COBISS: 1.08
Agris category code: Q01

EFFECT OF SALTING DURATION ON SALT CONTENT AND PROTEOLYSIS IN *KRAŠKI PRŠUT* DRY HAMS AFTER THE RESTING PHASE

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ABSTRACT

The objective of the present study was to reduce salt content in *Kraški pršut* dry ham by means of shorter salting period. Three groups of hams (equilibrated for weight, fatness and pH) were salted for 15 (HS), 11 (MS), and 8 (LS) days. After the end of salting, hams were submitted to resting (87, 91, 94 days for HS, MS and LS group, respectively). Processing losses were recorded at the end of each phase. After 102 days (end of resting), *semimembranosus* (SM) and *biceps femoris* (BF) muscles were sampled for determination of dry matter (DM, g/100g), salt (NaCl, g/100g DM), total nitrogen (N, g/100g DM), non-protein nitrogen (NPN, g/100g DM and proteolysis index (IP, %). Shortened salting was accompanied by lower ham weight losses at the end of this phase ($P < 0.0001$) but did not affect the losses in the resting phase and in total. As a result, slightly lower DM was observed in LS compared to HS hams (29.5 *vs.* 30.9, $P = 0.13$ in BF and 37.4 *vs.* 40.7, $P = 0.08$ in SM). A 7 day shorter salting period (LS) resulted in notable salt reduction (36.4% and 27.8% for BF and SM, respectively), while 4 day reduction (MS) had lesser impact (11.7% and 6.0% for BF and SM, respectively). There was no effect of salt reduction on the IP at the end of resting period. However, in SM a difference between LS and HS hams was significant for N (13.1 vs. 11.9, $P < 0.01$) and NPN (1.79 vs. 1.54, $P < 0.01$).

Key words: dry ham / salt reduction / proteolysis

1 INTRODUCTION

In *Kraški pršut* (a representative of the Mediterranean type of dry hams) sea salt is the only additive allowed. This key ingredient contributes to the development of characteristic dry ham sensory properties (texture, taste, flavour) and serves as a preservation agent by decreasing water activity and promoting water loss (Toldrá, 2002). High salt ingestion, however, presents increased risk for cardiovascular diseases; therefore health recommendations and consumers demand limited use of salt in meat products (Ruusunen and Puolanne, 2005). On the other hand, reducing salt in dry-cured hams, an important inhibitor of proteolytic enzymes (Sárraga, 1992), may lead to serious quality defects related to excessive proteolysis (Parolari *et al.*, 1994). Numerous strategies for reducing NaCl have been tested, including experiments with salt reduction, replacement of NaCl by other salts and different modifications of processing conditions, however, each strategy presents a new challenge to the processing technology and might also significantly alter dry ham quality (reviewed by Čandek-Potokar and Škrlep, 2012). The concentration of salt in commercially produced *Kraški pršut* is still relatively high. The *consortium* of *Kraški pršut* restricts a maximum values to 7.5% (in slice containing fat and muscle tissue), while in our recent research (Škrlep *et al.*, 2012) we determined the concentrations in lean tissue amounting to 7.6% and 6.7% of NaCl in *biceps femoris* and *semimembranosus* muscles, respectively. This is much higher than, for instance, in contemporary Italian *Parma* or *San Daniele* hams with 4–6% of NaCl in lean tissue (De Angelis, 2012; Manzocco

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et al., 2013). Reducing the time of salting represents an efficient measure for salt reduction. Therefore, the aim of the present study was to analyse the effect of different length of salting period on salt uptake, moisture loss and consequent proteolysis in two dry ham muscles at the end of the resting phase.

2 MATERIALS AND METHODS

2.1 HAM SELECTION, PROCESSING AND MEASUREMENTS

For the study, green hams were taken from one batch, trimmed according to the prescribed form for "*Kraški pršut*" and processed by a commercial dry ham producer. The selection of hams was based on trimmed ham weight, subcutaneous fat thickness (measured under *caput femoris*) and *semimembranosus* muscle pHu (measured in duplicate using a MP120 pH meter by Mettler-Toledo, GmbH, Schwarzenbach, Switzerland) in order to obtain three homogenous groups. After initial first salting phase (salting 1, 5 days) the hams were cleaned of the residual salt and salted again (salting 2). Three treatment groups were formed according to salting time, *i.e.* 8 (LS, n = 5), 11 (MS, n = 8) or 15 days (HS, n = 10, standard salting time). At the end of salting 2, the residual salt was removed, and the hams were left to rest (87, 91, 94 days for HS, MS and LS group, respectively) at 4–6 °C and 70–85% relative humidity allowing the salt to equilibrate in the product to reach microbial stability. Experimental period was thus 102 days for all treatment groups. Hams were individually weighed at the end of each phase, enabling us to monitor processing losses.

