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PROTEOLYSIS IN NORWEGIAN DRY-CURED HAMS; PRELIMINARY RESULTS

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Introduction

The processing of dry-cured hams is very complex and involves numerous biochemical reactions. One of the most important biochemical reactions in the development and processing of dry-cured ham is proteolysis. Along the dry-curing ripening process, numerous free amino acids are generated from peptides and protein fragments. It is speculated that the presence of some of these free amino acids and peptides in dry-cured hams make an important contribution to taste or even interact with volatile compounds to affect the whole flavor (Sentandreu and Toldra, 2000). Moreover, the combined effect of different proteolytic enzymes (cathepsin B, D, H, L, dipeptidases etc.) stimulate proteolysis, leading to flavor development and in reducing the hardness of dry-cured hams produced by a long ripening process.

Proteomics is a tool to study protein degradation in dry-cured hams and may be used to compare degradation patterns between different samples (Luccia *et al.*, 2005).

Objectives

1. Characterization of Norwegian dry-cured hams using chemical analysis.
2. To investigate and compare proteolytic degradation among different dry-cured hams produced in Norway using proteomics.

Methodology

A total number of 10 samples from Norwegian dry-cured hams from 6-month-old pigs from three different producers and four samples from non-processed fresh Norwegian hams were used in this study (Table 1). The dry-curing process consist of the traditional stage of salting between 0.8 and 1.0 days/kg ham, followed by post salting between 10 and 12 weeks. Finally, hams were allowed for ripening-drying period of 11-14 months, depending upon variable traditional production process and type of companies. Samples were analyzed for proteolytic index, protein content, salt content

and water activity (a_w) and compared for proteolytic degradation using proteomics. Salt content and a_w were measured in the dry-cured hams only.

Crude protein was determined by the Kjeldahl method and proteolytic index was calculated as percent ratio between nitrogen soluble in 5% trichloroacetic acid, determined by the Kjeldahl method after protein precipitation with trichloroacetic acid, and total nitrogen, as recommended by the Nordic Committee on Food Analysis standards (NMKL 6:2003). Salt (chloride) content in the dry-cured hams was measured according to the International Dairy Federation standards (IDF 179:1997). The water activity in the dry-cured hams was detected using the official AOAC method (AOAC 978.18). Extraction of muscle proteins were performed as described in Lametsch and Bendixen, (2001). 100 µg proteins were separated on 18 cm IPG pH 4 -7 in the first dimension and a 10% SDS-PAGE in the second dimension. Gels are silver stained according to the protocol in Blum *et al*. (1987) and aligned and quantitated using Image Master 2D Platinum v5.0 (Amersham Biosciences).

Multivariate statistical methods such as Principal Component Analysis (PCA) was used to interpret the variations in the data set obtained by 2DE using Unscrambler 9.0 (Camo, Norway).

Results & Discussion

Proteomics using two-dimensional gel-electrophoresis (2DE) is a powerful tool to study degradation of muscle proteins. However, to our knowledge only one study are published describing the use of proteomics to investigate protein degradation in dry-cured hams (Luccia *et al*., 2005). In this study, we describe the preliminary results of 2DE on Norwegian dry-cured hams and non-processed fresh ham. The results shown in Figure 1 showed that the protein patterns are generally changed between fresh non-processed and finished dry-cured ham during ripening. In the dry-cured hams, the main protein spots observed in Figure 1A are degraded as shown in figure 1B. In addition, a lot of proteins are observed in the upper part of the gel. This may be degradation products resulting from cleavage of the myofibrillar proteins. Furthermore, several protein spots appear in the lower part of the gels. These are also probably peptides generated from degradation of the structural muscle proteins in dry-cured hams during the ripening period. However, further analysis is needed to identify these proteins or peptides. The mechanism of protein degradation and effect of proteolytic enzymes were not studied. Previous investigators have demonstrated the release of free amino acids, peptides in the dry-cured hams are due to proteolytic enzymatic processes (Sentandreu and Toldra, 2000). The effect of proteolytic enzyme could be of great interest in stimulating proteolysis, in flavor development and in reducing the hardness of dry fermented sausages produced by a long ripening process.

Muscle samples from seven dry-cured hams were studied by 2DE (Samples S3, S4, S5, S7, S8, S9, and S10 in Table 1). Comparison of the protein patterns from the different samples showed a great variation in the degradation pattern between the different hams. Representative images of some of the 2DE protein patterns are shown in Figure 2. Multivariate statistical tools are useful in the analysis of complex data like 2DE protein patterns. A Principal Component Analysis (PCA) plot of the protein patterns are shown in Figure 3. The analysis demonstrated a great variation in the protein pattern observed in

the 2DE gels in the hams, and five principal components were needed to explain 88% of the variation in the data. However, a slight covariance is observed within the hams from the different production plants marked by circles in the PCA score plot. Further investigations are needed to identify the proteins and peptides that are related to the degree of proteolysis and end product quality of dry-cured hams.

