Denaturation of proteins under the influence of ultrasound. Hypothesis.

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Annotation. In the food industry, the use of ultrasound as a non-thermal treatment is expanding. Ultrasound is often used at the final stage of food production to improve rheological characteristics and prolong shelf life. However, an increase in temperature during ultrasonic treatment can negatively affect the quality of the final product. An increase in temperature during food processing causes the denaturation of enzymes. For most enzymes of animal origin, the denaturation temperature is 40-50 ºC, and for enzymes of plant origin it is 50-60 ºC. At the same time, at a temperature of 80 ° C and above, all enzymes are destroyed. In this regard, it was very important to investigate possible temperature changes during ultrasonic treatment. The essence of the hypothesis put forward by "Shoman" is the study of the fact that during ultrasonic treatment, thermal effects occur, at which protein denaturation begins. An increase in the temperature values during ultrasonic treatment above the denaturation temperature of proteins confirms our hypothesis. It is established that: 1) with ultrasonic exposure to a two-component oil/ water emulsion, the temperature rises to 83 °C with a duration of exposure of 28 minutes; 2) an increase in the proportion of oil relative to water contributes to an increase in temperature from 74 to 83 ºC; 3) with equal oil/water ratios, the content of mineral salts and organic substances in drinking water contributes to an increase in temperature under ultrasonic exposure compared to distilled water. The results obtained are valid for a two-component water-oil emulsion. It is of interest to study the influence of other food components on the dynamics of temperature changes during ultrasonic treatment. I would like to know the opinion of other researchers on the "Shoman" hypothesis.

1 Introduction

Today's consumer demand is for clean and safe food products that are processed without compromising their nutritional and organoleptic properties [1].

Ensuring the nutritional and organoleptic properties of food products is a necessity for every technological operation in the production of food products, especially for dietary products and functional products. But for some products, traditional heat-dependent

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processing methods, such as pasteurization and sterilization, can negatively affect their taste, nutritional value and appearance.

In this regard, the use of non-thermal processing technologies is expanding in the food industry. With non-thermal exposure, food is processed at a temperature close to room temperature, so the food is not damaged, since the heat-sensitive nutrients in the food remain intact, unlike heat treatment of food [2]. The absence of heat in these technologies makes it possible to obtain food products of higher quality due to the preservation of their sensory and nutritional properties.

Non-thermal methods of food processing, including ultrasonic exposure, have minimal impact on the nutritional and sensory properties of products and extend the shelf life by inhibiting or destroying microorganisms. They are also considered to be more energy efficient and retain largely qualitative characteristics than conventional thermal processes [3].

The most commonly used method for pasteurization and sterilization, which is effective for inactivating microorganisms and enzymes that cause spoilage, but at the same time it affects the appearance and organoleptic characteristics of the product, is heat treatment [4, 5]. Despite the effectiveness of reducing the microbial load and enzymatic activity, heat treatment leads to a deterioration in quality from the point of view of nutritional, functional, physico-chemical and organoleptic properties [6,7].

Ultrasonic processing of materials is widely used in various industries, such as medicine, food, pharmaceutical, machine-building and oil and gas industries [8,9]. It is known that ultrasound has a significant impact on the speed of various processes in the food industry [10,11]. One example of the use of ultrasound in the food industry is the creation of water-oil emulsions based on vegetable oils [12]. The use of ultrasound makes it possible to speed up food processes, thereby reducing the cost of processing, obtaining a more homogeneous mass and improving the quality of the final product. The use of ultrasound is a promising method in food technologies with low environmental impact, which is why it has become known as "green technology" [13].

Ultrasonic treatment, used alone or in combination with other processing methods, gives significant positive results in terms of food quality, therefore it is considered effective [14]. It is claimed that the food processes carried out under the influence of ultrasound are partially influenced by the phenomena of cavitation and increased mass transfer. It is a new and promising technology and is effectively used in the food industry for several processes such as freezing, filtration, drying, separation, emulsification, sterilization and extraction. Various studies have shown that ultrasound leads to an increase in the productivity of the process and improves food quality indicators [15, 16, 17, 18].

