FRACTIONATION OF HEN EGG AND OAT LIPIDS WITH SUPERCRITICAL FLUIDS

CHEMICAL AND FUNCTIONAL PROPERTIES OF FRACTIONS

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CONTENTS

ABSTRACT

Hen eggs and oats (*Avena Sativa*) are important materials for the food industry. Today, instead of merely satisfying the feeling of hunger, consumers are asking for healthier, biologically active and environmentally friendly products. The growing awareness of consumers' increasing demands presents a great challenge to the food industry to develop more sustainable products and utilise modern and effective techniques.

The modification of yolk fatty acid composition by means of feed supplements is well understood. Egg yolk phospholipids are polar lipids and are used in several applications including food, cosmetics, pharmaceuticals, and special nutrients. Egg yolk phospholipids are excellent emulsifiers, typically sold as mixtures of phospholipids, triacylglycerols, and cholesterol. However, highly purified and characterised phospholipids are needed in several sophisticated applications. Industrial fractionation of phospholipids is usually based on organic solvents. With these fractionation techniques, some harmful residues of organic solvents may cause problems in further processing.

The objective of the present study was to investigate the methods to improve the functional properties of eggs, to develop techniques to isolate the fractions responsible for the specific functional properties of egg yolk lipids, and to apply the developed techniques to plant-based materials, too. Fractionation techniques based on supercritical fluids were utilised for the separation of the lipid fractions of eggs and oats. The chemical and functional characterisation of the fractions were performed, and the produced oat polar lipid fractions were tested as protective barrier in encapsulation processes.

Modifying the fatty acid compositions of egg yolks with different types of oil supplements in feed had no affect on their functional or sensory properties. Based on the results of functional and sensory analysis, it is evident that eggs with modified fatty acid compositions are usable in several industrial applications. These applications include liquid egg yolk products used in mayonnaise and salad dressings.

Egg yolk powders were utilised in different kinds of fractionation processes. The precipitation method developed in this study resembles the supercritical anti-solvent method, which is typically used in the pharmaceutical industry. With pilot scale supercritical fluid processes, non-polar lipids and polar lipids were successfully separated from commercially produced egg yolk powder and oat flakes. The egg and oat-based polar lipid fractions showed high purities, and the corresponding delipidated fractions produced using supercritical techniques offer interesting starting materials for the further production of bioactive compounds. The oat polar lipid fraction contained especially digalactosyadiacylglycerol, which was shown to have valuable functional properties in the encapsulation of probiotics.

LIST OF ABBREVIATIONS

AA	aracidonic acid (20:4ω-6)
ALA	alpha-linolenic acid (18:3ω-3)
BC	black currant
DGDG	digalactosyl diacylglycerol
DHA	Docosahexaenoic acid (22:6ω-3)
EPA	5,8,11,14,17-eicosapentaenoic acid (20:5ω-3)
EYP	egg yolk powder
EYPro	delipidated egg yolk powder
FO	flax oil
HDL	high-density lipoprotein
GLs	glycolipids
GC	gas chromatography
LA	linoleic acid (18:2 ω6)
LDL	low-density lipoprotein
MUFA	monounsaturated fatty acids
LPC	lysophosphatidylcholine
NLs	neutral lipids
PLs	phospholipids
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositole
PS	phosphatidylserine
PUFA	polyunsaturated fatty acids
RESS	rapid expansion of supercritical solutions
RO	rapeseed oil
SAS	supercritical antisolvent process
SFA	saturated fatty acids
SC-CO_2	supercritical carbon dioxide
SF	supercritical fluid
SFE	supercritical fluid extraction
SFEE	supercritical fluid in the extraction from emulsion
SM	sphingomyelin
TAG	triacylglycerol

LIST OF ORIGINAL PUBLICATIONS

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II Aro, H., Järvenpää, E., Könkö, K., Sihvonen, M., Hietaniemi, V., Huopalahti, R. (2009). Isolation and purification of egg yolk phospholipids using liquid extraction and pilot-scale supercritical techniques. *European Food Research and Technology*, 228, 857-863.

III Aro, H., Järvenpää, E., Könkö, K., Huopalahti, R., Hietaniemi, V. (2007). The characterisation of oat lipids produced by supercritical fluid technologies. *Journal of Cereal Science*, 45, 116-119.

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1. INTRODUCTION

Lipids are found in all living cells. Together with proteins and carbohydrates, lipids are responsible for the structure and physical and chemical functions of food. Moreover, dietary lipids have an essential role in human nutrition. They possess high energy value, they have important functions as carriers of vitamins and other fat-soluble compounds, and their ability to create complex chemical structures is the background for many traditional functional properties of foods. Lipids possess many undesirable properties, too. For a long time, different kinds of diseases and toxicities have been connected to the lipids in food. Moreover, the consumer will not buy any food if the sensory quality is acceptable. For example, during food processing, lipids undergo many chemical changes that may be fatal to the sensory quality of the food.

Triacylglycerols (TAGs) make up to 99 % of the lipids (fats and oils) found in plants and animals. All basic foods (e.g., milk, meat, eggs, cereals, and vegetables) contain lipids. Hen egg yolk is a good example of a food rich in lipids. One egg yolk contains about 30-35% lipid structures of different kinds. After spray-drying, egg yolk powder contains up to 65% lipids. Especially in institutional kitchens, egg yolk powder and other powdered egg products are important ingredients in cooking. Moreover, when the crucial role of dietary cholesterol as a risk factor of unhealthy lipid profiles in humans is re-evaluated, eggs have been accepted as an important part of balanced everyday nutrition. In the non-food sector, egg lipids have been traditionally used in cosmetics and medical applications.

Among the cereals, oats are unique due to their high oil content, which varies between 4% and 12%. In Finland, oats have been typically considered as feed. During the last decades, the healthy effects of oat carbohydrate β-glucane have increased interest in oats as food. Especially now, when obesity has been recognised as an increasing risk factor for coronary heart diseases (CHD), the beneficial effects of oats and oat ingredients in controlling weight are under continuous investigation.

Knowledge about the biological activities of food ingredients is increasing. At the same time, the need for new, more sustainable separation techniques increases. The separation of food lipids is usually based on the use of organic solvents. However, the possible solvent residues in final products may incur unwanted effects in further processing, or even be harmful for consumers. Supercritical fluid extraction (SFE) has been utilised in many separation processes, and it has been proposed to replace organic solvents in industrial lipid separation processes. In SFE processes, the typical extraction medium is supercritical carbon dioxide (SC-CO_2). Recently, supercritical fluids have begun to be

also used as an antisolvent. The micronization of pharmaceutical compounds is a typical application of supercritical antisolvent (SAS) processes.

In this thesis, the impact of modified fatty acid composition of hen eggs on the functional properties of eggs was first evaluated. The modification of fatty acid composition of eggs was based on the modification of fatty acid composition in the diet of hens. For further investigation of functional properties, the pilot-scale supercritical techniques to separate the valuable lipid components hen egg yolk powder were studied, and similar pilot-scale techniques were further applied to the fractionation of oats. The yields, purities, and functional properties of the different lipid and delipidated fractions were determined. First, a technique resembling supercritical anti-solvent precipitation (SAS) was developed to produce pure phospholipids from dried hen egg yolk. Secondly, a similar technique was adapted to the separation of polar lipids from oats. Special attention was paid to create a process after which the further processing of the lipid fractions and delipidated by-products would be easy and economical.

In the literature review, data about the different lipid compositions of hen egg yolk and oat have been collected. The supercritical processes and applications are discussed. The discussion about the egg and oat-based ingredients are included in this review.

2. REVIEW OF LITERATURE

2.1. THE USE OF SUPERCRITICAL FLUIDS (SFs) IN PROCESSING OF HEN EGG YOLKS AND OATS

The first findings of the disappearance of gas-liquid boundaries of substances in high temperatures were made in the beginning of nineteenth century. Hanay and Hogarth (1879) reported observations of the solvent power of supercritical fluids. During the next decades, only a few progressive steps were done in the area of SFs. In 1970, a patent considering the decaffeination of green coffee using supercritical carbon dioxide (SC-CO_2) was launched (Zosel, 1974). This process was an important milestone in the industrial use of SC-CO_2.

2.1.1. Supercritical state and extraction process with supercritical fluids

Several books have been published considering the principles and practises of supercritical fluids (McHugh and Krukonis, 1994; King and List, 1996; Clifford and Clifford, 1999; Belinsky, 2010). In all supercritical extraction and/or precipitation processes, the temperature and the pressure of a medium are raised over their critical values, and the supercritical state is achieved. In this state, the distinction between the liquid and the gas phase has disappeared, and the fluid can no longer be liquefied by raising the pressure nor can gas be formed by increasing the temperature. In a supercritical state, the substance has both gas-like and liquid-like properties, which provide suitable conditions for extracting compounds in a short time with high yields (Sihvonen et al., 1999).

The major extraction medium is carbon dioxide (CO_2). It has several advantages: cheap and abundant worldwide, non-toxic, non-flammable, and environmentally friendly. The critical temperature of CO_2 (31°C) is close to room temperature, and the low critical pressure (74 bars) offers the possibility to operate with moderate pressures, generally between 100 and 450 bar (Guclu-Ustundag and Temelli, 2005). The major variables influencing the extraction efficiency are temperature, pressure, particle size and moisture content of feed material, time of extraction, flow rate of CO_2, and solvent-to-feed-ratio (Temelli, 2009).

The first commercial supercritical fluid extraction (SFE) application was performed in Germany in 1978 by Hag A.G. (Sahena et al., 2009). Until now, SFE has been used in several food fractionation processes, including hundreds of non-polar or volatile compounds in different kinds of foods (Hierro & Santa-Maria, 1992; Kerrola, 1995; Reverhcon et al., 1995; Reverchon, 1997; Marr and Gamse, 2000; Espinosa et al., 2002; King, 2002; Reverchon and De Marco, 2006).

In the food industry, new sustainable separation methods to obtain natural, highly purified, healthy components are under continuous investigation. The environment, clean technology, and the need for ultra-pure products create a great challenge for the food processing industry. Supercritical fluids give one alternative to conventional, usually solvent-based extraction technologies (Raventós et al., 2002; Sahena et al., 2009).

According to Temelli (2009), fractionation of the lipid mixtures can be achieved with three different approaches: collecting fractions as a function of time, using several separators in series, or using a fractionation column. The solubility of more polar lipids depends on the concentration of the co-solvent concentration, temperature, and pressure. The type of polar lipid has a critical role in the extraction efficiency (Catchpole et al., 2009). Using ethanol as a co-solvent, Cocero and Calvo (1996) found that the extraction efficiency of phospholipids (PLs) seems to be linearly correlated to the increasing ethanol concentration. Moreover, they reported that the solubility of PLs decreases if neutral lipids have been completely removed. This result suggests that neutral lipids (NLS) may have co-solvency effects on PLs extraction.

2.1.2. Supercritical fluids in precipitation and micronization processes of food ingredients

The first precipitation and micronization processes with SFs were originally developed for the pharmaceutical industry. The supercritical anti-solvent (SAS) process is one of the micronization techniques developed for micronic and nanometric particle production in supercritical conditions. In this process, SFs are used as an antisolvent to produce particles of a controlled shape and size without organic solvents. Several process arrangements and equipment have been tested, and a number of different acronyms for this process has been suggested (Reverchon, 1999).

Until now, a large number of antisolvent applications with SFs have been presented. These applications include, for example, the production of polymers, colouring matters, superconductors, catalysts, and inorganic compounds (Reverchon, 1999; Jung and Perrut, 2001; Hakuta et al., 2003; Adami, 2007; Yang et al., 2011; Zhao et al., 2011). Especially in food applications, by adjusting the solvent power and varying the process parameters, the precipitation of biologically active substances is possible (Pasquali et al., 2006; Marco and Reverchon, 2011).

