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RHEOLOGICAL PROPERTIES DESCRIPTION OF MYOFIBRILLAR PROTEIN HOMOGENATES AND CONCENTRATES OBTAINED BY DIFFERENT METHODS AND FROM DIFFERENT SPECIES

Floricel CERCEL[1], Mariana STROIU[1], Daniela IANIŢCHI[2], Petru ALEXE[1]

[1]Dunarea de Jos University of Galati, Faculty of Food Science and Engineering,
111 Domneasca St., 800201 Galati, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Animal Science, 59 Marasti Blvd, 011464Bucharest, Romania

Corresponding author email: fcercel@ugal.ro

Abstract

In our study we aimed the rheological behavior of fish myofibrillar proteins and of the homogenates of which these were obtained. For protein extraction and concentrates purification different methods were used. We tracked the variation of elastic modulus and phase angle according to temperature.
Myofibrillar proteins determine the water retention and meat hydration capacity, fat emulsifying and gelling capacity.
The source and the method of extraction influence the gelling properties of muscle protein. The protein concentration plays a key role in determining the heat induction properties of gels.
Gelling properties of the muscle proteins are also influenced by heating temperature, temperature rise speed and of various adjuvants.
Solubility and gelling properties are also important for obtaining edible films based on these proteins and also to obtain microcapsules.

Key words*: elastic modulus, fish protein concentrate, gelling properties, myofibrillar proteins, phase angle.*

INTRODUCTION

The manufacture of meat processed products involves the use of different types of meat, organs, edible subproducts and fat (as raw materials), derived mainly from cattle, sheep and swine, along with a large number of non-meat ingredients, with a very important role in the formulation of various products (Banu, 2009). These ingredients stabilize mixtures and add specific features and flavors.
The chemistry and functional behavior of meat, as a raw material for processing, are derived from the characteristics of muscle tissue. The muscle is a biological tissue, highly organized, with a complex intrinsic structure, a unique and very active composition from biochemical point of view.
The composition of the meat is very important for the final product. From a nutritional standpoint, muscle tissues are rich in protein, containing all essential amino acids and is also a good source of zinc, selenium, phosphorus, iron, vitamins B6, B12, niacin, choline (Lawrie, 2006), some meats being rich in vitamin K (Schürger et al., 2000).
The proteins functional properties are the physico-chemical properties that affect their behavior during processing, storage or consumption of food systems and contribute to the quality and sensory attributes of the food (Kinsella, 1976).
Gelling properties of the muscle proteins are influenced by:

- the source and type of muscle;
- the protein concentration plays a key role in determining the properties of thermal induction for gels;
- gelling properties of the proteins are dependent on pH;
- gelling properties of the muscle proteins are influenced by heating temperature, by the rate of temperature increase and by various adjuvants.

Gelling properties of the myofibrillar proteins have been studied in different species of animals.

Currently, the meat can be used in its natural state, or muscle proteins can be separated and used as functional ingredients, such as structural protein concentrates (surimi, surimi-like) protein isolates and hydrolysates (Lee, 1986; Ionescu et al., 2006).

MATERIALS AND METHODS

Raw materials

Hypophthalmichthys nobilis and Abramisbrama specimens were procured fresh from the local fish store. The fish was transported to the laboratory in a cool bag and then stored at 4°C until processing. After weighing the fish was descaled, gutted, beheaded and filleted. Fillets were boned and skinned by hand. Red muscles were detached manually and separate from the white muscles. White muscle, resulting after weighing, was minced using an electric mincing machine, fitted with a sieve with mesh size of 3 mm.

Minced meats were divided into equal parts, in order to obtain muscle myofibrillar protein concentrates by various methods: repeated washing of the meat with cooled water (3 washes), followed by centrifugation to remove the washing water; repeated extraction of minced meat with cooled solution of KCl 0.15M and 1 mM EDTA; acid solubilization of proteins and their precipitation from the solution at the pH of the isoelectric point of the muscle protein; alkaline solubilization of proteins and their precipitation from the solution at the isoelectric pH point of muscle protein.

