Developments in understanding and assessment of egg and egg product quality over the last century

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Centennial Paper

Developments in understanding and assessment of egg and egg product quality over the last century

M. ROSSI[1]*, Y. NYS[2], M. ANTON[3], M. BAIN[4], B. DE KETELAERE[5], K. DE REU[6], I. DUNN[7], J. GAUTRON[2], M. HAMMERSHØJ[8], A. HIDALGO[1], A. MELUZZI[9], K. MERTENS[5], F. NAU[10] and F. SIRRI[9]

[1]Università degli Studi di Milano, Dept. of Food, Environmental and Nutritional Sciences (DeFENS), via Celoria 2, Milano 20133, Italy; [2]INRA, UR83 Recherches Avicoles, Nouzilly, France; [3]INRA, UR1268 Biopolymères Interactions Assemblage Nantes, France; [4]University of Glasgow, Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow, United Kingdom; [5]Katholieke Universiteit Leuven, Dept. of Biosystems, Heverlee, Belgium; [6]Institute for Agricultural and Fisheries Research (ILVO), Technology and Food Science Unit, Melle, Belgium; [7]University of Edinburgh, The Roslin Institute and Royal School of Veterinary Studies, Midlothian, United Kingdom; [8]Aarhus University, Dept. of Food Science, Tjele, Denmark; [9]Università di Bologna, Dept. of Food Science (DISA), Ozzano Emilia, Italy; [10]INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, Rennes, France
***Corresponding author: Margherita.rossi@unimi.it**

The hen's egg, in the form of table eggs and egg products, forms a staple part of the world's total protein consumption. In the last century, there has been considerable research effort focusing on ways of improving egg production and enhancing the quality of eggs. More recently, and with the development and application of new molecular technologies, our understanding and knowledge of how an egg is formed, what it actually consists of, in terms of its major versus minor components, and what the functional roles of each of these components might be, have been greatly enhanced. For example, new previously unknown molecules with specific activity or functional properties have been discovered in the egg albumen and yolk, some of which have potential uses in pharmaceutical and other food related applications. This review paper, which is the collaborative effort of members of Working Group 4 - Quality of Eggs and Egg Products - of the European Federation of WPSA, describes the scientific research behind a number of these major advances and provides some insight to the focus of current research in this area.

Keywords: albumen; egg quality; eggs; shell; vitamins; microbiology; protein; yolk

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Introduction

Eggs have been used as food by humans since early antiquity (Romanoff and Romanoff, 1949), but it is only recently that the potential use of eggs and their related products has been fully realised and their production and quality maximized. The contribution of eggs to global protein consumption has shown a dramatic increase over the last 20 years (from 1.84 g/day in 1985 to 2.53 g/day in 2005) with the ratio of egg to milk proteins ranging from 0.4 in USA to 2.7 in China. In 2005, the yearly egg consumption was 145 eggs per capita in the world (Magdelaine, 2009). These trends are expected to continue to grow with increasing world population, especially in countries with improving income.

Intensive methods for egg production were only developed in the USA and, later on, in Europe just after the Second World War. Prior to this, production was limited to small scale production systems often run by families. By 1950, the USA became the world leader in intensive egg production with 60 billion eggs being produced, with 85% of these being destined for the table egg market. World egg production at this time was about 100 billion eggs, but by 1960 this figure had doubled, and by 1990 it had reached 550 billion. These dramatic increases in world egg production were mainly attributed to the large investment in production facilities which took place in European and Asian countries about this time (Japan 20-fold; Western Europe and Brazil four-fold). The rapid growth of egg production in China occurred more recently (four-fold between 1988 and 1998), indeed this trend has continued so that by 2008 China was producing one third of the world's eggs (60,700 t, Magdelaine, 2011). Since 2000, egg production in the majority of other countries has remained fairly stable; the only other exceptions to this are India, Indonesia and Mexico who collectively increased their contribution to global egg production by 26%.

Since the Second World War, there has also been a general shift from the consumption of whole eggs to liquid egg-products. This shift has varied in different countries, along with egg production and total egg consumption. The increased interest in the use of egg-products initially occurred in the USA in the 1950's (25%) reaching a stable level of 30% in the 1990's with Europe catching up later. However, some countries including China continue to have little interest in egg -products (< 5%).

