THE INFLUENCE OF TEMPERATURE ON PROTEIN EXTRACTION OF SALT TREATED PIG MUSCLE

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SUMMARY

The amount of protein extracted from pig M. semitendinosus was measured at different temperatures (0 - 20°C), in different salt concentrations (0.5, 1, 3 M NaCl) and in the presence or absence of pyrophosphate (PPi). At low salt concentration (0.5 M) the protein extraction increased with increasing temperature. In higher salt concentrations (1 and 3 M) more protein was solubilised at 0°C than at 3°C and 9°C. Extraction increased at higher temperatures up to 20°C. The addition of PPi resulted in a higher extraction yield but did not modify the result observed in salt alone. Protein analysis by sodium dodecyl sulfate polyacrylamide-gel-electrophoresis (SDS-PAGE) showed that with salt alone myosin extraction was greatest with 1 M at 0°C. Addition of PPi allowed myosin extraction in lower salt concentration (0.5 M) at 0° and 3°C and also in higher salt concentration (3 M) at 0°C, 3°C and 9°C. The highest extraction occurred in 1 M NaCl at 0°C with added PPi. Only in 1 M NaCl concentration myosin was extracted at 20°C in the presence of PPi.

INTRODUCTION

During the curing process several parameters influence the diffusion of curing ingredients in meat and subsequently the protein extraction. Among them the temperature is a parameter which can be controlled during cutting, injection, soaking or tumbling. During these processes the extracted proteins contribute to the emulsifying properties and to water retention in the final products. WISTREICH et al. (1959) observed sodium chloride diffusion in pork muscles. The amount of NaCl diffused into the muscle through one square centimeter of contact area was called the accumulation value and it increased exponentially with temperature. KRAUSE et al. (1978) studied the influence of tumbling on quality and yield of cured hams, they mentioned two tumbling temperatures but unfortunately they do not relate their influence on the results. Regarding protein extraction as a function of temperature, BARD (1965) and GILLET et al. (1977) reported contradictory results for minced meat. The first author observed a decrease in salt soluble proteins with increasing temperature whereas the second found an optimum extraction temperature at 7.2°C.

The purpose of this study was to measure the protein extraction of whole meat cubes soaked in different salt concentrations, in the presence or absence of pyrophosphate, incubated at different temperatures (0 - 20°C). The extracted proteins were identified by SDS-PAGE in order to determine at which temperature myosin extraction occured.

MATERIALS & METHODS

Pig semitendinosus muscles were collected 24 h post mortem, freed of visible fat and cut into cubes of ~ 5 g (~ 1.5 cm). Analytical grade NaCl and tetrasodiumpyrophosphate (PPi) were used for brine preparation. The composition of the brines was 0.5, 1 and 3 M NaCl in the presence or absence of 10 mM PPi, pH 6.0. The meat samples and the brines were equilibrated separately at the experimental temperature for 60 min. Samples were then soaked in 20 volumes of brine and incubated for 24 h at the experimental temperature with orbital shaking (100 rev/min). Brine samples were analysed for protein content following the method of ITZHAKI and GILL (1964) and were prepared for SDS-PAGE by precipitating proteins with 3 M trichloroacetic acid. After centrifugation the pellet was dissolved in 62.5 mM Tris/HCl pH 6.8, 0.4 % DTT (1,4-dithio-DL-threitol), 10 % glycerol, 2 % SDS and 0.01 % bromophenol blue, and heated for 5 min at 100°C. SDS-PAGE was performed with a Protean II (Bio-Rad) slab cell using a modified LAEMMLI (1970) discontinuous buffer system with a 12 % separating gel. The gels were stained with Coomassie Brillant Blue G 250.

