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SCIENTIFIC OPINION

Special measures to reduce the risk for consumers through *Salmonella* in table eggs – e.g. cooling of table eggs[1]

Scientific Opinion of the Panel on Biological Hazards

(Question No EFSA-Q-2007-198)

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PANEL MEMBERS

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SUMMARY

Following a request from the German Federal Institute for Risk Assessment the Scientific Panel on Biological Hazards was asked to deliver a scientific opinion on special measures to reduce the risk for consumers through *Salmonella* in table eggs, e.g. cooling of eggs.

As stated in the EFSA Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in 2007, the reported number of cases and incidence of human salmonellosis in the EU were, respectively, 154,099 cases and 31.1 cases per 100,000 inhabitants. The report also documents that the *Salmonella* prevalence in table eggs was 0.8%. According to an opinion from the Scientific Committee on Veterinary Measures relating to Public Health on *Salmonellae* in Foodstuffs (2003), eggs and products containing raw eggs are among the food categories most likely to pose the greatest risk to public health in relation to salmonellosis.

Table eggs are identified as a major source of *Salmonella*, and egg refrigeration has been suggested as one of many possible interventions along the food chain to reduce the incidence of salmonellosis in the human population. On the other hand, problems associated with this measure have long since been highlighted, including those resulting from an inability to maintain the cold chain and the consequential water condensation on the egg surface which facilitates growth and penetration of microorganisms into the egg. Additionally, rapid cooling may provoke cracks in eggs because of temperature gradients and this may further facilitate microbial migration through the shell.

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The Scientific Panel on Biological Hazards concludes that cooling of table eggs at 7˚C or below limits the growth of pathogens such as *Salmonella* spp. On the other hand, cooling does not reduce existing *Salmonella* contamination inside the egg, and can prolong the survival of *Salmonella* spp. on the egg shell.

Provided that the cold chain is maintained, commencing cooling at farm level has the highest beneficial effect with regard to the control of the growth of *Salmonella*. Cooling of table eggs is an additional control option complementing other measures applied at farm level and during processing in an integrated approach. Disruption of the cold chain is one factor that increases the risk of condensation and this could increase bacterial penetration into the egg.

There is evidence indicating that cross-contamination of egg shells can occur at the processing level (egg grading, packing, etc.). The probability of this cross-contamination depends on the proportion of *Salmonella*-contaminated eggs, and is further influenced by the type of technology used and the hygienic practices applied. There is however not sufficient data to evaluate the occurrence of trans-shell penetration and growth of *Salmonella* due to cross-contamination during processing and consequently to assess the related risk for consumers.

The estimation of the relative efficacy of egg cooling as an additional measure to reduce the risk of human salmonellosis would require a quantitative approach, taking into account *Salmonella* prevalence and contamination numbers on egg shell and in egg content. In addition, storage conditions and consumer practices have to be considered. Such data are highly variable and available only to a limited extent.

The Scientific Panel on Biological Hazards recommends that a quantitative approach should be initiated in order to assess the benefits of egg cooling. The collection of quantitative data on *Salmonella* contamination of egg shell and content, guided by preliminary modelling activities is recommended for different EU Member States to estimate the effectiveness of cooling as an additional risk reduction measure. Also an assessment of the efficacy of ongoing *Salmonella*-reduction measures at farm level is needed. More research is needed on the relevance of cross-contamination of eggs with *Salmonella* at processing level and its consequences for public health.

Key words: *Salmonella*, egg, cooling, consumer risk

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BACKGROUND AS PROVIDED BY THE GERMAN COMPETENT AUTHORITY

In the EU in 2006, salmonellosis was the second most commonly recorded bacterial zoonosis accounting for 160,694 confirmed human cases. Also in 2006, the majority of the reported food-borne *Salmonella* outbreaks were related to eggs and egg products[2] . In Germany, the number of human *Salmonella* cases has been reduced since 1992 (200,000 reported cases) to around 50,000 cases in 2006, which is still a high level of salmonellosis in the population.

According to a baseline survey regarding *Salmonella* prevalence in flocks of laying hens in each Member State of the European Union, the prevalence estimates varied largely, for a minimum of 0% to a maximum of 62.5%. In Germany, 24.8% of the holdings were infected with *Salmonella* Enteritidis and/or *Salmonella* Typhimurium. Moreover, findings of *Salmonella* in and on table eggs based on single sampling testing in six EU Member States (including Germany) ranged from 0% to 7.1% as reported in 2006[2]. Hence, *Salmonella* contaminated table eggs do still pose an important risk for the consumer. In particular, human *S.* Enteritidis cases are most commonly associated with contaminated eggs.

Control of *Salmonella* in the table egg sector is most effectively done by monitoring and controlling *Salmonella* in live hens in laying flocks. Regulation (EC) No 2160/2003[3] highlights that the number of contaminated table eggs is related to the number of human *Salmonella* cases within the EU and describes specific measures concerning the placing on the market of products originating from flocks that have not been tested free of relevant *Salmonella*. Regulation (EC) No 1168/2006[4] envisages a stepwise reduction of the contamination level as a complete eradication of *Salmonella* Enteritidis and/or *Salmonella* Typhimurium is currently not feasible.

Additionally, Regulation (EC) No 1237/2007[5] sets down the date 1 January 2009 when restrictions on the marketing of table eggs are to be applied. It sets rules on the marketing of eggs to guarantee that eggs from flocks which are subject to restrictions within the framework of a *Salmonella* control programme are marked in a way which easily distinguishes them from table eggs before being placed on the market. Within the control strategy, the flocks are regularly checked for the presence of *Salmonella*. However, as there is no method available to detect 100% of infection[6] (sensitivity) or to detect an early infection, the testing regime cannot guarantee completely that table eggs from infected flocks are not entering the market.

The fact that table eggs from different laying hen holdings within the EU and third countries (i.e. differing prevalence levels for *Salmonella*) are mixed within egg packing establishments entails the possibility of cross contamination between the eggs. Evidence exists that contaminations can occur at this stage[7]. While trans-shell penetration has been modelled

[2] EFSA, The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. The EFSA Journal, 2007. 130

[3] OJ L 325, 12.12.2003, p.1-15

[4] OJ L 211, 01.08.2006, p.4-8

[5] OJ L 280, 24.10.2007, p.5-9

[6] The WG understands hereby that based on currently available methods the risk of contaminated eggs entering the food chain can not be excluded

[7] Reu, K.d., *et al.*, Bacterial shell contamination in the egg collection chains of different housing systems for laying hens. British Poultry Science, 2006. 47(2): p. 163-172.

mathematically[8,9], this contamination mechanism and its possible impact on the contamination of fresh table eggs have not been considered in recent quantitative models[10,11].

In the current situation and despite the implementation of marketing restrictions from the beginning of 2009, *Salmonella* contaminated table eggs will enter the market due to the lack of sensitivity of the current detection methods. Therefore, additional measures need to be implemented to guarantee an effective protection of consumers and to reduce exposure to *Salmonella* through table eggs.

Several studies recommended cooling of table eggs to reduce the high numbers of human *Salmonella* infections. Experimental studies assessing the influence of time and temperature on the multiplication of *Salmonella* Enteritidis in table eggs showed a positive correlation between the number of *S.* Enteritidis and the storage temperature. It further showed a suppression of *Salmonella* penetration by cooling of the eggs prior to exposure to *Salmonella* suspensions. The value of prompt refrigeration for restricting the opportunities for *S.* Enteritidis to multiply to high numbers inside the yolk of contaminated eggs was also highlighted[12].

