content and the chemical composition. Finally, 10 samples of liqueurs of different trademarks obtained from the local market were analysed and the egg yolk contents were estimated.

Material. A sample of egg yolk was fully separated from the eggs (grade A) obtained from the local markets; the same sample of egg yolk was diluted with egg white to the final concentration of 90%. Twelve samples of liquid, pasteurised egg yolks were provided by egg liqueurs producers, seven of them were marked as the technological yolks, three of them were sweetened (with the addition of 45% of sucrose). Four samples of whole milk (lipid content 3.5%) were obtained from the local market. Ten samples of egg liqueurs and egg creams were obtained from the local markets. A set of model samples of liqueurs with the egg yolk contents of 70, 112, 125, 126, 140, 140, and 155 g/kg was prepared under laboratory conditions. One litre of liqueur emulsion that was produced by mixing whole milk (410 ml), ethanol (200 ml) and water with the acquired amount of egg yolks was stored in the refrigerator before analyses.

Analytical methods. For the moisture determination, a 10 g sample was dried at 105°C for 4 h.

The phosphorus content was determined according to the AOAC international method (Pulliainen 1996). The sample was dry-ashed and the acid-soluble inorganic residue was used for the colour reaction based on the formation of a blue complex ($MoO_2 \times 4\ MoO_3$) × H_3PO_4 in the presence of ascorbic acid. The intensity of the blue colour was measured spectrophotometrically at 823 nm.

Cholesterol was analysed directly after saponification by GC/FID (Kovacs 1990). To saponify

cholesteryl esters, the sample was heated at 60°C for 60 min with 50% KOH and ethanol mixture (1:9) and 5-α-cholestane as internal standard. Cholesterol was extracted with hexane, evaporated to dryness, diluted with 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 rate of 0.45 ml/min, temperature program: 260°C, 6°C/min, 290°C (8 min), injector: 300°C, split 1:1, detector: 300°C.

To determine the total content and the composition of fatty acids, the sample was hydrolysed and esterified by boiling with 2% solution of H_2SO_4 in methanol for two hours under reflux. Fatty acids esters were extracted with heptane and analysed by GC/FID. GC conditions: column DB-wax (30 m × 0.32 mm × 0.25 μm), carrier gas: nitrogen, constant flow rate of 1.7 ml/min, temperature program: 60°C (1 min), 10°C/min, 250°C (10 min), injector: 230°C, split 1:1, detector: 280°C.

Fat was extracted with chloroform/methanol mixture according to Boselli *et al.* (2001).

For the analysis of the lysozyme content (Kvasnicka *et al.* 2003), 2.5 g of sample with 30 ml 1M acetic acid was extracted at 40°C for 1 h. CITP-CZE (on-line coupled capillary isotachophoresis with capillary zone electrophoresis) conditions: UV detector LCD 2084 at 280 nm (ECOM, s.r.o.) and electrophoretic analysator EA 101 (Villa Labeco, SK). Operating conditions: capillaries 110 mm × 0.8 mm and 140 mm × 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).

To determine the lactose content in milk and liqueurs, the sample was homogenised, diluted with the mixture of H_2O/acetonitrile (25/75) and analysed by HPLC [column Maxsil 5 NH2 250 mm × 3.2 mm, mobile phase H_2O/acetonitrile (25/75), flow rate of 0.5 ml/min, refractive detector].

RESULTS AND DISCUSSION

The majority of the recent studies dealing with the estimation of the egg content in egg-containing products (egg pasta, pastry, mayonnaises, egg liqueurs) were based on the determination of a single analytical parameter, mainly cholesterol or specific egg white proteins (lysozyme, ovalbumine etc.). Cholesterol is a parameter used by official authorities for the evaluation of authenticity of these products; however, its content in egg is highly variable. To eliminate the disadvantages caused by a single chemical marker, several chemical markers were chosen and used for the analysis of raw materials and model samples.

