Hot air treatment for surface decontamination of table eggs

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ABSTRACT

A hot air assay was set up for the surface decontamination of table eggs experimentally contaminated by *Salmonella enterica* serovar Enteritidis. A hot air apparatus was built and a treatment of two shots of 8 s at 600 °C with an interval of 30 s of cold air was chosen and applied on contaminated eggs. The *S.* Enteritidis load on eggshells as well as the quality traits of the egg components of 190 treated and 190 not treated eggs was investigated during 24 days of storage at 20 °C. Hot air treatment reduced the *S.* Enteritidis load on eggshells of up to 1.9 log. No significant changes to any of the quality traits tested were recorded. These results suggest the usefulness of hot air pasteurisation for the surface decontamination of table eggs.

1. Introduction

For many years egg-associated salmonellosis has been and still is an important public health problem throughout the world (European Food Safety Authority, 2009). *Salmonella* Enteritidis represents the serovar most frequently associated with human salmonellosis due to the consumption of contaminated eggs and undercooked poultry meat as well as to cross contamination in catering and domestic environment. In particular this serovar caused 64.5% of human infections with *Salmonella* in Europe in 2007 (EFSA, 2009). Due to its unique capacity to persist between consecutively housed flocks in the environment and in the rodent population, *S.* Enteritidis represents the serovar most frequently isolated in laying hen holdings in Europe with 18.3% of prevalence in the period 2004–2005 (European Food Safety Authority, 2007).

By 2012, Council Directive 1999/74/EC, defining minimum standards for the welfare of laying hens, will abolish conventional cage systems in favour of enriched cages or barn systems in order to improve the laying hen welfare. However, keeping hens on the floor or outside may induce an increased risk to bacterial contamination. Eggshell contamination with total count of aerobic bacteria is generally significantly higher for nest eggs from non-cage systems compared to eggs from furnished or conventional cages (European Food Safety Authority, 2005).

In this regard the introduction of efficient measures to reduce eggshell contamination by *S.* Enteritidis or other bacterial pathogens, and thus to prevent any potential or additional food safety risk for human health, may be envisaged. In USA egg washing, together with the use of cold storage, is at the present time the most common decontamination treatment of egg surface. These treatments are forbidden in Europe for class A eggs (except for The Netherlands and Sweden where egg washing is applied on a voluntary basis) since egg washing is claimed to damage the cuticle of the egg thus favouring the penetration of bacterial pathogens if best practice washing procedures are not used.

Therefore alternative treatments should be investigated for the surface bacterial decontamination of table eggs. In this regard surface pasteurisation may represent one of the most promising decontamination techniques.

Water pasteurisation at high temperature (100 °C) for 3 s has been applied to pasteurise the surface of table eggs (Himathongkham, Reinmann, & Ernst, 1999). However this technique may induce shell microcracks (Himathongkham et al., 1999) thus increasing the risk of bacterial pathogen penetration. Water pasteurisation at temperatures ranging from 55 to 60 °C needs longer treatment times: 25 min are required in order to obtain 3 log reductions of a *S.* Enteritidis population on eggshells experimentally infected (Hou, Singh, Muriana, & Stadelman, 1996), 50–57 min are required for the complete inactivation of *S.* Enteritidis at 58 °C (Schuman, Sheldon, Vandepopuliere, & Ball, 1997). These treatment times are far too long in view of a possible industrial application and are sometimes associated with detrimental changes to quality traits of the albumen (Schuman et al., 1997).

In this regard hot air pasteurisation may represent a valuable alternative for the decontamination of egg surface also in relation to the EU ban of water use on table eggs. Few studies have been performed on the decontamination efficacy of this technique on table eggs. Hou and colleagues (1996) observed a 5 log reduction of the *S.* Enteritidis load on whole eggs treated with hot air oven at

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55 °C for 180 min. James, Lechevalier, and Ketteringham (2002) tested the applicability of a hot air gun treatment on egg surface pasteurisation. They investigated the temperatures of the interior and exterior of the egg and they identified 180 °C for 8 s as the best treatment corresponding to the highest surface temperature that can be achieved without detrimental changes to egg quality. Unfortunately no microbiological investigations were performed.

In the present study the decontamination efficacy of hot air generated by a new hot air apparatus was evaluated on shell egg surface of table eggs experimentally infected with S. Enteritidis. Moreover quality traits of treated eggs in comparison to non treated eggs were investigated.

