PE4.51 An Elucidation of Parameters in High Pressure Processing Using NMR Relaxometry 181.00

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***Abstract*—High-pressure processing (HPP) of raw sausage samples results in protein denaturation giving rise to alterations in water distribution and gel formation. In this study the effects of the HPP parameters start temperature (5 or 40°C), pressure level (500, 600 or 700 MPa), rate of pressure increase (3.3 MPa/sec or 46.7 MPa/sec) and holding time (1 sec, 2, 4 or 6 min) were elucidated using low-field proton NMR T_2 relaxation, and it was shown that start temperature and pressure level applied were the parameters that had the greatest impact on the distribution of water in HPP meat. Start temperature and pressure level together with rate of pressure increase all had influence on gel strength and texture, while holding time only influenced the gel strength. The combination of 3.3 MPa/sec, 40°C, 600 MPa and a holding time of 6 min gave rise to a high texture score and the highest gel breaking force, but still inferior to the strength of a heat-set gel. A correlation between subjective texture score of the products and NMR distributed T_2 data was established, revealing that water mobility and distribution is decisive for the texture of the HPP-treated products.**

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I. INTRODUCTION

HIGH-PRESSURE (HP) treatment of meat is known to Hinactivate microorganisms, but also results in major changes in meat texture [1]. Using nuclear magnetic resonance (NMR) techniques, alterations in water characteristics due to pressurization of meat have been shown [2, 3]. In addition, the sensory properties of pressured meat were reported to be quite different [3]. Consequently, high pressure processing (HPP) is a promising technology enabling the meat industry to introduce new products mildly preserved with HPP as sole conservation technology, and at the same time creating the textural properties necessary for functional and sensory acceptance by the industry and consumers [4]. However, presently HP technology is still in its initial stage regarding these new meat products, and optimization of the technique is needed to make the technique a success. In the present study we investigated the effects of varying processing parameters such as temperature, pressure level and holding time in HP treatment of a meat emulsion by using low-field proton NMR relaxometry and traditional compression analyses on sliceable samples.

II. MATERIALS AND METHODS

Raw sausage preparation

The meat emulsion used for the study was manufactured in a vacuum high speed cutter (V30L, Kilia, Germany) at 2880 rounds/min until an emulsion end temperature of 12°C, and stuffed on a vacuum filling machine (VF50, Handtmann, Germany) in poly amide casings (Naloflex, Kalle, Germany) Ø28 mm at lengths of 140 mm (80 g each). The meat emulsion consisted of (w/w) 40% fore-end and 30% rindless jowls, pregrinded through a kidney plate, 24.57% water, 2% potato starch, 1% dextrose, 1% nitrite salt (0.6% nitrite), 0.8% vacuum salt, 0.03% sodium-ascorbate and 0,6% spices. After manufacturing the raw sausages were kept at 2-5°C for 18-24 hours until high-pressure processing (HPP).

For comparison, raw sausages were heat-treated in a conventional cooking cabinet (CC1, DanfoTech, Denmark) to a core temperature of 72°C, holding time 10 min.

HPP treatment

The raw sausages were HPP treated at Deutsches Institut für Lebensmitteltechnik, Quackenbrück, Germany, in either Wave 6000/55 equipment (NC Hyperbaric, Burgos, Spain), pressure increase 3.3 MPa/sec or in 2l/7000 UHDE pilot equipment (Uhde High Pressure Technologies, Hagen,

Germany), pressure increase 46.7 MPa/sec. Tested levels of HPP: 500, 600 MPa on Wave equipment; 500, 600, 700 MPa on UHDE equipment, holding times 1 s, 2, 4, 6 min; starting temperature of sausages 5°C, directly from refrigerated storage, or 40°C, after 20-30 min in 40°C water bath. For the latter temperature, water with a temperature of 40°C was also used in the inner liner of the pressure vessel. After treatment the sausages were kept at 5°C for 1-8 days until analyses.

NMR-analysis

Proton NMR T_2 relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) operating at 23.2 MHz and equipped with an 18 mm variable temperature probe. From each HPP-treated sample, 4 sub-samples (approx. 4 cm long and 1 cm in diameter) were taken, and NMR measurements were carried out on these at a temperature of 25°C. The obtained T_2 relaxation decays were analysed using distributed exponential fitting analysis.