Additionally, EFSA has recommended in the Opinion of the Scientific Panel on Biological Hazards related to the Microbiological Risks on Washing of Table Eggs[13]: "*Storage of (washed) eggs below 8°C could be an option to prevent growth of pathogenic bacteria such as Salmonella spp. present in the egg.*"

In Germany, the current legislation requires to begin the cooling of eggs at 5-8°C at the latest 18 days after laying. This aspect seems to be crucial due to the fact that it is best practice[14] in Germany to mix table eggs from different laying hen holdings and also from other EU Member States and third countries within egg packing establishments.

TERMS OF REFERENCE

With regard to the fact that specific measures for the controlling of *Salmonella* in and on table eggs have been established and will be enforced in 2009 and against the background, that a harmonised approach for all EU Member States must be guaranteed, the European Food Safety Authority is asked to:

- assess whether cooling of fresh unwashed table eggs is an additional suitable measure to reduce the risk of salmonellosis for consumers and, if so,
- to recommend at what temperature, what time after laying and at which processing step the cooling of table eggs should be conducted;
- assess whether a cross contamination between eggs and trans-shell penetration and growth of *Salmonella* in the egg occurring during the egg processing may result in relevant increase of consumer risk due to *Salmonella* in the egg and on the egg shell.

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[8] Grijspeerdt, K., Modelling the penetration and growth of bacteria in eggs. Food Control, 2001. 12(1): p. 7-11.
[9] The WG points out that this publication discusses migration inside egg contents and does not model trans-shell penetration.
[10] FAO/WHO, Risk assessment for *Salmonella* in eggs and broiler chickens., in Microbiological risk assessment series No 2. 2002.
[11] FSIS, *Salmonella* Enteritidis Risk Assessment - Shell eggs and egg products. Final report prepared for the Food Safety and Inspection Service by the *Salmonella* Enteritidis Risk Assessment Team. 1998.
[12] Gast, R.K., P.S. Holt, and R. Guraya, Effect of refrigeration on in vitro penetration of *Salmonella* enteritidis through the egg yolk membrane. J Food Prot, 2006. 69(6): p. 1426-9.
[13] EFSA, Opinion of the Scientific Panel on Biological Hazards on the request from the Commission related to the Microbiological Risks on Washing of Table Eggs. The EFSA Journal, 2005. 269: p. 1-39.
[14] The WG assumes that what is meant here is "common practice"

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ASSESSMENT

1. Introduction

Table eggs are identified as a major source of *Salmonella*, and egg refrigeration has been suggested as one of many possible interventions along the food chain to reduce the incidence of salmonellosis in the human population. On the other hand, problems associated with this measure have long since been highlighted, including those resulting from an inability to maintain the cold chain and the consequential water condensation on the egg surface which facilitates growth and penetration of microorganisms into the egg. Additionally, rapid cooling may provoke cracks in eggs because of temperature gradients and this may further facilitate microbial migration through the shell.

Hygienic requirements and marketing of eggs are covered in the European legislation by Regulations (EC) No 557/2007[15], 853/2004[16] and 1020/2008[17]. For the purpose of this opinion, 'eggs', 'table eggs' or 'shell eggs' mean eggs in shell (not broken, washed, incubated or cooked eggs) that are produced by farmed birds and are fit for direct human consumption or for the preparation of egg products. Egg processing comprises the phases aimed at getting the egg ready for distribution (collection, inspection, sorting/grading, packing/packaging and shipping). Regulation (EC) No 557/2007 defines 2 classes of eggs (A and B) according to different physical characteristics as follows: (i) Class A eggs ("fresh eggs" or "table eggs") should have a "normal, clean and undamaged" shell and cuticle; they "shall not be washed or cleaned before or after grading", and "shall not be treated for preservation or chilled in premises or plants where the temperature is artificially maintained at less than 5°C. However, eggs which have been kept at a temperature below 5°C during transport for not more than 24 h or on retail premises or in annexes thereto for not more than 72 h shall not be considered as chilled". (ii) Class B eggs, i.e. eggs "which do not meet requirements applicable to eggs in grade A", may only be used by the food or non-food industries.

Implementation of preventive actions throughout the whole egg production chain is undoubtedly an effective approach to reduce *Salmonella* prevalence in the final product and hence salmonellosis incidence in the population. No single universal mitigation option capable of eliminating *Salmonella* entirely is currently feasible. Measures such as farm hygiene and biosecurity, feed control, improved poultry management, testing and removal of positive flocks from production, and poultry vaccination are effective tools that are implemented in *Salmonella* control programmes (EFSA, 2004). These measures should help MS comply with Regulation (EC) No 1168/2006 which, in the framework of a control programme, establishes a stepwise reduction of the prevalence level in *Salmonella* in laying flocks by setting targets for the prevalence reduction. MS should reduce the number of laying hens infected by a percentage each year, with bigger reduction targets for member states with higher prevalence levels. By setting incremental percentage reductions, the aim is to stimulate progress in those MS with a higher incidence of *Salmonella* in laying hens.

In the US, the *Salmonella* Enteritidis Risk Assessment for Shell Eggs and Egg Products (FSIS, 1998) is the result of a comprehensive effort carried out by the Food Safety and Inspection Service (FSIS). In this Risk Assessment exercise (where only *S.* Enteritidis (SE) internal contamination was modelled), egg cooling strategies during shell egg processing and

[15] OJ L 132, 24.5.2007, p. 5–20
[16] OJ L 139, 30.4.2004, p. 55–205
[17] OJ L 277, 18.10.2008, p. 8–14

distribution have been implemented as mitigation strategies in the model tested, estimating the reduction in human illness if eggs are cooled after lay to an internal temperature of 7.2ºC, and if this temperature is maintained throughout egg processing and distribution. The Risk Assessment of *Salmonella* in eggs and broiler chickens (WHO/FAO, 2002) has also evaluated egg refrigeration as a potential intervention along the food chain, calculating the risk reduction obtained by keeping retail storage temperature at no more than 7.7°C.

This opinion deals with food safety aspects of egg cooling from when the eggs are laid until they are processed or purchased as table eggs by consumers. Refrigerated storage of eggs by consumers at a domestic level is therefore not assessed in this opinion.

1.1. Epidemiological data

According to the European Commission (2003), eggs and products containing raw eggs are among the food categories most likely to pose the greatest risk to public health in relation to salmonellosis. In 2007, the reported number of cases and incidence of human salmonellosis in the EU were, respectively, 154,099 cases and 31.1 cases per 100,000 inhabitants (EFSA, 2009). Eggs and egg products were the most frequently reported source of foodborne outbreaks caused by *Salmonella* in 2006 (EFSA, 2007a). *S.* Enteritidis is the serovar causing more than 60% of the human *Salmonella* infections in the EU (EFSA, 2009), and also most often associated with eggborne infections (WHO, 2001).

