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Egg yolk oil as a source of bioactive compounds for infant nutrition

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Abstract. Egg yolk oil is a natural source of bioactive compounds such as DHA, fat–soluble vitamins, lutein, phospholipids and cholesterol. These important compounds are also found in breast milk: DHA for infant brain development, lutein for eye health, vitamins A and E to support developing cells. Egg yolk oil naturally contains vitamin D which is required for a normal bone development. Fatty acid profile of egg lipids is also close to human milk. The aim of this study was to evaluate the conformity of egg yolk oil for infant nutrition. In this study egg yolk oil extracted from liquid egg yolk using two-stage solvent extraction with polar and non-polar solvents was used. Extracted egg yolk oil was analyzed for fatty acids, fat-soluble vitamins, lutein, phospholipids and cholesterol using GC and HPLC methods. Results were compared with the chemical composition of human breast milk and nutritional recommendations for infant feeding. Fatty acid profile of egg yolk oil was similar to breast milk in terms of palmitic, stearic, linoleic and α-inolenic acids. Egg yolk oil used in this study was high in DHA, but low in ARA. Vitamin A, D and E content was sufficient for infant biological needs. Lutein and phospholipid content in egg yolk oil was lower than their content in breast milk fats, but cholesterol in opposite was in much higher concentration than available in breast milk. Chemical composition of egg yolk oil still makes it an excellent source of bioactive compounds for infant nutrition.

Key words: egg yolk oil, infant nutrition.

INTRODUCTION

There is no doubt that mother breast milk is the best diet for an infant. Breast milk contains all nutrients that are essential for normal development of the infant. But not all of mothers can or want to breast-feed their babies. For that reason artificial infant formulas containing many nutrients were created to fulfill biological needs of infants (Lawrence & Lawrence, 2016). All infant formulas try to replicate human milk in terms of nutritional and biological value. Some bioactive compounds, such as polyunsaturated fatty acids (PUFA), fat-soluble vitamins A, D and E, lutein, phospholipids and cholesterol are presented in lipids therefore the right choice of fats for infant formulas is so important (Berthold et al., 2005). In this study we evaluate the conformity of egg lipids (egg yolk oil) as a source of bioactive compounds for infant nutrition.

Bioactive compound content in human milk depends on the type of milk: colostrum, transitional and mature milk. Colostrum and transitional milk have a different and variable chemical composition and available for the infant for a very short period, but mature milk has more stable composition and is fed to infants for a longer time. Average fat content of mature milk is 4–5%. Breast milk fats provide 50% of total

calories consumed by infant (Sala-Vila et al., 2005). The content of fatty acids (FA) is very important in human breast milk, especially the content of polyunsaturated fatty acids (PUFA). PUFA content is about 1/5 from the total fatty acids (Lassek & Gaulin, 2014), where arachidonic (ARA) and docosahexaenoic (DHA) acids are the most important. PUFA content in breast milk depends mainly on mother's diet, wherein saturated fatty acid (SFA) content is relatively constant (Soleimani et al., 2013; Lassek & Gaulin, 2014). ARA and DHA play an important role for infant development, but their content in human milk is quite low and varies from 0.1 to 1.0% from total FA (Saphier et al., 2013). Both ARA and DHA are FA of phospholipids (PLs) and consumption of these FA by infant will depend on PL content in formulas (Heird, 2001). Egg yolk lipids are very close to human milk in terms of FA profile (Simopoulos & Salem, 1992) and contain about 10% of PL from total lipids (Ahn et al., 2006).

Without PUFA infants also need fat–soluble vitamins. Vitamins A, D and E are very crucial for infant development. Food must provide these essential vitamins to infants because human body cannot synthesize them.

Breast milk is rich in vitamin A, especially in the first postnatal days which probably relates to that fact that almost all children are born with very low stores of vitamin A. Later vitamin A content in human milk decreases (Fujita et al., 2011). Vitamin A is required for optimal health, growth and development of the infant therefore starting from 6 months of age consumption of vitamin A must be increased by additional supplement. Many children around the world are suffering from vitamin A deficiency that causes many deaths among babies (WHO, 2011). In the developed countries and countries where mothers consume a lot of leafy vegetables and other products rich in vitamin A (including eggs), deficiency of vitamin A in breast milk is not so actual, since vitamin A content in breast milk is correlated to its content in mothers' diet (Fujita et al., 2011). But vitamin A in high dosages can be toxic (Olson, 1989). There is a lot of studies about poisoning caused by vitamin A overdosing therefore vitamin A supplementation must be strongly controlled. Egg yolk naturally contains vitamin A and its content in eggs can be increased through hen's diet. Egg yolk oil can be used as a source of vitamin A for infant nutrition.

