Agricultural machinery used in the process of denaturation of minced fish proteine

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Abstract. The development of the marine industry and processing of raw materials is very important. Many floating marine transport platforms are engaged in processing and preparing raw materials. The paper presents an experimental study of the absorption method of the dependence of the degree of protein denaturation on the absolute value of external hydrostatic pressure, which was used to process minced fish for 5 minutes at a temperature of 295 K. A mathematical model of the process of denaturation of protein molecules in minced fish under the influence of high hydrostatic pressure has been developed. Based on experimental data, the equilibrium constant K = 0.0019439869 of denaturation of protein molecules after 5 minutes of action of external hydrostatic pressure at room temperature was determined. Extrapolation of experimental data using a model curve determined the pressure P = 520 MPa, at which the concentration of denatured protein molecules in minced fish for 5 minutes at a temperature of 295 K becomes equal to the concentration of denatured protein molecules in boiled minced fish. These studies are very important for the food and transport industry.

1. Introduction

The development of marine transport for processing raw materials is quite an urgent topic. The main task of the high-pressure technology is to obtain high-grade, highly attractive food with a long shelf life from protein and other food substances. Food technologies using high pressure are undoubtedly convenient for customers: basic food products sterilized by high pressure retain color, smell, consistency, structure, as it is observed in products that have been heat treated or frozen. It is well known that pressure affects protein and leads to protein denaturation, concentration, or gelation. The first systematic observation of protein denaturation under high pressure was made by Bridgman [1], who processed the egg albumin. It was noted that the occurrence of pressure-induced coagulant differs significantly from temperature-induced coagulant, and the ease of pressure-induced coagulation increases at low temperatures. More systematic studies on pressure-induced protein denaturation have been conducted using ovalbumin [2], chymotrypsinogen [3], and metmio-globin [4]. Researchers [5] applied high pressure to denature ovalbumin as the main component of egg protein. The results indicated that only conformational changes were caused by pressure treatment. Ovalbumin did not form a gel under pressure for 30 min at ~400 MPa. Presumably, this relative stability can occur due to
four disulfide bonds and strong non-covalent interactions that stabilize the three-dimensional structure of the protein.

2. Review of problematic issues of the study

High-pressure processing up to 1000 MPa affects the conformation of meat protein and causes its denaturation, aggregation or gelation, depending on the protein system of the meat, the pressure used, the temperature and the duration of processing. High pressure can change the structure and function of meat proteins. For example, the myosin of meat and fish denatures after pressure treatment and subsequently forms a gel-like texture [6]. These structural changes will affect the texture of the muscle tissue and cause a binding effect. Gelation of meat proteins under pressure depends on the protein system and the conditions of the high-pressure processing process [7]. Temperature is an important factor, and at the same time, the pressure and temperature are inextricably linked. The mechanism of protein denaturation is different and depends on pressure / temperature combinations [8].

Most studies related to the application of high pressure to seafood were conducted to identify its effects on fish proteins [9-13]. High-pressure treatment of surimi produced a new gel product with an excellent flavor, gloss, density and elasticity, quite different from those processed under temperature [14]. Moreover, the gels that were subjected to pressure retained their natural properties (i.e., color and flavor) of raw materials without forming the color and flavor of the prepared product [15,16]. Pressure - treated surimi gels from marine species such as saida, sardine, horse mackerel, tuna, and squid became softer, more elastic, and tastier compared to heat-treated [17-19]. Thus, the importance of studying changes in the protein structure at high pressure is undeniable, since only based on the obtained experimental results can be developed new, progressive (in this case, non-thermal) technologies for the production of protein products.

As the object of our research, we used minced fish from Ballerus (Abramis Ballerus). To obtain minced fish, they were cleaned of scales, butchered, separated muscle tissue from skin and bones, crushed with an electric meat grinder with a diameter of 2 mm holes in the grate, and thoroughly mixed. Under normal conditions, the minced fish obtained in this way is a transparent colored heterophase polydisperse system, whose particles consist not of individual molecules, but of their conglomerates (the dispersed phase) located in a liquid medium (the dispersion medium) that weakly absorbs visible light.

As we know [20], the amino acids that make up the native protein molecules in conglomerates absorb the energy of electromagnetic waves in the ultraviolet region of the spectrum, which is invisible to the naked eye. The size of protein molecules in conglomerates and individual structural elements of the dispersed phase, which form a phase inhomogeneity in minced fish, are smaller than the wavelengths of a high-energy section of visible light. Therefore, the layer of raw minced fish shows a weak molecular light scattering (Rayleigh scattering) and appears to have a bluish color of low intensity. When a visible light beam passes through a thin plane-parallel layer of minced fish, the intensity of the transmitted radiation decreases slightly. With an increase in external pressure, the polypeptide chains of amino acids begin to unfold and intertwine with each other, as a result of which the volume of protein molecules (the volume of light-scattering centers) increases, and the minced fish becomes a light-scattering cloudy (opaque) medium. At the same time, the greater the absolute value of the external pressure, the more turbid the minced fish becomes and the more it scatters the light.

