SOLUBLE PROTEIN DYNAMICS IN MUSCULAR FILLETS, DURING CONSECUTIVE FREEZING – THAWING CYCLES, FOR INDIVIDUALS OF *CARASSIUS GIBELIO* BLOCH. AND *CYPRINUS CARPIO* L. (*CYPRINIDAE)* SPECIES, SAMPLED FROM MOVILENI POPULATION

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Abstract: In the present paper, the main purpose was to establish soluble protein dynamics for muscular tissue, sampled from individuals of *Carassius gibelio* Bloch., 1782 and *Cyprinus carpio* L., 1758 (*Cyprinidae*) species, within consecutive freezing – thawing cycles. For protein dosing we used the Lowry method.

INTRODUCTION

Frozen storage is one of the most important preservation methods for maintaining microbiological and chemical stability and extending the shelf life of food products. Deteriorations in texture, flavor, and color, resulting from biochemical, enzymatic and functional changes in proteins, however, are problems associated with freezing and subsequent storage at subfreezing temperatures for many fresh and processed foods. Freeze-induced protein denaturation, enzyme inactivation and related functionality losses are commonly observed in frozen fish, meat, poultry, egg products and dough’s. Muscle proteins are particularly susceptible to freeze denaturation compared to plant-derived proteins, and this is especially true for fish species. Denaturation of proteins during freezing and frozen storage can be monitored by measuring alterations in protein surface hydrophobicity, amino acid composition, conformational stability, solubility, aggregation, and enzyme activity. Losses in functional properties of proteins are commonly assessed by comparing water–holding ability, viscosity, gelation, emulsification, foaming, and whipping properties (Erickson and Hung, 1997).

Freeze-induced protein denaturation has been attributed to the formation of ice crystals, dehydration, and concentration of solutes in the tissue or protein solution. For some marine fish, formaldehyde derived from trimethylamine oxide in frozen tissue has been shown to modify myofibrillar proteins and collagen by cross-linking adjacent polypeptides, thereby forming insoluble aggregates. Recent studies have also demonstrated that oxidative reactions, such as lipid peroxidation, are involved in denaturation and deterioration in functional attributes of muscle proteins during frozen storage. Thus, various cryoprotectants (sugars, sorbitol and polyols), and antioxidant additives are incorporated into food before freezing to minimize physicochemical changes in proteins and to prevent functionality losses. Moreover, the discovery of antifreeze proteins in fish has piqued considerable research interest in identifying alternate means for the preservation of protein stability and functionality during freezing and subsequent storage (Erickson and Hung, 1997).

Freezing is a physical process involving the transformation of water molecules from an amorphous state to highly structured ice crystals. The phase change can lead to protein denaturation caused by alterations in the chemical and physical environment of the protein. Freeze-induced damages to proteins are both mechanical and chemical. A conventionally accepted theory is that the damages involve three major alterations in the protein microenvironment: changes in moisture, changes in lipids or changes in certain cellular metabolites. According to Shenouda (1980), changes in moisture can be subdivided into the following: formation and accretion of ice crystals, dehydration and increases in solute concentration (Erickson and Hung, 1997).

Fish can form a very nutritious part of man’s diet; it is rich in most of the vitamins he requires, it contains a good selection of minerals, and the proteins contain all the essential amino acids in the right proportions, however, undergoing the storage and preparation processes, changes in its composition are induced (Murray and Burt, 2001).

For example, one of the most prevalent reactions to occur in fish muscle during freezing and frozen storage is the complex phenomenon of protein denaturation, but lipid oxidation, texture deterioration, loss of fresh odor and flavor, loss of volatile constituents, nutritional losses and changes in moisture also take place. The amplitude of all these changes depends on the time taken to freeze the fish, the freezing temperature and on the storage quality and period (Lester 1996).

MATERIALS AND METHODS

The biological material consisted of 5 individuals, for each of the two studied species: *Carassius gibelio* Bloch., 1782 şi *Cyprinus carpio* L., 1758 (*Cyprinidae*), sampled from Movileni population. All individuals were phenotypic investigated, towards establishing the homogeneity degree, thus, measurements have been made, following a series of phenotypic parameters (*lt*=total length; *ls*= standard length; *g* =individual weight; *C*=circumference; H_d=maximum height; H_a= minimum height; L_c=head length)

Towards obtaining information regarding the population's homogeneity degree as well as regarding the statistic signification level of the differences between the two species populations, biometrical measurements have been made, and some statistical parameters were calculated (Snedecor, 1968): $\overline{\mathbf{X}}$ = average; *S*= standard deviation and *Es*=standard error.

