Effects of Pressurization on Structure, Water Distribution, and Sensory Attributes of Cured Ham: Can Pressurization Reduce the Crucial Sodium Content?

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This study investigated the replacement of tumbling (intermittent vacuum tumbling for 6 h) with pressure treatment (7 MPa for 4 s) in the production of a cured ham product with the aim of elucidating if the pressure treatment could reduce the amount of salt added to obtain a satisfactory product. Confocal laser scanning microscopy (CLSM) revealed a pressure-induced loosening of the meat structure, and proton nuclear magnetic resonance (NMR) relaxometry revealed that this structural modification of the meat had an impact on water properties and water distribution in both cooked and cooked/fried products. Three salt levels (0.6, 1.1, and 1.7% w/w) were investigated, and sensory profiling revealed that the pressured-cooked meat obtained a significantly higher juiciness score at low and medium salt levels. In addition, sensory profiling assessments revealed that at the lowest salt concentration the pressured product was perceived to be saltier compared with the tumbled product; however, the difference was not significant and was absent upon frying. In contrast, in sensory time intensity analysis the maximum intensity and the total salt taste were significantly higher in the pressured samples at the low and medium salt levels in the cooked samples. In conclusion, the present study showed strong evidence that the structure and biophysical characteristics of cured ham is altered by pressurization and suggested that pressurization may reduce the critical amount of salt required in a ham product.

KEYWORDS: NMR; T_2 relaxation; CLSM; confocal microscopy; meat quality; pressure treatment; high pressure; pork; salt; curing; tumbling

INTRODUCTION

In recent years the focus on sodium intake has increased dramatically. Animal and human studies have indicated a relationship between sodium intake and blood pressure (*1*, *2*), and high blood pressure is known to be a risk factor for the development of cardiovascular diseases (*3*, *4*). Accordingly, guidelines for sodium intake have been put forward (*5*), and the food industry is interested in developing new products with low sodium content to fulfill the dietary recommendations. A requirement for this is an understanding of the basic structure of the food items and the interactions with the sodium ions. Cured ham is a low-fat product; however, a drawback is that it contains a relatively high amount of sodium.

Several investigations on high-pressure treatment of meat exist (*6*), and major changes in meat texture as a result of pressuriza-

tion of meat are reported (*7–9*). Effects of pressurization on the denaturation of myosin and actin (*10*, *11*) and the characteristics of titin (*12*, *13*) have been demonstrated. Using NMR microimaging, alterations in water characteristics due to pressurization of meat have also been shown (*14*). Moreover, an early study reported the sensory properties of pressured meat to be quite different, especially with regard to juiciness (*15*). During the curing of meat the salt binds additional water and thereby affects the juiciness of the meat. However, as pressurization seems to affect the water characteristics and juiciness of the meat, pressurization could also be expected to affect the relationship between added sodium and how juicy cured meat is being perceived. The aim of the present study was to investigate the effects of a pressure treatment on meat structure, water distribution, and sensory attributes of a cured, fried ham product using confocal laser scanning microscopy (CLSM) and proton nuclear magnetic resonance (NMR) relaxometry to elucidate if the sodium content of a cured product can be reduced if the tumbling procedure is replaced by a pressure treatment.

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MATERIALS AND METHODS

Animals and Sampling Procedure. A total of 18 female slaughter pigs were included in the study. The pigs were chosen at random at the slaughter line with a hot carcass weight between 74 and 79 kg, an average meat content between 57 and 61% [Commission Regulation (EC) 3127/94], and pHu in both thighs between 5.50 and 5.60. The day after slaughter left and right m. semimembranosus were excised. The animals were randomly divided into three treatment groups with six animals in each: low (0.6%), medium (1.1%). and high (1.7%) salt concentration.

