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CHARACTERIZATION OF MYOFIBRILLAR PROTEINS OBTAINED FROM FRESH ABRAMIS BRAMA (COMMON BREAM) MEAT

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Abstract

It must be pointed out that fish protein ingredients production has a growing trend all around the world because their low-cost, high nutritive quality and more concentrated protein levels. The current study refers to miofibrilar protein extraction and their characterization, from fresh carp meat. Acid pH dissolution, followed by precipitation at isoelectric pH was used as extraction method. The myofibrillar protein chemical characterization was made by taking into account the functional and rheological properties. Spray-dryer method for myofibrillar proteins solubilized at alkaline pH and acid pH and scanning electron microscopy (SEM) was used for dry. The solubility of the muscle proteins, constituent components of protein derivatives, is a critical property that controls the other functional characteristics of the protein (emulsifying capacity, foaming and gel formation). The protein concentrates/isolates, studied by their functional properties, protein solubility and gelling characteristics, can be suitable raw materials for protein films and biodegradable coatings generation.

Key words*: abramis brama, common bream, gelling characteristics of proteins, myofibrillar protein, solubility of the muscle proteins.*

INTRODUCTION

Fish myofibrillar protein concentrate is an important under utilised commodity in Romania even though they are known to be good source of protein and other nutrients. A variety of raw materials that come from aquaculture and marine are used for obtain fish protein concentrate. Abramis Brama (Common Bream)is in volume terms by far the most important aquaculture species in world fisheries. We focused on that food sources because they are most available to them.

Production of fish protein ingredients such as fish myofibrillar protein concentrate is growing throughout the world. It ingredient is a low volume but high value and relatively low prices and it can serve as active ingredients in functional foods, in edibles film and other health related products.

The pH of the protein environment are one the most important factor affecting protein solubility, conformation and functional properties, such as emulsification and foaming ability.

Protein is, after water, the most important constituent of animal bodies.

Myofibrillar proteins are located in myofibrils, contribute in the filamentous organization of the muscle and directly participate in the mechanochemical process of muscle contraction and stiffness. Structural proteins are the most abundant protein fraction of muscle tissue (54-70% of total muscle protein).

Myofibrillar proteins, from technological point of view, contribute to meat tenderness, determine the capacity of water retention and hydration of meat, fat emulsifying and gelling capacity. Myofibrillar proteins by high intake of essential aminoacids, contribute about 70% to the nutritional value of meat. (Ionescu et al., 2009).

Myofibrillar proteins have intermediate solubility between sarcoplasmic and stromal proteins (insoluble in water, but soluble in saline solutions with ionic strength higher than 0.3 or in solutions with controlled pH). They are fibrillar proteins that associate with each other to form complex parallel structures (Cuq et al., 1995).

Meat proteins have important functional properties, such as: water holding capacity, gelation and emulsification.

The factors which influence heat-induced gelation properties were studied for different myofibrillar proteins, in particular, beef, pork, poultry, fish and rabbit myosin (Fretheim et al., 1986; Smith et al., 1988; Hennigar et al., 1989; Chan et al., 1992; Xiong, 1992; Lan et al., 1995a, 1995b; Boyer et al., 1996a, 1996b). Gelation of muscle proteins involves partial denaturation followed by irreversible aggregation of the ends of myosin by the formation of disulfide bonds and the transition of the molecule's body from the helix form to spiral which results in a three-dimensional network structure (Samejima et al., 1981; Smith et al., 1988; Sharp et al., 1992; Stone et al., 1992). During gelation, myosin and other salt soluble proteins undergo complex changes of the rheological properties, depending on the temperature and pH at which they are exposed (Egelandsdal et al., 1986; Xiong, 1993).

MATERIALS AND METHODS

1. Raw materials

Fresh Abramis Brama (Common Bream) specimens (≈1000 g) were obtained from a commercial supplier. The samples were transported to the laboratory in ice. Immediately on reaching the lab, the fishes were thoroughly washed with cold water to remove blood, slime, dirt, etc. After decapitation and evisceration, fishes were de-skinned and filleted. The fillets were immediately used for myofibrillar proteins preparation.

