SWELLING PROPERTIES OF ISOLATED MYOFIBRILS FROM PSE AND NORMAL PORK MEAT

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SUMMARY

Large amounts of pure myofibrils from *M. longissimus dorsi* of normal and PSE pork meat were isolated in sucrose gradients using a zonal rotor. Swelling of the myofibrils was measured spectrophotometrically as decrease in light scatter and by phase contrast microscopy. The onset of swelling varied for different salts, and the swelling was observed over a concentration range, 0,2-0,6 M. PSE myofibrils showed very limited swelling under conditions where substantial swelling of normal myofibrils was observed. Swelling increased with increasing pH. When the ionic strength was raised by the chloride concentration, the swelling was independent of the cations Na^+, K^+ or Li^+. Anions known to affect the myosin-ATP-ase showed the greatest effect. A brine containing 10 mM lactate, 1 mM $MgCl_2$ and 0,35 M NaCl produced the greatest swelling. It was concluded that for practical purposes PSE meat has to be regarded as denatured.

INTRODUCTION

Porcine stress syndrome (PSS) is a problem to the industry, and pale soft and exudative (PSE) pork meat has a reduced water holding capacity. The water holding capacity in meat products depends on the ability of the myofibrils to swell and release their proteins when salt is added. Thermograms obtained by DSC (differential scanning calorimetry) revealed distinct differences between PSE and normal meat (STABURSVIK et al 1981). The low temperature myosin peak (around 58 °C) was more or less absent in PSE meat and had a significantly smaller apparent enthalpy at the thermal transition. This indicated that part of the myosin of PSE meat was denatured. OFFER and TRINICK (1983) pointed out that water losses in rigor and on cooking in the PSE condition may well result from shrinkage of the filament lattice. BOLES et al (1992) found a reduced sarcoplasmic and myofibrillar protein solubility and a reduced degradation of structural proteins in muscle from stress-positive animals. The myofibrils are assumed to contribute most to the water holding capacity. When pH or salt concentration (ionic strength) is increased, swelling of myofibrils occurs. Upon swelling myofibrillar proteins dissolve and a volume expansion and water uptake of the myofibrils results (OFFER and TRINICK, 1983). In practice only a limited number of salts and additives can be used in a brine. The aim of the present study was to compare the swelling properties of isolated myofibrils from normal and PSE pork meat in solutions of different ionic strength and composition in order to propose a brine that could be tested on pork meat.

MATERIALS AND METHODS

After storage of slaughtered pigs (4 °C, 48 h) meat cuts from the 5-7 ribs were characterized directly and after freezing and thawing. Drip, pH, colour reflectance (Gøfo), fiber optic probe (FOP), pork colour standard (PCS) and colour parameters (CIE 1976 L*, a*, b*) were determined (Table 1). Myofibrils were prepared essentially as described by HARBITZ et al (1982). Minced muscle was homogenized in buffer A (25 mM KCl, 39 mM boric acid, 5 mM EDTA, pH 7,4) and centrifuged. The sediment was resuspended in buffer B (100 mM KCl, 39 mM boric acid, 5 mM EDTA, pH 7,4) and centrifuged. To obtain a proper myofibrillar fraction the rotor was decelerated slowly. The myofibrillar supernatant was layered on a sucrose gradient (20-60 %, w/w) containing

Table 1. Characterization of pork loins (see Materials and Methods)

		Normal loin	PSE loin
pH		5,64 ± 0,04	5,15 ± 0,03
% drip		6,1	4,6
Gøfo -	fresh	67 ± 2	45 ± 2
	thawed	68 ± 1	53 ± 5
FOP -	fresh	123 ± 5	178 ± 10
	thawed	117 ± 2	152 ± 10
PCS		3	1
CIE 1976:	L*	54,2 ± 1,8	67,4 ± 3,0
	a*	10,1 ± 0,6	7,3 ± 0,6
	b*	10,9 ± 0,3	14,5 ± 0,5

2 mM EDTA, 10 mM HEPES, pH 7,4 in the Beckman zonal rotor. The gradient was run to equilibrium (15000 rpm, 18 h) and harvested. The myofibrillar fraction was washed twice with buffer A and used directly or stored in glycerol. Glycerol was removed by washing three times with buffer A. Swelling of myofibrils was carried out by mixing 0,1 ml myofibrils, 1 ml buffer A and different salt solutions and water to a total of 3 ml. Swelling was measured after 30 min at 540 nm (HUNTER and SMITH, 1967) using a Shimadzu UV 300 spectrophotometer. Swelling was expressed as percent reduction in light scatter, compared to myofibrils in buffer A; pH was adjusted with NaOH or the acid of the buffer system. Swelling was also studied by phase contrast microscopy. Protein was determined by the Biuret method.