2.1.2.1. Supercritical fluids in fractionation processes of eggs

Probably due to its high lipid content, dried egg yolk has been used as a starting material in many SFE applications. The first successful attempts to extract cholesterol and other lipids from dried egg yolk with SC-CO_2 was done over 25 years ago (Froning et al.,

1990; Rossi et al., 1990). In these studies, it was concluded that PLs are extractable from an egg matrix by adding an organic modifier to SC-CO_2. During the next few years, it was found that SC-CO_2 preferentially extracted the TAGs and cholesterol, with the relative cholesterol content being dependent on temperature and pressure. The total recovery of lipids was found to be 60-70 % (Bulley et al., 1992; Sun et al., 1995; Boselli et al., 1997; Paraskevopolou et al., 1997). Based on similar results, a dry egg yolk ingredient called Eggcellent was launched. In this product, the PLs and proteins are retained in the egg yolk. The structures and functional properties of this product were evaluated as being close to fresh egg yolk, and the nutritional value of the product was good (Bringe, 1997). In a more recent study, Boselli and Caboni (2000) showed that with neat SC-CO_2, it is possible to extract even more polar PLss from dried egg yolk on an analytical scale. Using the maximum extraction capacity of their analytical instrument (517 bar, 40°C), the yield of extract was 67 g of lipids per 100 g of egg yolk powder. With the traditional organic solvent (chloroform/methanol/water) -based extraction, the corresponding result was 63 g per 100 g of sample. Considering the yields of the PLs, the solvent-based extract contained 29 % PLs, and the neat SC-CO_2 extract consisted of 26 % PLs. In HPLC analysis, the PLs classes showed similar results both in SC-CO_2 extraction and organic solvent based extraction.

A mathematic model for the extraction of egg yolk oil with SC-CO_2 was published by Wu and Hou (2001). They evaluated the external mass transfer and the equilibrium between solvent and solute with three adjustable parameters: partition factor, overall mass transfer coefficient, and adsorption equilibrium constant. This model was criticised by Patel et al. (2011), who suggested that correlation of adsorption equilibrium constant as a function of temperature is not valid outside the specific range of temperature. The simple model developed by Patel et al. (2011) is based on solubility and diffusion related to mass transfer. This model agrees with the experimental results, but this is not tested with egg yolk.

Shah et al. (2004) described an analytical-scale method for the production of PLs-rich egg yolk fraction from dried egg. In this two step method, SC-CO_2 was used in a first step to extract the non-polar lipids, and ethanol as co-eluent in the second step to extract more polar PLs. The economical aspects of using supercritical fluids in fractionation of bioactive egg yolk components have been evaluated by Froning (2008).

2.1.2.2. Supercritical fluids in fractionation processes of oats

When compared to other cereals, the lipid content of oat is quite high, typically varying from 2 to 12 %. (Zhou et al., 1999). The majority of oat lipids are bound in the endosperm (Youngs et al., 1977). Moreover, the increase in total lipid content of different oat varieties seems to be accompanied by an increase in all individual fatty acids

(Zhou et al., 1998). In certain cases, the high lipid content makes oat lipids susceptible to rancidity problems during processing (Lehtinen et al., 2003). Oat lipids contain several antioxidants (Peterson, 2001), and oat lipids are typically highly unsaturated and contain essential fatty acids. For these reasons, oat lipids are regarded as nutritionally important (Youngs, 1986). Besides containing nutritionally valuable neutral lipids, oat is an important source of glycolipids providing a large proportion of digalactosyl diacylglycerol (DGDG) (Forssell et al., 1992; Andersson et al., 1997). Glycolipids are typical membrane lipids with sugar residues on the extracellular side of the membrane.

Oat lipids contain both non-polar and polar lipids, and the separation of these lipids is usually based on their different polarity. The polar lipids (mainly bound lipids) include glycolipids and PLs (Alkio et al., 1991). The traditional way to separate oat polar lipids is the extraction of oat flakes with polar organic solvents. The solvent itself, the extraction time, and the method used are important factors in process planning (Youngs, 1986).

So far, only a few applications are reported on the extraction of cereal lipids with SC-CO_2. The extraction efficiencies of wheat flour lipids with SC-CO_2, the AACC-approved Soxhlet method, and the AOCS official Butt method have been evaluated by Hubbard et al. (2004). The SC-CO_2 extract contained less neutral and more polar lipids than other methods. Qualitatively, all tested methods extracted lipids with similar components. SC-CO_2 extraction was evaluated for determining the surface lipid content of milled rice with two other extraction systems by Rohrer et al. (2004). According to their results, accelerated solvent extraction and Soxtec extraction produced more rice surface lipids than extractions with SC-CO_2.

Fors and Eriksson (1990) reported the extraction of oat lipids from two Swedish oat varieties with SC-CO_2. Andersson et al. (1997) described a semi-continuous batch process for the production of DGDG from oat oil. This process was similar to the rapid expansion of supercritical solutions (RESS) technique, which has been used in the production of fine particles. Alkio et al. (1991) removed the non-polar lipids from oat oil by using SC-CO_2, and Forssel et al. (1992) compared methods of removing polar lipids from oat oil. According to their analysis, polar fraction from SC-CO_2 extraction had the highest phospholipid and lowest tocopherol content. To isolate more glyco- and phospholipids, co-solvents have been added to the fluid. Suitable food-grade co-solvents for cereals are ethanol and water (Fors and Eriksson, 1990; Dunford and Zhang, 2003).

2.1.3. Other possible supercritical techniques in yolk and oat fractionation

One of the most challenging ways to utilise the supercritical fluids is the production of micronized particles with a controlled size and shape (Jung and Perrot, 2001). The classification of these techniques is presented by Adami (2007). In this process-based

classification, the role of SCF is evaluated to be solvent, solute, anti-solvent, or reaction media. Most of the processes are batch or semi-continuous processes, thus probably difficult to use in the food industry.

The use of supercritical fluid in the extraction from emulsion (SFEE) is a novel process presented by Chattopadhyay et al. (2004). In this process, the wanted compounds are micronized from typical, solvent containing oil-in-water or multiple emulsion. This method has been further developed to micronize cholesterol acetate (Shekunov et al., 2006) and to produce dry-encapsulated products (Chattopadhyay et al., 2006). An interesting advantage of this process is that, in principle, it is possible to modify the final particle size by modifying the size of the droplets in the emulsion (Mattea et al., 2009). SFEE-based applications related to food or natural substances have been presented by DellaPorta and Reverchon (2008), Kluge et al. (2009), and Mattea et al. (2009).

2.2. HEN EGG YOLKS AND THEIR LIPID COMPOSITION

2.2.1. Hen eggs

In the beginning of the 20th century, the production of the hen eggs started to increase dramatically. Before this, hens were kept in small groups on farms, but a new innovation – automatic hatching – enabled the production of eggs on an industrial scale. An entirely new industrial brand began.

Global egg production and trade in eggs and egg products have shown a remarkable dynamic during the last 35 years. When analyzing the period between 1970 and 2005, poultry meat and egg production increased faster than that of beef and veal or pig meat. In hen egg production, Asia was the only winner; the other continents lost market share. However, hen eggs are still mainly traded between European countries and between Asian countries (Windhorst, 2006).

In 2008, 6.27 million tons of eggs were produced in the EU-27 countries (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxemburg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom). This production is about 10 % of the world production. European egg consumption was reported to be 235 eggs per person per year, with strong variations among the different countries (Pascale, 2009). In Finland, the production in 2010 was 61.5 million kg, and the average consumption was 9.8 kg per person, corresponding to about 160 eggs per year (Ministry of Agricultural and Forestry).

Hen eggs are typically consumed as shell eggs. In industrial production, only a few egg ingredients are utilised in the manufacture of food or non-food products (Anton et al., 2006; Mine and Kovacs-Nolan, 2006). The primary aim of the egg is to give rise to a new generation of hens. For that reason, avian egg is a complete set of biological substances containing nutrients such as proteins, lipids, inhibitors, enzymes, and various biologically active substances, including growth-promoting factors as well as defence factors against bacterial and viral invasion (Mine and Kovacs-Nolan, 2004; Anton et al., 2006; Wu et al., 2010). The shell of the egg functions as a mechanical barrier against external strikes. Protein membranes on both sides of the shell matrix prevent the invasion of unwanted microbes inside the egg. Albumen contains several protein components with antimicrobial activity, and egg yolk is a reservoir of energy for the developing embryo (Hiidenhovi et al., 2004). Based on these facts, it is easy to conclude that eggs are an endless reserve for many bioactive compounds (Anton et al., 2006).

Hen eggs are one of the most versatile foods for humans. For a long time, hen egg was used as a nutritional standard to which other food was compared. Eggs are multifunctional (e.g., foaming, emulsification, and coagulation properties), and eggs can be stored as shell eggs for a long time (Burley & Vadehra, 1989). Moreover, eggs are an excellent source of high-quality protein and they give a balanced distribution of minerals and vitamins, particularly vitamins E, A, B_{12}, B_2, and folate (Surai & Sparks, 2001). In several studies, it has been shown that the fatty acid composition of egg lipids can be modified by changing the diet of the laying hens. These enriched eggs typically contain at least ten times more ω-3 fatty acids, especially linolenic acid (18:3ω3) (Farrell, 1994; Ferrier et al., 1995, Beynen, 2004; Woods and Fearon, 2009). In addition, the positive dietary effects of various functional feeds have been found at least on yolk carotenoids and vitamins (Hammershøj, 1995; Franchini et al., 2002; Mattila et al. 2003; Fredriksson et al., 2006). However, the possible effects of functional feeds on reducing the amount of cholesterol in yolks have remained unclear (Bovet et al., 2000; Elkin, 2006).

The average weight of an egg is about 60 g. Traditionally, an egg is divided into three parts; shell, albumen, and yolk. The shell consists of 10 **%** of the weight, albumen (egg white) 60 %, and yolk 30 %. Laying hens are typically brown or white, but the chemical composition of different colours of eggs are the same. An average egg provides about 6 g of lipids, which are exclusively positioned in the egg yolk. Lipids in the egg yolk are made up of 62 % TAGs, 33 % PLs, and less than 5 % cholesterol. Carotenoids give the typical colour to the yolk, and they represent less than 1 % of yolk lipids (Anton, 2007).

Schematic structure of a fresh egg is presented in Figure 1.

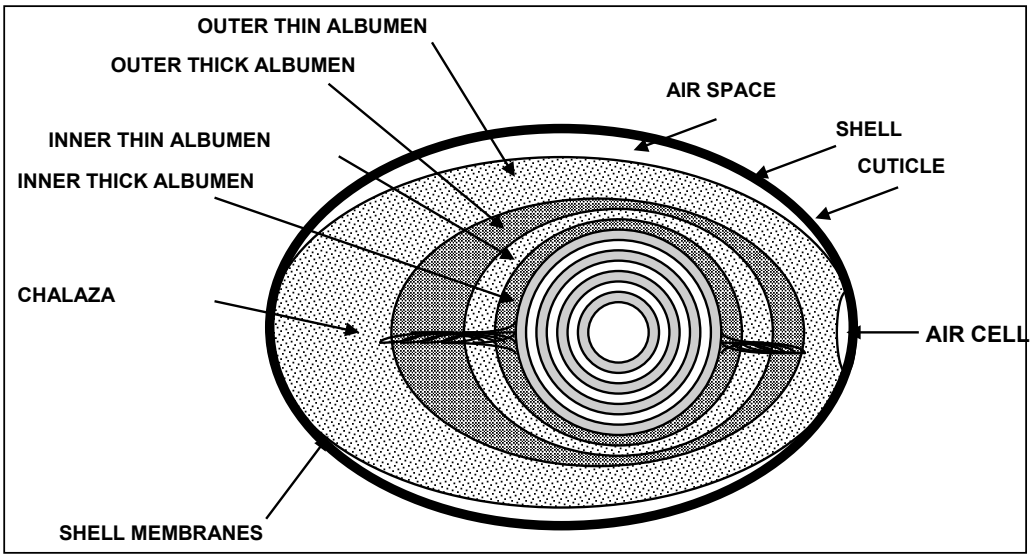


Figure 1: Schematic structure of a fresh hen egg (modified from Aro, 1998).

The eggshell – The eggshell is made of a protein fibre matrix and calcite or calcium carbonate crystals. The surface of the shell matrix is covered by a protein layer, the cuticle. And eggshell contains 98.2 % calcium, 0.9 % magnesium, and 0.9 **%** phosphorus.

Albumen - Albumen has four layers: outer thin white, thick white, inner thin white, and a chalaziferous layer. The albumen mainly consists in proteins (11-13 **%**) and water (87-89 **%**).

