Universitatea „Dunărea de Jos” din Galaţi
Școala doctorală de Inginerie

TEZĂ DE DOCTORAT

INVESTIGATIONS ON MYOFIBRILLAR PROTEINS: OBTAINING, CHARACTERIZATION AND APPLICATIONS IN FOOD INDUSTRY

(Rezumat teză)

Doctorand,
Ing. Floricel CERCEL

Conducător științific
Prof. univ.dr.ing. Petru ALEXE

Seria I4: Inginerie industrială Nr 39

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Key words:

- bread with protein,
- edible films,
- emulsifying capacity,
- fish,
- gelling properties,
- myofibrillar proteins,
- solubility of proteins.

CONTENTS

THESIS STRUCTURE

The doctoral thesis has 155 pages, 52 figures, 22 tables and is structured in two parts, namely:

I. **DOCUMENTARY STUDY** - it is presented - current state of knowledge through the study of literature;

II. **EXPERIMENTAL PART** - includes results, discussion, conclusions and personal contributions.Partea experimentală este structurată în patru capitole:

Chapter 2. Materials and methods - includes listing of feed materials, obtaining freeze-dried of protein concentrates and a description of the working methods used;

Chapter 3. Study of Functional concentrates / isolates of protein - myofibrillar proteins derived from different sources were characterized functionally by determining the solubility of proteins, properties of emulsifying, gelling and foaming;

Chapter 4. Improving the nutritional value of bread made from wheat flour - adding fish protein concentrate and lyophilized fish protein concentrate in the dough have improved the nutritional value of bread;

Chapter 5. Edible films - films have shown obtaining and characterizing edible / biodegradable.

EXPERIMENTAL PART ends with a chapter of conclusions and recommendations the synthesis conclusions drawn from research done.

DOCUMENTARY STUDY

Introduction

In the manufacturing of processed meat products, different types of meat, organs, edible subproducts and fat (as raw materials) are used, along with a large number of non-meat ingredients, with an important role in the formulation of various products. These ingredients stabilize the mixtures and add specific characteristics and flavors. Traditionally, there are used extension and thickening agents, water, salt, nitrite, nitrate, ascorbate/erisorbate, carbohydrates, antioxidants, mold inhibitors, spices, flavorings depending on the type of product.

Extension agents are represented by proteic additives, defined as non-meat proteins. Currently, various extension agents and thickeners are commercially available for using in emulsified sausages, salami with heterogeneous structure and restructured meat products to improve water binding and emulsifying capacity, slicing and consistency characteristics. They also increases the protein content, add specific flavors, improve the processing efficiency and reduce formulation costs. The most important extension agents for meat products are soy protein, milk protein, starch, flour and yeast. The maximum amount allowed for extension agents in sausage production is 3.5% and is strictly regulated by law in some countries. The meat composition is very important for the final product. Lean meat itself has a relatively constant chemical composition, similar to the muscle tissue, but the composition of the meats that include external fat is highly variable.

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, Aprodu, Alexe, 2009).

Myofibrillar proteins have intermediate solubility between sarcoplasmatic 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.

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

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, dependinging on the temperature and pH at which they are exposed (Egelandsdal, et al, 1986; Xiong, 1993).

The health status of many people could be improved by increasing protein nutritional value of wheat flour bread. The incorporation of ingredients like FPC and FPCL can lead to the production of nutritionally enhanced products like bread with high protein content. Supplement with FPC and FPCL improve bread protein content and compensate wheat deficiencies in lysine and threonine, two essential amino acids. This substitution can lead to decreases in loaf specific volume of bread [10].

This work studied the potential of supplementing bread with FPC and FPCL.

Materials and methods

Carp 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" (Figure 2.2). Total nitrogen was determined by Kjeldahl semimicro 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 ± 1oC.

Protein solubility

The solubility of proteins in wet protein concentrates was studied in the pH range from 3 to 11.0. Extraction of the proteins was carried out by stirring the suspension of protein concentrate (1o g per 1000 ml of buffered phosphate pH 7.0) using a magnetic stirrer for 1 h at room temperature. Aliquots

of the suspension were taken to determine the solubility of the proteins at different pH levels, adjusted with 2M HCl or with 2M NaOH and stirred for 1 hour. The samples were then centrifuged at 3000 rpm for 30 minutes and the nitrogen content in the supernatant was determined by the Kjeldahl semimicro method. Solubility was expressed as a percentage of the total protein of the original sample, present in the soluble fraction. For building solubility curve average values for each pH value were used. The determination was performed in duplicate.

