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Ensuring the safety of the lipid fraction of semi-finished products of a high degree of preparation from fatty fish raw materials

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Abstract: The relevance of the studies is caused by the need to improve the safety and extend the shelf life of semi-finished products from fatty fish raw materials. The study was carried out at the premises of the Saratov State Agrarian University. Study objects were fish mixed fodder; two-year-old carps; fish raw materials; carp semi-finished products. Physicochemical, histological, and organoleptic research methods were used in the study. The authors have developed a method for inhibiting the process of fish fat oxidation at all the stages of the life cycle of fish products using CO_2 rosemary extract and milk thistle oil meal as antioxidant components because they contain flavonolignans and carnosic and rosmarinic acids. Technological methods for obtaining complex fish mixed fodder with antioxidant properties were developed and the optimal dosages of the antioxidants added to a feed supplement were determined. A positive effect of an antioxidant supplement on the fish biological characteristics of a reared carp, as well as on the morpho-functional indicators of the obtained raw materials, was shown. The authors developed formulations of the fish semi-finished products made from the raw materials grown using antioxidant fish mixed fodder. The safety and quality indicators of the developed products were estimated. They showed that the use of an antioxidant component at the stage of fish rearing and in manufacturing process of fish semi-finished products makes it possible to significantly improve the stability of the fat phase both in fish raw materials and in the finished products.

Keywords: Fish products, oxidation, fat component, natural antioxidants, semi-finished products of a high degree of preparation, centralized production

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INTRODUCTION

The modern market of fish products of a high degree of preparation (fish cutlets, chopsticks, rissoles, meatballs, etc.) is characterized by the use of low-fat varieties of sea fish, mainly imported ones. The manufacturers of these products try not to use fatty fish varieties because of the possible loss of consumer properties of products at the circulation stage due to the rancidity (oxidation) of the fat component [15, 16]. It is known that the determining factor of the shelf life of fish and fish products that causes negative changes in the organoleptic properties and nutritional value, as well as the formation of toxic products, is lipid oxidation [1, 17, 18]. In addition, fatty sea fish varieties are characterized by a rather high cost, which makes it impossible to use them in manufacturing semi-finished products of a high degree of preparation and prohibitive to the majority of the population of our country [7].

Up to date, one of the most important tasks of the food industry is to meet the need and ensure the per capita consumption of the population of our country with quality and safe fish products taking into account the biological norms of consumption and the reduction of import dependence.

With the current pace of import substitution development, there is an increase in carp meat popularity, as the domestic pond fish raw materials are cheap and easy in rearing and in manufacturing.

However, when using it in industrial production, there is a problem to stabilize lipids since the unsaturated nature of carp lipids has a negative effect on oxidative stability during autolysis [11, 13, 19]. A large number of factors that initiate fat oxidation, as well as the chain mechanism of the process and the distinctive features of the technology for manufacturing various types of products, do not allow

for the development of a universal method for preventing the oxidative damage of products with a high proportion of the fat component.

For this reason, it is necessary to estimate the main factors of lipid oxidation and to select the appropriate methods for inhibiting them for a particular food system. An effective way to increase oxidative stability is the addition of antioxidants. Moreover, the approach to the antioxidant protection of each food system should be individual.

In this regard, the development of a method for the oxidative stabilization of carp lipids and the prolongation of the shelf-life of carp semi-finished products and culinary products is an urgent task since the widespread development of the fish production centralized industry is possible in case of the assured safety and quality of the products.

The presented study is aimed at developing a method for the oxidative stabilization of carp lipids and the prolongation of the shelf life of semi-finished products of a high degree of preparation by inhibiting the oxidation of the lipid fraction at all the stages of the manufacturing process.

STUDY OBJECTS AND METHODS

The objects of the studies were:

– standard mixed fodder for feeding two-year-old carps;

– the developed mixed fodder with antioxidant properties on the basis of standard mixed fodder with the addition of milk thistle oil meal (TU 9141-005-46899394-04) and CO_2-rosemary extract (TU 9169-001-10140736-03);

– two-year-old carps of the experimental and control groups;

– fish raw materials obtained after feeding with common feed and cultivated feed; and

– semi-finished products of a high degree of preparation from carp with the specified high antioxidant properties.