Egg yolk – The total solid content of egg yolk is about 50 **%**. Egg yolk contains about 32-36 **%** of lipids and 15-17 **%** of protein. One-third of the total lipids are PLs (Burley and Vadehra, 1989, Stadelman and Cotterill, 1995). The chemical composition of hen egg yolk is presented in Table 1.

Table 1. The chemical composition of hen egg yolk (Kovacs-Nolan and Mine, 2004)

Component	Proportion %	Sub-components	Proportion (%)
PROTEINS	**15.7-16.6**	Apovitellenin (I-VI)	37.3
		Lipovitellenin apoproteins α-lipovitellenin β-lipovitellenin	40.0
		Livetins α-livetin β -livetin γ-livetin	9.3
		Phosvitin	13.4
		Biotin-binding protein	tr.
LIPIDS	**32-36**	Triacylglycerol (TAG)	66
		Phosphatidylcholine (PC)	24.0
		Phosphatidylethanolamine (PE)	2.8
		Lysophosphatidylcholine (LPC)	0.6
		Sphingomyelin	0.6
		Cholesterol	5.0
		Others	1.0
CARBOHYDRATE	**0.2-1.0**		
ASH	**1.1.**		

2.2.2. Dehydration of egg yolks

Shell eggs are the most common way to sell eggs. However, egg products are also widely marketed in liquid, frozen, and dried form. These egg products are typically used by the food service industry and as ingredients in other foods. Especially in institutional kitchens, dried egg products are used as an essential part of recipes. Dehydration improves the storage time of food. The removal of water stops the growth of microorganisms and slows down chemical reactions. To ensure the edible food for the whole year, man has had dried fruits, meat, and other foods for centuries (Bergquist, 1995).

By spray drying, it is possible to remove water from liquid egg white, liquid egg yolk, or liquid whole egg. In the spray drying process, the evaporation of water is fast due to the large surface area of the particles (Bergquist, 1995). However, the conditions before and during processing and the quality of the raw material strongly affect the quality and safety of spray-dried egg products (Galobart et al., 2002). Most eggs are spray-dried by heating at an inlet temperature between 121°C and 232°C. Atomizing is accomplished by spraying with high–pressure nozzles into a hot air stream, which instantly evaporates water. Generally, indirect heating such as steam coils are preferred to prevent off-flavours or the formation of nitric oxides, which may be formed by direct heat using natural gas or propane (Froning, 2008b).

The yolk itself is a complex combination of proteins, lipids, and other constituents. Dried egg yolk typically contains 62,5 % lipids, 33.0 % proteins, 1.2 % carbohydrates, and 3.5 % minerals (Powrie and Nakai, 1986). Drying the egg yolk is challenging due to the high content of emulsifying lipids. Moreover, yolk lipids are exclusively associated with lipoproteins (Anton, 2007) and to some extent to other components of the yolk (Bergquist, 1995). In general, these interactions are largely responsible for the great functional properties of eggs. Drying the eggs reduces these functionalities; especially the foaming ability is lower in powder than that of the native yolk. Some off-flavours may also develop during long-term storage of egg yolk powder (Schultz et all., 1968).

2.2.3. Lipids in egg yolk

Egg yolk can be characterised as a complex system containing a variety of particles suspended in a protein solution (Li Chan et al., 1995). The five major structural constituents of egg yolk are: low-density lipoprotein (LDL, 68 %), high-density lipoproteins (HDL, 16 %), livetins (10 %), phosvitin (4 %), and other minor proteins (2 %). These constituents have a strong capacity to adsorb at the oil-water interfaces (Anton et al., 2001).

In hatching, neutral lipids act mainly as an energy supplying source, while the PLs and cholesterol are important ingredients promoting the formation of somatic cell structures and the cell membrane of cranial nerve cells (phospholipid bilayer) of the chick. In general, egg yolk lipids provide a high content of PLs and cholesterol as the constituent materials in the cell membrane (Juneja, 1997).

2.2.3.1. Triacylglycerols (TAGs)

TAGs represent the main part of the total fat in yolk. The main role of the TAGs is to provide energy for the developing embryo. According to Ternes and Leitsch (1997), the glycerol backbone is mainly esterified with palmitic acid (16:0, 24 %), oleic acid (18:1, 50 %), and linoleic acid (18:2, 11 %). Palmitic acid is typically found in sn-1 position, oleic and linoleic acids in sn-2 position, and oleic, palmitic and stearic (18:0) acids in sn-3 position (Kuksis, 1992). The susceptibility for oxidation of the yolk unsaturated fatty acids is exceptionally low (Burley and Vadehra, 1989).

2.2.3.2. Cholesterol

Cholesterol is found in all animal-based food. Cholesterol is a sterol, representing about 5 % of total lipids in yolk. It can be found either free or in esterified form. The cholesterol content of eggs has often been emphasised as a negative dietary factor (Froning, 1994). Many nutritional recommendations advise people to consume no more than 300 mg cholesterol per day, and limit the consumption of eggs (Krauss et al., 1996).

In recent studies, the nutritional role of egg cholesterol has been re-evaluated, and it has been suggested that the effect of dietary cholesterol on serum cholesterol levels is quite low (Hu et al., 1999; Kritchevsky and Kritchevsky, 2000; Houston et al., 2011).

Several attempts have been made to lower the cholesterol level of yolks. However, all these trials have been less than successful (Noble, 1986; Hargis, 1988; Punita and Chaturvedi, 2000; Chowdhury et al., 2002). And during the last 15 years, the dietary research concerning egg cholesterol has mainly concentrated on the interactions between cholesterol and other food components and their possible influence on the lipid profiles of humans (Jiang et al., 1991, McNamara, 2000). Genetic selection or alteration of the laying hen's diet with various nutrients, natural products, non-nutritive factors, or pharmacological agents as cholesterol-lowering have been tested during the past four decades. It has been suggested that in the future, the yolk cholesterol reduction will be based on the manipulation of key genes whose protein products mediate intestinal sterol absorption, hepatic cholesterol and lipoprotein synthesis, and/or lipoprotein uptake by growing oocytes (Elkin, 2006; Elkin, 2007). Several findings indicate that the absorption of cholesterol may be lower together with egg PLs than without PLs (Murata et al., 1982; Jiang et al., 2001; Noh and Koo, 2003).

2.2.3.3. Phospholipids (PLs)

Phospholipids are a group of complex lipids present in cell membranes. PLs may be classified into several sub-groups according to their molecular structure. Four basic components can be found in the structure of a phospholipid molecule: fatty acid(s), a platform to which the fatty acids are connected, a phosphate, and an alcohol. Fatty acids form a hydrofobic barrier while the other components are hydrophilic, enabling interactions in aqueous environments. The building platform may be glycerol (phosphoglycerides) or sphingosine. The common alcohol structures of phosphoglycerides are serine, ethanolamine, choline, glycerol, and inositol (Berg et al., 2006/Stryer).

In egg yolks the phospholipid fraction consists of phosphatidylcholine (PC, 76 %), phosphatidylethanolamine (PE, 22 %), and small amounts of phosphatidylinositol (PI), phosphatidylserine (PS), sphingomyelin (SM), cardiolipins (CL), lysoPC, and lysoPE. **PLs**, which surround the lipid core of TAGs and cholesterol, stabilise the LDL structure with hydrophobic interactions (Cook and Martin, 1969). PLs are important building material for cells, having essential roles in biological membrane functions. Yolk PLs are rich in arachidonic acid (AA), docosahexaenoic acid (DHA), and choline. The importance of DHA and AA in the maintenance of normal neural functions is well-known Choline is an important nutrient in brain development, liver function, and cancer prevention (Gutierrez et al., 1997). The typical main fatty acid composition of egg yolk phospholipid classes is presented in Table 2.

Table 2. Fatty acid relative composition (%) of egg yolk phospholipid classes (Rossi, 2007)

Fatty acid	PCs (total)	PEs (total)	PS	SM	TOTAL LIPID
16:0	31.6-43.6	16.7-33.4	29.6	37.9	24.9-26.3
16:1ω-7	0.6-2.8	0.5-2.0	4.8	5.9	3.4-3.7
18:0	11.6-17.3	24.3-31.5	24.1	16.0	8.1-9.8
18:1ω-9	27.5-31.4	15.0-27.6	14.0	21.5	35.9-39.6
18:2ω-6	5.3-16.2	5.4-11.2	6.4	8.2	17.0-18.6
18:3ω-3	0.1-1.0	-	-	-	0.8-0.9
20:4ω-6	2.7-4.3	12.5-15.8	8.4	-	2.2-2.5
22:6ω-3	1.7-5.3	6.6-16.3	-	-	0.9-1.1

Considering the nutritional applications, it has been suggested that in infant nutrition, PLs may enhance the immature intestinal functions of infants (Carlson et al., 1998). PLs are successfully used in the food industry and many pharmaceutical processes, especially in emulsion formation. However, the role of the egg yolk PLs in oil-water interactions is still poorly understood (Mine 1998a; Anton et al., 2000). The ability of yolk PLs to form liposomes has been utilised in several drug-carrying and delivery applications (Kobayashi et al., 1996; Hartmann and Wilhelmson 2001). There is also some evidence that egg yolk PLs may have a role in preventing diseases like Alzheimer's disease by improving memory retention and increasing acetylcholine concentrations (Juneja 1997, Masuda et al., 1998). Other biological activities related to yolk phospholipid are antiviral activity and reduction of bacterial or viral infections (Liu and Watson, 2002). Recently, several reviews are presented concerning all biological activities of the lipid and protein components of hen eggs (Mine & Kovacs-Nolan, 2004; Mine and Kovacs-Nolan, 2006; Anton et al., 2006).

2.2.4. Fatty acid composition of yolk lipids

The production of healthier eggs by enhancing the composition of polyunsaturated fatty acids in egg yolk is a modern way to improve the nutritional value of eggs. As early as 1934, Cruickshank presented that fatty acid composition of eggs can be altered by diet (Cruickshank 1934). It has even been suggested that eggs can be an alternative to fish and oilseed as a source of ω-3 fatty acids. Many health-conscious consumers find eggs rich in ω-3 fatty acids quite attractive (Marshall et al., 1994), and eggs rich in ω-3 fatty acids are commercialised in at least 31 countries around the world and have been widely accepted by consumers (Surai and Sparks, 2001; Yannakopoulos, 2007)

Since the work of Cruickshank, it has been shown in tens of studies that the fatty acid composition of egg yolk lipids is strongly dependent on the feed given to the laying hen. Naber (1979) and Stadelman and Pratt (1989) have described the dietary factors influencing the nutritive value of eggs. To increase the amount of PUFAs in egg yolks,

special oil supplements such as flax seed (Caston and Leeson, 1990; Cherian and Sim, 1991) and fish oils (Hargis et al., 1991; Farrell, 1994; Hammershøj, 1995; Farrell, 1998) are added to the diet of hens. With flax seed and flax oil, mainly the content of linolenic acid (18:3ω-3) increases manifoldly compared to the eggs of hens feed without flax supplement. Other possible oil sources include marine algae and canola oils (Van Elswyk, M. 1997). In Kivini et al., (2004), feeds were supplemented with vegetable-based or fish oils. The analysed phospholipid contents and compositions were similar in all feeding groups. Moreover, the fatty acid profiles of phosphatidyl cholines and sphingomyelins were similar to each other in each feeding group, and different from that of phosphatidyl ethanolamines. The supplements decreased the proportion of saturated fatty acids in total fat, but not in PLs.

In general, the total amount of saturated fatty acids does not change with the changes in the diet of hens (Naber 1979). When the level of the polyunsaturated fatty acids (PUFAs) in feeds is increased, especially the long-chain unsaturated fatty acids in hen diets are transferred to the egg yolk. Table 2 presents some recent results considering the changes in fatty acid compositions induced by feed. Comparisons are made between the typical major fatty acid composition of egg yolks from hens fed with basal diets and modified diets. Similar information from organic eggs is also presented.

