Agronomy Research **17**(S2), 1452–1459, 2019
https://doi.org/10.15159/AR.19.057

Effect of high pressure processing on raw pork microstructure and water holding capacity

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Abstract. High pressure processing (HPP) is widely used as an alternative to thermal food preservation technologies, including processed meats treatment. This technology affects food texture and water-holding capacity, which may have beneficial effect on product yield. After thermal treatment, meat partially releases water together with water-soluble proteins, which is concerned as a loss. It is very important not only because of changes in taste properties, but also economic aspects such as reduced final product weight. The aim of the study was to evaluate changes in the meat microstructure and water-holding capacity upon high pressure treatment. Pork samples were treated at various pressures and holding times, namely, 300 and 600 MPa with a 1 and 15 minutes holding time at each pressure. Untreated sample was regarded as a control. Microstructure of pork meat was evaluated after the paraffination of the samples. Fibre cross section area and space between fibres were measured and reported. Water-holding capacity was measured by centrifugation of meat samples over filter and calculating released amount of juice. Results indicated that fibre size did not change significantly after treatment at 300–600 MPa pressure comparing to the control sample – untreated meat. However, high pressure can affect hydrophobic properties of myofibrillar protein. The experimental results showed that water-holding capacity increases with the high pressure treatment. It is an important issue in meat processing industry, because HPP treatment allows reducing the water loss in fresh pork.

Key words: high pressure processing, histology, expressible water, pork.

INTRODUCTION

The structural changes in food systems caused by high pressure processing (HPP) depend on the pressure effect on specific food compounds. Thus, HPP can modify macromolecules or biopolymers, disrupting hydrogen bonds, resulting in the loss of enzyme and membrane activity of proteins (Farkas, 2016). It has been demonstrated that pressurization induce coagulation of proteins without drastic chemical changes, which are observed in heat treated products (Cao et al., 2012). HPP would not break covalent bonds, retaining flavours, pigments and other nutritionally important compounds.

From a physical point of view pressurization moves molecules closer to each other, leading to phase transitions which may be reversible after treatment (Hugas et al., 2002). An increased hydrostatic pressure induces structural changes in protein molecules. These structural changes may lead to conformations of molecules, resulting in new functional

properties of proteins such as gelation, coagulation, association, and dissociation (Chapleau et al., 2004), therefore enhancing the stability of meat gels. Pressure affects the functional properties of myofibrillar proteins related to meat texture, changing their solubility and water binding capacity (Duranton et al., 2012).

Meat is mainly constituted by water (approximately 75%). Lean meat contains also protein (15–21%), fat (0.5–25%), oligonutrients and vitamins, especially B group vitamins (Hugas et al., 2002). The most variable compound is fat. There exist close negative correlation (r = -0.99) between fat and water, while protein content has positive correlation with water. The structural organization of the muscle proteins is decisive for the distribution of the water within the meat.

An increased water retention results in reduced cooking loss without application of such additives as starch or phosphates, thus giving higher yield of final product (Ma et al., 2012). Additionally, changes in protein and starch structures may create natural compounds which possess emulsifying, stabilizing, texturizing properties used for water retention (Farkas, 2016). Water holding capacity (WHC) is closely related to other attributes such as meat texture, colour, juice loss etc. (Warner, 2017). On the other hand, low WHC can cause big water losses from meat and meat products due to exudation and evaporation, resulting in weight loss and reduced quality of the product. A thorough understanding of the water holding capacity is important as it affects quality, safety, and profitability.

Water in meat can be in three forms – bound, immobilized or free. Bound water makes only 1–2%, being bound to proteins it stays within meat through all processing steps. Immobilized water can make up to 80% from whole water. It has weaker link to proteins and other cell structures. Immobilized water may be lost or transferred to free water category. In meat processing it is important to immobilize as much water as possible, thus increasing WHC. Free water is easily lost in meat processing. However it may be trapped in meat structures such as cell membranes and capillaries, which are related to the space between myofibrils. Any type of processing which damages these structure will lead to increased loss of water. Although adequate processing allows changing free form to immobilized, which includes reduction in protein denaturation, increase in meat pH, increasing sarcomere length, minimizing damage of muscle structure, maintaining low storage temperatures (Huff-Lonergan & Sosnicki, 2005).

