SOLVENT EXTRACTION OF EGG OIL FROM LIQUID EGG YOLK

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Abstract

The egg yolk lipids have a very high nutritional value. Due to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition. There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method. The aim of this study was to compare two different solvent mixtures ethanol/chloroform (30/70 by volume) and 2-propanol/hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils. As a liquid egg yolk was used for extraction of oil, the choice of the solvents was based on solvents polarities. Also volatility and toxicity of the solvents were taken in account. The yield of extracted crude oil, water content, solvent residue, fatty acid profile and β-carotene content were determined and compared. The results show that extraction with 2-propanol/hexane gave higher yield of the crude oil than ethanol/chloroform, 28.90±0.27% and 26.37±1.04%, respectively. High water content and solvent residue in both oils mean that there is a purification of solvent extracted egg yolk oil needed. There was no significant difference in fatty acid profile in both oils, but β-carotene content was higher in oil extracted with 2-propanol/hexane 81.02±0.37 mg kg^{-1}, than in ethanol/chloroform extract 73.16±1.53 mg kg^{-1}.

Keywords: egg oil, solvent extraction method.

Introduction

The egg yolk lipids have a very high nutritional value. Thanks to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition (Lewis et al., 2000).

Nutritional properties in eggs can be affected through the laying hen feed (Surai et al., 2008; Stibilj et al., 1999). There are good ways known how to enrich egg yolk oil with nutrients, but how to extract them as much as possible and keep them in their natural condition after extraction remains the question.

There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method.

In case of solvent extraction high technological and safety requirements must be taken in account when choosing solvents for extraction. Solvents must extract as much as possible lipids from raw material, must have a low boiling temperature, for easier and economical removing from the product, and must be as less as possible toxic.

The difference of oil extraction from egg or egg yolk powder is that liquid egg yolk contains a lot of water and extraction with non-polar solvents is not efficient due to difference in solvent and egg yolk polarities. Polar solvents, such as lower alcohols, denature egg yolk proteins destroying hydrogen bonds or electrostatic interaction in protein structure opening the way to the neutral lipids, what makes extraction with non-polar solvent possible. Without protein denaturation, polar solvents will extract polar membrane-associated lipids from the egg yolk.

The combination of polar and non-polar solvents for better lipid extraction from liquid egg yolk can be chosen. The 2-propanol/hexane mixture can be a good solvent for extraction of egg oil from liquid egg yolk (Ahn et al., 2006).

2-propanol / hexane solvent

2-propanol, a quite polar solvent, can denature egg yolk proteins and extract polar lipids, but it is also non-polar enough to be soluble in non-polar solvent as hexane. 2-propanol is relatively low toxic and is accepted as a solvent in the processing of foodstuff (EFSA, 2005). Main disadvantages of 2-propanol is its boiling temperature which is 82.6 °C, but boiling temperature can be decreased by using vacuum in evaporation procedure.

Hexane is a most popular solvent for lipid extraction for food application. It is widely used in production of vegetable oils. In comparison with 2-propanol, hexane will extract mainly simple triglycerides – neutral lipids. Hexane boiling temperature is 69 °C, which makes it very economical in solvent extracted oil production. Taking in account all before mentioned advantages the choice of non-polar solvent was quite simple.

Because two solvents was mixed together to make extraction mixture, the ratio 30 : 70 between 2-propanol and hexane was chosen. Too much of 2-propanol will make mixture too polar to extract neutral lipids as much as possible.

Ethanol/chloroform solvent

One of the main tasks of oil extraction is the high yield of oil. The process where total lipid extraction is needed is an analysis of the fat content in food products. For fat analysis in egg containing food products methanol/chloroform is used as a solvent (Boselli et al., 2001).

Methanol usage for food grade oil extraction is not safe due to the toxicity of this solvent. For that reason methanol was replaced with ethanol. Ethanol is a lower alcohol, same as 2-propanol, it denatures egg yolk proteins and as a polar solvent extracts polar lipids.