Results from chemical investigation revealed that salt content among dry-cured hams are variable, ranging between 3.6 % and 7.8 % (Table 1). Theoretically, a lower salt content and less dehydration should result in dry-cured ham with more aroma and protein degradation. Generally, salt content and salt penetration the dry-cured hams are related to initial weight of the hams. Small hams have a large surface to mass ratio to receive more salt leading to salty product. Generally, our results suggest good agreement when salt content in dry-cured hams was calculated with initial weight of each ham. The results in the present study indicate that salt content is mainly related to the a_w, showing that high salt concentration enhances ham drying process (Figure 4). Protein content for the tested samples ranged between 20.8 % and 39.8 %. Proteolytic index among dry-cured hams varied between 0.20 and 0.36 (Table 1). Some of the dry-cured hams showed higher proteolytic index, indicating accelerated protein degradation. It is well known that proteolysis contribute positively to flavor and to the nutritional quality of matured ham, but also results in undesirable traits and disadvantages such as softness and color changes. The enzymes activity levels of the muscles, significantly depend on the properties of pre-slaughter meat properties (raw ham), such as age, and crossbreeding as well as the process conditions such as temperature, time, water activity, salt content (Sentandreu and Toldra, 2000). Thus, the control of the muscle enzyme systems plays important role for the standardization of the processing and/or enhancement of flavor quality of dry-cured ham.

Conclusions

The preliminary results showed that salt distribution, proteolytic index and protein degradation is variable among Norwegian dry-cured hams. However, the results are preliminary and based on few samples from different producers. Protein degradation giving rise to an important collection of free amino acids and small peptides that may directly contribute to flavor or indirectly contribute as precursors of other flavor compounds. Further investigation is needed to study the relation between protein degradation, and quality parameters such as sensory analyses.

References

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Table 1. Collection of samples from Norwegian dry-cured hams and preprocessed hams from three different companies.

Dry-cured hams	**Type**	**Analysis**	
		Protein	Proteolytic index
Company 1			
Sample S1	Dry-cured ham	27,9	0,29
Sample S2	Dry-cured ham	30,8	0,20
Sample S3	Dry-cured ham	38,0	0,25
Sample S4	Dry-cured ham	39,8	0,25
Sample S5	Dry-cured ham	34,0	0,28
Sample S6	Dry-cured ham	35,2	0,27
Sample R12	Preprocessed hams	21,7	0,14
Sample R13	Preprocessed hams	19,0	0,13
Sample R14	Preprocessed hams	22,3	0,13
Sample R15	Preprocessed hams	23,6	0,14
Company 2			
Sample S7	Dry-cured ham	26,3	0,27
Sample S8	Dry-cured ham	27,6	0,24
Company 3			
Sample S9	Dry-cured ham	26,3	0,20
Sample S10	Dry-cured ham	27,0	0,22

Figure 1. Representative 2DE maps of porcine muscle proteins. A. Non-processed muscle. B. Finished dry-cured ham. Proteins are separated on IPG 4-7 and 10% SDS-PAGE.

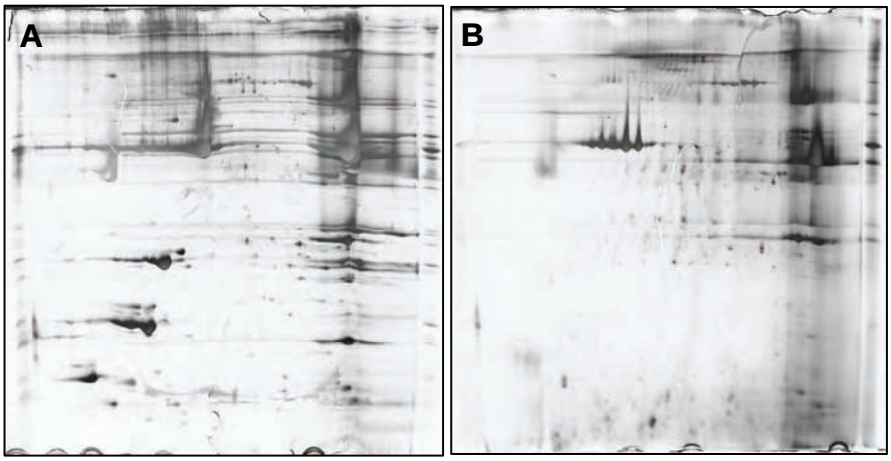

Figure 2. **Representative 2DE images of dry-cured hams from 3 different companies. A. Sample S4, B. Sample S5, C. Sample S8 and D. Sample S9. Proteins are separated on IPG 4-7 and 10% SDS-PAGE.**