2.2 CHEMICAL ANALYSES.

At the end of resting, samples (app. 200g) of *biceps femoris* (BF) and *semimembranosus* (SM) muscles were taken from the middle portion of the ham, cleaned of fat and connective tissue and ground in liquid nitrogen. Subsequent chemical analyses comprised:

- dry matter (DM, g/100g) determination (according to ISO 6496, 1999);
- salt (NaCl, g/100g DM) determination (by chloride potentiometric titration using DL53 General Purpose Titrator, Mettler-Toledo, GmbH, Schwarzenbach, Switzerland);
- total nitrogen (N, g/100g DM) determination (according to ISO 5983-2, 2005) using Kjeltec 2300 Nitrogen Analyser (Foss Analytical, Hileroed, Denmark);
- non-protein nitrogen (NPN, g/100g DM) determination, using the same protocol as for the total nitrogen on samples after the extraction (as described in Škrlep *et al.*, 2012);
- proteolysis index (IP, %) was subsequently calculated as a percentage of non-protein nitrogen in total nitrogen content.

2.3 STATISTICAL ANALYSIS

Data were analyzed using the GLM procedure of SAS statistical software (SAS Institute Inc., Cary, USA). The model included the effect of treatment group with ham trimmed weight and SM pH as covariates. When significant effect of the treatment group was detected ($P < 0.05$), least squares means (LSM) were compared using Tukey's test.

3 RESULTS AND DISCUSSION

As presented in Table 1, there were no differences among the groups in green ham traits. Due to shorter salting, lower ham weight losses were noted at the end of salting ($P < 0.0001$), whereas salting time did not affect moisture losses in the resting phase and in total. It is worth noting very small ham weight losses in the 2nd salting phase for LS hams indicating that salt uptake had similar rate as water loss in the first days of this phase. Moisture losses increased with prolonged salting for 4 or 7 days (*i.e.* by 1.0 and 1.6% in MS and HS, respectively) resulting in (although not statistically different) 2.7% point higher processing losses in HS compared to LS group at the end of the resting stage. This result confirms that higher ham weight losses are associated with higher salt uptake (Grau *et al.*, 2007).

Analysed chemical traits are reported in Table 2. Regardless of the muscle, a trend ($P = 0.13$) of decreasing dry matter content in both investigated muscles was observed along with the shortening of the salting period. Besides that, a strong effect on salt content was noticed. Shortening the salting period for 7 days (LS) resulted in 36.4% and 27.8% decrease in salt content (based on dry matter) for BF and SM, respectively. Such decrease already meets the demands of EC regulations (EC, 2006), requiring at least 25% less salt in products labelled "reduced salt" compared with their regular counterparts. Reduction of salting period for 4 days had less profound effect (11.7% and 6% in BF and SM, respectively). This confirms the fact, that salt uptake rate decreases in a nonlinear way with salting time (Picouet *et al.*, 2012) and indicates that noteworthy salt reduction can be achieved only by major shortening of the salting period (in our case for almost 50%).

Table 1: *Raw material properties and ham weight losses (LSM ± SE[1]) according to different salting duration*

	LS	MS	HS	P
Trimmed green ham				
Weight, kg	11.12 ± 0.25	11.80 ± 0.20	11.74 ± 0.18	0.116
Fat thickness, mm	15.5 ± 1.7	15.6 ± 1.3	15.6 ± 1.2	0.999
pHu SM	5.63 ± 0.05	5.53 ± 0.03	5.55 ± 0.03	0.220
Processing losses (%)				
Salting 1	2.1 ± 0.15	1.8 ± 0.11	2.1 ± 0.09	0.184
Salting 2	0.01 ± 0.23[a]	1.0 ± 0.22[b]	1.6 ± 0.15[c]	< 0.0001
End salting	2.1 ± 0.24[a]	2.8 ± 0.17[b]	3.7 ± 0.15[b]	< 0.0001
Resting	18.8 ± 1.93	18.2 ± 1.39	19.8 ± 1.23	0.664
End resting	20.8 ± 1.90	21.0 ± 1.37	23.5 ± 1.20	0.304