The fish were reared in the laboratories of the of Feeding, Zoohygiene and Aquaculture Department of the Federal State Budgetary Educational Institution of Higher Professional Education Saratov State Agrarian University in aquariums with a constant flow and water aeration. Aquariums had dimensions of 100 × 50 × 40 cm and a volume of 200 liters.

The experiment was conducted for 76 days. During the cultivation of two-year-old fish, the thermal conditions were favorable for their growth. The average water temperature was 19.3 and 19.5°C, respectively. The oxygen content was at least 5 mg/l.

The feeding rate was calculated based on the body weight, and water temperature according to the specially developed feed tables [10]. Manual feeding, twice a day during daylight hours, was applied. The daily feeding rate was 3% of the fish body weight.

The fish was weighed according to the recommendations of I.F. Pravdin [20]. All the fish used in the experiment was subjected to control weighing once every 10 days. At the same time, the daily ration was adjusted.

The growth rate was judged by the data of the absolute and average daily gain. The average daily growth rate of the juveniles was calculated using the following formula:

$$A = [(Мк/Мо)1/t – 1] × 100 (\%), \quad (1)$$

where A is the average daily growth rate, %;

Mk and Mo are the mass of the fish at the end and at the beginning of the experiment; t is the duration of the experiment, days.

The absolute gain was estimated using the formula:

$$Pab = Мк – Мо, \quad (2)$$

where Mk is the final mass of juveniles, g; M0 is the initial mass of juveniles, g.

To calculate the mass accumulation coefficient, the following formula was used:

$$Км = ((Мк1/3 – Мо1/3) × 3)/t, \quad (3)$$

where Км is a mass accumulation coefficient, units; Mk and Mo are the final and initial masses of the fish, g; t is the cultivation period, days.

The average daily gain was determined using the formula:

$$P = (Мк – Мо)/t, \quad (4)$$

where P is the average daily gain, g; Mk and M_O are the final and initial masses of the fish, g; t is the cultivation period, days.

The histological analysis of carp muscles was carried out according to the method described by of G.O. Merkulov [6] and M.P. Kokuricheva [5]. The pieces of tissues and organs were immediately placed in a fixing solution, namely a 10% aqueous neutral formalin solution. From the material fixed with the 10% aqueous neutral formalin solution, histological sections 15 μm in thickness were prepared using a model 2515 freezing microtome (ReichertWien). To detect neutral fats and phospholipids, the histological sections were stained with sudan black B.

For biochemical analysis, blood sampling was carried out according to the method of V.V. Limanskiy "Instruction on the physiological and biochemical analyses of fish".

The appearance and color of fish mixed fodder was determined organoleptically according to GOST R 51899-2002 "Granulated mixed feeds. General specifications". The smell of the experimental mixed fodder was evaluated according to GOST 13496.13-75 "Combined animal feeding stuffs. Methods for determinaton of smell, infestation by cereal parasites".

The granule size was determined according to GOST R 51899-2002 "Granulated mixed feeds. General specifications".

To determine the water resistance, GOST 28758-97 "Granular mixed fodders for fish. Methods for determination of water-proofness" was used.

Fats were extracted from the fish raw materials using an extraction-weight method according to GOST 54053-2010 "Confectionery. Methods for determination of fat weight fraction". The mass fraction of methyl esters of individual fatty acids of their total amount was determined by gas chromatography in accordance with

GOST R 51486-99 and GOST R 51483-99 using a Kristall 2000M gas chromatograph. The acidity index of the extracted fat was determined according to GOST R 52110 "Vegetable oils. Methods for determination of acid value". The peroxide number was determined using the method of N.A. Golovkin and R.L. Perkel, and peroxides in fish fats by potentiometric titration. [1.a.i.3].

The organoleptic indicators were estimated in accordance with GOST R 53161-2008. The average score was calculated in accordance with GOST 7631-85, taking into account the weight of individual indicators "Fish, marine mammals, marine invertebrates and products of their processing. Acceptance rules, organoleptic methods of quality control, sampling methods for laboratory tests". To obtain an unbiased evaluation, descriptors, which show the main characteristics of the product and characterize the technological methods that affect the preservation of food and biologically active substances were developed "ISO 11035 Organoleptic analysis. Methodology. General guidance for establishing an organoleptic profile".