At the same time, under the influence of pressure, a slight hydrostatic compression of protein molecules occurs [21], their volume decreases, the turbidity of the minced fish decreases, and the amount of scattered light decreases. When the pressure is released, the volume of molecules of the expanded (denatured) protein and the turbidity of the minced fish are restored and the amount of scattered light increases. The intensity of light passing through the layer of processed minced fish is related to the (number) concentration of protein molecules denatured under the action of external hydrostatic pressure.

The aim of this work is an experimental study by the absorption method of the dependence of the degree of protein denaturation on the absolute value of external hydrostatic pressure 0.1, 100, 200, 300
and 400 MPa, which was processed minced fish for 5 minutes at a temperature of 295 K (room temperature).

3. Process modeling

In the first approximation, considering the absorption of the layer in the region of electron transitions constant and independent of pressure, for qualitative analysis of the degree of denaturation of minced fish protein after the action of pressure, we can use the values of the light attenuation index (extinction index) $\mu$, which is equal to the sum of the absorption indicators $\mu_a$ ($\mu_a = \text{Const}$) and dispersion $\mu_p$ ($\mu = (\mu_a + \mu_p)$). At the same layer thickness $\mu$ will be proportional to the concentration of denatured protein molecules $c$ ($\mu \sim c$). Then, for the indicator of light attenuation $\mu$ and the concentration of denatured protein $c$ molecules, the equality will be observed:

$$c \cdot \mu = c \cdot (\mu_a + \mu_p) = c \cdot (\text{Const} + \mu_p),$$

(1)

In this case, the combined Beer–Lambert–Bouguer law for the intensity of light passing through a plane-parallel layer of minced fish with thickness $d$ will have the form:

$$I = I_0 \cdot \exp(-c \cdot \mu \cdot d).$$

(2)

where $I$ – the intensity of light that passed through the plane-parallel layer of minced fish; $I_0$ – the intensity of incident light; $\mu$ – the indicator of light attenuation; $c$ - the concentration of denatured protein molecules; $d$ – the thickness of the minced fish layer.

For processing samples of minced fish, a laboratory automated system of high pressure (ASHP) [22] of the piston-cylinder type with a working volume of 5 cm$^3$ was used, in which pressures from 0.1 to 1000 MPa are achieved with an accuracy of automatic pressure maintenance of $\pm$10 MPa. The operating temperature range of the ASHP from 278 to 397 K is also maintained automatically with an accuracy of $\pm$0.5 K. Polyethylsiloxanes-3 silicone oil is used as a pressure-transmitting medium in the ASHP.

Samples of minced fish were loaded into sealed plastic bags of low density. Each separate bag containing the sample was placed in the ASHP chamber and subjected to a set hydrostatic pressure of 0.1, 100, 200, 300 and 400 MPa for 5 minutes at a temperature of 295 K. The time to reach the set pressure and release the pressure was no more than 10 seconds.

For spectral measurements, a layer of minced fish with a thickness of $d = 0.3$ mm was formed between two plane-parallel quartz plates.

Minced fish processed at 373 K for 30 minutes at an atmospheric pressure of 0.1 MPa (boiled minced fish) was used as a reference for the concentration of denatured protein molecules.

Measurement of absorption spectra of samples were measured on an experimental setup using a single-beam upgraded PGS-2 spectrograph manufactured by “Carl Zeiss” company with a flat diffraction grating. A 170 W tungsten incandescent lamp with a color temperature of 1572 K was used as a source of continuous naturally polarized electromagnetic radiation. The radiation receiver was a photo-electronic multiplier PEM-118. To calibrate the measured spectra by wavelengths, a neon glow lamp TN-30 was used as a source for rappers of spectral lines, the radiation of which was directed to the entrance slit of the spectrograph using a special mirror. Continuous documentation of the measured spectra was performed using a differential bipolar ten-bit analog-to-digital converter based on the Kr1113PV1 processor and a personal computer. The analysis of the registered spectra was performed using the Origin program [23].

The measured spectral dependence of the intensity of incident radiation and radiation passed through the reference sample and samples of minced fish processed at a pressure of 0.1, 100, 200, 300 and 400 MPa for 5 minutes at a temperature of 295 K is shown in figure 1.
Figure 1. Spectral dependence of the intensity of incident radiation and radiation passed through the reference sample and samples of minced fish processed by pressure. (For clarity, the intensity of the incident radiation is reduced by 5 times).