2. Materials and methods

2.1. Table eggs

Table eggs were obtained from a flock of Hyline brown hens reared in conventional cages. The average weight of inoculated eggs was 68.05 (±4.23) g.

2.2. Hot air treatment technique and modelling

The aim of the technique was to gain the highest peak of temperature on the external surface of the shell, avoiding any egg quality traits. This result was possible alternating short heating and cooling phases. Both phases were realized using a couple of air jet impinging the shell on opposite faces of the egg. During the treatment the egg was posed on a couple of rolling cylinders to rotate around its main axis.

To study the problem, initially a simplified numerical model was developed describing the thermal interaction between the air and the egg, using a computational fluid dynamic tool (CFD) based on Finite Element Technique. The detail of the model development and validation are described by Fabbri, Cevoli, and Giunchi (2009).

The development of the numerical model required a thermophysical characterisation of the egg components. To carry out these determinations on the shell, a specific method, based on a mixture of water and eggshell splinters, was studied (Fabbri, Cevoli, & Sirri, 2007). This was made because the values reported in literature belong to pure calcium carbonate or just are poorly documented.

To validate the CFD model an egg simulant was set up equipped by a type-K thermocouple (Chromel/Alumel) fixed on the internal surface of the shell in equatorial area. The egg simulant was drilled and refilled with only albumen.

Tests were conducted at different combinations of air flow rate and treatment duration, acquiring temperature data using a PCI-6036E (National Instrument Corporate; Texas, USA) data acquisition system at a sampling rate of 250 Hz.

2.3. Hot air apparatus and thermal cycles

On the basis of the experience with the numerical model and the simulant, after some preliminary tests on experimentally inoculated eggs, a particular thermal cycle was selected. This treatment made possible to reach an estimated external shell surface temperature higher than 70 °C and an inner temperature always lower than 55 °C.

A specific apparatus was built to physically condition the eggs (Fig. 1). This was composed by two hot air generators (Bosh, model GHG 660 LCD-professional) in a fixed position over the roller conveyor. The rolling cylinders (wheelbase 35 mm) are moved by a transmission belt, linked to a stepping motor server by an electronic speed regulator. The air, cooled by expansion from high
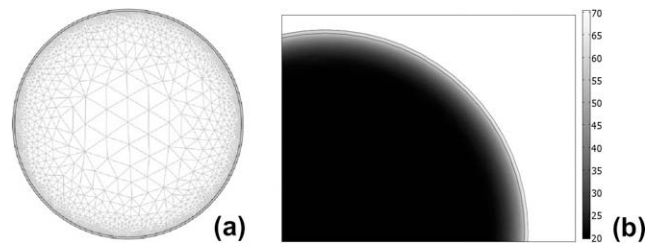

Fig. 1. Finite element model of the egg: (a) mesh of the equatorial plane of the egg, made with parabolic triangular elements and (b) thermal map in steady state condition.

pressure, flows from a nozzle (Φ 2 mm, 120 mm from the cylinders) in the lower part of the roller conveyor and it is served by a needle valve for flow adjustment. The cold air came from a compression plant, able to maintain the pressure in the range of 8.5–9 Bar.

For every egg the following procedure was applied: initially the hot air generators were switched on for at least 1 min, to reach stable working condition. Than an egg was exposed, on the rolling cylinders, to the hot and cold air flows simultaneously for 8 s. The hot air generators were then switched off and the egg was cooled by the cold air flow only for 32 s. Finally the heating treatment was re-applied for 8 s more.

2.4. Microbiological tests

2.4.1. Bacterial culture
S. Enteritidis (MB2509) strain streptomycin resistant was selected for experimental inoculation of table eggs.

2.4.2. Inoculum preparation
S. Enteritidis cells, stored on Protect Beads at −80 °C were resuscitated by incubation overnight at 37 °C in Brain Heart Infusion (BHI; Oxoid, Milan, Italy) containing 25 ppm of streptomycin. This culture was plated on BHI agar containing 25 ppm of streptomycin and again incubated overnight at 37 °C. Next, one colony was grown overnight at 37 °C in 10 ml of BHI supplemented with 25 ppm of streptomycin. An aliquot of the grown culture (0.1% inoculum) was subsequently transferred into 150 ml of BHI containing 25 ppm of streptomycin and incubated at 37 °C for 24 h with mild agitation. One hundred millilitres of the resulting broth-culture were centrifuged at 3000g for 10 min. Cell pellets were resuspended in sterile physiological saline at 22–25 °C. The Salmonella working suspension had an optical density at 600 nm (OD600) of approximately 0.4. Viable cells in the working suspension were enumerated by serial dilution in physiological saline, plating 100 μl of each dilution on Tryptone Soya Agar (TSA; Oxoid) and incubation overnight at 37 °C. The cell density was 2.8×10^8 CFU/ml.

