### The effects of early post-mortem pH and ultimate pH on level and amount of destructured zones in cooked cured hams

Gabriel Hugenschmidt a,b, Ruedi Hadorn b,d, Martin R.L. Scheeder c, Paolo Silacci b, Daniel Scherrer b, Caspar Wenk a

a Institute of Animal Science, Swiss Federal Institute of Technology Zurich, 8092 Zurich, Switzerland

b Agroscope Liebefeld-Posieux Research Station ALP, 3003 Berne-Liebefeld, Switzerland

c Swiss College of Agriculture, 3052 Zollikofen, Switzerland and SUISAG, 6204 Sempach, Switzerland

d Swiss Meat Association, 8032 Zurich, Switzerland

### 1. Introduction

Destructured zones in cooked cured ham have been reported to be a crucial problem for meat processors during the last decades. Balac, Bazin and Le Treut (1998) described the defect as "uncoloured", soft, and exudative zones right inside the raw ham, which became unsuitable for mechanical slicing after cooking. They reported that the defect appeared in 20-50% of the examined fresh hams and that it was positively related to a low pH at 30 min, 180 min and 24 h post-mortem (p.m.). Furthermore, a high temperature 30 and 180 min p.m. in the deep center of the affected hams and a large variation between slaughter series were observed. These findings were supported and extended by numerous studies focusing mainly on ultimate pH and early post-mortem pH fall as main causative factors. In the raw meat, the defect in these later studies appeared in 5-20% of the hams (Franck et al., 1999; Minvielle, Boulard, Vautier, & Houix, 2003; Minvielle, Houix, & Lebret, 2001), that may indicate a decreasing incidence of the defect during the last years. Mostly the defect was present in the Musculi (Mi.) adductor (AD), semimembranosus (SM), and biceps femoris (BF) (Laville et al., 2003). The presence of the n allele of the Hal-gene and the RN\_-allele of the RN-gene exacerbate the defect (Franck, Monin, & Legault, 2000; Le Roy et al., 2001). The appearance of destructured zones was negatively correlated with a high ultimate pH value (Aubry, Ligonesche, Guéblez, & Gaudré, 2000; Minvielle et al., 2003; Vautier, Minvielle, Boulard, Bouyssière, & Houix, 2004) and positively correlated with a high proportion of lean meat as well as a high slaughter weight of the animals (Bouffaud et al., 2002; Franck et al., 1999, 2000; Franck, Monin, Figwer, Poirel, & Legault, 2002; Minvielle et al., 2001; Vautier, Boulard, Bouyssière, Houix, & Minvielle, 2008). Chemical analyses conducted by Minvielle et al. (2001) showed that dry matter, protein and total collagen content of the raw meat were not affected by the defect, whereas glycolytic potential clearly was (P < 0.01) and thermosoluble collagen content was slightly (P < 0.05) higher in destructured zones. Furthermore, Voutila, Ruusunen and Puolanne (2008), studying the onset and the peak temperature of thermal shrinkage of intramuscular connective tissue as well as its content and solubility in M. semimembranosus, did not find a difference between normal and destructured zones. Concentrating on the biochemical properties of the defect, Laville et al. (2005) performed histochemical and electrophoretic techniques. They concluded that PSE-meat and destructured zones in raw meat show several similarities in their protein characteristics. Both defects seemed to result from an abnormally fast post-mortem glycolysis. Only little research has been done so far on destructured zones in the final product, the cooked cured ham itself, in which it was actually first discovered. A survey was conducted in seven different Swiss meat processing plants and observed that destructured zones caused up to a third of the losses in cooked cured ham production during slicing. The defect appeared in 7-8% of the slices and resulted in significant economic losses (Hugenschmidt, Hadorn, Suter, Scheeder, & Wenk, 2007). Thereby, a wide variance of the defect was observed between the different meat processing plants indicating that the processing of the raw meat to cooked ham also influences the emergence of

the defect. From Italy 1 to 2% of cooked cured hams are reported to be affected with destructured zones (Pedrielli, 2009) indicating that the frequencies of this phenomena vary among European countries. A subsequent study from Hugenschmidt et al. (2009) revealed destructured zones to be characterised by a higher dry matter and crude protein content as well as a lower crude ash, sugar and connective tissue content than normal cooked cured hams. Further attributes of the defect were a bright colour and a high myofibril fragmentation index. Even though several studies of the phenomenon of destructured meat have been performed and several influencing factors were identified, only few studies concentrated on the extent to which these factors account for destructured zones in cooked cured hams. Therefore the effect of the pH value as the most discussed factor for destructured zones in raw hams was examined in our study. In experiment 1, the influence of raw hams from different levels of early postmortem pH fall induced by electrical stimulation and different cooling methods on the frequency and the level of destructured zones in cooked cured hams was examined. In experiment 2, the effect of different levels of ultimate pH values in raw hams on the frequency and the level of destructured zones in cooked cured hams was investigated. Additionally, chemical analyses of cooked cured hams were performed in order to seek for characteristics of the defect, which may not be obvious in raw meat.

