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THE PROTEINS OF SQUID MEAT

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Introduction

The contents of the three main fractions of muscle proteins in squid mantle and tentacles differs a little from that in fish, red meats, and poultry. The sarcoplasmic proteins make only about 12 to 20% of the total proteins in Ommastrephes sloani pacificus and the stroma from 2 to 3% [1]. However, the available data regarding the protein fractions in squid of different species are rather scarce. A characteristic feature of the sarcoplasmic fraction is a high activity of proteinases which brings about extensive degradation of the myofibrillar proteins in the course of fractionation. This degradation of myosin was reported only in fresh unfrozen muscle of Ommastrephes sloani pacificus [2]. The myofibrillar proteins of squid differ from these of fish and mammals by being more water soluble. Up to 85% of the total protein in squid mantle can be extracted with distilled water by exhaustive extraction [3]. Paramyosin, the characteristic component of invertebratae muscles, makes up about 14% of squid myosibrillar proteins [4]. According to Iguchi et al. it may be involved in decreasing the rate of protein denaturation in frozen stored squid [5].

The collagen in the muscles and skin of squid contains, according to published data, about twice as many hydroxyproline residues per 1 000 residues as the cod skin. Different samples of collagen, prepared by exhaustive extraction from the mantles, skin, and muscle connective tissue membranes of Loligo and Illex, contain in dry matter according to Sadowska [6] from 0.3 to 4.9%, 3.5 to 6.1%, and 4.7 to 8.0% hydroxyproline, respectively. Other results of the same author indicate

that the collagens in squid belonging to different species differ in their cross--linking as determined by the number of bonds susceptible to hydroxylamine as well as in solubility in salt solutions and buffers. There are only few published data on the properties of different fractions of squid proteins. As the proteins have a great influence on the functional and rheological behaviour of squid meat it is necessary to learn more about them.

Experimental

Tubes of squid Illex argentinus, 23-28 cm long, and whole Loligo patagonica, 13-18 cm in length, frozen on board ship in blocks, glazed and packed in polyethylene and cordboard, stored about 1 year at -20°C were used in the experiments. Samples for the chemical analysis were minced in the frozen state. The main protein fractions were separated by selective extraction (Fig. 1). Collagen was calculated from the amount of hydroxyproline using the factor 22.9 and 20.4, respectively, for Illex and Loligo, after Sadowska [6]. The contents of myofibrillar nitrogen was calculated by subtracting the collagen nitrogen from the nitrogen of the myofibrillar proteins + stroma. The nonprotein nitrogen was calculated by subtracting the nitrogen of the sarcoplasmic proteins and of the soluble collagen from the nitrogen of the supernatant (Fig. 1). The purity of the sarcoplasmic fraction was checked by classical SDS PAG electrophoresis. The proteolytic activity of this fraction was determined against hemoglobin during 2 h at 35°C by assaying the degradation products soluble in 5% trichloroacetic acid according to Folin and Cioceltau.

Results

The contents of proteins separated as the sarcoplasmic fraction according to Fig.1 is in frozen stored squid meat about 15%. This result is in agreement with data known for fresh squid. The statistical significance of the differences in the contents of sarcoplasmic and myofibrillar proteins in the respective body parts of squid belonging to different species (Table 1) cannot be discussed yet as the number of the determinations was very limited and the biological factors like nutritional status and state of sexual development of the specimens could not be taken into consideration. The significant differences in the contents of collagen in squid of different species may be in some extent connected with the eating quality of the respective species. However, as yet there are no published data regarding the influence of particular proteins on the sensory quality of squid, although marked differences in the consumer preference towards various species of squid are known to exist. The proteolytic activity of the sarcoplasmic fraction of