2.4.3. Inoculation of shell eggs
Fresh, unfertilized eggs were obtained from hens reared in conventional cages from a local poultry farm. The decontamination effect of hot air was studied on 180 eggs experimentally contaminated with S. Enteritidis. Each egg was washed with distilled deionised water (22–25 °C). Washed eggs were subsequently brushed, to remove the cuticle, and then sanitised by dipping them in ethanol (70%, vol/vol) for 30 min as described by Hammack et al. (1993). Sanitized shell eggs were transferred to metallic grids and aseptically dried at room temperature for approximately 40 min before inoculation. Dried, sanitised shell eggs were dipped for 10 s into the Salmonella working suspension prepared as described in the previous paragraph. Both eggs and the Salmonella working suspension had a temperature of 20 °C. Contaminated shell eggs

were transferred to metallic grids and the shell permitted to dry for approximately 1 h until they were completely dried. Bacterial count on the contaminated eggshells ranged between 10^4 and 10^5 CFU/eggshell. Sanitized, uncontaminated shell eggs, dipped into sterile deionised water at 22–25 °C, were used as negative controls.

2.4.4. Quantification of eggshell and albumen contamination

The decontamination efficacy of hot air was investigated on experimentally inoculated eggs with S. Enteritidis. The contamination of eggshell was assessed on days 0, 1, 2, 3, 8, 10, 15, 21, 24 on 10 treated eggs per day, on 5 not treated eggs per day (positive control) and on 5 sanitised, not inoculated and not treated eggs per day (negative control). The eggs were stored at 20–25 °C. The contamination of eggshells was quantified by separating the eggshells from the other egg components. In a sterile plastic bag a volume of physiological saline equal to nine times the eggshell weight was added to the eggshell in order to dilute 1:10 the Salmonella load on the eggshell. The mixture of eggshell and diluent was mixed by rubbing the eggshell through the bag for 1 min to detach the bacteria. Enumeration of the bacteria was done by surface plating 100 μl of the diluent used to detach bacteria from the eggshells on Brilliant Green Agar (BGA, Oxoid). In case of 0–4 colonies, the value of 0 CFU/eggshell was assigned. This value corresponds to a concentration of bacterial cells on the shell below the detection limit of 10^2 CFU/eggshell. To allow a log transformation, counts were given of 1 CFU/eggshell upon observing no colonies.

2.5. Quality tests

Different quality traits were evaluated on egg treatment samples immediately after the hot air treatment (pH and turbidity of albumen, shell colour and the cuticle assessment) and after a storage of 28 days at 20 °C (yolk index, eggshell breaking strength and the shell membranes assessment). One hundred eggs were used as control (50 eggs stored for 28 days) and 100 eggs treated with hot air (50 eggs stored for 28 days).

The albumen pH was measured by a pH meter (CyberScan 510 pH-Eutech Instruments) on thick and liquid mixture of albumen. The turbidity of albumen was used to determine the thermocoagulation. Four milliliters were transferred to a translucent disposal PE cuvette (Sigma, Milan, Italy) and the transmission was measured by a spectrophotometer at 600 nm (Spectrophotometer UV-1601, Shimadzu Corporation, Japan). From the measured transmission spectra (three repetitions were made for each samples) turbidity of the albumen was calculated using the equations presented by Weijers, van de Velde, Stijnman, van de Pijpekamp, and Visschers (2006).

$$\tau_{\mathrm{alb}}(\lambda) = -\frac{1}{l} \ln\left(\frac{T(\lambda)}{100}\right)$$

where $\tau_{\mathrm{alb}}(\lambda)$ is the turbidity of the albumen in function of the wavelength λ, l is the length of the optical path through the albumen and $T(\lambda)$ is the transmission of the albumen in function of the wavelength.

The exterior L^*, a^*, b^* colour values of shell, before and after the treatment, were measured with a CIELab colorimeter (Konica Minolta Meter CR-400, Milan, Italy). In the $L^* a^* b^*$ colour space, colour difference was expressed as a single numerical value ΔE^*. Three measurements were done on each egg in the same point and the value ΔE^* was calculated as follows (Francis & Clydesdale, 1975):

$$\Delta E^* = \sqrt{(\Delta L^* + \Delta a^* + \Delta b^*)}$$

where ΔL^*, Δa^*, Δb^* were the differences of the average values measured before and after the treatment.