Vitamin E is extremely important in the early stages of life. Colostrum contains a huge amount of vitamin E while mature milk has much less. Being strong antioxidant the main function of vitamin E is to protect tissues from various destructive influences and stimulate infant immune system development. α-tocopherol is the most active form of vitamin E, but human milk has been found to contain β-, γ- and δ-tocopherols and γ-tocotrineols (Lima et al., 2014). Vitamin E in egg yolk lipids is presented by α-tocopherol and γ-tocopherol (Kovalcuks & Duma, 2013). There is a strong correlation observed between vitamin E content in eggs and in hen diet (Mori et al., 2003), so vitamin E is another example of adjustable bioactive compound.

Vitamin D helps to absorb calcium in human body and is required for a bone development of an infant. Lack of a vitamin D can cause rickets. Vitamin D also plays a role in muscle function and the immune system. Human breast milk contains insufficient amount of vitamin D therefore pediatricians recommend vitamin D as a peroral supplement (vitamin D droplets) for all breast-feed infants (Ballard & Morrow, 2013). But it needs to be aware with the dosage because vitamin D, similar to vitamin A, is toxic in high dosages. Instead of peroral supplement, food products rich in vitamin D, such as oily fish, eggs and others can be fed to the infant. But infants during the first year

of their life reluctantly consume such food and prefer liquid milk formulas. Vegetable fat-based artificial milk formulas do not contain vitamin D because vitamin D is an animal source compound. And usually vitamin D is added to infant formulas as a mono ingredient. Egg yolk oil, which is rich in natural highly bioavailable vitamin D, can be used in formulas and solve the problem with the absence of this vitamin.

Lutein is the major carotenoid found in breast milk. Lutein and its isomer are found in the neural retina and contribute to brain development. Similar to many other bioactive compounds, lutein content in human breast milk depends on mother's diet (Jewell et al., 2004). Eggs from hens fed by corn or green forage are rich in lutein. Lutein and other carotenoids are located in egg yolk lipids therefore egg yolk oil can be a good source of lutein for infant feeding. Usually infant formulas do not contain lutein since egg yolk lipids are not added (FSANZ, 2008).

The main phospholipids of human milk are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Phospholipids provide not only ARA and DHA, but also choline – an essential compound for infant brain development (Schneider, 2010). It is well known that egg yolk lipids are rich in PLs, which contain 30% of PLs from total yolk lipids (Ahn et al., 2006). PC is the major phospholipid of egg yolk meaning that egg yolk lipids are one of the richest sources of choline.

A lot of health concerns relates to cholesterol. High content of cholesterol in food results in high cholesterol level in blood and may cause a heart coronary disease (Paik & Blair, 1996). But it is totally opposite in case of infants. Cholesterol contribute to development of brain and nervous system of the infant and therefore it is one of the major compounds for infant nutrition. Moreover, infants fed with breast milk containing high cholesterol have a normal metabolism of this compound in later life (Owen et al., 2008). Cholesterol content in breast milk depends on an individual and can be affected by many other parameters, such as mother's age, diet, season and place of residence (Kamelska et al., 2012). Infant formulas contain a small amount of cholesterol that can be dangerous for a normal development of an infant. Egg yolk lipids contain 10% of cholesterol (Ahn et al., 2006) and can be used in infant formulas as a source of this vital component.

As mentioned previously, egg yolk lipids can provide many important bioactive compounds for the infant, but usage of egg products for infant nutrition often raises concerns about being allergic to eggs. Egg yolk oil does not contain egg proteins and there is no risk of allergy. Extraction of egg yolk lipids is very important in terms of safety. Usage of organic solvents in extraction process may cause a risk related to solvent residues in egg yolk oil therefore the choice of solvents and technological parameters must be made with respect to safety of the infant health.

MATERIALS AND METHODS

Egg yolk oil extraction

Hen eggs from Lohman Brown Classic breed were purchased in local grocery store. Egg yolks from 30 eggs were separated from egg whites and homogenized.

Extraction solvents (ethanol and hexane), used in egg yolk oil extraction, were analytical grade from Sigma-Aldrich (Germany). Compressed nitrogen gas with purity 99.999 % HiQ Nitrogen 5.0 was from Linde AG (Germany).

