Microbial spoilage of eggs and egg products

C. TECHER[1], F. BARON[1]* and S. JAN[1]

[1]Agrocampus Ouest, INRA, UMR1253 Science et Technologie du Lait et de l'Œuf, 65 rue de Saint Brieuc, 35042 Rennes Cedex, France
***Corresponding author: Florence.baron@agrocampus-ouest.fr**

Under healthy breeding conditions, the egg content is sterile. However, the eggshell surface can be contaminated by a diversified microbiota, including putative food spoilage microorganisms. Egg breaking systematically involves the contamination of egg white and egg yolk through contact with the spoiled shells, thus giving rise to highly perishable egg products. The aim of the present review is to propose a global overview on microbial spoilage of shell eggs and egg products. The spoilage characteristics, according to the type of product and the flora involved, and the methods already available for controlling, reducing or detecting spoilage events are described for both eggs and egg products.

Keywords: bacterial spoilage; eggs; egg products; psychrotrophy; rotten egg.

Introduction

The content of shell eggs is generally sterile, even if some cases of vertical contamination may occur. However, contamination occurs systematically during egg product processing, through the contact of the eggshells with the egg content at the egg breakage step. The microbial contamination can lead to sanitary and/or spoilage problems. The sanitary problem mainly concerns *Salmonella* Enteritidis, one of the most important agents involved in outbreaks in relation to shell eggs and egg products. Even if egg safety remains a key concern, the sanitary risk has been reduced particularly through the improvement of hygienic practices in the breeding environment and a better control of both pasteurization of egg products and respect of the cold chain for their storage. However, microbial spoilage of egg and egg products can still lead to high economic losses. Preventing egg product spoilage represents therefore a real challenge in responding to the ever-evolving consumer demand for natural, safe, nutritious and tasty food. In a first time, this article describes the spoilage characteristics due to the microbial flora involved in the spoilage of eggs and egg products. In a second time, several methodologies are highlighted, allowing controlling, reducing or detecting the spoilage of eggs and egg products.

Egg shell spoilage

The egg content can be contaminated during egg formation in the genital tract of infected hens as well as after laying. The former type of contamination is possible when the hen's reproductive tissues are highly contaminated but it remains sporadic and by far less frequent than contamination occurring after laying. Moreover, the levels of contamination are low. The latter corresponds to the contamination of the eggshell surface by the hen's faecal microorganisms, and by the flora of the environment of hen housing and egg conditioning centres. A diversified microbiota is involved, sometimes including pathogenic bacteria (essentially *Salmonella* Enteritidis) and also food spoilage microorganisms.

<u>Characteristic and species involved in the spoilage of shell eggs</u>

The microflora of the eggshell is dominated by Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Aerococcus* and *Micrococcus*. Other minor contaminants are Gram-negative bacteria, such as *Salmonella*, *Escherichia* and *Alcaligenes* sp., and also Gram-positive bacteria, such as *Bacillus* (De Reu *et al*., 2009; De Reu *et al*., 2008; Moats, 1980). Depending on the study, the level of mesophilic aerobic microbiota on the surface of the eggshells ranges from $10^{3.8}$ to $10^{6.3}$ cfu/egg, with an average level around $10^{4.5}$ cfu/egg (Moats 1980; Jones *et al.* 2004; De Reu *et al.*, 2005; Musgrove *et al.* 2005). Whereas the dominating flora present at the eggshell surface is composed of Gram-positive bacteria, the contamination of the egg contents mainly involves Gram-negative bacteria. These latter are described as better resisting the natural egg defences. It is recognized that bacteria involved in the spoilage of egg contents have the following characteristics: (i) a high mobility facilitating penetration through the eggshell and the eggshell membranes, (ii) ability to resist the growth-inhibiting properties of the albumen, and (iii) various enzymatic activities leading to the breakdown of complex nitrogen and carbon sources present in the egg fluids, thus rendering this matrix suitable for supporting bacterial growth.