Compression-analysis

Compression analyses were performed on sliceable samples, HPP treated at 600 MPa, 3.3 MPa/sec, 40°C, holding times 1 s, 2, 4 and 6 min and samples of heat-treated sausages on a Texture Analyzer TA-HD, 50 Kg load cell (Stable Micro Systems, Surrey, UK). For each HPP-treated sample, 3 sub-samples, 25 mm in height, 28 mm Ø were taken, and compression measurements were carried out at sample temperatures of 5°C with an Ø 35 mm cylindrical probe at test speed 1.0 mm/s, trigger force 0.05 N, compression depth 20 mm. From the compression curves the gel breaking force was determined, and the four holding times and the heat-treated control were statistically compared using analysis of variance and Students t-test

Subjective texture score

Texture of all the samples were subjectively judged by one assessor when cutting out samples for the T_2 relaxation measurements. The samples were given a score between 1 and 4 (1 = very soft; 4 = firm).

Data analysis

Distributed T_2 relaxation data was analysed using Principal Component Analysis (PCA) and Partial Least Square (PLS) regression models, using the Simca-P+ software (Umetrics AB, New Jersey, US), analysis of variance and Students t-test using the SAS 9.1.3 software (SAS Institute Inc, Cary, US). In PLS regressions the regression coefficient (R^2) and the root mean square error of the fit from the cross validated (RMSECV) observations in the model, is used for determining how good the fit is.

III. RESULTS AND DISCUSSION

Distributed T_2 relaxation data revealed the presence of three water populations in the raw, HPP-treated sausages with relaxation times of 2.6±1.0 ms (T_{2A}), 22.0±1.7 ms (T_{2B}), and 97.4±5.7 ms (T_{2C}), respectively, the latter being the dominant population with a relative area of 92.7%±1.9 (Figure 1).

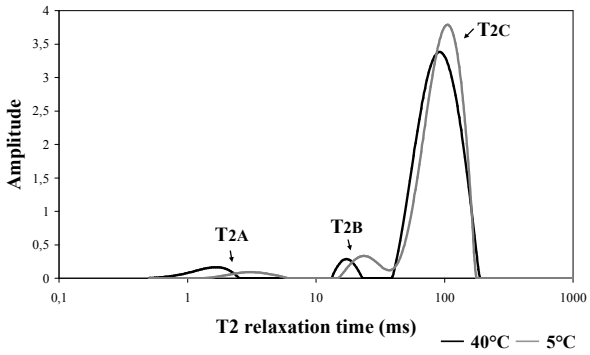

Figure 1: NMR distributed T_2 relaxation times obtained on representative raw HPP- treated samples, treated with rate of pressure increase 3.3 MPa/s, pressure level 500 MPa, holding time 1s, black line: 40°C and grey line: 5°C. Three populations of water that are designated T_{2A}, T_{2B} and T_{2C} are observed.

In order to investigate the magnitude of the different HP processing parameters, PCA was carried out on the distributed T_2 relaxation times, which revealed a clear separation between the two start temperatures (Figure 2), while no apparent effect of rate in pressure increase and holding time was seen (data not shown).

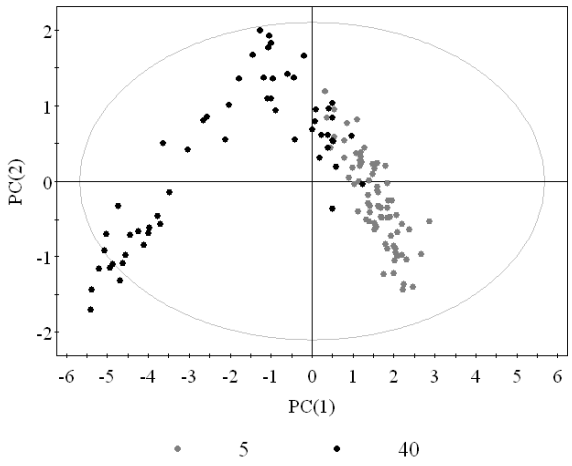

Figure 2: PCA score plot from NMR T_2 relaxation data obtained on raw samples of HPP-treated sausages. A clear separation of the samples according to start temperature is seen ● 5°C and ● 40°C, PC1 and PC2 explains 81% and 11% of the variation, respectively.