1.1.1. Monitoring of *Salmonella* in laying hen flocks

Regulation EC No 2160/2003 establishes a progressive reduction in prevalence for *Salmonella* serotypes of public health significance in farm animals. The baseline study (EFSA, 2007b) has determined the prevalence of *Salmonella* in different EU MS on commercial large-scale laying hen holdings with at least 1,000 laying hens (*Gallus gallus)* on the holding (5,310 holdings investigated), by analyzing samples from faeces and dust from laying hen flocks during the last nine weeks of their production cycle. *Salmonella* spp. was detected in an average of 30.8% of the laying hen holdings (range from 0% to 79.5% for specific MS). An average of 20.4% of the holdings was positive for *S.* Enteritidis / *S.* Typhimurium (range from 0% to 62.5% for specific MS). In the US, the prevalence of SE-infected flocks is assumed to be 19.2% with a standard error of 10.4%.

A flock detected positive does not necessarily mean a flock having currently infected birds because of the type of sample taken (faeces and dust in the environment) and although these types of environmental samples are apparently sensitive indicators of *Salmonella* infection in a flock, the current within-flock prevalence may be low (EFSA, 2007b). According to ICMSF (2005) the percentage of *Salmonella*-excreting birds in a flock can vary between less than 0.6 to 30%. The significance of positive environmental samples in terms of the risk of contamination of eggs at retail has not been fully elucidated, and the likelihood of *Salmonella*-contaminated eggs stemming from a *Salmonella* infected flock depends, amongst other factors, on flock prevalence, within-flock prevalence, numbers of organisms harboured and excreted by birds, the frequency with which infected hens lay contaminated egg, and the hygienic conditions in the laying house and egg processing facilities (EFSA, 2007b). According to several authors (Perales and Audicana, 1989; Humphrey *et al.*, 1991; de Louvois, 1993; Henzler *et al.*, 1994; Kinde *et al.*, 1996; Schlosser *et al.*, 1999), the proportion of contaminated eggs laid by naturally *Salmonella*-infected laying hen flocks is variable but often below 3%. It should also be taken into account that the proportion of contaminated eggs laid by an infected hen peaks immediately after *Salmonella* exposure and is followed by a period of lower *Salmonella*-positive egg production (FSIS, 1998). Some authors (Humphrey

et al., 1989b) have also reported egg contamination cycling in naturally-infected flocks (perhaps due to stress factors or natural cycling of re-infections), where *Salmonella*-positive eggs are laid by a flock at high frequency during specific times, intercalated by periods of low-frequency *Salmonella*-positive egg production.

1.1.2. *Salmonella* in eggs and egg products

The Community Summary Report on Zoonoses (CSR) in 2007 (EFSA, 2009) gathers data of *Salmonella* prevalence in the contents of table eggs for several Member States. The prevalence based on single or batch sampling testing has been estimated in the range from 0% to 5.8%. The average *Salmonella* prevalence was 0.8%, which corresponds to the level found in 2006. It should be noted that data are supplied by individual EU MS and the sampling procedures and microbiological analysis schemes are not standardized between MS and these factors can have an influence on results. There is no information provided whether this applies to internal and/or external contamination.

In 2006, six MS (AT, EE, DE, IT, SI, ES) have reported data on *Salmonella* prevalence in table eggs (*Salmonella* positive samples) consistently over the period 2004-2006 (EFSA, 2007a). For these six MS, no apparent trend in the weighted mean proportion[18] of positive samples is observed, while the proportion of positives is reported below 2%. Small sample sizes in some MS results in wide confidence intervals.

In the *Salmonella* Enteritidis Risk Assessment project carried out by FSIS (1998), the prevalence of internal *Salmonella* contamination in table eggs is estimated to be 0.00489%.

Most studies have found a higher prevalence of shell contamination compared to that of the egg contents (Anonymous, 2004; Davies and Breslin, 2004; Anonymous, 2007; Murchie *et al.*, 2007). Little is known about the number of cells in a internally contaminated egg at laying time, but data available indicate that it ranges from 1 to 400 SE bacteria, with most eggs containing less than 40 SE bacteria (Humphrey *et al.*, 1989a; Gast and Beard, 1992; Gast and Holt, 2000a; Chen *et al.*, 2002a).

1.1.3. Serotype distribution among isolates in table eggs

According to the CSR (EFSA, 2007a), which presents serotype distribution among isolates in table eggs and egg products for each MS, *S.* Enteritidis is the predominant serovar, with an average of 90.3% .In 2007, only five Member States reported *Salmonella* serovar distribution of ten or more isolates. *S.* Enterititis remained the most dominant serovar reported (66.5% of all reported serovars in EU) (EFSA, 2009).

1.2. Current regulatory situation for the storage of table eggs throughout the food chain

Recently, the Commission has amended Regulation (EC) No 853/2004, introducing an explicit indication for storage and transport temperature conditions for table eggs, by which "eggs must be stored and transported until sale to the final consumer at a temperature, preferably constant, that is best suited to assure optimal conservation of their hygiene properties, unless the competent authority imposes national temperature requirements for egg storage facilities and for vehicles transporting eggs between such storage facilities."

[18] Reciprocal of the ratio between the number of tested samples per MS per year and the number of laying hens per MS. Number of laying hens per MS were based on the population data reported for 2006, and supplemented with EUROSTAT data from 2005 (AT and IT)

Storage of eggs at low temperatures is observed in the European legislation[19], where eggs which have been kept at a temperature below 5°C during transport for not more than 24 h or on retail premises or in annexes thereto for not more than 72 h shall not be considered as chilled. According to Regulation (EC) No 853/2004, eggs must be delivered to the consumer within a maximum time limit of 21 days after laying. Exceptions for the French overseas departments are also permitted, whereby eggs intended for retail trade in the French overseas departments may be dispatched chilled to those departments. In that case, the sell-by date may be extended to 33 days.

In most European countries (e.g. Spain, Belgium, The Netherlands, and France), there are no specific obligations about egg refrigeration during commercialization. Producers or distributors can include a text on the label of fresh egg packages indicating that eggs should be kept refrigerated after buying, in accordance with European legislation[20] by which an indication advising consumers to keep eggs chilled after purchase can be included.

In Germany, according to Article 2 (20) of the German food law[21] shell eggs commercially put on the market must be kept at a temperature between 5 and 8°C from the 18th day after laying onwards for transportation and throughout storage.

In the US, according to regulations enforced by the Food Safety and Inspection Service (FSIS-USDA[22]), shell eggs packed for consumers must be kept at an ambient temperature not greater than 7.2°C after processing, during transportation, and throughout storage. Besides, all packed shell eggs are labelled with a statement that refrigeration is required; and any shell eggs imported into the United States, packed for consumer use, should include a certification that they have been stored and transported at an ambient temperature of no greater than 7.2°C.

2. Structure and defence mechanisms of the egg against microbial contamination

2.1. Egg structure and defence mechanisms

The structure of the egg has been thoroughly described elsewhere (EFSA, 2005).

2.1.1. The eggshell

The eggshell allows the exchange of water and gas between the exterior and the developing embryo while at the same time it acts as a barrier against microbial contamination.