RESULTS AND DISCUSSION

The aim of the studies was the development of a method for the continuous inhibition of lipid oxidation at all the stages: from rearing fish raw materials rich in lipids [12, 14] to manufacturing deep-fried semi-finished products of a high degree of preparation therefrom. The study stages included the solution of the following tasks:

– to develop special mixed fodder for fish (carp) with the use of antioxidants to improve the commodity and consumer properties of fish raw materials and increase their shelf life;

– to prove the positive effect of the developed feed on the antioxidant stability of fish raw materials on the basis of the biological studies;

– to develop formulations for fish products of a high degree of preparation for industrial manufacturing with the specified properties using the fish raw materials reared with the use of the new antioxidant mixed fodder.

It is known that some herbs, spices, and their extracts have the ability to slow lipid oxidation down [10, 16].

CO_2-herb extracts are of particular interest. For example, the antioxidant activity of rosemary extract is 10 times higher than that of ionol. Twenty-two substances, among which are phenolic acids, carnosol derivatives, and flavonoids, have been identified in Rosmarinus officinalis extract. In terms of inhibiting lipid oxidation, the most effective are carnosol, rosmarinic acid, carnosic acid, caffeic acid, rosmanol, and rosmadial. Carnosic acid and carnosol are strong lipid peroxidation inhibitors in microsomal and liposomal systems, as well as peroxide radical and superoxide anion absorbers. It was established that rosemary extract is highly resistant to high temperatures [8, 9]. Antioxidant mixtures, rather than individual antioxidants, are advisable for use in manufacturing.

The development of the feed formulation and the balance of the nutrient composition were carried out on the basis of the known carp needs determining the input rates for various components [10]. Milk thistle oil meal rich in antioxidants, flavonolignans and CO_2-rosemary extract, were used [8].

To determine the concentration of CO_2-rosemary extract in the composition of the feed, an experiment was carried out for 4 groups of fish. The study of the behavioral aspects of all the fish groups has revealed that an increase in the concentration of CO_2-rosemary extract results in a decrease in the values of fish-biological indicators (Table 1).

The group with CO_2-rosemary extract in a concentration of 0.05 g per 100 g carp mixed fodder had the best behavioral indicators. The growth rate of this group was equally high throughout the experiment, which confirms the efficiency of this rate of CO_2-extract in carp mixed fodder.

Proceeding from the experiment, to obtain antioxidant experimental feed, the feed mixture was enriched with the above-mentioned antioxidant consisted of 90.8% carp mixed fodder, 9.15% milk thistle oil meal, and 0.05% CO_2-rosemary extract. Table 2 presents the content of nutritional substances of mixed fodder with addition of milk thistle oil meal and CO_2-rosemary extract. The organoleptic and physical indicators of the experimental mixed fodder are represented in Table 3.

Table 1. Behavioral aspects of fish groups that consume various concentrations of CO_2-rosemary extract

Concentration of CO2-rosemary extract, g	Behavior of fish groups
0.03 (3 drops)	The group ate feed willingly in the morning, less willingly – in the afternoon; and active in the evening; gained weight moderately
0.05 (5 drops)	The group ate feed in the morning and in the afternoon willingly, very active; gained weight well
0.08 (8 drops)	The group eats feed unwillingly, low-active; gained weight slowly
Carp mixed fodder	The group ate feed willingly, active; gained weight moderately

Table 2. Nutrition content of the developed mixed fodder

Nutrients	Content, %	Normal value, %
Crude protein	36.8	30–38
Crude fat	4.2	2–5
Crude fiber	6.9	4–7
Crude ash	4.06	4–7
Lysine	1.82	1.8–2
Methionine	0.98	0.8–1.0
Tryptophane	0.23	0.2–0.3

Table 3. Organoleptic and physical indicators of the experimental mixed fodder

Indicator	Characteristic
Appearance	Cylindrical granules with a matte surface without cracks
Color	Corresponds to the color of bulk mixed fodder, dark brown
Smell	Corresponds to mixed fodder, the smell of CO_2-rosemary extract prevails
Water resistance, min	15–16
Swelling ability, min	27

Note. The choice of indicators is based on GOST R 51899-2002 requirements.

