2015 5th International Conference on Biomedical Engineering and Technology (ICBET 2015)
IPCBEE vol.81 (2015) © (2015) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2015. V81. 14

Decontamination of Shell Eggs by Using Non-Thermal Atmospheric Pressure Plasma

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Abstract. Aims: Salmonellosis is the major food borne illness in the European Union. For example, 90,000 reported cases have been observed in 2011. The serovar *S.* Enteritidis, strongly associated with eggs and egg products, can be held responsible for the most of the non-typhoid infections. Generally, bacteria are frequently heat inactivated. But a decontamination based on a heat treatment is not feasible for raw table eggs. Additionally, according to the European regulations, a treatment with gamma radiation or washing of eggs in order to reduce the bacterial load is not permitted. Therefore, a dry, non-thermal method is needed to preserve the sensory and technological properties of raw shell eggs during decontamination.
Methods: Whole table eggs were artificially contaminated with *S.* Enteritidis. Subsequently, the contaminated area was treated with non-thermal atmospheric pressure plasma. Various parameters such as the treatment time and the plasma composition were analyzed. Afterwards, the surviving bacteria were washed off the egg shell and spread onto agar plates. Finally, the colony forming units were counted to determine the reduction achieved.
Results: Reductions of up to 2.4 log steps, which conforms to a reduction of 99.63% of the initial *S.* Enteritidis population, were achieved.
Conclusion: While demonstrating that non-thermal atmospheric pressure plasma is successfully reducing the number of surviving bacteria on the egg shell, this technique has to be adapted to the industrial needs and is not applicable yet. That is, process technology has to be developed.

Keywords: Plasma, decontamination, salmonella, table eggs

1. Introduction

Salmonella infection (salmonellosis) is one of the most important food related illnesses. For example, the number of non-typhoid *Salmonella* infections in the US estimated by the Centers for Disease Control and Prevention (CDC) is 1 million cases per year [1].

Although the number of salmonellosis cases reported in the European Union continued to decrease in 2011, a total of 97,897 salmonellosis cases were still reported by the 27 EU member states. 95,548 of these reported cases were confirmed cases of salmonellosis. This translates into an EU notification rate of 20.7 cases per 100,000 population. Compared to 2010 this means a decrease in confirmed cases of 5.4 % [2].

Human salmonellosis has an incubation time of 12-36 hours. After that incubation period the characteristic symptoms are the acute onset of fever, abdominal pain, nausea and sometimes vomiting. These symptoms are often mild and most infections are self-limiting, lasting a few days. In some cases the illness does not proceed this mildly but turns into a very serious infection and the accompanying dehydration can be life threatening [2].

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In case *Salmonella* causes systemic infections, such as septicemia, the treatment with effective antimicrobials is essential. Salmonellosis may cause long-term and sometimes chronic sequelae, e.g. reactive arthritis. The mortality is usually low, less than 1 % of reported *Salmonella* cases have been fatal [2].

Although humans in general are vulnerable to this bacterial infection, the most vulnerable are children younger than 5 years of age, the elderly and immunocompromised people [3]. Infection with *Salmonella* often occurs when contaminated eggs or egg products are not thoroughly heated and consumed [3].

According to the community summary report, foodborne out breaks in the European Union in 2007 *Salmonella* of the serovar Enteritidis was responsible for 60% of all verified *Salmonella* outbreaks in the European Union [1]. *S.* Enteritidis is strongly associated with contaminated eggs and egg products [2].

The biggest risk of infection emanates from raw or undercooked food products of animal origin, especially eggs and egg products. In the year 2008 they were the cause for about 40,8% of all food borne *Salmonella* outbreaks in the EU [4]. According to the Robert-Koch-Institute and the Federal Institute for Risk Assessment (BfR) it is the Serovar *S.* Enteritidis that is identified in most cases of infections caused by eggs or egg products. This Serovar is the most prominent in contaminated eggs and was isolated in 78% of the typified *Salmonella* in table eggs [5].