## 2. Materials and methods

### 2.1. Animals, treatments, and slaughtering procedure

In experiment 1, 40 Large White pigs (20 gilts and 20 barrows) from 14 litters were fattened and slaughtered at Agroscope Liebefeld- Posieux Research Station ALP. After weaning between day 39 and 49 of age [13.07 ± 2.74 kg live weight (LW)], the piglets were reared in groups of 8-12 animals in an environmentally controlled shed (22 \_C, 60-70% relative humidity). The corresponding pens were equipped with individual transponders (Allflex RS 320 Series, Hauptner, Dietlikon, Schweiz) determining feed consumption from an automatic feeding system (MLP, Schauer Agrotronic AG, Sursee, Switzerland). The animals were fed restrictedly (calculated weekly from the individual LW) according to the recommendations of Agroscope Liebefeld-Posieux Research Station ALP (2004) with a standard starter (9-25 kg of LW), grower (25-65 kg LW), and finisher diet (65-105 kg of LW) until slaughter. On the day of slaughter after a fasting period of 16 h, the animals were moved to the abattoir. Slaughtering occurred in two batches with 10 gilts followed by 10 barrows each, using electrical stunning (310 V, 2 A, 5 s). Every second animal was treated by electrical stimulation (ES: 50 V, 14 Hz, 2 x 90 s) 2 min p.m. with electrodes being placed at the trunk and the right tarsal for 90 s in the first and at the left tarsal and the trunk in the second cycle. The left halves of all carcasses were stored at 2 C from 30 min p.m. on (conventional cooling, CC), whereas the right halves of all carcass were kept at 22 \_C until 120 min p.m. before being stored at 2 \_C (delayed cooling, DC). In both cooling treatments, carcasses were kept at 2 \_C until production of cooked cured hams began. Thus, four treatments were compared in a 2 \_ 2-factorial design with the two factors electrical stimulation and cooling as follows: treatment 1: electrical stimulation with conventional cooling (ES CC); treatment 2: electrical stimulation with delayed cooling (ES DC); treatment 3: no electrical stimulation with conventional cooling (NES \_ CC); treatment 4: no electrical stimulation with delayed cooling (NES \_ DC). In experiment 2, two batches of 800 (batch 1) and 1000 (batch 2) topsides (Mi. semimembranosus and adductor), originating from an industrial meat processing plant, were classified in two batches into three groups according to the ultimate pH (24 h p.m.) of SM: LpH group (pH < 5.5), M-pH group (pH 5.5–5.7), and H-pH group (pH > 5.7). After this classification, 24 topsides of the L-and M-pH group and 16 topsides of the H-pH group

in batch 1 and in batch 2 again 24 topsides of the L-and M-pH group and 32 topsides of the H-pH group were selected for further ham processing by remeasuring their ultimate pH.

### 2.2. Production of cooked cured hams

In experiment 1, cooked cured ham production started 96 h p.m. using topsides [Mi. semimembranosus (SM) and adductor (AD)] and silversides [B. femoris (BF)] as well as parts of Mi. semitendinosus and gracilis. Connective tissue, excess fat, bone and rind were removed from the muscles and weight of silversides and topsides recorded. Injection of  $12 \pm 1\%$  brine (67) g curing agent [scheid-salpökin-P: phosphates, citrate, ascorbate, dextrins, lactose, dextrose, spices], Scheid-Rusal AG, Gisikon, Switzerland), 170 g nitrite curing salt, 763 g water) was performed by the use of an automatic multi-needle injector (PR 20, Rühle GmbH, Grafenhausen, Germany). Muscles of the same hams were labelled using different combinations of coloured bands. Mechanical treatment was performed simultaneously for electrical stimulated animals and controls in two separate tumblers (Typ 250, Rewi Maschinenfabrik GmbH, Rothrist, Switzerland) in 34 interval cycles (26 min rotation and 4 min rest per cycle, 99% vacuum, 2 \_C, 3.0 km). After tumbling, silversides were placed above the corresponding topsides in the front part of standard aluminium molds for production of cooked cured hams, which are customary in the industry. The back part of the molds was filled with parts of Mi. gracilis and semitendinosus. The final weight of the cooked cured hams was aimed at 4.0 kg. Cooking was effected in a delta T process up to a core temperature of 62 \_C and a cabinet temperature of 74 C, followed by increasing the core temperature to 69 C at 74 C cabinet temperature. Finally, 69 \_C core temperature was held for 30 min leading to a cooking yield of  $92 \pm 2\%$ . The cooked cured hams were cooled using cold water for 2.5 h; then they were kept in a refrigerator at 2 \_C until slicing. In experiment 2, cooked cured hams were produced with the selected topsides of each pH-group as in experiment 1, except the mechanical treatment for which a different type of tumbler (MKR 160, Rühle GmbH, Grafenhausen, Germany) with the same program as in experiment 1 was used. The tumbling process was performed in a separate tumbler for each pH-group simultaneously. Filling the molds was effected using two identified topsides one upon the other. The rest of the mold was filled with parts of SM of the same pH group.