Chloroform is widely used in defatting of biological materials. The process of lipid extraction from tissues with methanol/chloroform first was mentioned in 1957 (Folch et al., 1957). The so-called Folch procedure requires addition of water to solvent mixture, but in

case of extraction of liquid egg yolk, water is already present in the egg yolk. Chloroform / methanol mixture still is used in laboratories for fat content determination in food products (Cizkova et al., 2004). But it is must be admitted that chloroform is toxic and even suspected of causing cancer (WHO, 2004).

The aim of this study was to compare two different solvent mixtures – ethanol/chloroform (30/70 by volume) and 2-propanol / hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils.

The quality of egg yolk oil during the storage can be decreased by lipid oxidation. In this case water content in the oil can affect oxidation process. Egg yolk contains approximately 50% of water and extraction of egg yolk lipids with polar solvents, without extraction of polar lipids, can also bring some water to the extract.

After oil extraction with organic solvents they must be removed from the final product. The solvent residue in extracted oil is an important quality parameter. All solvents used in food processing are strictly controlled by food safety regulators on its presence in food products (Directive 2009/32/EC, 2009).

The main nutritional value of egg yolk oil is unsaturated fatty acids. The extraction method can be evaluated as acceptable if it does not affect the fatty acid profile of egg oil.

β-carotene, as a vitamin A precursor, is a representative of oil soluble vitamins in egg yolk. Content of β-carotene in egg yolk oil can reflect the efficiency of extraction process. The very attractive colour of egg yolk oil is related to β-carotene and therefore its concentration can be easily detected by a simple photometric method.

Materials and Methods

Materials

Commercially available homogenized, pasteurized and chilled liquid egg yolk was obtained from JSC Balticovo (Iecava area, Latvia). Liquid yolk was stored at +4 °C and used with the same temperature for extraction. All solvents (ethanol, 2-propanol, chloroform, hexane) used in egg yolk oil extraction were with the analytical grade from Sigma Aldrich, Germany.

Egg yolk oil extraction

For egg yolk oil extraction two solvent mixtures - ethanol/chloroform (30/70) and 2-propanol/hexane (30/70) were used. For each extraction process solvents were mixed by volume and poured in beaker. Liquid egg yolk was added to solvent mixture with a thin squirt vigorously mixing. The ratio 2 : 1 between solvent mixture and egg yolk was used. Extraction was done at +21 °C temperature vigorously mixing for 30 minutes. Extracts were filtered using vacuum filtration and collected into a clean container. The oil was recovered by evaporation of the solvent mixture using rotary evaporator IKA RV 10 Control (IKA-Werke GmbH & Co. KG) at +70 °C for ethanol / chloroform extract and +80 °C for 2-propanol / hexane extract under the vacuum.

Analyses

The yield of egg oil after extraction with different solvent mixtures was expressed as a weight of total lipids extracted from liquid egg yolk in percent.

Fatty acid profile was determined in accordance with standard methods ISO 12966-2 and ISO 5508, GC-FID (gas chromatography with flame ionization detector). Shimadzu GC 2010 Plus gas chromatograph with flame ionization detector (Shimadzu Corporation, Japan) was used.

Water content in egg yolk oil was measured with Moisture balance MOC-120H scale from Shimadzu (Shimadzu Corporation, Japan). Water evaporated at 120°C until difference in the sample weight was less than 0.05 percent.

β-carotene content was determined in accordance with standard method ISO 17932:2012 using UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan).

Solvent residue in both oil samples was detected by combination of headspace and GC-FID (gas chromatography with flame ionization detector) methods (Restek, 2000; ISO 9832:2002; Stenerson, Verma, 2011; Tiscione et al., 2011).

The results are the means and standard deviation for three replicates. Means were compared by T-test and analysis of variance (ANOVA). Significance was defined at $p<0.05$. Statistical analysis was carried out by Microsoft Excel 2010 version software.