The analysis of the raw material, which is generally used for the production of egg liqueurs, was

Table 2. Chemical compositions of model samples of egg liqueurs and validation parameters of proposed methods for estimation of egg yolk content

Content of egg yolks (g/kg)	Phosphorus (mg/kg)	Cholesterol (mg/kg)	Fat (g/kg)	Fatty acids (g/kg)	Lactose (g/kg)	Estimated content of egg yolks (g/kg) according to			
						multiple regression equations	average values of all parameters	average values (P content correction I)	average values (P content correction II)
64	859	827	45	26	13,8	85	109	75	63
114	1088	1120	53	38	16	117	146	111	112
127	1185	1488	62	40	11.7	142	169	127	129
141	1335	1493	68	42	17.7	152	184	152	155
102	946	1024	64	24	12	103	139	106	91
127.2	995	1299	87	26	11.5	126	172	126	97
114.7	972	1123	77	25	16.2	113	156	99	104
Equation of correlation curve between real egg content (x) and method of estimation (y)						$x = 0.81y + 24.8$	$x = 0.99y + 41.7$	$x = 0.91y+11.3$	$x = 1.03y + 8.8$
Correlation between real content and method of estimation (R^2)						0.95	0.92	0.86	0.77

focused on the determination of the following chemical markers: phosphorus, fat, cholesterol, and fatty acids (only in the case of milk samples). The results obtained confirmed a relatively stable composition of milk that consists in average, of 1029 mg/kg, 35 g/kg, 88 mg/kg, and 25.5 g/kg of phosphorus, fat, cholesterol, and fatty acids, respectively. The results were in good agreement with the tabulated data (Simeonovová *et al.* 1999).

The compositions of egg yolk samples were rather variable and the values were generally lower than those mentioned in the literature. The concentration of phosphorus varied from 5439 mg/kg to 3138 mg/kg (average 3939 mg/kg), of fat from 31.6% to 19.1% (average 22.4%), of cholesterol from 17 406 mg/kg to 10 411 mg/kg (average 14 164 mg/kg) in all 11 unsweetened samples of yolks. To confirm the results obtained, subsequent analyses for dry matter (varied from 51.6% to 34.0%, average value 41.2%) and lysozyme (varied from 1487 mg/kg to 100 mg/kg, average value 724 mg/kg) contents were done. We estimated (Figure 1) the real content of clear egg yolk matter as the average of phosphorus, fat, cholesterol, and dry matter contents (egg yolk markers) and lysozyme content (egg white marker). The correlation between the two mentioned ways of calculation was high enough (R^2 = 0.93), similarly, the correlation between the easiest and most inexpensive method, dry matter determination, and the other measured parameters was rather good (R^2 varied from 0.72 to 0.97); thus, the method seems to be suitable for the fast checking of the quality control of technological yolks. To estimate the egg yolk content, the results obtained were recalculated according to the tabulated values for individual parameters (Simeonovová *et al.* 2001). Samples No. 1 and 2 represent the samples fully separated, clear yolk and 90% yolk, respectively, and the calculated egg yolk content corresponds to the tabulated value. Samples No. 3 to 11 are so-called "technological yolks" (yolk obtained by industrial separators) and their real content of yolk varies from 64% to 95%. The last three samples (No. 12, 13, 14) contained (due to high sucrose concentrations) only from 36% to 42% of yolks.

The result obtained showed that the producers of egg liqueurs have to analyse the technological yolks used in the production for at least dry matter content or have its minimal value stated in their supply contracts to fulfill the required content of yolk matter in their products. It is probable that more than 140 g/l of the technological yolk mixture has to be added into the liqueur to reach the level of chemical parameters that correspond to the presence of clear egg yolk as required by the official authorities.

