Effect of Pulsed Electric Fields on the main Chemical Components of Liquid Egg and Stability at 4°C

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Abstract: The effect of PEF on the whole liquid egg components, proteins and lipids, and the microstructure were studied and compared with pasteurisation. The effect of the refrigerated storage one week after the treatments was too studied. After all the treatments, only pasteurised samples showed water-soluble protein values significantly lower than the control and PEF treated samples, even after refrigeration. This could be related to the reinforcement of protein-protein interactions generated by the partial denaturalisation of proteins, observed by Cryo-SEM. Moreover, a water-soluble protein decrease was detected in the control and PEF treated samples after refrigeration, probably due to the aggregation of the egg lipoprotein during the storage. Furthermore, a slight lipolysis was observed in the control and PEF treated samples after refrigeration; but this effect was lower as higher was the PEF treatment. The study of the oxidation parameters showed an intermediate degradation of the lipids in treated samples, compared to the pasteurised eggs. These would reflect a higher microbiological stability of the PEF treated samples compared to the non-treated eggs.

Keywords: pulsed electric fields; egg; structure; proteins; lipids

INTRODUCTION

Nowadays, consumers are more and more demanding for minimally processed foods. This has led to the food industry to develop new technologies as Pulsed Electric Fields (PEF) that allows obtaining products with similar properties to fresh foods (Mañas & Pagán 2005). Specially, this technology is considered a very promising alternative to pasteurisation processes when processing high thermal sensitive liquid foods such as whole liquid egg (Sampedro *et al*. 2006). However, the main studies in egg treated by PEF are focused on inactivation of different target microorganisms. For that reason, the aim of this work has been to study the effect of PEF on proteins (N-Kjeldahl) and lipids (titration and spectrophotometric analysis) of whole liquid egg, and the microstructure of these components by Low Temperature Scanning Electron Microscopy. The effect of PEF treatment on whole liquid egg was studied and compared with pasteurisation, immediately after treatment and after one week of refrigerated storage.

MATERIALS AND METHODS

Materials. Whole liquid egg (WLE) samples not treated, pasteurised (66°C, 4.5 min) or treated by Pulsed Electric Fields (PEF) at 19 and 32 kV (5 pulses, 6 μs, 250 Hz) were supplied by the Swedish Institute for Food and Biotechnology (Göteborg, Suecia). All the samples were vacuum-packed in 250 ml plastic bags and kept under refrigerated storage at 4°C.

Low Temperature Scanning Electron Microscopy (Cryo-SEM).

A JSM-5410 SEM microscope (Jeol, Tokyo, Japan) coupled to a Cryo CT-1500C unit (Oxford Instruments, Witney, UK) was used. The sample was placed in the holder, fixed with slush nitrogen ($T \leq -210$°C), transferred frozen to the Cryo

unit, fractured, etched (–90°C), and gold-coated (2 mbar, 2 mA). The sample was then transferred onto the microscope and examined at 15 kV and –130°C.

Extraction and quantification of the water-soluble proteins. Water-soluble nitrogen were extracted according to AOAC 932.08 (AOAC 2000) and quantified by Kjeldahl, using a mixture of K_2SO_4, $CuSO_4$ and Se (10:1:0.1) as catalyser. All the results were expressed as protein using a conversion factor of 6.25.

Analysis of lipids. Lipids were extracted from the whole liquid egg samples according to Boselli *et al*. (2001). The acidity grade was determined in accordance with AOAC (1990) regulations. Hydroperoxides and secondary oxidation products were detected by spectrophotometric methods (UNE 55-047-73).

Statistical analysis. Statistical analysis of the results was performed using a one-way analysis of variance (ANOVA); the least significant differences (LSD) were calculated at the ≤ 0.05 significance level. The Statgraphics Plus 5.1 computer-assisted statistics program was employed.

