

The Influence of High Pressure and/or Antimicrobials on Some Functional Properties of Liquid Whole Egg

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ABSTRACT

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The influence of the high pressure treatment of 300 MPa/200 s, possibly combined with antimicrobial additives, on the quality of liquid whole egg (LWE) in terms of rheology, foaming and emulsification properties, colour changes, and microbial quality was studied and compared to the characteristics of commercially available pasteurised liquid whole egg (65°C, 3 min). It can be concluded that the above-mentioned regime of LWE pressurisation did not deteriorate its functional properties and can be used, after the addition of some antimicrobial agents, as a preservation technique keeping its organoleptic and nutritive qualities.

Keywords: liquid whole egg; high pressure; antimicrobials; functional properties

High pressure technology, defined as a pressure treatment between 100 and 1000 MPa, is of increasing interest to the food processing because of its potential to decrease the level of microbial contamination without any heat treatment and the opportunity to produce foods of high sensory and nutritional quality. The industrial high pressure food products are mainly manufactured in Japan (fruit jams and juices, sake, fish, and rice products), the USA (guacamole, ham, oysters, and fruit juices) and Spain (ready to eat meat meals, ham) (DE LAMBALLERIE-ANTON *et al.* 2002; NC Hyperbaric 2008).

The objective of this paper was inspired by the PhD thesis of LEE (2002) investigating the applicability of high pressure, as well as the utilisation of nisin, for the processing of liquid whole egg.

Liquid whole egg (LWE) is mainly used in the industrial production of bakery products, confectionery or ice-cream. LWEs, in addition to the nutritional value, contribute to such foods by their functional properties like foaming, coagulation, or emulsification. However, in the research of Lee mentioned above, no comparison of pressurised liquid egg and LWE processed by the most frequent technique – pasteurisation – appears. Therefore, the objective of this paper was to compare the non-treated, pressurised and pasteurised LWEs in terms of rheology, foaming, emulsification, colour and, after the addition of several types of antimicrobial agents, their microbial stability.

Although the heat process used to pasteurise LWE can ensure the food safety by eliminating

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heat sensitive pathogens from the egg products, some heat resistant spoilage microorganisms can survive these pasteurisation requirements and spoil LWE even under refrigerated conditions. Generally, only one to two (according to LEE 2002) or two to three log cycle reductions (according to MUCHOVÁ 2004) of viable cell counts are achieved by commercial thermal pasteurisation, depending on the pasteurisation regime, resulting in the content of as much as 10^3 microbial cells/g. Thus, the producers tend to apply double pasteurisation during the LWE processing (often combined with aseptic packaging) resulting in 10^1 CFU/g. However, changes in the functional and organoleptic properties of such egg products are often obvious. Non-thermal food processing technologies such as high hydrostatic pressure combined, according to a hurdle theory, with the addition of some antimicrobial agents, could overcome these limitations of the conventional heat treatment. Moreover, an instant and homogenous pressure treatment together with the exclusion of post-process contamination could be a considerable advantage of this technique as well.

MATERIAL AND METHODS

Samples preparation. Untreated and pasteurised (65°C , 3 min) LWEs were obtained from a commercial egg processing plant and were kept refrigerated at 4°C . One day later, the selected antimicrobial additives or their combinations were mixed with the untreated LWEs and two days later the high pressure treatment was applied.

Antimicrobial additives and their concentrations. The commercial preparation of nisin (Nisaplin[®]) containing 25 mg nisin/g was dissolved in sterilised deionised water and added to the LWE in a final concentration of 5 mg nisin/l. Monolaurin, glycerol ester of lauric acid (synthesised at the Department of Fat and Dairy Technology, ICT Prague, purity 99%) was first dissolved in a 96% ethanol; a dilution of this stock solution in sterilised water was added to the LWE in a final concentration of 50 mg/l. Lysozyme from chicken egg white (40 000 units/mg protein; Sigma) was dissolved in sterilised water and added to the LWE in a final concentration of 100 mg/l. The chelating agent EDTA was later dissolved in water, sterilised (121°C , 15 min) and added to the LWE in a final concentration of 75 mg/l.