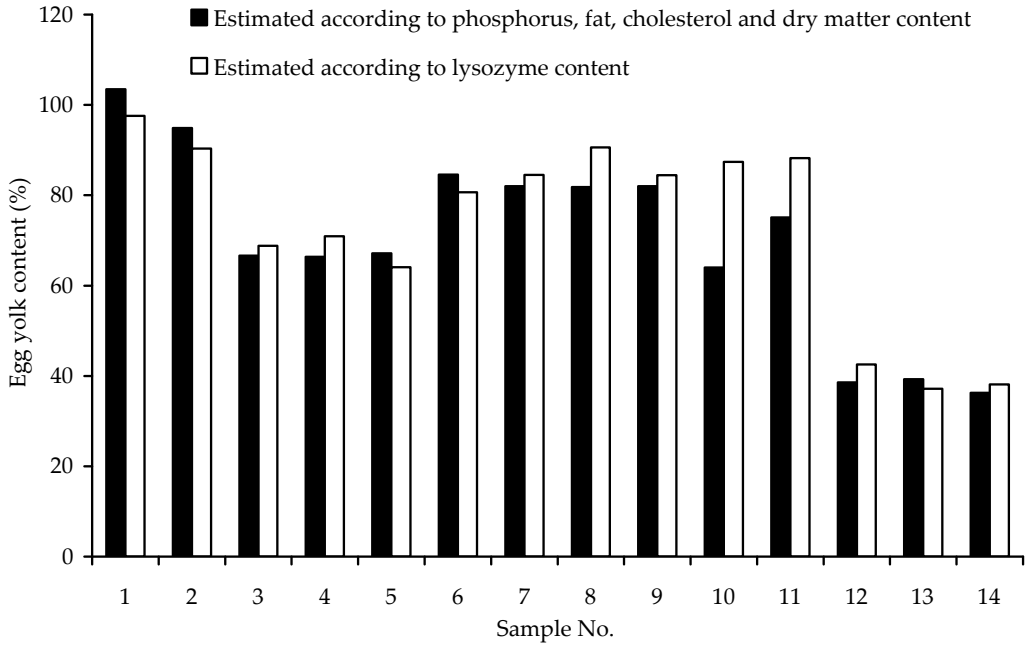

Figure 1. Estimated content of clear egg yolk matter in analysed egg yolk

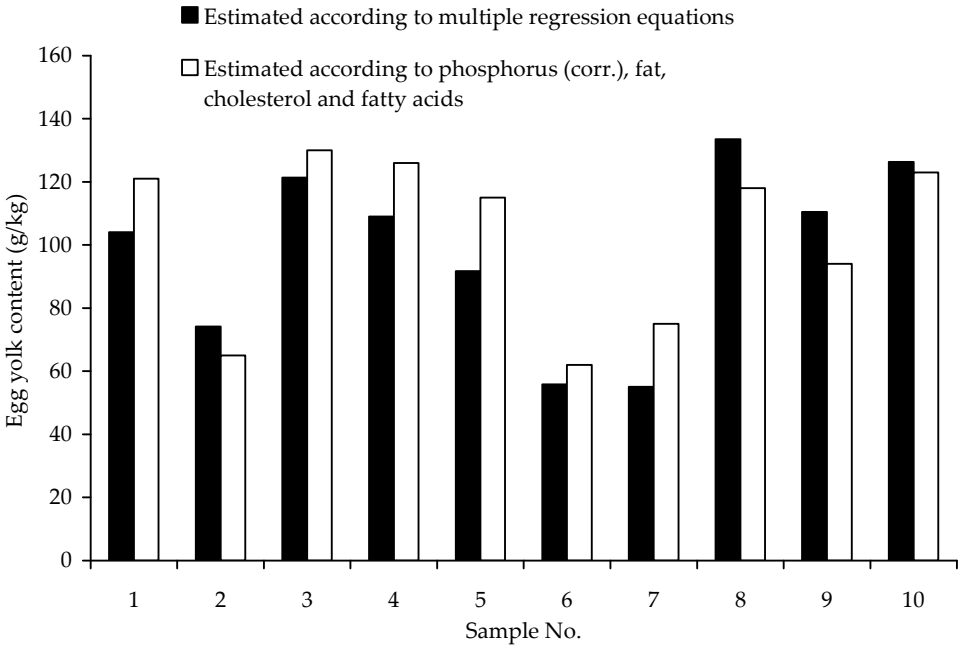

Figure 2. Estimated content of egg yolks in real samples of egg liqueurs and creams