Table 4. Biological indicators of the experimental and control carp groups

Indicator	Groups	
	Control	Experiment
Mass, g:		
initial	390	388
final	469	479
Total gain, g	79	91
Average daily gain, g	1.03	1.19
Average daily growth rate,%	3.94	5.73
Mass accumulation coefficient, U	1.04	1.19
Survivability, %	71.4	71.4

Table 5. Average values of biochemical analysis indicators of blood

Indicator	Units	Control group	Experimental group
Total bilirubin	μmol/l	–	4.6
Direct bilirubin	μmol/l	–	1.3
GOT	U/l	109.9	183.0
GPT	U/l	42.1	15.5
Total protein	g/l	62.6	56
Creatinine	μmol/l	–	49.9
Urea	mmol/l	6.7	2.8
Uric acid	μmol/l	257.8	115.7
Glucose	mmol/l	6.7	6.5
Calcium	mmol/l	2.90	3.25
Phosphorus	mmol/l	1.51	2.23
Magnesium	mmol/l	1.26	1.63
Sodium	mmol/l	–	189.9
Potassium	mmol/l	–	6.5
Iron	μmol/l	–	28.4

The developed fish mixed fodder with antioxidants is balanced in terms of the basic food substances and has good organoleptic and physical indicators.

At the next stage of the experiment, experimental and control groups of two-year-old carps were formed, 14 pieces each.

Table 4 presents the positive dynamics of the live weight of carps in the experimental groups compared to the control.

To study the physiological state of the reared fish groups, biochemical parameters of blood were investigated (Table 4). The results showed that the use of the developed antioxidant feed did not influence its biochemical parameters.

Thus, the use of antioxidant carp feed balanced in the basic nutrition elements positively affects the blood test and the general physiological state of the reared fish.

For detailed investigation of changes in the body, the authors carried out a histological analysis of fish muscle tissue.

The histological sections of fish tissues showed that fats and lipoids in the muscle tissue of fish from the experimental group are larger in size and higher in quantity in comparison with the control group. Thus, one can see from Fig. 1 that fats and lipoids of the control group are presented as single drops, while those in the tissues of the experimental group (Fig. 2) are evenly distributed in muscles; they are larger in size and are contained in larger amounts, which will improve the taste and technological properties of fish.

12 fish from the control group and 20 from the experimental group were involved in the study. Table 6 shows the minimum and maximum sizes of lipoids in both groups. Thus, fat drops (lipoids) in the tissues of the experimental group are bigger than those in the control by 60.385 micrometers, on average.

When manufacturing semi-finished products, the quality of fish raw materials were evaluated.

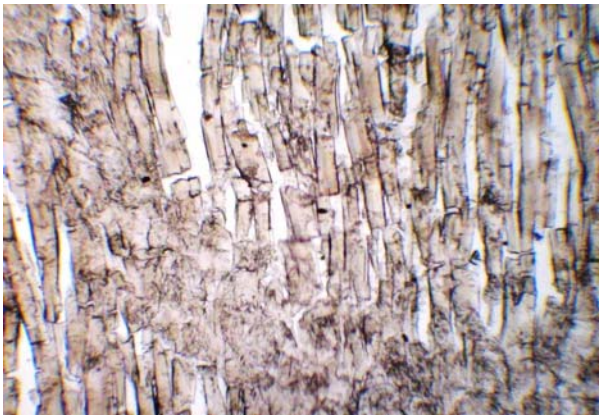


Fig. 1. Microphotograph of the carp tissue section. Small drops of fats and lipoids in the control group.