RESULTS & DISCUSSION

Protein content

Figures 1 and 2 show the protein extraction as a function of temperature at different salt concentrations. In the absence of pyrophosphate (Figure 1) the protein extraction increased with increasing temperature for the sample soaked in 0.5 M salt. In higher salt (1 and 3 M) more

Figure 1 : Protein extraction as a function of temperature in different salt concentrations

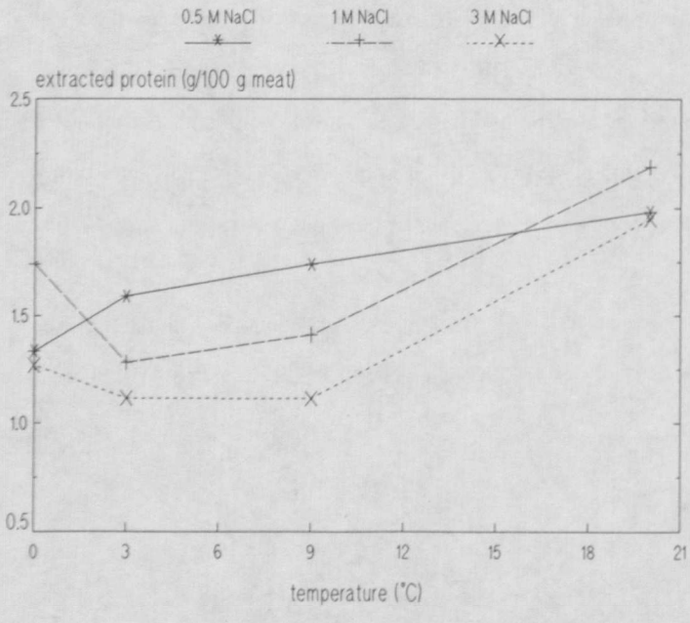
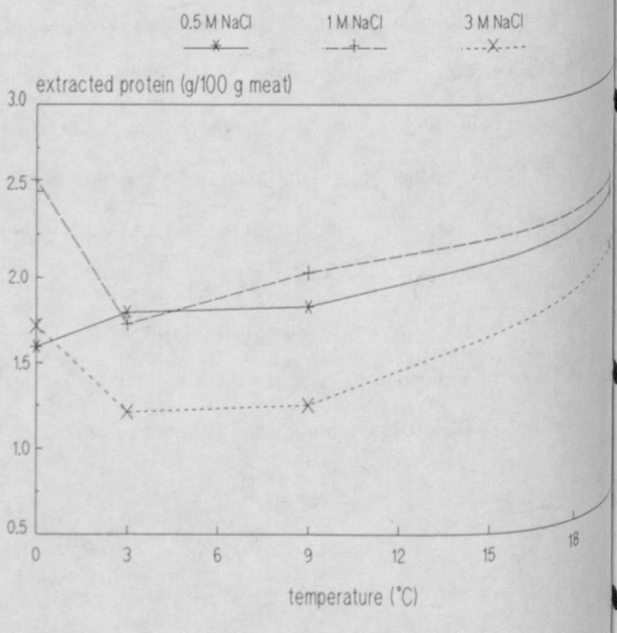


Figure 2 : Protein extraction with added pyrophosphate (10 mM)



protein was solubilised at 0°C than at 3°C and 9°C, then extraction increased again towards 20°C. The effect of temperature on protein extraction has been observed for minced meat by BARD (1965) who reported a gradual decrease of salt soluble proteins when temperature was increased from - 5 to 30°C. The extraction was performed with 3.9 % NaCl (0.67 M). In contrast GILLET et al. (1977) found that maximum extraction was at 7.2°C in 1.28 M NaCl.

The addition of pyrophosphate (Figure 2) resulted in a higher extraction but did not modify the result observed in salt alone, therefore the shape of the extraction curves as a function of temperature was NaCl concentration dependent.

SDS-PAGE

Protein patterns obtained by SDS-PAGE are shown in Figures 3 and 4. In salt alone (Figure 3) a thick myosin band was clearly visible in 1 M NaCl at 0°C. This brine sample also had the highest amount of protein. Only little, or no myosin at all, was extracted from samples soaked in the other salt concentrations at different temperatures. The protein pattern at 1 M NaCl indicated that the amount of salt soluble proteins decreased with increasing temperature, this was in agreement with the result obtained by BARD. As expected, the addition of pyrophosphate enhanced mainly the myosin extraction. OFFER and TRINICK (1983) showed that the presence of pyrophosphate caused myosin to be extracted in lower salt concentrations than salt alone. As shown in Figure 4 myosin extraction occurred in 0.5 M NaCl at 0°C and 3°C whereas no myosin extraction could be observed in 0.5 M NaCl in the absence of PPi. In 1 M NaCl strong myosin bands were present at 0°C, 3°C, 9°C and also at 20°C. At 20° C the myosin band was clearly visible, but the extraction was lower compared with the other temperatures. In 1 M NaCl with added PPi the extraction of creatine kinase and of glyceraldehyde-3-phosphate dehydrogenase was also enhanced compared to salt alone. However no major changes in sarcoplasmic proteins were observed as a function of temperature. In higher

