Determination of the effect of microbial transglutaminase on technological properties of common carp (*Cyprinus carpio* L.) meat

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ABSTRACT: The aim of this study was to evaluate the effect of microbial transglutaminase (TG) on processed meat of common carp (*Cyprinus carpio* L.). Three levels of microbial transglutaminase (0.5, 1.0 and 1.5%) combined with three levels of NaCl (0, 1 and 2%), added to support the binding reaction, were examined. For the evaluation of quality changes in restructured fish meat, we used the textural property hardness and water holding capacity (WHC). The results confirmed a strong improvement of texture and water holding capacity after the addition of transglutaminase and salt, whereas the addition of 1% TG + 1% NaCl seems to be the best combination from the economic aspect. Another increase in the addition of TG and NaCl to the fish meat did not lead to further improvement of qualitative properties, as it was in the groups with 0% and 1% NaCl.

Keywords: processing; common carp; enzyme; NaCl; transglutaminase

Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme capable of catalysing acyl-transfer reactions creating covalent cross-links between proteins (Nonaka et al., 1989) as well as peptides and various primary amines. When the ε-amino groups of lysine residues in proteins act as acyl acceptors, ε- (γ-glu)-Lys bonds are formed both intra- and inter-molecularly, which results in the improvement of textural properties (hardness, elasticity, cohesiveness, etc.) and in a decrease in weight losses of the product (improvement of water holding capacity). This type of chemical bond is much stronger than other types like ionic or hydrogen bonds and cannot be broken through the influence of standard temperatures and other physical forces.

Different literary sources have documented that the addition of salt improves the effect of transglutaminase and other binding agents (containing fibrinogen, thrombin, modified food starch, carrageenan and others). NaCl helps to release water from fish meat, which can be used for the restructuring of fish meat (Ramírez et al., 2002). Fish meat shows a natural capability of hardening after the addition of 2–3% of salt in a temperature range between 5 and 43°C. This phenomenon is called "setting" and depends on the presence of endogenous transglutaminase (Tgase).

The ability of meat to bind water (WHC – water holding capacity) is one of the most important technological properties that affect the quality of the product. WHC is also important from the eco-

Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. MSM 6007665809).

nomic aspect, especially in connection with water losses during processing, storage and heat treatment. WHC is defined as the capability of meat to hold its own and/or added water during the action of a certain force or other physical stress (pressure, heating, etc.). The higher the force, the higher the amount of water that can change from the immobilised condition to the free-moving condition (Pipek, 1995).

The methods of measuring WHC can be divided into 3 groups according to the type of physical method. They are methods without the use of a force (evaporation and drip losses), methods depending on the use of a force (compression method, centrifugation method, capillary volumetry, extractive refractometry) and methods using thermal effects (evaporation losses).

One of the most frequently used methods is a compression method in which WHC is calculated by the formula:

$$\text{WHC} = 100 \times \frac{W_1 - W_2}{W_1} \times 100$$

where:

W_1 = initial weight

W_2 = final weight

The meat of common carp was treated by two procedures: cutting meat into small pieces and mechanical separation. Separation is a process when fish meat is crushed and then separated from bones, scales, skin and tissues. The quality of mechanically separated meat (separate) depends on the quality of the raw material and especially on the whole separation process. The process of separation, used equipment and conditions have a considerable effect on the quality of the final product (Pavlíček, 2001).

Most of the principles of meat separation are based on mechanical separation of muscle tissues from non-muscle ones through a perforated steel cylinder. There are three basic separation methods. Regenstein and Regenstein (1991) described the principle of the first method that consists in pushing the meat by a rubber band into a perforated steel cylinder. The rubber band fits tightly with a surface of about 25% to the cylinder circumference. The band and cylinder move against each other at different speeds and with the raising rate of the cut the final yield increases. The second method is based on the principle of a round die stock and a perforated cylinder when the meat is pushed through the cylinder by a round die stock instead of a rubber band (Taylor, 1972). The principle of the third method of separation is the effect of two interlocked concentric cylinders, where the inner cylinder is perforated, rotating and by using the external cylinder, the meat is pushed through the inner cylinder (Miller, 1980).

Through the separation process a separate consisting of meat from different parts of the fish body is produced that more or less affect the texture, flavour and overall appearance of the separate (Grantham, 1981). Among the most visible components in fish meat that can be perceived sensorially are bone parts. Fish bones account for about 15% of the whole fish body and over 30% from the rest after filleting, which can negatively affect the sensorial properties of the separate and the following perception by the consumers.