Studies have shown that ultrasound accelerates processing without compromising the quality of food. In food products, ultrasound can improve enzyme activity and metabolism, as well as promote the Mayer reaction, oxidation, esterification and hydrolysis of protein, which leads to maturation and improvement of the texture, color, aroma and taste of fermented foods [19]. It has been found that ultrasound improves the tenderness of meat products and effectively removes bound water, which reduces the drying time, but this negatively affects the color of meat [20]. Accelerated lipid oxidation leads to a decrease in the content of unsaturated fatty acids. In addition, it was found that ultrasound treatment significantly increased the content of essential fatty acids and the total content of fatty acids [21]. Ultrasound treatment leads to an increase in the total level of free amino acids in apples [22,23].

Ultrasound treatment alone is not very effective for killing bacteria in food, but the use of ultrasound in combination with pressure and/or heat is promising. Thermosonic (heat plus ultrasound treatment), manosonic (pressure plus ultrasound treatment) and manothermosonic (heat and pressure plus ultrasound treatment) treatment are probably the best methods of inactivation of microbes, since they are more energy efficient and effective in destroying microorganisms [24].

Ultrasound and pulsed electric fields, as well as microwaves, are considered effective tools for improving the interaction of food ingredients in various technologies [25].

In the last decade, more attention has been paid to ultrasonic treatment than to other non-thermal processing methods [26,27]. This is due to the fact that it often has a significant beneficial effect on the functionality of various food components [28]. In addition, ultrasound is a promising, efficient and environmentally friendly technology.

It has been established that protein is one of the most sensitive components to the effects of cavitation in many different food media [29]. Thus, the response to ultrasound differed between proteins isolated from food and proteins present in the food system, and these reactions differed depending on the type of food. These studies confirm the effect of ultrasound on the interaction of food components in a protein food environment.

In recent years, high-energy ultrasound has been used as an alternative to improve the functional properties of various proteins, such as milk, eggs, soy and poultry meat [30]. The advantages of implementing this technology depend on the characteristics of the protein source, the intensity and amplitude of ultrasound, as well as on pH, temperature, ionic strength, time and all variables affecting the physicochemical properties of proteins. Therefore, it is necessary to establish the optimal parameters of ultrasonic treatment and the ratio of components for each type of nutrition.

The above-mentioned research results by various authors using ultrasound were carried out taking into account the fact that the temperature during processing does not increase or increases slightly. There are no data from studies of temperature changes during ultrasonic treatment. And also, the influence of certain components of food products on temperature changes during ultrasonic processing, which can contribute to the occurrence of negative side effects, such as fat oxidation, inactivation of valuable enzymes and denaturation of proteins.

Ultrasound has been found to activate the inactivation of microorganisms and enzymes, crystallization, drying, degassing, extraction, filtration, homogenization, softening of meat, oxidation, sterilization, etc. [31]. Ultrasound is often used at the final stage of food production. In this regard, it is very important to investigate possible temperature changes during ultrasonic treatment that affect the quality of the product. The hypothesis we propose is that with ultrasonic exposure, the temperature rises to the values at which protein denaturation begins. A significant increase in temperature during ultrasonic processing affects the change in the functional properties of ingredients that are very sensitive to temperature changes in food media.

According to the "Shoman" hypothesis put forward by us, the temperature with the "non-thermal" method of food processing rises above the temperatures, the beginning of protein denaturation. To confirm our hypothesis, it is necessary to conduct studies of temperature changes in the process of ultrasonic food processing. It is very important to investigate the effect of ultrasound on the interaction of food ingredients at different ratios of the components of the medium under study. Thus, although ultrasound is used in the food industry to develop innovative products, it still needs additional research before implementation.

2 The purpose and objectives of the research

The purpose of this work is to study the dynamics of temperature rise at which protein denaturation begins during ultrasound exposure.
The objective of the study is to confirm the hypothesis of "Shoman", about a significant increase in temperature during ultrasonic processing of food products on the example of a water-oil emulsion, as well as the effect of the water-oil ratio on temperature changes.