From its inner surface, the eggshell is made up of a mammillary layer, cone layer, palisade layer, vertical crystal layer and cuticle. Pores present in the shell are 12-20 μm in diameter, and their number vary according to egg size and location (6,000-10,000; (Bruce and Drysdale, 1994)). Some of the many matrix proteins described (such as ovocleidin, ovocalyxin, ovotransferrin, ovalbumin, osteopontin, lysozyme, clusterin) (Hincke *et al.*, 2000) have antibacterial properties (e.g. lysozyme, ovocalyxin). An uneven layer (cuticle), which is made up of a protein carbohydrate complex and which contains a small amount of the crystal complex hydroxyapatite, is covering the outer surface of the eggshell. This layer is secreted in the shell gland pouch during the last hour of shell formation. Its function is either to bridge the outer pore openings or to extend down into the pore canals, plugging them (Cooke and

[19] OJ L 132, 24.5.2007, p. 5–20
[20] OJ L 109, 6.5.2000, p. 29–42 and OJ L 163, 24.06.2008, p6-23
[21] Verordnung zur Durchführung von Vorschriften des gemeinschaftlichen Lebensmittelhygienerechts as of 8th August 2007 (BgBl 2007 part I No. 39)
[22] Code of Federal Regulations, 2003. Title 21, Chapter 1, Part 115 Refrigeration of shell eggs held for retail distribution. Washington, DC. www.access.gpo.gov/nara/cfr/waisidx_03/21cfr115_03.html

Balch, 1970; Board, 1982). The cuticle may also link the lumina of pore canals to the egg's exterior and, thereby, serve as a pathway for gas diffusion (Board and Scott, 1980). Numerous factors such as the age of the animal, feeding regime, number of eggs laid, etc, influence the extension and composition of the cuticle (Nascimento *et al.*, 1992; Messens *et al.*, 2007).

2.1.2. The shell membranes

The shell membranes are build up of three distinct layers: (1) the inner shell membrane (ISM; lies immediately over the albumen), (2) the outer shell membrane (OSM; is attached to the true shell) which consist of a network of randomly oriented fibres and (3) a homogeneous third layer of electron-dense material called the limiting membrane (Bruce and Drysdale, 1994). This limiting membrane intermeshes with the innermost region of the inner membrane fibres rather than forming a separable and distinct layer (Wong Liong *et al.*, 1997). The shell membranes are approximately 70 µm thick and held firmly together, except at the blunt end of the egg, where they separate to enclose the air space (Simons and Wiertz, 1963). The diameter of the membrane fibres ranges from 0.4 to 3.6 µm, the smaller ones being more numerous in the inner membrane. The fibres have a protein core surrounded by a mucopolysaccharide mantle (Tranter *et al.*, 1983). The composition of the shell membrane fibres is still not fully disclosed. However, the shell membrane protein contains the cross-linking amino acids desmosine and isodesmosine and are different from the other fibrous proteins such as keratin, connectin, collagen or microfibrillar protein (Roberts and Brackpool, 1994). The membranes consisting of a network of branched fibres have pores of approximately 1 µm diameter (Tung and Richards, 1972).

2.1.3. The vitelline membrane

The vitelline membrane surrounding the yolk is made up of two main layers: (1) the inner layer formed in the ovary and (2) the outer layer deposited in the oviduct (Li-Chan *et al.*, 1995). Fromm (1967) noted that the outer surface of the vitelline membrane in fresh eggs is composed of fibres connected to the chalaziferous layer. The vitelline membrane strength decreases during storage (Fromm, 1964; Kirunda and McKee, 2000; Jones and Musgrove, 2005) and has been related to a loss of structural integrity (Back *et al.*, 1982).

The outer layer of the vitelline membrane from hen egg yolk consists of ovomucin, vitelline membrane outer layer protein I (VMOI) and lysozyme (about 60% dry weight). In addition, some minor constituents are present. Ovomucin appears to form the skeleton of the outer layer, but especially lysozyme is responsible for its integrity. The function of VMOI is unknown. The inner layer consists largely of the proteins GPI, GPII and GPIII as isolated before (Kido *et al.*, 1975) from the whole membrane (Back *et al.*, 1982). Also another protein was isolated from the outer layer, which was designated as vitelline membrane outer layer protein II (VMOII) (Kido *et al.*, 1992).

2.1.4. Defence mechanisms

In a well structured shell, the cuticle and the calcite complex of the shell itself provides an effective barrier to horizontal bacterial contamination, mechanically as well as chemically (cuticle that plugs pores and antibacterial proteins within the shell matrix). If the shell is breached, then invading bacteria must progress through the web of membrane fibres before they reach the albumen mass. These fibres too have a complement of antibacterial proteins.

Egg white serves as a barrier because of its properties (viscosity impairs bacterial motility), lack of nutrients (albumen nutrients are not easily metabolised by microorganisms), presence of several enzyme inhibitors (ovoinhibitor, ovostatin), vitamin sequesters (avidin) or

antibacterial proteins (lysozyme), and iron-limiting environment (ovo-transferrin). When the egg is laid, pH of the albumen ranges between 7.6-7.8, but it increases to 9.1-9.6 after three days of storage at ambient temperature due to loss of carbon dioxide. Other factors that function as barrier against microbial growth are the presence of antibodies in egg white as well as the yolk. Yolk contains only IgG and IgM. IgA is absent from yolk but present in the white. Conversely, IgG is either absent from or present in very low concentration in egg white (Rose and Orlans, 1981).

2.2. Mechanisms of primary and secondary contamination and possible cross-contamination along the food chain

The contamination of eggs with *Salmonella* can occur either before shell formation (primary contamination) or after oviposition through contamination of the shell surface and following penetration of bacteria from the shell into the egg content (secondary contamination)(Meyer, 2001). For the purpose of this opinion cross-contamination is considered as transmission of *Salmonella* from the surface of one egg to another.

2.2.1. Primary contamination

Primary bacterial contamination of shell eggs can occur when *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. This may happen if a hen is systemically infected with *Salmonella* spp. through an oral infection, and is the result of a direct deposition of *Salmonella* spp. into the follicles or yolk, while still present and/or attached to the ovary. Serotypes associated with poultry reproductive tissues include *Salmonella* Enteritidis, *S.* Gallinarum (biovars Gallinarum and Pullorum), *S.* Typhimurium, *S.* Heidelberg, and some strains of *S.* Arizonae. Bacteria may also enter the oviduct from the cloacal area and move towards the upper part, the magnum. There they can be incorporated into the albumen or even directly on the yolk membrane Thirdly the *Salmonella* could be introduced through insemination of the hen with infected sperm cells.

2.2.2. Secondary and cross-contamination

At the moment of lay, the wet egg enters an environment with a temperature of approximately 20ºC below the hen's body temperature. While cooling gradually, the egg contents contract and a negative pressure establishes inside the egg, thereby moving contaminants through the shell (Padron, 1990). This contamination will deposit at the shell membranes, and pose a future threat to the egg. Grijspeerdt *et al.* (2005) show in a migration/growth model how such a contamination can develop inside an egg.

Contamination of the egg shell can occur through contact with contaminated surfaces (nesting material, dust, feed, shipping and storage containers), handlers and animals (pets, rodents, insects). The extent and likelihood of trans-shell penetration depends, e.g. on the length of contact time, shell quality (specific gravity) (Sauter and Petersen, 1974), evenness and nature of cuticle and dynamic stiffness values (Messens *et al.*, 2007).

Cross-contamination between eggs and equipment along the food line can also lead to the presence of *Salmonella* on the eggshell. It has been shown by Davies and Breslin (2003) that contamination in egg-packing plants may be a significant contributory factor to external contamination of shell eggs, also those obtained from *Salmonella*-free flocks. Sterilized eggs that passed through five farm-packing plants showed a contamination rate of at least 0.3%. De Reu *et al.* (2005) showed that the moment the eggs enter the candling, grading and packing area can be regarded as a critical point for a significant increase (approx. 1 log) of bacterial

eggshell contamination. At that point, all eggs passed the same small surface, a short conveyor with metal grid.

