

THESIS OF THE DOCTORAL DISSERTATION

Gábor Jónás

Budapest

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Hungarian University of Agriculture and Life Sciences

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**INVESTIGATION OF THE EFFECTS OF HIGH HYDROSTATIC
PRESSURE TREATMENT DURING MEAT BRINING**

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Hungarian University of Life Sciences – Doctoral School of Food Sciences

Doctoral school: Doctoral School of Food Sciences

Discipline: Food sciences

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Approval signature of Head of the doctoral school and supervisors:

The candidate has fulfilled all the conditions prescribed by the doctoral school of Szent István University, the comments and suggestion at the thesis workshop were taken into consideration when revising the thesis, so the dissertation can be submitted to a public debate.

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INTRODUCTION AND OBJECTIVES

The aim of salting and brining is to introduce salt and other additives into the meat. The traditional techniques of salting and brining are well known, where the salt penetrates into the meat due to the difference in concentration between the meat and brine. It is a slow and time-consuming process that depends on several factors. Techniques such as tumbling and injection have been developed to accelerate salt penetration and equilibration in the meat by mechanically destroying the meat tissue. Technological developments in this field have mostly been aimed at making these techniques more economical. In the last 20 – 30 years, the focus of research and development has become wider and turned towards gentler technological solutions, e.g. ultrasound, vacuum, pulsed electric fields or high hydrostatic pressure. The high hydrostatic pressure is increasingly used to increase microbiological stability and shelf life. At present few information is available on the effects of pressure treatment on meat brining, so my doctoral thesis aimed to study the effects of high hydrostatic pressure treatment on curing and on some technological properties of meat.

The pressure treatment is carried out in a hydrostatic pressure medium, usually water, possibly propylene glycol. In order to avoid leaching and cross-contamination, the meat should be packaged before pressure treatment. Packing is usually done by vacuum packing, but the packing can also be filled with e.g. brine (salt solution), so that curing can be done together with the pressure treatment. In some research papers I have found that moderate pressure treatment (≤ 300 MPa) has been effective in accelerating diffusion in mass transfer operations (Rastogi and Niranjana, 1998; Sopanangkul et al., 2002; Villacís et al., 2008). Based on this, my objectives are to study the effect of the sequence of brining and pressure treatments, the pressure levels, brine concentrations and the duration of brining on the salt equilibrium between meat and brine, salt and water diffusion, meat proteins, microstructure, water binding capacity, texture and colour in case of

- a) the pressure treatment is carried out on meat packaged in brine
- b) the meat is pressure treated followed by brining
- c) the brining is carried out under normal conditions (without pressure treatment)
- d) the a) and b) are carried out at pressures of 100, 200 and 300 MPa in brine solutions with a salt concentration of 5 m/m% and 10 m/m%.

MATERIALS AND METHODS

Pork loin (*Longissimus dorsi*) obtained from a local slaughterhouse was used for all investigations (pH $5,56 \pm 0,02$). Cylinder shaped meat samples (diameter (d)=15 mm, height (h)=80 mm) were cut from the meat parallel to the longitudinal axis of the meat fibers. Meat cylinders were divided into the following treatments groups **(i)** pressure treatment in brine, **(ii)** brining after pressure treatment and **(iii)** brining without pressure treatment (control sample).

In **(i)** meat cylinders were pressure treated in PE bags filled with brine at pressures of 100, 200 and 300 MPa at 5°C for holding times of 1, 3, 5, 10 and 15 min. In **(ii)** first meat was treated at pressures of 100, 200 and 300 MPa at 5°C for 5 min then they were brined. In **(iii)** brining was carried out at 5°C without pressure treatment of the meat.

In (i-iii) experiments, brining was carried out in brine of 5 m/m% and 10 m/m% salt concentration, prepared from sodium chloride. In all cases meat – brine ratio was 1:4. Pressure treatments were

carried out in a Hiperbaric 135 (Hiperbaric, Burgos, Spain) high hydrostatic pressure treatment unit.

I have grouped the measurements and calculations around the following themes:

- Calculation of salt and water diffusion coefficients
- Protein structure and microstructure analysis
- Analysis of technological properties

The salt content of meat samples for the salt diffusion was determined by Mohr's method and the moisture content for the water diffusion was determined by drying at 105°C to constant weight. For the salt diffusion calculation, the equilibrium salt content was determined both experimentally and by calculation based on Körmendy (1991). The equilibrium moisture content for water diffusion was determined experimentally. To describe the diffusion of salt and water, I used models derived from Fick's law II ([1] - [3]).

$$\frac{C_t - C_0}{C_\infty - C_0} = 1 - e^{-\left(\frac{tD_s}{l^2}\right)^\beta} \quad [1] \text{ (Martuscelli et al., 2017)}$$

$$\frac{C_t - C_0}{C_\infty - C_0} = 1 - \sum_{n=1}^{\infty} \frac{4}{\mu_n^2} \exp\left(-\mu_n^2 \frac{D_s t}{R^2}\right) \quad [2] \text{ (Abbasi Souraki et al., 2012)}$$

$$\frac{C_t - C_0}{C_\infty - C_0} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(\frac{-D(2n+1)^2 \pi^2 t}{R^2}\right) \quad [3] \text{ (Telis et al., 2003)}$$