The knowledge of transglutaminase and possible methods of its utilization has made a rapid progress recently. In food processing for human nutrition the utilization of this enzyme plays a major role, so a lot of large companies and organizations are engaged in its research. Transglutaminase can be used in the food processing industry in various ways which include the processing of products from animal and plant production. In addition, transglutaminase partakes in the development of different types of products where meat can be replaced with plant raw materials. In the near future these products might be produced with the same qualitative parameters as the real meat products have.

Transglutaminase is one of the new enzymatic products in the food processing industry. It is an enzyme commonly occurring in nature that has been found in animal and plant tissues (Folk, 1980; Falcone et al., 1993; Yasueda et al., 1994) and microorganisms (Ando et al., 1989). As mentioned above, transglutaminase can be divided into endogenous (calcium dependent) and microbial transglutaminase (calcium independent).

Endogenous transglutaminase is present for example in liver (Zhu et al., 1995; Ohtsuka et al., 2001), muscles (Nozawa et al., 1997), fish eggs – mainly in the envelope of fish eggs (Ha and Iuchi, 1998; Kudo and Shigeharu, 1998), blood plasma (Hall et al., 1999) and haemocytes – blood elements (Yeh et al., 1999; Soederhaell et al., 1999).

Currently, fermentation by means of microorganisms is the most frequently used and economically optimal method of obtaining transglutaminase; mainly the genus *Streptoverticillium* (Motoki et

al., 1989) and *Streptomyces* is used (Ando et al., 1989, 1992). The enzyme can be extracted from a culture medium. During the process, the rest of the culture and the medium are fully separated from the enzyme. In the last processing step, the enzyme is transferred into a product in powder form.

There are two possible ways of the enzyme application that result from the form of the product. It can be used either in loose or in rehydrated form. It depends especially on the properties of the raw material to which transglutaminase is added.

The reaction time depends on the temperature. Higher temperatures require less time for the reaction between transglutaminase and free protein groups, whereas lower temperatures require a longer reaction time. An optimal temperature for transglutaminase activity is in the range from 50°C to 55°C (the reaction is stable at temperatures in the range of 0–60°C) and an optimal pH is in the range from 6 to 7 (stable at pH 5–9). Another advantage of this enzyme is its easy inactivation that starts above 60°C. At 75°C the enzyme is inactivated within 5 minutes, at 80°C within 1 minute. The duration of inactivation also depends on the type of foodstuffs.

In fishery, transglutaminase is currently used mainly in the processing of saltwater fish and their products (surimi, fillets, separate, etc.).

The forecast of fish processing development in the Czech Republic is positive. At present, only 8–11% of the total fish production (in live weight) is processed in the Czech Republic and the rest of it is realised by sales of live fish, abroad (47–51%) or inland sales (40–44%).

MATERIAL AND METHODS

Approximately 30 kg of fish meat from the common carp (*Cyprinus carpio* L.) of market size (scaly form) were processed, which came from the Fishery Třeboň, a.s. At first, the fish were scaled, gutted, cut into halves and then a half of them was cut into small pieces and the rest was mechanically separated. Nine groups of samples were prepared (Table 1), needed for the evaluation of the performance of restructured fish meat under the following thermal processing (freezing, frying and smoking). Textural properties were evaluated before and after the heating of fish meat. The hardness values were measured three times for each sample.

In parallel with the restructured fish meat a control group without addition of transglutaminase and NaCl was monitored that underwent the same heating processing.

The instruments used for the texture analysis must meet the requirements for objective food quality evaluation. Therefore an apparatus TA.XT2 of the company Stable Micro Systems (one of the latest mobile texturometers) was used. Among other things, this apparatus is able to measure the effect of emulsifiers, thickening agents, stabilizers and other treatments used in different fish meat processing technologies. The apparatus works with a force of calibration weight of 5 kg or 25 kg, which

Table 1. Values of meat hardness (in N) measured with a cylindrical probe 25 mm in diameter