If the shell is properly structured (with regard to shell thickness, cuticle uniformity, presence of antibacterial proteins, and absence of cracks) bacterial migration to the egg contents will be minimised. Pores do not represent the prime route of transfer and, according to Nascimento (1992), shell imperfections are more significant in this respect. Once through the shell, invading microorganisms must breach the shell membranes barrier. The latter possess antibacterial properties, but they are a temporary barrier when faced with a heavy bacterial challenge.

3. The refrigeration practice

In the US refrigeration of shell eggs is required after processing, during transportation and throughout storage. Refrigeration can be applied at the farm level in integrated facilities (in-line systems) or in separate industrial coolers (off-line system). In the industrial layer facilities, eggs are laid onto a wire floor which rolls the egg toward the front of the cage onto a belt, which transports eggs out of the house either to the egg processing facility (in-line) or to a storage cooler (off-line). In the modern in-line layer facility, laying and packing (and even processing) of eggs is integrated at one location, where freshly laid eggs are taken directly into a processing system (within minutes to 12 h post-lay) where they are visually inspected (checked for eggshell problems, dirt, cracks, blood spots) cleaned, washed, rinsed, sanitized, dried and oiled, where permitted sorted/graded, and packed (in cartons and crates onto shipping pallets) for distribution. Following packaging, eggs are moved to a cooler room, from where they are delivered, commonly within one week of lay. In the off-line facilities operations are not integrated, and fresh eggs are collected and shipped away (usually once a day but sometimes less often) from laying facilities to egg processing facilities where they are processed and packed. These eggs are frequently placed in coolers (for two to three days) at the laying facility before shipment to the processing premises.

For transportation, eggs usually are loaded into refrigerated or more frequently thermally isolated transports for shipment to distribution centres or retail outlets. Some are delivered directly to retail outlets, and others to warehouses and other intermediate distribution points before going to the retail store or food service facility where they reach the consumer.

3.1. Temperature

Patterson *et al.* (2008) conducted a study in the US during which they sampled eggs from the egg belt immediately after oviposition. The initial internal temperature of these eggs averaged 37.5ºC. According to this study internal egg temperature can vary considerably depending on ambient temperature, time since oviposition, and the conducting surfaces to which the eggs are exposed. Information about cooling of eggs for the European situation is scarce and focuses on cooling of hatching eggs (Meijerhof and Van Beek, 1994). Recently, Braun *et al.* (2008) demonstrated that it takes about 120 min until the table eggs have cooled from 41°C (hen's body temperature) to the ambient temperature of 20°C and 210 min to an ambient 10°C.

In-line eggs have been reported to enter the processing centre at an average internal temperature of 21ºC whereas off-line eggs are received at temperatures around 13ºC (Koelkebeck *et al.*, 2008). These values can be very variable depending on many aspects, for example whether eggs are washed (normally a water temperature of 46ºC is used, but also cool washing water has been used (Jones *et al.*, 2005)). When washing was used, the internal temperature at packaging time was reported to be in the range 26.7-34.4ºC (for in-line

facilities) and 24.4-26.7ºC (off-line). After packing, most processors hold eggs in coolers at an ambient air temperature of 7-15ºC (when cooling systems are used). Storage and transport can take place at varying ambient air temperatures, but this temperature does not correlate to internal egg temperature. Eggs at the centre of a pallet can take up to 142 h or more to equilibrate with an ambient temp of 7ºC (Anderson *et al.*, 1992; Anderson, 1993). Eggs in cardboard cases require nearly one week to cool down from 27ºC to 7ºC (Czarick and Savage, 1992). Cooling rate depends on the temperature differential between ambient and egg, the egg weight, air speed, the egg position in the pack, the packaging materials, and how the crates are packed and stacked. Transport for delivery is carried out usually employing isothermal containers, and no temperature reduction is generally achieved in this stage. Similarly, in most warehouses and supermarket display cases in Europe eggs are kept at room temperature, and they are not refrigerated. Eggs received by retail stores are frequently at temperatures well above 7ºC. During storage and transport excessive temperature fluctuations (which provoke water condensation and subsequent microbial growth) at all stages until consumption should be avoided.

There are new developments aimed at accelerating the cooling rates of eggs at the laying and/or packing centre. For example, new stacking procedures or designs which allow an adequate air flow around the eggs achieve a quicker cooling. Other ways of accelerating cooling include the use of specially vented, view windows, corrugated fibreboard cases and packing egg cartons in returnable wire baskets. This can be complemented by systems to cause air to flow through packaging so that each piece is exposed to moving cold air. High capacity cooling systems need a dedicated refrigeration equipment and fan component, while small-scale systems can use existing cold room refrigeration with a fan. Rapid cooling systems use forced convection at temperatures around 0ºC and air velocity 30 m/min. Rapid cryogenic cooling methods using liquid or gaseous nitrogen and carbon dioxide have been tried (Jones *et al.*, 2002b).

For rapid cooling systems, the number of microcracks in the egg shell has been reported to increase due to thermal stress. Thermal stress has been cited to be much greater in rapid cooling conditions (Lin *et al.*, 1996). Anderson *et al.* (1995) reported that cryogenic systems based on liquid carbon dioxide were able to effectively reduce internal egg temperature from 43.3°C to 7.2°C within 80 to 90 seconds. Mermelstein (2001) indicated that a cryogenic system, based on liquid carbon dioxide cooling, increased the strength of the eggshell membranes, and in addition, reduced *Salmonella* Enteritidis contamination by 2 $\log_{10}$. In these systems the egg quality is generally higher (higher Haugh units and reduced internal and external bacterial counts). Fajardo *et al.* (1995) found by scanning electron microscopy that the eggshell pieces from rapidly cooled eggs (air speed, 30.5 m/min; air temperature, 0°C) had more microscopic cracks, which were wider, compared to the eggshell pieces from the control (no cooling). It could be seen that the shell from the cooled egg had longer and wider microscopic cracks than the cracks found on the control. In the rapidly cooled eggs, some microscopic cracks had openings as large as 5 to 10 µm wide allowing microorganisms to penetrate into the shell membranes.

3.2. Condensation

Water condensation on the egg shell is dependent on the surrounding temperature, relative humidity (RH) and actual egg shell temperature (Zeidler, 1994) and could be avoided by optimising the cooling process, storage and distribution of eggs. Generally condensation or "sweating" of eggs occurs when eggs are transferred to a higher temperature environment without adjusting the RH. Measuring the RH in the new situation and adjusting the

temperature and/or RH according to a psychrometric chart or Mollier diagram may avoid condensation.