Determining the approximate chemical composition

The contents of water, protein, fat and ash were determined using standard method of analysis (AOAC, 1990; Ionescu et al., 1992). Also, moisture was determined by fast drying to constant weight using the thermobalance "Precisa XM 60". Total nitrogen was determined by Kjeldahlsemi micro method, mineralization being performed in the "Trade Raypa" facility. Total proteins were calculated by multiplying the total nitrogen content by a factor of 6.25. All chemical analyzes were carried out in duplicate.

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

Protein solubility

The solubility of proteins in wet protein concentrates was studied in the pH range from 3.0 to 11.0.

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. Samples were run in duplicate.

RESULTS AND DISCUSSIONS

Determining the approximate chemical composition

Table 1 describes the type of concentrates/isolates of myofibrillar proteins, separation methods used and their concentrations in the dry matter and protein.

Table 1. Chemical composition of fish meat (Abramis brama, Hypophthalmichthys Nobilis) and a protein concentrate obtained

Indicators	ABRAMIS BRAMA				HYPOPHTHALMICHTHYS NOBILIS			
	MHAB	CPMAH 1	CPMAH 2	CPMAH 3	MHAB	CPMAB 1	CPMAB 2	CPMAB 3
Water, %	76.52	82.05	84.38	85.82	80.86	83.82	85.23	83.55
Proteins, %	17.21	16.42	13.84	12.75	17.83	14.66	13.36	13.75
Fat , %	4.28	0.52	0.59	0.56	2.38	0.12	0.17	0.46
Ash, %	1.26	0.06	0.05	0.09	1.06	0.16	0.05	0.12
Other, %	0.71	0.93	1.12	0.75	0.53	1.24	1.19	0.75

MHAB - Muscle homogenate of Abramisbrama
CPMAB 1 -Protein concentrate of Abramisbrama - Alkaline extraction
CPMAB 2 - Protein concentrate of Abramisbrama – Acid extraction
CPMAB 3 - Protein concentrate of Abramisbrama - KCl and EDTA extraction
MHAH - Muscle homogenate of Hypophthalmichthys nobilis
CPMAH 1 -Protein concentrate of Hypophthalmichthys nobilis - Alkaline extraction
CPMAH 2 - Protein concentrate of Hypophthalmichthys nobilis- Acid extraction
CPMAH 3 - Protein concentrate of Hypophthalmichthys nobilis - KCl and EDTA extraction.

Myofibrillar proteins, derived from different sources, were functionally characterized by determining the proteins solubility and gelling properties.

The quality and stability of the final protein product are affected by the functional properties of the proteins (Xiong, 2000). The most important functional properties, when muscle protein concentrates or isolates are obtained, for use in food products are the following: solubility, viscosity, water retaining capacity, emulsifying and gelling capacity (Hultin et al., 1999).

Solubility characteristics of myofibrillar proteins are interesting due to their relationship with other functional properties, especially the gelling and water retention properties (Hultin et al., 1995;Dagher et al., 2000).

Protein solubility

Myofibrillar proteins are generally soluble in solutions with ionic strength >0.3. Modification of muscle protein solubility can be obtained in different ways, by changing the ionic strength, types of ions, pH and/or temperature, this way being affected the hydrophobic and/or ionic nature of proteins. For a long time, there was a general conviction that in order to form good protein gels, the solubilization of mofibrilare proteins in high saline concentration (0.3 - 0.6 M) is required, characteristic for meat products with added salt and polyphosphates. However, Stefansson and Hultin (1994) showed that code myofibrillar proteins are soluble in solutions with less than 0.3M ionic strength, at both neutral pH and acidic pH, because the repulsion forces due to negative charges of the side chains of the proteins are sufficient to maintain the protein molecules separated, when there is enough water available (Stefansson et al., 1994).

The solubility of proteins depends on the species of animal, the type of muscle, postmortem changes, the exposure to pH values lower than 6.6 (Hultin et al., 2002; Ionescu et al., 2002, 2003, 2006), the treatment applied (freeze-thaw).

Protein solubility curves are shown in Figures 1 and 2. The solubility profiles were similar for all protein pastes which were analyzed.

The fish concentrates showed minimum solubility in isoelectric domain, with pH range between 5.5 - 7.0, characteristic for most muscle proteins (Xiong, 1997), the lowest protein solubility values were observed at pH 5.5. For protein concentrates/isolates obtained by alkaline and acid solubilizing, higher solubility values were observed at pH 5.5, than for protein concentrates obtained from washing with water or various solutions of minced meat.