Table 3. Major fatty acid compositions of yolks from hens fed with different type of feed (% of total fatty acids)

	Fish oil[1,a]		**Flax oil[2]**		**Algae[3]**		**Organic feed[4]**	
Fatty acid	basal	modified	basal	modified	basal	modified	basal	modified
16:0	24.9	24.9	22.8	20.8	20.1	19.6	25.1	25.5
18:0	9.0	8.4	7.5	8.0	4.4	4.5	8.4	8.8
18:1ω-9	44.5	44.4	44.7	36.6	50.5	50.9	46.7	46.0
18:2ω-6	12.2	10.3	13.6	16.1	14.5	14.6	13.1	13.1
18:3ω-3	0.3	0.4	0.5	8.2	2.1	2.5	0.5	0.5
20:4ω-6	1.9	1.8	1.8	1.1	0.3	0.2	1.8	1.9
20:5ω-3	0.0	0.2	0.0	0.1	0.1	0.1	nd	nd
22:6ω-3	0.9	4.1	1.2	2.2	0.3	0.3	0.9	0.8
ω-3 total	13.3	49.9	1.7[a]	10.5[a]	2.5[a]	2.9[a]	1.4[b]	1.3[b]
ω-6 total	146	112	15.4[a]	17.2[a]	14.8[a]	15.1[a]	15.0[b]	15.0[b]
ω-6/ω-3 ratio	11.0	2.3	9.1[a]	1.6[a]	5.9[a]	5.2[a]	11.0[b]	11.2[b]

a) *calculated from original data*
b) *all the data not available, indicative results*
1) Cachaldora et al. (2008)
2) Beynen (2004)
3) Fredriksson et al. (2006)
4) Samman et al. (2009)

From a nutritional point of view, the families of ω3 and ω fatty acids are considered to be the most important. The principal fatty acids of these families are alpha-linolenic (ALA, 18:3ω) and linoleic (LA, 18:2ω) acids. These fatty acids are considered essential fatty acids because they cannot be synthesised by the body. A balanced ω6/ω3 ratio in the diet is essential for normal growth and development and should lead to decreases in cardiovascular disease and many other chronic diseases, and improve mental health. According to nutritional recommendations, the optimal ω6/ω3 ratio varies a little, but values close to 1 are suggested (Simopoulos, 1991; Simopoulos, 2002).

The main problem is usually the unwanted changes in the sensory properties of eggs. To ensure the acceptable sensory quality of eggs and to avoid oxidation of PUFAs in ω3-enriched egg yolks, active antioxidants such as vitamin E are added to the diet of the hens (Jiang et al., 1994; Leeson et al., 1998). However, there are results which suggest that certain vegetable lipids (palm butter, grape seed, and flax seed), ω3 PUFA (flax seed and marine algae), and rosemary may be used in the hens› diet without affecting the sensory properties of eggs (Parpinello et al., 2006). There is also evidence that egg yolk PLs have antioxidative activity. Sugino et al. (1997) showed that in DHA rich oil and squalene, the increase in antioxidative activity of different type of yolk lipid fractions was proportional to the PLs concentration. Over 50 years ago, Rhodes (1957) noticed that the addition of cod-liver oil to the diet of the laying hen increased the unsaturation of PLs fatty acids. The housing system of the hens seems to have only a minor influence on fatty acid composition of egg yolks (Cherian et al., 2002; Hidalgo et al., 2008).

The enrichment of eggs is not only limited to the manipulation of fatty acid in the composition of the yolk. Other applications, such as enrichment with selenium (Surai, 2000), lutein, iodine, and folate (Surai and Sparks, 2000) have proven to be possible ways to incorporate health promoting components to egg yolks.

2.2.5. The use of egg yolk lipids in food and non food applications

The use of eggs is not limited to just traditional food uses. Egg yolk contains many substances that can be produced on an industrial scale and be used as ingredients in commercial products. Several scientific papers discussing these applications have been published recently (Hartmann and Wilhelmson, 2001; Mine and Kovacs-Nolan, 2004; Kovacs-Nolan et al., 2005; Anton et al., 2006; Mine and Kovacs-Nolan, 2006). From a commercial point of view, the most promising applications are related to egg yolk antibodies, PLs, carotenoids (lutein), lipoproteins, phosvitin, and sialic acid.

2.2.5.1. Food applications

The food industry uses eggs mostly as egg white, egg yolk, or whole egg. Commercial egg yolk contains up to 20 % albumen due to the interactions of albumen and yolk in the separation process (Powrie and Nakai, 1986). To ensure microbial safety, egg processors generally pasteurise yolk at 60-68 °C for 3.5 to 4.5 minutes (Le Denmat et al., 1999). Egg yolk and fractions of it are important constituents in the manufacture of mayonnaise, salad dressings, bakery products and cakes, noodles, baby foods, creams, chocolate and candy products, ice cream, and "ready-to-drink" products. Yolk is responsible not only for high sensory properties but also for the excellent functional properties are necessary to optimise the rheological and textural quality of products (Kiosseoglou, 2003a; Shah el al., 2004).

Emulsions - The yolk itself can be characterised as an emulsion in which the water is the continuous phase to which the lipid components are dispersed (Baldwin 1986). The emulsifying components in egg yolk are PLs, lipoproteins, livetins, and phosvitin (Mine 1998b). The main constituents of egg as an emulsifying agent are LDL, HDL, phosvitin, and livetin (Mizutani and Nakamura, 1985; Li-Chan et al., 1995). Egg yolk is a complex structure organised in large spheres, granules and low density lipoprotein micelles and globular proteins. In this structure, lipids and proteins form a dispersion in the water phase, where the apolipoproteins act as emulsifying and stabilizing agents (Kiosseoglou, 2003b). The emulsions made of egg yolks have many unique properties. They seem to have exceptional stability against droplet coalescence and contribute to emulsion stability against creaming, depending on the pH value and particle structure disorganisation (Kiosseoglou and Sherman, 1983a; Kiosseoglou and Sherman 1983b). Kurt and Zorba (2009) showed that ionic strength and pH had significant effects on the emulsion characteristics of egg yolk.

Aluko and Mine (1998) and Mine (1998b) concluded that the main contributor to egg yolk emulsions in different pH values is granules, and the granules stabilise the emulsion (Anton and Gandemer, 1997, Anton et al., 2000). Le Denmat et al. (1999) evaluated that intact granules can stand more severe heat treatments than native egg yolk without lessening their emulsifying properties. Sirvente et al. (2007) concluded that natural granule aggregates persist at least partially at the oil–water interface. Laca et al. (2010) studied the use of granules as a replacement for whole yolk in mayonnaise preparation and found that the test mayonnaise showed similar characteristics to those of typical mayonnaise.

The emulsifying activity of yolk proteins is higher than that of yolk PLs (Kiosseoglou and Sherman, 1983a), and LDL is considered the most important contributor to the emulsifying properties of yolk. Mizutani and Nakamura (1985) and Bringe et al. (1996) showed that only a minor amount of PLs and cholesterol coming from liporoteins is adsorbed

at the oil-water interface. Kiosseoglou and Sherman (1983a; 1983b; 1983c) suggested that LDL is disrupted during the adsorption and the PLs are liberated together with apoproteins. In the adsorption mechanism, yolk proteins tend to form a strong film at the oil-water interface, supporting the stability of emulsions (Kiosseoglou, 1989).

Egg yolk can be fractionated to plasma and granules by centrifugation without any denaturation (McBee and Cotterill, 1979). The precipitated granules (19-25 % of the yolk dry matter) consist of 42-48 % protein and 15 % PLs. Moreover, granules consist of 70 % HDL, 16 % phosvitin, and 12 % LDL. Plasma is composed of 85 % LDL and 15 % livetins. In several studies, it has been concluded that the components in yolk plasma are the main contributors to yolk emulsifying properties (Anton, 1998, Anton and Gandemer, 1997, Dyer-Hurdon and Nnanna, 1993). So in food applications, yolk may act as an emulsifier and as a structure–forming material (Kiosseoglou, 1989). According to Anton (2007), the main contributors of the emulsifying and interfacial properties are LDL components. In the LDL structure, the interactions between amphiphilic apoproteins and PLs are the main factors of these properties. Proteins and PLs are the main surface-active compounds coming from hen egg yolk.

Gel formation – Gelation of yolk can be envisaged as a process of particle destabilisation brought about by denaturation, upon heating, of particle-stabilizing yolk protein molecules, leading to an interparticle network formation (Kiosseoglou, 2003). Anton et al. (2001) showed that the gelation of yolk solutions seems to have a similar model as in yolk plasma, and the role of granulas is less effective. So, the apoproteins of LDL have the main role in yolk gelation. Pasteurisation, freezing, drying, or cholesterol extraction influence the degree of disorganisation and gelation behaviour (Kiosseoglou, 1989, Paraskevopoulou and Kiosseoglou, 2000).

PLs are promising candidates for functional foods, but have some problems to overcome. Careful formulation strategies are needed for the production of PLs, the texture and viscosity problems should be solved as well as the problems related to hydrolysis of PLs in moisture environment (Davis and Reeves, 2002; Schneider 2001). The modification of PLs for industrial applications and human nutrition, together with the evolution of modern bioengineering, has brought a number of novel concepts for the utilisation of PLs and its derivatives (Guo et al., 2005).

2.2.5.2. Pharmaceutical and cosmeceutical applications

The use of emulsions and liposomes based on egg PLs in pharmaceutics and cosmetics is recently reviewed by Cansell (2007). The main applications of emulsions are related to parenteral administration or non-parenteral delivery systems for oral, ocular, and topical drug administration. Similar applications can be envisaged for drug-loaded lipo-

somes. The applications and functions of egg yolk based emulsions and liposomes in pharmaceutical and cosmeceutical applications are described in Tables 4 and 5.

Cosmetics - Eggs and egg yolk lipids are considered non-toxic and biodegradable, and they resemble skin lipids (Davis and Reeves, 2002) . Egg lipids have been added to shampoos, rinses, skin creams, lotions, and soaps to increase the effectiveness of the product (Juneja, 1997, Lautenschläger, 2001). The skin feel and absorption, emollient, spreading, and emulsifying properties of “egg oil” are the main factors for the use of egg lipids in cosmetic products (Baker, 1979).

Yolk PLs form liposomes that help the transport of cosmeceutical compounds at membrane surfaces. Yolk PC and PE, (and in some cases, PS and PI), together with sphingomyelin and cholesterol, are the main constituents of yolk liposomes. In liposome structures, various biologically active ingredients are encapsulated, thus showing better stability in cosmetic products (Van Biervliet et al., 1991). Liposome formulations prepared from egg PLs have shown to be effective in skin hydration (Betz at al., 2005). The increased encapsulation capacity and an improved stability of liposomes was found with using hydrogenated egg yolk PLs as phospholipid component (Nuhn et al., 1985).

Some preliminary cosmeceutical tests of egg yolk PLs produced with supercritical techniques are presented. The Sun Protecting Factor (SPF) and Mean Irritation Index (M.I.I) of egg yolk PLs based products were determined together with typical standard products used in cosmeceutical testing. Yolk-based products showed slightly better SPF values and the M.I.I. value was 0, which means that the yolk-based product did not cause any skin irritation. The emulsion made of yolk PLs were smooth and showed high technological quality (Aro et al., 2006; Aro, 2007).

Pharmaceutical applications - Yolk PLs have high entrapment efficiency, stability, and low cost. To increase the use of egg components in the pharmaceutical industry, a rapid and economical method to produce different yolk lipid fractions without toxic solvents has been presented by Sim (1994; 1995). Thus the use of liposomes is an important part of drug delivery in pharmaceutical treatments. Only a few egg yolk based liposomal products are on the market or in advanced clinical studies (Cansell, 2007). When lactoferrin was tested as an anti-inflammatory agent, it was found to be more efficient when encapsulated with yolk PC than without the encapsulation (Ishikado et al. (2005). The increase in the degree of saturation of egg yolk lecithin tended to show better encapsulation efficiency when testing the encapsulation of water-soluble and insoluble drug formulations (Nii and Ishii, 2005). Besides the traditional use of egg PLs, a new application is in composite film. In this application, PLs are formulated with hydrocolloids formulation to be used in localised drug delivery (Grant et al., 2005). In intravenous

administration, the physical behaviour of emulsifying components has an important role in final pharmaceutical formulations.

Table 4. The applications and functions of egg yolk based emulsions (adapted from Cansell, 2007).