2. Myofibrillar proteins extraction.

Myofibrillar proteins were extracted from fish muscle according to following methods: alkaline pH method; acid pH method and KCl + EDTA. The resulting concentrates myofibrillar proteins were used immediately for determination of their physicochemical properties and for protein recovery, also. The physicochemical properties of fish protein concentrateswere determined according to Association of Official Methods of Analysis (AOAC, 1990; Ionescu et al., 1992). Crude protein contentwas calculated by multiplying N by 6.25. The results were expressed as g/100g protein concentrate. Nitrogen (N) was calculated using the Kjeldahl method (AOAC, 1990; Ionescu et al., 1992) (Raypa, Spain). Crude lipid contentwas determined according to Soxhlet method described in (AOAC, 1990; Ionescu et al., 1992) (Solvent extractor VELP Scientifica SER148, USA). The results were expressed as g/100g protein concentrate. Crude ash contentwas obtained by heating the protein concentrates in a furnace at 550°C for 24 h. Crude ash was determined according to method. The results were expressed as g/100g protein concentrate. Protein recovery (% yield) of the washed mince from different washing methods was determined according to the method of Kim and al. The recovery of protein was expressed as % in DM (dry matter).

The pH was measured potentiometric, using the pH meter type "Hanna" using protein dispersions with a concentration of 10% (G/V)), at a temperature of 22 ± 1^0C.

All chemical analyzes were carried out in duplicate.

3. Myofibrillar proteins characterization.

Finally, after finding the bestmyofibrillar proteinsextraction methods for fresh fish, the functional properties of myofibrillar proteins obtained by these methods were determined. Functional properties such as foaming capacity, emulsion capacity and solubility, are important factors if fish proteins are to be incorporated into a food or dish as additives during preparation.The solubility of protein obtained from best method of extraction was measured according to the method of Choi and Park with slight modifications. The protein solubility was calculated on the basis of 100% solubility of the protein.The emulsion capacity was calculated by dividing the emulsion volume after centrifugation by the original emulsion volume and then multiplying by 100. Emulsifying stability was determined by the same procedure except that, before centrifugation, the emulsion was heated at 90°C for 30 min followed by cooling in tap water for 10 min.The method of Miller and Groninger wasused to determine foaming properties. The foam was calculated as the volume of mixture after blending compared to the original volume. The foaming stability was the ratio of the foam capacity after time

observation divided by the original foam capacity.
The determination was performed in duplicate.
4. Gelling properties
The gelation properties were determined by dynamic rheological measurements at oscillations of small amplitude, performed by a voltage-controlled rheometer (AR 2000, TA Instruments, New Castle, DE), attached to a control software computer (Rheology Advantage Data Analysis Program, TA , New Castle, DE). The temperature was monitored using a Peltier temperature control system. All rheological measurements were made using a cone plate geometry of 40 mm with an angle of 2° and a gap of 2000 μM. For each test, about 2 g of protein suspension was placed at the base of the rheometer plate. To prevent dehydration low viscosity silicone was added around the edges of the plate. The measurements were made at a constant angular frequency of 0.3142 rad / min (0.05 Hz frequency) by scanning the temperature ranges 4.3 – 74.9° C and 31-80°C. Changes in storage modulus (G') and phase angle or deformation (δ) were recorded depending on the temperature. The heating rate was programmed to 1°C/min. For all samples the linear viscoelasticity domain was established at a constant temperature of 20°C and at a frequency of 0.10 Hz. For each test, the sample was kept for 5 minutes for temperature equilibration. Samples were running in duplicate.

RESULTS AND DISCUSSIONS TNR 12

1.Determining the approximate chemical omposition

Table 1. Proximate chemical composition of Abramis Brama (Common Bream) meatand a protein concentrated obtain.

Sample nature	Water (%)	Proteins %	Fat %	Ash %	Other
MHAB	76.52	17.21	4.28	1.26	0.71
CPMAB 1	82.05	16.42	0.52	0.06	0.93
CPMAB 2	84.38	13.84	0.59	0.05	1.12
CPMAB 3	85.82	12.75	0.56	0.09	0.75

MHAB - Muscle homogenate of Abramis Brama
CPMAB 1 -Protein concentrate – Alkaline extraction
CPMAB 2 - Protein concentrate – Acid extraction
CPMAB 3 - Protein concentrate - KCl and EDTA extraction
Depending on the method of extraction is higher protein concentrate (more pure) the method of extraction by acid leaching at pH compared with other methods, as shown in the (Table 1).
2.Protein solubility
The solubility characteristics of the myofibrillar proteins are interesting because of the relationship with other functional properties, particularly the gelling and water retention properties (Hultin et al., 1995). Muscle proteins are properly differentiated by their solubility.
To find the proper pH values for maximum solubilization and recovery of muscle protein, we constructed the solubility curves (protein concentration versus pH) for myofibrillar protein concentrates and isolates.
Protein solubility curves are shown in the Figure 1. Solubility profiles were similar for all analyzed protein paste.