Processing. All muscles were cured using multineedle injection to a weight gain of approximately 10%. The pressure of the injection was 1.1 bar, and the speed was 85−90 strokes/min. The composition of the brines was optimized in preliminary investigations to give a final yield of 85% and consisted of 7, 14, and 20% (w/w) curing salt (0.6% nitrite in NaCl) resulting in a final salt concentration in the product of 0.6% (low), 1.1% (medium), and 1.7% (high), respectively. A salt level of 1.7% corresponds to the level in a standard commercial ham product, which was the rationale for the choice of salt levels. Subsequently, one muscle semimembranosus from each animal was pressured. Pressure treatment was carried out across the fiber direction using a manual mechanical pressurizer (in-house made), and when a pressure of 7 MPa was obtained, the pressure was kept for 4 s, resulting in a total treatment time of approximately 10−12 s. The other muscle semimembranosus from each animal was intermittently vacuum-tumbled for 6 h using a speed of 6 revolutions/min. Next the muscles were cooked at 78 °C to an internal temperature of 72 °C in a cooking cabinet (Danfotech, Aalborg, Denmark), and after cooling to room temperature, the muscles were shredded into strips with a length of 5−8 cm and a cross-sectional area of 1×1 cm. Half of the strips from each muscle were fried (Phillips Comfort Plus, Phillips, The Netherlands) in grapeseed oil (FoodLine, Denmark) at 175 °C for 1.5 min. Meat strips for sensory analysis were packed in 80% N_2/20% CO_2 using oxygen impermeable bags, whereas strips for chemical analyses, NMR, and CLSM measurements were vacuum-packed using oxygen impermeable bags (oxygen permeability < 3 cm^3/m^2/24 h/atm). All samples were stored at 4 °C until further analysis.

Chemical Analyses. The total water content of the cooked meat samples was determined according to a gravimetric analysis by drying the meat in an oven at 102−105 °C until a constant weight was reached. Salt content (Cl$^-$) was determined according to a potentiometric titration using an ABU90 autoburette (Radiometer, Copenhagen, Denmark) and a titration manager TI900 (Radiometer). The sample was extracted with hot, basic buffer solution. Proteins were precipitated, and the solution was filtrated. Nitric acid was added, and the content was determined by a potentiometric end point titration with silver nitrate (ABU 901 autoburette and TIM 900 titration manager, Radiometer).

NMR Measurements. Water distribution in a total of 96 cooked and fried meat samples was examined using ^{1}H NMR relaxometry. The NMR relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, U.K.) with a resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18 mm variable-temperature probe. The meat strips were cut and placed in cylindrical glass tubes for the NMR measurements and tempered in a 25 °C water bath for 15−20 min prior to measurement. Transverse relaxation, T_2, was measured using the Carr−Purcell−Meiboom−Gill sequence (CPMG). The T_2 measurements were performed with a τ value (time between 90° pulse and 180° pulse) of 150 μs. The 90° and 180° pulses were 8.2 and 16.4, respectively. The repetition time between two scans was 3 s. Data from 4096 echoes were acquired as 16 scan repetitions, with 1 dummy scan. Only data from even-numbered echoes were used in further data analysis to avoid the influence of imperfect pulse settings. The obtained T_2 relaxation decays were analyzed using distributed exponential fitting analysis (*16*) using in-house-made scripts in Matlab.

CLSM. Cooked and fried meat samples were examined with CLSM at two different salt concentrations: 0.6 and 1.7%. Thick transverse sections (slice thickness ~ 1 mm) were sliced free-hand with a scalpel from the cooked and the fried meat samples. The samples were placed in a 24-well microtiter frame and were covered with Alexa Fluor 488

phalloidin (0.22 μM) in PBS buffer (pH 7.2) for a minimum of 20 min. Subsequently, the samples were mounted on microscope glass slides and covered with coverslips. The samples were examined with a laser scanning confocal fluorescence microscope (Bio-Rad Radiance 2100, AGR-3Q AOTF, Hertfordshire, U.K.), attached to a Nikon Eclipse E800 upright microscope. The argon laser beam was set at 488 nm excitation and 500 nm long-pass emission filter. The images were acquired with $10\times$ (0.45 NA, Nikon Plan Apo) and $60\times$ (1.20 NA, WI Plan Apo, water immersion) objectives, and only samples with a proper transverse cut were examined further. Z-series of images of representative areas were collected, restored, and projected using the software LaserSharp2000 (Bio-Rad, Hermel Hempstead, U.K.), and the images were further processed with the software LaserPix 2000 (Bio-Rad, Hermel Hempstead, U.K.).