The concentrations of the chemical markers in model samples are given in Table 2. The values (the contents of phosphorus, cholesterol, fat, and lactose, and fatty acids content and composition) obtained with the samples with known egg yolk contents were used for suggesting and confirming the ways of yolk matter estimation in real samples of egg liqueurs. The results of the estimations and correlation coefficients are also given in Table 2.

Firstly, the results obtained from the analyses of model samples were used to propose the multiple regression equation which was used to estimate the egg yolk content in real samples. The calculated equation was: egg yolk content (g/kg of liqueur) = –16 + 0.0431 × phosphorus (mg/kg) + 0.0485 × cholesterol (mg/kg) + 0.28 × fat (g/kg) + 0.4368 × fatty acids (g/kg). The results of equation correlated well with the real yolk contents and the ac-

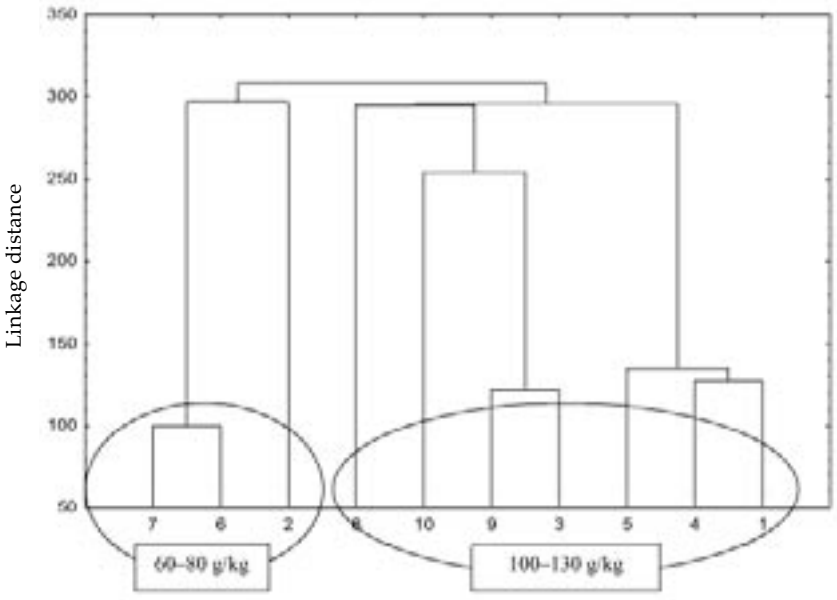

Figure 3. Cluster analyses of egg liqueurs and creams (samples divided into two groups: with 60–80 g/kg and 100–130 g/kg egg yolk content)

curacy of the estimation was acceptable (R^2 = 0.95, RSD = 6.8%).

Secondly, the egg yolk content was calculated as the average of the results obtained with all markers after recalculation of the concentration to the yolk content according to the tabulated values for clear egg yolk (phosphorus 5900 mg/kg, cholesterol 10 000 mg/kg, fat 330 g/kg, fatty acids 220 g/kg) (Simeonovová *et al.* 1999). In general, the results of calculation correlated well with the real yolk content (R^2 = 0.92) but were about 30% higher, probably because of the presence of some markers in other usual components used for the liqueur production. Milk contains also all of the markers followed; mostly they are in negligible concentrations considering the amount of milk in the recipe. However, phosphorus (phospholipids etc.) is present in such concentrations which can affect the estimation. For the correction, the subsequent approaches were suggested:

(1) The milk content of liqueur was firstly estimated by the determination of lactose, the results, however, did not correspond to the real content of milk in model samples and varied from 11.5 to 17.7 g/kg for the same milk content (42.4 vol. %).