The models are based on the assumption that only radial, cylinder-symmetric diffusion occurs due to the geometric size and the salt concentration of the brine is constant during the brining. The effect of diffusion in the direction parallel to the meat fibers was reduced by cutting off the end blades of the cylinders to determine the salt and moisture content. Changing salt (C) for moisture (X) in the equations [1]-[3], water diffusion coefficient (D_v) can be calculated. The diffusion coefficient (D) was optimized using MS Excel 365 SOLVER using a non-linear method to minimize the Root Mean Squares of Error (RMSE) between measured and calculated salt and water content values. The "goodness of fit", was calculated by the coefficient of determination (R^2).

Protein denaturation analysis was carried out in a SETARAM MicroDSC III (SETARAM Instrumentation Caluire, France) thermoanalytical apparatus in the temperature range of 25-90°C at a heating rate of 1°C/min. In vitro digestion model experiments were performed for the protein state according to Minekus et al. (2014). Molecular separation of digested meat samples from the stomach and intestine phase was performed by polyacrylamide gel electrophoresis (SDS PAGE). Microstructural mapping of meat samples was performed on fixed, dehydrated sections using a Thermo Scientific™ Prisma™ E SEM (Waltham, Massachusetts, USA) scanning electron microscope.

Among the technological indicators, one of the most important ones is the water binding capacity, known in the international literature as "Water Binding Capacity" (WBC). It indicates the ability of meat samples to absorb water or brine [%] per initial weight as a result of different combinations of pressure treatment and brining operations.

Texture profile analysis (TPA) was performed to characterize the texture of meat samples using an SMS TA.XT Plus (Stable Micro Systems Ltd. Godalming, Surrey, UK) equipped with a 500 N load cell. From the TPA curves hardness and cohesivity were used. Color was measured with a Konica Minolta CR400 (Konica Minolta Inc., Japan) colorimeter under C65 illumination in CI-E Lab color space. In addition to the color factors L^* , a^* and b^* , the hue of the meat samples was characterised by the hue angle (h°), since the color of meat and consumer perception is essentially determined by its red appearance. The hue is determined by the ratio of the yellowness (b^*) and redness (a^*) $h^\circ = \arctan(b^*/a^*)$. The $h = 0^\circ$ represents the red color and moving away towards $+90^\circ$ or -90° the redness decreases and the yellow or blue color becomes dominant.

Statistical analysis of variance (ANOVA) was performed by IBM SPSS Statistics 20.0 (IBM Corp., USA) with 95% confidence interval ($p < 0.05$). The independent variables for the evaluation were the sequence of pressure treatment and brining, the pressure level (100, 200, 300 MPa) and the salt concentrations of the brine (5 m/m% and 10 m/m%). In the statistical evaluation, I used the partial eta square as an indicator of effect size, which shows how much of the variance of the dependent variable is explained by the independent variables. From this I inferred the magnitude of the effect of the independent variables.

RESULTS

As a first step of the experiments, the equilibrium salt and water contents of the meat samples were determined (C_{eq} , X_{eq}). These are necessary to characterize the salt and water equilibrium dynamics (C_t/C_{eq} , X_t/X_{eq}) between meat and brine and to calculate the diffusion coefficient (D , m^2/s) describing the mass transport rate. Based on the time evolution of C_t/C_{eq} and X_t/X_{eq} , the most dynamic equilibration was observed in the 100 MPa (5 min, $5^\circ C$) pressure treated meat samples, indicating faster salt and water penetration, both in comparison to the 200 and 300 MPa treated meats and to the meat cured without pressure treatment. The salt and water diffusion coefficients were determined by using mathematical models derived from Fick's Law II (Abbasi Souraki et al., 2012; Martuscelli et al., 2017; Telis et al., 2004). The accuracy of the models was assessed by fitting the salt and water content measurements and the 'strength' of the relationship between them. Model of Martuscelli et al., (2017) showed the closest fit and strongest relationship. This means that this model is able to predict with high accuracy the changing of salt and water content of the meat, thus determining the brining time, which is important for the production technology.

The salt (D_s) and water diffusion (D_v) coefficients confirmed the relationships observed for the equilibration dynamics. Meat brined after pressure treatment at 100 MPa showed 1.7 times higher salt and 1.9 times higher water diffusion coefficients in case of 5 m/m% brine and 1.4 times higher salt and 1.7 times higher water diffusion coefficients in case of 10 m/m% brine compared to meat brined without pressure treatment. Lower diffusion coefficients were observed for brinings following pressure treatments of 200 and 300 MPa, as well as for all pressure treatments in brine.