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 ran in duplicate.

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. Solubility profiles were similar for all analyzed protein paste.

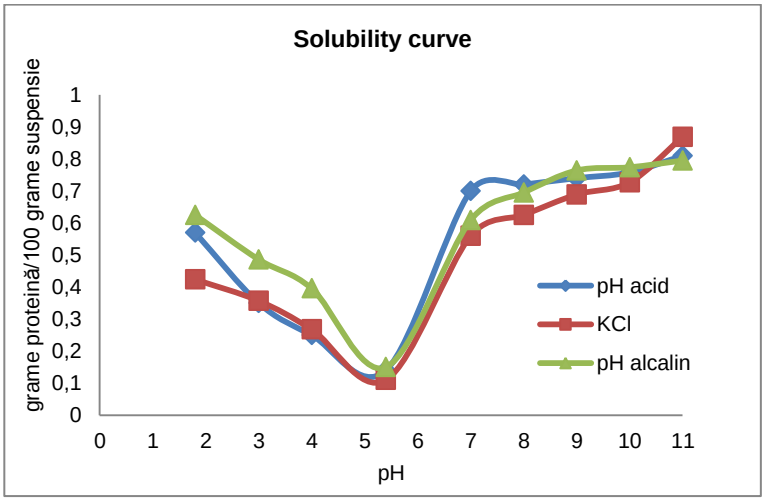


Figura. 3.7. Protein solubility curves

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 (17.89 to 21.25% per s.u.) than protein concentrates obtained by washing the minced meat with water with or with different solutions (KCl and EDTA) (12.45 to 14.31% per s.u.). We explain this by the presence, in the composition of those concentrates, of sarcoplasmatic 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, Aprodu, Alexe , 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 87% 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 leads to increased solubility, suddenly up to 7, then 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).

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 carp.

In our study, we followed the rheological behavior of protein suspensions by scanning a wide temperature range (4,3-74,8oC or 31-80oC) 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

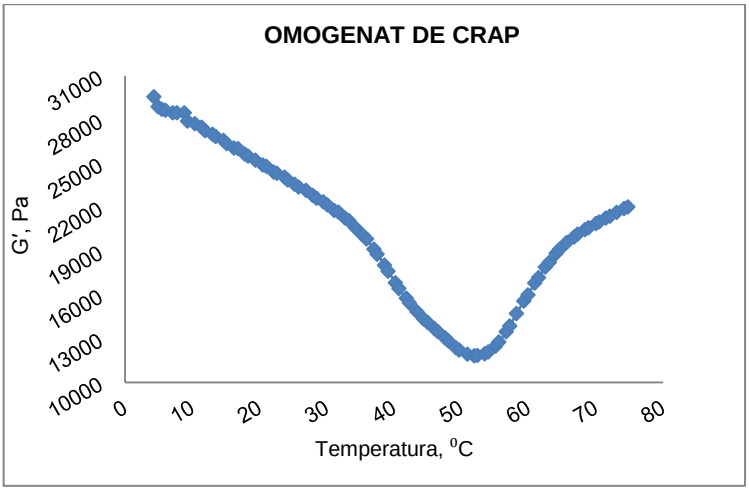
deformation per unit volume and the property which makes the correllation with the elastic nature of the material.

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.

The rheological behavior of fish protein

As can be seen, the values of the elastic modulus and phase angle (δ) of the homogenate and the carp muscle protein derivatives have evolved differently depending on the temperature domain and the nature of the sample.

In the case of homogenated carp muscle (pH 6.3), elastic modulus had a moderate downward trend in the temperature domain between 4,3-35,9oC, characterized by high values of G ', 29580 Pa at 4,3oC and 19850 Pa at 35.9oC. This interval is followed by another temperature domain (35,9-51,9oC) characterized by a more significant reduction of this parameter to a minimum of 11970 Pa (51,9oC). 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 noncovalent 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.