1. Average

$$\bar{x} = \frac{\sum xi}{n}$$, where: Σxi = individual values sum
n = number of individuals

2. Estimating standard deviation from sample towards population

$$S = \sum x^2 - \frac{\left(\sum x\right)^2}{n}$$, where:

Σx^2 = average of squares' sample mean;
$(\Sigma x)^2$ = square of means' samples average;
n = number of individuals

3. Standard error

$$S_{\bar{x}} = \sqrt{\frac{S^2}{n}} = \frac{S}{\sqrt{n}}$$

S^2=variance

Using the average $(\bar{x})$ and standard deviation *(S)* values, calculated based on the samples from one population, the values for the entire population (or species) can be estimated. Estimating the population's average implies standard error and average $(S_{\bar{x}})$ calculation, according to the sample's variability and size (Varvara et. al., 2001).

It is necessary to fiend a critical value for a certain degree of confidence α and a certain number of liberty degrees *t(α, t-1)*, than, the estimation's probability will be given by *1-α*, meaning *α=0,05. t* value represents t distribution's critical value for a certain confidence level or significance level α and *n-1* liberty degrees. The significance level represents the arbitrarily cosen probability, for the calculated confidence interval to contain the population average. For the majority of studies, a 95% probability, meaning *α=0,05*, is considered to be satisfactory.

The average's standard error is used for calculating the population's average confidence interval, which includes, with a certain probability, the population's average

$\mu = \bar{x} \pm t(\alpha, n-1) \cdot S_{\bar{x}}$, were μ represents the population's average, and $\bar{x}$ the samples' average values,

The interval limits: inferior limit *(LI)* and superior limit *(LS),* are given by the following formulas:

$$LI = \bar{x} - S_{\bar{x}} \cdot t(\alpha, n-1)$$

$$LS = \bar{x} + S_{\bar{x}} \cdot t(\alpha, n-1)$$

Thereby, the LI-LS interval includes the population's average, with a *1-α* probability.

From all individuals of the analyzed species, muscle fillets were sampled, which afterwards underwent 15 consecutive freezing – thawing cycles, with a freezing temperature of -18^0C. After each freezing – thawing cycle, from each sample, an amount of 100mg of muscular tissue was taken, towards albumin extraction with 5ml of distillated water. The samples were than centrifuged for 15 minutes at 3000 rpm and afterwards 1ml of the supernatant was taken.

For protein dosing, we used the Lowry method (Lowry et. al., 1951) , which is one of the most common colorimetric assays performed by biochemists. This procedure is particularly sensitive and it relies on two different reactions. The first is the formation of a copper ion complex with amide bonds, forming reduced copper in alkaline solutions, (this is called a

"Biuret" chromophore). The second is the reduction of Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate) by tyrosine and tryptophan residues. The reduced Folin-Ciocalteu reagent is blue and thus detectable with a spectrophotometer in the range of 500-750 nm. The Biuret reaction itself is not all that sensitive, but using the Folin-Ciocalteu reagent to detect reduced copper makes the assay nearly 100 times more sensitive than the Biuret reaction alone.

Reagents: 1. Na_2CO_3 2% in NaOH 0,1N;
2. $CuSO_4$ crystallized with 5 H_2O 1%;
3. Sodium tartrate 2%;
4. Working solution (50ml reagent 1 with 0,5ml reagent 2 and 0,5ml reagent 3);
5. Diluted Folin – Ciocalteu reagent (1ml of concentrated reagent with 2ml of distillated water).

At 1ml protein solution 5ml of reagent 4 were added, afterwards the test tubes were agitated and than left for 10 minutes at room temperature. In the next step 0,5ml of diluted Folin – Ciocalteu reagent were added, and after stirring the tubes were left for 30 minutes at room temperature. After those 30 minutes the absorptions were read at 500nm using as control sample distillated water.

RESULTS AND DISCUSSIONS

Based on the values distribution around averages (Figure 1 and Figure2) and from the phenotypic investigation and biometrical measurement, resulted the existence of a phenotypic similarity for the individuals of both studied genera.