Results and Discussion

Extraction process

It needs to be admitted that in the extraction process the particle size of denatured egg yolk proteins was different. In ethanol/chloroform extraction protein fraction had smaller aggregates and, due to the high density of chloroform, protein fraction was positioned in the top layer. Vacuum filtration of ethanol/chloroform extract requires a longer period of time comparing to 2-propanol / hexane. In case of 2-propanole / hexane extraction, yolk proteins had a bigger particle size and proteins were deposited in bottom layer. This information is important for choosing filtration equipment for egg yolk oil in large scale production.

The results presented in Table 1 show quality parameters of both extracted egg yolk oils. The yield of crude egg yolk oil extracted from liquid egg yolk with 2-propanol / hexane solvent was significantly higher than from ethanol / chloroform extraction ($p<0.05$). The determined water content was high in both oils. This can be explained by the fact that liquid egg yolk contains approximately 50% of water and extraction with mixture of polar and non-polar solvent causes the phase separation where egg yolk phospholipids dissolve in polar phase and start to swell absorbing the water from the egg yolk. The presence of water in egg yolk oil can affect quality of egg oil during storage

resulting in rancidity. Egg oil needs to be purified to increase its storage time.

After solvent removal from the extracts by means of evaporation under the vacuum, crude egg yolk oil was analyzed for solvent residues (Table 1). Ethanol, 2-propanol and hexane, can be used as extraction solvents in food processing in compliance with good manufacturing practice, but anyway these solvent residues in food product are specified by a maximum residue limits in extracted foodstuff or food ingredient, where 2-propanol is limited to 10 mg kg^{-1} and hexane to 1 mg kg^{-1} (Directive 2009/32/EC, 2009; EFSA, 2005). The residue of 2-propanol and hexane in egg oil exceeds the limits allowed in food products and it means that the solvent recovery process must be optimized for better solvent removal. The allowed daily intake of ethanol varies, but its residue in ethanol/chloroform extracted egg yolk oil must not exceed the dangerous limits for human health. Chloroform is a very toxic chemical and its presence in food products even in a very small concentration can have influence on human health therefore chloroform containing food is prohibited under any circumstance in most countries.

Table 1

Egg yolk oil quality parameters

Quality parameters	Extraction solvent	
	2-propanol / hexane	ethanol / chloroform
Yield, %	28.90±0.27	26.37±1.04
Water content, %	14.75±1.05	12.76±2.05
2-propanol, mg kg^{-1}	264.14±7.18	–
Hexane, mg kg^{-1}	2.03±0.02	–
Ethanol, mg kg^{-1}	–	86.08±12.38
Chloroform, mg kg^{-1}	–	22.04±0.77

β-carotene content

β-carotene content in egg oil extracted with 2-propanol/hexane solvent was 81.02±0.37 mg kg^{-1} and 73.16±1.53 mg kg^{-1} in ethanol / chloroform extracted oil. The higher content of β-carotene in 2-propanol / hexane extracted egg yolk oil can be explained by usage of particularly non-polar solvent hexane. β-carotene is a non-polar compound and it dissolves better in non-polar solvents such hexane. Hexane is usually used for extraction of β-carotene from carotenoid containing products.

Fatty acid profile

The fatty acid profile of 2-propanol / hexane and ethanol / chloroform extracted egg yolk oils presented in Table 2. The results show that there is no difference in fatty acid content for both oils. Both 2-propanol / hexane and ethanol/chloroform gave the same fatty acid profile of egg yolk oil.