Besides being an energy food and the source of valuable proteins and lipids, the egg finds its way into many other food and non-food applications thanks to the activities of its components. Ovalbumin, egg lecithin and lysozyme have a long history of use as technological coadjuvants, surface active or antimicrobial agents in the food, pharmaceutical and cosmetic industries. In addition, many other molecules bearing biological activities have been identified either in yolk or albumen fractions (Huopalahti *et al.,* 2007). The potential future applications of these egg-derived compounds in designing new functional foods look promising and attractive to consumers and producers alike (López-Fandiño *et al.,* 2007) and might even further increase the popularity of eggs both as a food and as a source of health promoting components.

Changes in hen performance and selection for improved egg quality

In the early 20th century, hens matured between at six and nine months and produced 100 to 180 eggs per annum depending on the month in which they laid their first egg (Romanoff and Romanoff, 1949). A combination of improvements in genetics and nutrition, the use of artificial lighting programmes and appropriate rearing systems has

seen the number of eggs per bird rise from 130 per annum in 1940, to 260 per annum by 1980, and to > 300 per annum by 2000.

In the 1950's, the focus of breeding companies was on increasing egg numbers and improving feed efficiency. Egg quality traits have received attention only more recently, mainly to avoid any deterioration in eggshell quality or yolk content. Brown egg laying lines, in contrast, have been selected over a shorter time period than American white lines, and so for the European market, where consumers prefer brown eggs, breeding companies continued to focus on improving egg production and feed conversion throughout the 1980's. By the early 1990's, improvements in the region of +30 eggs, +2 kg egg mass and -35 g/kg eggs/yr feed conversion were obtained by selecting for earlier sexual maturity and decreased feed consumption resulting in lower hen weight gain (Nys *et al.,* 2008).

Concerning egg quality, the main effort of breeding companies between 1981 and 1991 was to control egg weight with hen age. Improvements in eggshell colour and thickness were considered during this period, principally to avoid any detrimental effects resulting from selection on egg number. During the next 10 years, feed efficiency was further improved by slightly lowering the hen weight but mainly by increasing the egg mass. Egg number was increased (+26 eggs) because of earlier sexual maturity, higher peak production and better persistency in lay (25 weeks with more than 90% production in 2001). The improvement in egg mass (180 g/hen/year) and reduction in feed intake (24 g/kg of eggs) was obtained without changing mean egg weight.

From 1995, as a result of the increased demand for liquid egg products but also as a consequence of the requirements from certified production systems, including higher quality products (*e.g.* 'label' appellation), breeding companies started to include the technological properties of egg white (Haugh unit; HU) in their selection programmes. Any decrease in egg yolk/white ratio which might result from selection for egg quality has been continuously controlled. From 2000, the focus has been on improved bird viability in alternative systems of management in response to the proposed European ban on the traditional layer cage which came into force on 1st January 2012. The current focus is now on improved sustainability of production systems, through selection for improved persistency in lay over a longer laying period (more than 80 weeks). It is well established that egg quality (shell, Haugh unit) decreases with bird age, therefore a prerequisite for keeping hens in production longer will be to reduce the number of downgraded eggs by selecting for older hens (more than 65 weeks) which exhibit good eggshell and albumen quality (HU) characteristics including a lower incidence of blood spots, a criterion considered as a priority for certified alternative production.

Recently, breeding companies have started to show interest in the development of genetic markers for novel eggshell quality traits and to localise genetic variability for egg quality traits to specific regions of a chromosome using genome scans to identify loci using medium density microsatellite maps or candidate gene approaches (Tuiskula-Haavisto *et al.,* 2002; Hocking, 2005; Honkatukia *et al.,* 2005a; Dunn *et al.,* 2009; Dunn, 2011; Dunn *et al.,* 2012). One of the great successes of this approach was for a Mendelian trait. The discovery of a mutation on chromosome 8 (SNP) in the FMO3 gene associated with fishy odour (Honkatukia *et al.,* 2005b) has been used for commercial elimination of this defect. It seems likely that the next advance will be the use of genome-wide breeding values for individual birds using low cost genotyping combined with large scale phenotyping of egg quality traits (Albers, 2010; Dunn, 2011; Preisinger, 2012).