4. Effect of temperature on microbiological growth and survival in eggs

4.1. Effect of temperature on the survival of *Salmonella* on the egg shell

The survival of *Salmonella* on the surface of eggs is dependent on temperatures of storage (Humphrey, 1994) and may be increased by storage at low temperatures (Baker, 1990; Radkowski, 2002; Messens *et al.*, 2006). *Salmonella* on eggshells can die rapidly during storage but survival is enhanced as the temperature (10°C, 15°C, 23°C) is lowered farther from the optimum for growth (Simmons *et al.*, 1970). At a RH of 85-88%, *Salmonella* survived longer at lower temperatures (2°C or 10-13°C) than at 22-25°C (Rizk *et al.*, 1966). In the study by Baker *et al.* (1990), *S.* Enteritidis only survived for one day at room temperature, while contaminated eggshells could still be found at 12 days of storage at 7°C. Braun *et al.* (1999) observed that *S.* Enteritidis survived on the shell for up to 4 weeks at 5°C, but could not be recovered later than 2 weeks at 21°C. Survival can also be enhanced due to the slower metabolism induced by the disadvantageous conditions on the dry eggshell surface. In the study by Rizk *et al.* (1966) moisture retention on the shell may also have accounted for the greater survival of *Salmonellae* at low temperature because the eggs were placed in storage when still wet. In the study by Messens *et al.* (2006) the survival of *S.* Enteritidis was examined upon storage for up to 20 d at temperatures ranging from 15 to 25°C and RH of 45 to 75%. At all storage conditions, mean counts decreased over time, but a limited proportion of shells carried high numbers of *Salmonella*. Radkowski (2002) observed better survival of *Salmonella* on shells of egg at lower temperatures (2ºC, 20 ºC, 30ºC tested).

When the eggshell was inoculated with faeces containing *Salmonella* and stored at 4°C, numbers also decreased over time (Schoeni *et al.*, 1995) but *S.* Enteritidis still survived for 4 weeks (Braun *et al.*, 1999). A longer survival at 21°C on eggs contaminated with faeces compared to clean eggs was also observed by Braun *et al.* (1999). At 25°C, growth of *Salmonella* on the eggshell was observed; faeces may thus have provided the required nutrients (Schoeni *et al.*, 1995).

Salmonella penetration can occur either after lay (most likely) or at later stages in the distribution chain, if eggs are subject to environmental changes resulting in temperature differential across the shell, or condensation. Certain combinations of temperature and RH can lead to condensation on the eggshell (see above), and influence survival, penetration and growth of *Salmonella*. Several studies report that low RH together with low temperature increases to a certain extent the probability of survival on the eggshell as compared to high RH (Radkowski, 2002; Messens *et al.*, 2006). On the other hand, high RH leads more easily to condensation on the eggshell, and facilitates penetration and possible growth inside the egg (Braun *et al.*, 2005; Messens *et al.*, 2006). In this regard, penetration and growth are favoured when the inoculation dose is high and temperatures are high. When the temperature is below 10ºC, penetration and growth are severely restricted.

4.2. Effect of temperature on the growth of *Salmonella* in eggs

Freshly laid eggs can be contaminated with *Salmonella* at various sites within the egg (the yolk, the vitelline membrane, the albumen near or far from the yolk, and the inner shell membranes). They are typically reported to contain no more than few hundred *Salmonella* cells (Humphrey *et al.*, 1989a; Gast and Beard, 1992; Gast and Holt, 2000a; Chen *et al.*, 2002b). Both the site of deposition of *Salmonella* and the storage temperature will influence

the growth of *Salmonella*. Most common *Salmonella* contamination was observed on the yolk membrane (Timoney *et al.*, 1989; Shivaprasad *et al.*, 1990; Gast and Holt, 2000a; Gast *et al.*, 2002) or in the albumen (Humphrey *et al.*, 1989a; Mawer *et al.*, 1989; Gast and Beard, 1990; Shivaprasad *et al.*, 1990; Gast and Beard, 1992). In all these studies however *S.* Enteritidis has been isolated from the yolk and albumen.

Upon artificial inoculation in separated albumen, survival upon three days storage was observed for *S.* Enteritidis strains at 10°C, 17.5°C (Gast and Holt, 2000b) and 25°C (Gast and Holt, 2000b; Gast and Holt, 2001b) and for *S.* Typhimurium at 8°C and 22°C (Hu *et al.*, 2001). At 20°C, *S.* Enteritidis did not grow well in separated albumen that was removed from the area near the yolk (within 1 cm around the yolk) and further away from the yolk of eggs previously stored at 20°C for up to 6 weeks (Humphrey and Whitehead, 1993). Among *Salmonella* serovars and phage types, differences in their capacity to remain viable or grow feeble at 20°C were found (Lock and Board, 1992). Some studies however reported growth in separated albumen. Cogan *et al.* (2001) observed that upon a higher inoculum dose of *S.* Enteritidis PT4 in separated albumen, a pronounced growth (levels > 4 log cfu per ml) occurred upon storage for 8 days at 20°C. This was supported by Messens *et al.* (2004) and it was observed that a pronounced growth occurred more frequently when fresh albumen was inoculated compared to stored albumen due to the influence of the pH of the albumen or by its influence on albumen constituents. According to Kang *et al.* (2006), a major factor that controlled *S.* Enteritidis growth in egg albumen was iron restriction, since egg albumen supplemented with iron allowed *S.* Enteritidis to grow, and iron acquisition mutants of *S.* Enteritidis showed decreased survival in egg albumen. In addition, lowering the albumen pH from 9.0 to 8.0, high concentrations of bacteria and low incubation temperatures increases the chances of survival.

When *Salmonella* is inoculated in the albumen of intact eggs, growth can occur in the albumen itself and the cells can migrate to the egg yolk as modelled by Grijspeerdt *et al.* (2005). Braun and Fehlhaber (1995) observed that *Salmonella* can be found in the egg yolk within 1 or 2 days at 20°C and 30°C but also within 14 days at 7°C. Schoeni *et al.* (1995) found that the serovars Enteritidis, Typhimurium and Heidelberg increased ≥ 3 log units upon one day at 25°C in albumen. Even upon inoculation with only 8 cells of *S.* Enteritidis in the albumen of intact eggs, generalized growth was observed upon storage at 20°C in 50% of the samples when the eggs where fresh at the moment of inoculation. When the eggs were stored for 2 and 3 weeks prior to inoculation, less than 10% of the samples showed growth (Messens *et al.*, 2004). This is in contrast to a previous study (Humphrey and Whitehead, 1993) in which growth at 20°C took place more quickly when the eggs were stored prior to inoculation for > 3 weeks at 20°C. Okamura *et al.* (2008) inoculated the albumen with *S.* Enteritidis and simulated field conditions from farm to home situation. They found no growth of *S.* Enteritidis during 10 week storage at 10ºC. At 25ºC or higher rapid growth occurred after 2 weeks of storage.

Egg yolk is an excellent medium for *Salmonella* growth at supportive temperatures. Rapid multiplication occurred at 20°C (generation time of 1.23-1.82 h) (Guertler and Fehlhaber, 2004) and above (Gast and Holt, 2000b; Guertler and Fehlhaber, 2004). Also at 17.5°C (Gast and Holt, 2000b) and 15°C (generation time of 3.01-3.87 h) (Bradshaw *et al.*, 1990; Guertler and Fehlhaber, 2004) growth occurred. At 10°C such growth occurred far more slowly (Gast and Holt, 2000b) while no multiplication was observed in eggs incubated at 7°C for 94 h (Bradshaw *et al.*, 1990). Fortunately, Gast and Holt (2001b) found only occasionally *S.* Enteritidis contamination of the yolk itself and more frequently the presence of *S.* Enteritidis on the yolk membrane. Migration through this yolk membrane however to reach the yolk

contents could allow rapid and extensive bacterial multiplication. An in-vitro egg contamination model was used (Gast *et al.*, 2007) to study the penetration into and multiplication within the yolk contents upon inoculation of 4 *S.* Enteritidis strains and 4 *S.* Heidelberg strains onto the exterior (vitelline) membrane surface of egg yolks (dose of 100 cfu). All strains were able to penetrate to the yolk contents. After 36 h storage at 30°C, 45.1% of the samples were penetrated and growth occurred to high levels. The ability to migrate by the 4 *S.* Enteritidis strains was significantly greater than by the 4 *S.* Heidelberg strains. Penetration occurred infrequently at 20°C (in 4.69% of all samples). Using the same in-vitro egg contamination model, multiplication of *S.* Enteritidis within the interior yolk contents occurred in 10% of samples after 6 h of incubation and in 75% of samples after 24 h at 25°C (reaching mean levels of about 4 log cfu/ml) but in only 20% of samples incubated for 72 h at 15°C (Gast *et al.*, 2007).

