BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Achieving sustainable production of eggs

Volume 1: Safety and quality

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SCIENCE PUBLISHING

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Introduction

The quality of the egg encompasses its chemical composition, nutritional quality as a human food source, sensory qualities including appearance and special nutraceutical benefits for human health. Eggs contain all essential nutrients for human health except for dietary fibre and vitamin C. This volume also discusses handling, packaging and storage of eggs, conditions that affect the quality of the product that reaches the consumer. The microbiological safety of eggs is of paramount importance as eggs have been implicated in outbreaks of food-borne illness, mainly caused by *Salmonella*. Fortunately, by virtue of its role in nature, the egg possesses many anti-microbial properties, and these properties can be maintained and enhanced for the benefit of consumers. This volume, *Achieving sustainable production of eggs Volume 1: Safety and quality*, explores the wealth of research addressing these themes.

Part 1 Egg composition and chemistry

Part 1 consists of three chapters on egg composition and chemistry of the eggshell, egg white and egg yolk, respectively. The integrity of the eggshell is of primary importance because it ensures the safe delivery of the egg's contents from the point of production to the consumer, as well as influencing the microbiological safety of the egg's contents, as outlined in **Chapter 1**. The layers of the eggshell perform different functions. The mammillary layer and its bond with the shell membranes is the foundation of the eggshell. The palisade layer forms the bulk of the thickness of the eggshell. The surface cuticle is a protective layer that impedes the ingress of bacteria into the egg's contents. The eggshell consists of both the inorganic calcite and an organic matrix, with the synthesis of the organic components determining the dynamics of eggshell production. At the time of publication, 699 proteins have been identified in the eggshell. Most characteristics of eggshell quality have moderate to high heritability, so genetic selection by breeder companies plays a vital role in maintenance and improvement of eggshell quality. In recent years, the availability of high-throughput technologies in fields such as proteomics and transcriptomics has facilitated the study of the organic components of the eggshell. The goal of the commercial industry is to extend worldwide the life span of layer flocks to around 100 weeks, at the same time maintaining eggshell quality.

Chapter 2 describes the structure and role of the egg white (albumen). As for the eggshell, modern techniques have facilitated the identification of many major proteins in egg white (78 identified to 2007), including ovalbumin, ovotransferrin, ovomucoid, ovoglobulins and ovomucin. In addition, there are many 'minor' proteins. Various proteins influence the properties of egg white, from textural and functional to anti-microbiological properties. The allergens in egg white are ovalbumin and ovomucoid. Ovomucin is largely responsible for the jelly-like structure of egg white and plays a major role in the egg white thinning during storage. Egg white contains many antimicrobial substances including lysozyme, ovotransferrin, ovomucin, ovocystatin and avidin, as well as carbohydrates, minerals and vitamins. In the food industries, egg white is used for its functional properties of gelation and foaming.

Egg yolk composition and chemistry is outlined in **Chapter 3**. Egg yolk consists of 15.7–16.6% proteins (lipoprotein, livetin, phosvitin, riboflavin-binding protein, biotin-binding protein, thiamine-binding protein, egg yolk transferrin, proteins with cholinesterase activity), 32–35% lipids (neutral lipids, phospholipids, cholesterol), minerals and vitamins, antibodies and bioactive substances such as sialic acid. Egg yolk immunoglobulins (IgY) can be selectively produced in eggs for human therapeutic purposes. Egg yolk can be separated into two main fractions by dilution and centrifugation: plasma (supernatant – 85% low-density lipoproteins, 15% livetins) and granules (precipitate – 70% high-density lipoprotein or lipovitellin,16% phosvitin). Egg yolk can be fortified with desirable substances such as omega-3 fatty acids, folic acid and vitamins by enriching the diet of the hens. Continuing development in proteomics of egg yolk proteins has great potential for identifying novel egg proteins and their biological functions for use in human health. Egg yolk lipids are used in human infant formula because they closely mimic the composition of human breast milk. In the food industries, egg yolk is used as an emulsifier. Recent studies have shown that earlier concerns about consumption of egg yolk causing elevation of plasma cholesterol in humans are unfounded as eggs contain low levels of saturated fatty acids which are the main cause of elevated plasma cholesterol.