The eggshell – new insights into its formation and structure

The eggshell of the hen is a highly ordered polycrystalline structure and was first described by Von Nathusius (1821-1899) and translated and edited by Tyler (1964). The development of scanning electronic microscopy in the 1970's confirmed the early observations of these authors (Simons, 1970; Hamilton, 1986; Solomon, 1991, Arias *et al.,* 1993; Nys *et al.,* 1999). In essence, the hen's eggshell is composed of six layers. The inner and outer shell membranes form the innermost two layers. The inner zone of the calcified part of the shell is composed of irregular cones or mammillary knobs, the tips of which are penetrated by the outer membrane fibres. The palisade layer, which gives rise to the thickest part of the calcified shell, extends beyond the fused bases of the mammillary (knob) layer and ends in a thin vertical crystal layer where the crystallite crystals are aligned perpendicular to the shell surface. The outermost layer of the shell, the cuticle, is an organic layer deposited on the surface of the vertical crystal layer. It contains a thin film of hydroxyapatite crystals in its inner zone (Dennis *et al.,* 1996), and the bulk (two-thirds) of the superficial eggshell pigments (Nys *et al.,* 1991). All avian eggshells share the same mineral component, namely the trigonal phase of calcium carbonate ($CaCO_3$) known as calcite, which is the more stable polymorph at room temperature. The calcite crystals within the hen's eggshell show a preferred orientation (Sharp and Silyn-Roberts, 1984), the C axis tending to be perpendicular to the eggshell surface in the upper region of the shell (García-Ruiz and Rodríguez-Navarro, 1994). This high degree of control of size, shape and orientation of the crystals of calcite is responsible for the eggshells unique ultra structure and exceptional mechanical properties (egg breaking strength for a domestic hen egg is 35 N for a mean eggshell thickness of 0.33 mm). Recent evidence indicates that there is a strong genetic basis to the crystal size and orientation in eggs from pedigree laying lines (Dunn *et al.,* 2012), suggesting that it may be possible to incorporate these traits into selection programmes in the future.

The thickness of the eggshell, its form and size and structural elements, as well as features of the porous system varies among different species, however, the general structure of the eggshell is basically the same in all birds (Romanoff and Romanoff, 1949; Board, 1982; Mikhailov, 1997). The eggshell forms at body temperature in a confined space, within the distal segment of the oviduct in an acellular uterine fluid that is super-saturated with calcium and bicarbonate (80 - 120 times greater than the solubility product of calcite; Nys *et al.,* 1991) and which contains the organic precursors of the shell matrix. Calcium carbonate precipitates spontaneously from this saturated milieu in the form of calcite, the polymorph promoted by the presence of the organic constituents (Hernandez-Hernandez *et al.,* 2008). The initial radial crystal growth occurs at specific seed sites on the outer shell membranes which consist of organic aggregates, and then continues outwards and upwards to form the mammillary knob layer. Subsequently, adjacent mammillae fuse to form the base of the palisade layer where radial growth is inhibited due to competition for space. Only crystals growing approximately perpendicular to the surface can continue to elongate, explaining the observation of a preferred orientation in the upper region of the shell (García-Ruiz and Rodríguez-Navarro, 1994). The anisotropic growth of calcite crystals, which is a prerequisite for this hypothesis, is due to a control of crystal morphology by the components of the organic matrix (Chien *et al.,* 2008; Hernandez-Hernandez *et al.*, 2008). The characterisation of the eggshell matrix proteins and the organic precursors in the uterine fluid was initiated in the 1990's. These matrix proteins, including the specific uterine ovocalyxins and ovocleidins, have since been described in numerous

reviews (Nys *et al.,* 1999; 2004; Hincke *et al.*, 2008; 2012) as has the *in vitro* and *in vivo* evidence which demonstrates their role in controlling the formation of the eggshell.