The aim of the study was to examine changes in pork microstructure and water holding capacity depending on the applied pressure and treatment time.

MATERIALS AND METHODS

The study was done in the scientific laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

Chilled pork obtained from *Musculus longissimus lumborum* was purchased from the meat processing company 'Kurzemes Gaļsaimnieks' (Latvia) unpackaged; stored under chilled condition at temperature 3 ± 1 °C ; maximal storage time 24 h. No breed, age, sex or premortal handling was recorded.

Meat treatment

The obtained chilled pork meat was cut in 2.0 ± 0.2 cm thick slices across the muscle fibre and slices were divided into portions with the weight of 80.0 ± 0.2 g each,

packed in the vacuum pouches made from polyamide/polyethylene film (film thickness 60 ± 3 μm). The final pressure in the vacuum-packaging was 8 mbar. Samples were stored in the refrigerator at 4 ± 2 °C till experiment. The pH of chilled pork immediately after purchase was 5.51 ± 0.06. Samples of pork meat were treated in a high-pressure processor ISO-Lab S-FL-100-250-09-W (Stansted Fluid Power Ltd., UK) with a pressure chamber of 2 L and a maximum operating pressure of 900 MPa. The pressure transmitting medium was a mix of propylene glycol with water (1:2 v/v) at room temperature.

Vacuum-packed meat samples were randomly assigned to one of the treatment pressures (300 and 600 MPa), each pressure level was applied for three meat samples for durations of 1 and 15 min. while untreated sample served as the control. Totally we had five batches of samples: 1) control – raw meat; 2) 300 MPa 1 min; 3) 300 MPa 15 min; 4) 600 MPa 1 min; 5) 600 MPa 15 min. The pressurisation experiment was repeated 4 times.

Expressible water

The amount of expressible water of pork samples was determined according to the modified centrifugation method described by Januškevičienė et al. (2012). 10.00 ± 0.01 g of minced meat were placed on a plastic funnel, which was lined with large – pore filter (0.45 μm) of known weight. The funnel with the sample was placed into centrifugation test-tube. Centrifugation in a centrifuge Z 206 A (Hermle Labortechnik GmbH, Germany) was carried out for 20 min at a speed – 6000 revolutions per min. Then, the sample was weighed together with a filter and the amount of expressible water was calculated. The reported results are average of 12 independent measurements.

Preparation of histological samples for light microscopy

Meat samples were cut into 5 × 5 × 5 mm pieces along muscle fibres. Then samples were placed into the perforated cassettes for treatment in a 10% formalin solution for 24 h at room temperature, followed by holding at 37 °C 2 h in 70% ethanol, 2 h in 80% ethanol, and 24 h in 98% ethanol. The next preparation step was soaking of sample in xylene for 0.5 h and another 0.5 h in fresh xylene. Next, the sample was moved to solution of xylene and paraffin (ratio 1 : 1) and held for 1.5 h at 57 °C, then transferred to melted paraffin for 1 h, and another time to new liquid paraffin for 1 h. After this, the samples were removed from cassettes, placed in the plastic moulds and poured with paraffin. After paraffin solidified, samples were cut into thin slices (5 μm) by Microtom (Microtom GmbH, Germany) and dropped into water bath. Prepared samples were placed on the glass slides and dried for 10 min at 37 °C. For removal of paraffin, the slide with the sample was soaked in xylene for 10 min, then in 98% ethanol for 10 min and let dry (Kondratovics, 1976; Ramane et al., 2008). Ten slides per condition were observed using a microscope Leica DM300 LED (magnification 10 × 40), photos were taken by camera Leica DFC 290 HD and ten measurements per sample were completed using software Leica Application System (LAS) V4.2. (Leica Microsystems, Germany).