Sensory Analyses. A trained sensory panel consisting of nine assessors—one male and eight females—was used. All assessors came from the area around Roskilde and were familiar with pork assessments. Two sensory sessions were carried out, one on cooked samples and one on fried samples. The following attributes were assessed in a profiling analysis: salt taste, juiciness, and tenderness. The scale was a 15-point nonstructured line anchored at the extremes (0 = slight and 15 = intense). The profiling analysis was followed by a time intensity analysis assessing salt taste in 60 s on a 15 cm nonstructured line scale. The position was logged every 2 s. The assessors were instructed to chew at their own speed and to end the assessment when they found the sample to be ready for swallowing. The data were collected in FIZZ Biosystems (ver 2.10H, Counternon, France) and the following parameters calculated: maximal intensity (I_{max}), area under the increasing phase (area Inc), area under the plateau phase (area Pl), area under the decreasing phase (area Dec), and total area (area Tot).

Statistics. Principal component analysis (PCA) was performed on the obtained T_2 relaxation decays using the Unscrambler software vers. 9.2 (CAMO, Trondheim, Norway). PCA is a descriptive method, which allows the main variability aspects of a data set to be visualized without the constraint of an initial hypothesis concerning the relationship between samples and between samples and variables. The analysis aims at finding relationships between the different parameters (samples and variables) and displaying possible clusters within samples and/or variables. The data are arranged in a matrix, $X_{(n,m)}$. To find the main sources of data variability and the relationship between or within samples and variables, the data are decomposed into a few orthogonal principal components by a least-squares method. The decomposition is formulated by eq 1

$$X_{(n,m)} = T_{(n,k)}P_{(k,m)}{}^{T} + E_{(n,m)} \tag{1}$$

where T is the sample-score matrix, P is the variable-loading matrix, E is the error or residual matrix, n is the number of samples, m is the number of variables, and k is the number of principal components.

Statistics were performed using the SAS system 9.1 (SAS Institute, Cary, NC). To analyze the statistical differences between treatments, an analysis of variance model was used (PROC Mixed) including the fixed effect of treatment (tumbling versus pressurization) and salt level (low, medium, high) and the interaction between these and for the chemical data animal as random effect. Moreover, assessor and the interactions between assessor and salt level, assessor, and treatment and the three-way interaction between these were included as random effects.

RESULTS

Cooking Loss and Salt and Water Contents. Cooking loss for the different combinations of salt and treatment is given in **Table 1**. Cooking loss decreased with increasing salt concentration and was significantly higher in pressured samples compared with tumbled samples. **Tables 2** and **3** show the NaCl and water contents in the cooked samples. At the low and medium salt levels an identical salt concentration was obtained in pressured and tumbled samples, whereas at the high salt concentration the salt level was significantly lower in pressured samples

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Table 1. Cooking Loss (Percent) as a Function of Salt Level[a]

	low	medium	high	SE
tumbling	23.0a	18.3c	13.0d	0.5
pressurization	24.3b	22.2b	19.7c	0.5

[a] Different letters indicate significant differences ($P < 0.05$).

Table 2. NaCl Content (Percent w/w) in the Cooked Samples as a Function of Salt Level[a]

	low	medium	high	SE
tumbling	0.60a	1.14b	1.89c	0.04
pressurization	0.62a	1.07b	1.66d	0.04

[a] Different letters indicate significant differences ($P < 0.05$).

Table 3. Water Content (Percent w/w) in the Cooked Samples as a Function of Salt Level[a]

|	low	medium	high	SE
tumbling	69.2b	69.7b	71.08c	0.3
pressurization	67.8a	68.0a	69.3b	0.3

[a] Different letters indicate significant differences ($P < 0.05$).

compared with tumbled samples. The water content was generally lower in pressured samples compared with tumbled samples, which can be ascribed to a higher cooking loss in pressured samples.

