Research Article

The Influence of Transglutaminase on Minced Muscular Fish Tissue Structure Formation After the Application of Various Protein Substrates

Tatyana Pivnenko, Julia Karpenko, and Viktoria Krashchenko

Far Eastern State Technical Fisheries University, Vladivostok 690087, Russia
ORCID
Tatyana Pivnenko; 0000-0002-0330-489X

Abstract. This research aimed to examine the effect of a microbial transglutaminase preparation (Activa® GS, Ajinomoto Co., Inc, Japan) on the structure formation of the myofibrillar protein system of a deep-sea fish species – the giant grenadier – after the addition of various protein substrates. The low content of proteins and their low water-holding capacity in this fish, subjected to various processing methods, leads to significant losses in the initial mass and decreased gelation ability in the muscle tissue system. Various concentrations of transglutaminase were used but these did not ensure the restructuring of the initial muscle tissue of the grenadier. Additional protein substrates with different molecular weights and amino acid composition were added, including gelatin, milk casein, hydrolysates of the skin and milt of the fish, and whole bivalves, which were used to create a firm structure. It was shown that the introduction of gelatin and casein at a concentration of 5% led to the formation of a firm, thermostable structure under the action of the enzyme, while hydrolyzed proteins with low molecular weight at their various concentrations enhanced the expression of water and formation of the fluid consistency. The ability of gastrointestinal tract proteases (pepsin and trypsin) to digest did not depend on the formation of protein-to-protein cross-linking in these combined products. The influence on the growth of the *Tetrahymena pyriformis* ciliates test culture also showed the high degree of product availability. The technology of molded products based on fermented minced muscle tissue of grenadier with added casein, both in the form of semi-finished products and in the form of ready-to-eat products, was developed.

Keywords: transglutaminase, muscle tissue, structure formation, deep-sea fish

Corresponding Author: Tatyana Pivnenko; email: tnpivnenko@mail.ru

Dates
Published 13 January 2022

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the 8th Scientific and Practical Conference Conference Committee.

1. Introduction

Many species stocks of aquatic biological resources are plummeting because of the growing demand for fish and fish products. At the same time, there are some species that are rarely used because of their size, taste, smell, color or texture. Low-value species and side components of processing (muscle trimmings, shavings from sawing frozen fish, etc.) can be converted into new products using restructuring technology [1–3]. A number of additives are used to improve mechanical and functional properties

of new products, a special place being occupied by an enzyme which forms thermo stable interprotein covalent crosslinking – transglutaminase (TG) [4]. The most common commercial preparations are microbial (MTG). When using them, the physicochemical properties of fish muscle proteins should be taken into account in order to obtain high-quality products with high commercial value.

One of the most common deep-sea fish species in the northern part of the Pacific Ocean is the giant grenadier *Albatrossia pectoralis* which production is growing steadily. At the same time, the processing of muscle tissue of the giant grenadier causes significant difficulties associated with the high water content and low concentration of myofibrillar proteins. Therefore, the main problem fishermen face is not deep sea giant grenadier fishing, but the difficulty of further technological processing of this object and the associated sales volumes. Balanced way to process muscle tissue of this object is to bind water in the tissue by creating structured dispersed systems with the addition of structure-forming agents which create a continuous spatial framework throughout the entire volume of the product [5]. MTG can be used as this agent. However, in order to produce catalytic effect and obtain solid texture of the final product it must be provided with the necessary concentration of proteins. Amino acid composition of these proteins should correspond to the substrate specificity of the enzyme, primarily glutamic acid and lysine.

2. PURPOSE OF THE STUDY

In this study the aspects of creating structured products based on low-protein muscle tissue of giant grenadier employing various protein substrates affected by MTG (ACTIVA, Ajinomoto Co., Japan) are considered.

3. MATERIALS AND METHODS

The following protein substrates were used: gelatin, casein, soluble collagen from fish skin, protein hydrolysates from hydrobionts, nucleoprotein complex from salmon milt. The amino acid composition of protein hydrolysates corresponds to the substrate specificity of TG [6]. It was found that the critical gelation concentration for all samples corresponds to 1% of the MTG preparation relative to the mass of the samples. It should be taken into consideration that the preparation contains only 1% of the enzyme itself; the rest is the filler - maltodextrin. Fermentolysis conditions are as follows – temperature

40°C, time 4 hours, pH 7.0 ± 0.5. To inactivate the enzyme, the samples were kept for 20 min at a temperature of 90 ° C.

To conduct the experiment, previously prepared additives were added to the minced grenadier fillet. The optimal amount of the additives is presented in Table 1. The main indicators characterizing the properties of the obtained protein gels are also given there. Water-holding capacity (WHC) was determined by pressing. The dynamic viscosity of the samples before heat treatment was measured on a Fungilab ALPHA Series Brookfield Rotational Viscometer. The breaking strength of the samples after heat treatment was measured on a Valent's tester with hemispherical indenter (VTs-1).

4. RESULTS AND DISCUSSION

Table 1 show the concentrations of substrates whereby significant differences were obtained in relation to the control samples (without substrates). The results obtained allow us to characterize the qualitative and quantitative aspects of the enzyme-substrate interaction of the myofibrillar proteins of giant grenadier and additional protein substrates affected by MTG.