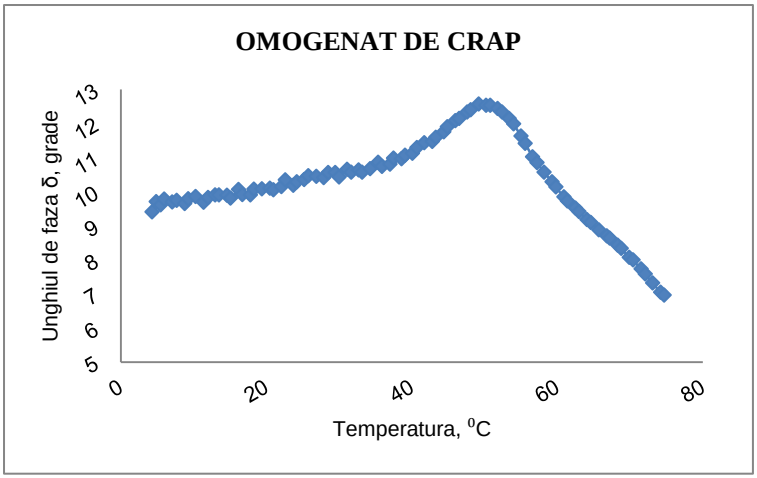


Fig. 3.17The elastic modulus change depending on temperature and changing of the phase angle depending temperature - (protein concentration of 16.12%, 6.3 pH, 1 ^{0}C / min heating rate)

The thermo-rheogram, shows below, a portion close to a plateau in the 50,9-59,9oC 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 50-57oC, for myofibrillar protein samples with a 6.0 pH and lower concentration.

Starting with the inflection point of the curve (51,9oC), elastic modulus values increased very slowly at first, then the increase was accelerated when the temperature was raised above 59,9oC to the finalization of the heating process at 74,8oC. This rheological behavior is typical for the thermal gelation of carp 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 carp 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 6,96-12,57o, across all the temperature domain of 4,3-74,8oC are 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,> 12,0o.

Below are presented the thermo-rheograms of the elastic modulus and phase angle for wet protein concentrates,

extracted from carp 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 carp muscle homogenate except for the elastic modulus values which were different, being much higher for the muscle homogenate (see the table). 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 (50,8oC), slightly lower than the one registered for the muscle homogenate (51,9oC).