# 2.3. Slicing and quantification of destructured zones in cooked cured hams

Slicing was performed in an industrial meat processing plant with a standard slicer (250 slices per minute, 1.25 mm thickness) 14 days after cooking. Destructured zones in silversides and/or topsides of the cooked cured hams were classified in 1st, 2nd, and 3rd level according to Hugenschmidt et al. (2007), cut out from the affected slices, weighed and indicated as g/kg raw muscle because individual muscle weights could not be determined in the cooked cured hams being placed in standard molds. During slicing, the evaluation of the destructurations in the final product was performed per mold and restricted to that part of the cooked cured hams, in which the tested muscles (topsides and/or silversides, respectively) could be clearly recognised visually.

### 2.4. Meat quality measurements

In experiment 1, temperature and pH were recorded in SM and BF 1, 3 and 24 h p.m. at 4 cm depth with four pH meters (826 pH mobile, Metrohm, Switzerland; WTW pH 197 and WTW pH

340, Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany: Portamess 911 pH, Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany). For technical measurement reasons, another pH meter was used for each of the four treatments (ES \_ CC; ES DC: NES CC: NES DC), but all of the pH meters were equipped with the same type of glass spearhead electrode (Metrohm, 6.0226.100) combined with a Pt 1000 temperature probe (Metrohm/Knick/WTW) and calibrated with the same standard solutions of pH 4.01 and 7.00 at 20 C. Additionally, their accordance was tested with meat samples at the beginning and regularly during the measuring period. In experiment 2, pH was determined after 24 h p.m. in SM as well as 72 h p.m. in SM and AD. Temperature and pH 24 h p.m. were measured using three pH meters (826 pH mobile, Metrohm, Switzerland; WTW pH 197 and WTW pH 340, Wissenschaftlich- Technische Werkstätten GmbH, Weilheim, Germany) for a preselection. Topsides for cooked cured ham production according to ultimate pH were finally selected using a 4th pH meter (Portamess 911 pH, Knick Elektronische Messgeräte GmbH & Co. KG, Berlin, Germany). The level of destructuration in raw muscles was determined in experiment 2 according to the IFIP quotation scale (IFIP, 2005) 72 h p.m. and meat colour measured on the surface of SM using a Chromameter (CR 300, Minolta, KONICA Minolta Sensing, Inc., Osaka, Japan).

#### 2.5. Chemical analyses of cooked cured hams

At least 60 g of destructured or normal cooked cured ham were needed in order to perform all chemical analyses. After homogenisation (Vertec; Edmund Bühler GmbH, Hechingen, Germany) samples were freeze dried (Christ - Delta 1-24 LSC, Martin Christ, Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and stored at 5 \_C until further analysis. Crude ash content (550 \_C until constant weight) and dry matter (105 \_C, 160 min) were determined gravimetrically (Leco TGA-601, Leco Corporation, MI-St Joseph, USA). Crude fat was measured gravimetrically after an automatic extraction (Soxtec Avanti 2050, Gerber Instruments AG, Effretikon, Switzerland) with petroleum ether, distillation of the solvent and drying of the fatty extract (SLMB, 1999). Crude protein content was analysed after the procedure of Kjeldahl (factor: 6.25) using an auto sampler system (Kjeltec 2400/2460, Gerber Instruments AG, Effretikon, Switzerland) and digestor (Foss Digestor Auto 20, Gerber Instruments AG, Effretikon, Switzerland). Total and insoluble connective tissue content were determined following the method of Arneth and Hamm (1971). As a slight change of this method, the extraction of soluble collagen was performed at 65 C for 60 min instead of 90 C for 120 min, in order to determine the already solubilised collagen without forcing further solubilisation. Total and insoluble collagen were calculated from the hydroxyproline content (factor: 8). Concentration of connective-tissue-free muscle protein resulted from the difference between crude protein and connective tissue protein. The myofibrillar fragmentation index (MFI) was determined according to the method of Culler, Parrish, Smith and Cross (1978). Apart from the dry matter content, which was indicated as g/kg fresh matter, and the myofibrilar fragmentation index, all laboratory results were expressed as g/kg dry matter.