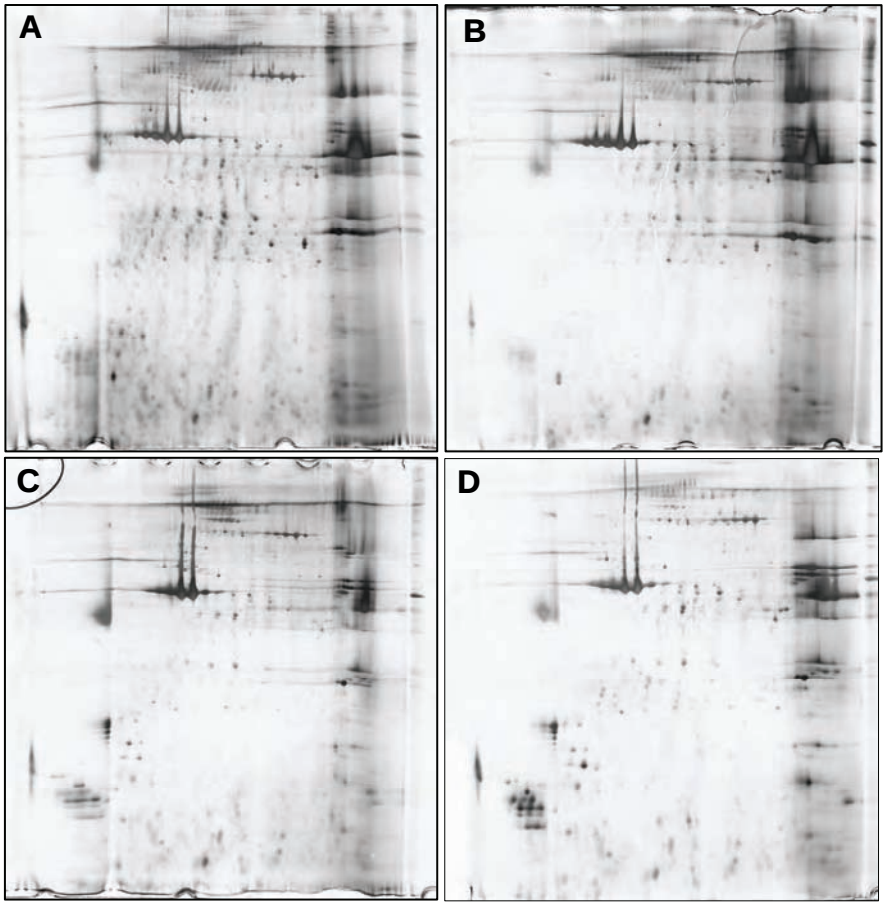

Figure 3. A principal component analysis (PCA) of the protein patterns in Norwegian dry-cured hams. The figure shows the variations in PC1 and PC2. Hams from the three different companies are circled.

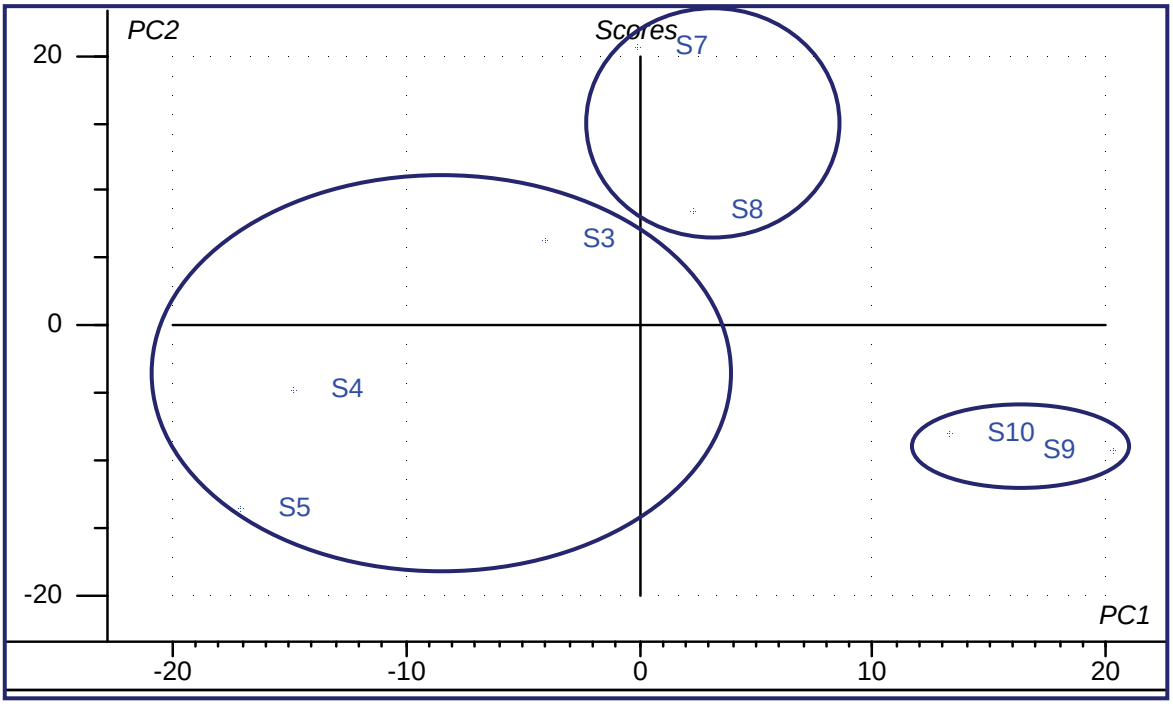

Figure 4. Protein content, proteolytic index, salt and water activity (A_w) analysis for dry-cured hams and non-processed Norwegian hams.