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3 Materials and methods of research

Food emulsions of the "water- vegetable oil" type were used as objects of research. Tap water was used according to GOST R 51310-2003, which had the following qualitative characteristics: a hydrogen index of 6.5, a total hardness of 6.8 mg–eq/l, a phenolic index of 0.20 mg/l. Safflower oil was used as vegetable oil. Physico-chemical parameters and fatty acid composition of safflower oil are presented in Table 1.

Table 1. Physico-chemical parameters and fatty acid composition of safflower oil.

№	Name of the indicator, unit of change.	Values of indicators
1	Acid number, mgKON/g	1.07
2	Peroxide number of IF, mmol/kg O_2	8.09
3	Anisidine number AH	3.25
4	Saturated fatty acids, %	8.0
5	Monounsaturated fatty acids, %	10.6
6	Polyunsaturated fatty acids, %	81.3
7	Linoleic acid content, %	76

For the experiment, food emulsions with different ratios of ingredients were used: the first option – 20% vegetable oil + 80% water; the second option – 50% vegetable oil + 50% water; the third option – 80% vegetable oil + 20% water; the fourth option – 50% vegetable oil + 50% distilled water. The duration of the ultrasonic exposure was 28 minutes, with a capacity of 100 ml, with an exposure power of 400 watts. The temperature of the food emulsion before ultrasonic exposure was 25 ° C.

Fig.1. Ultrasonic homogenizer Omni Sonic Eruptor 4000.

The ultrasonic treatment was carried out using the Omni Sonic Ruptor 4000 ultrasonic homogenizer shown in Fig.1.

4 Results and discussion

As a result of ultrasonic treatment of water-oil emulsion at different ratios of oil/water, drinking and distilled, for 28 minutes with a temperature fixing interval of 4 minutes, the following data were obtained, presented in Table. 2. The temperature was measured using Camera parts models TG165-X, manufactured by FLIR.

Table 2. Results of ultrasonic treatment of water-oil emulsion

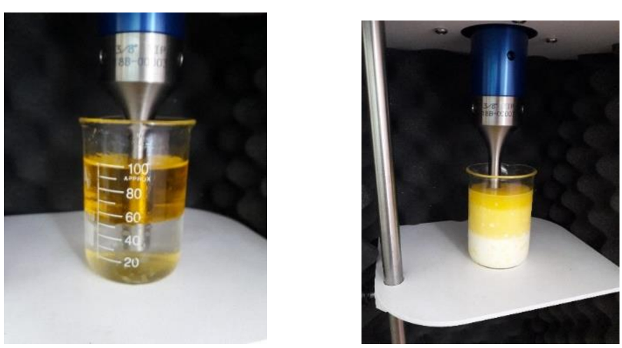
Duration, min.	Oil/water ratio, %			
	20/80	50/50	80/20	50/50, distilled water
4	39.5	46.2	44.3	39.1
8	51.8	54.0	61.5	51.8
12	60.5	62.1	69.2	61.4
16	64.2	67.0	74.6	65.6
20	68.3	72.1	78.7	69.6
24	71.2	74.8	80.9	74.0
28	74.2	77.1	82.9	76.8

Figures 2,3,4 show the figures before and after processing the emulsions at different percentages of oil/water.

a b

Fig. 2. Ratio -20% vegetable oil + 80% water

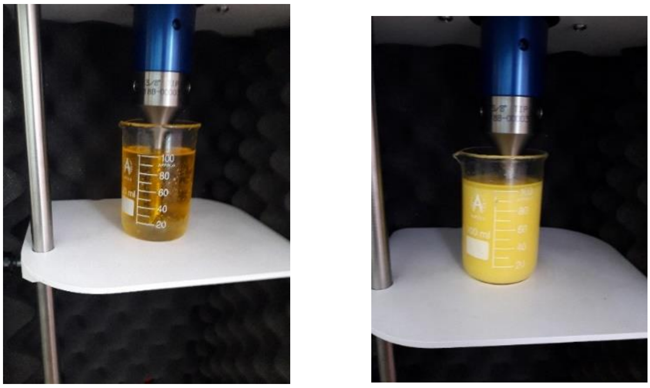
Where: a- is the beginning of processing; b -is the end of processing



a b

Fig. 3. Ratio – 50% vegetable oil + 50% water

Where: a -is the beginning of processing; b -is the end of processing



a b

Fig. 4. Ratio – 80% vegetable oil + 20% water

Where: a is the beginning of processing; b is the end of processing

As a result of processing the obtained temperature data during ultrasonic treatment of water-oil emulsion at different ratios of oil/water and distilled water, the following dynamics of temperature change was observed, shown in Fig. 5.