### 2.6. Statistical analyses

In order to test equality of populations among independent groups, the Kruskal–Wallis test was applied. If the null hypothesis of equal population was rejected, multiple pairwise comparisons (without correction for multiple testing) were performed by Mann–Whitney U-tests. In tables average means of the parameters measured followed by the accordant Kruskal–Wallis P-values are indicated. For binary logistic regressions, destructured zones of 1st, 2nd and 3rd level were weighted by the factors 1, 10 and 100, respectively, and summed up resulting in a new variable,

which was transformed into a binary variable separating at its 80th percentile. A stepwise forward/backward elimination of variables was performed, before the final model was calculated using the variables with the lowest "probability to remove" values. For experiment 1, the pH value and temperature 1 h p.m., 3 h p.m., bodyweight and gender, and for experiment 2, pH 24 h p.m., pH 72 h p.m., L\*- , a\*-, b\*-value in SM and pH 72 h p.m., L\*-, a\*-, b\*-value in AD as well as the level of destructuration in raw hams were used in the stepwise model. In the logistic model for the chemical analyses total and insoluble connective tissue, crude ash, crude fat, dry matter and connective-tissue-free muscle protein entered in the stepwise model. However, due to labelling problems in experiment 2 (several coloured ribbons adhered to the paddles of the tumbler, ruptured and could not be used for identification of the corresponding topsides anymore) identifying individual hams without having an influence of assigning them to the accordant treatments only a reduced model containing 50 of the totally 144 topsides could be included in the calculations of binary logistic regressions. P-values lower than 0.05 were considered as significant in the present study. All statistical analyses were performed by Systat (Version 12, Systat Software Inc., San Jose, California, USA).

## 3. Results

# 3.1. Effect of early post-mortem pH on level and amount of destructurations in cooked cured hams (experiment 1)

As intended, electrical stimulation accelerated the pH-decline (Table 1). In topsides as well as in silversides of both batches, pH values 1 h p.m. were significantly lower in electrically stimulated carcasses (ES) than in controls (NES). After 3 h p.m. topsides in batch 2 and silversides in electrically stimulated carcasses of both batches still showed significantly lower pH values, whereas after 24 h p.m. no difference in pH was observed between treatments anymore. The temperature measured 3 h p.m. in topsides of the carcass halves cooled 30 min p.m. (CC) and 120 min p.m. (DC) differed significantly in batch 1 and 2, whereas the temperature measured 1 h p.m. as well as 24 h p.m. was not affected by the different cooling procedures (Table 1). In silversides already 1 h p.m. temperature differed significantly between carcass halves cooled 30 min p.m. and 120 min p.m. The temperature measured 3 h p.m. was again significantly lower in carcass halves cooled 30 min p.m. than 120 min p.m. apart from the non-stimulated carcass halves batch 1. After 24 h p.m., no significant differences could be observed in the temperature of raw ham comparing the two cooling procedures. In both batches of cooked cured hams. destructurations of all three levels appeared in silversides as well as in topsides (Table 2). However, topsides showed a much higher susceptibility for the defect resulting in a higher amount of destructured zones than silversides. Between the four different treatments of the carcass halves, only small differences could be observed. In batch 2, the amount of 1st level destructurations in silversides was significantly higher for treatment 3 (NES CC) than for the other ones. For all the other levels of destructuration in both batches and both muscle types, the amount of the defect did not differ significantly between the different treatments of the carcass halves. The binary logistic regression (Table 3) for topsides indicated temperature 1 h p.m. (P = 0.054) as the most important predictor for the defect followed by the gender barrow (P = 0.118), whereas in silversides the temperature at 60 min p.m. (P = 0.067) and at 180 min p.m. (P =0.096) ranked first.

Muscle	Attribute	Batch 1					Batch 2				
		$ES \times CC$ ( $n = 10$ )	$ES \times DC$ (n = 10)	$NES \times CC$ (n = 10)	$NES \times DC$ (n = 10)	P-value	$ES \times CC$ ( $n = 10$ )	$ES \times DC$ (n = 10)	$NES \times CC$ (n = 10)	$NES \times DC$ (n = 10)	P-value
Topside	pH 1 h p.m. [-] pH 3 h p.m. [-]	5.68° 5.49	5.65 <sup>a</sup> 5.54	6.21 <sup>b</sup> 5.65	6.10 <sup>b</sup> 5.71	0.002 0.079	5.63 <sup>a</sup> 5.48 <sup>a</sup>	5.59 <sup>a</sup> 5.47 <sup>a</sup>	6.18 <sup>b</sup> 5.76 <sup>b</sup>	6.19 <sup>b</sup> 5.65 <sup>a,b</sup>	0.002 0.013
	pH 24 h p.m. [-]	5.57	5.50	5.55	5.52	0.368	5.58	5.57	5.65	5.62	0.450
	T1h p.m. [℃]	38.36	38.99	37.79	39.10	0.620	38.01	38.47	37.90	38.18	0.570
	T 3 h p.m. [°C]	30.43ª	36.39 <sup>b</sup>	31.39ª	36.28 <sup>b</sup>	< 0.001	30.78ª	34.50 <sup>b</sup>	30.83*	35.12 <sup>b</sup>	< 0.001
	T 24 h p.m. [°C]	5.43	5.25	5.68	5.56	0.088	4.87	3.48	5.23	2.75	0.615
Silverside	pH 1 h p.m. [-]	5.54ª	5.60 <sup>a</sup>	6.23 <sup>b</sup>	6.32 <sup>b</sup>	< 0.001	5.51ª	5.56ª	6.07 <sup>b</sup>	6.22 <sup>b</sup>	< 0.001
	pH 3 h p.m. [-]	5.46 <sup>a</sup>	5.50 <sup>a</sup>	5.78 <sup>b</sup>	5.78 <sup>b</sup>	< 0.001	5.59 <sup>a,b</sup>	5.48ª	5.81 <sup>b</sup>	5.70 <sup>b</sup>	0.012
	pH 24 h p.m. [-]	5.59	5.60	5.55	5.65	0.396	5.63	5.55	5.67	5.56	0.059
	T 1 h p.m. [°C]	39.74ª	39.89ª	38.79 <sup>b</sup>	38.24 <sup>b</sup>	0.020	38.24ª	39.94°	38.62 <sup>a,b</sup>	39.33 <sup>b,c</sup>	0.006
	T 3 h p.m. [°C]	32.62ª	35.72 <sup>b</sup>	32.44ª	34.77 <sup>a,b</sup>	0.009	31.02 <sup>a</sup>	35.50 <sup>b</sup>	31.91*	35.18 <sup>b</sup>	< 0.001
	T 24 h p.m. [°C]	5.06	5.18	5.57	5.40	0.131	4.78	3.16	5.09	2.74	0.930