^{1}H NMR Measurements. Figure 1 displays PCA score plots for low, medium, and high salt concentrations (panels **A**, **B**, and **C**, respectively). Independent of salt concentration, the samples are strongly discriminated into cooked and fried samples in the score plot, whereas a grouping according to tumbling and pressurization also is observed. **Figure 2** shows distributed T_2 relaxation times for the tumbled and pressured samples after only cooking and after cooking and frying in samples with low and high salt concentrations (panels **A** and **B**, respectively). In the cooked meat a pronounced difference is observed in the water distribution between tumbled and pressured meat as pressured meat is characterized by a much broader distribution of relaxation times compared with tumbled meat. Frying causes a shift toward lower relaxation times, and fried samples are characterized by narrower populations; the difference in water distribution between tumbled and pressured meat is less evident upon frying.
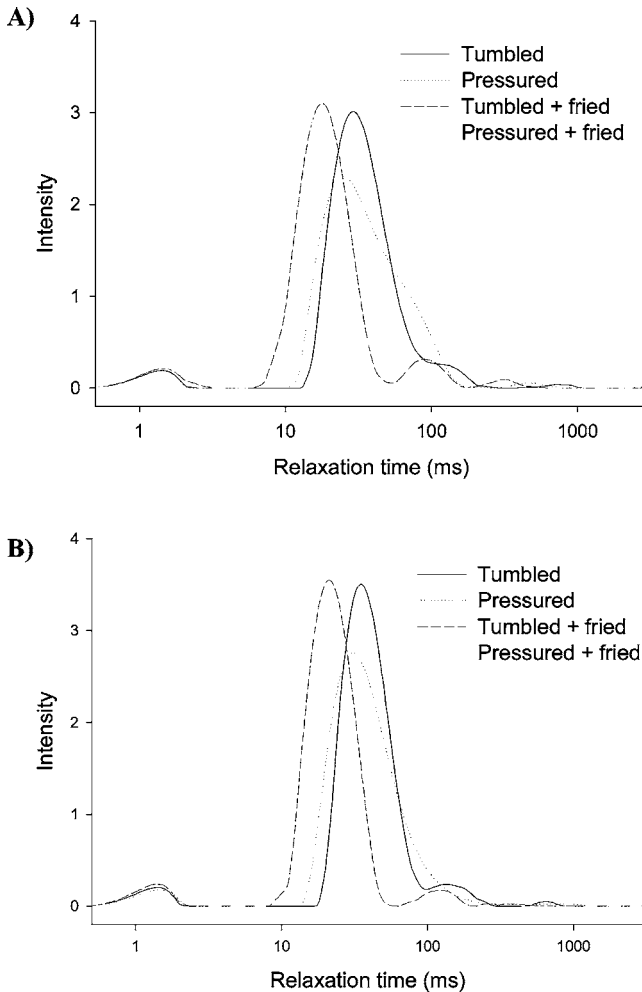
CLSM Examinations. CLSM images of cooked tumbled and pressured meat with low salt concentration are displayed in **Figure 3**. The CLSM images reveal a much looser and more open meat structure in pressured meat compared with tumbled meat, causing the distance between adjacent fibers to be bigger in pressured than in tumbled meat, and this effect was observed for all salt levels (data not shown). The looser structure in pressured meat was also evident by a better penetration of the phalloidin dye, causing a greater staining intensity of this meat compared with tumbled meat.

