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OPTIMIZATION OF THE PURIFICATION PROCESS OF ACTOMYIOSIN AND G-ACTIN FROM POST-RIGOR PORCINE SKELETAL MUSCLE

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Introduction

In processed meat products the majority of used meats are in post-rigor state and the separation of the constituents of muscle is necessary for various physiological and biochemical studies. Therefore, the isolation of myofibrillar proteins from post-rigor muscles is important to evaluate the functional properties of post-rigor proteins at the molecular level. Also, in order to study their effect on the interaction with other molecules such as volatile components presents in meat products (Gianelli et al, 2003), it is necessary to obtain high yields of myofibrillar proteins using simplified purification processes. In this sense, there are many extraction procedures for isolation of myofibrillar proteins from muscle (Dudziak and Foegeding, 1988; Pardee and Spudich, 1982) which are very tedious including many extractions processes (Syrovy, 1984). Therefore, it is important to obtain a simplified isolation process for both proteins actomyosin and actin with a purity enough to be used in studies of molecular interactions with volatile compounds.

Objectives

The objective of this investigation was to optimize the isolation of actomyosin and actin from post-rigor porcine muscle based on differential solubility, gel filtration chromatography and extraction times in order to use them for further studies on molecular interactions of myofibrillar proteins with volatile compounds.

Methodology

The methodology used for the purification of proteins (Figure 1) consists on the extraction of pork meat with a buffer containing 20 mM EDTA for three times to eliminate the sarcoplasmic proteins. The extraction of myofibrillar proteins is made with Hasselbach- Shneider solution (0.6 M KCl, 10 mM $Na_4P_2O_7 \cdot 10H_2O$, 1 mM $MgCl_2$ 20 mM EGTA 0.1 M KH_2PO_4/K_2HPO_4 pH= 6.4) (Dudziak and Foegeding, 1988). The pellet obtained is used to prepare the acetone powder by successive three extractions with acetone for 20 min and further filtration. The acetone powder is used for the extraction of G-actin by using buffer A (2 mM tris Cl 0.2mM ATP 0.5 mM 2-mercaptoethanol 0.2mM $CaCl_2$ 0.005% sodium azide pH=8.0 at 25 ºC) with different extraction times.

SDS-PAGE electrophoresis. The purity of the isolated fractions was monitored by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 10 % gels and staining with Coomassie blue R-250 (Laemmli, 1970). The protein concentration in the fractions was determined according to the method of the biochinconinic acid (Smith et al., 1985) using bovine serum albumin as standard.

Results & Discussion

The actomyosin was purified from post rigor muscle using differential solubility processes and gel filtration chromatography (Figure 1). The successive steps of the isolation process were followed by SDS-PAGE electrophoresis as it is shown in figure 2 and the protein concentration is shown in table 1. The first steps extracted the majority of the sarcoplasmic proteins and afterwards, the myofibrillar proteins were extracted using a high ionic strength solution (Dudziak and Foegeding, 1988). The fraction containing the actomyosin protein (M4p) was purify using a gel filtration column in order to obtain a purified actomyosin fraction (M8, Figure 3). The yield obtained for this purification process was 0.14 mg of actomyosin per g of meat. This process is shorter than the proposed by Dudziak and Foegeding (1988) although with a similar yield. However, we did not be able to separate actomyosin from myosin in the chromatography step.

In the same process, the second major myofibrillar protein (actin) was isolated. In order to purify the protein G-actin, the pellet obtained after the extraction with the high ionic strength solution (M3p) was used to acquire the acetone powder instead of using the extractions proposed by Pardee and Spudich (1982). Furthermore, the acetone powder was used for the isolation of actin by successive extractions with buffer A (Figure 1) instead of using depolymerization and repolymerization steps as indicated Pardee and Spudich (1982). A filtration step was added to eliminate the presence of myosin contamination in fraction A4S obtaining a final fraction (A4Sw) highly concentrated in G-actin. The successive steps of the isolation process of G-actin were followed by SDS-PAGE electrophoresis as it is shown in figure 4 and the protein concentration is shown in table 1. The yield of the process was 18.6 mg of G-actin per 1 g of acetone powder or 2.5 mg per g of meat.

Conclusions

Actomyosin and G-actin were isolated from post-rigor porcine skeletal muscle reducing the extraction times in both proteins purification processes. Both proteins were obtained with a recovery of 0.014 g and 0.25 g per 100 g of meat, respectively. However, during the actomyosin purification process it was not possible to separate actin from myosin although an enriched fraction of G-actin was obtained on the further purification process.