Lipid extraction with ethanol and hexane from liquid egg yolk was made by following steps. First, polar lipids were extracted with ethanol from liquid egg yolk and then neutral lipids were extracted from precipitate with hexane (Fig. 1).

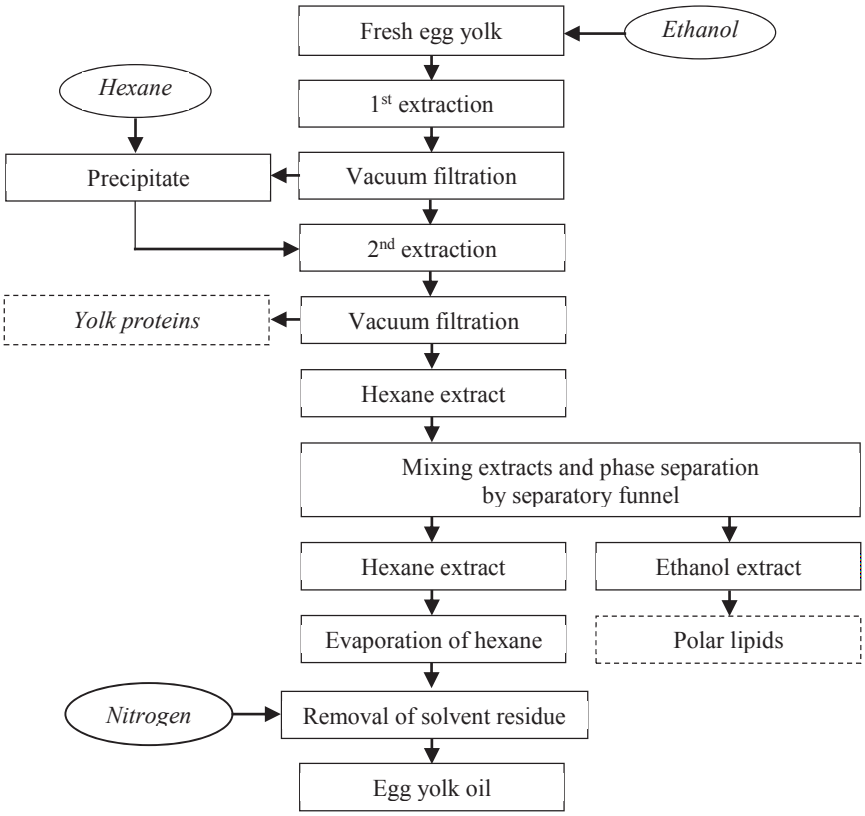

Figure 1. Egg yolk oil extraction process from liquid egg yolk.

For polar lipid extraction 200 g of homogenized liquid egg yolk was added to 400 ml of ethanol and stirred until egg yolk proteins denature and completely disperse. Extraction was done at 20 °C for 30 minutes. Then, the mixture was filtered by vacuum filtration, and the supernatant was collected and transferred to a separatory funnel. The precipitate containing egg yolk proteins and non-polar lipids was extracted with 400 ml hexane vigorously mixing for a 30 minutes at 20 °C using a magnet stirrer. The extract was filtered by vacuum filtration and supernatant was collected and added to the same separatory funnel. Both ethanol and hexane extracts were thoroughly but gently, to avoid emulsion formation, mixed to extract polar lipids and impurities to a polar ethanol/water phase and neutral lipids to a non-polar hexane phase. Then the mixed extracts were left for 1 hour for phase separation. Bottom ethanol/water layer, containing polar lipids and water soluble compounds was drained from the separatory funnel through the open stopcock. Hexane extract was collected in clean container. Egg yolk oil was obtained from the hexane extract by evaporation of the hexane in the rotary evaporator IKA RV 10 Control V (IKA®–Werke GmbH and Co. KG, Germany) at the temperature of 70 °C and 400 mbar pressure. After solvent evaporation in the rotary evaporator, as the last

step of the solvent removal, the pure nitrogen gas was laid through the egg oil for 10 minutes in the same rotary evaporator with the same evaporation conditions by means of a plastic tube immersed in the oil (Kovalcuks, 2014).

Analysis

Fatty acids

Fatty acids of egg yolk oil were determined in accordance with the standard methods ISO 12966–2:2011 and ISO 12966–1:2014, GC-FID (gas chromatography with flame ionization detector).