It is known from the literature that hydrophobic, ionic, non-covalent bonds forming the tertiary and quaternary structures of meat proteins can be affected by pressures as low as 100-600 MPa, resulting in reversible or irreversible changes in protein structure (Balny and Masson, 1993; Heremans and Smeller, 1998; Knorr et al., 2006). In addition, salt can also produce similar structural changes above a certain concentration, caused by the phenomenon of salting-out (Gou et al., 2003). Calorimetric measurements were performed to investigate the changes in meat proteins structure

during pressure treatment and brining. Pressure treatments as low as 100 MPa resulted in a shift and decrease in the peak denaturation temperatures of the major protein fractions, myosin and actin. For the 200 and 300 MPa pressure treatments, the peak of myosin was not detectable, while that of actin fused with the peak of sarcoplasmic proteins. The effect of the applied pressures on the denaturation of the meat proteins was found to be more pronounced than the concentration of brine. The change in the structure of meat proteins provides a larger surface area available for human organism, which is important for digestion. The pressurization in brine resulted meat samples that were degraded during in vitro digestion into peptide-sized components, which are more favourable for intestinal absorption than larger complex molecules.

The water binding capacity, which is important from a technological point of view, was highest in meat samples brined after pressure treatment of 100 MPa. This means that they absorbed the most brine during brining. This means an uptake of 9.3 – 10.1% of brine, based on the initial weight. The effect of this, the swelling of the fibers, was also visible in scanning electronmicroscopic micrographs. The meat fibre thickness was found to be the highest in these meat samples (>40 μm). However, none of the pressure-brining combinations caused morphological changes in the meat tissue in the observed size range (100 μm). Meat samples with higher water binding capacity resulted in softer texture. This may be explained by the fact that the higher amount of brine "diluted" the meat constituents, which resulted in a lower hardness value, i.e. softer meat. The sequence of pressure treatment and brining had the greatest effect on the development of hardness. Meats brined after pressure treatment showed significantly lower hardness, i.e. they were softer than pressure treated in brine samples. The results on the effect of pressure treatment on meat colour were consistent with the literature. The effect of pressure level on meat colour was found to be the strongest factor. As the applied pressure increased, a lightening (L^*) of the meat and a decrease in the intensity of the red colour were observed, independently of the sequence of the pressure treatment and brining. Meat treated at 300 MPa showed an appearance similar to cooked meat. Based on the hue angle (h°), brining after pressure treatment was found to be preferable to pressure treatment in brine in terms of 'maintaining' the red colour of the meat.

The results show that pressure treatment of meat before brining can be a useful addition to the brining process to improve salt and water diffusion, water binding and/or brine absorption and to achieve a softer meat.

NEW SCIENTIFIC RESULTS

1. I demonstrated that the sequence of pressure treatment and brining influences salt and water diffusion in pork loin.

Pressure treatment at 100 MPa for 5 min at 5°C then brining in 5 m/m% brine results in 1.7 times faster salt and 1.9 times faster water diffusion, and brining in 10 m/m% brine results in 1.4 times faster salt and 1.7 times faster water diffusion compared to brining without pressure treatment.

2. I found that the most accurate prediction of salt and water diffusion is provided by the model of Martuscelli et al. (2017).

My findings were based on fit and strength of relationship (R²) tests, which were confirmed by comparison with measured values, and thus this model is best suited to accurately predict salt and moisture content of meat during brining and to determine brining time.

3. I have found that pressure treatment and curing do not cause morphological changes in the microstructure of pork loin.

I base my findings on scanning electron microscopic images of 100 µm intact fibers of pork loin pressure treated at 100-300 MPa in 5 m/m% and 10 m/m% brine and brined in 5 m/m% and 10 m/m% brine after 100-300 MPa pressure treatments.

4. I have found that pressure treatment in the brine has an effect on the *in vitro* digestibility of pork loin.

The 200 and 300 MPa pressure treatment in 5 m/m% brine breaks down the light chain proteins of myosin, troponin-T, actin and the heavy chain proteins of myosin into peptide-sized components below 10 kDa, which are more favourable for absorption in the alimentary canal than the higher molecular weight proteins, during *in vitro* gastric digestion.

5. I have found that the sequence of pressure treatment and brining has an effect on the water binding capacity of pork loin.

I base my findings on the fact that pressure treatment at 100 MPa before the pork loins are brined results in significantly higher water binding capacity compared to both the 200 and 300 MPa treatments and the 100-300 MPa pressure treatments in brine.

6. I found that the sequence of pressure treatment and brining, the pressure level, the salt concentration of brine and the duration of pressure treatment and brining affect the hardness of pork loin.

I found that the pressures of 100-300 MPa and the salt concentrations of 5 m/m% or 10 m/m% have significant effects on hardness and I proved from the effect size test that the effect of the sequence and duration of pressure treatment and brining is the strongest factor on the hardness of pork loin.

7. I have found that the sequence of pressure treatment and brining, the pressure level, the salt concentration of brine and the duration of pressure treatment and brining affect the lightness and redness of pork loin.

I base my findings on the results of effect size test, which showed that the effect of the pressure level is the strongest factor on the lightness (CIELab, L*) and on hue angle (h°) which expresses the red colour of pork loin

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