Rows with different indices within a line differ significantly. ES, electrical stimulation; NES, no electrical stimulation; CC, conventional cooling; DC, delayed cooling; T, temperature; p.m.: post-mortem.

Muscle	Defect	Batch 1					Batch 2				
		$ES \times CC$ ( $n = 10$ )	$ES \times DC$ (n = 10)	$NES \times CC$ (n = 10)	$NES \times DC$ ( $n = 10$ )	P-value	$ES \times CC$ (n = 10)	$ES \times DC$ (n = 10)	$NES \times CC$ ( $n = 10$ )	$NES \times DC$ ( $n = 10$ )	P-value
Topside	1st level	31.4	22.0	34.0	22.8	0.483	24.1	26.7	41.3	14.0	0.799
	2nd level	17.2	16.3	16.1	11.9	0.680	16.8	13.6	24.7	36.6	0.596
	3rd level	33.1	44.9	39.6	31.7	0.877	17.5	30.6	54.1	39.4	0.256
	Total	81.7	83.2	89.6	66.4	0.760	58.4	70.8	120.0	89.9	0.398
Silverside	1st level	3.1	1.5	4.5	1.5	0.371	2.6ª	0.4ª	26.3 <sup>b</sup>	3.0 <sup>a</sup>	0.021
	2nd level	1.3	0.6	2.3	0.0	0.517	2.7	1.3	8.9	0.0	0.267
	3rd level	1.3	0.0	0.0	0.0	0.392	0.0	0.0	0.0	0.0	1.000
	Total	5.7	2.1	6.8	1.5	0.307	5.3	1.7	35.2	3.0	0.036

# 3.2. Effect of different ultimate pH (24 h p.m.) on level and amount of destructurations in cooked cured hams (experiment 2)

The amount of destructurations did not differ significantly between pH-groups in batch 1 on any level of destructurations (Table 4). However, the 3rd level of destructurations tended to increase from the H-pH to the L-pH group. In batch 2, pH varied more widely than in batch 1, particularly in the H-pH group, where pH was higher. At the same time the amount of destructurations differed significantly in batch 2. With the exception of the 3rd level, the highest amounts of defect zones were found in the L-pH group, followed by the M-pH and the H-pH group on all levels as well as on the total of destructurations. Meat quality variables in the raw hams indicated decreasing levels of destructuration as well as L<sup>\*</sup>-, a<sup>\*</sup>- and b<sup>\*</sup>-values from the L-pH to the H-pH group in both batches (Table 5). pH 24 h p.m. in SM as well as pH 72 h p.m. in SM and AD were increased from the L-pH to the H-pH group as would be expected. Of the total 82 destructured zones, 63 appeared in the AD, while SM was affected 19 times by the defect (data not shown). The destructured zones were mostly located in the center of the ham close to the bos femur. According to the binary logistic regression (Table 3) the two strongest predictors for the defect in cooked cured ham were pH at 24 h p.m. (P = 0.135) and the b<sup>\*</sup>-value in the SM (P = 0.205), although even those were not significant.

xperiment	Parameter	Estimate	Standard error	Ζ	P-value	95% Confidence	e interval
						Lower	Upper
°opside (experiment 1)	Constant	-21.44	10.19	-2.11	0.035	-41.41	-1.48
	T 1 h p.m.	0.51	0.26	1.93	0.054	-0.01	1.02
	Barrows	0.95	0.61	1.56	0.118	-0.24	2.15
Silverside (experiment 1)	Constant	-18.74	13.56	-1.38	0.167	-45.33	7.84
	T 1 h p.m.	0.62	0.34	1.83	0.067	-0.04	1.28
	T 3 h p.m.	-0.21	0.13	-1.67	0.096	-0.46	0.04
Experiment 2	Constant	23.18	17.92	1.29	0.196	-11.95	58.32
	pH 24 h p.m.	-4.75	3.18	-1.50	0.135	-10.98	1.48
	b* 72 h p.m.	0.37	0.29	1.27	0.205	-0.20	0.94
Chemical analyses	Constant	-18.46	12.50	-1.48	0.140	-42.96	6.04
-	Crude ash	-0.11	0.05	-2.16	0.030	-0.20	-0.01
	Dry matter	0.11	0.05	2.40	0.016	0.02	0.20