All chemicals and reagents used for analysis were of analytical grade and purchased from Sigma-Aldrich (Germany). 37 components FAME mix 47885-U (Supelco, Germany) was used as standard in the fatty acid content analysis. Shimadzu GC 2010 Plus gas chromatograph equipped with flame ionization detector (Shimadzu Corporation, Japan) and Nukol™ (Sigma-Aldrich, Germany) capillary GC column (30 m × 0.25 mm, d_f 0.25 μm) were used. The GC analysis conditions were from ISO 12966–1:2014. Compounds were identified by comparison of the retention times of 37 components FAME mix 47885–U (Supelco, Germany).

The results were the means of three replicates with standard deviation and expressed in g 100 g^{-1}.

Vitamins A, D, E

Fat–soluble vitamins (α-tocopherol, cholecalciferol and retinol) content determination in egg yolk oil was made according to the Latvian standards: LVS EN 12823–1:2014; LVS EN 12821:2009 and LVS EN 12822:2014 respectively.

All solvent used in the chromatography analysis were for HPLC grade and purchased from Sigma-Aldrich (Steinheim, Germany). A-tocopherol, retinol and cholecalciferol standard solutions were purchased from Sigma-Aldrich (Germany) and then they were diluted in hexane. Vitamins were quantified using internal standard method where peak areas were identified by comparisons of retention times with those of standards used for vitamins determination.

For HPLC analysis Shimadzu Nexera X2 liquid chromatograph equipped with UV and fluorescence detector and Intersil 5 silica column (5 μm, 250 mm × 4.6 mm) was used. Temperature of the column oven was 30 °C. Mobile phase contained 3% of 1,4-dioxane in n-hexane. Flow rate of the mobile phase was 2 ml per min, and the injection volume was 20 μl. The following wavelengths for vitamin detection were used: 325 nm for retinol, 265 nm for cholecalciferol and 290 nm for α-tocopherol.

For vitamin HPLC analysis egg yolk oil sample was diluted with hexane and injected in the HPLC system. Vitamins A and E was identified by comparing of peaks with those of authentic standards and their contents were calculated on a weight basis. Peak identification and purity was operated with normal-phase chromatography system with UV (retinol) and fluorescence detections (α-tocopherols). For vitamin D analysis reverse-phase chromatography with UV detection was used after the semi-preparative clean-up procedure.

The results were the means of three replicates with standard deviation and expressed in mg kg^{-1}.

Lutein

1 g of egg yolk oil was accurately weighed in 50 ml glass vial and 5 ml of methanol was added. The sample with methanol was homogenized and then left overnight (16 h)

in a refrigerator at 4 °C. Then the sample was centrifuged 800 × g for 10 min. The methanol layer was transferred to a 25 ml volumetric flask. 5 ml tetrahydrofuran (THF) was added to the glass vial with sample and vial was vortexed for 30 seconds, then centrifuged at 800 × g for 5 min. The THF layer was transferred into the methanol containing volumetric flask. The sample was extracted three more times and the THF layers were combined into the volumetric flask. THF was added to make the final volume 25 ml 10 ml of extract was dried under nitrogen. The extract was resuspended in 500 ml of ethanol and vortexed for 30 seconds (Perry et al., 2009). 20 µl were injected into the HPLC system (Shimadzu Nexera X2, Japan) for lutein analysis.

All chemical for lutein analysis and solvents for the HPLC mobile phase were of HPLC grade and obtained from Sigma-Aldrich (Germany). The lutein was determined using a C30 column (3 µl, 150 mm × 4.6 mm, YMC). Lutein was monitored at 445 nm with Shimadzu SPD-M20A photodiode array detector. The mobile phase was methanol : *tert*-Butyl methyl ether : water (95 : 3 : 2, v/v, with 1.5% ammonium acetate in water) – solvent A, and methanol : *tert*-Butyl methyl ether : water (8 : 90 : 2, v/v, with 1.0% ammonium acetate in water) – solvent B. The flow rate was set at 0.4 ml min^{-1} (10 °C). Gradient procedure: start at 100% solvent A; a 21-min linear gradient to 45% solvent A and 55% solvent B; 1-min hold at 45% solvent A and 55% solvent B; an 11-min linear gradient to 5% solvent A and 95% solvent B; a 4-min hold at 5% solvent A and 95% solvent B; a 2-min linear gradient back to 100% solvent A, and a 28-min hold at 100% solvent A (Perry et al., 2009). Peak identification in sample was based on comparisons with retention time and absorption spectra of known lutein standard from Sigma-Aldrich Germany. Lutein was quantified by integrating peak area in the HPLC chromatograms. The results were the means of three replicates with standard deviation and expressed in mg kg^{-1}.