Figure 3 : Pattern of extracted proteins at different temperatures and in different salt concentrations

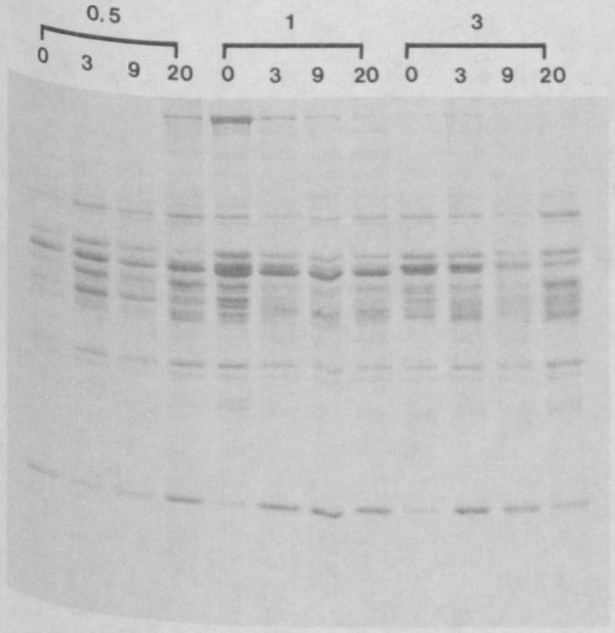
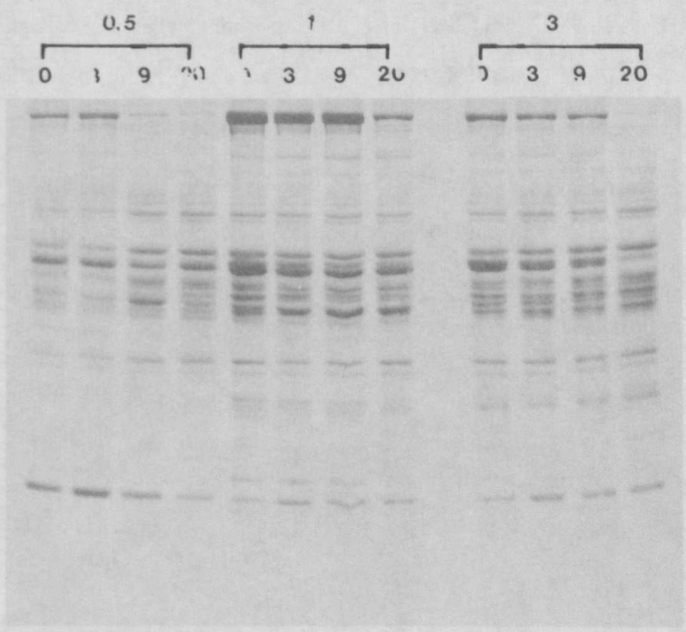


Figure 4 : SDS-Page of brine samples with 10 mM added pyrophosphate



salt concentration is given in (M), the temperature is indicated at the top of each slot in (°C)

salt (3 M) the addition of pyrophosphate also enhanced the myosin extraction compared to salt alone , extraction occured at 0°C, 3°C and 9°C.

Figures 2 and 3 showed an increase in the total amount of extracted proteins at 20°C. The amount of protein was equal, or higher, to the amount measured at 0°C, but the protein pattern observed in the SDS-PAGE gels did not show an enhancement in the extraction of a specific protein. Staining of the stacking gel did not show the presence of a high molecular weight protein, which could indicate myosin aggregation.

CONCLUSION

The temperature influenced the protein extraction from meat but the amounts of protein extracted were salt (NaCl) concentration dependent. The shape of the protein extraction curves changed for low (0.5 M) and high salt (1-3 M) concentrations. The temperature also played an important role in myosin solubilisaton: in salt alone (1M) myosin extraction occurred mainly at 0°C, higher temperatures did not contribute to improve extraction. The addition of pyrophosphate enhanced the myosin extraction in low and high salt and also the extraction at higher temperatures.

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