RESULTS AND DISCUSSION

The meat quality measurements shown in Table 1 are typical of normal and PSE loins. The absorbance at 540 nm of normal and PSE myofibrils purified from the loins was proportional to protein concentration. The ratio absorbance/mg protein per ml was 1.6 and 1.7 for normal and PSE myofibrils respectively, indicating that the contraction of PSE myofibrils was higher than that of normal

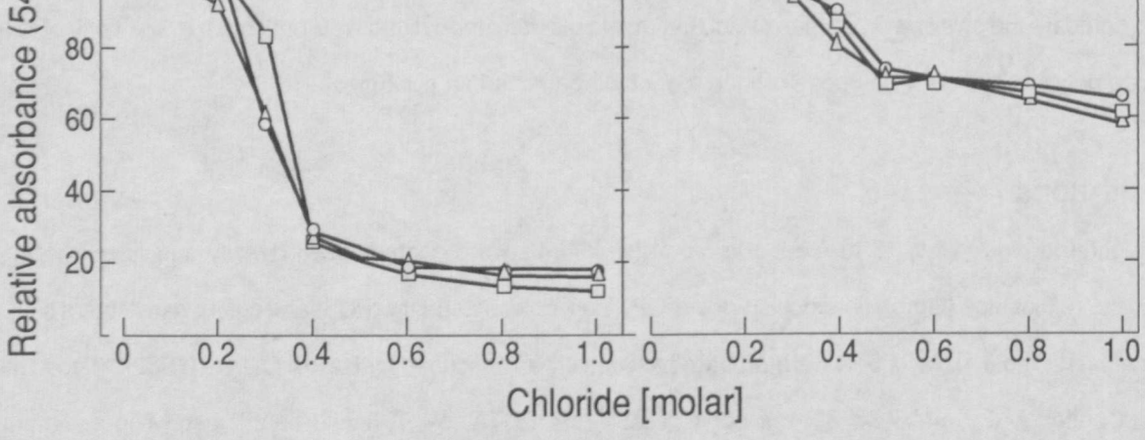

Figure 1. Swelling at pH 7,2 of isolated myofibrils from normal (A) and PSE (B) meat in the presence of (○) NaCl, (△) KCl and (□) LiCl.

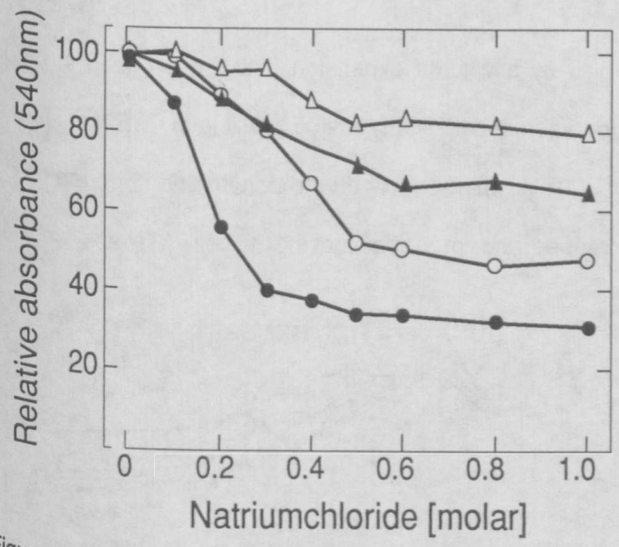

Figure 2. Swelling of isolated myofibrils in the presence of 25 mM sodiumpyrophosphate at pH 5,6,(△) from PSE meat; (○) from normal meat; and at pH 7,0, (▲) from PSE meat; and (●) from normal meat.