Pressure treatment. The high pressure treatments were performed in an isostatic press CYX 6/0103 (Žďas, Ždár n. Sázavou, Czech Republic) located in the Food Research Institute, Prague. The pressure unit was equipped with a cylindrical pressure chamber of a 2 l volume, 90 mm in diameter and 320 mm high. As a compression fluid, water was used. The samples were pressurised at 300 MPa for 200 s; during the samples treatment, the control samples were maintained at 4°C . Soon after the treatment, all the samples were stored under atmospheric pressure at 4°C until required.

Rheological measurement. Prior to the rheological measurements, non-treated, pasteurised and pressurised samples of LWE were allowed to equilibrate to 10°C . Viscosity was then measured with the Haake-RS 150 rheometer equipped with a concentric cylinder measuring system. The relative deviation of the rheological parameters was 10%.

Foaming property. Foams were prepared by whisking 100 ml of LWE for 5 min using a mixer equipped with a whipping beater. The foaming property was expressed as the foaming capacity *FC* and the foam stability *FS* as follows:

$$FC = \frac{\text{foam volume}}{\text{LWE volume}} \times 100 (\%)$$

$$FS = \frac{\text{foam volume} - \text{liquefied LWE volume after 30 min}}{\text{LWE volume}} \times 100 (\%)$$

Emulsifying property. The emulsifying property of LWE was examined by preparing an oil-in-water emulsion according to LEE (2002). Initially, 1 ml of LWE and 5 ml of commercial sunflower oil were blended at 5000 rpm for 20 s using an immersion homogeniser. Then 5 ml of deionised water were added and the mixture was homogenised at 800 rpm for 1 minute. Immediately after the preparation, the oil droplets in the emulsion were observed under a microscope connected to a camera using 100× magnification.

Colour measurement. The CIEL**a***b** values for lightness (*L**), redness/greenness (*a**) and yellowness/blueness (*b**) were measured using a Minolta Chromameter 2600d (Minolta, Japan). Each measurement was repeated ten times and the average values were calculated. The numerical values of total colour difference were calculated using the equation:

$$\Delta E^* = [(L^* - L_{\text{ref}}^*)^2 + (a^* - a_{\text{ref}}^*)^2 + (b^* - b_{\text{ref}}^*)^2]^{1/2}$$

Table 1. Coefficient of consistency (K) and flow behaviour index (n) for non-treated, pasteurised (65°C, 3 min), and pressurised (300 MPa, 200 s) liquid whole eggs at 10°C and shear rate between 1 and 400 s⁻¹

	Non-treated	Pasteurised	Pressurised
n (-)	0.91	0.88	0.89
K (Pa.s ^{n})	0.0184	0.0278	0.0265

Microbiological analysis. The sample of LWE was homogenised in physiological solution using a Stomacher type homogeniser. Serial 10-fold dilutions were prepared and for the enumeration of the total count Plate Count Agar (Merck, Germany) was used.

RESULTS AND DISCUSSION

Rheological measurement

The main objective of the rheological study was to confirm that the pressure treatment of 300 MPa/200 s chosen according to the results of LEE *et al.* (2000) does not cause a significant change in physico-chemical properties such as coagulation (resulting in altered rheological behaviour) of pressurised versus common pasteurised (65°C/3 min) LWE. Also, in that case all the settings of the processing technology (mixing, pipeline transport, filling line parameters) may remain the same as those for a standard pasteurised product.

Flow curves of each LWE, non-treated, pasteurised and pressurised (not presented here), were established by expressing the shear rate ($\dot{\gamma}$ in s⁻¹) versus the shear stress (τ in Pa). All the three LWE types showed a non-linear dependence, representing a non-Newtonian behaviour. The transformation

to logarithmic coordinates led to a linear dependence of $\log \tau$ versus $\log \dot{\gamma}$ which was expressed by the power-law model ($\tau = K\dot{\gamma}^n$) with $R^2 > 0.999$ for all the LWE types; the values of K (coefficient of consistency, Pa.s ^{n}) and n (flow behaviour index) are seen in Table 1. As $n < 1$, it reveals a shear thinning behaviour. The flow of the apparent viscosity μ (calculated according to equation (1), see below) versus the shear rate was modelled (Figure 1), showing higher viscosity for the pressurised versus the non-treated sample, which is in agreement with the results of (HERALD *et al.* 1988). However, all the parameters for pasteurised versus pressurised liquid eggs were found to be very similar.