Sensory Analyses. Figure 4 shows the assessed salt taste in the meat as a function of salt concentration. In cooked meat and at the lowest salt concentration the pressured meat attained a higher score in salt taste than the tumbled meat; however, the difference was not statistically significant (**Figure 4A**). At the medium salt concentration the two treatment groups attained identical scores for salt taste, whereas for the highest salt concentration the pressured meat was characterized by a lower salt taste score, which probably can be ascribed to the lower
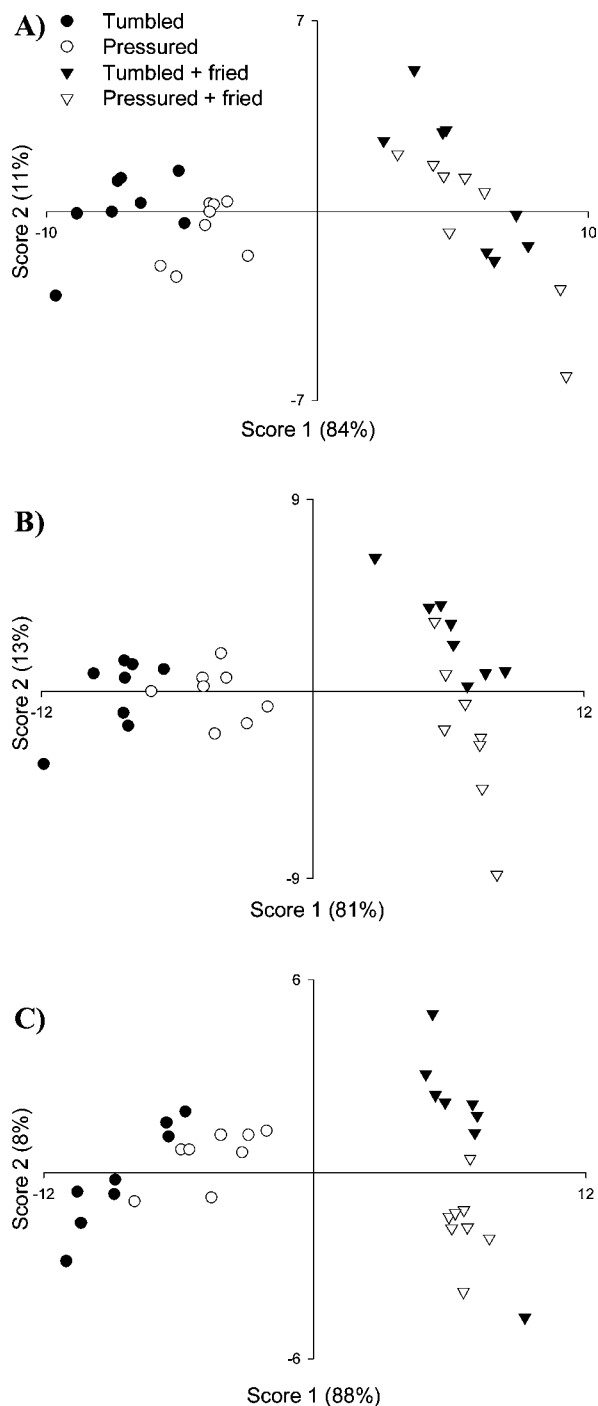

Figure 1. PCA score plot showing the two first principal components for (**A**) low-salt samples, (**B**) medium-salt samples, and (**C**) high-salt samples.

salt concentration for this group compared with tumbled meat at same high salt concentration (**Table 2**). In the fried meat a pattern equivalent to that for cooked meat was observed (**Figure 4B**). In cooked meat the salt taste increased significantly with increasing salt concentration, and an increase in salt taste with increasing salt concentration was also observed in the fried meat; however, the difference in salt taste between low and medium salt concentrations was not statistically significant.

Figure 5 shows the assessed juiciness in the meat as a function of salt concentration. For cooked meat at low and medium salt concentrations the pressured samples attained a significantly higher juiciness score compared with the tumbled samples (**Figure 5A**). In the fried meat no significant difference in juiciness was observed between the two treatments at the

