Influences of pig breed and slaughter age on cathepsin B+L activity in fresh meat and dry-cured hams

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Abstract— Cathepsin are group of muscle proteinases that are suggested to play a major role in the quality of dry-cured hams. Cathepsins B and L are two of the main muscle endopeptidases. They are cysteine proteinases, which are active through the entire dry-curing process. The main aim of this study was to investigate cathepsin B+L activity in fresh meat and in mature dry-cured hams produced from three different pig breeds (Duroc, Norwegian Landrace and Hampshire) at three different slaughter ages (6, 7.5 and 9 months). Significant differences of cathepsin B+L activity was observed in fresh meat between the breeds (p<0.05) and the highest activity was observed in the samples from the Hampshire breed. In matured drycured hams produced from Hampshire pigs, the cathepsin B+L activity was significantly lower than in hams produced from Duroc (p<0.05). No significant differences among the age groups neither in fresh meat nor in dry-cured hams were observed. However, a tendency towards a decrease between the 6 months and 9 months old pigs were observed in the fresh meat samples.

Keywords— cathepsin B+L, pig breed, slaughter age.

I. INTRODUCTION

Cathepsins play a major role in muscle proteolysis post mortem and the proteolytic activity is involved in obtaining high quality dry-cured hams [1]. Cathepsin B and L are two of the main muscle proteinases (endopeptidases). Their optimum pH is around 6, which is the pH that hams obtain during processing [2]. This allows the cathepsin B + L to be active during the entire dry-curing process.

Several studies have shown the influences of pH [3, 4], NaCl concentration [3, 4], temperature [4], pig weight [5], sex [6], and sire [6] on the cathepsin activity. One study indicated differences in cathepsin activities among different pig breeds [7]. To our

knowledge, however, the effect of age and breed on cathepsin B+L activity in fresh meat as well as drycured hams have not been extensively investigated.

The objective of this study was to investigate the effects of pig breed and slaughter age on the cathepsin B+L activity in fresh meat and dry-cured hams.

II. MATERIALS AND METHODS

Experimental design: A total of 90 samples of *adductor* muscle, collected 45 min after slaughter, of three breeds (Norwegian Landrace, Duroc and Hampshire) slaughtered at three ages (6, 7.5 and 9 months) were used in this study. All animals were from the same farm and slaughtered the same day at the same abbatoir. Dry-cured hams were produced from the same pigs at Nortura Tynset, Norway.

Sample extraction: Sample extraction was performed mainly as previously described with minor modifications [8]. Briefly, 300 ± 5 mg of muscle/drycured ham samples were homogenized with 1000 ± 15 µl of extraction buffer (50 mM sodium acetate pH 5.0 containing 1 mM Na₂EDTA and 0.2 % (v/v) Triton X-100 at 4°C) using the Precellys 24 tissue homogenizer. The extracts were stirred at 4°C for 30 min and centrifuged at 13000 rpm for 30 min. Supernatants were stored at -80°C until use.

Cathepsin B+L activity: Assay was performed as described by Toldra and Etherington [8]. Briefly, the activity of cathepsin B+L was determined at 37°C and pH 6.0. Samples were incubated with fluorescent substrate (Z-Phe-Arg-NMec) for 10 min. The reaction was stopped by adding 2 ml of 100 mM monochlor acetic acid in 100 mM acetate buffer, pH 4.3. A standard curve was made using 25 - 200 nM 4-amino-7-methyl coumarin dissolved in a stop buffer. Fluorescence was measured with excitation and emission wavelengths at 355 and 460 nm, respectively. All samples were analyzed in triplicates.

Statistical analysis: The data were analyzed using Minitab 16 software and p-values less than 0.05 were considered statistically significant.

III. RESULTS

In the present study a total of 178 samples (90 fresh meat and 88 dry-cured ham) were analyzed for cathepsin B+L activity.

A significant effect of breed on cathepsin B+L activity was observed in samples from both fresh meat and dry-cured hams. In fresh meat the highest cathepsin B+L activity was observed in Hampshire and it was higher than both Duroc and Norwegian Landrace (p<0.05) (Fig 1.). However, dry-cured hams from Hampshire had the lowest cathepsin B+L activity and the results was lower than in the samples from the Duroc breed (p<0.05). No difference was observed in cathepsin B+L activity between the Norwegian Landrace and Duroc as well as between Norwegian Landrace and Hampshire (Fig 2.).

No differences among the age groups neither in fresh meat nor dry-cured hams was detected (not shown). However, a tendency towards a modest decrease in cathepsin B+L activity with slaughter age in fresh meat was observed (p-value??).



IV. DISCUSSION

Fig 1. Cathepsin B+L activity in meat samples collected from adductor muscle 45 min post-mortem. Bars with letters A and B differ significantly.



Fig 2. Cathepsin B+L activity in dry-cured hams from *B*. *femoris*. Bars with letters A and B differ significantly.

In the present study significant differences in cathepsin B+L activity among the breeds were observed. Hampshire pigs had a higher cathepsin B+L activity than Norwegian Landrace and Duroc (p<0.05). The higher cathepsin B+L activity in Hampshire pigs could be due to the higher body weight in this breed compared to the Norwegian Landrace and Duroc. We observed a tendency in reduction of cathepsin B+L activity with slaughter age.

V. CONCLUSIONS

The results show that there are differences in cathepsin B+L activity among the three breeds that has been investigated in this study. Further work is in progress to relate cathepsin B+L activity with sensory attributes in these dry-cured hams.

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