RESULTS AND DISCUSSION

Table 1 shows the protein values obtained by N-Kjeldahl. After the treatments or 1 week of storage, pasteurised samples showed water-soluble protein values significantly lower than the control and PEF treated samples. This could be attributed to the partial insolubilisation of the proteins caused by the high temperatures reached during the thermal treatment. No changes were observed in the pasteurised samples after 1 week of storage, although an N-soluble decrease was observed in the control and PEF treated samples. The egg natural colloidal emulsion was destabilised during the refrigerated storage, and a partial insolubilisation of proteins was produced in the control and in the PEF treated samples.

The lipid fraction was analysed to determine chemical changes in this fraction due to a possible lypolitic activity. After one week of storage, a slight lipolysis occurred in all the samples, except in the pasteurised ones. This was attributed to the growing of microorganisms, but this effect was lower as higher was the PEF treatment applied (32 kV).

Furthermore, an increase in the K_{232} parameter was observed in all the samples, after one week of storage, although the highest effect was produced in the pasteurised samples. After one week of refrigerated storage, the K_{270} parameter decreased in all the samples.

The values of K_{232} and K_{270} oxidation parameters after the treatments and one week of storage showed an intermediate degradation effect of the lipidic fraction in PEF treated samples compared to the pasteurised ones. It would be related to a higher microbiological stability of these samples, compared to the control whole liquid egg. The thermal treatment during the pasteurisation affected in a greater level the chemical stability of the lipidic fraction during the storage.

Figure 1 shows the Cryo-SEM micrographs of the control, pasteurised and treated by PEF WLE

Table 1. Water-soluble proteins analysed by Kjeldahl, in just treated samples (A) and after one week of refrigerated storage (B): control WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P)

	A	B
C	8.72 ± 0.06^{a}	$8.11 \pm 0.06^{ab*}$
19 kV	8.46 ± 0.15^{a}	$7.96 \pm 0.15^{a*}$
32 kV	8.69 ± 0.33^{a}	$8.15 \pm 0.09^{ab*}$
P	7.02 ± 0.19^{b}	6.80 ± 0.25^{c}

Different letters in the same column indicate significant differences at $P < 0.05$ according to the LSD multiple range test; *means that N-soluble values of the samples at week 0 differ from those at week one ($P < 0.05$) according to the LSD multiple range test

Table 2. Acidity grade of the control WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P), in just treated samples (A) and after one week of refrigerated storage (B)

	A	B
C	1.21 ± 0.06^{c}	$1.42 \pm 0.02^{c*}$
19 kV	1.06 ± 0.04^{a}	$1.33 \pm 0.03^{b*}$
32 kV	1.12 ± 0.05^{b}	$1.29 \pm 0.04^{b*}$
P	1.10 ± 0.04^{ab}	1.09 ± 0.02^{a}

Different letters in the same column indicate significant differences at $P < 0.05$ according to the LSD multiple range test; *means that acidity grade values of the samples at week 0 differ from those at week one ($P < 0.05$) according to the LSD multiple range test