Eggs may get contaminated with *Salmonella* during egg formation inside the body of an infected laying hen [1], as poultry do not show any sign of infection [2], or through the processing after lay which includes the transportation, collection, sorting and packaging. In most of the cases *S.* Enteritidis was positively detected on the egg shell [5]. By breaking the egg shell in order to process the egg, it may come in contact with the contaminated exterior of the shell and get contaminated as well. Lasagabster et al. (2011) conclude therefore, that the external contamination of the egg shell represents a health hazard to the consumers. The thermal treatment of eggs is not possible, because of the denaturation and coagulation of the proteins and the associated loss of desired technological properties. The washing of eggs is a common method of decontamination and is allowed and practiced in the USA, Canada, Australia and Japan, but forbidden in the European Union [6]. As well as any kind of washing of eggs the use of gamma radiation is also banned [7]. Thus, a dry non thermal method is needed for the successful decontamination of raw shell eggs. Plasma occurs if matter in a gas state is energized further. It becomes a mixture of highly reactive species including the original gas molecules, charged particles like positive and negative ions, free radicals and electrons as well as electromagnetic and UV radiation. In non-thermal atmospheric pressure plasma all these reactive species exist at near room temperature and under atmospheric pressure [8].

Because of this, non-thermal plasma is a suitable technology for the decontamination of heat sensitive products. It shows promising results in the inactivation of microorganisms on food and could possibly offer such a dry, non-thermal method needed for the decontamination of shell eggs.

2. Material and Methods

2.1. Microorganisms and Specimen

For the reduction of *Salmonella* on shell eggs, *Salmonella enterica* ssp. *enterica* serovar Enteritidis (RKI: S.E.: 12-03959) were used. These microorganisms were isolated directly from an outbreak by the Robert Koch Institute (RKI, Berlin, Germany) and were kindly provided by the University of Leipzig.

The microorganisms were dispensed in Nutrition Broth 1(Sifin diagnostics GmbH, Berlin, Germany) and used in concentrations of 10^8 cfu $\cdot ml^{-1}$ (colony forming units per millilitre).

Artificially contaminated, undamaged, dry, white table eggs, seize M (53-63g) were investigated. The contaminated area on the egg shell was 1 cm^2.

2.2. Methods of Contamination of Table Eggs

The eggs were artificially contaminated with *S.* Enteritidis. Therefore, areas of 1 cm^2 were marked on the shells of the eggs. Into these areas 10 µl of a suspension with approximately 10^8 cfu $\cdot ml^{-1}$ were dropped and evenly spread. The solution was left to dry at ambient air under aseptic conditions for 15 minutes.

2.3. Recovery of Contamination from Shell Eggs

In order to recover the surviving (active?) microorganisms after the plasma treatment from the egg shell, a cuvette with 1 ml Nutrition Broth 1 was fixed above the contaminated and treated area of the egg. To fix the cuvette and to prevent any leakage of fluids, the gap between egg and cuvette is sealed tightly. Modeling clay was used as sealing mass.

The egg is turned upside down once and kept in this position for 30 seconds, so the nutrition broth is able to loosen the bacteria from the egg shell. For a further detachment of the microorganisms, the eggs were repeatedly turned upside down and vice versa for 3 minutes each.

In the next step the surface-spread plate-count method with Xylose-Lysin-Desoxichlorat-Agar (XLD-Agar) plates is used. The detection limit of this procedure was 200 cfu $\cdot ml^{-1}$.

2.4. Treatment with Plasma Jet kINPen 09®

The non-thermal plasma treatment was performed with the use of the commercially available Plasma Jet kINPen 09® [9]. The eggs were adjusted beneath the jet with a distance of 8 mm ± 1 mm between the nozzle and the contaminated area of egg shell.

Three different feeding gases, pure Argon 5.0, Argon 5.0 + 0,5% O_2 and Argon 5.0 + 1% O_2 (Linde gas AG,12347 Berlin, Germany) were applied. A gas flow of 5 slm was used for the different feed gases. With pure Argon 5.0 the effectivity of a second gas flow rate of 10 slm was analyzed. The treatment time was varied between 15 to 300 seconds.

3. Results

The effectivity of non-thermal atmospheric pressure plasma in inactivating *Salmonella* Enteritidis on egg shells was investigated. Three different feed gases or gas mixtures as well as two gas flow rates were analyzed. The Results of these investigations are shown in Fig. 1.