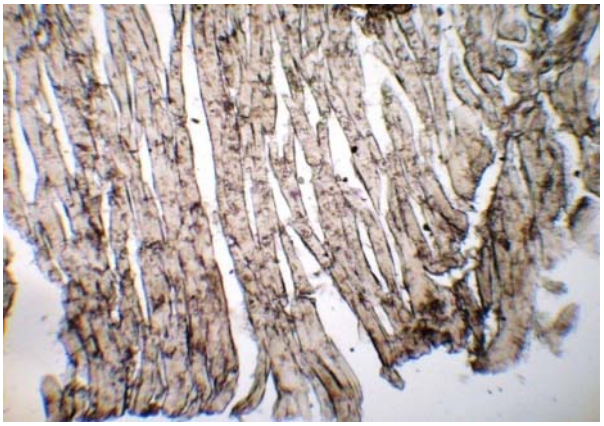


Fig. 2. Microphotograph of the carp tissue section. Fats and lipoids in the experimental group.

Table 6. Indicators of the linear and geometric values of lipoids in carp

Group	Area, μm^2		Perimeter, μm		Average size, μm	
	Min	Max	Min	Max	Min	Max
Control group 1	5.679 ± 0.003	9.872 ± 0.003	8.486 ± 0.003	11.142 ± 0.003	2.794 ± 0.003	3.694 ± 0.003
Control group 2	0.084 ± 0.003	9.521 ± 0.003	1.828 ± 0.003	11.433 ± 0.003	2.156 ± 0.003	3.688 ± 0.003
Experimental group 1	3.708 ± 0.003	13.279 ± 0.003	7.155 ± 0.003	13.552 ± 0.003	2.344 ± 0.003	4.316 ± 0.003
Experimental group 2	4.376 ± 0.003	16.135 ± 0.003	7.586 ± 0.003	14.604 ± 0.003	2.458 ± 0.003	4.747 ± 0.003

Table 7. Fatty acid composition of the lipid fraction isolated from carp

Fatty acid notation	Fatty acid	Test results		Test method reference	Method error
		Control	Experiment		
C 12:0	Dodecanoic	0.1	0.1	GOST R 51483-99	At the content of the substances sought: less than 5% – 0.28%; equal to or more than 5% – 1.42%
C 14:0	Myristic	1.2	1.6		
C 16:0	Palmitic	15.4	15.9		
C 16:1	Palmitoleic	3.8	4.2		
C 18:0	Stearic	3.8	4.7		
C 18:1	Oleic	31.5	32.4		
C 18:2	Linoleic	41.9	39.0		
C 18:3	Linolenic	0.2	0.3		
C 20:0	Arachic	2.0	1.7		

Table 8. Peroxide number content and acidity index in the fish tissues

Indicator	Unit	Test results		Method error
		Control	Experiment	
Acidity index	mgKOH/g	10.3	6.4	± 7% rel.
Peroxide number	mEq of active oxygen/kg	2.92	2.78	± 14% rel.

The fatty acid composition of the fat phase (Table 7) isolated from the product showed that the ratio and amount of fatty acids slightly varies. In the experimental group, the content of saturated acids slightly increases with respect to unsaturated ones, which favorably influences the storage of fish raw materials. The content of linoleic acid decreases. At the same time, there is an increase in the amount of oleic acid that has a beneficial impact on the maintenance of body immunity.

Thus, changing the diet of carp, it is possible to vary the ratio and the quantitative composition of fatty acids.

Of the indicators that characterize the safety of the product, the peroxide number and acidity index were determined.

During storage, free fatty acids accumulate due to the hydrolysis of muscle lipids under the influence of tissue lipases.

Table 8 shows the content of peroxides and free fatty acids of the fat component in experimental and control groups of fish.

Table 8 shows that the content of free fatty acids in the experimental group is almost 2 times less than that in the control group. This suggests that the hydrolytic processes in the tissues of the experimental group of fish are slower during storage. Nevertheless, the accumulation of free fatty acids in fish lipids is quite intense.

The content of peroxides in both groups corresponds to the norms (no more than 10 mEq/kg) and in the experimental group it is less than in the control group.

Thus, the change in the quality of the lipid fraction of fish after giving them the feed that contain natural antioxidants was characterized by a decrease in the peroxide number and acidity index.

The results of the study testify to the effect of the antioxidants added to the feed on a change in the fatty acid composition and the quality of the lipid fraction of the experimental group of fish.

Deep-fried semi-finished products (fish croquettes) without and with the addition of CO_2-rosemary extract (the control and the experimental sample, respectively) were manufactured in order to develop a formulation for semi-finished fish products of a high degree of preparation with a prolonged shelf life under laboratory conditions for industrial manufacturing.