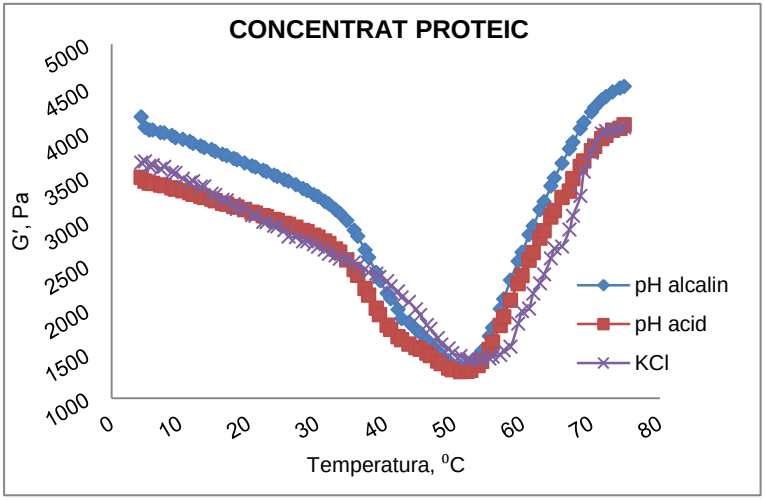


Fig. 3.18.The influence of temperature on the elastic modulus values

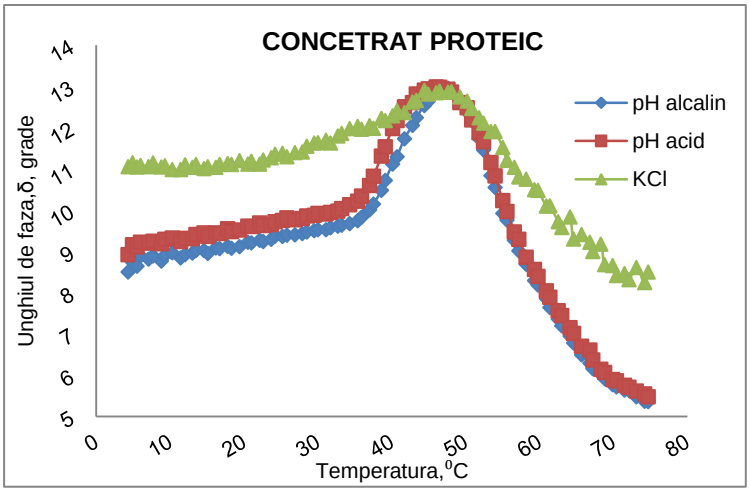


Fig. 3.19. The influence of temperature on the phase angle values

The modifications of the rheological properties on heating of the carp protein concentrates compared to the carp 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. 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.

Table 3.2The temperature dependence of the elastic modulus and the method of extraction of muscle proteins

Sample nature	Elastic modulus, Pa			
	4,3°C	50,8°C	51,9°C	74,7°C
Muscle homogenate	29.580	-	11.840	22.020
Protein concentrate – Alkaline extraction	4.178	1.382	-	4.523
Protein concentrate – Acid extraction	3.453	1.269	-	4.070
Protein concentrate - KCl and EDTA extraction	3.660	1.476		4.058

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 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 sarcoplasmatic 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 (30-71,9oC) showed values above 0.930, values slightly higher at lower protein concentrations.

The analized 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.

Nutritional Effects of Added Fish Proteins in Wheat Flour Bread

The wheat flour contained: 0.48% ash (SR ISO 936:2009), 10% protein (STAS 9064/4-8), 0,9% lipids (SR 9065-10:2007) and 13,8% moisture (SR ISO 1442:2010).

The fish protein concentrate (FPC) is obtained by extraction of fish meat composition mixed in a Blixer at 3000 r. p.m. and 60 s. with KCl 0,2 M(1:5), EDTA 1mM (1:3), KCl 0.14 M (1:4)solutions and distilled water (1:4) followed by three centrifugations at 3000 r.p.m..

The FPC contained: 15.12% protein (STAS 9064/4-8), 83.45% moisture (SR ISO 1442:2010), 0.1 lipids (SR 9065-10:2007) and 0.3% ash (SR ISO 936:2009).

The fish protein lyophilized (FPCL) is obtained by lyophilization of FPC in Alpha 1-4 LD PLUS apparatus.

The FPCL contained: 89.7% protein(STAS 9064/4-8), 7,1% moisture (SR ISO 1442:2010), 0.5% lipids (SR (9065-10:2007) and 1.7% ash (SR ISO 936:2009).

Bread baking

Baking tests were carried out according to SR 91-2007. A baking station equipped with a fermenter and electrical oven was used for fermenting and baking. Dough kneading was carried out using Kitchen Aid (Model 5 KSM 150, England) with the following conditions: 1 min. (280 r.p.m.), 1 min. (360 r.p.m.) and 20 s.(440 r.p.m.) that was settled as a preliminary study. After mixing, the dough was fermented for 60 min. at 30^0C and 85% RH. Then, the dough was removed and divided into 2 portions of 320 g each. Each portion was formed into bread, placed in a baking-tin, and put back into the fermenter for another 60 min. Finally, the bread was baked for 30 min. at 230^0C. After being removed from the oven, the bread was

cooled for 2 h., remove from the baking-tin, and stored at 20^0C, 50% RH. before their functional properties were analysed. Each recipe produced two loaves of breads, and all bread recipes were baked twice, thus for each recipe four loaves of breads were produced.

Determination of functional bread properties

Loaf specific volume

Each loaf of bread was weighed and then measured for volume using a rapeseed displacement volumeter. Three measurements were made for each loaf of bread and the values were related to 1g bread (cm^3/g) .

Crumb relative elasticity

Three cylinders with dimensions 4.15 x 6 cm. (D x H) were prepared from each loaf of bread using cylindrical cutter. They were pressed until to half from initial height (1 min.) and then was removed the press (1 min.) and was noted the final height. The relative elasticity (REL) in percent was calculated by dividing the final height at the initial height and multiplied by 100 [1] .