Emulsions				
Function	**Applications**	**Advantages**	**Pharma-ceutical**	**Cosmet-ics**
Parenteral administration	plasma clearance		x	
	red cell hemolysis	easier penetration and transport of vesicles	x	
	intravenous delivery system	incorporation of water insoluble drugs, drug bioavailability and sta-bilisation, reduced number of drug-based side effects, controlled drug delivery	x	
Non-paren-teral delivery systems	oral administration	increased drug absorption	x	
	ocular administration	increased local bioavailability, no toxic or inflammatory response	x	x
	topical drug administration	improved drug delivery	x	x

Table 5. The applications and functions of egg yolk based liposomes (adapted from Cansell, 2007).

Liposomes				
Function	**Applications**	**Advantages**	**Pharma-ceutical**	**Cosmet-ics**
Model mem-brane systems	testing drug molecules in phospholipid bilayers		x	
Pharmaco-logical and therapeutic activities	antibiotics	modulation of bilayers	x	
Drug delivery	encapsulation with liposomes	new delivery characteristics (“lipo-somes in liposomes”)	x	x
Use as drug carriers		better solubility, drug stability and permeability, modification of pharma-cokinetic properties, better drug tar-geting, reduction in drug side effects		
Parenteral and non-paren-teral delivery systems	drug loaded-liposomes, oral administration of pharma-ceutical com-pounds	enhancement in administration, bet-ter stability and effectiveness	x	
Skin regeneration	supplying ma-terial for skin	increased humidity, elasticity and bar-rier functions.		x

2.3. Oats and its lipid compositions

2.3.1. Oats and oat products

Oat (*Avena sativa* L.) is a crop produced mostly in northern Europe and North America, especially Canada, with a total production volume of 23.0 million tons in 2010-2011. In Europe, EU-27 oats production is estimated at 7.62 million tonnes for 2010-11 (8.52 million tons for 2009-2010). Poland is the largest producer of oats in the EU-27 with a volume of 1.27 million tons. Finland’s oats production is expected to be 0.81 million tonnes. Together with Germany and Sweden, Finland is one of the largest oat producers in Europe. In the EU, the average annual consumption of oats is approximately 1.2 kg/person (European Commission, 2005; Foreign Agricultural Service, USDA, 2011).

Oats are typically used in cattle feed, but during the studies of the last decades, oats have been found to contain several components beneficial for human health (Mattila et

al., 2003). Oats are a rich source of soluble and in-soluble fibre components, proteins, vitamins, and minerals. (Charalampopoulos et al., 2002; Peterson et al., 2002; Esposito et al., 2005). Moreover, many bioactive components, such as tocopherols, alk(en)ylresorcinols, and avenanthramides avenalumic acids, are present in oats (Liu et al., 2004; Brennan and Cleary, 2005; Mattila et al., 2005). These compounds possess antioxidant activities and thus contribute to the stability and sensory properties of food (Peterson, 2001). Oat β-glucans are included in the soluble dietary fibre fraction, and they have shown to decrease serum cholesterol levels in humans (Johansson et al., 2000; Delaney et al., 2003).

The main constituents of oat grains are starch (39-55 %), protein (9-16 %), lipids (2-18 %), and dietary fibre (20-39 %, including non-starch polysaccharides and Klason lignin) (Åman, 1987; Frey and Holland, 1999; Zhou et al., 1999). Oat oil consists of polar and non-polar fractions. The non-polar fraction is comparable to traditional vegetable oils. It contains nutritionally beneficial fatty acids together with a lipid soluble antioxidant (Peterson and Wood, 1997). Oat oil consists primarily of palmitic, oleic, and linoleic acids, with a variation in genotypes and growing environments. The high content of oleic acid favours the use of oat oil in food applications, but for economical reasons, oat oil is less processed for food use (Peterson, 2004). Polar lipids of oats have a great potential to be used as an emulsifying agent, even beyond traditional food use (Forssell et al., 1998; Erazo-Castrejon et al., 2001; Peterson, 2002).

Several oat-based consumer products are available in different forms. Other cereals like wheat, rye, and barley are usually used as flour. The oat groat is that portion of the oat kernel that remains after removing the hull, botanically known as the caryopsis. Wholegrain oat products are typically used as oat flakes, which are made of a dehulled form of oat groat. The main reason for that is the high lipid content of oats, which makes the handling and storage of oat flour difficult (Zhou et al., 1999; Lehtinen et al., 2003). The quality of the final oat product is strongly dependent on every step in the production and processing of oats (Molteberg et al., 1996; Lapveteläinen et al., 2001;).

The outermost layer of the oat kernel is called oat bran, which is rich in dietary fibre and β-glucan. Oat bran is usually separated from endosperm components by sieving or classification processes (Wu and Doehlert, 2002).

2.3.2. Lipids in oats

In general, plant seeds store cellular triacylglycerols (TAGs) in droplets called oil bodies, which are typically 0.5–2.5 mm in diameter. They are composed of a TAG (94-98 %) core surrounded by a monolayer of PLs (0.5-2.0 %) embedded with small basic proteins (0.5-3.5 %), specific to oil bodies (Tzen et al., 1993). Compared to other cereals, oat

grains are rich in oil content typically varying from 2 % to 18 % or even up to 18 % of the grain weight (Sahasrabudhe, 1979; Youngs et al., 1979; Åman, 1987; Frey and Holland, 1999; Banas et al., 2007,). In certain studies, the naked oat varieties seem to have higher oil content compared to husked oats (Givens et al., 2004), although the results are not clear (Brindzova et al., 2008). Cool temperatures increase the oil content of oats (Saastamoinen et al., 1989).

Using column chromatography, Sahasrabudhe (1979) found the typical lipid composition of oat to be 51 % TAG, 7 % free fatty acids, 3 % sterols, 3 % steryl esters, 8 % GLs, and 20 % PLs. In animal feed, the high lipid content has the advantage of providing high energy, but in human food use, high lipid content is less beneficial in technological processes (Zhou et al., 1997).

The classification of oat lipids is usually based on solubility, chemical structure, or location, but the classifications are not mutually exclusive (Zhou et al., 1999). Based on solubility, oat lipids are divided into free and covalently bound forms. The covalently bound lipids (polar lipids) include typically glycolipids and PLs (Alkio et al., 1991).

With non-polar solvents, about 80 % of oats lipids are extracted. These lipids are usually called free lipids, whereas the remaining bound lipids require more polar solvents for extraction (Youngs et al., 1977). Up to 90 % of the oat lipids are located in the endosperm (Banas et al., 2007; Price and Parsons, 1979). Peterson and Wood (1997) reported that the higher oil content is inversely correlated to the starch content of oats. During grain development, the major part of TAGs are aggregated in the endosperm, and the differences in oil content between a medium-oil and a high-oil cultivar are in practise based to an increase on the oil content of the endosperm in the high-oil cultivar (Banas et al., 2007). In general, during the seed formation, the main synthesis of oat lipids takes place while the seed contains more than 50 % water (Youngs, 1978). The oat lipids are partly involved in the pasting properties of oat starch, thus influencing the functional properties of oats. Lipids have a role in the flavour/off-flavour attributes of oats, too (Zhou et al., 1999). Due to the high polar lipid content of oats, several biological activities are related to oat lipids, but the published scientific data in this area is sparse. Moreover, many of these potential biological activities are only partly characterised, and the applications related to these properties are under continuous investigation.

Oat lipids are typically fractionated to TAGs, PLs, GLs, free fatty acids, and sterols by chromatographic methods, using oat groats or flour as starting materials (Forssell et al., 1992; Frey and Hammond, 1975; Price and Parsons, 1975). Typical extraction media for total lipids of oats are mixtures of alcohol, chloroform, hexane, and water. The increase in solvent polarity produces increasing yields of polar lipids. Several SF-CO_2-based applications are presented, too. Using chromatographic methods, the extracted oil can

be further fractionated to purer non-polar (neutral lipid) and polar lipid (phospholipids and glycolipids) fractions (Sahasrabudhe, 1979; Moreau et al., 2003; Forssell et al., 2006). However, it is obvious that oat is not competitive in the production of "bulk" vegetable oil. Frey and Hammond (1975) estimated that in order to be an economically feasible oilseed crop in Iowa, the oil percentage of oats should be 16 %.

2.3.3.1. Neutral lipids (free lipids)

According to Price and Parsons (1975), neutral lipids of oats constitute TAGs, FFA, partial glycerides, steryl esters, and free sterols, corresponding to about 73 % of the total lipids of oats. TAG is the most abundant lipid class. Karunajeewa et al. (1989) found significant differences between oat cultivars in free and bound lipid contents, and in the proportion of fatty acids in these lipids. Sahasrabudhe (1979) investigated seven different solvent systems in extracting the oat lipids and noticed a significant correlation between the total lipid and the neutral lipid content of oats. Moreau et al. (2003) stated that the increase in solvent polarity resulted in increasing yields of polar lipids, and in a temperature of 100°C, more of each lipid class was extracted than at 40°C.

Palmitic, oleic and linoleic acids are the major lipid components in all lipid fractions of oats. (Zhou et al., 1998; Liukkonen et al., 1992), but neutral lipids seemed to contain less palmitic and more oleic acid than the glycolipids or phospholipids (Sahasrabudhe 1979).

SC-CO_2 is used for the separation of neutral lipids from complex lipid mixtures (Acosta et al., 1994), and its use in fractionation of oat lipids has been extensively studied (Fors and Eriksson, 1990; Forssell et al., 1992). However, it is quite obvious that oat is not competitive in production of "bulk" vegetable oil. Frey and Hammond (1975) estimated that in order to be an economically feasible oilseed crop in Iowa, the oil percentage of oats should be 16 %.

2.3.3.2. Phospholipids

The accurate information on the phospholipid (and glycolipid) contents of oats is sparse, and the determinations of the phospholipid content of oats show large variation, ranging from 6 to 26 % of the total lipids (Zhou et al., 1999). The main phospholipid is phosphatidylcholine, consisting of 45-51 % of the total phospholipids (Youngs et al., 1977). The other main components are phosphatidylethanolamine and phosphatidylglycerol, but oat oil also contains several minor polar components (Holmbäck et al., 2001). Sahasrabudhe (1979) compared the total phospholipid content of oats and found the content to be similar in all oat cultivars. Earlier, de la Roche et al. (1977) noticed that high

lipid strains contained a greater proportion of triglyceride and a lower proportion of phospholipid.

2.3.3.3. Glycolipids

Polar lipid fractions from cereals show a high content of glycolipids. The dominating compounds in higher plants are monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (Kates, 1990). DGDG is one of the main lipid components of the various membranes of chloroplasts and related organelles. It is found in all photosynthetic tissues, including those of higher plants, algae, and certain bacteria. Oat oil (obtained by extracting oat flour with hexane or ethanol) contains unusually high levels of DGDG, and it is the most abundant glycolipid in oat kernels (Hauksson et al., 1994; Andersson et al., 1997; Moreau et al., 2003; Moreau et al., 2008).

DGDG is amphiphilic, and it can form lamellar crystalline phases (Brentel et al., 1985). DGDG can therefore be used to prepare lipid aggregates such as liposomes and oil-in-water emulsions. Oat-based liposomes are of interest for nutritional, cosmetological, and pharmaceutical applications (Hauksson et al., 1994).

2.3.4. Fatty acid composition of oat lipids

About 95 % of the oat fatty acids are palmitic (16-22 %), oleic (28-40 %), and linoleic (36-46 %) acids. Typically, linoleic and palmitic acids tend to vary inversely, and oleic acid tends to vary directly with the total lipids or total fatty acids (Youngs, 1978; Liukkonen et al., 1992; Zhou et al., 1998). Compared to other cereals, oats have the greatest varietal variation in total fatty acid content and in the proportion of its component fatty acids (Welch, 2006). The free and bound lipid content, and the fatty acid composition of these lipids, in 11 oat (*Avena sativa* L) cultivars grown in three Australian states were determined in Karunajeewa et al. (1989). The proportion of linolenic acid in oat cultivars grown in Western and South Australia was greater than that in the same cultivars grown in Victoria. A positive correlation between the free lipid content and the proportion of linoleic acid in one cultivar was found.

2.3.5. The use of oat lipids in food and non-food applications

Recently, together with the new findings of health-promoting properties in oats, many new technologies for oat processing have been developed (Brennan and Cleary, 2005). Especially the beneficial fibres together with the bioactive co-passengers make oats suitable for both common diets and a gluten-free diet (Sontag-Strohm et al., 2008).