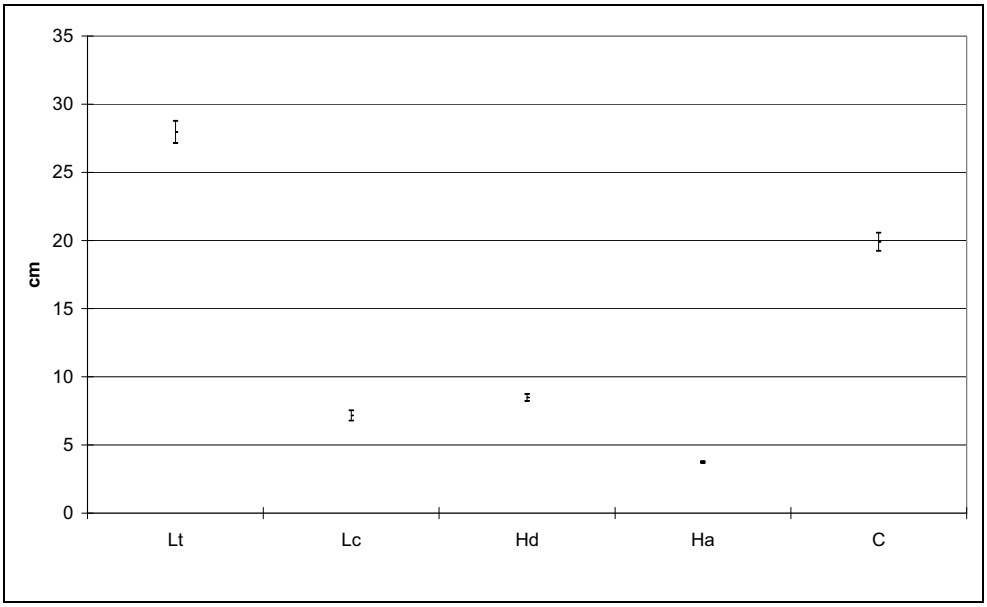

Figure 1 The investigated phenotypic parameter for *Cyprinus carpio* L.

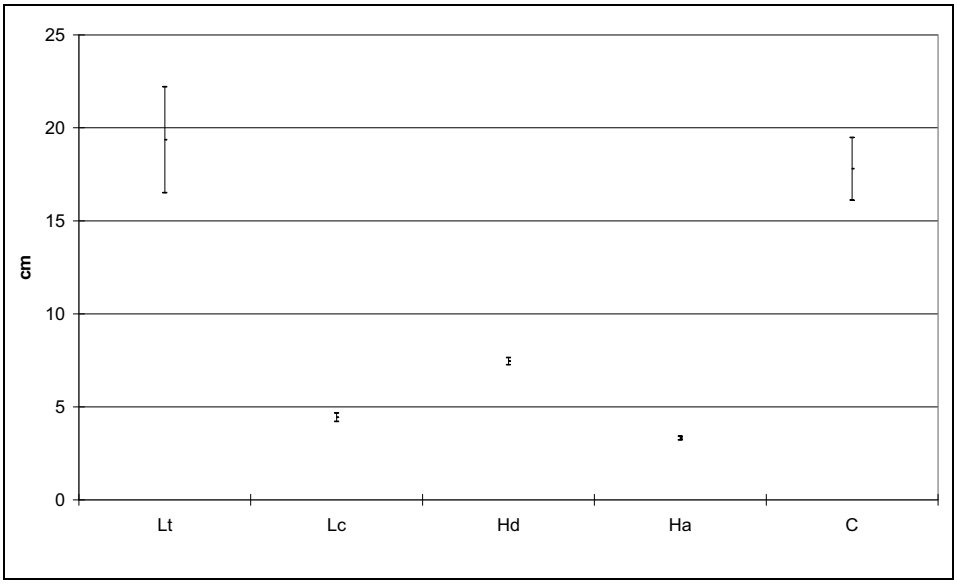

Figure 2 The investigated phenotypic parameter for *Carassius gibelio* Bloch.

From the investigated phenotypic parameters variability intervals graphic, for individuals of *Cyprinus carpio* L., as shown in Figure 1, we can observe a uniformity of the studied individuals, with very small differences for the head length (Lc), height at the dorsal fin (Hd), height at the caudal fin (Ha) and slightly higher for the total length (Lt) and individuals' circumference (C).

Compare to individuals of the previous species, individuals of *Carassius gibelio* Bloch species have the same low variation degree for this three parameters: head length (Lc), height at the dorsal fin (Hd), height at the caudal fin (Ha). Even if for the other two remaining phenotypic parameters variations are larger compared to the previous species, the average values for the two studied lots are close, showing the individuals' uniformity. Further more, tracing the lines between the individuals' average values two similar curves can be observed.

As the freezing – thawing processes took place a particular high variation of albumin was observed (Figure 3).