Crumb relative porosity

Three cylinders with dimensions 4.15 x 6 cm. (D x H) were prepared from each loaf of bread using cylindrical cutter. They were pressed until to disappeared the goals and the compact balls were introduced in a graduated cylinder with oil established volume. It was noted the final oil volume. The relative porosity (RPO) in percent was calculated by dividing the initial volume at the final volume and multiplied by 100.

Sensorial analysis

Sensorial analysis of samples was achieved by a group of 10 panelists belonging Sensory Analysis of Department of Faculty of Food Science and Engineering, Dunarea de jos University of Galati, Romania. Each loaf of bread was verified on the quality scheme of 30 points. This scheme was included physical indexes (specific volume, porosity, elasticity) and sensorial indexes (bread volume - 4 points , crust colour - 4 points, crumb texture - 6 points, crumb porosity - 6 points, flavor - 4 points, taste - 6 points).

Experimental protocol

Detailed recipes was summarized in Table 4.1. The amount of water addition was established by the consistency dough sample 1 (control C) at the Konsystograf Typ SZ-5 of Sadkiewicz Instruments.

Content of essential amino acids (mg/g) of flour and FPC (Table 4.2.) was calculated according to [ww.nutritionvalue.org]. The chemical score (CS) was calculated for each essential amino acid by the following formula: [milligrams of amino acid in 1g test protein / milligrams of amino acid in 1g reference pattern] x 100. The reference pattern used were those established by FAO [8].

Table 4.2. *. The recipes of bread containing FPC and FPLC*

Sample	Flour, g.	FPC, g.	FPCL, g.	Oil (O) g.	Water, ml.	Yeast, g.	Salt, g.
I	200	-	-	-	120	6	3
II	200	140	-	10	61	6	3
III	200	140	-	-	61	6	3
IV	200	-	20	-	146	6	3

Table 4.3.. *Content of essential amino acids (mg/g) of flour and fish carp*

Sample	Val	Iso	Leu	Lys	Met+ Cys	Thr	Trp	Phe+ Tyr
Flour	49	43	84	18	44	31	12	92
Fish carp	51.5	46	81	92	41	44	11	83

Statistical analysis

Treatment effects were evaluated by analysis of variance using a completely randomized design. Treatments means from two replicates were compared using least significant difference.

Chemical composition of wheat flour bread

As shown in Table 4.3. bread samples with fish proteins addition is characterized by high protein, lipid and ash contents. Level of proteins (% d.b.) improved from 9.73 in C to 19.26 in FPC bread and to 10.45 in FPCL bread samples.

Table 4.4. *Chemical composition of wheat flour bread samples*

Sample	Moisture (%)	Proteins		Lipids		Ash	
		%	% d.b.	%	% d.b.	%	% d.b.
I	42.98	5.55	9.73	0,25	0,43	0,50	0,87
II	48.58	9.90	19.26	3,83	7,46	0,53	1,03
III	49.29	9.66	19.09	0,35	0,69	0,60	1,17
IV	46.03	10.45	19.37	0,34	0,63	0,78	1,45

Nutritional value of wheat flour bread supplemented with FPC and FPCL probably improved because of better balance in amino acid content.

The improvement of nutritional value of bread supplement with different sources of proteins reported by various workers.

Essential aminoacid content

The complementary effect of fish proteins improved the protein quality of wheat flour bread samples by calculation of essential amino acids content (Table 4.5.) and chemical scores for each essential amino acid from each wheat flour bread samples (Table 4.6).

Table 4.5. Content of essential amino acids (mg/g) of wheat flour bread samples and FAO

Sample	Val	Iso	Leu	Lys	Met + Cis	Thr	Trp	Phe + Tyr
I	43,00	38,00	69,00	24,00	39,00	28,00	11,50	77,00
II	47,00	42,00	74,00	54,50	40,00	35,00	11,00	75,00
III	46,50	41,20	74,00	52,80	40,00	34,80	10,70	75,00
IV	47,00	41,60	74,90	56,10	40,00	35,60	12,50	75,00
FAO	50,00	40,00	70,00	55,00	35,00	40,00	10,00	60,00

In control samples (1) lysine is the first limiting amino acid and threonine the second. Addition of FPC and FPCL improved the lysine and threonine content but threonine was still an limiting amino acid.