Although *Salmonella* is deposited in the egg content rarely, refrigeration of eggs as soon as possible after lay is important for inhibition of rapid multiplication of *Salmonella* (Gast and Holt, 2001a).

4.3. Comparison of growth and survival of *Salmonella* in refrigerated and non-refrigerated eggs

Simmons *et al.* (1970) observed better survival of *Salmonella* on the shell surface at low RH (75%) and at low temperature (10ºC). However when *Salmonella* had reached the shell membrane at temperatures above 23ºC growth of *Salmonella* was observed.

Kim *et al*. (1989) demonstrated that *Salmonella* in eggs at 4ºC did not multiply in contrast with 10ºC or higher. Pless (1993) found similar results at 4ºC. However, there was a relation with the inoculation dose (Kim *et al.*, 1989).

Dolman and Board (1992) inoculated the air cell membranes with *S.*Enteritidis and some other bacteria (Gram-positive and Gram-negative). Both at 4ºC and at 37ºC on the membrane *Salmonella* could not compete with *Pseudomonas,* whereas in albumen *Salmonella* became prevalent. There was a difference in the survival of *Salmonella* at 37ºC in relation to the position of the egg: survival of bacteria could not be observed with air cell down.

In the study by Chen *et al.* (2002b), the albumen or yolk were inoculated with *S.* Enteritidis (*ca.* 10 cells) and cooled to 7.2°C using three different cooling techniques (traditional, two-stage-air or liquid CO_2). Especially upon inoculation of the yolk contents, slow cooling or temperature abuse (cooled storage for 30 days followed by storage for 6 days at 37°C) promoted the growth of *S.* Enteritidis in eggs. When the eggshell was inoculated, cooled using the three cooling techniques and stored cooled, rapid cooling did not affect the penetration of *S.* Enteritidis through the eggshell.

Zeidler (1999) exposed eggs to different cooling procedures (slow or fast), and found no transmission of *Salmonella* from the shell or membranes into the egg contents. Catalano and Knabel (1994) found less *Salmonella* penetration through the shell in rapidly cooled eggs. Miyamoto *et al*. (1998) observed that the penetration of *Salmonella* was significantly reduced when the eggs were cooled prior to inoculation. Eggshell contaminations during or after storage when refrigerated temperatures were reached, are therefore of minor importance. Wang and Slavik (1998) studied penetration of *S.* Enteritidis after washing eggs with water or chemicals. No difference in penetration between storage temperatures of 4ºC and 23ºC was found. Consequence of egg cooling is that more microscopic cracks occur in the surface of the egg (Fajardo *et al.*, 1995). Eggshell penetration by *S.* Enteritidis was studied when eggs were at room temperature after cooling by natural convection or forced convection. Using both

cooling techniques eggshell penetration was significantly increased compared to the uncooled eggs. Interruption of the cooling chain may lead to condensation on the shell (Kaess, 1960 in Kiefer, 1976) and penetration of bacteria through the shell. Ernst *et al*. (1998) divided commercial shell eggs into intact and cracked groups. Half the eggs were inoculated by *S.* Enteritidis, air dried, and returned to refrigeration. Half of each group was removed from refrigeration and allowed to sweat for 3 h. The eggs were then quantitatively cultured to determine levels of *S.* Enteritidis penetration. Sweating did not seem to increase the number of *S.* Enteritidis-positive eggs or the number of *S.* Enteritidis present. Dramatically higher numbers of *S.* Enteritidis were found in *S.* Enteritidis-contaminated cracked eggs than in sound eggs. This suggests that sweating for 3 h does not increase egg contamination with *S.* Enteritidis; however, bacterial penetration through the shell is more likely to occur in cracked eggs.

In the study by De Reu *et al*. (2006) the condensate group was stored at 6°C for 24 h followed by storage for 20 days at 20°C and 60% RH (giving 30 min of condensate on the eggs) while the control group was continuously stored at 20°C. Using eggs filled with agar to visualise penetration, more frequent egg shell penetration of *S.* Enteritidis was observed following condensation on eggs, but no effects could be shown using intact eggs. A higher *Salmonella* shell contamination was observed for the agar filled eggs of the condensate group compared to the control group which may explain the higher probability of penetration. In an earlier study (Fromm and Margolf, 1958) penetration was observed more frequently in the eggs allowed to sweat. In this study however, wet eggs kept at 22-24°C were subsequently stored at 10-12°C and 80% RH. The higher probability of penetration in the condensate group could be due to the negative pressure in the eggs allowing contaminated moisture to be drawn into the egg contents.

Another significant change that occurs as the egg gets older is the enlargement of the air chamber. This in turn increases the pressure difference between inside and outside the egg that occurs when temperature differences exist, thereby increasing the likelihood of microbial penetration through the eggshell (ICMSF, 2005).

4.4. Possible side effects of refrigeration on microorganisms other than *Salmonella*

There is a complex microflora colonizing or entering the shell, amongst which pathogens such as *Salmonella*. Probably because of their tolerance to dry conditions, Gram-positive bacteria form the majority of the microflora of fresh clean eggs (Moats, 1980; Mayes and Timoney, 1983). However in spoiled eggs Gram-negative rods are predominant (Board and Board, 1968; Kiefer, 1976; Mayes and Timoney, 1983; Board *et al.*, 2008). Some of the most common contaminants are members of the genera *Alcaligenes*, *Pseudomonas*, *Escherichia*, *Proteus* and *Aeromonas* (Mayes and Timoney, 1983; Board *et al.*, 2008). Many of the spoilage microflora may grow at relatively low temperature such as *Pseudomonas* spp. and fungi.

When the egg is being laid there is a natural temperature gradient between the warm egg (around 40ºC) and the relatively cold environment (ambient temperature). When free water is present, with help of capillary action and temperature differences bacteria can move into the pores and colonize on the shell membrane (Board *et al.*, 2008). Pfennig (1974) observed that motile coliform bacteria could easily penetrate the shell, but Board and Tranter (2008) have not seen direct evidence to support this observation. They merely observed that a very motile strain of *Proteus* increased the possibility for other (non motile) bacteria to pass through the shell. However, penetration of bacteria through the shell is a possible side effect of refrigeration. Kaess (1960 in Kiefer, 1976) states that the presence of water facilitates the

invasion of bacteria and fungi. This may happen when the refrigeration chain of eggs is interrupted and condensation on eggs appears. Vedehra *et al.* (1970) found that the exposure to *Pseudomonas* of the blunt end of the egg caused more spoilage than other areas.