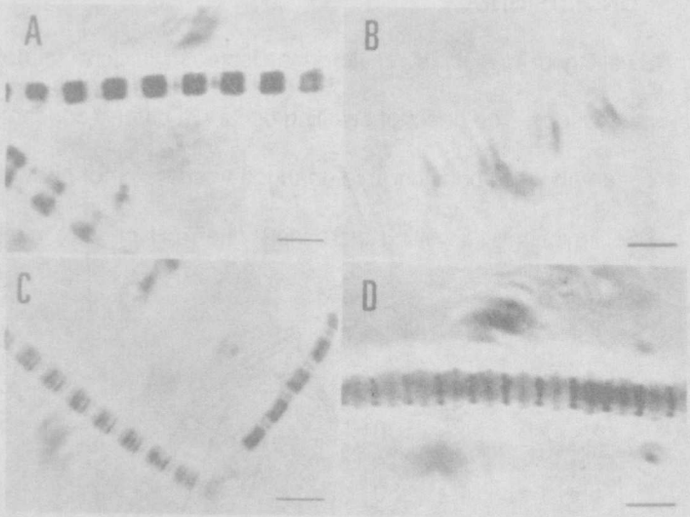

Figure 3. Light microscopic picture of myofibrils. (A) and (B) normal myofibrils at 0,2 and 1M NaCl pH 5,6; (C) and (D) PSE myofibrils at 0,2 and 1 M NaCl pH 7,2. The bar represents 3 μm.

myofibrils. When the ionic strength was raised by the chloride concentration, the difference in swelling properties of normal and PSE myofibrils at pH 7.2 (Figure 1A and B) was independent of the cations Na^+, K^+ and Li^+. Thus, the chloride ion with its negative charge affects the swelling (OFFER and TRINICK 1983). The swelling of normal and PSE myofibrils in the presence of pyrophosphate at pH 5,6 and 7,0 is shown in Figure 2. Myofibrils consist of 50-55 % myosin and 25 % actin with isoelectric points of 5,4 and 4,7, respectively (BAGSHAW, 1982). The increase in negative charges on the myofibrils at the higher pH values explains their increased ability to swell. The limited swelling of PSE myofibrils under conditions where substantial swelling of normal myofibrils was observed can also be seen in the light microscope (Figure 3). This indicated that PSE myofibrils were denatured (STABURSVIK et al 1981), and cannot be properly solubilized by ionic forces. The effect of different ions was tested on normal and PSE myofibrils (Figure 4A and B), and a high influence of lactate on the myofibrils ability to swell was observed. Figure 5A and B show the effect of different ions. Mg^{2+} ions seem to enhance swelling, while the effect of the so called "good buffer" HEPES was limited. It was a general observation throughout the experiments that ions affecting the myosin-ATP-complex increased the swelling ability of the myofibrils. However, none of the salts tested had significant effects on the swelling ability of PSE myofibrils.