Groups	NaCl (%)	Control (%)	TG (%)		
		0	0.5	1	1.5
Samples before thermal processing	0	132.07 ± 2.96	230.3 ± 17.05	245.63 ± 12.22	267.04 ± 5.06
	1		310.44 ± 1.53	430.67 ± 2.48	470.05 ± 0.78
	2		330.65 ± 1.34	445.76 ± 4.69	473.24 ± 5.76
Freezing	0	127.05 ± 3.17	195.34 ± 11.16	213.68 ± 3.1	242.12 ± 2.16
	1		210.44 ± 2.15	330.97 ± 4.79	390.05 ± 2.12
	2		240.65 ± 2.15	370.76 ± 1.07	398.24 ± 2.04
Frying	0	137.08 ± 2.38	235.3 ± 4.9	240.93 ± 2.24	250.14 ± 6.22
	1		296,45 ± 2.17	453,97 ± 1.63	480,05 ± 4.92
	2		325.35 ± 4.21	470.36 ± 2.12	515.24 ± 1.92
Smoking	0	140.08 ± 4.43	230.54 ± 4.9	245.78 ± 6.96	254.14 ± 5.1
	1		254.47 ± 4.23	340.85 ± 1.91	420.37 ± 2.87
	2		270.95 ± 2.79	410.66 ± 1.03	430.24 ± 2.27

fully meets the requirements for the testing of texture in fish products.

Microbial transglutaminase

Microbial transglutaminase in the product called "Activa" EB (powdery form) of the company Ajinomoto CO. was used for our test. The composition of the product is as follows: sodium caseinate 60%, maltodextrin 39.5% and transglutaminase 0.5%.

Small pieces

Meat pieces were obtained by cutting the fish halves without spine. From the cubes made, three subgroups of samples were created (without heat treatment, chilling/frying and smoking) with different contents of transglutaminase and salt as it is shown in Table 1. Transglutaminase was rehydrated with cold water (0°C) at the ratio of 1 (TG):5 (H_2O). This transglutaminase with a given amount of salt was added to the cut meat, which was left to stand for a while. After about 10 minutes, the meat was stuffed into synthetic guts of various types. The type of gut depended on the following heat treatment of the sample. The individual weight of the samples was about 150 g. The gut Betan 40 was used in the samples that were to be frozen; it was put into cold water for 30 minutes before application. The samples designed for smoking were stuffed into a gut R2LD S2 (inedible collagen), which was immersed in a 15% salt solution for 1 hour. We put an emphasis on the expression of all air between the pieces of meat. Then the meat was stored overnight at 4–5°C. After the reaction took place, the samples were cut into slices (approximately 1 cm) and their hardness was measured. The point temperature in the meat core was not monitored.

In the first subgroup, textural properties (hardness) and water holding capacity (WHC) were measured immediately after the treatment with transglutaminase and compared with the first control subgroup.

The second subgroup contained a spectrum of samples which were arranged in the same way (Table 1). All samples from this subgroup were frozen at –26°C for 48 hours. After thawing, changes in textural properties were determined. After the necessary data were acquired, the samples underwent another heat treatment – frying (185°C). After frying, the measurements of textural properties were conducted again. The results were compared with the second control group that underwent the same heat treatment (freezing and frying).

The third subgroup contained a spectrum of samples which were arranged in the same way, but were treated with hot smoke without previous freezing. The smoking runs in three phases:

at 90°C for 30 min
at 100°C for 30 min
at 120°C for 60 min

The smoking duration depends on the size and properties of smoked raw material. After smoking, the textural properties were measured again. The results were compared with the third control subgroup that underwent the same heat treatment.

Separate

The separate was made from halves of common carp of market weight on the apparatus TR-6 1HP. It is a horizontal type separator of the performance 250 kg of separated fish meat per hour. The samples of the separate were divided into equal subgroups (Table 1) and treated in the same way as the cubes of meat.

Statistical analysis of the results

The results were analyzed using the program Statistica 6.0. For their interpretation the analysis of variance (ANOVA) was used. The significance of individual factors was evaluated with Duncan's test.

RESULTS AND DISCUSSION

Monitoring of fish meat hardness

In the first subgroup without heat treatment, the highest value of hardness 473.24 ± 5.76 N was measured in the sample containing 1.5% TG and 2% of sodium chloride (Table 1). The lowest value of 230.3 ± 17.05 N was reached by a sample that was treated only with 0.5 % TG. An addition of 1% of salt into the sample increases the value of hardness noticeably up to 310.44 ± 1.53 N. During a further

increase in the dose of TG and sodium chloride to 1%, the value of hardness increased rapidly up to 430.67 ± 2.48 N. The following increase of TG to 1.5% caused an increase in the hardness value to 470.05 ± 0.78 N, but this increase was not as intensive as at the dose of 1% TG and 1% sodium chloride. We can also observe the same pattern of the increasing value of hardness even in groups with the content of 0.5% TG + 2% sodium chloride (330.65 ± 1.34 N) and 1% or 1.5% of TG + 2% sodium chloride. The samples in the control group reached a hardness value of 132.07 ± 2.96 N.