The protein composition of the egg

The egg, including the shell, contains a large range of nutrients and bioactive molecules that are essential for the development of a reproductive cell into a mature chick. Until 2000, less than 15 of these proteins had been biochemically characterised in the albumen, and less than five in the yolk (Burley and Vadehra, 1989; Li-Chan *et al.,* 1995; Sugino *et al.,* 1997). Up until this point, egg-related proteins could only be studied using classical biochemical techniques although this was later expanded by the development of molecular tools in the 1990's. The functional relevance of only a fraction of these proteins had been determined, for example, of the 13 major egg white proteins identified, lysozyme had been functionally assigned as an glycoside hydrolase, ovomucoid and ovoinhibitor, cystatin as a protease inhibitor and ovotransferrin, ovoflavoprotein and avidin were functionally categorised as having either mineral- or vitamin-binding capacity (Li-Chan *et al.,* 1995). For the egg yolk proteins, lipoproteins (Low Density Lipoproteins, HDL), livetins and phosvitins were successfully purified and characterised. IgG-like antibodies (IgY) found in the γ-livetin fraction have since been discovered and numerous applications for these have been developed in pharmacology and diagnostics (Schade *et al.,* 2007; Hatta *et al.,* 2008).

The development of high-throughput methods (proteomics and transcriptomics) used in combination with the chicken genomic sequence (International Chicken Genomic Sequence Consortium, 2004) together with the development of bioinformatics tools to identify genes and ascribe function has since revealed that there are actually hundreds of minor proteins in the egg and its shell (Gautron *et al.,* 2011). For example, molecular biochemistry has revealed more than 10 specific matrix proteins, the ovocalyxins and ovocleidins (Nys *et al.,* 2004), in the eggshell. However, the most surprising information has came from a recent study which examined the entire eggshell proteome and revealed the presence of 500 proteins in the eggshell matrix (Mann *et al.,* 2006). A transcriptomic study has revealed that there are 600 over-expressed genes in the uterus (responsible for eggshell formation) (Jonchère *et al.,* 2010). The same technologies have also revealed about 200 different proteins in the egg white (Mann, 2007; D'Ambrosio *et al.,* 2008) and more than 800 specifically expressed genes in the magnum (Gautron *et al.,* 2011), about 300 proteins in egg yolk (Mann and Mann, 2008) and vitelline membrane (Mann, 2008) and hundreds of specifically expressed genes in the liver and the infundibulum. Many of these proteins are currently classified according to their putative biological function using bioinformatics by analogies with molecules previously characterised in various tissues of birds or mammals (Mine and D'Silva, 2008; Rehault-Godberg *et al.,* 2011). These putative functions include a large range of activities (cell metabolism and regulation, protein synthesis and folding, ionic transport, antimicrobial or antioxidant activities, proteases and antiproteases). This screening of egg proteins will mobilise numerous scientists to examine the putative function of these components in coming years. The following step will be to purify a selection of molecules identified as having high potential to confirm their functional properties and to provide new insights into egg-related biology and technology (embryonic development, protective or technological properties). This strategy would allow the future development of novel proteins which could be used by the pharmaceutical and food industries. Alternatively, some of these proteins might also be used as markers to select hens producing eggs of higher quality, using single nucleotide polymorphisms (SNP) of corresponding genes, in particular to

reduce the risk of food-borne disease outbreaks for humans. Some success in this respect has been made with the association between ovocleidin 116, shell thickness (Dunn *et al.*, 2009) and crystal orientation (Dunn *et al.,* 2012) as well as ovocalyxin 32 with crystal orientation (Dunn *et al.,* 2012), mammillary layer thickness (Dunn *et al.*, 2009) and deformation (Takahashi *et al.,* 2010).

Improvements in our knowledge relating to egg microbiology

Eggs contents are generally sterile at oviposition, but can become contaminated by micro-organisms, including spoilage organisms and pathogenic bacteria. There are two possible and thoroughly studied routes causing bacterial egg contamination: transmitted either vertically or horizontally. In the vertical or transovarian route, the egg content becomes contaminated during its formation in the ovary or oviduct. In the horizontal route, after oviposition, micro-organisms present on the eggshell surface penetrate the eggshell.