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Figure 2. Average distributions of T_2 relaxation times for the four combinations of tumbling, pressurization, cooking, and frying for (**A**) low-salt samples and (**B**) high-salt samples.
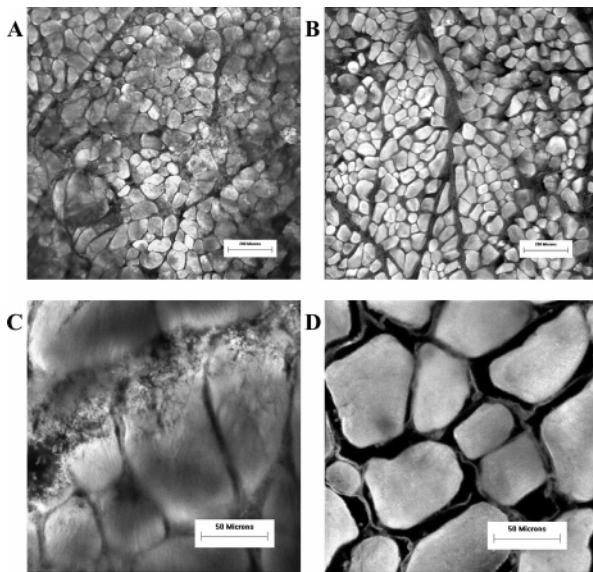
Figure 3. CLSM images of transverse sections of cooked ham (**A**) tumbled meat, 10× magnification; (**B**) pressured meat, 10× magnification; (**C**) tumbled meat, 60× magnification; and (**D**) pressured meat, 60× magnification.

low and medium salt concentrations; however, at the highest salt concentration the tumbled meat attained a significantly higher juiciness score compared with pressured meat (**Figure**
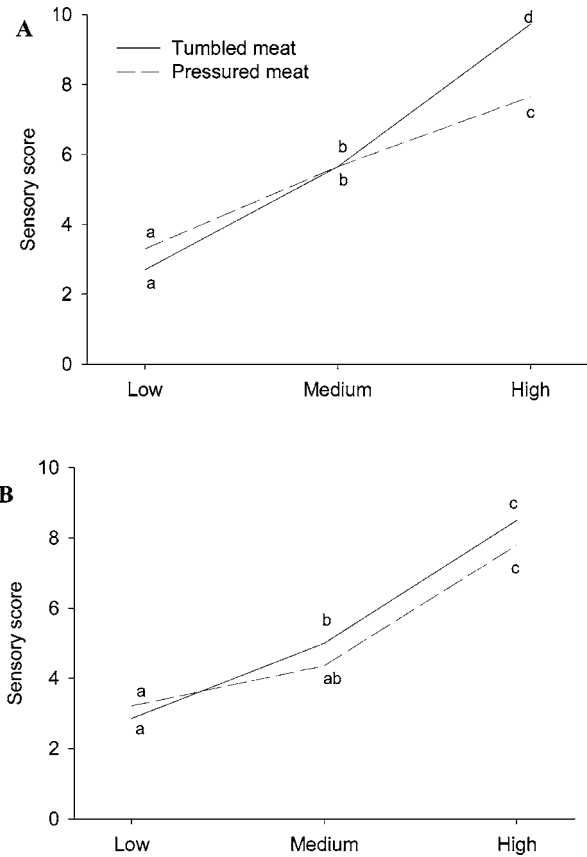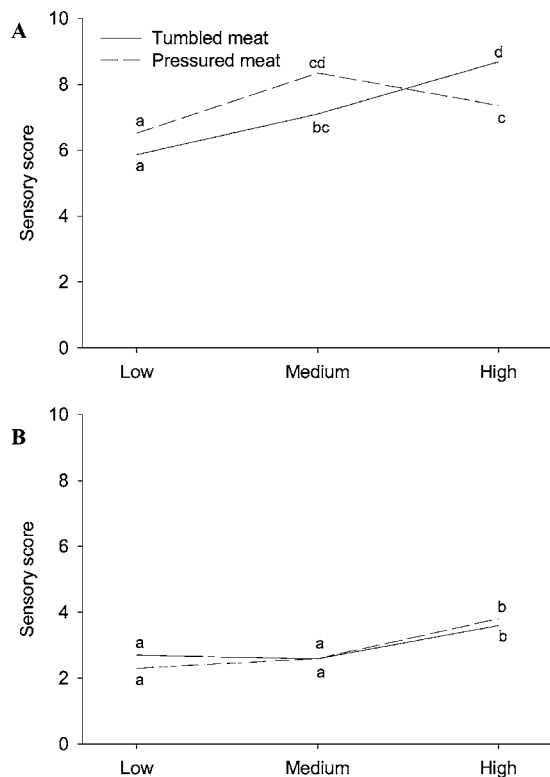

Figure 4. Salt taste as a function of salt level as assessed by the sensory panel: (**A**) cooked product; (**B**) cooked and fried product. Letters a–d indicate significant differences.