Table 4.6. Content of CS (% FAO) of wheat flour bread samples

Sample	Val	Iso	Leu	Lys	Met + Cis	Thr	Trp	Phe + Tyr
I	86,0	95,0	98,5	44,0	111,0	70,0	115,0	128,0
II	94,0	105,0	106,0	99,0	114,0	88,0	110,0	125,0
III	93,0	103,0	106,0	96,0	114,0	87,0	107,0	125,0
IV	94,0	104,0	107,0	102,0	114,0	89,0	125,0	125,0

Effect of fish proteins on bread quality

Wheat flour bread control and wheat flour bread with FPC and FPCL are presented in Fig.4.1 and Fig. 4.2. It observed a diminution of volume loaf in order : C(1), FPCL (4), FPC (3), FPC+O (2).

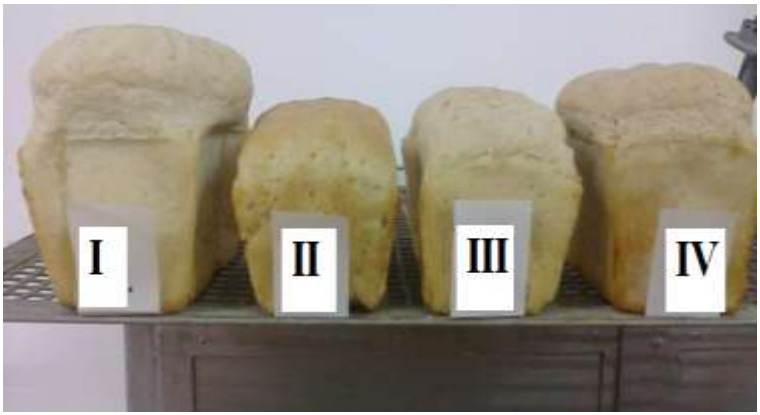


Fig.4.1 External characteristics of wheat flour bread samples

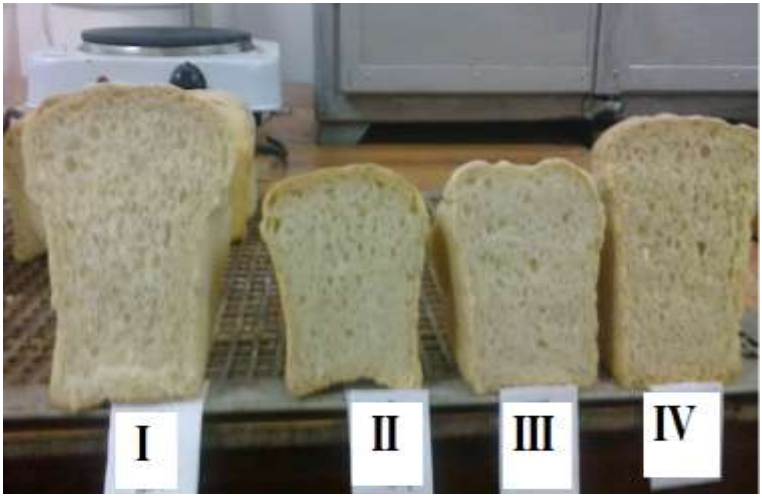


Fig. 4.2 Internal characteristics of wheat flour bread samples

The physical indexes of wheat flour samples are presented in Tabel 4.7..

Table 4.7.. Physical indexes of wheat flour bread samples

Sample	Specific volume g /cm^3	Porosity %	Elasticity %
I	3.9	76	97
II	2.5	75	98
III	3.1	74	97
IV	3.2	75	97

The specific volume decreased in order: C (1), FPCL (4), FPC (3) and FPC+O (2);
The porosity decreased in order: C (1), FPCL (4) = FPC+O (2) and FPC (3);
The elasticity decreased in order: FPC+O (2) and C (1) = FPC (3) = FPCL (4).
Sensorial evaluation of wheat flour bread samples are presented in Table 4.8.