The same tendency in the rise of the value of hardness was observed in the second subgroup that was processed in the same way with TG and salt as it was in the first subgroup without heat treatment, but it was subsequently frozen for 48 hours. Table 1 shows how the value of hardness gradually increases with the increase in the dose of TG and sodium chloride. Generally the values of hardness are somewhat lower than they are in the first subgroup, which could have been caused by the influence of low temperature. The following frying raised the values of textural properties again and the values were comparable with the first subgroup without heat treatment, they were even a little higher in most cases (Table 1). The frozen control group reached the value of hardness 127.05 ± 3.17 N but the addition of 0.5% transglutaminase and NaCl to the meat increased the value of hardness up to195.34 ± 11.16 N. The greatest difference in the increase of textural properties was between subgroups with 0.5% and 1% NaCl (Table 1). For comparison, a control sample reached the value of hardness 137.08 ± 2.38 N, but after addition of the lowest dose of TG (0.5%) it markedly increased up to 235.3 ± 4.9 N.

The third subgroup was smoked. Measurements of textural properties were carried out after smoking. The measured value in the control group was 140.08 ± 4.43 N. On the other hand, after addition of 0.5% TG, hardness reached the value of 230.54 ± 4.9 N. Generally, the samples reached a little lower values of hardness than in the first subgroup without heat treatment (Table 1), but the dynamics of the growth of hardness values after addition of transglutaminase and NaCl was the same as in the previous two groups.

WHC determination

The determinations were conducted in the control group and in the group without heat treatment. The control group without treatment reached the value of WHC 52.97 ± 1.51%. Subsequently, there was a tendency of WHC increase with the increasing addition of TG and NaCl. As expected, the best WHC values were reached by the sample with the content of 2% NaCl and 1.5% TG addition (Table 2).

During the study, the effect of different amounts of microbial transglutaminase and salt on textural properties (hardness) and water holding capacity (WHC) of common carp meat was examined. Hardness was found to increase in the samples treated with TG and salt. For example similar experiments were conducted by Ramírez et al. (2002) on the silver carp (*Hypophthalmichthys molitrix*) meat where they also used three levels of TG (0, 0.3 and 0.6%) and salt (0, 1 and 2%). Essentially they reported the same results that the addition of TG and salt increased the qualitative parameters of restructured fish meat. They insisted on the need of salt addition for a considerable improvement of qualitative properties of meat.

Separate

In the first control subgroup the value of hardness 56.31 ± 0.41 N was measured. After addition of 0.5% TG the value of hardness noticeably increased up to115.75 ± 1.54 N. The addition of 1% of salt increased the hardness of the sample up to 195.75 ± 2.06 N again. A further increase in TG and salt

Table 2. The values of WHC (%) in the control and samples before thermal processing

Groups	NaCl (%)	Control (%)	TG (%)		
		0	0.5	1	1.5
Samples before thermal processing	0	52.97 ± 1.51	55.4 ± 1.51	57.1 ± 0,93	62.9 ± 0.3
	1		64.4 ± 1.03	67.3 ± 2.05	77.5 ± 2.09
	2		76.5 ± 1.8	79.8 ± 5.24	81.1 ± 1.61

Table 3. Values of separate hardness (in N) measured with a cylindrical probe 25 mm in diameter

Groups	NaCl (%)	Control (%)	TG (%)		
		0	0.5	1	1.5
Samples before thermal processing	0	56.31 ± 0.41	115.75 ± 1.54	135.72 ± 1.14	170.43 ± 7.73
	1		195.75 ± 2.06	230.57 ± 4.86	245.72 ± 1.26
	2		205.74 ± 4.90	235.81 ± 1.09	250.43 ± 21.56
Freezing	0	49.47 ± 0.35	95.63 ± 4.75	120.56 ± 4.29	133.84 ± 4.70
	1		110.65 ± 5.01	200.57 ± 7.76	235.76 ± 4.87
	2		120.79 ± 2.67	230.86 ± 2.48	242.81 ± 2.51
Frying	0	65.23 ± 0.31	120.84 ± 3.06	140.98 ± 2.09	199.77 ± 34.25
	1		200.54 ± 0.96	250.71 ± 1.96	260.73 ± 4.54
	2		210.71 ± 6.00	255.82 ± 2.55	265.93 ± 2.41
Smoking	0	58.32 ± 0.11	117.91 ± 0.99	150.73 ± 3.94	179.84 ± 14.99
	1		155.83 ± 1.15	232.42 ± 1.65	250.81 ± 1.86
	2		207.78 ± 2.57	237.75 ± 4.61	257.95 ± 2.20

addition contributed to a repeated improvement of textural properties, but the growth of hardness was not so rapid (Table 3).