Egg contamination by *Salmonella enteritidis* (SE) is currently one of the most important causes of foodborne gastroenteritis in humans throughout the world. SE seems to have a special capability to colonise the hen's reproductive tract and to survive in the egg albumen (Raspoet *et al.*, 2011). Although studies have showed that eggs can be contaminated by SE via penetration of the eggshell (De Reu *et al.*, 2006a), several studies support the idea that the transovarian route may be more important (Raspoet *et al.*, 2011).

The eggshell surface becomes bacterially contaminated with 10^4 to 10^6 CFU/egg as it passes through the vent and comes into contact with the environment (De Reu *et al.,* 2008). Because of their tolerance for dry conditions, the microflora on the surface of the eggshell are dominated by Gram-positive bacteria. With the introduction of alternative housing systems for laying hens in the EU, recent research has focussed on the influence of housing system on contamination of the eggshell (De Reu *et al.*, 2008).

The internal contents generally remain sterile after oviposition because of eggs' natural defences. Many researchers have shown that the cuticle, the shell and shell membranes are important barriers preventing micro-organisms entering the internal egg environment at this time (De Reu *et al.*, 2006a). In addition, the egg albumen has important antimicrobial defences (lysozyme, ovotransferrin, physico-chemical properties). Besides *Salmonella* (especially SE) other major contaminants of the egg contents include Gram-negative bacteria, including *E. coli*, *Alcaligenes spp*., *Pseudomonas spp.* and *Proteus spp.,* and, to a lesser extent, Gram-positive bacteria such as *Staphylococcus spp*. and *Bacillus spp.* (De Reu *et al.,* 2006b). It is generally considered that that Gram-negative bacteria are better equipped to overcome the antimicrobial defences of the albumen.

The microbiota of egg products depends essentially on that of the raw material (egg), the nature of the product (*e.g.* liquid, dried, concentrated, added salt) and is strongly influenced by the processes of transformation and stabilisation that are applied. Pasteurisation, for example, significantly reduces the number of Enterobacteriaceae (*e. g. E. coli, Pseudomonas*, *Salmonella*). The egg product industry focuses particularly on controlling heat-resistant and spore forming psychotropic bacteria, depending on the transformation and/or stabilisation processes used (Baron and Jan, 2011).

New developments in measuring egg freshness

Freshness makes a major contribution to egg quality and grading; however, the only quantitative parameter for egg freshness evaluation provided by European Union regulation is air cell height (EU, 2003), whereas U.S. grading requirements also consider thick albumen height, expressed as HU (USDA, 1995). These indices have been questioned because of their dependence on egg weight and laying-hen age, respectively.

Chemical methods of assessing egg freshness, such as HPLC quantification of uridine and pyroglutamic acid in albumen, are available but the high natural variability in these parameters has limited their application (Rossi *et al.,* 1995). Very promising results however can be obtained by measuring furosine in albumen, as an index of Maillard reaction development during egg aging (Hidalgo *et al.,* 1995), and by applying low resolution proton nuclear magnetic resonance (LR-H-NMR) to evaluate the thinning phenomenon of albumen (Capozzi *el al.,* 1999). These methods, although sensitive, both suffer from the need for specific and expensive analytical equipments, and the former is also time consuming. Better potential as a routine control analysis is found in the spectrophotometric rapid test of the reaction between albumen and 3,3',5,5'-tetramethylbenzidine (Rossi *et al.,* 2001), which gives a response proportional to the level of iron passing from yolk to albumen during egg storage. Karoui *et al.* (2006), using front-face fluorescence, observed that fluorescent spectra recorded on thick and thin egg albumen can potentially be used to differentiate between fresh and aged eggs.

Non-destructive methods for determining egg freshness in on-line sorting machines have recently been reviewed by Mertens *et al.* (2011). Ragni *et al.* (2008) has developed PLS models based on the dielectric spectra for the prediction of the air cell height and thick albumen height. Wang *et al.* (2009) monitored the internal egg quality (HU) using an electronic nose while Kemps *et al.* (2007) evaluated the possibility of combining LR-H-NMR and visible near-infrared (VIS-NIR) transmission spectroscopy for the assessment of albumen freshness. These authors concluded that the combination of both techniques did not improve the accuracy of prediction when compared to the sole VIS-NIR. Giunchi *et al.* (2008) used Fourier transformed (FT) NIR reflectance spectroscopy to predict air cell height, thick albumen height and HU, and obtained 100% eggs prediction of days of storage. Zhao *et al.* (2010) measured the NIR reflectance spectra of eggs and, using a support vector data description algorithm, found that this correctly discriminated 93.3% of fresh and aged eggs according to their HU. VIS-NIS transmission spectroscopy was successfully applied by Abdel-Nour *et al.* (2011) - the spectral data in this case was compared to the egg HU and albumen pH. Prediction correlation coefficients up to 0.94 were obtained in this study. In conclusion, spectroscopic techniques appear to have the potential for measuring egg freshness, potentially on the packing line.