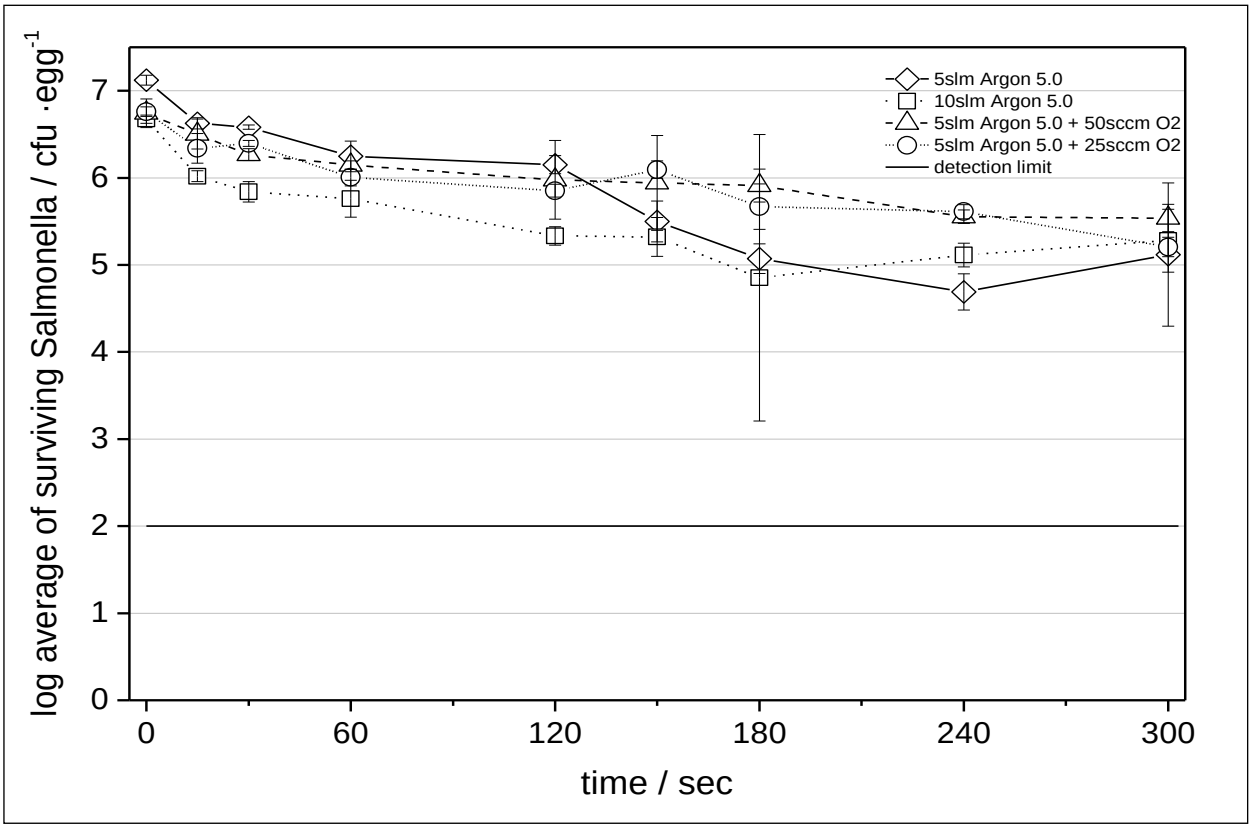

Fig. 1: Results of the plasma treatment of artificially contaminated shell eggs

Reductions of *S.* Enteritidis from 1.2 up to 2.4 log were accomplished. The highest inactivation of 2.4 log steps, which translates to an inactivation of 99.63 % of the microorganisms, was achieved by using pure Argon at a flow rate of 5 slm. A higher flow rate did not improve the inactivation and caused a reduction of

1.8 log. By the combination of 0.5% or 1% of oxygen in argon reductions of 1.4 and 1.2 log steps were accomplished, respectively. When pure argon was used as feed gas, the reductions were achieved after 240 seconds of plasma treatment. For the gas mixtures the result was obtained after 300 seconds of plasma treatment.

On the one hand, the lower inactivation of *S.* Enteritidis seems not to be encouraging enough to vindicate the use of a plasma-based egg shell densification as a commercially used process. Additionally, the costs for the feed gasses are an important factor for the commercial use of plasma. An additional drawback is the impossibility of the used plasma jet to widespreadly treat the whole egg, which leads to a low throughput. On the other hand, the plasma jet might not be the plasma source of choice and for the treatment of whole eggs the technique has to be adapted to the industrial needs. That is, process technology has to be developed. In order to decontaminate whole eggs a new plasma source specific for this sample is to be developed during this research project.

4. Acknowledgements

We thank the Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke" e.V. (project funding reference number: AiF 178177 BR) for financial support.

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