References

Dudziak, J.A. and Foegeding E.A. (1988). Journal of Food Science vol 53 nº 5 1287–1290.

Gianelli M.P. Flores, M. Toldrá, F. (2003) J Agric. Food Chem. Vol 51, 6828–6834.

Laemli, U.K. (1970). Nature 227:680.

Pardee J.D. and Spudich A. (1982). Methods in enzymolgy 85, 164–179.

Smith, P.K.; Krohn R.I.; Hermanson g.T.; Mallia A.K.; Gartner F.H.; Provenzano M.D.; Fujimoto E.K.; Goeke N.M., Olsen B.T.; Klenk, D.C. (1985) Analytical Biochemistry, 150, 76–85.

Syrovy, I (1984). Journal of chromatography 300, 225–247.

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Tables and Figures

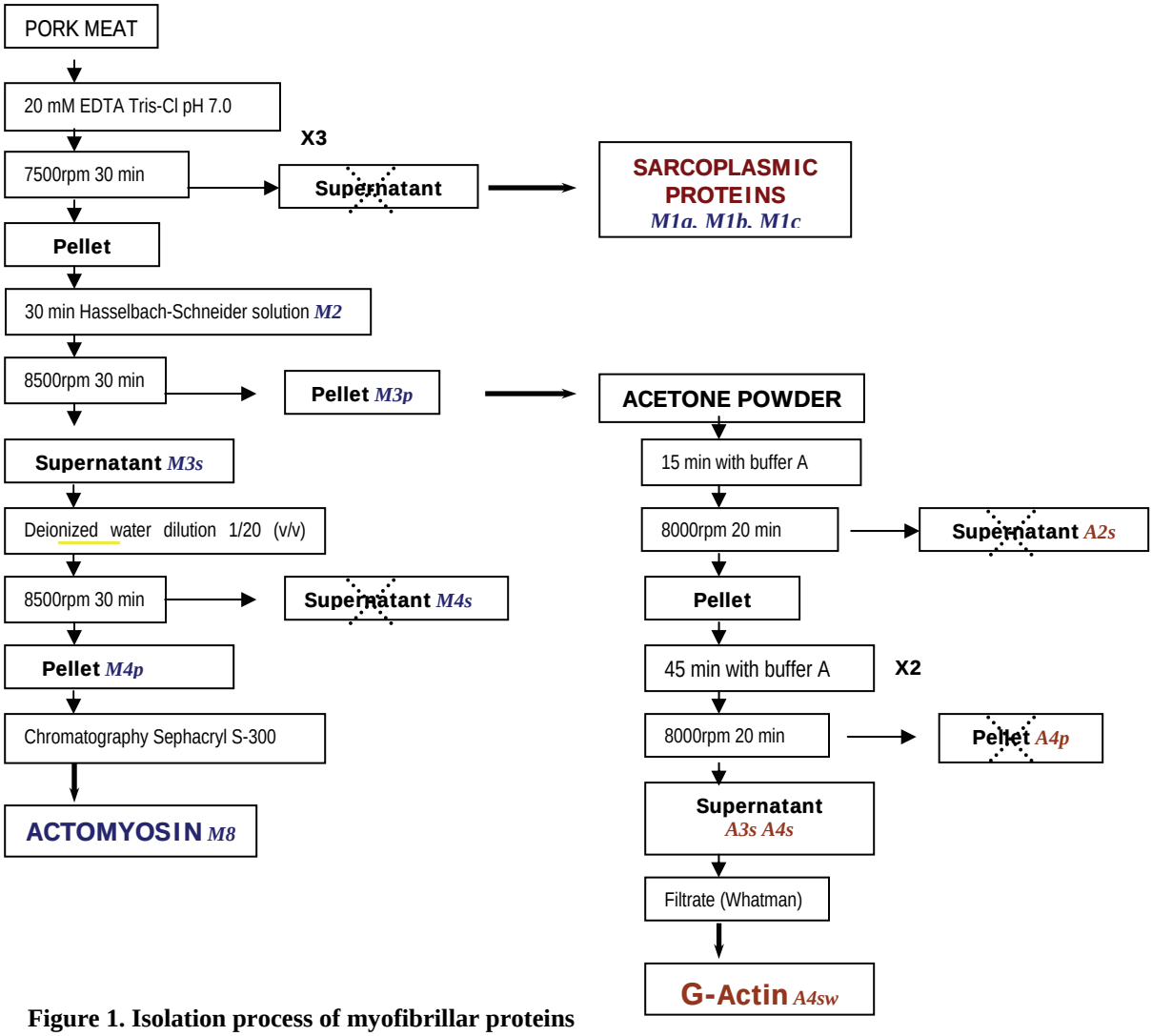

Figure 1. Isolation process of myofibrillar proteins