LS – 8 days of salting; MS – 11 days of salting; HS – 15 days of salting; Ham fat – subcutaneous fat thickness measured under caput femoris; pHu SM – pH in semimembranosus muscle. [1] LSM with different superscripts within a row are significantly different (P < 0.05).

The results obtained for IP (either in BF or SM) indicate no significant effect of salting time (and consequently salt reduction) on the degree of proteolysis. On the other hand, shorter salting period resulted in higher NPN concentration in SM muscle. This could either be indicative of increased proteolysis (*i.e.* proteolytic enzymes less inhibited in LS) or merely a consequence of higher N concentration in LS than HS hams. Significantly higher N and NPN in LS than HS hams (with intermediate position of MS) can be related to the lower loss of soluble proteins with leaked muscle juice. Regarding the extent of the proteolysis, it is worth noting that present results do not relate to end product, since biochemical process of proteolysis is just at the beginning. Entire dry ham processing lasts long time (minimum of one year is prescribed for *Kraški pršut*), with some proteolytic enzymes (*i.e.* cathepsins) remaining active for a long time (Toldrá and Etherington, 1988). Also, BF muscle is the site of more intensive proteolysis. As indicated by Théron *et al.* (2011) in their proteomic study on dry ham, the SM, as a superficial muscle, encounters a direct contact with salt and rapid desiccation, which blocks the proteolysis relatively shortly after the start of processing. On the other hand, proteolytic degradation in internal muscles (like BF) was reported to be relatively intensive and long lasting.

Table 2: *Chemical composition (LSM ± SE1) according to different salting time*

	LS	MS	HS	P
M. biceps femoris				
DM, g/100 g	29.5 ± 0.56	30.2 ± 0.40	30.9 ± 0.36	0.130
NaCl, g/100 g DM	8.2 ± 1.08[a]	11.4 ± 0.78[ab]	12.9 ± 0.69[b]	0.008
NPN, g/100 g DM	1.89 ± 0.06	1.89 ± 0.04	1.91 ± 0.03	0.880
N, g/100 g DM	13.19 ± 0.60	12.73 ± 0.43	12.07 ± 0.38	0.268
IP, %	14.3 ± 0.97	14.9 ± 0.70	16.2 ± 0.61	0.192
M. semimembranosus				
DM, g/100 g	37.4 ± 1.20	38.9 ± 0.87	40.7 ± 0.76	0.077
NaCl, g/100 g DM	10.9 ± 0.98[a]	14.2 ± 0.71[ab]	15.1 ± 0.62[b]	0.008
NPN, g/100 g DM	1.79 ± 0.06[b]	1.61 ± 0.04[ab]	1.54 ± 0.04[a]	0.012
N, g/100 g DM	13.10 ± 0.30[b]	12.23 ± 0.21[ab]	11.85 ± 0.19[a]	0.011
IP, %	13.7 ± 0.43	13.2 ± 0.31	13.0 ± 0.27	0.494

LS – 8 days of salting; MS – 11 days of salting; HS – 15 days of salting; DM – dry matter; NaCl – salt content; NPN – non protein nitrogen content; N – total nitrogen content; IP – index of proteolysis, percentage of NPN in total N; [1] LSM with different superscripts within a row are significantly different (P < 0.05).

4 CONCLUSION

A considerable reduction of salt content in dry ham can be achieved only with a major shortening of salting period. This reduction of salt had however no major impact on processing yields or extent of proteolysis at the end of the resting stage. Although this study provides very informative results, they should be treated as preliminary, and final conclusions are only to be drawn after the processing has been finished, because proteolysis process is still in progress in this stage of processing and some degree of salt distribution is still expected.

5 ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Slovenian Research Agency (program P4-0133 and project L4-5521) and AGROTUR – Karst agrotourism, a project co-founded by Interreg Program Italy-Slovenia 2007–2013 and European Regional Development Fund.

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