Developments in non-destructive methods for eggshell defect detection, and automated sorting and grading

Methods for measuring and grading egg quality have evolved from sample based, destructive, subjective and/or slow methods towards fast, non-destructive and objective techniques that allow the rapid measurement of all eggs on a packing line (Mertens *et al.,* 2011).

The first critical aspect of shell quality has to be the inherent strength of the shell. Table eggs have to be able to resist all of the impacts they are subjected to during routine

grading and handling. Broken eggs cannot be sold as class A eggs. The occurrence of hair line cracks also raises the risk for bacterial contamination forming a threat to external and internal quality, and even food safety. A novel, non-destructive measure of shell strength, 'the dynamic stiffness', based on the acoustic response techniques, was developed during the 1990s. This measure is based on the resonant frequency or vibrational response of intact eggs after being subjected to a small, non-destructive impact. The dynamic stiffness of an egg has subsequently been shown to predict whether an egg will remain intact or broken during routine egg handling (Mertens *et al.,* 2006). Alternatively, the complete frequency spectrum can be used to predict shell strength in terms of the maximum breaking force (F_{max}).

The second issue related to eggshell quality is the presence of cracks. Novel methods for crack detection include the acoustic response of the egg itself or by the vibration of a piezoelectric sensor. These techniques, or indirect derivations of them, are already applied in online egg graders. Commercial crack detectors use either impactors (small metal balls or hammers) or use the egg as an impactor on a sensor (piezoelectric).

Consumers perceive the visual aspect (homogeneous colour and cleanliness) as a major issue in terms of eggshell quality. In industry, shell colour is measured using reflectometry, but novel methods for defining the brown colour of eggs, such as VIS/NIR spectroscopy (Mertens *et al.,* 2010) and Front Face Fluorescence Spectroscopy (Karoui *et al.,* 2006), have been evaluated. In the former, the Transmission Colour Value (TCV) is defined using the specific absorption peak (643 nm) of the molecule mainly responsible for the shell colour *i.e.* protoporphyrin IX (PPIX), with higher TCV values for paler eggs. The latter method measures the fluorescence at 635 and 672 nm, also specifically related to PPIX, after ultra violet lightening. Shell cleanliness is usually defined using camera vision systems that are currently operational in industrial grading systems. The last aspect of the shell is the *cuticle quality*, an important factor for protection against bacterial penetration. The presence or absence of a cuticle can be visualized by means of Tartrazine and Green S staining. Methods to quantify the resulting colour change using reflectance spectroscopy have met with some success (Bain *et al.,* 2009)

Modifying the nutritional value of eggs

In 1934, Cruickshank observed that the hen's diet modifies the unsaturated fatty acid composition of the yolk, whilst the saturated fatty acid composition was less affected (Cruickshank, 1934). Since then, there have been many attempts to increase the proportion of unsaturated fatty acids, mainly linoleic acid, and the unsaturated/saturated fatty acids (U:S) ratio. During the eighties, human nutritionists and health experts advised consumers to increase the U:S ratio in their diet by increasing their polyunsaturated fatty acid (PUFA) intake. As a consequence, several studies were undertaken in the egg sector to achieve this goal. During the nineties, the British Nutrition Foundation and the Department of Health, stressed that not all the PUFA's had the same effect and that all saturated fatty acids were atherogenic. The recommendation at that time was therefore to increase the intake of n-3 PUFA instead of n-6 PUFA in order to reach a n-6:n-3 ratio lower than 6:1. As a result, research was directed towards the enrichment of eggs with n-3 PUFA by feeding hens with vegetable or animal products rich in these specific types of fatty acid.