Food applications – The contribution of cereal-based foods to human nutrition is significant. Highly unsaturated oat lipids are regarded as nutritionally important due to the high content of some essential fatty acids and a high level of antioxidants (Youngs, 1986; Peterson, 2001). In traditional cereal processing, the high oil content of oats was considered to be problematic. Difficulties in milling, baking, and storage of oats and oat-based products have been the main reason for the limited use of oats in foods. Recently, a method of preparing functionally valuable products, such β-glucan, protein, starch, and lipid concentrates from oat by supercritical extraction, dry milling, sieving, and air classification has been presented (EP20080709317 20080207). Oat fractions produced with the modern separation methods are well suited for beverages, dairy applications, baking, snack products, and fermented products. The cosmetics and pharmaceutical industries can also utilise them (Sibakov et al., 2011).

Oat-based emulsifiers are believed to have economical potential in the food industry due to their better physical and sensory properties compared to soy-derived products. Erazo-Castrejón et al. (2001) showed that especially oat polar lipids can improve loaf volume, grain, and texture, and delay staling in bread.

Non-food applications -The non-food use of oats has a long history, both in research activities and in applications. Today, modern fractionation techniques offer the possibility to increase the use of oats in non-food applications. Naturality is usually the main advantages for the use of oat-based functional ingredients, and the use of sustainable raw materials attract consumers.

Even a relatively crude oat oil has proven to have useful properties in cosmeceutical and dermatological applications A process to prepare oat oil compositions with beneficial dermatological properties has been patented by Potter et al. (1994). This patent includes oat oil based formulations having antioxidant and other dermatologically beneficial properties. Applications for the cosmetic industry for inhibiting ultraviolet irradiation-induced skin damage and other beneficial dermatological properties were presented.

Oat polar lipids have been used to make liposomes for pharmaceutical applications. Due to its surface-active properties, oat oil rich in polar lipids has been shown to form a fine particle emulsion, which has been used in self-emulsifying systems in drug delivery (Gren and Kaufman, 2002; Odeberg et al., 2003). Additionally, oat polar lipids form microcapsules in aqueous media and therefore are able to encapsulate various sensitive substances inside capsular structures. The presence of these new types of chemical structures help to explain some of the pharmaceutical properties of liposomes prepared with oat lipids (Moreau et al., 2008).

2.4. SUMMARY OF LITERATURE REVIEW

Hen eggs and oats (*Avena Sativa*) are important materials for the food industry. Both of these food stuffs are eaten in several different forms all over the world. Instead of only satisfying the feeling of hunger, consumers are asking for more healthy, biologically active, and environmentally friendly products. The increasing awareness of consumers increasing demands presents a great challenge to the food industry to develop more sustainable products and to utilise modern and effective techniques.

The fatty acid composition of hen eggs is mostly dependent on the feed of the hens. For that reason, the modification of yolk fatty acid composition by means of feed supplements is well understood. Typically, the increase in the content of alfa-linolenic acid in eggs is induced with vegetable oils supplements in the feed of hens. Eggs enriched with polyunsaturated fatty acids may show some unwanted sensory properties, especially fishy taints. To prevent these changes, the levels of the oil supplements should be controlled and the use of antioxidative compounds in feed should be favoured. Moreover, enrichment of eggs is not only limited to fatty acids: the contents of vitamins, minerals, and other nutrients in eggs can be manipulated. In egg processing, egg yolks modified with different feed supplements seem to have similar functional properties (emulsification, foaming, hardness, and gelling) than regular eggs.

Egg yolk phospholipids are polar lipids, and they are used in several applications including food, cosmetics, pharmaceuticals, and special nutrients. Egg yolk PLs are excellent emulsifiers, and they are typically sold as mixtures of PLs, triacylglycerols, and cholesterol. However, highly purified and characterised PLs are needed in several sophisticated applications. Industrial fractionation of PLs are usually based on organic solvents. With these fractionation techniques, some harmful residues of organic solvents may cause problems in further processing.

As an alternative fractionation technique, the separation processes of egg yolk PLs with supercritical fluids have been developed. With this technique, the purities of the separated products are extremely high and no solvent residues are left in the final products. Supercritical fluid techniques are an environmentally friendly alternative to conventional organic solvents. Supercritical fluids are neither gas nor liquid, and they can be utilised in the production of bioactive compounds from food-based sources.

Oat is rich in nutrients with potential health-promoting properties. Typical, commercially available oat nutrients contain protein and carbohydrate fractions, which are shown to be beneficial for a balanced human diet. Earlier, oats were considered mainly as a feed, but today the biological activities and health-promoting properties of oats are well-known. The oil content of oats is high, which is unique among the cereals. Oat lipids contain several nutritionally important, highly unsaturated fatty acids. The polar

lipid fraction of oats consists of glycolipids and phospholipids, which are typically extracted from oat flakes with polar organic solvents. With supercritical fluid techniques, the extraction of polar lipids from oat flakes produces pure and solvent-free products.

Liposome formulations prepared from egg yolk PLs and oat PLs and glycolipids have been shown to be effective in cosmeceutical and food applications. With the modification of lipid composition in liposome preparations, the increase in encapsulation capacity and liposome are achieved.

3. AIMS OF THE PRESENT STUDY

The objective of the present study was to investigate the methods to improve the functional properties of eggs, to develop techniques to isolate the fractions responsible for the specific functional properties of egg yolk lipids, and to test these techniques with plant-based materials. The purification techniques based on supercritical fluids were utilised for the separation of the lipid fractions of eggs and oats. The chemical and functional characterisation of the fractions were also performed. Finally, the produced oat polar lipid fractions were tested as a protective barrier in encapsulation processes.

The study focused mainly on:

- the modification of egg yolk fatty acids and their effect on the functional properties of eggs
- the fractionation of egg yolk and oat lipids with supercritical techniques
- the characterisation of lipid fractions produced with supercritical techniques

4. SUMMARY OF MATERIALS AND METHODS

Detailed information on materials and methods is described in the original publications (I-V).

4.1. Sample material for the evaluation of the functional and sensory properties of eggs with a modified fatty acid composition (I)

Laying hens (White Leghorn) from the Agrifood Research Finland (MTT) henhouse were used in feeding experiments. Hens were fed a standard laying diet (control group) or a diet supplemented with 5 % flax oil (FO), 5 % rapeseed oil (RO), 5 % fish oil (FISH), or 20 % dried black currant byproduct (BC) containing 2.3 % oil, and the metabolisable energy and protein content of the experimental diets were determined. The eggs were collected twice a day, and the eggs for the analysis were randomly chosen from both feeding groups. Eggs intended for later analyses were stored at 12°C in 60 % humidity. The behaviour of the hens were routinely monitored daily.

The fatty acid analysis of feed samples, eggs, and mayonnaise samples were performed from fresh eggs and from eggs storage for 21 days. For mayonnaise preparations, six fresh egg yolks from both feeding groups were pooled, and the mayonnaise was prepared according to a general recipe.

4.2. Sample material for the fractionation of egg yolk phospholipids with SF-CO_2 (II, IV)

Egg yolk powder was purchased from a commercial manufacturer (Scanegg Suomi Ltd, Piispanristi, Finland), and stored refrigerated. The powder was mechanically granulated prior to use in order to enhance the extraction process. The diameter of the granules was about 2.5 mm and their length was from 3.5 mm to 7 mm.

4.3. Sample material for the fractionation of oat lipids with SF-CO_2 (III, V)

The oat variety “Roope” used in the experiments was cultivated on a commercial basis in southwestern Finland. The oat seeds were dehulled and mechanically flattened into flakes in order to enhance the extraction process. The thickness of the flakes was approximately 0.5 mm and their diameter varied from 3 mm to 7 mm. The flakes were kept at +4°C until used.

4.4. Extraction of lipids of feed, egg, mayonnaise, and oat samples for fatty acid analysis(I-V)

The total lipids from all the samples were extracted according to the modified method of Röse-Gottlieb (IDF 22B, 1987, I) or the Twisselman method (II-V).

4.5. Fatty acid analyses (I-V)

Fatty acids were analysed as their methyl esters. The analysis was carried out using a Perkin-Elmer 8700 capillary gas chromatograph equipped with a CP-Sil-88 column (50 m * 0.25 id). A mixture of standard fatty acids (C4:0 – C24:1) purchased from Nu-Check-Prep Inc. (Minnesota, USA) was used as the standard to identify the peaks.

4.6. Sensory analysis of eggs and mayonnaise preparations (I)

The eggs were cooked for 10 minutes, cooled to room temperature, and analysed immediately. In a multiple comparison test, twelve trained panellists evaluated the odour, taste, and general acceptance of the eggs. In triangular tests, six trained panellists were informed that two of three eggs are similar. Panellists were asked to compare the odour, taste, and other sensory properties of the egg yolks, and according to these properties, indicate which egg is different. In every test, two of the eggs came from a control group and the third egg came from a modified group.

In the mayonnaise analysis, six trained panellists were asked to evaluate the odour and taste of different mayonnaises and evaluate the superiority of the samples. The panellists were also asked to describe the differences between the samples.

All of the trials of the sensory properties of eggs and mayonnaise preparations were performed according to the Heiniö (1993).

4.7. Analysis of functional properties of eggs (I)

Foam capacity and foaming stability were analysed according to Rokka et al. (2002), and Phillips et al. (1987). The emulsifying and gelling properties of the samples were analysed by a modified method of Sathe and Salunke (1981) and Quinn and Paton (1979), as described in Rokka et al. (2002).

4.8. Pilot scale lipid extractions with ethanol and supercritical fluids (II-V)

The supercritical fluid treatments were performed with a pilot scale plant built by Chematur Ecoplanning, Rauma, Finland. The schematics of different supercritical processes are presented in Figure 2.

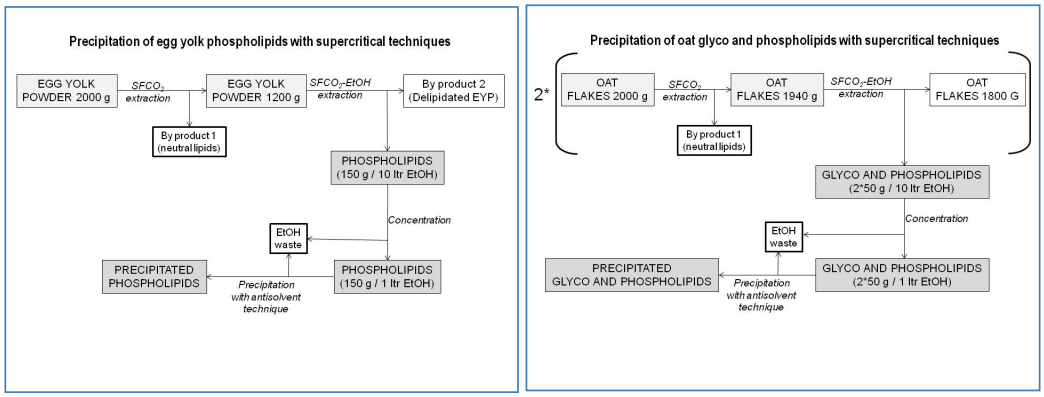


Figure 2: Processing of egg yolk powder and oat flakes with supercritical fluid technologies.

The pilot scale extractions of egg yolk lipids with ethanol (II) were performed with a slightly modified method of Juneja (1997).

4.9. The analysis of lipid classes (II-V)

Lipid classes of the products were analysed by the liquid chromatographic method (Kivini et al., 2004). The estimation of the phospholipid contents of the precipitated egg yolk polar lipid fractions were based on ICP analysis by multiplying by 30 the phosphorus content of samples. The phospholipids (PC and PE) used for preparation of the standard curvex were purchased from Sigma-Aldrich (Steinheim, Germany).

4.10. The analysis of egg yolk proteins and peptides (III)

The solubility of different egg yolk based protein fractions were evaluated in different ionic strengths and pH values, as described in Anton et al. (2003). The protein analysis of the yolk samples was performed with two combined Superose 6 gel filtration HR 10/30 columns (Amersham Biosciences) and monitored by a UV detector at 214 nm.