Fig. 1. Flow sheet of protein fractionation

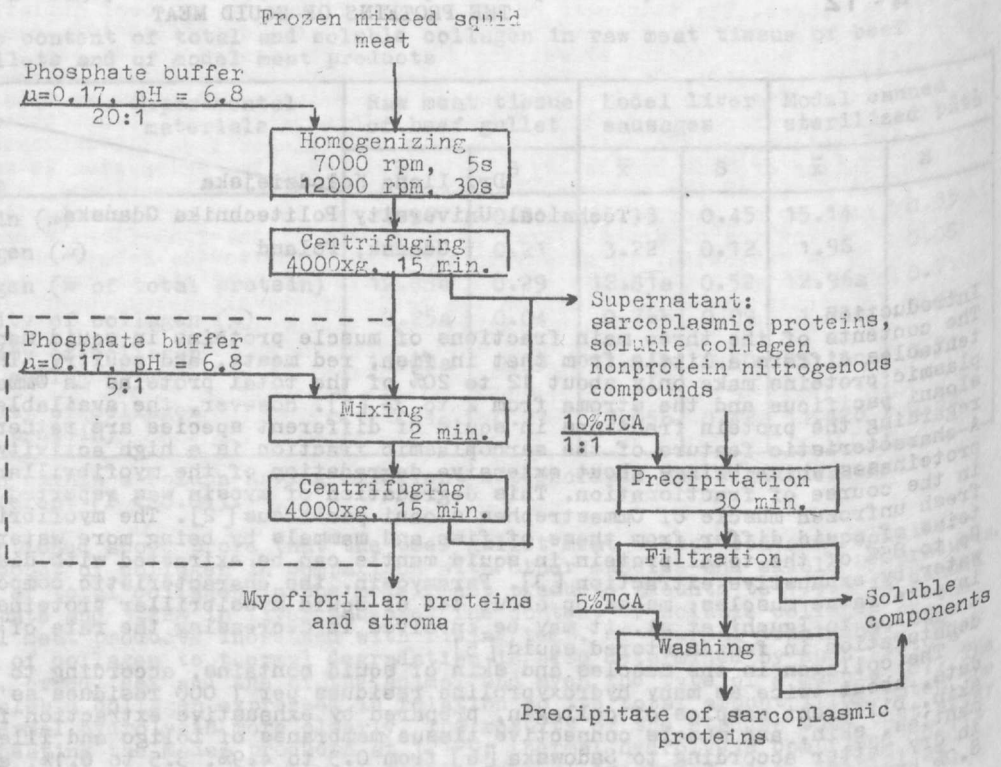

Table 1. The protein and nonprotein nitrogen in squid meat

Species and body part	Total protein nitrogen gN/100g	Protein fractions' nitrogen in total protein nitrogen %			Nonprotein nitrogen in total nitrogen %
		Myofibrillar	Sarcoplasmic	Collagen	
Illex, tentacles	1.95	64.8	15.2	16.0	38.8
Illex, mantle	2.01	74.6	11.5	11.1	38.1
Loligo, mantle	1.85	79.0	14.9	3.0	36.2

All data represent mean values of results obtained by analysing 4 samples taken from one batch of mince prepared from the meat of several squid. The standard deviation for the Kjeldahl nitrogen does not exceed 1.5% and that for hydroxyproline 9%.

frozen stored squid has a sharp maximum at pH about 3 (Fig. 2). It is probably of the cathepsin D type, as there was no significant influence of the inhibitors of thiol and serine proteinases (Table 2). The components of the sarcoplasmic fraction from frozen squid mantle were separated by electrophoresis into 20 distinct bands. The electrophoretic pattern is, however, more influenced by the state of freshness of the squid prior to freezing, as visualized by discolourations of the skin and the muscle surface, than by the species and thus cannot be used for species identification of skinned frozen mantles.

The myofibrillar fraction, obtained by screening the collagen fibers from the meat homogenate on a cloth followed by extraction of the sarcoplasmic fraction, is considerably pure as the residual collagen is not detectable by electrophoresis. The loss of the myofibrillar components during isolation, reported in the literature, can be effectively prevented by using a phosphate buffer at pH 6.8 and ionic strength 0.17 for extraction of the sarcoplasmic fraction. In Illex mantle stored 1 year at -20°C no proteolytic activity in the course of protein fractionation was observed.

Fig.2 The influence of pH on the proteolytic activity of squid sarcoplasmic proteins against hemoglobin

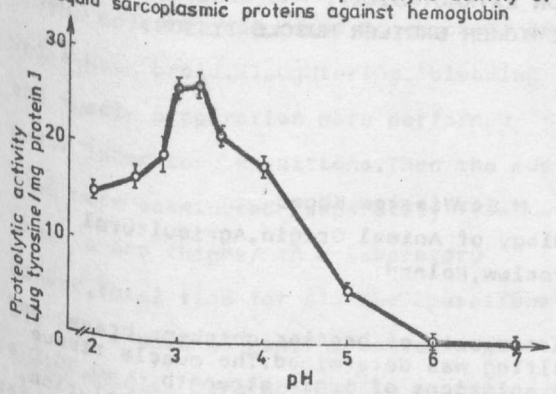

Table 2. The influence of activators/inhibitors on the proteolytic activity of squid sarcoplasmic proteins against hemoglobin at pH 3.0[x]

Activator/inhibitor	$(A^{xx} \pm G)100$
Control	16.2 ± 0.3
EDTA, 1mM	15.4 ± 0.4
Iodoacetamide, 1 mM	14.9 ± 0.4
Soybean trypsin inhibitor, 100 $\mu g/dm^3$	15.1 ± 0.2

[x] mean values of results from 6 determinations in one batch of sarcoplasmic fraction.

[xx] $A = A_1 - A_0$

A_1 and A_0 = absorbancy of the investigated and blank sample, respectively.

Conclusions

The electrophoretic pattern of the sarcoplasmic proteins from squid differing in freshness prior to freezing cannot be used for species identification of the muscles. Several published data regarding collagen in fish and squid meat represent rather the contents of insoluble proteinaceous material (stroma) than true collagen. In case of frozen tissues the stroma may be composed not only of collagen and elastin but also of various, mainly myofibrillar, proteins denatured due to prolonged storage. Our results based on the concentration of hydroxyproline indicate that the muscles of some squid are indeed very rich in collagen. Further research is needed to supply more data on the contents of collagen in various body parts of squid and on the role of the connective tissues in the sensory properties of squid of different species. The fractionation procedure applied in our experiments can furnish reliable results.

References

1. Matsumoto J.J.: Studies on muscle proteins of the squid. Bull. Tokai Reg. Fish.

Res. Lab., No 23, 51-63, 1959.
2. Tsuchiya T., Yamada N., and Matsumoto J.J.: Extraction and purification of squid myosin. Bul. Jap. Soc. Sci. Fish., 44, 175-179, 1978.
3. Matsumoto J.J.: Identity of M-actomyosin from aqueous extract of the squid muscle with the actomyosin - like protein from salt extract. Bull. Jap. Soc. Sci Fish., 25, 38-43, 1959.
4. Horie N., Tsuchiya T., and Matsumoto J.J.: Studies on ATPase activity of actomyosin of squid mantle muscle. Bull. Jap. Soc. Sci. Fish., 41, 1039-1045, 1975.
5. Iguchi S.M.M., Tsuchiya T., and Matsumoto J.J.: Studies on the freeze denaturation of squid actomyosin. Bull. Jap. Soc. Sci. Fish., 47, 1499-1506, 1981.
6. Sadowska M.: Unpublished, Politechnika Gdańska, 1984.

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