The main egg spoilage event is described as rotten egg, which appears as a coloured egg (black, blue, pink, red, green) mostly developing a rotten odour. The bacteria described as being involved are *Pseudomonas, Proteus, Alcaligenes, Enterobacter, Serratia, Stenotrophomonas, Cloaca, Acinetobacter*, *Moraxella* and *Citrobacter* spp. Other egg spoilage events are described as leading to a yellow pigmentation of the shell membranes, due to the action of *Flavobacterium* or *Cytophaga* species.

The relationship between the level of contamination of the eggshell surface and the occurrence of spoilage events is not obvious. Several predominant eggshell bacteria, such as *Micrococcus* and Aerobacter, are rarely found in rotten eggs. On the contrary, several bacteria, such as *Aeromonas* and *Proteus*, are found at low levels on eggshells, while they are amongst the highest occurring bacteria in rotten eggs (Mayes and Takeballi, 1983).

Factors influencing the contamination of shell eggs

The spoilage of eggs is related to eggshell contamination and the ability of some bacteria to penetrate the egg. The type and level of egg contamination of the eggshell surface are related to the hygienic conditions in which the hens are reared, the breeding environment, the breeding practices, the housing system, the geographical area, and the season. Contamination may also occur during egg transport and/or packaging in farms or in the conditioning centre, either through the environment or from one egg to another. Even though the microflora of the eggshell surface varies, the spoilage flora of the egg content tends to be less diversified, highlighting the fact that the intrinsic egg barriers have a strong influence on the invasiveness of spoilage bacteria. Firstly, the cuticle, shell and shell membranes are barriers preventing the penetration of microorganisms from the surface into the egg content. Nevertheless, the cuticle which is resistant to water and microorganism penetration, may crack rapidly over time or during egg manipulation. The effectiveness of this protective coating is therefore limited. The shell, a calcified proteic layer, represents a physical barrier but is rather ineffective because of the possible transfer of microorganisms through the pores, particularly if condensed water is present on the eggshell. The presence of eggshell cracks or micro-cracks increases the risk of contamination. The manipulation of eggs, especially in the conditioning centres, increases the risk of egg cracking. The external and internal shell membranes represent effective filters composed of anti-bacterial glycoprotein fibres, which may play a role in protection against penetration. In addition to these physical barriers, egg white, similar to an intracellular fluid, is an important line of defence against invading bacteria because it represents a not favourable environment for microbial development (nutrient-poor, exhibiting an alkaline pH, and high viscosity and heterogeneity), and because it contains several molecules expressing antimicrobial activities, such as lysozyme, ovotransferrin, proteinase inhibitors (cystatin, ovomucoid and ovoinhibitor), and vitamin-binding proteins (riboflavin binding protein, avidin- and thiamin-binding proteins). The integrity of these barriers (cuticle, shell, shell membranes, egg white, vitelline membrane) is essential to prevent microbial penetration and proliferation.

Controlling, reducing and/or detecting egg spoilage

Various techniques have been explored for the reduction of the level of eggshell contamination and/or for preserving or enhancing the natural defences of the egg itself. They include both the upstream steps (hen selection, improvement of the practices of breeding and farm management) and the downstream steps (packaging, transport and storage of eggs) of egg production and management.

Since the bacterial level on the surface of eggshells is related to the level of air contamination in the breeding environment, it appears crucial to limit dust formation and to promote good hygienic and building decontamination practices in order to prevent the spreading of microorganisms. The microflora and also the number of cracked or broken eggs, are related to the type of hen housing systems. Most of worldwide egg production farms use conventional cages but, due to animal welfare considerations, these conventional cages were banned from the EU in 2012 (E.U. Directive 1999/74/EC) and replaced by aviaries, on-floor and free range systems or furnished cages. While the design of conventional cages was optimized for achieving high hen performance with the additional advantage of hygienic egg quality, the use of a nestes and dust baths generally increases the incidence of dirty eggs and microbial contamination. Nowadays, there remain many unknown details about the incidence of such systems on the egg flora and, consequently, on subsequent egg spoilage events. Therefore, attention should be paid in designing cages ensuring the best egg hygiene quality. The practices of egg collection on the farm, as well as the sorting, packaging, storage and delivery, must also be improved to reduce contamination. Cross-contamination must be avoided by preventing contamination of the staff and the environment.