The addition of rapeseed or linseed oils to the hen's diet resulted in a substantial increase in the n-3 PUFA content, mainly consisting of alpha-linolenic acid in the egg yolk with a small increase in long chain n-3 PUFA's such as eicosapentaenoic (EPA) and

docosahesaenoic acids (DHA) (Caston and Leeson, 1990; Cherian and Sim, 1991; Meluzzi *et al.,* 2001).

Several attempts have been then made to enhance the proportion of long chain n-3 PUFA (mainly DHA and EPA) in the yolk by including fish oil or marine algae in the diet of laying hens (Farrell, 1994; Sirri *et al.,* 2001, Sirri and Meluzzi, 2011). However, when the yolk was enriched with these long chain n-3 PUFA's, the arachidonic acid (n-6 PUFA) content, which is synthesised from linoleic acid (n-6 PUFA), decreased due to the larger utilisation of delta-6-desaturase in the n-3 pathway rather than in the n-6 pathway.

The concept of enriched food has been applied to the enhancement of the vitamin content of eggs. In general, as the fat-soluble vitamin concentration of the feed increases, so does the vitamin content of the yolk. In this respect vitamin E has been studied most due to its important role in human health because of its antioxidant properties. It has been shown that the alpha-tocopherol (AT) content of fresh eggs can be increased by the inclusion of alpha-tocopheryl acetate (ATA) in the diet up to doses of 20 g ATA/kg feed (Surai *et al.,* 1995; Grobas *et al.,* 2002). Above this level the AT transfer efficiency from the diet to the yolk decreases. This also happens when high levels of unsaturated fats are added to the hen's diet (Meluzzi *et al.*, 2000; Sirri and Barroeta, 2007).

Vitamin A is important since it is involved in numerous processes (vision, integrity of mucous membrane, reproduction, immune response, bone development). Vitamin A levels in the egg yolk can be increased using diets supplemented with beta-carotene and retinyl acetate (Naber, 1993; Jiang *et al.,* 1994).

Vitamin D is important in the formation and maintenance of bone. Mattila *et al.* (2003) increased the yolk vitamin D content by enhancing the supplementation of dietary cholecalciferol in the hen diet.

In summary, the fortification of the hen diet with PUFA and vitamins has resulted in the production of eggs with higher nutritive value which had, in turn, a direct positive impact on not only satisfying human daily nutrients requirements, but in improving the health of consumers.

The cholesterol story

The first observation of a link between blood cholesterol and death from coronary heart disease (CHD) was made a century ago, but this hypothesis was not confirmed until 50 years later by large scale epidemiological studies (Martin *et al.,* 1986; Griffin, 2011). Eggs contain a large amount of cholesterol (around 200 mg/egg). In the 1970s-80s it was demonstrated that a very high daily consumption of eggs significantly increased plasma LDL cholesterol in humans. This form of cholesterol is a determinant of health risk due to its regulation by cell internalisation through the LDL receptor pathway (Brown and Goldstein, 1986). The link between eggs and dietary cholesterol resulted in a dramatic reduction in egg consumption in the 1980s and, at the same time, stimulated numerous experiments to try and reduce the level of cholesterol in eggs. However, while enrichment of egg is easily achieved by modifying the hen's diet, it was soon discovered that it was much more difficult, even impossible, to decrease the cholesterol content of eggs by nutritional or alternative means (Griffin, 1992).

Lipids and protein precursors in the yolk issue from intact lipoproteins synthesised in the liver, which have a very stable composition in the hen (Nys and Guyot, 2011). Over 95% of yolk cholesterol is associated with the yolk triglyceride-rich lipoproteins, 80% being non-esterified and linked to phospholipids on the surface of very low density lipoproteins (VLDL). In otherwords, the cholesterol content of yolk is determined mainly by the cholesterol content of the VLDL, and not by the cholesterol levels in