Part 2 Safety

Part 2 comprises Chapters 4–10 on the food safety of eggs and egg products. **Chapter 4** lists the main pathogenic bacteria associated with eggs. Although a large number of microorganisms (bacteria, yeasts, moulds) may be found on the outside of eggs, the organisms of greatest concern are *Salmonella* spp. and, to a lesser extent, *Campylobacter* spp. In many parts of the world, *Salmonella* Enteritidis is the main cause of egg-associated food-borne illness. *S.* Enteritidis is capable of entering the egg during its forming in the hen's oviduct. It is also able to pass across the shell of a fully-formed egg. To date it appears to be the only type of *Salmonella* capable of such vertical transmission. In Australia, where *S.* Enteritidis is not endemic in commercial laying hen flocks, *S.* Typhimurium is the main cause of egg-associated illness. **Chapter 5** explores the mechanisms by which pathogens of concern to human health are transmitted into the contents of eggs, with emphasis on *S.* Enteritidis. Although *S.* Enteritidis is able to enter an egg via the hen's oviduct (primary or vertical transmission), transmission of other pathogens appears to occur exclusively by horizontal transmission from the outside of the eggshell into the egg contents. Other pathogens associated with eggs are discussed including other serovars of *Salmonella* such as Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Campylobacter jejuni* and *Listeria monocytogenes.* Mechanisms of trans-shell penetration (horizontal transmission) are discussed, including risk factors such as cracks or defects in the shell and/or cuticle, storage conditions and water condensation on the eggs. Chapter 5 also covers the natural antimicrobial properties of all regions of eggs.

Chapter 6 reviews the literature on methods of sampling and detection of *Salmonella* in eggs. This information is critical to effective monitoring of commercial eggs. Emphasis is on *S.* Enteritidis, although other bacteria associated with eggs are mentioned. In commercial production systems, pathogens such as *Salmonella* arise mainly from environmental sources. It is difficult to detect bacteria inside eggs so sampling needs to involve large numbers of eggs. Storage time and temperature influence the ease with which bacteria can reach the egg contents. Bacteria are detected on the outside of eggshells by immersing the whole eggs in a nutrient broth in special bags. Shells may be crushed to release bacteria contained in the pores of the shells. Detection of bacteria in egg contents requires pooling and incubation of samples to increase the chances of detection. Following pre-enrichment, samples are plated onto selective agar, incubated and colonies selected for further analysis. Rapid detection methods have increased the speed at which the process can be conducted. Egg yolk antibody detection provides additional information. **Chapter 7** provides a more detailed description of the antimicrobial properties of egg white and addresses ways of enhancing these natural defences. Detailed information is provided on the properties and role of lysozyme; ovotransferrin; the proteinase inhibitors ovomucoid, ovoinhibitor, ovostatin and cystatin; the vitamin-binding proteins avidin and riboflavin-binding protein; the defensins AvBD11 and Gallin (OvoDA1) and others including Tenp (Transiently Expressed in Neural Precursor) (Ovoglobulin G2); Ovalbumin-related protein X (OVAX) and recently identified proteins VMO-1, pleiotrophin and beta-microseminoprotein-like. Factors influencing bacterial growth and spread in egg white include temperature, pH, viscosity, carbon dioxide levels and ionic composition. This chapter also reports on the effects of physiology, genetics, age and immune status of the hen on egg white antimicrobial properties. The effect of changes occurring during egg storage, on the antimicrobial properties, of the egg white are also discussed in detail. The use of modified atmosphere packaging (MAP) and cryogenic cooling of eggs can improve the properties of stored eggs. Also discussed are the changes that occur during incubation of embryonated eggs and how these affect the antimicrobial properties of the egg white.

The egg industry, all around the world, has experienced a trend of moving away from conventional cages, towards cage-free production systems. Conventional cages are banned in the EU and egg production in Australia has been moving progressively towards free-range production. **Chapter 8** reviews the effect of housing system on egg quality and safety. Social pressures resulting from concerns about hen welfare have resulted in this shift towards cage-free production, although this production system does not result in a product of higher quality. The chapter reviews the types of microorganisms that are found on eggs. Some of these are indicators of general cleanliness and others are potentially pathogenic for humans. Research studies comparing housing systems have produced contradictory results, although non-cage systems are more likely to result in higher levels of bacteria on the outside of eggs and this is particularly the case for floor eggs. Strain of hen, flock management, management of the nest boxes and stocking density have all been shown to influence egg cleanliness. Challenge studies using *Salmonella* indicate that non-cage production systems do not necessarily result in worse outcomes for food safety. Similar results were obtained when commercial farms were surveyed for the presence of *Salmonella* and *Campylobacter* with housing system not having a consistent effect on flock microbiological status. Various studies have investigated the effect of production system on eggshell quality and egg internal quality. Although some studies have reported best-quality eggs from conventional cages, others have found no consistent relationship. It appears that some of the earlier egg quality problems that occurred in non-cage production systems have been overcome by improvements in the design of the production systems and in flock management. Ensuring that hens lay in the nest boxes in cage-free systems is essential for the maintenance of egg quality and safety.