According to Board and Halls (1973) the cuticle assessment was evaluated by a solution containing Tartrazine and Green S (Cuticle Blue; MS Technologies Ltd, Northants, UK), able to stain the cuticle of the egg. The eggs were immersed in the solution for 1 min and then were rinsed in distilled water to remove excess dye, before drying. The visual assessment of the egg was based on the degree of uptake of the dye with dark green colour (good cuticle) to pale (poor cuticle). Moreover the green colour was quantified using CIELab colorimeter. Three measurements were done on each egg in the same point and the value ΔE^* was considered where the ΔL^*, Δa^*, Δb^* were the differences of the average values measured on the treated and control sample.

The yolk index was calculated as the height of the yolk divided by the width of the yolk measured using a decimal digital calliper. The eggshell breaking strength was carried out by using universal testing machine (Egg Shell Force Gauge Model-II, Robotmation Co. Ltd., Tokyo, Japan) with a 50 N load cell and strain rate set to 20 mm/min. The eggs were laid down horizontally between two parallel steel plates and, in order to prevent rolling of the egg, a ring made of soft synthetic was used to hold the egg.

The shell membranes assessment was carried out by using an indirect method based on the evaluation of water loss of egg through the shell pores. The water loss was evaluated by weighing the eggs after 7, 14 and 28 days of storage.

3. Results and discussions

3.1. Hot air treatment technique and modelling

Fig. 2 represents the finite element model of the egg. In particular the geometry discretization of the calculus domain and a calculated thermal map in an example condition are shown.

The development of the numerical model required a thermophysical characterisation of the egg components. Moreover to validate the CFD model an egg simulant was set up. Tests conducted at different combinations of air flow rate and treatment duration compared with simulated results obtained a correlation index higher than 91% (Fabbri et al., 2009).

The numerical model, once validated, permitted a quick evaluation of a large number of testing conditions. Particularly it was solved in transient conditions, as a virtual bench, sampling the parameters space: air temperature; air speed; duration of the treatment; revolving speed of the egg; rest interval between successive treatments.
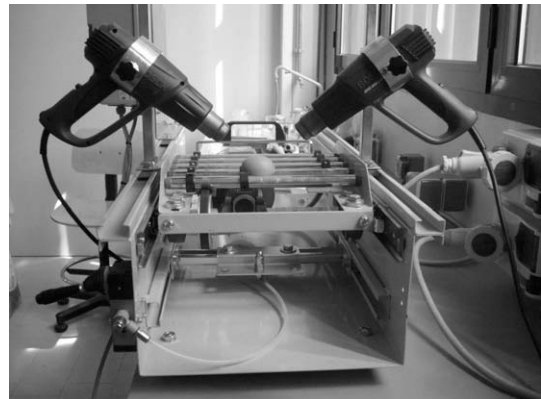

Fig. 2. The prototype for hot air treatment of eggs: the hot air generators in the upper part, the rolling cylinders for egg support, moved by a transmission belt and, in the lower part, the cold air pipe.

3.2. Selection of the best thermal treatment

The thermal cycle selected made it possible to reach an estimated external shell surface temperature higher than 70 °C and an inner temperature always lower than 55 °C. The thermal and dynamic parameters of the tested thermal cycle were: hot air temperature: 550–650 °C; cold air temperature: 20–25 °C; mean hot air speed: 10 m/s; mean cold air speed: 40 m/s; duration of the heating shot: 8 s; number of heating shots: 2; duration of the cooling phase between two shots: 32 s; revolving frequency: 1.2 Hz. Data of the first four parameters were measured at the outlet section of the generator.

3.3. S. Enteritidis load on hot air treated and not treated table eggs

Overall the load of S. Enteritidis on treated eggs was lower than not treated eggs from day 0 to day 21 post infection (Fig. 3). The mean values of $\log_{10}$ CFU/eggshell reduction of the S. Enteritidis load on treated eggshells ranged between 0.1 and 1.9 from day 0 to day 21 post-infection.

The S. Enteritidis load on treated eggs was lower than the positive control until day 21. In particular the $\log_{10}$ reduction ranged between 0.6 of day 0 and 1.9 of day 10.