Phospholipids

For separation of phospholipids approximately 5 g of egg yolk oil was fractionated on a 5-g column of silica gel (60–200 mesh), by sequential elution with 200 ml chloroform, 100 ml acetone, 100 ml methanol and 100 ml 0.1% phosphoric acid in methanol. The methanol fractions were combined for recovery of the total phospholipids. Solvent was removed in a rotary evaporator. The sample residue was dissolved in chloroform, washed with saturated salt solution, and then sodium bicarbonate was added until neutral. The sample was dried with sodium sulfate and filtered. The solvent was removed by rotary evaporation at room temperature. Samples were diluted with chloroform to give a 1 mg $µl^{-1}$ solution for analysis (Seri et al., 2010).

The standards of phosphatidylethanolamine and phosphatidylcholine, chloroform, methanol, ammonium hydroxide and water for the mobile phase were of HPLC grade and obtained from Sigma-Aldrich (Germany). For determination of phospholipids Shimadzu Nexera X2 with evaporating light scattering detector ELSD-LTII (Japan) was used. As the nebulizing gas, N_2 was used at a flow rate of 4 l min^{-1}, and a nebulizing temperature of 40 °C (Mounts et al., 1992).

A 125 × 4.0 mm Si – 60 column with 5 µm particle diameter (Lichrospher) was used. The elution program was a linear gradient with 80 : 19.5 : 0.5 (v/v) chloroform : methanol : ammonium hydroxide (NH_4OH) at t = 0 min to 60 : 34 : 5.5 : 0.5 (v/v) chloroform : methanol : water : ammonium hydroxide (NH_4OH) at t = 22 min and the column was allowed to equilibrate until the next injection at t = 27 min (Yalçyn et al.,

2007). The results were the means of three replicates with standard deviation and expressed in mg $100g^{-1}$.

Cholesterol

For the egg yolk and egg yolk oil cholesterol analysis the standard method of AOAC 994.10 was used with some modifications. 10 g of the egg yolk oil was transferred to a 250 ml flask, then 40 ml of ethanol-methanol-2-propanol (90 : 5 : 5) solution and 10 ml 60% KOH were added. The flask was connected to the water-cooled condenser and refluxed for 1 hour. After cooling the mixture to room temperature, 100 ml of hexane were added and the mixture was stirred for 10 min. Then 25 ml of deionized water were added and the mixture stirred for a further 15 min. After the layers were separated, hexane layer was collected in an Erlenmeyer flask. An aliquot of 25 ml from the hexane layer was evaporated in a rotary evaporator at 40 °C. The residue was dissolved in 2 ml of ethanol and 3 µl were injected into a gas chromatograph (Chung et al., 2004). For analysis Shimadzu GC 2010 Plus with flame ionization detector was used. GC conditions: column DB-5 (30 m × 0.32 mm × 0.25 µm), carrier gas: nitrogen, constant flow 0.45 ml min^{-1}, temperature program: 260 °C, 6 °C min^{-1}, 290 °C (8 min), injector: 300 °C, split 1 : 1, detector (FID): 300°C. The results were the means of three replicates with standard deviation and expressed in mg 100 g^{-1}.

RESULTS AND DISCUSSION

Egg yolk oil used in this study was obtained by the solvent extraction from liquid egg yolk. Ethanol and hexane were used as less toxic solvents which are widely used in food processing. The parameters of extraction process were chosen to produce safe and qualitative product. Extraction solvent residue in the ready product was below acceptable limits (Kovalcuks, 2014). But egg yolk oil extraction from liquid egg yolk met some problems, therefore it was necessary to separate polar egg yolk lipids from non-polar lipids, so as a result non-polar lipid fraction became the egg yolk oil (Kovalcuks, 2014). Most of egg yolk bioactive compounds are non-polar (fat-soluble), but some are polar causing losses of these compounds in non-polar egg yolk oil.

Egg yolk oil bioactive compounds were compared to mature humane breast milk because colostrum and transitional milk are highly variable in terms of chemical content and their feeding to infants lasts only for few weeks.

Fat is the most variable macronutrient of mature human milk and its content varies average from 3 to 5% (Sala-Vila et al., 2005). Bioactive compound content of egg yolk oil was expressed in mg $100g^{-1}$ fat or mg kg^{-1} fat, but data, found in literature, about micronutrient in human breast milk or infant formula are mostly expressed in 100 ml of milk, therefore results of human milk and infant formulas were recalculated on 100% fat content where average fat content of human breast milk and formulas was accepted as 4%.