Consequently, the HPP parameter with most significant influence on water mobility and distribution was start temperature. When PCA analyses were carried out individually on each start temperature, clear tendencies for separation of samples according to pressure level was also observed, however, this was more profound for 40°C than for 5°C (data not shown). These findings illustrate that the start temperature as well as pressure level are important for the mobility and distribution of water upon HPP treatment.

In accordance with NMR data, texture score was significantly affected by start temperature ($P<0.001$) and pressure level ($P<0.01$). In addition, a significant effect of rate of pressure increase ($P<0.01$) was observed. Thus, a rate of pressure increase 3.3 MPa/sec, start temperature 40°C, and a higher pressure level of either 600 or 700 MPa resulted in significant higher texture scores (mean 3.0 – 3.4). An earlier study has revealed similar findings in respect to effect of pressure level, where higher pressure level of 400 MPa compared to 200 MPa, increased the measured penetration force and gel strength [5]. Additionally Fernandez-Martin et al. observed no effect of temperature in the range 10-40°C when working with holding times of 30 min [5]. Compared to start temperature and pressure level we observed that holding time ($P=0.06$), rate of pressure increase*holding time ($P=0.09$) and pressure level*holding ($P=0.05$) had less pronounced effect on texture, where a holding time of 1 s, resulted in a lower score compared to longer holding times. The correlation between NMR T_2 relaxation times and subjectively assessed texture was investigated, and PLS regression showed a significant correlation between the entire distribution of T_2 relaxation times and the texture score for all samples ($n = 128$: $R^2 = 0.61$, RMSECV = 0.57). Hence, water mobility and distribution is essential for the texture of the samples. Furthermore, analysis of correlation between the specific relaxation populations and texture revealed that the correlation between NMR T_2 relaxation data and texture mainly could be ascribed to the relaxation time of the main relaxation population, T_{2C} ($n = 128$: $R^2=0.57$, RMSECV=0.64). Thus, a lower T_{2C} relaxation time value reveals a tighter structure resulting in a more firm texture.

Gel breaking force was analyzed on all samples where the texture allowed it, and also on this parameter a strong effect of start temperature appeared, as it was not possible to measure gel breaking force on any samples with a start temperature of 5°C due to very limited slice ability of these samples. Gel breaking forces for the HPP-holding times 1 s, 2, 4 and 6 min are shown in Table 1.

Table 1: Gel breaking force of raw sausage samples (n=3) treated with HPP at 600 MPa, 40°C, pressure increase 3.3 MPa/s, for 1s, 2, 4, 6 min or treated by heat to a core temperature of 72°C.

Pressure holding time	Gel breaking force (N)
1 s	11 ±0.5[a]
120 s	17 ±2.3[b]
240 s	16 ±2.1[b]
360 s	30 ±0.9[c]
Heat treated control	77 ±5.1[d]

Means with different letters are statistically different ($P<0.05$)

The gel breaking force measurements indicate that increasing pressure holding times creates gels with increased strengths. This finding illustrates that HPP used as sole protein denaturing process is capable of creating gels, though not as strong as heat-set gels from the same emulsion. In addition, preliminary compression tests seem to correlate to the distributed T_2 relaxation data, even though the amount of data was limited ($n = 16$: $R^2 = 0.55$, RMSECV = 3.08), indicating that the gel breaking force, like subjective texture score, is dependent upon the water mobility and distribution.

IV. CONCLUSION

The study reveals that HPP treatment through protein denaturation causes changes in the macromolecules, which give rise to gel formation and changes in water mobility and distribution in the sausages. Additionally, the strength of the gel is determined by the water mobility and distribution that are highly affected by the microstructure of the product, where start temperature, rate of pressure increase, the pressure level and holding time is of importance. The study illustrates that it is possible to predict the texture of the HPP sausages using proton NMR T_2 relaxation, which is a rapid non-destructive method. Furthermore it seems that proton NMR T_2 relaxation data are related to gel breaking force, revealing that the water distribution is essential for the strength of the gel.

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