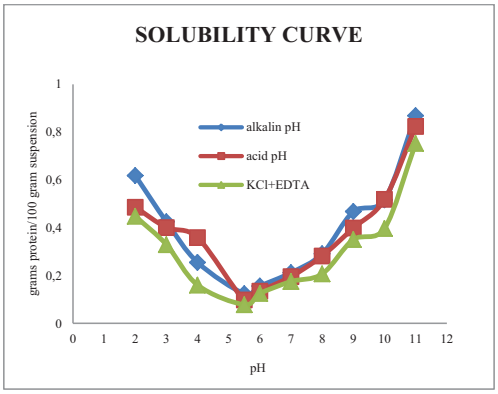

Figure 1. Myofibrillar protein solubility of Abramis Brama (Common Bream)

Fish concentrates showed minimum solubility in isoelectronic range with pH ranging from 5.5 to 6.0, characteristic for most muscle protein (Xiong, 1997), the lowest values of protein solubility being observed at pH 5.5. For protein concentrates / isolates obtained by alkaline and acid solubilisation, higher values of solubility at pH 5.5 were recorded (12.46 to 9.88 % per s.u.) than protein concentrates obtained by

washing the minced meat with water with or with different solutions (KCl and EDTA) (7.85 to 7.31% per s.u.). We explain this by the presence, in the composition of those concentrates, of sarcoplasmic proteins soluble in water and in solutions of low ionic strength and which represent 20-30% of the muscle proteins (Haard et al., 1994; Ionescu et al., 2009).

Lowering the pH to the isoelectric point resulted in a substantial increase in the protein solubility up to a pH of 2.0 where the proteins exhibited a solubility of more than 45% for all the samples we tested. The maximum solubility was reached at pH 2.0 (for the concentrate obtained by solubilization in alkaline pH and the one obtained in acidic pH).

Increasing the pH value relative to pI (isoelectric point) leads to increased solubility, suddenly up to 7, than we have a gentle slope to reach the maximum solubility at pH 11.

By changing the value of the pH of the protein solution, the protein gains a net negative or positive charge at which the moisture of the charged residues and electrostatic repulsion causes an increase in solubility (Damodaran et al., 1996). At pH values close to the isoelectric pH of the protein, the repulsion between the chains of the proteins is reduced and their association occurs. As a result, most of the proteins have minimum solubility at the isoelectric point (pI), since the lack of electrostatic repulsion promotes hydrophobic interactions (protein-protein) and aggregation of the protein molecules. Because of the protein aggregation under these conditions, they can be separated from the solution by means of an appropriate centrifugal force.

At pH below 5.5, the proteins become negatively charged resulting in electrostatic repulsion which facilitates protein to bind water and swell. Also, at pH higher than the isoelectric point, proteins gain positive net charge resulting in repulsion, hydration of the proteins and increase in their hydrodynamic size, viscosity of the protein solutions (Damodaran et al., 1996).

3.Gelling properties

Myofibrillar proteins are responsible for the textural properties of the processed meat products (Yasui et al., 1980; Asghar et al., 1985).

Among the myofibrillar proteins, myosin and actomyosin contribute most to the development of gel characteristics of salted meat processed products.

We studied the gelling properties of some carp homogenized muscle and wet protein concentrates obtained from Abramis Brama (Common Bream).

In our study, we followed the rheological behavior of protein suspensions by scanning a wide temperature range (4.3-74.8^{0}C or 31-80^{0}C) and monitoring parameters: elastic modulus and phase angle (delta). Rheological measurements were determined by dynamic rheological method at small deformation, non-destructive, conducted in the linear region of viscoelasticity, which enables the determination of the elasticity and viscous nature of the tested sample.

Elastic shearing modulus (storage or storage facilities, G') is a measure of the released energy per cycle of deformation per unit volume and the property which makes the correlation with the elastic nature of the material.

Phase or deformation angle (δ) is a measure of the prevalence of viscous properties (characteristic to the liquids) and elastic properties (characteristic to the solids) in the viscoelastic behavior of a material. The phase angle is related to the formation of bonds in the gel during the heating/deformation, mainly in temperature increase/oscillation frequency decrease.