$$\mu = \frac{\tau}{\dot{\gamma}} = \frac{K\dot{\gamma}^n}{\dot{\gamma}} = K\dot{\gamma}^{n-1} \quad (1)$$

Foaming property

No publication was found concerning the foaming property of pressurised LWE except that of LEE (2002) which summarises that foaming power (foaming capacity) of egg white can be either decreased or increased by the extent of the applied pressure, and that these contrary effects were observed not only at high pressure treatment but also at thermal treatment of LWE, probably owing to the fact that the foam formed by whipping the LWE has not the characteristics of a real foam

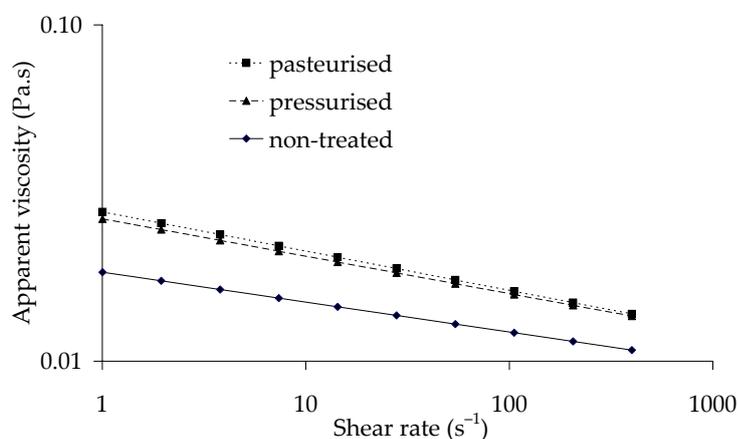


Figure 1. Apparent viscosity vs. shear rate for non-treated, pasteurised and pressurised liquid whole egg

Table 2. Foaming capacity (FC) and foam stability (FS) for non-treated, pasteurised and pressurised liquid whole eggs

	FC (%)	FS (%)
Non-treated	343 ± 8.6 ^a	70 ± 5.6 ^a
Pasteurised	395 ± 6.6 ^b	84 ± 3.8 ^b
Pressurised	337 ± 6.1 ^a	68 ± 2.9 ^a

All values are expressed as mean ± standard deviation ($n = 6$); Means in column with different letters are significantly different ($P < 0.05$)

which can be seen in the case of egg white. The unstable LWE foam is caused only by the incorporation of air into the mass whereas in the case of egg white the air bubbles are incorporated into the denatured film of proteins which makes the foam stable. For the foaming capacity and foam stability of pressurised and pasteurised LWEs as compared to fresh LWEs (Table 2). Minimal differences between all the tested liquid eggs confirm the minimal influence of the chosen pressure treatment on the LWE protein denaturation. However, measuring the baked volume would probably be another useful test to evaluate the foaming properties of the tested materials.

Emulsifying property

The principal problem of food emulsions is their instability which is caused by the coalescence of droplets. Because the coalescence is affected by the properties of the interfacial film and the emulsion droplets size, the average droplet diameter and the size distribution provide useful information about the emulsifying property. Generally, smaller droplets are favoured regarding the emulsion stability. (DAS & KINSELLA 1993; LEE 2002).

Photographs representing oil in water emulsions prepared from non-treated, pasteurised and pres-

surised LWEs are shown in Figure 2. The droplet size of emulsions prepared from non-treated and pressurised LWEs did not vary significantly; the same results were also obtained with LWEs mixed with all kinds of antimicrobial additives used in this study (pictures not presented). On the other hand, the emulsifying property visualised by the size distribution of the emulsion prepared from the pasteurised LWE showed better results for the way used for the emulsion preparation.