An important indicator for the enzyme-substrate interaction was the molecular weight of the introduced proteins. The presence of low molecular weight proteins, regardless of their amino acid composition did not ensure the formation of protein gels, and even led to almost complete expression of the water contained in the tissue, both with and without MTG. In this case, the samples became runny or very runny. Rheological properties of the grenadier muscle gels affected by MTG slightly improved only using a nucleoprotein complex of salmon milt containing DNA derivatives and protamines. The introduction of MTG into the samples in the absence of substrates made it possible to obtain structure compaction, an increase in the WHC, and dynamic viscosity, however, fluidity retained, so it was impossible to measure the strength of these samples.

Strong, dense and elastic structure was formed after fermentation when casein and gelatin were added. In this case, an increase in viscosity by 10-15 times was observed in comparison with samples containing combined substrates, but without MTG. WHC also increased significantly, almost threefold. After heat treatment (90°C, 20 min), these samples did not lose their properties. Losses during heat treatment did not exceed 5% of the mass, while for control samples in respect of minced muscle tissue they amounted to 24.8% without additives and without enzyme and to 16.9% applying MTG.

When studying the obtained samples for digestibility by digestive enzymes in model experiments, it was shown that the amount of low molecular weight protein components

Table 1: The properties of gels from the muscle tissue of the giant grenadier affected by various protein substrates and MTG (1% by weight).

No.	Substrate, % to the mass of minced fish	WHC, %	Dynamic viscosity, Pa.s	Breaking strength, N	Description
without MTG					
1	Absent	22.3±4.4	1.75±0.24	-	Very soft with pronounced fluidity
2	Gelatin, 5 %	53.14±2.9	2.87±0.32	0,7±0,2	Durable, brittle, granular
3	Casein, 5 %	40.5± 6.0	2.03±0.21	-	Soft, homogeneous, with low fluidity
4	Soluble collagen from fish skin, 7 %	9.4±2.1	0.75±0.07	-	Runny
5	Hydrolysate from bivalve mollusks, 7 %	7.1±1.9	0.63±0.07	-	Very runny
6	Nucleoprotein complex from salmon milt, 7 %	15.8±2.0	0.93±0.06	-	Runny
1% MTG to minced fish					
1	Absent	34.9±2.7	12.76±1.77	-	Compacted, friable, with slight fluidity
2	Gelatin, 5 %	53.4 ±2.6	32.72±2.70	1.5± 0.2	Firm, resilient, sliceable
3	Casein, 5 %	50.5 ± 5.1	21.96±3.31	0.7±0.1	Durable, homogeneous, resilient
4	Soluble collagen from fish skin, 7 %	0	0.65 ± 0.10	-	Runny
5	Hydrolysate from bivalve mollusks, 7 %	0	0.78 ± 0.07	-	Very runny
6	Nucleoprotein complex from salmon milt, 7 %	33,3 ± 4,4	2.16 ± 0.12	-	Compacted, with slight fluidity

formed after the sequential action of pepsin and trypsin on the samples obtained with and without MTG fermentation were almost the same. Consequently, the obtained products retain good digestibility in the gastrointestinal tract.

When studying the influence of the samples on the growth of the ciliate *Tetrahymena pyriformis* test culture, their total biological value ranged from 78 to 134% with respect to the control (collagen hydrolyzate). The highest indicator was obtained for the sample containing minced muscle tissue of giant grenadier, casein and MTG.

5. CONCLUSION

Thus, protein substrates such as gelatin and casein are the most promising for the creation of structured food products based on the muscle tissue of giant grenadier. Low molecular weight proteins of various origins do not provide gelation in this system.

The experimental work and the data obtained made it possible to substantiate the technology of molding products based on fermented mince muscle tissue of giant grenadier with the addition of casein, both in the form of semi-finished products, and in the form of ready-to-eat products.

References

[1] Ramírez J, Uresti R, Téllez S, Vázquez M. Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. Journal of Food Science. 2002;67(5).1778-1784 doi: 10.1111/j.1365-2621.2002.tb08722.x

[2] Moreno HM, Borderías AJ, Baron C. Evaluation of some physicochemical properties of restructured trout and hake during cold gelation and chilled storage. Food Chemistry 2010;120.410-417 doi.org/10.1016/j.foodchem.2009.10.021

[3] Domoratsky SS, Domoratskaya MV. Fermenting fish trimmings using enzyme-based drug transglutaminase. Scientific Journal NRU ITMO University. 2014;4. 30-38

[4] Rachel NM, Pelletier JN. Biotechnological applications of transglutaminases. Biomolecules. 2013;3. 870-888 doi: 10.3390/biom3040870

[5] Ramírez JA, Uresti RM, Velázquez G, Vázquez M. Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review**.** Food Hydrocolloids. 2011;25. 1842-1852doi.org/10.1016/j.foodhyd.2011.05.009

[6] Pivnenko TN, Kovalev NN, Zaporozhets TS, Besednova NN, Kuznetsova TA. Enzymatic hydrolysates from hydrobionts of the Pacific Ocean as the basis for the creation of biologically active food additives and functional food products. Vladivostok: Dalnauka; 2015.