FUNCTIONAL AND PHYSICAL-CHEMICAL PARAMETERS OF ACTOMYOSIN FROM PORK MUSCLE OF DIFFERENT CHARACTERISTICS.

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INTRODUCTION

The relationship between functional properties and structural characteristics has frequently been studied in order to explain the varying functional behaviour of myosystems (Kinsella, 1982; Nakai and Li-Chan, 1988). In experiments conducted at our laboratory, a large degree of variability has been found in the results from measurement of functional parameters such as apparent viscosity, in myofibrillar proteins extracted from the identical muscle in different pork carcasses.

Protein functionality has been studied depending on species, muscle type and technological treatment of the myosystem (Jiménez Colmenero and Borderías, 1983; Ashgar et al, 1984; Morrissey et al, 1987; Whittle et al, 1989), but there is scarcely any information available on the influence of the post-mortem characteristics of the same muscle extracted from different carcasses. Factors such as breed, variability among individual animals, ante-mortem treatments, conditions in which post-mortem processes proceed, etc., could all give rise to myosystems of differing characteristics, which in turn might explain certain modifications in some protein functional properties.

This work tries to determine the influence exerted on both functional (essentially apparent viscosity) and physical-chemical parameters as a function of the characteristics proper to pork muscle (evaluated as by means of pH and water holding capacity), especially as the meat of this species typically presents varying characteristics.

MATERIAL AND METHODS

The study was conducted on **Longissimus dorsi** muscle of pork in post-rigor condition. Samples were selected in such a way as to provide varying characteristics of pH and water holding capacity (WHC).

The pH was determined on a homogenate of 5 g of muscle in 100 ml distilled water. Water holding capacity was analysed according to Montero and Borderías (1990): 1 g of minced meat is centrifuged with a small cylindrical filter (Gilson) at about 5000 rpm for 5 min. The amount of moisture collected on the filter was calculated in terms of expressed moisture on a sample moisture content basis. The results are expressed as percentage of water retained.

Natural actomyosin was isolated by the procedure of Kawashima et al (1973) and the extraction process was performed throughout at between 0°C and 4°C. Protein concentration was quantified by the biuret method (Gornall et al, 1949).

The actomyosin (10 mg/ml, NaCl 0.6 M, pH 7.0) was dialysed against 0.2 M NaCl (Tris-maleate, pH 7.5) with 4 buffer changes and analysed as described below. Ionic strength and pH were selected from those displaying the highest levels of apparent viscosity (V) (Cofrades et al, 1992).

Apparent viscosity was measured on a RHEOMAT RM15 MEASURING SYSTEM MS-O (Contraves, Switzerland) at a shearing rate of 28 sec-1. The results are shown in centipoises (cP). Determination of this parameter was performed in triplicate.

Solubility (S) was determined by centrifuging 10 ml of the protein extract for 30 min. at 5000 rpm (temp. 4°C) and quantifying the protein fraction in the supernatant. The results are expressed as percentage of soluble protein. Measurements were performed in duplicate.

Aromatic hydrophobicity (ANS) and aliphatic hydrophobicity (CPA) were evaluated respectively by the procedure of Hayakawa and Nakai (1985) and the procedure of Kato and Nakai (1980) as modified by Hayakawa and Nakai (1985).

ATP-ase activity was measured at 25°C and 3 mg/ml actomyosin according to the method of Kawashima et al (1973) and the inorganic phosphorus released (Pi) was measured by the procedure of Fiske and SubbaRow (1925). Results are given as μmol of Pi released min^{-1} mg^{-1} of protein. Discontinous 10% SDS-PAGE was performed according to Hames (1985).
All analyses were performed on actomyosin from two separate extractions. The level of significance of averages was determined by analysis of variance using a F test.