(2) The milk content, and subsequently the phosphorus content added by milk, was estimated from the composition and content of fatty acids (C8, C10, C12, C14 and C16) in the analysed samples. From the estimated milk content (presumably milk with 3.5% of fat), the amount of "milk phosphorus" was calculated. The results obtained by this procedure correlated well with the yolk content in all model samples [R^2 = 0.86 for the correction according to (C10)/C16 ratio and R^2 = 0.77 for the correction according to (C8 and C10 and C12 and C14)/C16 ratio].

Ten samples of egg liqueurs and egg creams were analysed for the phosphorus, cholesterol, and fat contents and fatty acids content and composition. The egg yolk content was estimated according to multiple regression equations and the average of all parameters measured, i.e. phosphorus (corrected according to the fatty acids composition), fat, cholesterol, and fatty acids contents. The results of the estimation of egg yolk content in yolks in liqueurs are presented in Figure 2. The differences between the results obtained by different methods used varied from 0 to 15% (for 7 samples) and from 11 to 26% (for 3 samples). The reason for the high variation in the second group of samples can be explained by the differences in the composition and lower variability of target markers in the model samples in comparison to the real egg liqueurs, which can be improved in the future by analysing a larger set of model samples. As the final method for the determination, we suggested the calculation according to all markers where phosphorus content is corrected according to the milk content.

The cluster analysis of samples based on the concentrations of the chemical markers measured was done (Figure 3). The samples can be divided into 2 groups according to the egg yolk content per kg of liqueur: (1) Samples with the egg yolk content in the range from 100 to 130 g/kg (samples No. 1, 3, 4, 5, 8, 9 and 10) and (2) Samples with a very low egg yolk content (samples No. 2, 6, 7), i.e. the content of yolks in the range from 60 g/kg to 80 g/kg. Only 6 from 10 samples analysed met the limit of 127 g/kg (corresponding approximately to 140 g/l, calculated with the 10% standard deviation error of estimation) required by the recent Czech legislation.

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Received for publication January 21, 2004
Accepted after corrections February 13, 2004

Souhrn

Čížková H., Voldřich M., Prokorátová V., Kvasnička F. (2004): **Stanovení obsahu vaječného žloutku ve vaječných likérech**. Czech J. Food Sci., **22**: 9–15.

Česká legislativa (vyhlášky č. 264/2003, 93/2000 a 57/2003 Zákona o potravinách č. 110/1997 Sb. v původním znění) určuje požadavky na minimální obsah vajec nebo vaječných žloutků ve vaječných výrobcích (majonézy, vaječné těstoviny, vaječné likéry). Vhodné analytické metody ani způsoby stanovení tohoto podílu však nejsou specifikovány. Prezentovaná studie se zabývá vývojem a ověřením analytických metod vhodných pro stanovení obsahu vaječného žloutku v likérech. Vzhledem k vysoké variabilitě ve složení vajec a předpokládanému vlivu použitého technologického postupu na výsledné složení likéru byl analyzován následující soubor chemických parametrů (markerů vaječného podílu): obsah sušiny, celkového fosforu, tuku, cholesterolu, lysozymu a složení a obsah mastných kyselin. Obsah žloutků v reálných vzorcích vaječných likérů byl stanoven podle multiregresní rovnice získané pro soubor modelových vzorků o známém obsahu žloutků a podle dat o složení používaných surovin experimentálně naměřených a publikovaných v literatuře. Z 10 analyzovaných vaječných likérů pocházejících z tržní sítě pouze 6 splnilo limit 140 g žloutků na 1 litr likéru (počítáno s 10% chybou odhadu), požadovaný českou legislativou.

Klíčová slova: vaječný žloutek; stanovení vaječného obsahu; vaječný likér; falšování; autenticita

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