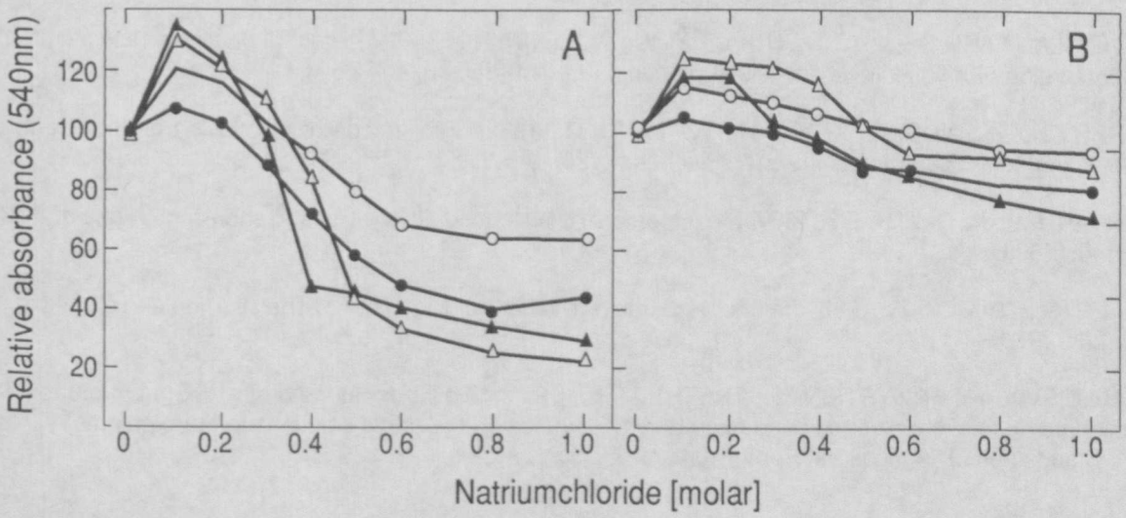

Figure 4. Swelling at pH 5,6 of isolated normal myofibrils (A) and isolated PSE myofibrils (B) in the presence of 25 mM (○) sodium citrate, (●) sodium pyrophosphate, (△) sodium acetate and (▲) sodium lactate.

CONCLUSIONS

Swelling of myofibrils results in a decrease in light scatter parallelled by a volume expansion that can be seen in the lig microscope. The onset of swelling occurs at differnt concentrations for different salts. Only limited swelling of PSE myofibrils wa observed while considerable changes were seen for normal myofibrils. This was probably due to denaturation of proteins in th PSE myofibrils. Swelling increased when pH or ionic strength increased, and ions affecting the myosin-ATP-ase were mo effective.

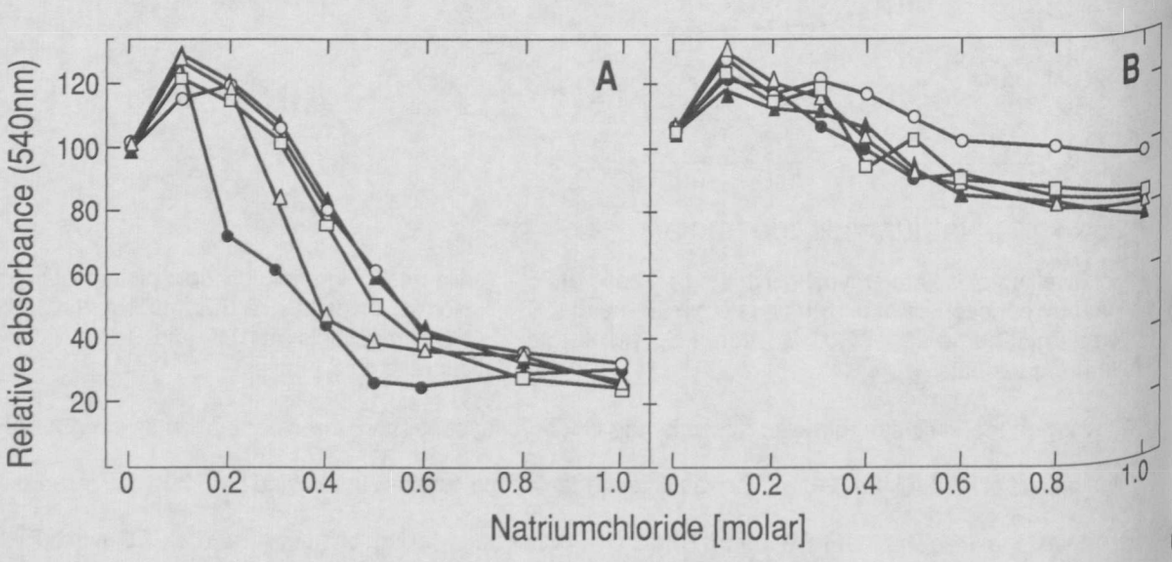

Figure 5. Swelling at pH 5,6 of isolated normal myofibrils (A) and isolated PSE myofibrils (B) in the presence of (□) I mM MgC and 10 mM HEPES (○) 10 mM pyrophosphate, (△) 10 mM lactate, (●) 1 mM $MgCl_2$, 10 mM HEPES and (▲) 10 $MgCl_2$ 10 m HEPES and 10 mM lactate.

In practice the water holding capacity can be increased by NaCl, KCl and magnesium salts of the ions acetate, lactate polyphosphates. A brine containing 10 mM lactate, 1 mM $MgCl_2$ and 0.35 M NaCl may be of practical interest.

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