RESULTS AND DISCUSSION

The characteristics of the muscles studied are given in table 1, which shows differing values for pH and WHC, indicating the existence of post-mortem biochemical differences between the samples analysed. These differences may be attributed to a variety of factors such as variation in individual animals, ante-mortem treatment, conditions in which post-mortem biochemical processes develop, etc. WHC can be justified essentially on the basis of the effect of pH.
The functional properties and physical-chemical characteristics of pork actomyosin are shown in table 2. The results indicate that levels of solubility, ATP-ase activity and hydrophobicity in the proteins from both samples do not appear to be dependent upon those factors which are responsable in the muscle of variations in pH and WHC values. Apparent viscosity, on the other hand, is significantly higher ($P<0.05$) in the protein from the muscle with higher pH.
Both solubility and viscosity are properties that depend on factors relating to the characteristics of the protein (composition, sequence and type of amino acids, diameter and size, number, etc.) and to both protein-solvent and protein-protein interaction (Kinsella, 1982). The differences in the intrinsic characteristics of the two actomyosin samples studied could be due to the existence of differing degrees of inter- and intra-molecular interaction; although these are not apparent in terms of solubility, they are readily appreciable in the viscous behaviour of the system, enhanced perhaps by the conditions in the medium, in which this functional property, under the given experimental conditions, attains optimum levels (Cofrades et al, 1992).
It has been noted that in myofibrillar proteins and concentrated solutions, the importance of the protein-protein interaction is such that its effect on viscosity predominates over all other factors (Borderías and Montero, 1988). When ionic strength is above 0.3 and pH is neutral, myosin molecules are dispersed as monomers but they are assembled into filaments upon lowering the ionic strength. The equilibrium between monomers and filaments shifts towards higher ionic strength with lower pH (Kaminer and Bell, 1966). Thus, the ionic conditions in which the measurements were performed favoured a high degree of protein-solvent interaction, prompted by a slightly-alkaline pH (7.5), and a high degree of protein-protein interaction as a result of low ionic strength and the consequent formation of filaments (0.2M). Again, in AM gels made from PSE and normal pork muscle, Camou and Sebranek (1991) found less protein in the water expelled from normal samples as compared to that from PSE samples, an effect they attributed to a lesser protein-protein interaction in PSE samples.
Other authors (Ashgar et al, 1984) have observed that although there are no significant differences, the specific viscosity of myosin from leg and breast muscle of nutritional stressed chicken tended to be lower than that of "ad libitum" feeding.
The fact that in this study protein solubility did not change in the actomyosin from two muscles with differing pH and WHC, may be due to two factors: firstly, the conditions in which solubility-based protein extraction was carried out (any actomyosin molecule having undergone any change that makes it insoluble is not extracted); and secondly, the ionic strength of the medium was not the best suited for this functional property. Under such conditions, the myosin which constitutes 45-50% of AM (results not shown) is largely insoluble (Huxley, 1963) at either of the two pH levels, and it is precisely in this protein that the greatest change may be assumed to have occurred.

Camou and Sebranek (1991) found no differences in salt-soluble protein levels between normal and PSE muscle, although the range of values for the latter was wider. López-Bote et al (1989) reported a smaller quantity of myofibrillar protein extracted in PSE muscle than in normal or DFD muscle, due to a degree of myofibrillar protein denaturation in the former. It has been found in the present study that, although there were no significant differences, yield in actomyosin extraction was slightly greater in the case of the higher-pH muscle (sample 1 = 9.1%, sample 2 = 8.3%). This may indicate some degree of initial protein denaturation.

Since no significant differences were detected in ATP-ase activity or in aliphatic (CPA) or aromatic (ANS) surface hydrophobicity (Table 2), located in the head region of the myosin molecule (Borejdo, 1983; Cheung and Morales, 1969), it may be deduced that the changes occurring in the head region of the molecule were not sufficiently marked for significant alterations to ensue in the enzyme activity located there or in hydrophobic interactions. The molecular changes giving rise to significant differences in viscosity appear to occur in the tail region of the myosin molecule or to affect the myosin molecule's capacity to form filaments due to irreversible changes in the protein resulting from a reduction in muscle pH. This would concur with the findings of Starsbursvik et al (1984), who reported that part of the myosin muscle from PSE pork underwent extensive denaturation mainly in the light meromyosin segment.

In this study, no significant differences have been found in the AM electrophoretic profiles (results not shown), which is consistent with the findings of Camou and Sebranek (1991) for AM derived from the same muscle but with differing characteristics (PSE and normal). These results indicate that the type of inter- or intra-molecular interactions which might explain the differing behaviour of the samples analysed are susceptible to break down in the denaturing conditions required for SDS-PAGE electrophoresis.

The fact that changes occur in some functional properties of a myosystem, depending on its characteristics, should be borne in mind when considering experiments for the study of functionality, as in some cases this may lead to results of limited significance, or even to erroneous results.

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Table 1. Characteristics of the muscles studied[1].

	pH	WHC
Sample 1	6.0[a]	91.8[a]
Sample 2	5.4[b]	82.5[b]

1. Each value is the average of two determinations.
Different letters in the same column indicate significant differences ($P<0.05$).

Table 2. Functional properties and physical-chemical characteristics of pork actomyosin[1].

	S	V	ATP-ase	ANS	CPA
Sample 1	50.5	117[a]	18.0	195	113
Sample 2	47.5	86[b]	18.9	220	126

1. Different letters in the same column indicate significant differences ($P<0.05$).
S- solubility; V- apparent viscosity; ATP-ase- ATP-ase activity; ANS- aromatic hydrophobicity; CPA- aliphatic hydrophobicity.