Environmental scanning electronic microscopy (ESEM)

Sample for ESEM were prepared according to Das Murtey M. & Ramasamy P. (2016) with some modifications. First, small samples of meat are placed in PE containers

and fully coated with 4% formaldehyde solution. After 2 h, the formaldehyde solution was replaced by deionised water, which has been exchanged 3 times every 5 minutes. The sample were dehydrated by immersion in ethanol baths of increasing content until 96% EtOH, then samples were frozen in liquid nitrogen and then lyophilised for 72 h.

In order to improve the electrical conductivity of the surface of the samples, their surface shall be coated with a thin layer of Au (~ 15 nm), using the Emitech K550X materials. A surface inspection of samples has been performed with the Schottky-type field emission electron microscope Tescan Mira/LMU, under high vacuum conditions, at an electron acceleration voltage of 15 kV using a back-scattered electron detector.

Data processing

Microsoft Excel v16.0 for Windows was used to process the obtained data; mean ± standard deviation was calculated. Cross-comparison of data was performed using ANOVA and Tukey's test. For data analysis, confidence level was 95% ($\alpha = 0.05$). The factors have been evaluated as significant, if P-value < $\alpha_{0.05}$.

RESULTS AND DISCUSSION

Water holding capacity for high-pressure treated meat

Water holding capacity was significantly affected by high pressure treatment. Centrifugation of pork samples demonstrated that the highest amount of expressible water was in the control sample (16.00 ± 2.98%), which was about 5-fold higher than from HPP samples (Fig. 1).

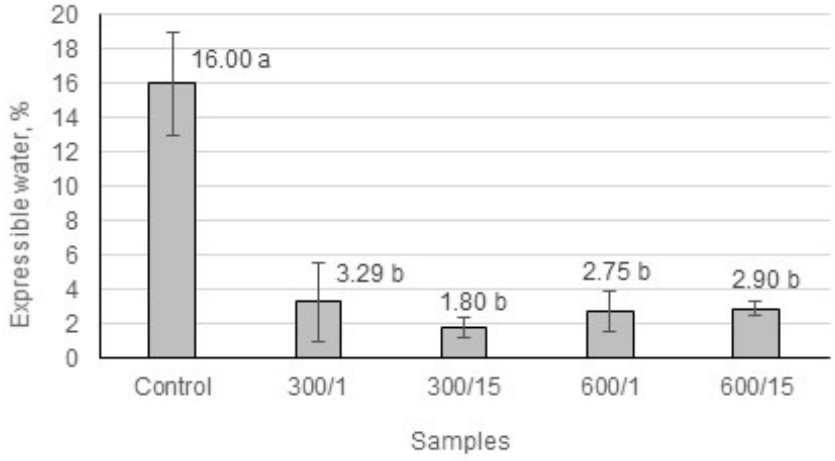

Figure 1. Expressible water in pork detected by centrifugation method.
Different letters indicate significant differences ($P < 0.05$), n = 12.

Among HPP samples the highest WHC exhibited pork treated at 300 MPa for 15 min, having the smallest released water amount (1.8 ± 0.55%), but the lowest WHC was for pork treated at 300 MPa for 1 min. Expressible amount of water for pork treated at 600 MPa was not affected by treatment time.

The differences between untreated pork and HPP pork samples were statistically significant ($P < 0.05$), which coincides with other research results (Ros-Polski et al., 2015; Xue et al., 2017) who found that water became more tightly bound to the meat

matrix. Pressure greatly influences functional properties of myofibrillar proteins, such as solubility and their water binding and gelling ability. These properties are related to meat ability hold water (Chapleau et al., 2003). It may be influenced also by pH, which is changed during HPP treatment, as it was described in our earlier research (Sazonova et al., 2017). There was not established significant differences among expressible water in HPP treated samples ($P > 0.05$) irrespective of applied pressure or time.