Chapter 9 evaluates the effect on food safety of the egg-washing process. Egg washing is not permitted for first quality eggs in Europe, but is commonly practiced in other countries including the United States and Australia. Whereas properly conducted egg washing can improve the microbiological safety of eggs, it can actually make the situation worse if washing is not carried out correctly. A series of steps need to be carried out and important considerations are temperature; time; type, concentration and application of the cleaning chemical used (including agitation, use of brushes); rinsing; application of sanitizer; and drying of the eggs. This needs to be followed by monitoring, process control and verification. Care needs to be taken to avoid damage to the cuticle of the eggshell and to avoid ingress of the egg washing solutions into the egg contents.

The type of packaging used for transport of eggs from the processing plant to the consumer is important, as reviewed in **Chapter 10**. Most egg packaging used throughout the world is ‘low barrier’, meaning that it is very permeable to air and water vapour, but has the advantage of minimizing the build-up of water condensation on the eggs. The loss of carbon dioxide and water vapour for eggs housed in low barrier packaging results in changes in egg quality during storage. These losses can be reduced by using high barrier packaging but at the expense of increased condensation on the eggs. MAP enables maintenance of egg quality. Carbon dioxide atmosphere packaging improves egg albumen quality in terms of Haugh unit, coagulation and foaming, whipping and gelling properties. Although studies have shown no adverse effect of the MAP on the microbiological safety of the eggs, it is recommended that an absorbent be included in the packaging to reduce condensation of water vapour on the outside of the eggs.

Part 3 Sensory and nutritional quality

Part 3, Chapters 11–17, covers the sensory and nutritional quality of eggs. Maintenance of egg quality is of fundamental importance to the egg industries as consumers are increasingly discriminating in their choice of food items. **Chapter 11** examines consumer preferences for egg quality and the ways in which quality can be measured. Quality indicators of concern to consumers are cleanliness, egg size, shell colour and shell colour uniformity, shell strength and integrity (absence of cracks and defects), albumen quality, yolk colour, absence of meat and blood spots. Preferences for some of these measures vary among countries. Some consumers are strongly influenced by animal welfare considerations and the type of production system. Others focus on health benefits and are prepared to pay a premium for specialty eggs such as organic, or eggs fortified with nutrients such as omega-3 fatty acids. A range of different equipment types are available for measurement of eggshell quality and egg internal quality in research studies and in quality assurance laboratories associated with commercial facilities. However, in commercial egg-processing operations, equipment for measuring quality needs to be compatible with the in-line processing equipment used. Such methodologies are being extended to detection of microbiological agents, as well as of eggs produced in one production system (e.g. conventional cage), but are being sold as having been produced in a different production system (e.g. free range). Measurement of egg appearance and colour is further explored in **Chapter 12**. Egg consumption per annum varies greatly among countries, with Mexico and Japan having the highest consumption at more than 300 eggs per year per person. The attractiveness of eggs as a food item is influenced by many of the quality measurements described in Chapter 12 and also by packaging and marketing. This chapter discusses the regional differences in consumer preferences and outlines ways in which egg appearance and colour can be achieved.

Shell eggs are regarded as having a limited shelf life and **Chapter 13** investigates ways of improving the shelf life of eggs. Shelf life is important both in terms of the sensory qualities of eggs and also in terms of their microbiological safety. This chapter outlines some of the ways in which shelf life is determined by regulators in different countries and ways in which it may be extended. Of primary importance is the handling and storage of eggs, with refrigeration being one of the most important factors. Rapid cooling has been investigated, although it is important that this process does not induce microcracks in the shells of the eggs. Pulsed visible light, electrolysed water, MAP, ultraviolet radiation, ionizing radiation, whole egg pasteurization and ultrasonic treatment have all been investigated, at least experimentally. A range of egg coating substances have been tested: oils and waxes, carbohydrate coatings such as those based on cellulose or chitosan, emulsions of oils and chitosan, proteins and synthetic polymers. Oils, both mineral and vegetable, have excellent moisture-proofing properties, whereas chitosan coatings have antimicrobial properties, which make the combination of these two substances promising for use in extending the shelf life of eggs. One commercial provider reports use of whole-egg pasteurization followed by coating with food-grade wax as extending the shelf life significantly.