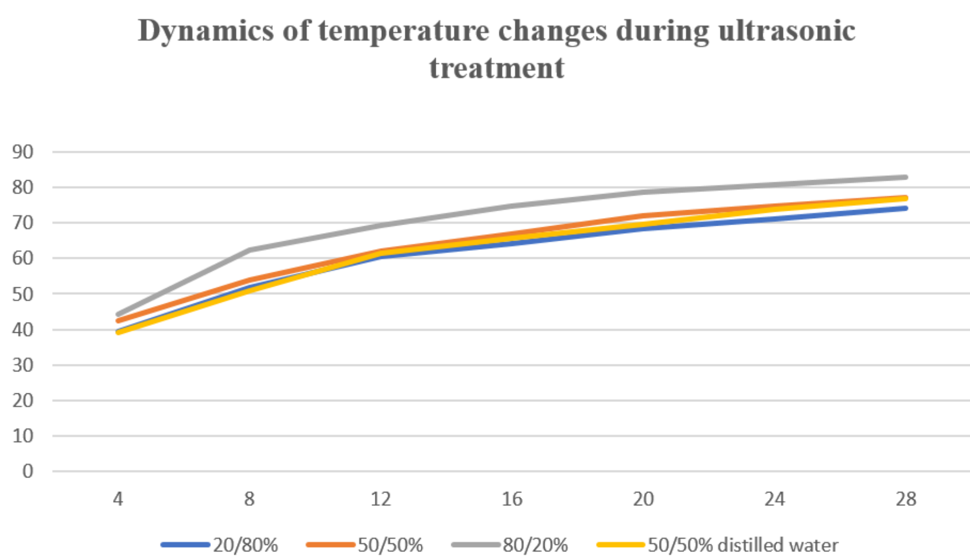


Fig. 5. Dynamics of temperature changes at different oil/water ratios.

Analysis of the data obtained shows that during ultrasonic treatment the temperature increased significantly from 25 °C above 70 °C for 28 minutes at all ratios oil/water. At the same time, with an oil/water ratio of -80/20%, the temperature was higher (82.9 oC) compared to other ratios. The lowest (74.2 oS) at a ratio of 20/80%.

The results obtained show that an increase in the proportion of oil relative to water increases the temperature under ultrasonic exposure. Also, a comparison of the results of oil/water in relation to 50/50% with drinking water and distilled water shows that the temperature of the emulsion during ultrasonic exposure when using drinking water is slightly higher than when using distilled water.

Based on the data obtained and in accordance with the "Shoman" hypothesis, the following conclusions can be drawn that ultrasonic treatment cannot be attributed with full confidence to non-thermal processing methods, especially when processing dietary products and functional products, because the enzymes contained are denatured when the temperature rises. For most enzymes of animal origin, it is equal to 40-50 oC, for plant origin 50-60 oC. Almost all enzymes are destroyed at a temperature of 80 ° C and above.

5 Conclusion

An increase in the temperature values during ultrasonic treatment above the denaturation temperature of proteins confirms our hypothesis.

It is established that:

-with ultrasonic exposure to a two-component oil/ water emulsion, the temperature rises to 83 ° C with a duration of exposure of 28 minutes;

-an increase in the proportion of oil relative to water contributes to an increase in temperature from 74 to 83 oC;

-with equal oil/water ratios, the content of mineral salts and organic substances in drinking water contributes to an increase in temperature under ultrasonic exposure compared to distilled water.

The results obtained are valid for a two-component water-oil emulsion. It is of interest to study the influence of other food components on the dynamics of temperature changes during ultrasonic treatment. I would like to know the opinion of other researchers on the "Shoman" hypothesis.

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