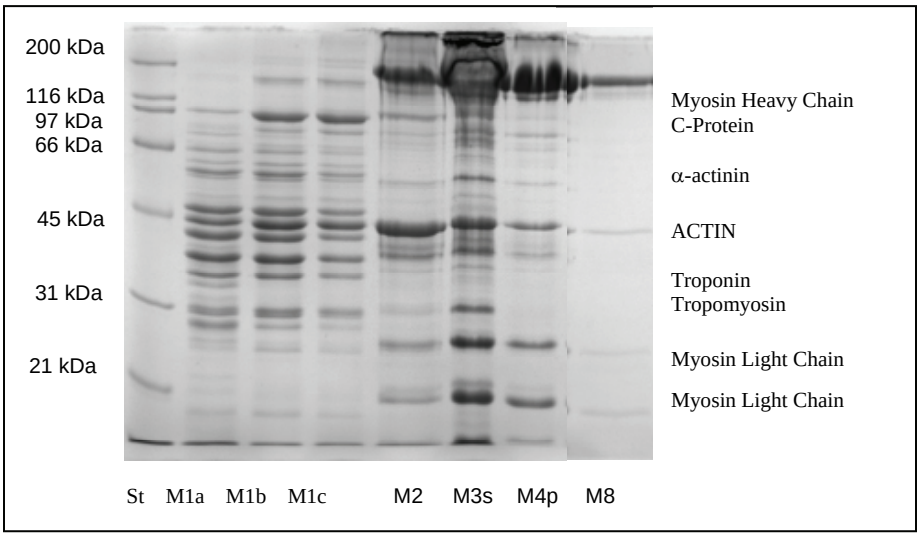

Figure 2. Ten percent SDS-PAGE gel of the actomyosin purification process.

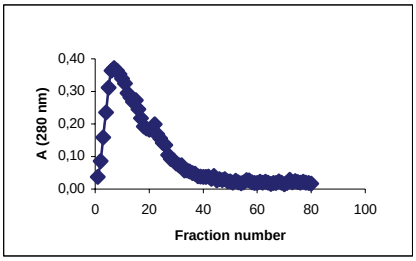

Figure 3. Gel filtration chromatography (Shephacryl S-300) of M4P fraction.

Table 1. Protein concentration (mg/ml) in the isolation steps.

Protein Isolation Step					
Sarcoplasmic proteins					
M1a	M1b	M1c			
18.40	4.70	2.80			
Actomyosin purification					
M2	M3S	M4p	M8		
7.30	6.50	7.10	0.31		
Actin purification					
A2s	A2sw	A3s	A3sw	A4s	A4sw
0.84	0.86	0.45	0.42	0.86	0.86

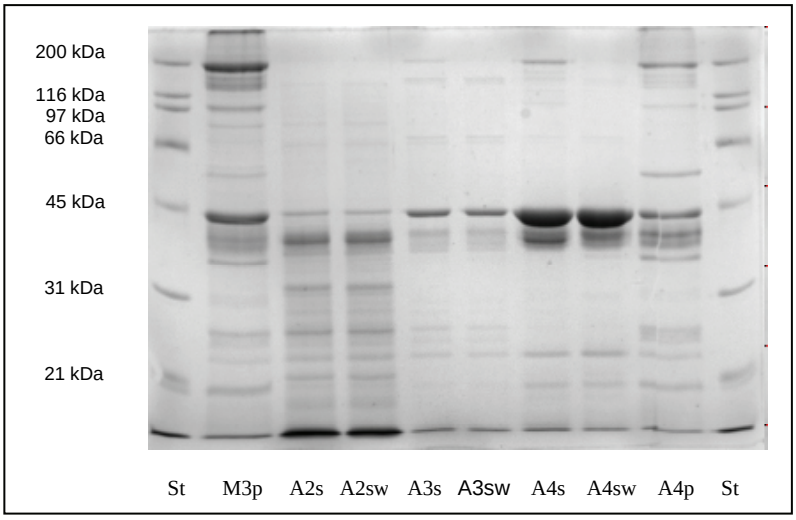

Figure 4. Ten percent SDS-PAGE gel of the G-actin purification process.