Colour measurement

The effects of different technologies on the colour of LWE immediately after the processing and 7 days later are shown in Table 3. The L^* value for lightness remained the same for the pressurised sample compared to the non-treated one, whereas with pasteurised egg the L^* value increased as the result of the so called whitening process caused by the denaturation of heat labile proteins (RHIM *et al.* 1988). The main driver of the overall colour change besides the L^* value was also the a^* value for redness, decreasing after the pressure and heat treatment and increasing in respect to time, probably as the result of the protein denaturation and its precipitation and sedimentation during the time. After seven days of storage in refrigerated

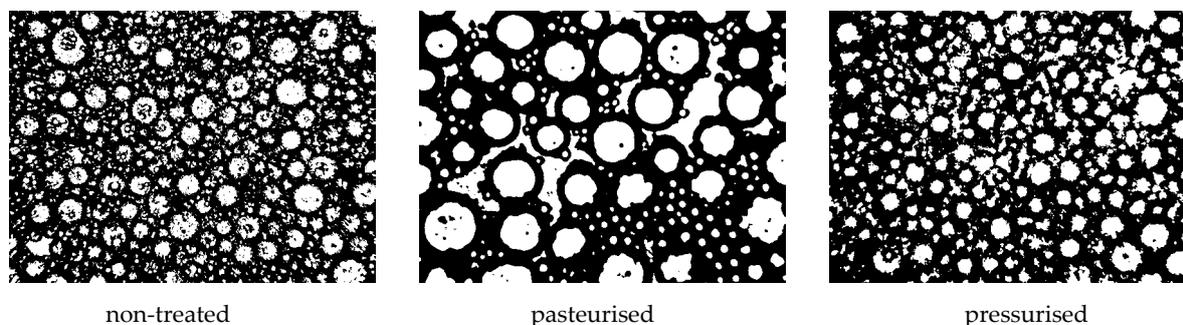


Figure 2. Emulsifying properties of non-treated, pasteurised and pressurised liquid whole eggs visualised as the emulsion droplet size (magnification: 100×)

Table 3. CIEL**a***b** colour coordinates and total colour difference (ΔE^*) for non-treated, pressurised and pasteurised liquid whole egg

	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE^*
24 h after treatment				
Non-treated (control)	63.81	6.37	24.51	0.000 ^a
Pressurised	63.36	4.83	22.78	2.359 ^b
Pasteurised	70.11	3.14	26.54	7.365 ^c
7 days after treatment				
Non-treated (control)	68.02	12.66	32.88	11.285 ^d
Pressurised	66.47	8.88	25.71	3.849 ^b
Pasteurised	72.07	5.05	33.23	12.083 ^d

All values are expressed as mean of $n = 10$; Means in column with different letters are significantly different ($P < 0.05$)

conditions, only the pressurised sample retained its colour relatively similar to that of the control (fresh, non-treated) LWE.

Microbiological analysis

The initial microbial spoilage of the non-treated LWE was determined as 5.4 log CFU/g. The

pressure treatment caused a decrease of the total count by 1.6 log to 3.8 log CFU/g. After the addition of antimicrobial agents and subsequent pressure treatment (300 MPa, 200 s), the total count decreased, on average, by three logs (Figure 3). Comparing the decrease of the total flora with the two combinations (i) monolaurin + pressure and (ii) nisin+pressure, there was a synergistic effect identified if monolaurin (or nisin) was combined with pressure. As to the (iii) lysozyme + pressure