The purpose of adding vegetable ingredients to the formulation was to improve the safety and organoleptic characteristics of fast food products from fish raw materials, as well as to increase their shelf life.

As a research model was chosen deep-fried fish croquettes, since those are popular among consumers, who prefer quick-frozen fish culinary products, such as fish sticks, cutlets, rissoles, and meatballs [7], as well as because of the expansion of the assortment of this group of products.

The semi-finished products of a high degree of preparation (French croquettes) were prepared from the fish raw materials grown with the use of the antioxidant mixed fodder we had developed to produce fish raw materials with the specified properties according to the formulation given in Table 9. CO_2-rosemary extract was used as an antioxidant.

The formulation considers the following losses: 1% when mixing, 1% when molding, 20% when frying, and 5% the weight gain when crumbing.

The optimum concentration of rosemary extract in mincemeat was selected experimentally. Table 10 and Fig. 3 show the organoleptic characteristics of fish croquettes with the addition of various concentrations of CO_2-rosemary extract.

Table 9. Croquette formulation

Component	Net weight, kg		
	Concentration of CO_2 – rosemary extract in minced meat, %		
	0.03	0.04	0.05
Fish (carp)	80	80	80
Wheat bread	6.2	6.2	6.2
Milk	20	20	20
Eggs	12	12	12
Cooking fat	3.1	3.1	3.1
Yellow onion	6.2	6.2	6.2
Bread crumbs	–	–	–
Salt	2.5	2.5	2.5
Ground pepper	0.02	0.02	0.02
CO_2-rosemary extract	3	4	5
Yield	100	100	100

As one can see from Fig. 3 and Table 9, the sample with a concentration of rosemary extract of 0.05% has the best indicators: a pleasant fresh smell of fish and rosemary, a homogeneous and smooth texture, a pleasant taste, and a homogeneous color.

Fig. 4 shows the process stream of fish croquette production. During the manufacture, CO_2-extract with calculated concentration was mixed with the fish mince. Then, the rest of the ingredients (black pepper and salt) were added and also mixed. The resulting mass was molded into round molds and crumbed twice. The weight of one unit was 60 g. The semi-finished products were fried in a large amount of oil at a temperature of 140–150°C for 5–7 minutes until a brown crust was formed, then cooled to a temperature of not higher than 15°C.

It is important to note that the developed technology does not require the re-equipment of enterprises, and therefore will not make the manufactured products more expensive.

Table 11 shows the safety parameters of deep-fried fish croquettes after 3 months of storage at low negative temperatures (-18–24°C).

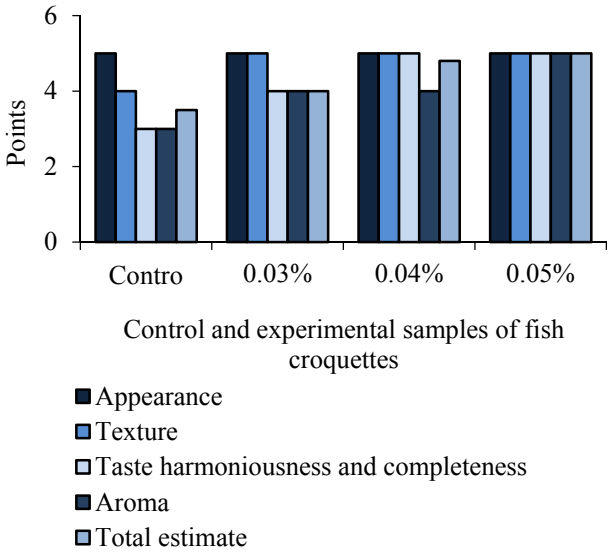
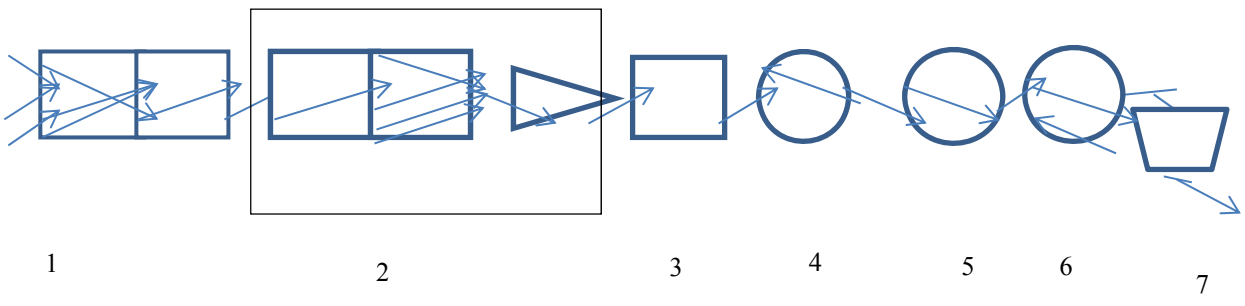