Egg decontamination practices could reduce the number of bacteria on the eggshell surface. There are various technologies which are employed, depending on the country. Table eggs (Class A eggs) are washed in many countries, including the USA, Canada, Japan, Australia and Russia. This practice is not used in Europe, except in Sweden. Other ways of egg decontamination are being investigated, such as pasteurization, "flash" treatment based on hot water or steam, ultraviolet (UV), ozone, irradiation, ultrasonic, electrolyzed water or ionized air (plasma) treatments (Baron and Jan, 2010). Attention should be paid in improving egg storage conditions, including temperature and duration conditions, both these factors being particularly involved in the preservation of the natural egg defences. These improvements should prevent penetration and growth of bacteria under the eggshell or inside the egg. The storage of eggs at refrigeration temperatures is an effective way of reducing the liquefaction of egg white, the loss of integrity of the vitelline membrane, and consequently, the bacterial growth.

The presence of cracked or "flowing" eggs increases the risk of contamination. The egg conditioning step is hence critical. The integrity of the eggshell appears amongst the best defences against spoilage bacteria. It is thus reasonable to assume that the preservation of the natural egg barriers, and mainly the integrity of the eggshell, would allow an overall reduction in the occurrence of egg spoilage problems. In the packaging centres, egg candling is a key step for controlling the microbiological quality of eggs. Advanced technologies, utilizing computerized integrated cameras and sound wave technology, is also being applied for egg sorting. The use of UV light has been particularly efficient in detecting the fluorescent pigment due to the spoilage of the albumen by *Pseudomonas* (Elliot, 1958). Other methods have been developed for evaluating egg freshness at research or industrial levels, namely sensory evaluation, physicochemical methods (pH, Haugh Units), infrared or front-face fluorescence spectroscopy, and other methods involving microwave sensors, electronic nose based systems, and Nuclear Magnetic Resonance (Karoui *et al*., 2006).

Spoilage of egg products

Because of their various functional properties (foaming, binding, gelling, or dying properties), the egg products are particularly used in the food industry and enter in the composition of a wide range of food, such as sauces, pasta, biscuits, cakes, processed meats, creams and refrigerated desserts. The process of egg product manufacturing induces a systematic contamination of the egg content, since, once broken, the egg loses its natural antimicrobial defences. Whole egg and egg yolk are indeed ideal environments for the development of microorganisms. Furthermore, egg products, themselves liable to contamination, can be added to the composition of susceptible foodstuffs, which in turn can be spoiled even if these products are kept at refrigerated temperature. Monitoring the microbiological quality of egg contents appears therefore essential, especially when they are used as ingredients in susceptible products. Hence, the processes of separation and stabilization have crucial effects on the quality of egg products.

<u>Species involved in spoilage and spoilage characteristics</u>

The predominant microorganisms surviving pasteurization are Gram-positive bacteria, such as *Streptococci*, *Enterococci* and *Bacillus* spores (Protais *et al*., 2006). Nevertheless, the spoilage of egg products is most commonly due to post-pasteurization contaminations by Gram-negative bacteria, such as *Pseudomonas* or *Enterobacteriaceae*. The food spoilage due to non spore-forming psychrotolerant bacteria generally arises from inadequate heating or post-pasteurization contamination, that can be controlled by corrections of the pasteurization protocols and by improved sanitation processes. Therefore, excluding post-process contaminants, *Bacillus* species appear as one of the main flora involved in spoilage events in this sector. Even if *Bacillus* spores are present at low levels in both raw and pasteurized egg products, they resist the pasteurization step. The *Bacillus* genus, and notably the *B. cereus* group, is able to multiply in liquid whole egg where it causes enzymatic spoilage events. These ubiquitous bacteria are difficult to eliminate because of their heat-resistance and their strong adhering capacities, which allow them to form biofilms on industrial surfaces. Finally, some psychrotrophic strains are able to grow at temperatures around 4 to 6°C (Baron *et al*., 2007; Jan *et al*., 2010).