Table 2

Fatty acid profile of egg yolk oil

Fatty Acid	Extraction solvent	
	2-propanol / hexane g 100 g^{-1}	ethanol /chloroform g 100 g^{-1}
(C14:0)	0.14±0.02	0.09±0.01
(C14:1)	0.04±0.01	0.03±0.01
(C15:0)	0.08±0.01	0.09±0.01
(C16:0)	22.72±0.04	22.27±0.03
(C16:1)	0.28±0.02	0.29±0.01
(C17:0)	0.19±0.02	0.21±0.02
(C17:1)	0.12±0.01	0.11±0.01
(C18:0)	6.20±0.21	6.10±0.03
(C18:1)	52.61±0.06	53.21±0.13
(C18:2)	13.67±0.03	13.65±0.01
(C18:3)	1.72±0.01	1.77±0.01
(C20:1)	0.23±0.01	0.22±0.01
(C20:2)	0.01	0.01
(C20:3)	0.19±0.02	0.15±0.01
(C22:1)	0.03±0.01	0.03±0.01
(C20:4)	0.07±0.01	0.08±0.02
(C20:5)	0.03±0.01	0.03±0.01
(C24:1)	0.02	0.02
(C22:4)	0.08±0.02	0.09±0.01
(C22:5)	0.05±0.01	0.06±0.01
(C22:6)	1.02±0.02	0.95±0.02
Other	0.46±0.01	0.50±0.01
SFA	29.37	28.80
MUFA	53.33	53.91
PUFA	16.84	16.79

Egg yolk for an egg yolk oil extraction was obtained from laying hens which were fed with supplementation of canola oil and can be considered as a product enriched with unsaturated fatty acids. In comparison with other researchers (Stibilj et al., 1999; Souza et al., 2008) our extracted oil contains more oleic acid, but less palmitic and linoleic acids. But the difference in fatty acid content must be related more to the hen feed than to the extraction method or used solvents. Anyway extracted egg yolk oil due to the high content of monounsaturated and polyunsaturated fatty acids is a high value product which after purification can be used as an additive to a human nutrition providing the health benefits.

Figure 1 shows the content of main fatty acids yolk and total content of saturated, monounsaturated and polyunsaturated fatty acids in egg yolk oil and egg.

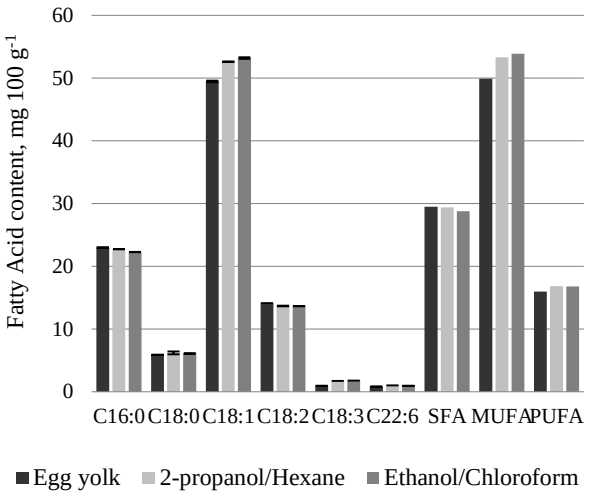

Figure 1. Fatty acid content in egg yolk and egg yolk oils extracted with 2-propanole / hexane and ethanol / chloroform

C16:0 – Palmitic Acid, C18:0 – Stearic Acid, C18:1 – Oleic Acid, C18:2 – Linoleic Acid, C18:3 – α-linolenic acid, C22:6 – Docosahexaenoic acid, SFA – Saturated Fatty Acids, MUFA – Monounsaturated Fatty Acids, PUFA – Polyunsaturated Fatty Acid

Conclusions

Solvent extraction of egg yolk oil from liquid egg yolk can be applied with combination of a polar solvent, as lower alcohols, and a non-polar solvent. As a result egg yolk oil rich in monounsaturated and polyunsaturated fatty acids and β-carotene can be obtained.

Better extraction results were achieved with 2-propanol / hexane solvent which gave higher yield of oil and β-carotene content. Extraction of egg yolk oil from liquid egg yolk with ethanol / chloroform also is possible, but for the health safety reasons usage of chloroform must be prohibited.

Both 2-propanol / hexane and ethanol / chloroform extracted oils have the same fatty acid profile.

Due to the high water content and solvent residue crude egg yolk oil must be purified.

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