One log reduction is an important result and corresponds to a bacterial population reduction of 90%. This result is even more important if we consider that Salmonella on eggshell is often reported to be approximately no more than 10^2 up to 10^3 CFU/ml (or CFU/eggshell) only in rare cases (Humphrey, 1994; Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991) in eggs collected from hens reared in conventional cages. One log reduction would therefore be sufficient to decrease the S. Enteritidis load on eggshell to values corresponding to a significant foodborne risk reduction for human health.

A decrease of the S. Enteritidis load on eggshells from day 8 to day 24 was observed in not treated eggs but not in treated eggs. The increase of 1.7 log of S. Enteritidis load on treated eggs from day 21 to day 24 may be due to resuscitation of heat injured not culturable cells which may have found optimal conditions of growth at room temperature.

Other authors reported the complete inactivation of S. Enteritidis on eggshell after immersing contaminated shell eggs in boiling water for 3 s, unfortunately this approach showed side effects on the quality of the egg due to crack formation (Himathongkham et al., 1999).

In comparison to other thermal inactivation techniques, this hot air treatment showed a low decimal reduction time at 600 °C (6.7 s). Higher values of decimal reduction time in liquid whole egg were reported (D50 °C = 16.5 min, D57.5 °C = 0.7 min, Stadelman, Singh, Muriana, and Hou (1996) D55 °C = 7.04 min, D57 °C = 3.39 min, D60 °C = 0.63 min, Alvarez, Niemira, Fan, &

Table 1
Quality traits of hot air treated and untreated eggs immediately after the treatment.

	Treated eggs	SD[a]	Untreated eggs	SD[a]
pH	8.96	0.2	8.97	0.30
Turbidity [/m]	41.60	7.30	43.44	7.40
L^*	63.01	2.80	62.85	2.79
a^*	15.83	1.55	16.45	1.65
b^*	29.87	2.13	30.02	2.19
ΔE^* (shell colour)	0.65		–	
L^* (cuticle)	52.70	4.69	51.80	3.98
a^* (cuticle)	−11.46	7.73	−10.00	10.21
b^* (cuticle)	29.67	2.01	29.23	1.82
ΔE^* (cuticle)	1.76			

[a] SD – standard deviation.

Table 2
Quality traits of hot air treated and untreated eggs after 28 days of storage at 20 °C.

	Treated eggs	SD[a]	Untreated eggs	SD[a]
Breaking strength (N)	39.56	5.79	38.71	5.82
Yolk index	0.17	0.03	0.16	0.02
Weight loss 7 days (%)	1.41	16.2	1.40	20.9
Weight loss 14 days (%)	2.72	14.7	2.71	13.8
Weight loss 21 days (%)	4.91	14.4	5.16	13.2
Weight loss 28 days (%)	6.77	10.8	6.88	12.4

[a] SD – standard deviation.

Sommers, 2006) demonstrating the promising applicability of this assay at the egg packaging plants thank to the short treatment time needed.

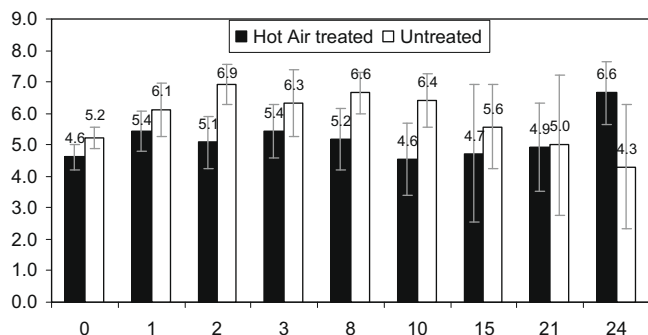## 3.4. Quality traits of hot air treated and not treated eggs

The quality traits, measured on treated eggs as well as those of untreated eggs immediately after the treatment, are reported in Tables 1 and 2 after 28 days of storage at 20 °C. All the quality indexes showed no statistically significant differences between treated and untreated eggs so that it can be assumed that the treatment does not exert negative effects on the main quality traits of egg. This finding together with the microbial results on experimentally inoculated eggs, suggests the useful industrial application of the hot air treatment on eggs before packaging in order to reach an approximately 90% reduction of S. Enteritidis population naturally infecting the surface of the eggs.

Acknowledgments

Fig. 3. Comparison of S. Enteritidis load ($\log_{10}$ CFU/eggshell) on eggshell of hot air treated and untreated eggs.

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