Egg yolk proteins were hydrolysed by using a combination of Alcalase and Flavourzyme enzymes. The peptide profile of the hydrolysed EYPro sample was determined using

high-performance liquid chromatography (HPLC) equipped with a Superdex Peptide HR 10/30 column (Amersham Biosciences) and monitored by a UV detector at 214 nm. An SDS-PAGE was performed with a slightly modified method of Anton et al. (2003).

4.11. Solubilities and determinations of emulsion properties (IV)

The solubility of egg yolk protein (EYPro) fraction in different pH and ionic strength values was determined according to the method of Anton et al. (2003). The solubility of hydrolysed EYPro (EYPro-H) was determined as described in Hiidenhovi et al. (2005).

The volume-surface area's average diameter distribution (d_{32}) of the emulsion droplets was determined by laser light diffraction. Emulsions were kept at an ambient temperature (18°C) for 4 hours, and then the volume frequency distribution was measured. To detect the flocculation process, emulsions were diluted in buffers with and without SDS, and the flocculation index was evaluated.

4.12. Tests using oat polar lipids as encapsulative agents in protecting probiotics (V)

A suspension of the precipitated oat polar lipids and water was prepared in a ratio of 3 to 100 (w/v). Freeze=dried *Bifidobacterium breve* pellets were added to the suspension at the level of 1,7 x 10^8 CFU. A similar suspension was prepared with the addition of maltodextrose to the suspension in a ratio of 3 to 100 (w/v).

The tested products were: the pure test media (negative control), *Bifidobacterium breve* alone, encapsulated with oat polar lipids or with a combination of oat polar lipid encapsulation and maltodextrose as a prebiot. The level of amended Bifidobacteria was approximately 1.3 x 10^8 bacterial cells per simulation.

The storage part of the simulations was run separately for either one day or one week, resulting in a total of 8 treatments. To mimic the stomach conditions, the samples were shaken for 2 hours with a pepsin-HCl solution at a pH below 2.5, and for 3 hours with a pancreatin solution at a pH of 6.5 to mimic duodenum conditions.

A supernatant of swine colon digesta was used to provide substrates for *Bifidobacterium breve* and to leave out the vast majority of the microbes of colon digesta. Gas production and changes in PH were measured at the end of the colon. The total microbial numbers were enumerated by a flow cytometre.

4.13. Statistical analysis (I, V)

In the statistical analysis for evaluating the functional and sensory properties of eggs with modified fatty acid composition (I), the results from the control group eggs were analysed against the results of different feeding groups using one-way ANOVA.

In tests using oat polar lipids as an encapsulative agent in protecting probiotics (V), the statistical analysis consisted of one-way ANOVA within each combination of medium and time point. The results from different media were then compared to a negative control within the storage time using Dunnett's *post hoc* test.

5. SUMMARY OF RESULTS AND DISCUSSION

5.1. The changes in functional and sensory properties of eggs enriched with PUFAs (I)

5.1.1. Fatty acid compositions in feed, eggs, and mayonnaise

The main fatty acid composition of feeds and egg yolks in all feeding groups are presented in Table 6. Compared to the control group, typical changes were found in eggs analysed in oil supplementation groups. In the FO group, the amount of a-linolenic acid increased more than 11-fold compared to the control group, and in the FISH group, the amount of DHA increased to 3.74 % fatty acids. These results agree with the results of other experiments with plant oil or fish oil supplementation (Jiang et al., 1991; Botsoglou et al., 1998; Sari et al., 2002).

In the BC group, the feed contained 4.69 % gamma-linolenic acid. However, in eggs, the corresponding value was only 0.30, indicating that the BC seed oil was only partly available for the hens. In general, flax, rapeseed, and fish oil supplements in hen's diets changed the fatty acid composition of eggs to be nutritionally more beneficial.

Table 6. The main fatty acid compositions of feed, eggs, and mayonnaise preparations in control and test groups. Statistically significant differences between the control group and each test group are marked with letters a ($p<0.05$) and b ($p<0.005$).

	FEED (n=3)				
	Control %	FO %	RO %	FISH %	BC %
C16:0 (palmitic)	19.0	11.1[b]	11.2[b]	15.5[b]	15.6[b]
C16:1 / C17:0 (anteiso. tr)	1.4	0.6[a]	0.6[a]	1.5	0.9
C18:0 (stearic)	3.1	3.6[a]	3.1	3.9[a]	4.1
C18:1 (oleic)	22.9	18.2[a]	42.3[b]	36.9[b]	21.1[a]
C18:2 ω-6 (linolic)	42.6	24.5[b]	26.6[b]	27.9[b]	40.0[a]
C20:4 ω-6 (arachidonic)	0.0	0.0	0.00	0.0	0.00
C18:3 ω-3 (alfa linolenic)	6.8	39.7[b]	11.0[b]	5.7	8.6[b]
C18:3 ω-6 (gamma linolenic)	0.0	0.0	0.0	0.0	4.7[b]
C20:5 ω-3 (EPA)	0.0	0.0	0.0	0.9[b]	0.0
C22:6 ω-3 (DHA)	0.0	0.5[b]	0.50[b]	1.4[b]	0.0
ω-3 tot.	6.8	40.2	11.5	7.9	9.0
ω-6 tot.	42.6	24.5	26.6	27.9	44.7
W-6/W-3	**6.3**	**0.6**	**2.3**	**3.5**	**5.0**

	EGGS (n=3)				
	Control %	FO %	RO %	FISH %	BC %
C16:0 (palmitic)	26.6	23.6[a]	23.4[a]	27.2[a]	25.0
C16:1 / C17:0 (anteiso. tr)	2.5	2.1[a]	1.7[a]	3.0[a]	2.0
C5610					
18:0 (stearic)	10.1	10.5	8.8[a]	9.4[a]	9.9
C18:1 (oleic)	41.6	37.0[a]	41.4[a]	35.1[a]	37.9[a]
C18:2 ω-6 (linolic)	12.1	13.9[b]	12.5[a]	11.00[b]	15.9[b]
C20:4 ω-6 (arachidonic)	1.6	0.9[b]	1.5[a]	0.9[b]	1.6[a]
C18:3 ω-3 (alfa linolenic)	0.7	7.8[b]	1.9[a]	0.8[a]	1.9[a]
C18:3 ω-6 (gamma linolenic)	0.0	0.0	0.0	0.0	0.3[b]
C20:5 ω-3 (EPA)	0.0	0.0	0.1	0.8	0.1
C22:6 ω-3 (DHA)	1.5	1.8[a]	1.9[a]	3.7[b]	1.7[a]
ω-3 tot.	2.2	9.6	4.1	6.00	3.1
ω-6 tot.	13.7	14.8	14.00	11.8	18.1
W-6/W-3	**6.2**	**1.5**	**3.4**	**2.0**	**5.8**

	MAYONNAISE (n=3)			
	Control %	FO %	RO %	FISH %
C16:0 (palmitic)	7.1	6.8	6.8	6.9[a]
C16:1 / C17:0 (anteiso. tr)	0.2	0.2[a]	0.2[a]	0.1[a]
C18:0 (stearic)	4.7	5.0[a]	4.8	4.5[a]
C18:1 (oleic)	23.3	19.7[a]	21.9	24.8
C18:2 ω-6 (linolic)	60.3	65.0	62.7	59.1[a]
C20:4 ω-6 (arachidonic)	0.2	0.3	0.1[a]	0.1 [a]
C18:3 ω-3 (alfa linolenic)	0.6	0.6	0.6	1.0[a]
C18:3 ω-6 (gamma linolenic)	0.0	0.0	0.0	0.0
C20:5 ω-3 (EPA)	0.3	0.0[a]	0.1[a]	0.0[a]
C22:6 ω-3 (DHA)	0.1	0.1	0.2	0.1
ω-3 tot.	1.0	0.8	0.8	0.7
ω-6 tot.	60.5	65.3	62.8	59.2
W-6/W-3	**62.0**	**87.6**	**80.5**	**81.7**

5.1.2. Foaming, emulsification, and gelling properties of eggs

In albumen foams, the index values varied from 900 to 950 in all oil-supplemented feeding groups in fresh eggs. Compared to the value of the control group (950), the data from these experiments indicate that supplementation with RO and FISH oils or a BC byproduct have no influence on the foaming capability or foam stability of fresh egg albumen. A statistical difference was found with FO oil-supplementation ($p<0.05$).

Between the fresh and stored eggs, slight but not significant changes in albumen foam volume were found in all groups.

Whole egg foam indexes showed values of 425-500 for fresh eggs in the control group and in all test groups. For stored eggs, the corresponding values were 400-530. All of the whole egg foams were weak, and the measurements were difficult to carry out. Compared to control group, the best foam stability was found in the BC group. In the FISH oil group, the whole egg foams of stored eggs lost part of their capability to maintain foam stability.

The results of the emulsifying and gelling measurements are presented in Table 7. In general, only small variations between the fresh eggs from the control group and FO, RO, and FISH groups in emulsion activity, emulsion stability, and gel-forming capacity were found. However, in the FISH group, the results show worse emulsion activity ($p<0.005$) and emulsion stability ($p<0.005$) with fresh eggs, and we can't exclude the possibility that the changes in fatty acid composition affects the emulsifying properties of these eggs. With stored eggs, statistically significant differences were found in emulsion activity in the FO ($p<0.005$), RO ($p<0.05$), and FISH ($p<0.005$) groups, but from a technological point of view, these differences are negligible.

In gelling, albumen proteins typically create a network via non-covalent cross-linkages, such as hydrophobic interactions, hydrogen bonds, or electrostatic interactions, and (to some extent) covalent interactions (Gosal and Ross-Murphy, 2000). When modifying the fatty acid composition of egg yolk, it should be evident that the relationships between albumen proteins don't change and the gelling properties remain equal, both in control and modified eggs. However, the reasons for the wide variations in gel forming capacities between the eggs stored for different times and from different feeding groups remain unclear.

Table 7. Mean values of emulsion activity (g oil emulsified/g), emulsion stability, and gel-forming capacity (hardness/N) of eggs in a control and the FO group. Statistically significant differences between the control group and each test group are marked with letters a ($p<0.05$) and b ($p<0.005$).

	CONTROL				FO			
	Albumen fresh	Albumen 21 days	Whole fresh	Whole 21 days	Albumen fresh	Albumen 21 days	Whole fresh	Whole 21 days
Emulsion activity (n=5)			67.4	63.9			67.2	65.2[b]
Emulsion Stability (n=5)			53.9	23.8			42.9[a]	36.5[b]
Gel-forming capacity (n=5)	4.2	6.4			5.4[b]	7.7[a]		

	RO				FISH			
	Albumen fresh	Albumen 21 days	Whole fresh	Whole 21 days	Albumen fresh	Albumen 21 days	Whole fresh	Whole 21 days
Emulsion activity (n=5)			67.1	64.8[a]			69.5[b]	63.2[a]
Emulsion Stability (n=5)			57.4	24.4			35.4[b]	23.1
Gel-forming capacity (n=5)	5.2[b]	6.7			4.9[a]	8.2[b]		

5.1.3. Sensory properties

Eggs from the RO group, FO group, and BC group showed similar sensory properties as the eggs from the control group. All these eggs were acceptable to the senses. Eggs from the FISH group showed clearly worse sensory properties in all evaluations. In this study, only the level of flax oil in feed was optimised to avoid sensory problems, but according to the panellists' comments, flax oil induced some fishy-like aromas in eggs.

In triangle tests, 9 of 10 panellists recognised the right egg in the FO group. The corresponding result for the RO group was 1 out of 10; for the FISH group, 9 out of 10; and for the BC group, 6 of 10. These results corroborate the results of multiple comparison tests.

The flax oil supplementation in the diet of the hens did not change the sensory properties of the PUFA-enriched eggs or the mayonnaise preparations based on these eggs. In practise, if the level of fax seed oil supplement in feed is less than 5 %, the changes in sensory properties are difficult to find. If the level of oil supplement in feed is higher, sensory problems like "fishy" or "paint-like" taste or odour may arise. In addition, the age and the storage of eggs may have an effect on unwanted changes in the sensory properties of eggs. No changes between the control group and test groups were found in the sensory properties of mayonnaise preparations. These results suggest that the egg processing industry may produce egg-based products using oil-supplemented eggs without major problems in functional or sensory properties.