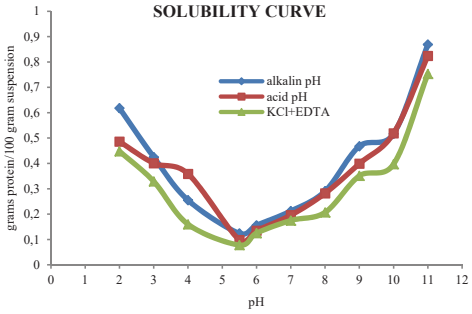

Figure 1. Protein solubility curves - Abramis brama

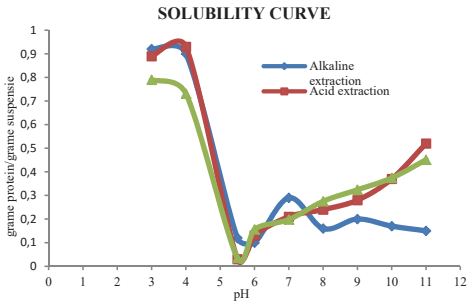

Figure 2. Protein solubility curves – Hypophthalmichthys nobilis

This can be explained by the presence in the constitution of this protein concentrates of sarcoplasmatic proteins soluble in water and low ionic strength solution and which represents 20-30% of the muscle proteins (Haard et al., 1994; Ionescu et al., 2009). The proteins soluble in isoelectric point domain were mostly sarcoplasmatic proteins and possibly dissociated actin. As can be seen from Figures 1 and 2, over 20% of the proteins were soluble in the physiological pH range (6.5-7.0), pH found in some of the meat products salted with added polyphosphates.

The decrease of solubility at very low pH values (1.5) could be due to aggregation induced by anions, as more hydrochloric acid into the environment will increase the ionic strength of the solution and can reduce the electrostatic repulsion between proteins (Goto et al., 1994; McClements, 1999; Damodaran, 1989).

Gelling properties

Myofibrillar proteins are responsible for the textural properties of the processed meat products (Yasui et al., 1980; Asghar et al., 1984). In general, the proteins extracted in saline solutions with high ionic strength (0.3-0.6M), also known as salt-soluble proteins (SSP) represent 55 to 60% of the total muscle protein or 10% of the skeletal muscle weight (Asghar et al., 1985). Among the myofibrillar proteins, myosin and actomyosin contribute most to the development of gel characteristics of the processed products obtained from salted meat (Ionescu et al., 2008, 2010).

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.

As can be seen from Figures 3 and 4, values of the elastic modulus and phase angle (δ), in case of the homogenate and protein derivates from Abramis brama and Hypophthalmichthys nobilis muscle, have evolved differently depending on the temperature domain and on the nature of the sample.

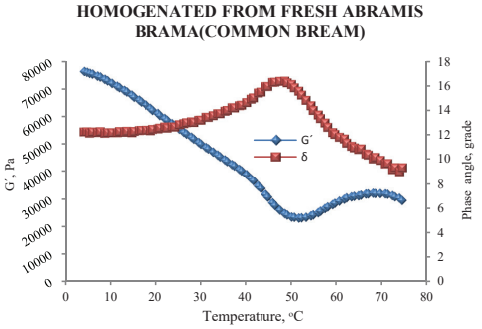

Figure 3. Shows the rheological behavior of Abramis brama (Common Bream)

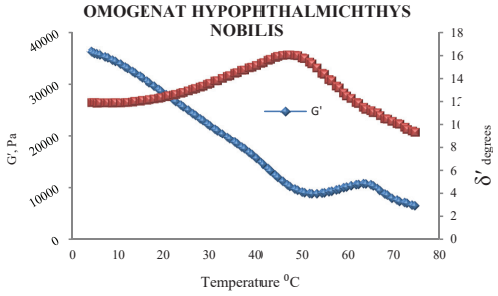

Figure 4. Shows the rheological behavior of Hypophthalmichthys nobilis

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. For the homogenated of Hypophthalmichthys nobilis 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', 36240 Pa at 4.3°C and 17620 la 36.8°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) for homogenated Abramis brama (Common Bream) muscle and 8972 Pa(51.8ºC) for Hypophthalmichthys nobilis muscle. 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.