According to Huff-Lonergan & Lonergan (2005) the majority of water in muscle is held within the myofibrils, between them and within other structural elements of muscle – sarcolemma, cells, muscle bundles. When pressure is applied at ambient temperature, little to no changes to connective tissue is observed, probably due to collagen stabilization by hydrogen bonds (Warner et al., 2017). However, HPP treatment can cause destabilization of non-covalent interactions between proteins, also little unfolding occurs, with formation of hydrophobic and disulphide bonds after pressure release (Chapleau et al., 2004; Sun & Holley, 2010). Thus, non-covalent interactions between amino acid residues, which support the protein tertiary structure is first destabilized and then replaced by protein-water interaction. Our previous study (Sazonova et al., 2019) indicated that FTIR spectra showed the intensity decrease in bands representing collagen type I, which was proportional to the pressure and to the treatment time. Thus suggesting denaturation of collagen and release into meat juice, which could bind water.

Microstructure of high pressure treated pork

Microscopy of histological samples (Fig. 2) showed that the fibre size in untreated pork was slightly smaller compared to treated samples (Table 1). After HPP treatment fibre cross section area was slightly increased which correlates with improved water holding capacity. It can be related to protein denaturation. Fig. 2 shows the connective tissue in a transverse section of meat (the connective tissue is light, the fibre are dark).

There was not observed statistically significant ($P > 0.05$) differences in fibre cross section area and extracellular space. It indicates that observed muscle structures retained their shape after pressurization. Similar conclusion was drawn by Jemeljanovs et al. (2007), who described nucleus, which were retained under sarcolemma after HPP. Researchers have described changes in the protein secondary, tertiary and quaternary structures, which brings modifications in both structure and function of proteins (Zhang et al., 2018). These effects typically are magnified with increased pressure.

Table 1. Fibre area and extracellular space in pork

Sample	Fibre area, μm²	Extracellular spaces line length, μm
Control	1,074.58 ± 453.97 a	12.79 ± 5.32 a
300/1	1,159.06 ± 454.73 a	10.32 ± 4.19 a
300/15	1,395.00 ± 599.53 a	15.84 ± 6.45 a
600/1	1,501.13 ± 595.76 a	18.41 ± 8.46 a
600/15	1,267.58 ± 510.90 a	11.57 ± 4.67 a

Values within the same column sharing the same letters are not significantly different ($P > 0.05$), $n = 100$.

ESEM's observations (Fig. 2) were helpful in supplementing histology data and providing evidence that the samples retained their fibre shape and size.

Transmission electron microscopy completed by Kaur et al. (2016) showed the presence of aggregates, resulting probably from protein denaturation of sarcoplasmic proteins, in the subcellular space and between myofibrils.