The same dynamics of the effect of TG and salt on the samples was observed in the second subgroup as was determined in the cubes of meat. The drop in temperature led to a small decrease in hardness values again. The following frying raised the values of textural properties again.

In the third subgroup, the addition of TG and salt led to subsequent improvement of the texture as it was in the cubes of meat.

Statistical analysis

The primary data analysis of fish meat hardness was carried out on the level of four factors (effects).

Table 4. The primary data analysis of variance (ANOVA) of fish meat hardness

Effect	*df*	*MS*	*F*
1	1	10 200 71	22 281
2	3	38 269	836
3	2	298 611	6 522
4	2	174 495	3 811
12	3	5 329	116
13	2	22 258	486
14	2	13 684	299
34	4	15 406	337
Residual	144	46	

The most marked influences are given in Table 4. It follows from the results that the significance of single factors decreases in the order: raw material > salt > TG > treatment.

Considering the large difference in significance of the influence of raw material and treatment, these two factors were interpreted by Duncan's test. The results are shown in Table 5. On the basis of the acquired data it is possible to claim that the influence of raw materials is so large that the whole set can be divided according to the raw material. From the aspect of the treatment, the most favourable influence was exerted by frying, the worst by freezing. But in comparison with other factors, the influence of the treatment is only minimal.

Table 5. Correlations between raw materials and treatment

Raw material	Method of treatment	Hardness (N)	
Separate	freezing	165.7	a[1]
	before treatment	198.4	b
	smoking	199.0	b
	frying	211.8	c
Meat	freezing	288.0	d
	smoking	317.6	e
	before treatment	356.0	f
	frying	363.1	g

[1]values with different letters in the column indicate significant differences at $P < 0.05$

Table 6. The influence of single factors, Duncan's test

Effects		Hardness of meat		WHC		Hardness of separate	
Salt (%)	Transglutaminase (%)	(N)		(%)		(N)	
0	0	134.1	a[1]	53.0	a[1]	18.8	a[1]
0	0.5	222.9	b	55.4	a	112.5	b
0	1	236.5	bc	57.1	a	137.0	c
0	1.5	253.4	bc	62.9	b	171.0	de
1	0.5	267.9	cd	64.4	bc	165.7	de
2	0.5	292.0	d	76.5	d	186.3	e
1	1	389.1	e	67.3	c	228.6	f
2	1	424.4	f	79.8	de	240.1	fg
1	1.5	440.1	f	77.5	de	248.3	fg
2	1.5	454.2	f	81.1	e	254.3	fg

[1]values with different letters in the column indicate significant differences at $P < 0.05$

Subsequently, the analysis of variance (ANOVA) was carried out, separately for cut meat and mechanically separated meat, containing the factors salt (NaCl) and TG. It follows from the results that the content of NaCl has the most significant ($P < 0.05$) influence on hardness while the influence of TG content is smaller. There is also a statistically significant interaction between both factors. The same relations were also provided by the analysis of bound water content (WHC).

The influences of single factors were evaluated by Duncan's test. The results are shown in Table 6. It is evident that the influence of salt and TG shows similar dependences for each of the three groups. The influence of salt is very expressive and in the variants that contain no salt, the influence of TG is minimal. On the other hand, at the highest level of used salt (2%), the further strong increase in the content of TG has no evident effect.

CONCLUSION

Among all the groups, the best results were achieved at the content of 1% TG and 1% NaCl. If TG and NaCl doses further increased, the differences between hardness values were lower. With the heat treatment or freezing, the dynamics of the influence of TG and salt remained the same. It means that the higher the dose of TG and NaCl, the higher the value of hardness. It was found that the freezing process and subsequent thawing reduced the hardness values of the samples only a little, which was expected. On the contrary, during the frying and smoking process, the values of hardness increased.

In addition to the textural properties, the determination of water holding capacity (WHC) was evaluated, which confirms the efficiency of transglutaminase addition – with increased doses of TG and NaCl the amounts of expressed water decreased.

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Received: 2005–09–20
Accepted after corrections: 2006–09–06

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