		101 0		nom topsides	(experiment 2).			
Defect level	Batch 1				Batch 2			
	L-pH $(n = 24)$	M-pH (n = 24)	H-pH $(n = 16)$	P-value	L-pH $(n = 24)$	M-pH (n = 24)	H-pH (n = 32)	P-valu
1st level	8.3	12.2	13.8	0.593	19.1ª	10.5 <sup>b</sup>	6.8 <sup>c</sup>	< 0.001
2nd level	5.6	6.9	4.4	0.707	32.6ª	15.2 <sup>b</sup>	4.4 <sup>c</sup>	< 0.001
	9.4	3.2	2.0	0.142	10.1ª	12.3*	1.2 <sup>b</sup>	0.001

Rows with different indices within a line differ significantly. L-pH, ultimate pH < 5.5; M-pH, ultimate pH > 5.7.

Muscle	Attribute	Batch 1				Batch 2			
		L-pH (n = 24)	M-pH (n = 24)	H-pH (n = 16)	P-value	L-pH (n=24)	M-pH (n = 24)	H-pH (n = 32)	P-value
SM	L* [-]	48.9	47.9	47.1	0.119	48.9ª	47.0 <sup>b</sup>	43.4 <sup>c</sup>	< 0.001
a* [-] b* [-] pH 24 h p.m. [-]	a* [-]	7.0	6.7	6.0	0.209	7.2ª	6.1 <sup>b</sup>	5.8 <sup>b</sup>	0.002
		4.5	4.1	3.5	0.038	4.3ª	2.0 <sup>b</sup>	1.4 <sup>c</sup>	< 0.001
	5.32ª	5.59 <sup>b</sup>	5.81 <sup>c</sup>	< 0.001	5.42ª	5.59 <sup>b</sup>	6.00 <sup>c</sup>	< 0.001	
	pH 72 h p.m. [–]	5.45ª	5.61 <sup>b</sup>	5.83°	< 0.001	5.43ª	5.58 <sup>b</sup>	5.89 <sup>c</sup>	< 0.001
٩D	pH 72 h p.m. [–]	5.49ª	5.78 <sup>b</sup>	6.16 <sup>c</sup>	< 0.001	5.46ª	5.65 <sup>b</sup>	6.13 <sup>c</sup>	<0.001
AD <sup>A</sup>	Defect level [-]	1.7ª	1.5*	1.0 <sup>b</sup>	< 0.001	1.5*	1.1 <sup>b</sup>	1.0 <sup>b</sup>	0.001

semimembranosus; L\*, L\*, -value (lightness); a\*, a\*-value (redness); b\*, b\*-value (yellowness); p.m., post-mortem.
According to the IFIP quotation scale (IFIP, 1995).

### 3.3. Chemical analyses

The destructured ham samples of both studies could be characterised by higher dry matter and lower crude ash contents compared to the normal hams (Table 6). Connective-tissue-free muscle protein did not vary between normal and destructured zones in experiment 1, but was significantly increased in the destructured zones in experiment 2. Crude fat in the destructured zones of experiment 1 was slightly higher and in experiment 2 significantly lower than in the normal zones. Only in experiment 2, but not in experiment 1, total connective tissue and insoluble connective tissue protein tended to be higher in the normal zones compared to the destructured ones. Furthermore, myofibrillar fragmentation index (MFI) in the destructured zones was significantly higher than in the normal ones. The binary logistic regression showed that crude ash (P = 0.030) and dry matter (P = 0.016) were the most important characteristics describing the defect (Table 3).

Attribute	Experiment 1			Experiment 2		
	Destructured $(n = 8)$	Normal $(n = 8)$	P-value	Destructured $(n = 4)$	Normal $(n = 5)$	P-value
Dry matter <sup>a</sup>	281.5	271.5	0.022	300.6	281.1	0.103
Crude ash	107.5	115.3	0.005	118.2	138.9	0.039
Crude fat	54.0	43.6	0.261	29.6 <sup>d</sup>	55.6	0.007
Crude protein	832.6	834.0	0.861	855.6	817.0	0.018
TP total <sup>b</sup>	22.2	22.0	0.349	21.4	26.0	0.094
TP insoluble <sup>b</sup>	17.2	17.0	0.395	17.5	21.5	0.091
CTFP <sup>C</sup>	810.4	812.0	0.852	834.2	791.0	0.007
MFI	143.7	101.8	0.002	n.d.	n.d.	-