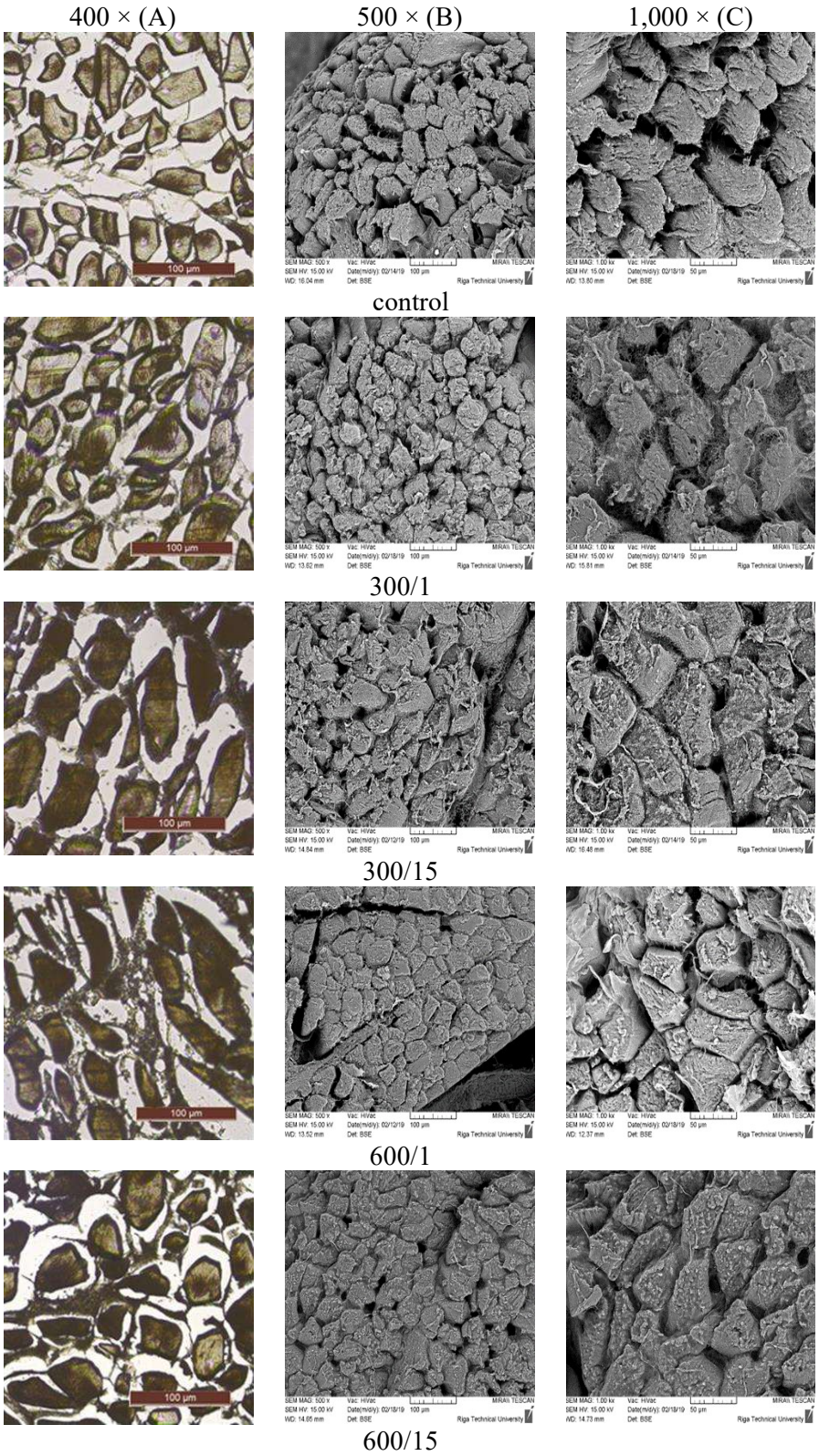

Figure 2. Cross section of pork muscle (*Musculus longissimus lumborum)* tissue after HHP treatment. The Histological cross sections (column A) were observed at 400 × magnification. ESEM cross sections were observed at 500 × magnification (column B). ESEM cross sections were observed at 1,000 × magnification (column C).

There were no statistically significant differences among samples, although the untreated sample had slightly smaller fibre cross section area. Literature analysis revealed that there is a lack of data on changes in raw meat upon HPP. Majority of the recent researches deal with meat supplemented with various additives (Duranton et al., 2012; Ma et al., 2012). For more advanced understanding of changes occurring in HPP, further studies on cross sections would be suggested.

CONCLUSIONS

The study revealed, that HPP treatment at 300–600 MPa for 1 and 15 min influenced pork meat water holding capacity, increasing it approximately 5-fold comparing to untreated meat, irrespective of applied pressure and time. Since microstructure analysis indicated that fibres in treated samples were not different from untreated pork, the increased water holding capacity could be due to macromolecular changes caused by HPP treatment.

ACKNOWLEDGEMENTS. Research was funded by the grant from the project Z13 of the programme 'Strengthening Research Capacity in the LLU'.

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