

Main Chemical Changes in Proteins and Structure of Egg Treated with High Pressure Homogenisation

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Abstract: The aim of this work was to study the main chemical changes in the protein fraction (SDS-PAGE) and the microstructure (TEM) of whole liquid egg treated with HPH and stored one week at 4°C. In the electrophoretic study, no changes were observed after the treatments neither after one week of refrigerated storage in the water-soluble proteins of the samples, indicating that no proteolysis was produced. The typical structure of the egg lipoprotein matrix was maintained after the HPH treatments and one week of refrigerated storage. However, a progressive disaggregation of the protein granules was observed, especially when high intensity HPH treatment was applied. It was attributed to the HPH treatment and the microstructural changes observed could affect the functionality of the whole liquid egg when it is used as an ingredient in foodstuffs.

Keywords: proteins; structure; egg; High Pressure Homogenisation Electrophoresis

INTRODUCTION

During the last years, non-thermal food processing technologies are regarded with special interest by the food industry. Among them, High Pressure Homogenisation (HPH) is gaining popularity with food processors because it inactivates microorganisms, preserving the nutritional and sensory properties of foods, and maintaining their shelf life. Furthermore, it has the capacity to develop "value-added" food products or foods with interesting functional properties (TEWARI & JUNEJA 2007). The egg is an ingredient commonly used in the food industries because of their chemical components functionality. These properties have to be preserved when new technologies are applied. The aim of this work has been to study the main chemical changes in proteins and the microstructure of whole liquid egg treated with HPH at different pressure levels (0, 1000, 3000, and 5000 bar), and stored one week at 4°C. Egg soluble proteins were studied by Polyacrylamide Gel Electrophoresis with Sodium Dodecil Sulphate (SDS-PAGE) and the microstructure was observed by Transmission Electron Microscopy (TEM).

MATERIALS AND METHODS

Materials. The egg samples were treated with HPH at different pressure levels: 1 cycle (1000 bar), 3 cycles (1000 bar) and 5 cycles (1000 bar) by means of a continuous homogeniser equipped with a PNSA valve. All the samples were packed at sterilisation conditions and kept under refrigerated storage (4°C). Non-treated egg samples were also studied and compared to those treated by HPH. All the samples were supplied by the Department of Food Science of the University of Bologna (Italy).

Transmission Electron Microscopy (TEM). The liquid egg samples were stabilised by mixing with a low gelling temperature agarose solution (3%) at 30°C, which facilitates fixation and embedding prior to TEM observation (SHARMA *et al.* 1996). Next, samples were cut into cubes (1 mm³), fixed (primary fixation with 2.5% glutaldehyde and secondary fixation with 2% osmium tetroxide), dehydrated with 30, 50, and 70% ethanol, contrasted with uranyl acetate (2%) and embedded in epoxy resin (Durcupan ACM, FLUKA, Buchs, Switzerland). The blocks thus obtained were cut using a Reichert-Jung ULTRACUT ultramicrotome

(Leica, Barcelona, Spain). The ultrathin sections obtained (~ 100 Å) were collected in copper grids and stained with 4% lead citrate to be observed in the Philips EM 400 Transmission Electronic Microscope (Eindhoven, Holland) at 80 kV.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE). The water-soluble proteins were extracted as follows. First, samples were freeze-dried (TELSTAR Lioalfa-6, Terrassa, Spain) for 24 h at 10^3 Pa and -45°C . After freeze-drying, they were defatted in a continuous extraction method with *n*-hexane-isopropanol, 77:23 (CHUNG & FERRIER 1991). The extraction of the water-soluble fraction was carried out with distilled water (15 ml) from the defatted samples (2.5 g). Next, these mixtures were centrifuged at 3500 rpm for 20 min in a Sorvall Super T₂₁ centrifuge (KENDRO Laboratory Products, Hanau, Germany). Protein concentration of these water-soluble extracts was determined by the Bradford method (1976) using BSA for the preparation of the standard curve.