## 4. Discussion

## 4.1. Effect of early post-mortem pH on level and amount of destructurations in cooked cured hams (experiment 1)

Application of electrical stimulation resulted in significantly different pH values in silversides as well as in topsides 60 min p.m. and partly at 180 min p.m. The delayed cooling process led to a higher temperature 3 h p.m. in silversides as well as in topsides and partly already 1 h p.m. in silversides compared to conventional cooling. Temperature and pH at 24 h p.m. were not affected by the different treatments. Therefore, the intended conditions to examine the influence of early post-mortem pH and temperature settings on destructured zones were achieved by the applied experimental factors. The amount of destructured zones in cooked cured hams varied between the four treatments; however, these differences were not significant due to the large variations within the treatments. The combination of conventional cooling (CC) without electrical stimulation (NES) led to an increase of destructurations. This is surprising because several studies claim a fast early-p.m. pH decline as key factor for the development of destructured zones, however determined in raw hams (Balac et al., 1998; Franck et al., 2002; Le Roy et al., 2001). Only few studies on destructured zones have been carried out so far in cooked cured ham, the actual processed product. Le Roy et al. (2001) found in two of three batches lower slicing losses for cooked cured hams processed from fast pH fall M. semimembranosus (SM) than from normal pH fall SM. Only in the third batch, the slicing loss was lower for slow pH fall SM, as would have been expected. However, because the animals within the three batches of cooked cured hams originated from different breeds, a separation of pH-effects from other genetic effects was impossible. Our binary logistic regression revealed increased temperature at 60 min p.m. in both, BF and SM, as important predictor for destructurations in cooked cured hams, which is in accordance with the results of Balac et al. (1998). They found higher temperatures in the deep center of destructured raw hams than in the ones of normal hams. In this study, the temperature in 4 cm depth 60 min p.m. was 40.4 C in topsides and 41.0 C in silversides, respectively; they were even higher in the center of the hams. Freise, Brewer and Novakovski (2000) reported that already a temperature between 35 and 42 C even with normal pH-values could cause PSE-similar colour changes due to protein denaturation (Zhu and Brewer, 2002). These results lead to the suggestion that due to a high temperature in the early post-mortem stage, a partial denaturation of meat proteins (mainly sarcoplasmatic proteins, incl. myoglobin) occurs, supporting the development of destructurations in cooked cured hams. The positive relation of the defect in raw meat to the slaughter weight and lean meat content (Bouffaud et al., 2002; Franck et al., 2002; Minvielle et al., 2001; Vautier et al., 2008) may also

be temperature-dependent. An increased thickness of the muscle layer around the center, due to larger carcasses and heavy muscling may lead to a reduced heat efflux. This may also explain the higher susceptibility of barrows to the defect in cooked cured hams revealed by the binary logistic model as they normally have thicker subcutaneous fat layers than the gilts leading to a slower chilling rate in the center of the raw hams.

## 4.2. Effect of different ultimate pH (24 h p.m.) on level and amount of destructurations in cooked cured hams (experiment 2)

The production of cooked cured hams selected according to ultimate pH in SM did not result in different amounts of destructured zones in batch 1, whereas for batch 2 the amount of the defect was significantly higher in low-ultimate-pH hams. In the cooked cured hams of each ultimate pH group of both batches, all levels of destructuration appeared. Furthermore, three times more destructurations were recorded in AD than in SM, which is consistent with the results of Laville et al. (2003), who reported most of the defect in the raw material in AD, followed by SM and BF. Referring to the binary logistic regression of the two batches, a low ultimate pH was the most important predictor for the destructured zones in cooked cured hams. This is in accordance with former studies claiming low ultimate pH to be the main cause of destructured zones recognised in raw hams (Minvielle et al., 2001; Vautier et al., 2008). In cooked cured hams, Le Roy et al. (2001) have shown that using SM of a high ultimate pH leads to a reduced slicing loss in cooked cured ham, which is also in accordance with the recommendations of Brauer (2002). And a study of the French Institute for Pig and Pork Industry (IFIP, 1996) revealed that using SM of an ultimate pH higher than 5.5 can reduce the slicing losses by up to 20% compared to SM of ultimate pH beneath 5.5. To what extend these slicing losses included destructured zones is unknown. The fact that the destructurations of batch 1 were obviously independent of the ultimate pH in SM in the present study, indicates that ultimate pH alone cannot be used as the only reliable indicator for the defect in cooked cured hams, without considering also the course of pH and temperature fall early post-mortem. In the raw material, indicators and grades of the defect were higher with a lower ultimate pH. In the HpH group of both batches, no destructured zones appeared in the raw muscles [indicated by quote 1.0 in the IFIP quotation scale (IFIP, 2005), Table 5], but all levels of the defect were present in the cooked cured hams. Consequently a low level of the defect in the raw material does not guarantee cooked cured hams without any destructured zones, which was already demonstrated by Pizza and Pedrielli (2002). They showed that insufficient cooking and excessive mechanical treatment lead to a loss of intramuscular cohesiveness as it is typically observed in destructured zones.