5B). **Figure 6** shows the assessed tenderness in the meat as a function of salt concentration. Tumbled meat attained a significantly higher tenderness score compared with pressured meat; however, in the fried meat an interaction between treatment and salt concentration was observed.

Table 4 shows the time intensity parameters. As can be seen, the I_{max} is assessed higher in the pressured meat compared with the tumbled meat. The total area under the curve expresses the total salt experience during chewing. There was a tendency to a significant interaction between treatment and salt level in the cooked samples ($P = 0.08$) as the area was higher in the pressured meat for the low and medium salt levels, but not at the high salt level. As for the sensory attributes, the differences were more pronounced in the cooked samples compared with the fried samples.

DISCUSSION

Sodium intake is gathering increasing attention, and there is an increasing demand for the food industry to develop low-sodium products. Pressure treatment has been put forward to be the most important emerging technology with applications relevant to meat processing (*17*), and several investigations on the impact of various pressure applications on the structure and biophysical properties of muscle foods have been reported (*7– 13*). However, in the present study it was for the first time investigated if pressurization of a ham product potentially can reduce the need for sodium in the product. Three salt levels were investigated; the highest concentration corresponds to the level in a traditional commercial ham product. Low-field ^{1}H NMR relaxometry revealed pronounced effects of pressurization on the T_2 relaxation time in the cooked and fried product

Figure 5. Juiciness as a function of salt level as assessed by the sensory panel: (**A**) cooked product; (**B**) cooked and fried product. Letters a–d indicate significant differences.
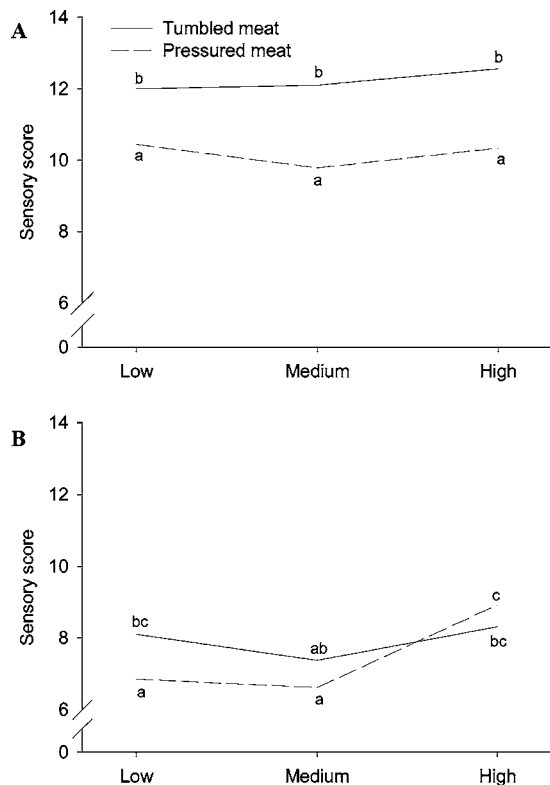
Figure 6. Tenderness as a function of salt level as assessed by the sensory panel: (**A**) cooked product; (**B**) cooked and fried product. Letters a–d indicate significant differences.

