PROTEOLYSIS OF SARCOPLASMIC PORK MEAT PROTEINS EVALUATED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract – A reverse-phase high performance liquid chromatography method that enables simultaneous analysis of peptides and proteins was applied for routine monitorization of proteolytic changes that occur in sarcoplasmic pork meat proteins due to the contribution of endogenous and exogenous enzymes from yeast origin. The chromatograms obtained for sarcoplasmic protein extracts, with and without addition of yeast enzyme extracts were analyzed at 0 min incubation and after hydrolysates at 37ºC during 24 h. Qualitative and quantitative differences were observed in the chromatograms. Hydrolysis of sarcoplasmic extracts by endogenous enzymes lead to 33% of protein degradation and an increase of 124% in polypetides of big dimensions, no significant formation of low molecular peptides and medium size peptides was observed. Yeast enzymatic extracts promoted increased hydrolysis of sarcoplasmic extracts (up to 57% of protein degradation) and an increase of 245% in polypetides of big dimensions, 170% in low molecular peptides and 244% in medium size peptides.

Key Words – Endopeptidases and Exopeptidases, Muscle proteins, Proteolysis monitorization

I. INTRODUCTION

Meat industries are constantly searching for methods that improve meat tenderness and upgrade meat products quality [1]. In general, muscle proteolysis causes degradation of both sarcoplasmic and myofibrillar proteins contributing to meat tenderness. Proteolysis can be due to muscle endopeptidases and by the action of certain groups of exopeptidases [2].

The physical features of myofibrillar and sarcoplasmic proteins also play an important role in tenderness. Most methods of improving tenderness will have more impact if exogenous enzymes are used, namely those from plants, bacteria, and fungal sources. According to Sullivan *et al.* [3] the United States federal agencies only recognize five exogenous enzymes – papain, ficin, bromelain, Aspergillus oryzae protease, and Bacillus subtilis protease – as Generally Recognized as Safe (GRAS). However, the use of yeast proteases opens new possibilities of exogenous sources of enzymes with different proteolytic features as recognized by Chaves-López *et al.* [4].

Different analytical methodologies can be used for evaluation of proteolysis of myofibrillar and sarcoplasmic meat proteins; the most traditional are quantification of low to medium molecular weight peptides present in the water-soluble nitrogen (WSN) and non-protein nitrogen (NPN) fractions. Other methods, such as, gel permeation chromatography or sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and reversed phase high performance liquid chromatography (RP-HPLC) can be applied to obtain important information about evolution of proteolytic process.

The aim of this work was the optimization of a RP-HPLC method that enables simultaneous analysis of peptides and proteins for routine monitorization of proteolytic changes that occur in sarcoplasmic pork meat proteins due to the contribution of endogenous and exogenous enzymes from yeast origin.

II. MATERIALS AND METHODS

a. Materials

All reagents used were of analytical grade purity. Eluents for HPLC were filtered through 0.22μm

NL 17 filters and degassed under vacuum for at least 15 min before use

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b. Meat extracts

Pork meat samples (Lombmeat, *Longissimus Dorsi (LD)*) were bought in a local market (butcher). Fat and connective tissue were discarded, and clean pork meat was used to prepare protein extracts.

Sarcoplasmic extracts were prepared according to the method described by Patel *et al.* [5] with few modifications. Muscle samples were minced using a Moulinex A320 Chopper during 5s. Five grams of the minced sample were homogenized in a Ultraturrax T25 Homogenizer (Jankel & Kunkel, Lisboa, Portugal) using 45 ml of homogenization media [HM consisting of 0.05 M Tris (Tris-HCl) with 1.5 mM dithiothreitol and 1.5 mM tetra sodium ethylene diamin tetraacetic acid (EDTA), pH 7.0]. The resulting homogenate was filtered through two layers of cheesecloth to remove large particles, residual fat and connective tissue. A 30 ml aliquot of filtrated was centrifuged at 2,800g for 15 min at 4°C. The pellet was discarded and the supernatant was centrifuged at 11,500g for 40 min at 4 °C and kipped frizzed at -20°C until used.

c. Pork sarcoplasmic hydrolyses

Sarcoplasmic extracts (0.1 ml) were placed in an eppendorf and mixed with 0.7 ml of phosphate buffer 0.1M pH 7. These extracts were analyzed by RP-HPLC at 0 min incubation and after 24h at 37ºC. Other sarcoplasmic extracts (0.1 ml) were placed in an eppendorf and mixed with 0.3 ml of yeast homemade enzymatic extract with proteolytic activity of 0.07 UNITS/ml and 0.4 ml of phosphate buffer 0.1M pH 7 to reach a final volume of 0.8 ml. These samples were also analyzed by RP-HPLC at 0 min incubation and after 24h at 37ºC to promote proteolysis.