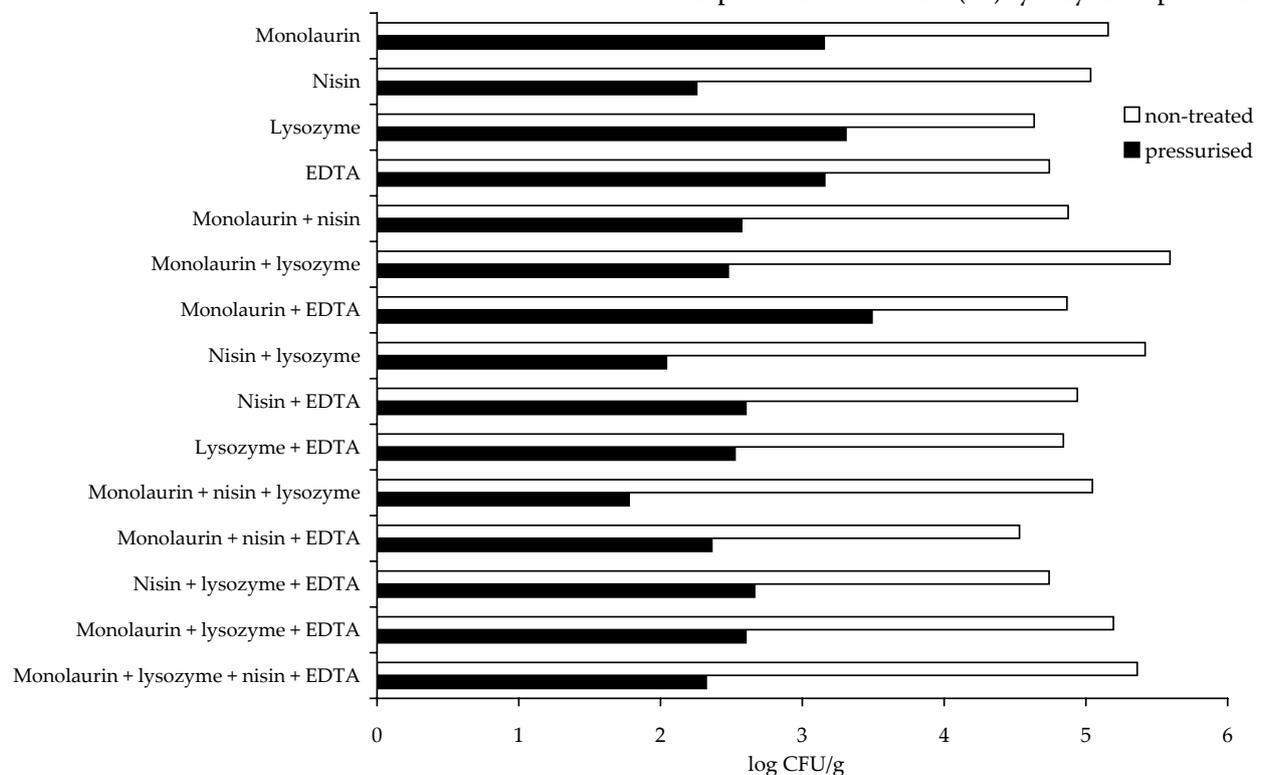


Figure 3. Influence of antimicrobial additives and pressure treatment on the total flora of liquid whole egg (for control, i.e. unpressurised sample without any antimicrobial, log CFU/g = 5.4)

and (iv) EDTA + pressure, only an additive effect was noticed.

According to many authors, e.g. CUTTER and SIRAGUSA (1995) and BRANEN and DAVIDSON (2004), the antimicrobial effects of nisin, monolaurin and lysozyme, active mainly against Gram positive organisms, can be extended to Gram negative organisms by combining with EDTA. However, in our study, this phenomenon was not confirmed. The reason resides probably in the fact that the majority of the studies reporting the positive influence of EDTA were based upon (1) the observation of organisms suspended in a buffer, rather than growing in nutrient media, (2) the use of unrealistically high levels of antimicrobial agents for food applications (GILL & HALLEY 2003).

CONCLUSIONS

On the basis of the evaluation of the rheometric data, it was stated that the liquid whole egg tested behaved as non-Newtonian pseudoplastic liquid. Pressurisation at 300 MPa/200 s did not significantly change its rheological properties in comparison to pasteurised LWE (65°C, 3 min). The appropriateness of the pressurisation regime was also confirmed by the fact that no differences were seen in the emulgation properties between the non-treated and pressurised liquid egg. The foaming properties were not found dramatically different either. On the other hand, the pasteurised LWE showed better emulsifying properties than the non-treated or pressurised LWEs. The addition of microbial agents or their combinations induced decrease of the initial microbiological contamination for three logs on average. The combinations of nisin or monolaurin with subsequent pressure treatment caused synergistic effects on the total flora decreasing. Quite a widespread idea that the effects of nisin, monolaurin or lysozyme can be enhanced by the addition of EDTA was not confirmed.

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