Fig. 3. Organoleptic estimation of fish croquettes with the addition of various concentrations of CO_2-rosemary extract.

Table 10. Organoleptic characteristics of fish croquettes with the addition of CO_2-rosemary extract in various concentrations

Concentrations of CO_2-rosemary extract	Appearance	Color	Smell	Taste	Textute
0.03	The structure is of a regular round shape, the surface is evenly crumbed	A brown crust, white meat on the cut	The distinctive smell of fried fish	A distinctive fish taste	Homogeneous, smooth
0.04	The structure is of a regular round shape, the surface is evenly crumbed	A brown crust, white meat on the cut	A fresh fried fish aroma	Pleasant taste	Homogeneous, smooth
0.05	The structure is of a regular round shape, the surface is evenly crumbed	A gold brown crust, white meat on the cut	A pleasant fresh fried fish aroma	A pleasant taste with a pleasant aftertaste	Homogeneous, smooth



1 – combining components
2 – grinding
3 – croquette molding
4 – croquette roasting
5 – packing
6 – cooling
7 – freezing
8 – storage and sale

Fig. 4. Fish croquette process stream.

Table 11. Safety parameters of the extracted fat component of fish semi-finished products of a high degree of preparation

Indicator	Test results		Concentration of oxidation by-products – petroleum ether insoluble copolymers, %
	Acidity index, mg KOH/g	Peroxide number, mEq of active oxygen/kg	
Croquettes without antioxidants	3.9	23	1.4
Croquettes with antioxidants	2.9	9.9	0.45

In terms of safety parameters, the croquettes produced from unstabilized fish raw materials and mincemeat are more than twice the established norms for a peroxide content and 1.5 times – oxidation by-products – petroleum ether insoluble copolymers. The croquettes for the production of which stabilized fish raw materials and CO_2-rosemary extract were used correspond to the required safety parameters.

During the study, the expediency of antioxidant stabilization of fish lipids for manufacturing industrial products from fish raw materials was proved. Special antioxidant mixed fodder for fish (carp) was developed to improve commodity-consumer properties and to increase the storage and sales terms in the following component ratio: 90.8% of carp mixed fodder; 9.15% of milk thistle oil meal; and 0.05% of CO_2-rosemary extract. The clinical and physicochemical studies of fish and fish raw materials showed that the use of an antioxidant feed supplement had a positive influence on the fish-biological characteristics of fish, as well as on the antioxidant stability of the fish raw materials obtained after providing the developed feed. A number of formulations of fish semi-finished products of a high degree of preparation for industrial manufacturing with functional properties and a prolonged shelf life was developed with the rosemary concentration of 0.05g of per 1 kg of mincemeat for fish croquettes.

CONCLUSION

The technology of antioxidant stabilization of carp lipids has been developed at the stage of rearing and carp raw material processing, which makes it possible to increase the safety the finished product and improve the commodity-technological properties. The use of CO_2-rosemary extract in fish mixed fodder and then in the fish mincemeat produced from the fish reared with the use of antioxidant stabilized mixed fodder (silymarin and carnosic and rosmarinic acids) allows us to significantly improve the stability of the fat phase, both in fish raw materials and in the finished product.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The results of the research are of no commercial interest to legal entities or individuals. The article does not describe objects of patent rights or any other type of rights, except for copyright.

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