The spectral dependence of the natural optical density $D(\lambda)$ of samples was calculated from the measured spectral dependences of the intensity of incident and transmitted radiation using the formula:

$$D(\lambda) = \ln \left( \frac{I_0(\lambda)}{I(\lambda)} \right) = c \cdot \mu \cdot d,$$

(3)

The spectral dependence, calculated using the formula (3), of the natural optical density of the reference sample and samples of minced fish processed at a pressure of 0.1, 100, 200, 300 and 400 MPa for 5 minutes at a temperature of 295 K is shown in figure 2.

Figure 2. Spectral dependence of the natural optical density of the reference sample and samples of minced fish processed by pressure.

As can be seen in figure 2, the spectra of all measured samples have a single absorption band with a maximum of 4000 Å, the relative integral intensity of which is practically independent of pressure and is the same on all curves. The maximum values of the optical density of all samples coincide with the
maximum of the corresponding absorption band, the absolute value of which depends on the pressure and is associated with an increase in the corresponding curve. With increasing pressure, the concentration of denatured protein in minced fish increases, and the sample begins to scatter light to a greater extent, which leads to an increase in experimental curves [23,24].

The integral natural optical density \( D_\Sigma \) of samples of minced fish processed at a pressure of 0.1, 100, 200, 300 and 400 MPa for 5 minutes at a temperature of 295 K was normalized to the value of the integral natural optical density of the reference sample. The experimental dependence of the normalized integral natural optical density \( D_\Sigma \) on the pressure \( P \) is shown in figure 3.

![Figure 3](image)

**Figure 3.** Experimental dependence of \( D_\Sigma(P) \).

The dashed line corresponds to the value of the normalized integral natural optical density of the reference sample.

As can be seen in figure 3, with increasing pressure, the dependence \( D_\Sigma(P) \) grows monotonously and does not reach the maximum possible value corresponding to the reference sample. Since the normalized integral optical density \( D_\Sigma \) is proportional to the concentration \( c \) of denatured protein molecules in minced fish samples \( (D_\Sigma \sim c) \), then within the assumptions made in the work, the experimental curves in figure 2 directly display the functional relationship \( c(P) \).

Thus, the concentration of denatured protein molecules in samples of minced fish processed for 5 minutes at room temperature with the highest hydrostatic pressure of 400 MPa in the experiment is 1.37 times less than their concentration in a sample of boiled minced fish.

### 4. Mathematical processing

For mathematical modeling of the process of denaturation of protein molecules from the absolute value of the pressure of processing minced fish, we will write the equilibrium constant \( K \) as the ratio:

\[
K = \frac{c_D(P)}{c_N(P)},
\]

where \( c_N(P) \) and \( c_D(P) \) – the concentrations of the native and denatured protein molecules, respectively, at the pressure of \( P \).

The equation of change of the equilibrium constant under the action of pressure at a constant temperature is represented as the equation of the isotherm of a biochemical reaction:

\[
\left( \frac{\partial \ln K}{\partial P} \right)_T = -\frac{\Delta V^*}{P},
\]

where \( \Delta V^* \) – the change in the activation volume of the biochemical reaction, \( P \) - is the pressure.

The solution of equation (5) has the form:
\[
\ln \frac{K_P}{K_{P_0}} = \ln \frac{P}{P_0},
\]

(6)

where: \(K_P\) and \(K_{P_0}\) – the values of the equilibrium constant, respectively, at pressures \(P\) and \(P_0\).

From equation (6) follows the linear dependence of the equilibrium constant \(K\) on the pressure \(P\).

To fit the model curve to the experimental data in figure 5, we used linear least squares regression (Gauss-Newton method). The model linear curve has the form:

\[
y = a \cdot x,
\]

(7)

where: \(a\) – a constant numerical coefficient.

According to the solution of equation (5), the calculated value of the constant coefficient of the model curve (7) \(a = 0.0019439869\) is equal to the equilibrium constant \(K\) \((K = 0.0019439869)\).

5. Conclusions

The value of external hydrostatic pressure at which the concentration of denatured protein molecules in the samples of processed minced fish becomes equal to the concentration of denatured protein molecules in the reference sample was found by extrapolation of experimental data from the model curve. The extrapolation result is shown in figure 4.

![Figure 4. Extrapolation of experimental data of the model curve.](image)

As can be seen in figure 4, the model curve crosses the dashed line of the reference sample at approximately 520 MPa. This means that if the minced fish is processed with a pressure of 520 MPa for 5 minutes at a temperature of 295 K, the concentration of denatured protein molecules in it will become equal to the concentration of denatured protein molecules in boiled minced fish.

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