Fatty acids

Fatty acid content of human breast milk varies by country (Lassek & Gaulin, 2014). Some authors (Ballard & Morrow, 2013; Soleimani et al., 2013) explain variety of fatty acid content by difference in mothers' diets. Fatty acid content of human breast milk of mothers from different countries is summarized in Table 1 (Saphier et al., 2013; Yuhas et al., 2006).

Table 1. Composition of essential fatty acid in egg yolk oil and breast milk of mothers from different countries*

Fatty Acids	Fatty acid content, g $100g^{-1}$ total lipids	
	Egg yolk oil	Human breast milk**
Myristic acid (14:0)	0.16 ± 0.02	3–12
Palmitic acid (C 16:0)	20.74 ± 0.07	20–23
Palmitoleic acid (C16:1)	2.08 ± 0.18	3–4
Stearic acid (C 18:0)	6.58 ± 0.32	5–7
Oleic acid (C18:1n9)	50.43 ± 0.89	31–38
Linoleic acid (C18:2n6)	15.57 ± 0.02	12–20
α-linolenic acid (C18:3n3)	1.83 ± 0.02	1–2
Arachidonic acid (20:4n6)	0.02 ± 0.01	0.4–0.5
Docosahexaenoic acid (C22:6n3)	1.17 ± 0.19	0.17–0.30

* – Israel, Australia, Canada, Chile, China, Japan, Mexico, Philippines, United Kingdom, United States;
** – fat content 4.0%.

Recommendations on fatty acid content for infant formulas usually are based on average fatty acid content of human breast milk. The best recommendation could be given if data were collected from each country and were applied for this particular country.

Saphier et al. (2013) declare that about 70% of total fatty acids in the human breast milk comprised palmitic, oleic and linoleic acids. Palmitic, oleic and linoleic acids in egg yolk oil contain more than 85% of total fatty acids. The saturated fatty acids (SFA) in breast milk are stable regardless of mother's diet (Yuhas et al., 2006). The same is observed for egg lipids, where hen's diet does not affect SFA content in eggs (González-Muñoz et al., 2009). Palmitic and stearic acid content was similar in both breast milk and egg yolk oil. Concentration of polyunsaturated fatty acids (PUFA), in opposite, depends on human and hen's diet (Saphier et al., 2013; Soleimani et al., 2013). Linoleic and α-linolenic fatty acid content was at the same level in breast milk and egg yolk oil, but oleic acid content in egg yolk oil was higher than in human milk. As PUFA content in eggs can be 'designed' through hen's diet, it is possible to produce eggs which will more precisely mimic PUFA content of human breast milk (Simopoulos & Salem, 1992). Arachidonic (ARA) and docosahexaenoic (DHA) acids always raise special attention due their high biological value. ARA and DHA content in human breast milk is very low and usually do not cover infant needs of these compounds (Lassek & Gaulin, 2014). Egg yolk oil used in this study was high in DHA, but low in ARA. These two essential fatty acids come from phospholipids and their content depends on phospholipid content in egg lipids (egg yolk oil).

Fat–soluble vitamins

Fat–soluble vitamin A, D and E content in egg yolk oil, human breast milk and recommendations for the content of these vitamins in infant formulas are presented in Table 2.

Babies are born with low stores of vitamin A. Probably due to this reason colostrum contains the highest concentration of vitamin A. Later concentration of vitamin A decreases (Fujita et al., 2011). According to Lawrence & Lawrence (2016) vitamin A concentration in mature milk is on average 18.75 mg kg^{-1} fat. Recommendations for infant formulas propose to use vitamin A at levels of 15–45 mg kg^{-1} fat (Berthold et al., 2005). A lot of children around the world feel the lack of vitamin A that causes many

deaths of young children (WHO, 2011). But in the same time high dosages of vitamin A can be toxic. There are strong recommendations regarding upper limits of vitamin A for infant nutrition (Olson, 1989). Particular egg yolk oil contained two times lower vitamin A concentration than human breast milk, but taking into account that eggs can be enriched with vitamin A through hen diet (Jiang et al., 1994), egg yolk oil could be the good source of vitamin A for infants.