Chapter 14 reviews the nutritional role of eggs for humans. Eggs are one of the most affordable, accessible and nutrient-dense foods available around the world. The proteins, lipids, carbohydrates, vitamins, minerals and other functional nutrients in an egg not only are sufficient to support the transition from fertilized cell to newborn chick, but also provide unique nutrient contributions to the human diet. Eggs contain proteins (12.6%), lipids (9.5%), vitamins and minerals. There have been concerns about the association between egg consumption, coronary heart disease (CHD) and blood lipids. However, recent research and analyses of data from a large number of studies have failed to support any association between egg consumption and CHD. There is also no clear evidence to implicate egg consumption in the development of type II diabetes. Because of their effect on satiety, eggs can play a useful role in weight loss. In addition, eggs are the source of many bioactive compounds which promise to be useful for human health. The uses of eggs are further explored in **Chapter 15**, which reviews the nutraceutical benefits of eggs. Biological functions of eggs, reported in the literature, include anti-inflammatory, anti-hypertensive, anti-microbial, anti-oxidant, blood cholesterol/lipid-lowering, immune modulatory, anti-toxin/allergen and skin/cartilage/bone strengthening. Eggshell powder is a natural source of calcium and microelements (magnesium, fluorine, strontium, copper, manganese and zinc), which have a positive effect on bone density. Components of eggshell membranes can assist in maintenance of healthy joints and as treatment for wounds. The section on egg white complements Chapter 2. Egg white proteins that have nutraceutical benefits are ovalbumin, ovotransferrin, ovomucin, ovomucoid, avidin, lysozyme and cystatin. Egg yolk proteins with nutraceutical benefits are lipoprotein, livetins and phosvitin, and lipids with nutraceutical properties are fatty acids and phospholipids. Efficient methods for large-scale isolation and purification of biologically active constituents of eggs are needed for these products to be commercially viable.

Chapter 16 describes how the nutritional profile of eggs can be enhanced by supplementing the diet of hens with bioactive substances such as omega-3 fatty acids, vitamins, minerals and carotenoids that are then deposited into the eggs. Various sources of omega-3 fatty acids may be used including plant sources such as flax and marine sources such as microalgae and fish. Attention needs to be paid to sensory qualities of the eggs produced. Water-soluble B vitamins are deposited into the egg albumen whereas the lipid-soluble vitamins (A, D, E, K) are deposited into yolk. Carotenoids are deposited into yolk. Egg enrichment with the minerals selenium, iodine, manganese, chromium, zinc, copper, cobalt and boron has been investigated. Combinations of supplements have also been trialled. Enriched eggs are generally viewed as a specialist food item, but have the potential to increase dietary intake in selected groups of humans.

Chapter 17 addresses molecular breeding techniques for improving egg quality. Since the release of the chicken genome sequence in 2004 (Hillier et al., 2004), the range of technologies available for genetic improvement of the multiple traits that define egg quality has increased greatly. The genetic improvement techniques include whole-genome methods, genomic selection, the targeted gene approach and investigations of gene expression and regulation. Genome-wide association studies have identified regions of the genome associated with variation in the different egg quality traits. These regions are called quantitative trait loci as it is presumed that they contain genes that influence the quantitative traits. These studies are able to identify candidate genes, leading to marker-assisted selection. Egg production, along with the various indicators of eggshell quality and egg internal quality, can be considered individually and in combination. Genomic selection relies on using whole-genome information to predict breeding values of animals rather than trying to identify specific genomic regions. In the targeted gene approach, genes with potential to influence the traits of interest are identified. These candidate genes are then investigated in more detail. The thousands of genes that constitute the chicken genome do not work independently. They work together within genetic regulatory networks of interacting genes that influence gene expression. Proteomics is the study of large numbers of proteins all of which are expressed at one time or in one tissue. By combining information from genetic networks with proteomic studies of specific tissues, it is possible to understand when genes are turned on or off, and in which tissues or regions of individual organs this occurs. This knowledge can help in understanding traits of interest for improving egg production. The recently developed genetic manipulation tool CRISPR (Cong et al., 2013) allows rapid and highly specific editing of genes. The genome is altered to produce a very specific and desired change in the genes to improve a particular trait. All these methods complement each other in achieving better understanding of genetic determination of egg-quality traits.

Summary

The chapters in volume 1 outline the structure and function of the main egg components: eggshell, egg white and yolk. The microbiological challenges to the food safety of eggs are discussed, as are ways of detecting pathogens, enhancing the egg's natural defences against pathogens and ways of minimizing food safety risks in the egg industries. The appeal of eggs as a food product is critical to the profitability and sustainability of the egg industries, so ways of maintaining and enhancing the sensory and nutritional quality of eggs are outlined. Finally, the way in which modern molecular breeding techniques improve egg quality is discussed.

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