On the one hand, considering the antimicrobial properties of egg white described above, this medium does not favour bacterial growth. Events of egg white spoilage mainly imply defects in the breaking process, providing egg yolk nutrients in the case of poor separation between egg white and egg yolk. Visual spoilage of egg white is difficult to evaluate. Liquefaction may occur throughout storage times, mainly due to protein denaturation accompanying microbial growth (Correa *et al*., 2008). On the other hand, the bacterial spoilage of liquid whole egg and egg yolk often involves visible modifications such as coagulation and/or colour changes. The other spoilage characteristics concern the consistency/viscosity and flavour. Whole egg and egg yolk are particularly rich in proteins and lipids including phospholipids. These molecules can be metabolized by bacteria, giving rise to colour or texture changes. Except the work of Miller *et al*. (2010), assuming the expression of proteolytic and lipolytic activities by *Enterococcus* sp. strains isolated from whole egg products, the involvement of bacterial enzymatic activities in the spoilage of egg products has not received sufficient attention for establishing a clear relationship between a type of bacterial enzyme and a specific characteristic of spoilage. Moreover, the putative involvement of heat resistant enzymes in spoilage events, well-known in the dairy industry, has never been investigated in the egg product environment.

Dried egg products are scarcely affected by spoilage because of their low water activity (a_w). In dried egg products, spoilage events may occur only in the case of mishandling leading to an increase in the a_w value. If we turn to frozen egg products, microbial growth is also prevented by low water activities. Processed egg products such as hard-boiled eggs, scrambled eggs, and toaster eggs are generally cooked under temperatures higher than 71°C, the process involving the coagulation of egg proteins. These temperatures kill the vegetative forms of spoilage microorganisms. Moreover, these foods are often sold frozen, avoiding subsequent microbial growth.

Monitoring, reducing and/or detecting the spoilage of egg products

In the egg product industry, the destruction of microorganisms is mainly carried out by heat treatments at temperatures around 65 to 68°C for 5 to 6 min for both whole egg and egg yolk. The treatments are milder for egg white (around 55 to 57°C for 2 to 5 min), due to the higher thermal sensitivity of egg white proteins. These treatments are designed for the destruction of the vegetative microbiota, but they are ineffective on heat-resistant forms. Practices of sugaring, salting, freezing, concentration, or drying are efficient measures which reduce spoilage, in preventing bacterial growth by decreasing the a_w values. Other essential way for monitoring spoilage bacteria is the respect of the temperature of storage and delivery of pasteurized liquid egg products, which should not exceed 4°C. The use of peracetic acid in solution with hydrogen peroxide and acetic acid on the shell eggs intended for specific applications (such as desserts), or of some additives (e.g nisin), reduces the spoilage risk in some specific types of egg products.