d. RP-HPLC conditions

The HPLC equipment consisted on a Gilson chromatograph (Gilson Medical Electronics) equipped with a type 302 pump, a type 305 pump, and a type 7125 Rheodyne Injector with a 500 µL loop. A Gilson 118 variable wavelength ultra violet detector was used. The equipment was controlled by Gilson 712 software that controlled the solvent gradient, data acquisition, and data processing. The column was a reversed-phase Chrompack P 300 RP column that contains polystyrene-divinylbenzene copolymer based packing (8 mm, 300 A°, 150 x 4.6 I.D.). A Chrompack P RP (24 x 4.6 mm I.D.) was used as a precolumn maintained at room temperature with a flow rate of 1 ml/min. The eluted peaks were monitored at 214 nm and gradient elution was carried out with a mixture of two solvents, where the Solvent A consisted of 0.1% TFA in water (TFA, Aldrich Chemicals Co., Milwaukee, WI) and solvent B consisted of 0.1% TFA in acetonitrile (EM scientiRc, Gibbstown, NJ). Run gradients and conditions were: 5% B for 0-5 min, 10% B for 5-10 min, 55% B for 10-30 min and 5%B for 30-45 min.

III. RESULTS AND DISCUSSION

Chromatograms obtained by RP-HPLC analyzes of sarcoplasmic protein extracts, with and without addition of yeast enzyme extracts at 0 min incubation and the respective hydrolysates obtained after 24 hs at 37ºC are shown in Figures 1 and 2. Qualitative differences were observed in the chromatograms. Major meat proteins from sarcoplasmic extracts eluted between 31 and 35 min of retention time (Fig 1A). After 24 h at 37 ºC new peaks were observed in the chromatograms with a retention time between 27 and 30 min and less than 5 min (Fig 1B). Under the chromatographic conditions used polypetides of big dimensions close to original proteins eluted before 30min. Thus, the chromatogram highlights slight proteolysis of meat proteins due to endopeptidases originating polypetides with dimension and polarity close to that of sarcoplasmic protein. The formation of molecules with high polarity and low molecular weight was also observed, probably due to free amino acids. Mixture of meat extracts with yeast enzymatic extracts at 0 min incubation eluted between 31 and 35 min of retention time similar to the profile obtained for meat sarcoplasmic proteins (Fig 2A). Increased proteolysis was observed after 24 h incubation evaluated by the formation of chromatographic peaks with retention time between 27 and 30 min (polypetides of big dimensions), between 15 and 25 min (medium size

peptides), between 5 and 15 min (low molecular peptides) and lower than 5 min (free amino acids) (Fig 2B). The excessive formation of low molecular peptides and free amino acids is undesirable because it can cause modification in

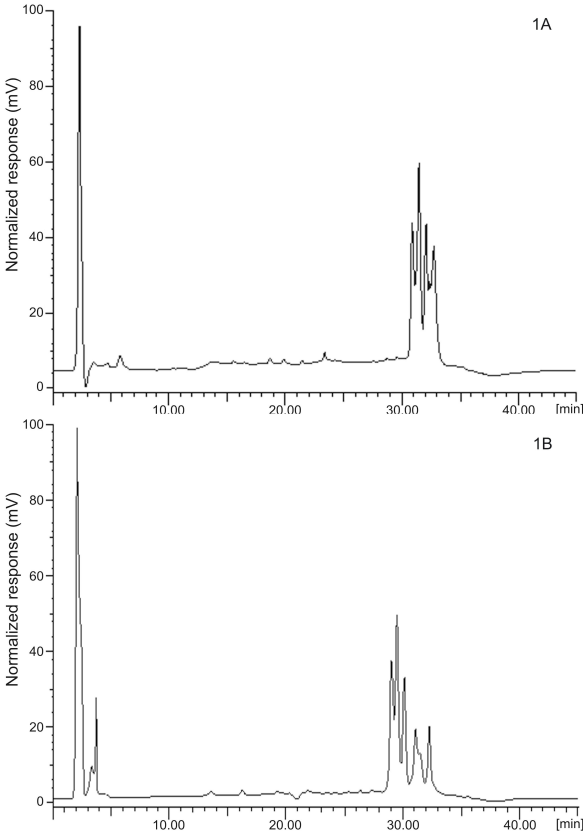

Figure 1. Typical chromatograms of pork meat protein extracts mixed with phosphate buffer 0.1M pH 7: 1A) sarcoplasmic proteins at 0 min incubation; 1B) hydrolysate of sarcoplasmic proteins after 24 h at 37ºC

structural characteristics such as texture and firmness as Fidel *et al.* [6] explained, that an excessive protein breakdown may substantially affect the texture, resulting in an excessively soft meat product and in taste characteristics because an accumulation of peptides and free amino acids that may result in strange tastes, for example, bitter or metallic tastes.