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, 2006), 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°C), elastic modulus values increased very slowly at first, then the increase was accelerated when the temperature was raised above 59.7°C to the finalization of the heating process at 74.6°C.

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 is specific to the visco-elastic 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°.

Figures 5 and 6 are presented thermo-rheogram elastic modulus and phase angle for wet protein concentrates extracted from moss Abramis brama and Hypophthalmichthys nobilis, by the process of alkaline acid and wash with KCl.

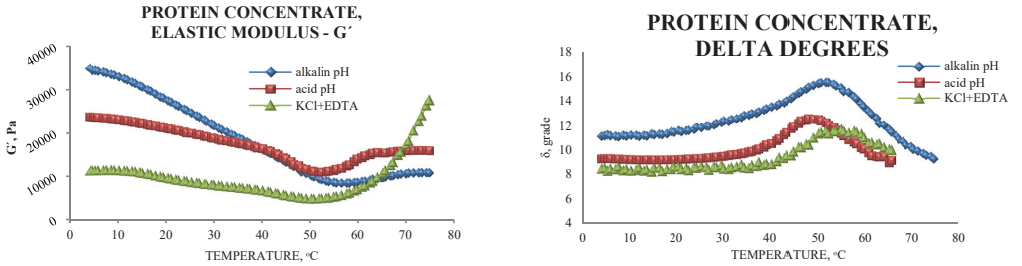

Figure 5. The elastic modulus change depending on temperature and changing of the phase angle depending temperature for Abramis brama

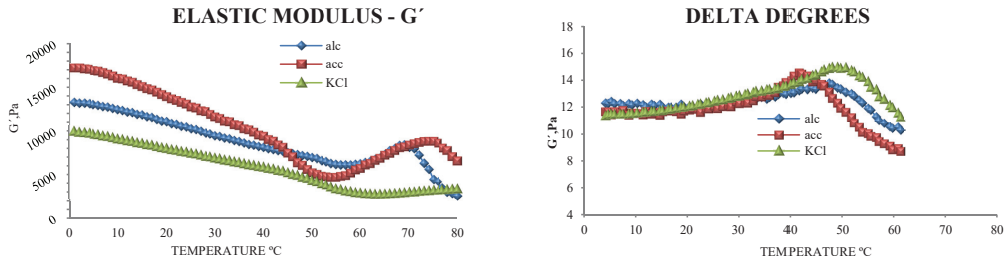

Figure 6. The elastic modulus change depending on temperature and changing of the phase angle depending temperature for Hypophthalmichthys nobilis

Protein concentrates thermograms profile was similar to that of muscle homogenate except that the elastic modulus values were different, being much higher in the muscle homogenate. If we compare the three types of protein concentrates (acid, alkalin and wash with KCl) it can be seen that the values of G' were higher for alkaline protein concentrate relative to the acid and not to KCl. For the two types of protein concentrate transition temperature of the ground to the gel was the same (50.8^{o}C), slightly lower than that recorded in the muscle homogenate (51.9ºC).

The modifications of the rheological properties on heating of the Abramisbrama (Common Bream) protein concentrates compared to the Abramisbrama (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). Protein concentration and pH are very important parameters in thermal gelation of meat protein (Lesiów et al., 2003). In addition, it is 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 sarcoplasmatic protein that are retained in the acid process, but not in the alkaline one (Ingadottir, 2004).

CONCLUSIONS

The functional properties of protein derivates were dependent on the protein source and separation techniques

The solubility profile of the muscle protein concentrates/isolates varied depending on the nature of the product and the pH of the protein solution. All protein derivates showed a minimum protein solubility at pH 5.5 and two areas of maximum solubility in strongly alkaline pH (11) and strongly acid pH (3).

The protein concentrates/isolates obtained by alkaline or acid solubilization and precipitation at pI are characterized by better solubility in the isoelectric range 5.5-6.5, pH values commonly encountered in various sausages formulations or in restructured products, where they may be used as functional ingredients.

Studied protein concentrates behaved, from rheological point of view, as viscoelastic systems with high elastic component, but variable depending on the temperature, protein source and extraction method.

For the protein concentrate, lower values of the elastic modulus were found at the beginning of the heating treatment, than for the homogenate at the same temperature, and higher values at end of the heating process for the concentrate compared to the homogenate.

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