the hen's plasma (Griffin, 1992). The proportion of yolk versus white in an egg appears to be important in this situation. The presence of free cholesterol is crucial for cell metabolism and reproductive physiology, and any drastic reduction of plasma cholesterol by pharmacological means can impair egg formation and production (Griffin, 1992; Nau *et al.,* 2010). In 2000, the scientific evidence linking dietary cholesterol with serum cholesterol and CHD was re-appraised. Moreover, it was demonstrated that dietary cholesterol exerts a relatively minor and clinically insignificant effect on serum LDL cholesterol and the risk of CHD risk compared to the role of saturated fats and other CHD risk factors (Kritchevsky, 2000; Howell, 2000, Herron *et al.*, 2003; Griffin, 2011). In addition, it was shown that some of the components of eggs *e.g.* ovomucin (Nagaoka *et al.,* 2002) and sphingomyelin (Noh and Koo, 2003), were able to reduce the intestinal cholesterol absorption in mammals. In summary, the daily intake of one to two eggs is no longer associated either with an increased risk of cholesterolemia or CHD (McNamara, 2002) in the majority of the human population. Hyper-responders however still need to be cautious (Knopp *et al.,* 1997; Herron *et al.,* 2003) with eggs as with all foods, and consumers should follow the national safety recommendations provided by their country's food standard agency or the equivalent.

Advances in egg product processing and functional properties

The egg is a major multifunctional food ingredient. The proteins, lipids and lipoproteins are responsible for these functionalities, due to their biochemical and structural characteristics, and the supramolecular assemblies they form either naturally or after processing. Nowadays, the major challenge is to control these supramolecular assemblies, to understand the structure-functional relationship, to enhance the quality of existing products and to imagine innovative products.

EGG WHITE FUNCTIONALITIES

The egg white mainly consists of proteins (almost 90% of dry matter) in water. Among the many proteins identified (Guérin-Dubiard *et al.,* 2010), the major ones have been extensively described, and characterised. Their interfacial properties and their ability to stabilise foam make egg white a very effective foaming agent (Vani and Zayas, 1995). Egg white proteins are also responsible for the gelling properties of the egg white, but all these properties largely depend on environmental parameters (Mine, 1995; Hammershøj *et al.*, 1999), as well on technological treatments (Hammershoj *et al.,* 2004). In 1989, Kato *et al.* (1989) discovered that heating egg white powder improves its foaming and gelling properties. This discovery led to a wide range of industrial applications (Hammershøj *et al.,* 2006) but the fundamental question still remained: which protein characteristics govern the functionalities of egg white?

The development of analytical chemical techniques has since linked functional performance to gel microstructure (Croguennec *et al.,* 2002), and specific molecular structures (Lechevalier *et al.,* 2007; Desfougères *et al.,* 2011) but there is still much to learn in this field.

EGG YOLK FUNCTIONALITIES

For many years it has been recognised that the yolk is a complex system consisting of granules (22% of yolk) held in suspension in a plasma (78% of yolk) (Chang *et al.,* 1977). The plasma has been shown to consist mainly of low density lipoprotein (LDL), spherical particles (about 35 nm in diameter) with a lipid core in a liquid state

(triglycerides and cholesterol esters) surrounded by a monolayer of phospholipids and proteins (Evans *et al.,* 1973). More recently it was discovered that the LDL plays an essential role in the main technological properties of egg yolk (emulsifying properties). Indeed, the LDL serves as a vector, until the interface, of surfactant constituents (apoproteins and phospholipids), insoluble in water (Le Denmat *et al.,* 2000; Martinet *et al.,* 2003; Dauphas *et al.,* 2006; Jolivet *et al.,* 2006). Once near the interface, the LDL structure is broken up to release surfactant constituents. The anchorage of the apoproteins present on the LDL surface is the initial step of the LDL disruption mechanism, because of the induced unfolding of the protein, followed by the destabilisation of the external layer of the LDL. These results clearly demonstrate how the functionalities of egg yolk are largely due to the supramolecular assemblies it naturally contains.

Conclusions

The science regarding egg production has changed a great deal since 1912. Scientific research and technological developments continue to play a pivotal role in providing us with new insights and understanding about how eggs form, precisely what they consist of, and what the function of their individual components might be. This will not only ensure that eggs and their products will continue to significantly contribute to the worlds protein consumption, but that they will be used in a much more diverse way in the future.

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