Chromatographic peak areas were used for quantitative measurements and divided in four groups. Peptides formation was evaluated by the sum of peak area with retention time between 5 and 15 min (for low molecular peptides), between 15 and 25 min (for medium size peptides), between 27 and 30 min (for polypetides of big dimensions close to proteins), whereas protein degradation was evaluated by the sum of peak area with retention time between 31 and 35 min. Table 1 presents results from RP-HPLC analyzes of sarcoplasmic protein extracts, with and without addition of yeast enzyme extracts at 0 min incubation and the respective hydrolysates obtained after 24 h at 37ºC.

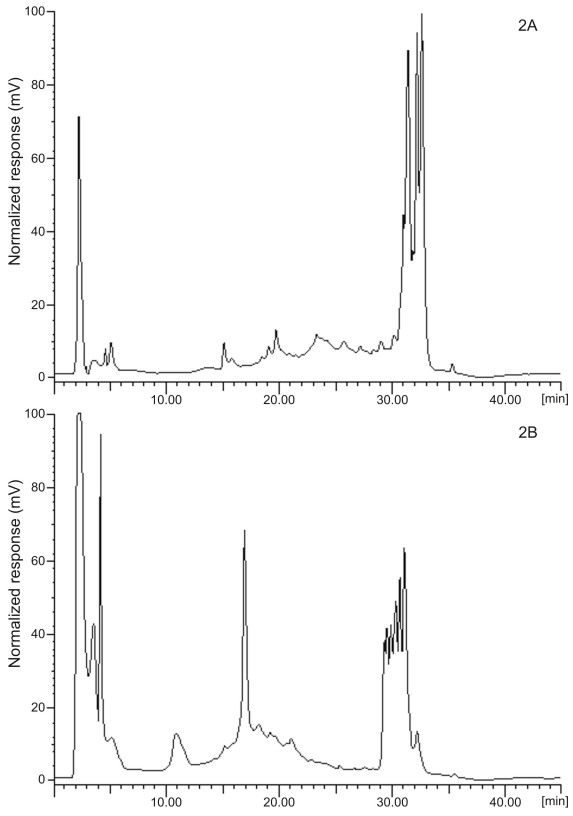

Figure 2. Typical chromatograms of pork meat protein extracts mixed with yeast enzymatic extracts in phosphate buffer 0.1M pH 7. 2A) sarcoplasmic and enzyme proteins at 0 min incubation; 2B) hydrolysate of sarcoplasmic and enzyme proteins after 24 h at 37ºC

Hydrolysis of sarcoplasmic extracts by endogenous enzymes at 37ºC during 24 h lead to 33% of protein degradation and an increase of 124% in polypetides of big dimensions, no significant formation of low molecular peptides and medium size peptides was observed.

Table 1. Representation of peptides and proteins peaks areas of sarcoplasmic proteins and hydrolysates of sarcoplasmic protein extracts.

Rt intervals	Sum of peak areas (arbitrary units x 10^3)			
	Sarcoplasmic proteins at 0 min	Hydrolysate of sarcoplasmic proteins after 24 hs	Sarcoplasmic and enzyme proteins at 0 min	Hydrolysate of Sarcoplasmic proteins and enzyme after 24 hs
5 to 15	2,092	2,194	3,983	13,708
15 to 25	4,175	4,594	16,773	45,382
27 to 30	6,046	13,590	7,405	27,744
31 to 35	11,232	7,540	37,164	15,990

Yeast enzymatic extracts promoted increased hydrolysis of sarcoplasmic extracts at 37ºC during 24 h lead to 57% of protein degradation and an increase of 245% in polypetides of big dimensions, 170% in low molecular peptides and 244% in medium size peptides.

IV. CONCLUSION

The RP-HPLC method is a useful approach for routine qualitative and quantitative evaluation of proteolytic changes that occur in sarcoplasmic pork meat proteins due to the contribution of endogenous and exogenous enzymes and to simultaneous analysis of different classes of peptides and native proteins.

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