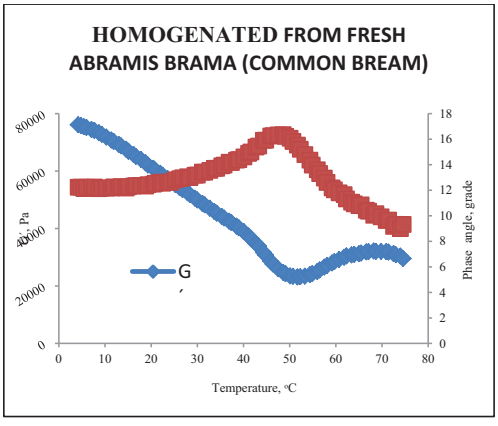

Figure 2. Shows the rheological behavior of Abramis Brama (Common Bream)

As can be seen, the values of the elastic modulus and phase angle (δ) of the homogenate

and the Abramis Brama (Common Bream) muscle protein derivatives have evolved differently depending on the temperature domain and the nature of the sample.

In the case of homogenated Abramis Brama (Common Bream) muscle (pH 6.3), elastic modulus had a moderate downward trend in the temperature domain between 4.3-35.9°C, characterized by high values of G ', 76140 Pa at 4.3°C and 43700 Pa at 35.8°C. This interval is followed by another temperature domain (35.9-51.7°C) characterized by a more significant reduction of this parameter to a minimum of 23150 Pa (51.7°C). In these temperature ranges, the reduction of storage module can be attributed to the complex structure of fish muscle proteins due to denaturation of certain protein fractions. Denaturation of the quaternary structure, tertiary and secondary when applying external stress (heating) possibly involved subunit dissociation of protein filaments, breaking of the disulfitic bonds (-S-S-), dipole-dipole non-covalent interactions between polar aminoacids and interactions between non-polar aminoacids in the side chains, as well as partial conversion of α-helix structures and ß-folded at the configuration of random twisted spiral.

The thermo-rheogram, shows below, a portion close to a plateau in the 50.7-59.7°C domain, possible characteristic to the denaturation and simultaneous aggregation of some protein fractions, given the complex nature of the system investigated. Our findings are in agreement with those reported by Westphalen, etc. (2005), who found the existence of the plateau in the range of 50-57°C, for myofibrillar protein samples with a 6.0 pH and lower concentration.

Starting with the inflection point of the curve (51.7^{o}C), elastic modulus values increased very slowly at first, then the increase was accelerated when the temperature was raised above 59.7^{o}C to the finalization of the heating process at 74.6°C. This rheological behavior is typical for the thermal gelation of Abramis Brama (Common Bream) muscle proteins and for the increase of the formed gel strength. The gel formation involves irreversible aggregation of denatured proteins to form new disulfitic bonds, in particular, between the globular myosin ends and the transition of the helical spiral the myosin molecule rod to a three-dimensional network structure (Stone et al., 1992; Sharp, et al, 1992; Samejima et al., 1981). Changes in rheological characteristics depending on the temperature of the Abramis Brama (Common Bream) homogenate are confirmed by the evolution of the phase angle. The thermo-rheogram of the phase angle indicates a reverse trend relative to the elastic modulus. Low values of the phase angle, between 8.998-16.34 grade, across all the temperature domain of 4.3-74.6°C are specific to the viscoelastic bodies at which elastic component was permanently predominant relatively to the viscose component. The base zone of the elastic modulus in the thermo-rheogram corresponds to the highest value of phase angle,> 12.0°.

Below are presented the thermo-rheograms of the elastic modulus and phase angle for wet protein concentrates, extracted from Abramis Brama (Common Bream) muscle by the alkaline and acid procedure and extracted by washing with KCl and EDTA. The thermo-rheogram profile of the protein concentrates was similar with the one of the Abramis Brama (Common Bream) muscle homogenate except for the elastic modulus values which were different, being much higher for the muscle homogenate (see the table).