Table 4.8. Sensorial evaluation of wheat flour bread samples

Sample	Bread Volume	Crust colour	Crumb texture	Crumb porosity	Flavor	Taste	Total points
I	4	3	5	6	3	4	25
II	2	4	6	5	4	5	26
III	3	3	5	4	4	5	24
IV	3	4	5	5	2	3	22

By sensory evaluation the samples of breads were favourited in the following order: FPC+O (2), C (1), FPC (3), FPCL (4)

Conclusions

The addition of fish protein concentrate and fish protein concentrate lyophilized to wheat flour dough improved the nutritional value of bread. These products were achieved an acceptable specific volume and a crumb structure (texture and porosity) and were a good acceptation by consumers.

Dissemination of research results

A. Articles published in ISI

1. **Cercel,F.,** Stroiu, M., Alexe, P., 2015 Characterization of myofibrillar chicken breast proteins for obtain protein films and biodegradable coatings generation. Journal of Agriculture and Agricultural Science Procedia, 6, 197-205;

2. **Cercel, F.,** Burluc, R.M., Alexe, P., 2016. Nutritional effects of added fish proteins in wheat flour bread. Manuscris în pregătire, 2016.

B. The patent application:

1. **Floricel Cercel**, Petru Alexe, Romulus Marian Burluc, 2016, Obţinerea pâinii echilibrată nutriţional în proteine, Cerere de brevet de invenţie nr. A/00602 din 31.08.2016, România.

C. Articles published in journals listed BDI

1. Cercel, F., Stroiu, M., Ianiţchi, D., Alexe, P., 2016, Rheological properties description of myofibrillar protein homogenates and concentrates obtained by different methods and from different species. *International Conference "Agriculture for Life, Life for Agriculture"* 9 - 11 iunie 2016, Book of Abstracts Section 3 Animal Science University of Agronomic Sciences and Veterinary Medicine of Bucharest Faculty of Animal Science, ISSN 2501-7160 (CD-ROM), ISSN-L 2457-3221;

2. **Cercel,F.,** Stroiu, M., Alexe, P. 2015 Characterization of myofibrillar proteins obtained from fresh abramis brama (common bream) meat**.** International Conference "Agriculture for Life, Life for Agriculture", 4-6 of June, 2015 at University of Agronomic Science and Veterinary Medicine of Bucharest, Romania series D. animal science volume LVIII, 2015;

3. Tudose,C., Iordachescu, G., Stan, F., **Cercel, F.**, Alexe, P., 2014, Influence of animal fat replacement with vegetable oils on the sensorial perception of meat emulsified products, The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology, 38(2);

4. Dima, C., Neagu, C., **Cercel,F.,** Alexe, P. 2014, Sensory, physico-chemical and microbiological properties of cooked ham with β-ciclodextrin loaded with coriander and pimento essential oils. Journal of Agroalimentary Processes and Technologies 2014, 20(4), 319-329;

5. Cercel, F., Stoica, M., Popescu, A., Stroiu, M., Alexe P., 2010. The extraction and characterization of myofibrillar fish proteins. International Conference "Agricultural And Food Sciences, Processes And Technologies" Sibiu, December 9 - 12, 2010;

6. **Cercel,F.,** Stroiu, M., Alexe, P., 2010. Characterization of myofibrillar pork proteins for obtain the comestibles films. The 39th international session of scientific communications of the Faculty of Animal Science, Series D, vol III, ISSN 1843-6048, Bucharest 2010;

7. **Cercel,F.,** Stroiu, M., Alexe, P.,2010. Characterization of myofibrillar fish and beef proteins for obtain the comestibles films. The 39th international session of scientific communications of the Faculty of Animal Science, Series D, vol III, ISSN 1843-6048, Bucharest 2010;

8. **Cercel,F.,** Pătraşcu, L., Alexe, P., Stroiu, M., 2009. Preliminary characteristics of myofibrillar proteins obtained for edible byofilms realization. Proceedings of the 2nd international symposium "New Researches in Biotechnology" Series F, ISSN 1224-7774, Bucharest 2009.

9. **Cercel,F.,** Stroiu, M., Alexe, P., 2009. Preliminary study concerning the production of biofilms from myofibrillar proteins. Food Science and Food Industry in the Recession Era, Paper of the International Symposium, ISSN 1843-5114, Galati, October 9-10, 2009.