(**Figures 1** and **2**), which implies altered water distribution upon pressurization. Upon pressurization the T_2 relaxation times were characterized by much broader distributions, which probably can be ascribed to a larger spatial variation in protein structures

Table 4. Time Intensity Parameters in the Cooked and Fried Samples as a Function of Treatment and Salt Level[a]

	tumbled			pressure		
	low	medium	high	low	medium	high
cooked						
I_{max}	3.8d	6.3c	11.9a	5.3c	8.3b	11.5a
area Inc	39.9c	66.8c	126.7a	47.3c	86.5b	136.7a
area Pl	11.0c	18.3bc	67.4a	16.2bc	41.9abc	51.9ab
area Dec	39.4b	79.2b	259.9a	55.4b	109.5b	235.3a
area Tot	89.9c	167.3c	454.3a	117.8c	242.1b	430.5a
fried						
I_{max}	3.0d	6.0c	11.6a	4.2c	5.7c	9.9b
area Inc	31.0b	58.0b	122.1a	36.8b	52.1b	114.5a
area Pl	6.2c	29.3bc	81.8a	11.7bc	22.0bc	59.4b
area Dec	25.9b	62.4b	202.1a	37.6b	56.8b	143.5a
area Tot	62.6c	159.5c	422.1a	86.1c	133.0c	317.9b

[a] Different letters indicate significant differences ($P < 0.05$).

(*18*). A previous MRI study investigating the effect of high pressurization (300 MPa) of beef likewise observed effects on the T_2 relaxation times and thereby water properties in the meat (*14*). Accordingly, evidence exists that pressurization of meat changes the interactions between the muscle proteins and water molecules. In the present study high-resolution CLSM images were also obtained on meat samples using minimal sample preparation and no prior fixation, thereby minimizing the introduction of artifacts. The CLSM examinations revealed that this can be ascribed to the fact that pressurization causes a loosening and opening of the meat structure (**Figure 3**), which has also been observed in an earlier study using immunoelectron microscopy (*13*). Hence, the CLSM results comply with the observed broadening of the T_2 distribution in this meat, which in association with the Brownstein−Tarr theory (*19*) would correspond to a wider range of distances between water molecules and protein protons. Consequently, the NMR and CLSM examinations revealed that pressurization causes a loosening of the meat protein matrix with concomitant changes of the interactions between muscle proteins and water molecules and thereby alterations in the water distribution in the meat. To investigate if this structural modification also affects the properties of added salt and the general sensory attributes of the meat product, sensory assessments were also included in the present study. Three salt levels were investigated, and in cooked meat at the lowest salt concentration the pressured meat was actually perceived to be saltier than the corresponding tumbled meat; nevertheless, the difference was not statistically different in the sensory profile (**Figure 4**). In contrast, in the time intensity analysis, which had a special focus on the salt taste, the maximum intensity and the total area were significantly higher in the pressured samples at the low and medium salt levels in the cooked samples (**Table 4**). In addition, if the cooked meat was subsequently fried, pressurization could not enhance the salt taste. However, noteworthy for cooked samples the pressurized meat was perceived to be significantly juicier compared with the tumbled meat at low and medium salt concentrations (**Figure 5**). This positive effect of pressurization on juiciness is in agreement with an earlier investigation (*13*) and reveals that the pressure-induced alteration in meat structure and water distribution is affecting how juicy the meat is being perceived during consumption. The tenderness of the meat products was also affected, as pressured meat was perceived to be significantly less tender than tumbled meat. Sensory studies on sausages and fish fillets pressed at 500 MPa have reported improved texture of the pressured products compared with the control products (*20, 21*). In contrast, at lower pressure levels

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studies have reported a lack of tenderizing effects of pressurization in beef (*8, 22*), which is consistent with the findings in the present study.

In conclusion, the present study demonstrated that pressurization of meat alters meat structure and water distribution in cooked ham. The pressure treatment improved the juiciness of low- and medium-salted products when compared with tumbled products. However, even though the pressured ham product at a low salt level was perceived to be saltier than the corresponding tumbled product, no significant change in salt taste in a sensory profiling was obtained using pressurization. In contrast, in sensory time intensity analysis the maximum intensity and the total salt taste were significantly higher in the pressured samples at the low and medium salt levels in the cooked samples. Therefore, it remains uncertain if the used pressurization technique enables a reduction in the critical amount of salt required in a ham product, and supplementary studies are needed. In addition, studies attempting to find other alternatives for sodium reduction and/or replacement in ham products could be of great interest.

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