The methods used to predict spoilage events during egg product processing, including the final product, or inside the foodstuffs composed of egg products among other ingredients, can be divided into two groups: those targeting bacteria and those targeting markers of spoilage (end product compounds coming from the bacterial metabolism). Classical microbiological methods can be carried out in the egg product factory, in order to estimate the shelf-life of their products. They should focus, for example, on the search for psychrotrophic bacteria or for well-known spoilage bacterial species such as *Bacillus* or others. Nevertheless, the microbiological methods are time-consuming, especially when a psychrotrophic flora is tested for. Alternative methods, based on molecular tools such as PCR, have been developed, which identify spore-forming bacteria among the broad range of whole egg flora (Postollec *et al*., 2010). Nevertheless, this method targets only spore-forming bacteria and does not discriminate between spoilage from non-spoilage strains. The search for a specific species does not necessarily imply that this species is involved in spoilage events. In fact, the ability to spoil the product can vary from one species to another and from one strain to another, as already described in the dairy industry (Baglinière *et al*., 2010). Other alternative approaches use optical methods (red LED light) or calorimetrical methods for studying the growth of spoilage bacteria involved in the spoilage of egg products (Riva *et al*., 2001; Correa *et a*l., 2008). Nevertheless, the lack of sensitivity or the use of specific technical equipment cannot be applied for routine prediction of egg product shelf-life. In the USA, the acceptability of the liquid egg products intended for consumption is partially based on the odour of the product, as perceived by the trained and licensed USDA egg product inspectors. The measurement of dimethylsulfide (DMS) has been reported as an objective method for the acceptability of liquid or frozen egg products destined to human consumption, in addition to odour analysis (Brown *et al*., 1985). Other studies have proposed uracil as an efficient marker of unacceptable microbial development (Alamprese *et al*., 2004; Hidalgo *et al*., 2004; Hidalgo *et al*., 2008). However, the use of this marker as a relevant spoilage marker in pasteurized products seems difficult to achieve, since the detection threshold is associated with a microbial concentration greater than 10^6 cfu/ml, and is detectable after the egg samples are heavily spoiled (from a sensory point of view), hence no longer suitable for human consumption. In the same way, the use of organic acids (lactic and acetic acids, 3-hydroxybutyric and succinic acids) as spoilage indicators allows detecting spoilage events that have already occurred, but cannot be relevant for the prediction of egg product spoilage.

Conclusions

Even if the pathogenic species, including *Salmonella* Enteritidis are under control in the egg product sector, there is a need to develop relevant methodologies for the control of spoilage bacteria, and particularly psychrotrophic and heat-resistant species which are potentially selected by the transformation and stabilization processes of liquid egg products stored at low temperatures. Nowadays, the establishment of sensitive and convenient methods, allowing for an early prediction of spoilage events, still remains a real challenge for the egg product industry and for the food industry in general.

References

ALAMPRESE, C., ROSSI, M., CASIRAGHI, E., HIDALGO, A., and RAUZZINO, F. (2004). Hygienic quality evaluation of the egg product used as ingredient in fresh egg pasta. *Food chemistry* **87** : 313-319.

BAGLINIERE, F., TANGUY, G., JARDIN, J., MATEOS, A., BRIARD, V., ROUSSEAU, F., ROBERT, B., BEAUCHER, E., HUMBERT, G., DARY, A., GAILLARD, J.L., AMIEL, C. and GAUCHERON, F. (2012) Quantitative and qualitative variability of the caseinolytic potential of different strains of Pseudomonas fluorescens: implications for the stability of casein micelles of UHT milks during their storage. *Food Chemistry* **135**: 2593-2603

BARON, F. and JAN, S. (2010) Qualité microbiologique de l'œuf coquille, in NAU, F., GUERIN-DUBIARD, C., BARON, F. & THAPON, J.L. (Eds) Science et technologie de l'œuf, vol.1, pp315-361 (Paris, Lavoisier).

BARON, F., COCHET, M.F., GROSSET, N., MADEC, M.N., BRIANDET, R., DESSAIGNE, S., CHEVALIER, S., GAUTIER, M. and JAN S. (2007) Isolation and characterization of a psychrotolerant toxin producer, Bacillus weihenstephanensis, in liquid egg products. *Journal of Food Protection* **70:**2782-2791.