### 4.3. Chemical analyses

The low crude ash and the higher dry matter content of the destructured zones as well as their higher connective tissue protein content (only in experiment 2) confirm findings of a previous study of Hugenschmidt et al. (2009). The reason for the high dry matter and the low crude ash content, respectively, was hypothesised to originate from a decreased brine-binding capacity in the defect zones. Therefore, the brine containing salt, nitrite and other additives cannot be bound completely in the destructured zones resulting in a lower brine-component content. The low brine-binding capacity may be due to a denaturation of proteins in the defect zones, mainly influenced by low pH and a high temperature early post-mortem as well as low ultimate pH (Schäfer, Rosenvold, Purslow, Andersen, & Henckel, 2002). This also corresponds to the high MFI of the destructured zones in cooked cured ham as an indicator for the occurrence of protein denaturation. The connective tissue and especially its insoluble part were found to be

significantly lower in the destructured zones in the former study of Hugenschmidt et al. (2009). Therefore, they were assumed to play an important role in the development of the defect zones in cooked cured hams due to their structural properties in the muscle. In the present study normal cooked cured hams tended to show higher total and insoluble connective tissue content than destructured zones in experiment 2, whereas no difference was observed in experiment 1. Voutila et al. (2008) studying the onset and peak temperature of thermal shrinkage of 24 h p.m. intramuscular connective tissue in raw ham as well as its content and solubility and Minvielle et al. (2001) analysing its total and thermosoluble content did not find significant differences between normal and destructured zones either. The higher content of insoluble connective tissue in normal cooked cured ham compared to destructured ones in the present study is in accordance to findings of Minvielle et al. (2001), who found slightly more thermosoluble collagen in destructured than in normal raw meat. This should result in a higher content of insoluble collagen in normal cooked cured ham. Consequently, the higher content of insoluble collagen could explain the increased hardness of normal compared to destructured cooked cured ham reported by Hugenschmidt et al. (2009). However, a causative relation between destructured zones and connective tissue traits remains unclear. Crude protein content was higher and fat content was lower in destructured than in normal zones of cooked cured hams in experiment 2. A high crude protein content usually correlates with a low intramuscular fat content and is associated with a high meatiness of the carcass and a low ultimate pH value in the muscles (Florowsky, Pisula, & Kamyczek, 2007) both leading to destructured zones in raw meat (Franck et al., 2002). However, in experiment 1 neither crude fat nor crude protein content differed between normal and destructured zones in cooked cured hams and in the former study of Hugenschmidt et al. (2009) crude protein was increased in destructured cooked cured hams but crude fat was not. These results indicate that according to studies in raw ham (Minvielle et al., 2001; Voutila et al., 2008) crude protein content, crude fat content as well as total and insoluble collagen content do not play a major part in the development of destructured zones in cooked cured hams. In this study, the processing technology was standardised and the yields of the cooked cured hams from the different treatments in experiment 1 or 2 did not differ significantly. However, it should be kept in mind that apart from raw material characteristics, processing conditions such as tumbling effects and cooking method strongly influence brine-binding capacity and consequently yield and structure of cooked cured ham (Pizza and Pedrielli, 2000).

## 5. Conclusions

Considering early post-mortem temperature and pH-value, an increased temperature early postmortem in the raw muscle was identified as an important predictor for the occurrence of destructurations in cooked cured hams. This leads to the suggestion that the temperature course early post-mortem, when it can reach over 41 C in the center of hams, is directly involved in the development of the defect by protein denaturation. This assumption is supported by the higher myofibrillar fragmention index and a lower brine-binding capacity in destructured zones, both characteristics of denaturated muscle proteins. Considering ultimate pH, uncooked hams with pH-values below 5.5 can also produce more destructured zones in cooked cured hams than raw hams with ultimate pH values above 5.7. Nevertheless, choosing meat with a high ultimate pH or a low level of destructurations may not necessarily prevent cooked cured hams from developing the defect. Consequently, the technology of cooked cured ham processing also has to be taken into account as a potential factor contributing to the defect. It can be concluded from the present study that a combination of high early p.m. temperature and/or low ultimate pH-values are important causative factors for destructurations in cooked cured hams. Therefore, influencing factors of post-mortem pH and temperature like pig breed, nutrition, transport, fasting, slaughtering, cooling, storage and processing technology may also

contribute to the defect. Thus, the destructured zones are PSE–pork-like not only due to their development at a high temperature early post-mortem, but also regarding their chemical and physical characteristics such as low water binding capacity and bright colour. Future studies should be focused on the combined effects of early post-mortem temperature and pH-value on the occurrence of destructured zones in both, cooked cured hams and raw hams simultaneously. This should be investigated preferably in M. adductor, because this muscle was shown to be the most susceptible for the defect.

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