Table 2. Fat-soluble vitamin and lutein content in egg yolk oil, breast milk and recommendations of these compounds for infant formula

	Concentration, mg kg^{-1} fat		
Bioactive compound	Egg Yolk Oil	Breast Milk	Recommended**
Vitamin A	9.80 ± 1.18	av. 18.75	15–45
Vitamin D	0.127 ± 0.015	av. 0.015	0.25–1.25
Vitamin E	205.56 ± 24.67	25–246	125–1.250
Lutein	0.33 ± 0.07	0.375–1.425	max 6.25

* – fat content 4 %
** – for infant formulas (4% fat content in ready product)

Vitamin D is a very important compound, but its content in human breast milk is very low and inadequate for normal infant development. Therefore pediatricians recommend additional peroral supplement of vitamin D for all infants till they are 1 year old (Lawrence & Lawrence, 2016). Recommendations for vitamin D in infant formulas also offer higher concentrations than naturally observed in human milk (Berthold et al., 2005). Egg yolk oil contains highly bioavailable vitamin D in concentration of 0.127 ± 0.015 mg kg^{-1} fat and can be used as a natural source of vitamin D for infant nutrition.

A-tocopherol is the most active form of vitamin E therefore comparison of vitamin E content in egg yolk oil and human breast milk were made in regards of α-tocopherol. Vitamin E concentration in human breast milk depends on an individual and ranges between 25–246 mg kg^{-1} fat (Lima et al., 2014). Infants from 0 to 1 year require a higher dosage of vitamin E than in later life and based on this fact recommendations for vitamin E allow to use 125–1.250 mg kg^{-1} fat for infant formulas (Berthold et al., 2005).

Egg yolk oil contains vitamins A, D and E in concentrations similar to their content in mother milk and is an excellent source of these vitamins for infant nutrition.

Lutein

Lutein is a polar compound and its majority was extracted from liquid egg yolk with ethanol therefore lutein concentration in egg yolk oil (non-polar fraction of egg lipids) was at a level much lower than its possible content in egg yolk. According to Canfield et al. (2003) lutein content in human breast milk from mothers of different countries varies from 0.375–1.425 mg kg^{-1} fat and is considered to be very low and insufficient for infants. Therefore recommendations of lutein content for infant formulas allow using them in concentration up to 6.25 mg kg^{-1} fat (FSANZ, 2008). The difference between breast milk lutein and recommendations also lies in the fact that breast milk lutein is more bioavailable than lutein added to infant formulas (Lewis, 2014). Lutein content in human milk depends on mother's diet. Consumption of product rich in lutein increases lutein content in milk (Sherry et al., 2014). Egg yolk oil used in this study contains 0.33 ± 0.07 mg kg^{-1} of lutein that was less than the lower limit of lutein in breast

milk. Purified polar fraction of egg yolk lipids can be used as a valuable source of lutein for infant nutrition.

Phospholipids

Comparing to the human breast milk egg yolk lipids contain high amount of phospholipids (PLs), approximately 30% from total lipids. Main PLs of egg yolk are phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Ahn et al., 2006). Egg yolk oil contains 0.582 ± 0.009 mg $100g^{-1}$ of PC and 0.015 ± 0.002 mg $100g^{-1}$ of PE. The low concentration of PLs, the same as lutein, is connected to the polarity of these compounds. PLs are highly polar compounds and majority of PLs was extracted from egg yolk with ethanol to a polar lipid fraction. Due to the low content of PLs egg yolk oil cannot be considered as a good source of these bioactive nutrients. Relative absence of PLs negatively affects ARA content in egg yolk oil. According to Guiffrida et al. (2013) human breast milk contains 77–295 mg $100g^{-1}$ fat of PC and 80–240 mg $100g^{-1}$ fat of PE. PLs content in infant formulas is calculated taking in account desired ARA and DHA, and also choline concentration in ready product. Average dosage of total PLs can be at level of 2 g per L formula, or 5,000 mg $100g^{-1}$ fat.

Cholesterol

The average content of cholesterol in mature milk is convincingly stable at 240 mg $100g^{-1}$ fat (Lawrence & Lawrence, 2016). But according to Kamelska et al. (2012) cholesterol content in human milk is 84–492 mg $100g^{-1}$ fat. Cholesterol content in breast milk depends on an individual and changes during breast-feeding. Commercially produced infant formulas have very low cholesterol content (23–137 mg $100g^{-1}$ fat) (Kamelska et al., 2012). Cholesterol is important in proper development of nervous system, hormone and vitamin synthesis in the growing infant; therefore lack of this compound can have negative effect on his/her normal development. Egg yolk lipids naturally contain a high amount of cholesterol. Egg yolk oil presented in this study contains 3,105 mg 100 g^{-1} fat of cholesterol and it is 10 times more than presented in breast milk. For a developing infant probably it can be considered as a benefit, but it must be discussed with pediatricians.