BROWN, M. L., HOLBROOK, D. M., HOERNING, E. F., LEGENDRE, M. G., and STANGELO, A. J. (1986) Volatile indicators of deterioration in liquid egg products. *Poultry science* **65**: 1925-1933.
CORREA, E. C., DIAZ-BARCOS, V., FUENTES-PILA, J., BARREIRO, P., and GONZALEZ, M. C. (2008) Modeling ovoproduct spoilage with red LED light. *Acta Horticulturae* **802:** 265-272.
DE REU, K., GRIJSPEERDT, K., HEYNDRICKX, M., UYTTENDAELE, M., DEBEVERE, J. and HERMAN, L. (2006) Bacterial shell contamination in the egg collection chains of different housing systems for laying hens. *British Poultry Science* **47**: 163-172.
DE REU, K., GRIJSPEERDT, K., HEYNDRICKX, M., ZOONS, J., DE BAERE, K., UYTTENDAELE, M., DEBEVERE, J. and HERMAN, L. (2005) Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. *British Poultry Science* **46**: 149-155.
DE REU, K., MESSENS, W., HEYNDRICKX, M., RODENBURG, T.B., UYTTENDAELE, M. and HERMAN, L. (2008) Bacterial contamination of table eggs and the influence of housing systems. *Worlds Poultry Science Journal* **64**: 5-19.
DE REU, K., RODENBURG, T.B., GRIJSPEERDT, K., MESSENS, W., HEYNDRICKX, M., TUYTTENS, F.A.M., SONCK, B., ZOONS, J. and HERMAN, L. (2009) Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An international on-farm comparison. *Poultry Science* **88**: 2442-2448.
ELLIOTT R. P. (1958) Determination of pyoverdine, the fluorescent pigment of pseudomonads, in frozen whole egg. *Applied Microbiology* **6**:247–251.
HIDALGO, A., FRANZETTI, L., ROSSI, M., and POMPEI, C. (2008) Chemical markers for the evaluation of raw material hygienic quality in egg products. *Journal of Agricultural and Food Chemistry* **56**: 1289-1297;
HIDALGO, A., ROSSI, M., POMPEI, C., and CASIRAGHI, E. (2004) Uracil as an index of hygienic quality in egg products. Italian Journal of Food Science **16**: 429-436.
JAN, S., BRUNET, N., TECHER, C., LE MARECHAL, C., KONE, A.Z., GROSSET, N., COCHET, M.F., GILLARD, A., GAUTIER, M., PUTERFLARN, J. and BARON, F. (2010) Biodiversity of psychrotrophic bacteria of the *Bacillus cereus* group collected on farm and in egg product industry. *Food Microbiology* **28:**261-265.
JONES, D. R., MUSGROVE, M. T. and NORTHCUTT, J. K. (2004). Variations in external and internal microbial populations in shell eggs during extended storage. *Journal of Food Protection* **67**: 2657-2660.
KAROUI, R., KEMPS, B., BAMELIS, F., DE KETELAERE, B., DECUYPERE, E., and DE BAERDEMAEKER J. (2006). Methods to evaluate egg freshness in research and industry: A review. *European Food Research and Technology* **222**:727-732.
MAYES, F. J. and TAKEBALLI, M. A. (1983) Microbial contamination of the hen's egg: a review. *Journal of food protection* **46**: 1092-1098.
MOATS, W.A. (1980) Factors affecting bacterial loads on shells of washed eggs. *Poultry Science* **59**: 1641-1641.
MUSGROVE, M. T., JONES, D. R., NORTHCUTT, J. K., COX, N. A. and HARRISON, M. A. (2005). Shell rinse and shell crush methods for the recovery of aerobic microorganisms and Enterobacteriaceae from shell eggs. *Journal of Food Protection* **68**, 2144-2148.
POSTOLLEC, F., BONILLA, S., BARON, F., JAN, S., GAUTIER, M., MATHOT, A. G., HALLIER-SOULIER, S., PAVAN, S., and SOHIER, D. (2010) A multiparametric PCR-based tool for fast detection and identification of spore-forming bacteria in food. *International Journal of Food Microbiology* **142**: 78-88.
PROTAIS, J., P. GERAULT, QUEGUINER, S., BOSCHER, E., CHIDAINE, B., ERMEL, G., RIVOAL, K, SALVAT, G., PAGES, J., THUAULT, D., HUCHET, V., COIGNARD, M., BOURION, F., FEDERIGHI, M., JUGIAU, F., THOUVENOT, D., EFSTATHIOU, T., and LORTHIOIR, P. (2006) Identification et comportement des bactéries d'altération dans la matrice œuf entier liquide. *Sciences et Techniques Avicoles* **57** : 4-13.
RIVA, M., FESSAS, D., and SCHIRALDI, A. (2001) Isothermal calorimetry approach to evaluate shelf life of foods. *Thermochimica acta* **370**: 73-81.