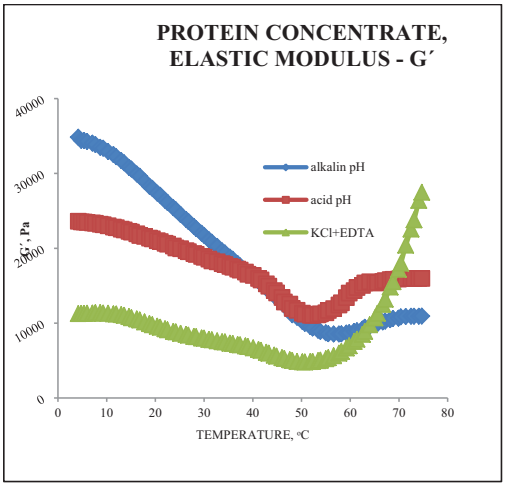

Figure 3. The elastic modulus change depending on temperature and changing of the phase angle depending temperature

If we compare the three types of protein concentrates (acid, alkalin, and KCl) it can be observed that the values of G' were higher for the alkaline protein concentrate compared with the acid one and the one extraced by washing with KCl. For the three types of protein concentrates, the transition temperature from ground to gel was the same (50.8°C), slightly lower than the one registered for the muscle homogenate (51.9°C).

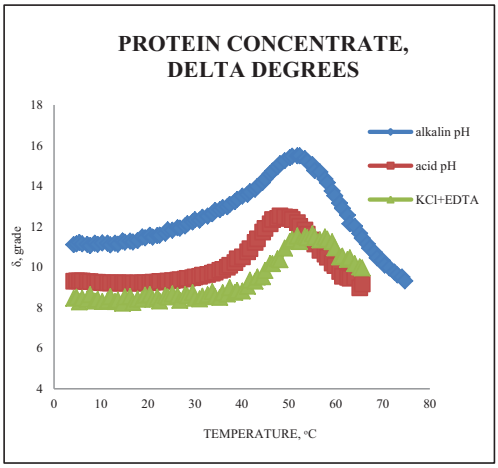

Figure 4. The influence of temperature on the phase angle values

The modifications of the rheological properties on heating of the Abramis Brama (Common Bream) protein concentrates compared to the Abramis Brama (Common Bream) muscle homogenate we ascribe on the greater complexity of the homogenate, differences in protein content and characteristic pH values and potential denaturing changes in the protein system during extraction treatments (Yongsawatdigul and Park, 2004).

Table 2. The temperature dependence of the elastic modulus and the method of extraction of muscle proteins

Sample nature	Elastic modulus (G´), Pa			
	4.2°C	50.7°C	51.7°C	74.6°C
MHAB	76140	-	23150	29560
CPMAB 1	34840	9975	-	10940
CPMAB 2	23620	11210	-	15990
CPMAB 3	11280	4809		27490

MHAB - Muscle homogenate of Abramis Brama

CPMAB 1 -Protein concentrate – Alkaline extraction

CPMAB 2 - Protein concentrate – Acid extraction

CPMAB 3 - Protein concentrate - KCl and EDTA extraction

Protein concentration and pH are very important parameters in thermal gelation of meat protein. In addition, it is a well-known fact that during the extraction of muscle proteins by the acid procedure, due to the high concentration of hydrochloric acid suffers modifications which influence the functional and rheological properties.

Reduced capacity to form gels of acid treated protein, when compared to those treated under alkaline conditions may be attributed to conformational changes (partial loss of myosin heavy chain) or due to the unfavorable conformation of the protein during the acid treatment (several hydrophobic groups leading to larger aggregates and to a less ordered gel). Another explanation could be that related to the presence of denatured sarcoplasmic protein that are retained in the acid process, but not in the alkaline one (Ingadottir, 2004).

CONCLUSIONS

The protein content of protein derivatives was conditioned by the extraction technique applied.

Solubilization of muscle proteins, in a strongly alkaline medium, followed by their precipitation in the solution at the pH of isoelectric point (pI) also ensures the recovery of sarcoplasmic proteins which precipitate at 5.5 pH.

The solubility of muscle proteins, components of protein derivatives, is a critical property it controls the other functional characteristics of the protein (emulsifying, foaming and gels formation capacity).

The variation of the elastic modulus (G') and phase angle (δ) during thermal treatment of protein suspensions reflects profound changes in the protein system (denaturation, dissociation and reassociation) depending on the temperature.

All concentrates/isolates of muscle protein behaved, from rheological point of view, as viscoelastic systems with high elastic

component, but variable depending on the temperature, source of proteins, extraction method and drying process through lyophilization.

Elastic modulus values were directly proportional to the protein concentration from proteic suspension. The correlation coefficients between protein concentration and elastic modulus during heating (30-71.9^{o}C) showed values above 0.930, values slightly higher at lower protein concentrations.

The analyzed protein concentrates/isolates have functional capabilities suitable for use in various systems based on meat, bringing products added nutritional value through their protein component but their production is only justified economically for species of inutilisable fish, inferior quality meats and some organs.

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