5.2. The fractionation of polar lipids with supercritical techniques (II-V)

5.2.1. Supercritical fluid extraction (SFE) with CO_2 and CO_2 with ethanol as an entrainer

In pilot scale extractions, the extraction chamber of the SFE pilot plant was filled with egg yolk powder or oat flakes. The quantity of extract was measured every 30 minutes.

In SFE extractions with CO_2, the extract consisted mostly of neutral lipids, and no polar lipids were observed in the extract. In second step extractions using ethanol as an entrainer, the extract contained polar lipids and a small amount of neutral lipids.

5.2.2. The characterisation of products produced with SF techniques

Egg yolk powder

Approximately 960 g of a mixture of oil and water was obtained from a 2-kilogram batch of EYP granules using SF-CO2. When storing overnight at + 4°C, a 150-200 ml volume fraction of water was separated at the bottom of the storage bottles. The rest of the fraction was the neutral lipid fraction containing 750-800 g of fat. According to the analysis of phosphorus content, the phospholipid content of this fraction was evaluated to be 0.9 g in 1000 g of the sample. With the HPLC method, less than 0.4 g of phospholipids could be detected in this fraction.

Using ethanol as an entrainer in SFE processes, a pale yellow ethanol solution was collected in the separator of the pilot plant. In theory, about 300-350 g of PE and PC could be found in 2 kg of EYP. With our process, only 50-55 g of PE and PC were recovered in the separator.

In the precipitation process, the PL-rich ethanol solutions were used as a feed in the SAS process. In this process step, from the condensed ethanol solutions the recovery of phospholipids was about 85-95 %, so in practise almost all phospholipids in ethanol solutions were precipitated. The purity of the PL fraction was very high: the total phospholipid content of the precipitated SF-EtOH-PLs fraction was evaluated to be 1034 g in 1000g of sample.

The fatty acid compositions of the different egg yolk based fractions produced with SC-CO_2 are presented in Table 8.

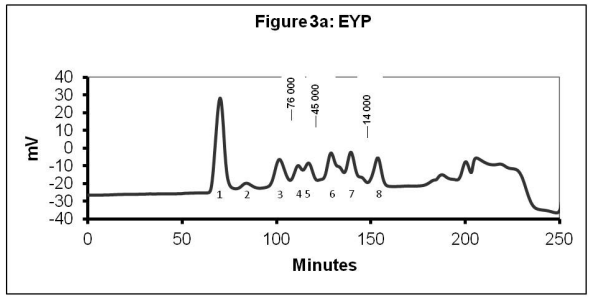
Table 8. Fatty acid relative composition (%) of egg yolk based fractions produced with SC-CO_2

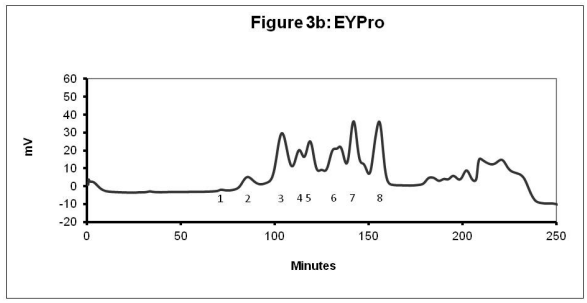
Fatty acid	EYP	NLs	PLs	EYPro
16:0	24.1	25.5	36.7	28.7
16:1ω-7	2.6	2.1	0.2	0.8
18:0	6.7	5.2	14.7	15.1
18:1ω-9	44.9	54.7	29.3	29.7
18:2ω-6	15.5	12.2	15.0	14.6
18:3ω-3	0.5		0.0	0.1
20:4ω-6	1.9		2.5	5.7
22:6ω 3	0.8		1.7	2.6

Functional properties of EYP fractions

The maximum solubility of the EYPro fraction was reached at pH 7 and at an ionic strength of 0.3-0.4 M NaCl. In general, the solubility of the EYPro fraction was less than 30 % in all tested pH and salt concentrations. Enzymatic hydrolysis with a series of Alcalase and Flavourzyme enzymes digested EYPro into peptides. The average yield of solubilised EYPro peptides was 83 %, so the solubilisation of EYPro-H was quite efficient.

Protein profiles of EYP and EYPro are displayed in Figure 3a and 3b. When comparing the profiles, lipid extraction with SF-CO_2 and SF-CO_2-EtOH induced only slight changes in the profile of EYPro compared to EYP. The main changes were found in peak 1, corresponding to low density lipoprotein (LDL). Figure 3c illustrates a Superdex Peptide GFC chromatogram of EYProH. The hydrolysis with the Alcalase-Flavourzyme combination digested EYPro into peptides showing MWs mainly <1 350 kDa. These hydrolysates contained a high amount of carbohydrates, up to 17.1 g/100g of dry weight.





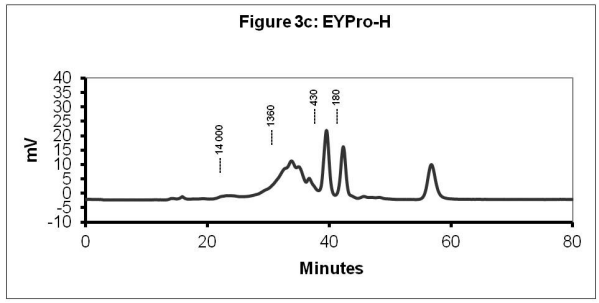


Figure 3: Protein profiles of EYP, EYPro, and EYPro-H samples.

Oat flakes

In general, with oat flakes, the extraction efficiency was clearly more effective compared to groated oats. However, the shape and thickness of the oat flakes have a crucial role in the SC-CO_2 extraction efficiency of oats. The extraction efficiency did not improve by increasing the surface area of the flake. It can be concluded that when the surface area of the flake reaches its maximum, the area-mass ratio becomes so high that the fluid no longer penetrates into the flakes but creates "channels" through them. Moreover, the use of non-heat-treated or slightly heat-treated oat flakes instead of strongly heat-treated oat flakes showed clearly better extraction efficiency. Based on that finding, a patent application concerning the preparation of functionally valuable products, such as β-glucan, protein, starch, and lipid concentrates from oats was applied by Kaukovirta-Norja et al. (2007).

The mixture of 62 % lipids, 32 % water, and 6 % carbohydrates and other components was collected from the separator after SC-CO_2 extraction. The HPLC lipid analysis showed that the extract contained neutral lipids (triacylglycerols, sterols, and carotenoids) and free fatty acids. Similarly as in egg yolk extractions, a clear water phase separated at the bottom of the storage bottles when storing at + 4°C.

The removal of lipids with SC-CO_2 was quite effective. About 87 % of the original crude fat was removed in the two extraction steps (SC-CO_2 extraction and SC-CO_2ethanol extraction). In theory, about 53 g of neutral lipids and 106 g of water would be extractable from 1.0 kg of oat flakes. With the SC-CO_2 treatments, about 108 g of yield was obtained from oat flakes. The oil phase was about 50-60 % of the total volume. For comparison with extractions with organic solvents presented in the literature, the values of 52 g kg^{-1} have been presented (Andersson et al., 1997).

The fatty acid composition of the different oat-based fractions produced with SC-CO_2 is presented in Table 9.

Table 8. Fatty acid relative composition (%) of oat-based products produced with SC-CO_2

Fatty acid	Oat flakes	Oat oil	Polar lipids
14:0	0.1	0.1	0
16:0	15.1	17.4	26.8
18:0	0.8	0.7	0.8
18:1ω-9	37.1	38.6	17.7
18:2ω-6	45.2	42.9	54.1
18:3ω-3	1.1	0.3	0.7
20:1ω-9	0.2	0	0

5.3. Oat polar lipids in encapsulation probiotics (V)

Encapsulative properties of oat polar lipid fractions were tested with the pure test media (negative control), *Bifidobacterium breve* alone, encapsulated with oat polar lipids or with a combination of oat polar lipid encapsulation and maltodextrose as a prebiot.

The results with a phosphate-buffered control medium suggest that the colon microbes from swine colon supernatant are using oat polar lipids and maltodextrose as substrate. The results of the tests using yoghurt as an environment were more complicated to evaluate. Yoghurt itself hosts large microbial communities, providing a suitable environment with enough nutrients for its microbial population. Thus, most probably the addition of oat polar lipids and probiotic maltodextrose would not result in a significant effect.

5.4. Some environmental aspects (II-V)

In several industrial processes, supercritical fluids are suitable as substitutes for organic solvents. Moreover, supercritical fluids offer a range of unusual chemical variations in chemical processes. In the food industry, however, the number of industrial applications is limited; typical applications include oil plants and marine oils.

The main advantages of supercritical fluids are related to sustainability: after processing, the products contain no solvent residues. Due to rapid changes in regulations, the concepts of “Green Chemistry” and “Sustainable Development” are under continuous public discussion. Within this context, the use of supercritical fluids in processing offers a interesting step in the right direction (Aymonier et al., 2007).

According to Arai et al. (2008), in a sustainable society, the design philosophy of chemical processes will be changed from large-scale mass production systems to decentralised local-scale production systems. With this philosophy, chemicals and energies can be supplied from diverse biomass and other renewable resources. SC-CO_2 allows selective separations, efficient transformations, and low-energy processing of many types of materials. Supercritical fluid technology is essential for developing compact systems for decentralizing chemical processes and local production. Supercritical water oxidation (SCWO) of biomass will be one key energy conversion technology for material recycle.

Ramsey et al. (2009) reviewed some examples of industrial applications with sustainable processing using supercritical fluids. These applications include: chemical extraction and purification, synthetic chemical reactions including polymerisation, and inorganic catalytic processes. Moreover, biochemical reactions involving enzymes, particle-size engineering, textile dyeing, and advanced material manufacture provide further illus-

trations of vital industrial activities where supercritical fluid technology processes are being implemented or developed.

In a recent review article, Sahena et al. (2009) listed the main techno-chemical advantages of supercritical fluids as follows:

(1) Relatively low viscosity and high diffusivity, thus penetrating into porous solid materials more effectively than liquid solvents.

(2) In extraction with supercritical fluids, a continuous flow through the samples providing quantitative or even complete extraction.

(3) High selectivity by changing pressure and/or temperature of a fluid.

(4) Easy separation of solutes dissolved in supercritical CO_2 by depressurisation.

(5) Suitable technique to study thermally labile compounds.

(6) Sample mass. In optimal conditions, only 0.5–1.5 g of sample is needed in SFE methods.

(7) No (or significantly less) environmentally hostile organic solvents compared to typical extraction procedures.

(8) Possibility for direct coupling with a chromatographic method

(9) Recycling of fluids in large-scale SFE processes

(10) Scalability from analytical scale to pilot plant scale

6. CONCLUSIONS

Modification of fatty acid composition of egg yolks with flax oil, rapeseed oil, fish oil black currant byproduct did not have an effect on their functional or sensory properties. Foaming, foam stability, emulsion formation, and gelling properties were quite similar in eggs with an ordinary diet compared to eggs with a modified fatty acid composition. Based on the results of sensory analysis, it appears evident that eggs with a modified fatty acid composition could be used in the egg industry. Possible applications include liquid egg yolk products used in mayonnaises or salad dressings, or egg yolk powders used in different kinds of fractionation processes.

An environmentally-friendly SF-based method for the production of value-added egg products was developed. With pilot scale supercritical fluid processes, non-polar lipids (i.e., TAGs and cholesterol) and polar lipids (mainly PC and PE) were successfully separated from commercially produced egg yolk powder. Compared to processing with liquid ethanol, a two-step purification process with supercritical fluids produced lipid fractions of higher purity. However, the yield of the lipids was clearly lower than with pure ethanol extraction. The egg-based polar lipids and delipidated egg yolk powder produced with supercritical techniques offer interesting starting materials for the further production of bioactive compounds.

The lipid fractions of oats were separated into non-polar (mainly TAGs) and polar lipid (mainly phospholips and glycolipids) fractions using supercritical fluids. The precipitation method developed in this study resembles the supercritical anti-solvent method, which is typically used in the pharmaceutical industry. The polar lipid fractions contained polar lipids, especially digalactosyadiacylglycerol, which were shown to have valuable functional properties in the encapsulation of probiotics.

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