Phospholipids and cholesterol content in egg yolk oil, breast milk and infant formula is mentioned in Table 3.

Table 3. Phospholipids and cholesterol content in egg yolk oil, breast milk and infant formula

	Concentration, mg $100g^{-1}$ fat		
Bioactive compound	Egg Yolk Oil	Breast Milk*	Infant formula*
Phosphatidylcholine (PC)	0.582 ± 0.009	77–295	av. 5,000
Phosphatidylethanolamine (PE)	0.015 ± 0.002	80–240	
Cholesterol	3,105	84–492	23–137

* – fat content 4%

CONCLUSIONS

- Fatty acids of egg yolk lipids more closely mimic the fatty acid composition of human breast milk. Moreover, fatty acid profile of egg yolk lipids can be affected by a hen diet which allows providing the product for specific customer needs. Fatty acid profile of egg yolk oil used in this research was similar to breast milk in terms of palmitic, stearic, linoleic and α–linolenic acids. DHA content was

1.17 ± 0.19 mg $100g^{-1}$ being in compliance with infant needs, but content of ARA was much lower than found in human breast milk. Content of ARA in egg yolk oil was 0.02 ± 0.01 mg $100g^{-1}$ in comparison with 0.4–0.5 mg 100 g^{-1} fat in human breast milk.

- Egg yolk oil contains essential fat-soluble vitamins such as vitamins A, D and E. There is no doubt about their importance for a developing organism. But concentration of these vitamins in infant food is of utmost importance. Vitamin A concentration in particular egg yolk oil was twice lower (9.80 ± 1.18 mg kg^{-1}) than observed in human milk, but it can be easily improved by enrichment of eggs with vitamin A through hens' feed. Vitamin D concentration in egg yolk oil was in the middle between its content in breast milk and recommended by pediatricians for infant nutrition. Usually the lack of vitamin D in infant nutrition is compensated by additional oral supplement via food additive (vitamin D drops). But more important is to receive vitamin D in natural and highly bioavailable form. Egg yolk oil used in this research can provide 0.127 ± 0.015 mg kg^{-1} of natural vitamin D. Vitamin E content in egg yolk oil was 205.56 ± 24.67 mg kg^{-1}. This natural antioxidant content in human breast milk can be found in concentration 25–246 mg kg^{-1} therefore egg yolk oil can fully compensate infant needs of this vitamin.
- Breast milk contains a high amount (84–492 mg 100 g^{-1} fat) of cholesterol, while infant formulas contain much less (23–137 mg $100g^{-1}$ fat). Egg yolk, being an animal source product, naturally contains a high amount of cholesterol that is perceived as a disadvantage for adult nutrition, but not for infants. High content of cholesterol (3,105 mg 100 g^{-1}) in egg yolk oil can be considered as a benefit in terms of infant nutrition, since it is responsible for brain and central nervous system development of an infant.
- Lutein content in egg yolk oil, due to specifics of extraction process, was low (0.33 ± 0.07 mg kg^{-1}) comparing to its content in human milk (0.375–1.425 mg $100g^{-1}$ fat). Lutein is a polar compound and in two solvent system, containing ethanol and hexane, majority of lutein was extracted into polar ethanol phase. Bioavailability of egg yolk lutein is much higher than from other sources therefore addition of egg yolk lutein in infant formulas has an important benefit for infant health and development.
- The main egg yolk oil phospholipids are phosphatidylcholine and phosphatidylethanolamine whose summary content in extracted egg yolk oil was 0.597 mg 100 g^{-1}. The same as lutein, phospholipids are polar compounds and their content in egg yolk oil depends on solvents used for extraction process. Phospholipids provide infants with ARA, DHA and choline, essential nutrients for normal infant development, therefore low content of phospholipids in egg yolk oil must be compensated by other ingredients.
- Purified polar fraction of egg yolk lipids, containing polar bioactive compounds such as lutein and phospholipids can be used as a separate additive for infant formulas increasing their biological value.
- High content of bioactive compounds of egg yolk oil makes it an ideal ingredient for infant formulas, supplying infants with PUFA, fat-soluble vitamins and cholesterol.

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