In a study already mentioned above Dolman and Board (1992) *Pseudomonas* became the dominant bacteria on inoculated air cell membranes which where stored at 4ºC and at 37ºC for 20 days. In the albumen *S.* Enteritidis and *Staphylococcus xylosus* were prevalent.

De Reu *et al.* (2006) compared the eggshell penetration of 7 selected bacterial strains (*Staphylococcus warneri*, *Acinetobacter baumannii*, *Alcaligenes* sp., *Serratia marcescens*, *Carnobacterium* sp., *Pseudomonas* sp. and *S.* Enteritidis, recovered from egg contents) using the agar and whole egg approach. Results of the first approach indicated that the Gram-negative, motile and non-clustering bacteria penetrated the eggshell most frequently; *Pseudomonas* sp. and *Alcaligenes* sp. were primary invaders followed by *S.* Enteritidis. However, particularly *S.* Enteritidis was a primary invader of whole eggs.

5. Effect of refrigeration on the egg quality

There are no specific studies available aimed at the effect of refrigeration on the egg properties like effect on egg shell pores, permeability of the vitelline membrane, possibility of condensation, enhancement of microscopic cracks, interior egg quality properties. Haugh Units (HU; Haugh, 1937) are considered as a standard for interior egg quality. Studies including a consideration of refrigeration effects on these properties are often focused on other factors, like nutritional values or consumer acceptability. However, some data can be taken from research reports.

There is some literature on the weight loss of eggs during storage and the directly related air cell size. Jones and Musgrove (2005), Cepero *et al.* (1995), Baur (1975), Bell (1996), Braun (2000) and many others have found that weight loss of eggs stored at low temperatures is lower than of those stored at room temperature. In a study regarding effects of extended storage on egg quality factors (Jones and Musgrove, 2005) eggs were stored at 4°C with 80% RH for 10 weeks. Shell strength and vitelline membrane strength did not significantly change during cold storage. However vitelline membrane elasticity did decrease resulting in possible yolk breakage when used in the household (Jones and Musgrove, 2005). Also albumen height and HU decreased during cold storage in this study. The authors conclude that refrigeration did not compromise physical quality factors or consumer satisfaction. HU values of eggs stored under refrigeration (7.2ºC and 4ºC) were better than those of eggs stored at room temperature (22ºC and 17-18ºC) (Cepero *et al.*, 1995; Bell, 1996). Jones *et al.* (2002a) state that cryogenic cooling can lead to increased HU values. Additionally, Haugh unit values were greater for cryogenically treated eggs as compared to traditionally cooled eggs. Vitelline membrane strength was greater for the cryogenically cooled eggs versus traditional processing. Vitelline membrane breaking strength decreased over storage time. Vitelline membrane deformation at rupture was significantly ($P < 0.05$) greater for the cryogenically cooled eggs compared to the traditionally cooled eggs in each. Bell (1996) also found more frequent yolk rupture when eggs were stored at room temperature.

6. Advantages and disadvantages

6.1. Advantages

Refrigeration is an important tool to limit bacterial growth, especially in fresh products, and the lower the temperature, the more efficient is the control. Consequently cooling of table eggs is an effective tool to control the growth of pathogenic bacteria, especially *Salmonella*

Enteritidis, in both internally and externally contaminated eggs. It also helps restrict eggshell and vitelline membrane penetration by *Salmonella* spp. (Chen *et al.*, 1996; Gast *et al.*, 2007).

Additionally, cooling of table eggs is an efficient tool to control the growth of spoilage microorganisms and to maintain the chemical and physical properties of the egg components (yolk and albumen) and consequently to maintain its internal quality.

6.2. Disadvantages

Eggs are a very fragile product, therefore some technical procedures used for cooling could damage the shell integrity, i.e. microscopic cracks can occur when rapid cooling systems are used. This could be the first step of developing visible cracks allowing the penetration of microorganisms (bacteria, moulds). Penetration of psychrotrophic microorganisms into the egg could be an important risk of rapid physical and biochemical alterations of the internal components of the egg, even if stored at low temperature.

It is recognised that the interruption of the cold chain during trade (collection, sorting, grading, packaging and distribution) can lead to condensation on the eggshell. This condensation can occur under certain combinations of actual eggshell temperature on one hand and temperature and relative humidity of subsequent storage on the other hand. Consequently there may be an increased risk of growth and penetration of microorganisms, including *Salmonella* spp., present on the surface of the eggshell.

It has also been demonstrated that *Salmonella* spp. can survive longer on the egg shell at lower temperatures depending on the relative humidity.

6.3. Balancing advantages and disadvantages

Ideally, cooling early after laying decreases the risk of microbial growth but may increase the opportunities for disruption of the cool chain during trade: at the packaging centre, during distribution, at the retail level and during the transport from the shop to the consumer's kitchen. In addition, multiplication of *Salmonella* is inhibited under refrigeration but survival on the egg shell might be enhanced.

Cooling of eggs does not reduce existing *Salmonella* prevalence in eggs (number of contaminated eggs), nonetheless consumers' exposure to *Salmonella* (number of cells ingested) due to egg consumption would be reduced.

Consequently the balance between the advantages of strategic cooling of eggs from the food safety perspective and any disadvantages arising with regard to logistics requires to be reached with due regard to the safety of the product, i.e. the egg, for the consumer.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Cooling of table eggs at 7˚C or below limits the growth of pathogens such as *Salmonella* spp. but does not reduce existing *Salmonella* contamination inside the egg
- Cooling can prolong the survival of *Salmonella* spp. on the egg shell
- Provided that the cold chain is maintained, commencing cooling at farm level has the highest beneficial effect with regard to the control of the growth of *Salmonella*
- Cooling of table eggs is an additional control option complementing other measures applied at farm level and during processing in an integrated approach.
- The relationship between environmental temperature, relative humidity and actual egg shell temperature affects the development of condensation. Cold chain disruption is one factor increasing the risk of condensation and this could increase bacterial penetration into the egg.
- The likelihood of developing microcracks in the shell increases when using rapid cooling methods. This may limit the range of technologies (time/temperature combinations) that can be applied.
- There is evidence indicating that cross-contamination of egg shells can occur at the processing level. The probability of this cross-contamination depends on the proportion of *Salmonella* -contaminated eggs, technology and hygienic practices. There is however not sufficient data to evaluate the occurrence of trans-shell penetration and growth of *Salmonella* due to cross-contamination during processing and consequently to assess the related risk for consumers.
- The estimation of the relative efficacy of egg cooling as an additional measure to reduce the risk of human salmonellosis would require a quantitative approach, taking into account *Salmonella* prevalence and contamination numbers on egg shell and in egg content. In addition, storage conditions and consumer practices have to be considered. Such data are highly variable and available only to a limited extent.

RECOMMENDATIONS

- A quantitative approach should be initiated in order to assess the benefits of egg cooling.
- The collection of quantitative data on *Salmonella* contamination of egg shell and content, guided by preliminary modelling activities is recommended for different EU MS to estimate effectiveness of cooling as an additional risk reduction measure. Also an assessment of the efficacy of ongoing *Salmonella*-reduction measures at farm level is needed.
- More research is needed on the relevance of cross-contamination of eggs with *Salmonella* at processing level and its consequences for public health.

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