10. Patrascu,L., **Cercel,F.,** Alexe, P., 2009. Influence of extraction method on rheogical properties of myofibrillar proteins from different sources. Challenges for Food Science and Food Industry in the Recession Era, Paper of the International Symposium, ISSN 1843-5114, Galati, October 9-10, 2009;

11. **Cercel,F.,** Pătraşcu, L., Alexe, P., 2009. Myofibrillar proteins; optimization of extraction method and characterization of different myofibrillar proteins obtained. Challenges for Food Science and Food Industry in the Recession Era, Paper of the

International Symposium, ISSN 1843-5114, Galati, October 9-10, 2009;

12. Patrascu,L., **Cercel,F.,** Alexe, P., 2009. Influence of extraction method on rheogical properties of myofibrillar proteins from different sources – The Annals of the University Dunarea de Jos of Galati, Fascicle VI Food Technology, New Series Year III (XXXII), ISSN 1843-5157, Galati University Press 2009;

D. Participation in international conferences

1. **Cercel, F.,** Stroiu, M., Ianiţchi, D., Alexe, P., 2016, Rheological properties description of myofibrillar protein homogenates and concentrates obtained by different methods and from different species. International Conference “Agriculture for Life, Life for Agriculture” 9 - 11 iunie 2016, Bucureşti, România, (prezentare orală);

2. **Floricel Cercel**, Romulus Marian Burluc, Petru Alexe 2016. Nutritional effects of added fish proteins in wheat flour bread. International Conference “Agriculture for Life, Life for Agriculture” 9 - 11 iunie 2016, Bucureşti, România, (prezentare orală);

3. **Floricel Cercel**, Mariana Stroiu, Petru Alexe 2015 Characterization of myofibrillar chicken breast proteins for obtain protein films and biodegradable coatings generation. International Conference "Agriculture for Life, Life for Agriculture", 4-6 of June, 2015 at University of Agronomic Science and Veterinary Medicine of Bucharest, 2015 (prezentare orală) ;

4. **Floricel Cercel,** Mariana Stroiu, Petru Alexe , 2015. Characterization of myofibrillar proteins obtained from fresh abramis brama (common bream) meat. International Conference "Agriculture for Life, Life for Agriculture", 4-6 of June, 2015 at University of Agronomic Science and Veterinary Medicine of Bucharest, 2015;

5. **Floricel Cercel**, Mariana Stroiu, Petru Alexe. 2014. Carp miofibrilar protein concentrate dry by using spray dryer technology and elemental mapping of microstructures by scanning electron microscopy. The 8th International Conference on WATER IN FOOD Politehnica ”University of Timişoara, Romania, 25-27 Mai, 2014, Timişoara, Romania;

6. **Floricel Cercel**, Maricica Stoica, Anca Popescu, Mariana Stroiu, Petru Alexe 2010. The extraction and characterization of myofibrillar fish proteins, International Conference “Agricultural And Food Sciences, Processes And Technologies” Sibiu, December 9 - 12, 2010. (prezentare orală)

7. Floricel Cercel, Maricica Stoica, Mariana Stroiu, Petru Alexe, 2010. The extraction and characterization of myofibrillar fish proteins – International Workshop on ″ Fishery and Aquaculture – a view point upon the sustainable management of the water resources in the Balkan Area″, Galati, May 26-28, 2010, – ROMANIA (poster);

8. **Floricel Cercel**, Mariana Stroiu, Petru Alexe., 2010. Characterization of myofibrillar pork proteins for obtain

the comestibles films - Department of Biochemistry and Technologies, Faculty of Food Science and Engineering “Dunarea de Jos” University of Galati of the 39th international session of scientific communications of the Faculty of Animal Science, Bucharest 2010; (prezentare orală)

9. **Floricel Cercel**, Mariana Stroiu, Petru Alexe 2010. Characterization of myofibrillar fish and beef proteins for obtain the comestibles films - Department of Biochemistry and Technologies, Faculty of Food Science and Engineering “Dunarea de Jos” University of Galati of the 39th international session of scientific communications of the Faculty of Animal Science, Bucharest 2010 (poster);

E. Participation in national conferences

1. **Floricel Cercel**, Mariana Stroiu, Petru Alexe 2014, Characterization of edible films made from myofibrillar proteins obtained from fresh Abramis brama (Common bream) meat. Scientific Conference of Doctoral Schools from “Dunărea de Jos” University of Galati Second Edition - Galaţi, 15-16 Mai 2014.

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