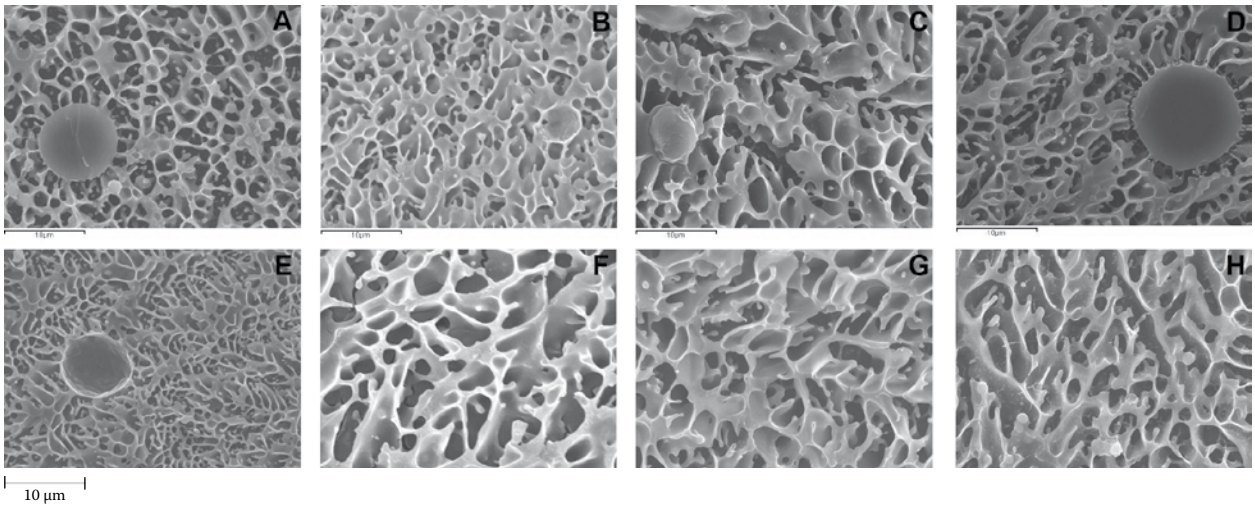

Figure 1. Cryo-SEM. A: control WLE; B: pasteurised WLE; C: WLE treated by PEF at 19 kV; D: WLE treated by PEF at 32 kV; E: control WLE, after one week of refrigerated storage; F: pasteurised WLE, after one week of refrigerated storage; G: WLE treated by PEF at 19 kV, after one week of refrigerated storage; H: WLE treated by PEF at 32 kV, after one week of refrigerated storage 3500×

samples, respectively. In the control, the lipoprotein matrix is observed as a continuous network in which the protein granules are immersed and closely interacting with the other components of the matrix (Figure 1A). The empty areas would be mainly occupied by water in the original sample. Pasteurisation (Figure 1B) produces a thickening of the lipoprotein matrix, which is observed closer in these samples; this is probably due to the intensification of the protein-protein interactions, caused by the partial denaturalisation or insolubilisation of the proteins during the thermal treatment. In the PEF treated samples, some discontinuities could be observed in the lipoprotein matrix and it was even broken in some areas (Figures 1C and 1D). This effect could be related to a weakening of the interactions among the liquid egg components.

After one week of refrigerated storage, an aggregation effect can be observed in all the samples (Figures 1E, F, G, and H). However, this effect was greater in the control egg (Figure 1E), in which the areas originally occupied with water had practically disappeared. These observations could explain the N-soluble decrease observed in the water-soluble protein analysis, due to an aggregation effect during the pasteurisation and the PEF treatments.

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Table 3. Oxidation spectrophotometric parameters (K_{232} and K_{270}) of the control WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P), in just treated samples and after one week of refrigerated storage

	Just treated		One week at 4°C	
	K_{232}	K_{270}	K_{232}	K_{270}
C	2.05 ± 0.09^{b}	3.19 ± 0.31^{b}	$2.52 \pm 0.17^{b*}$	$2.27 \pm 0.27^{b*}$
19 kV	2.70 ± 0.19^{d}	2.02 ± 0.07^{a}	2.90 ± 0.23^{c}	1.66 ± 0.23^{a}
32 kV	1.61 ± 0.07^{a}	3.46 ± 0.08^{b}	1.80 ± 0.06^{a}	2.72 ± 0.23^{c}
P	2.12 ± 0.09^{bc}	2.63 ± 0.33^{c}	$3.49 \pm 0.79^{d*}$	$1.71 \pm 0.54^{a*}$

Different letters in the same column indicate significant differences at $P < 0.05$ according to the LSD multiple range test; *means that K_{232} and K_{270} values of the samples at week 0 differ from those at week one ($P < 0.05$) according to the LSD multiple range test

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