The preparation of the samples for the electrophoretic study was carried out adjusting the protein concentration to 1.25 mg/ml with Laemmli buffer.

Electrophoresis was performed using the method of LAEMLI (1970) on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, USA), using 12.5% polyacrylamide gels ExcelGel SDS Homogeneous (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 600 V, 38 mA, 23 W and 15°C

for 1 h 30 min, and 8 μl of each sample were loaded in the gel in duplicate.

The standard proteins were an Amersham low molecular weight calibration kit (GE Healthcare, UK) consisting of: phosphorylase b (97 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 000 kDa), trypsin inhibitor (20.1 kDa) and α -lactalbumin (14.4 kDa).

Proteins were stained with Coomassie Brilliant Blue tablets (PhastGel Blue R., GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Destaining was performed in an aqueous solution of 25% ethanol and 8% acetic acid. Gels were preserved in a solution of 10% glycerol and 7.2% acetic acid.

Gels were scanned with an ImageScanner III LabScan 6.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and analysed with the ImageQuant TL Image Analysis Software v7.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) to determine the molecular weight of each band.

RESULTS AND DISCUSSION

Figure 1 shows the electrophoregram obtained from the water-soluble proteins of the treated eggs and those after refrigeration during one week. 10 bands were identified in the non-treated samples; the majority bands were 5 and 8 corresponding to ovotransferrin and ovalbumin, respectively, main proteins in the egg white.

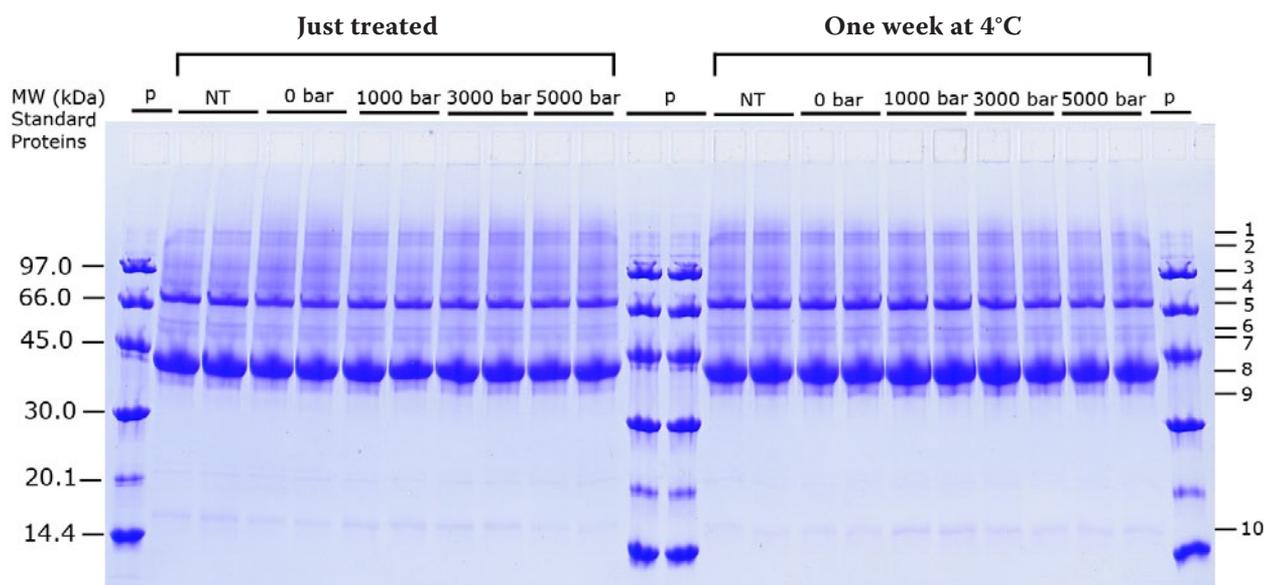


Figure 1. Water soluble protein electrophoregram of the whole liquid egg treated by HPH (0, 1000, 3000, and 5000 bar), and after 1 week of refrigerated storage. NT: non-treated samples; p: standard proteins