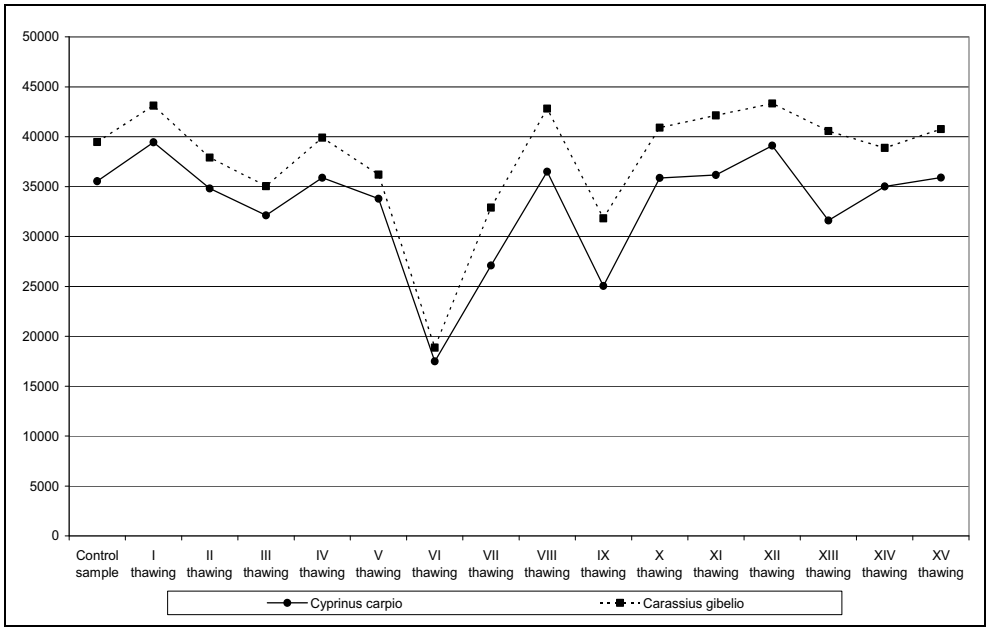

Figure 3 Total albumin quantity variations from to species muscle tissue

From the results presented in Figure 3, we can observe an albumin initial growth (after the first thawing), which we consider to be the result of membranes and other cellular structures disrupter as a consequence of ice crystals formation which leads to the soluble proteins release. The subsequent decrease registered after the second and third thawing can be the result of loss and degradation of the released proteins after the first two thawing processes. We consider the albumin's concentration decrease after the fifth thawing as being the moment in which the majority of the cellular structures and also the tissue are destroyed marking the maximum point of soluble protein loss, as the result of their release from the containing structures, of their dissolution as well as tissue liquid loss, liquid which contains soluble proteins.

We consider that albumins concentration growth after thawing in the sixth and seventh fazes, as a result of tissue dehydration from the anterior stage, which made necessary the prelevation of a bigger quantity of tissue towards respecting the weighting proportions, quantity that had a larger concentration of soluble proteins.

In conclusion, all ulterior fluctuations are the result of tissue's liquid quantity fluctuations. Thus while more subsequent freezing – thawing cycles take place, the tissue looses more and more liquids, and it will concentrate soluble proteins.

Protein denaturation can be related with the freezing out of water; the conformation of most native proteins has the hydrophobic side chains buried inside the protein molecule, however, some of this hydrophobic side chains are exposed at the surface of the molecule, where they interact with water molecules, which form a network of hydrogen that contributes to the stability of the highly organized three-dimensional structure of the proteins. During freezing, as water molecules freeze out, they migrate to form ice crystals, inducing the disruption of the organized H – bonding system that stabilizes the protein, and implicitly the deformation of the protein's three-dimensional structure (Lester 1996).

As the thawing process begins, the ice crystals melt, leaving behind ruptured cells and cellular organelles, as well as a small amount of whitish thaw drips, which lead to the leaching out of the dissolved materials, including water – soluble proteins (Lester 1996).

In addition to this, moisture is also lost through its transfer to the air and then on the refrigeration coils, where it builds up as ice. Hereby, the remaining tissue, after 15 freezing – thawing cycles is highly dehydrated and it has a rubbery texture and a modified smell and color.

CONCLUSIONS

During the freezing process, one of the most prevalent chemical reactions to occur in fish muscle is the complex phenomenon of protein denaturation.

When the fish is frozen and subsequently thawed, the formation of thaw drips occurs, inducing the loss of dissolved materials, including water – soluble proteins.

Even if freezing is the most important commercial process for preserving fish, especially in fillet form, it causes serious changes of quality indices such as color, texture, flavor and it induces massive dehydration.

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