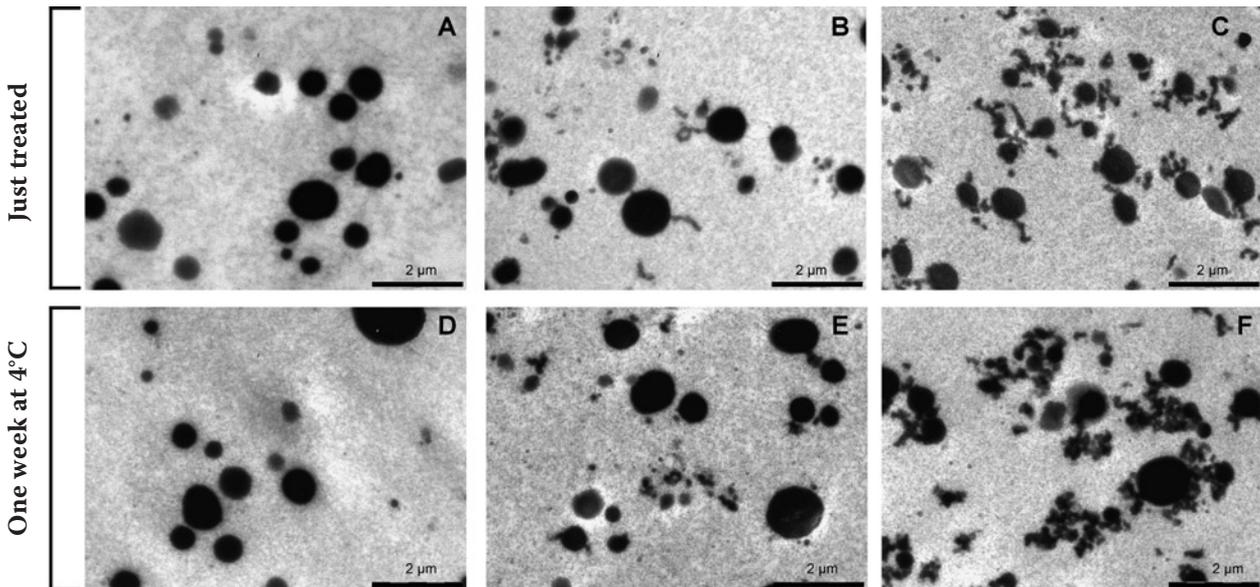


Figure 2. TEM. A: whole liquid egg (non-treated); B: HPH treated (1000 bar) whole liquid egg; C: HPH treated (5000 bar) whole liquid egg; D: whole liquid egg (non-treated), after one week of refrigerated storage; E: HPH treated (1000 bar) whole liquid egg, after one week of refrigerated storage; F: HPH treated (5000 bar) whole liquid egg, after one week of refrigerated storage (2950 \times)

Changes were observed neither after the treatments nor after one week of refrigerated storage in the water-soluble proteins. This would indicate that these treatments do not produce proteolysis in the egg proteins. Furthermore, endogenous proteolytic activity was not detected after one week at 4°C, because no changes were observed in these electrophoreogram after the refrigerated storage.

Figure 2 shows the TEM micrographs of the non-treated liquid egg and HPH treated samples (0, 1000, 3000 and 5000 bar). The characteristic structure of whole liquid egg consists of a continuous lipoprotein matrix in which protein granules are dispersed (Figure 2A). These protein granules are strongly electrodense, round shaped and have different sizes (1–1.3 μm). The structure of the lipoprotein matrix was not affected by the HPH treatments and it was also maintained after one week of refrigerated storage. However, a progressive disaggregation of the protein granules was observed, especially when high pressure levels (Figure 2C) were applied. This microstructure does not change after one week of refrigerated storage. This is in conformance with the results obtained in the electrophoretic study in which no proteolysis was observed.

It could be concluded that the structural changes observed in the protein granules were due to the HPH treatment. These changes could affect the whole liquid egg functionality when it is used as a raw material in foodstuffs.

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