

The object of the study is the analytical values of lipids of milk thistle seeds of a modified composition, namely acid and peroxide values, as well as the induction period of lipids of chocolate mass with the addition of milk thistle seeds. The paper substantiates rational parameters of thistle seed processing for the inactivation of lipases and lipoxygenases. Approximate dependences of the acid and peroxide values of thistle seed lipids on the pH of the wetting solution and the wetting degree of seeds were obtained. This makes it possible to substantiate such rational processing parameters of thistle seeds that inhibit the accumulation of free fatty acids and primary oxidation products in seeds during storage. An increase in the induction period of the oxidation of the lipid component of the chocolate mass with the use of thistle seeds of a modified composition was proven, which is 2.5 times higher than the induction period of the chocolate mass sample with thistle seeds with a native enzyme complex. The research results make it possible to develop a technology of healthy chocolate mass using thistle seeds of a modified composition, the lipid component of such mass is stable to oxidation and hydrolysis. The data obtained in the work are explained by an increase in the ability to inactivate lipolytic and lipoxygenase enzymes of milk thistle at high humidity in an acidic environment under the influence of microwave radiation. The advantage of the obtained results is the possibility of not violating the integrity of thistle seeds during the inactivation of the enzyme complex, which allows extending the shelf life of this raw material. An applied aspect of using the scientific result is the possibility of expanding the range of healthy chocolate masses with the use of thistle seeds

Keywords: *thistle seeds, chocolate mass, oxidation stability, acid value, peroxide value*

USE OF THISTLE SEEDS OF MODIFIED COMPOSITION IN CHOCOLATE MASS TECHNOLOGY

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1. Introduction

Chocolate mass has numerous consumer appeals that make it popular and desirable in the food industry. In particular, it is a rich, delicate taste and texture that melts

in the mouth. The technology of chocolate mass allows an unlimited number of opportunities for expanding the range. Manufacturers can add various ingredients to it, such as nuts, fruits, caramel, coffee, or other additives in order to create different flavor combinations, increase nutritional

value, or provide health benefits [1]. This makes it possible to satisfy the individual tastes and preferences of consumers. Innovations in the formulation of chocolate masses can help manufacturers attract the attention of consumers who are looking for new flavors and health products. Oilseeds attract attention as a component of chocolate masses. They contain a significant amount of useful substances, such as polyunsaturated fatty acids, proteins, fiber, vitamins, minerals and antioxidants [2]. The addition of oilseeds to the chocolate mass can significantly increase the nutritional value of the product, making it more useful for consumption. In addition, oilseeds add a unique taste and texture to the chocolate mass. The structure of such chocolate products can vary from crunchy to juicy, which will make the product more interesting for consumers.

Among oil crops, milk thistle attracts special attention, which is a source of valuable oil characterized by a high amount of polyunsaturated fatty acids, as well as protein and dietary fiber. In addition, this plant includes a complex of biologically active substances, the most significant of which are flavolignans, flavonoids, essential oils, sterols, organic acids, resins, sugars, amines and a number of others. The plant is rich in B and E vitamins, β -carotene, micro- and macroelements. These compounds determine important physiological properties of the plant – hepatoprotective and antioxidant [3]. Thistle seeds are a promising ingredient for the food industry [4]. Milk thistle belongs to nutraceuticals, which are used for therapeutic and preventive nutrition. In addition, the seeds are used as an ingredient in health products [5].

However, an endogenous enzyme complex plays an important role in the formation and quality change of both thistle seeds themselves and the oil obtained from them. When storing and processing oilseeds in the production of vegetable oils, hydrolytic and redox enzymes are the most important in terms of the impact on the quality of the obtained products [6]. The enzyme complex of oilseeds (lipoxygenase, lipase) is localized in the gel part of the seeds. There are no enzymes in the oil part, and they are also absent in filtered pressed and extracted oils [7].

Since triacylglycerols are insoluble in water, lipases and lipoxygenases act at the boundary of the phase separation: water – lipids, and are lipoproteins with hydrophilic-hydrophobic groups. With an increase in the moisture content of seeds and their storage temperature, the complex of lipases and lipoxygenases catalyzes the rapid hydrolysis of triacylglycerols and oxidation, first of all, of free fatty acids that are removed. Hydroperoxides of fatty acids that are formed have a high oxidizing capacity due to the presence of peroxide oxygen and can further oxidize unsaturated fatty acids and carotenoids. This leads to an increase in the acid and peroxide values of oil in seeds and a deterioration of their quality [8]. The search for ways to regulate the activity of the specified enzyme complex is becoming an increasingly urgent task in terms of preserving the oxidative stability of seed lipids and, therefore, expanding the range of products based on them, in particular chocolate masses. But it should be noted that before introducing such developments to the market, it is important to conduct research on the oxidative properties of milk thistle lipids, justify the formulation of the chocolate mass, and take into account the relevant technological requirements.

It should be noted that, despite the rather well-studied composition of milk thistle seeds, there is almost no

comprehensive information on the chemical composition of its seeds in scientific and technical sources, with limited data on the activity of their enzyme complex. Given the high content of milk thistle oil prone to enzymatic oxidation [3], it is of interest to develop ways to reduce the activity of native lipases and lipoxygenases. This technological treatment will help inhibit the processes of hydrolytic and oxidative destruction of the lipid complex of seeds.

Therefore, research aimed at finding and substantiating factors affecting the activity of thistle seed enzyme complex allows us to reveal the dependence of the shelf life of seeds on the features of technological treatment. The obtained data will rationalize the production technology of chocolate masses with the addition of thistle seeds. The obtained scientific results of the specified area are relevant for food production, as there is a need to increase the shelf life of milk thistle seeds as a raw component and products based on them, in particular chocolate masses. This will make it possible to expand the range of healthy chocolate masses, which, in addition to biological value, have increased resistance to oxidative deterioration.

2. Literature review and problem statement

In the study [9], it was found that lipases (triacylglycerol lipase, diacylglycerol lipase, monoacylglycerol lipase) of oil crops such as sunflower and sesame differ not only in the nature of the substrate, but also in the optimum pH and temperature. The issue of the optimum pH of enzymes of other oil crops remains unresolved. The question posed was somewhat revealed by researchers in [10], where water-soluble and water-insoluble lipase was identified in different types of oilseeds. In particular, water-insoluble lipase is contained in castor seeds and has an optimum effect at pH 3.6. Soluble lipase is found in the seeds of most oil crops, the optimum pH is 8.0. It has been proven that the optimum pH for lipase action varies significantly depending on the physiological state of seeds: for example, in resting soybeans it is equal to 5, in germinating beans – 7. This pattern is also valid for seeds of other oil crops (hemp, cotton, flax). But there are still unresolved issues related to determining critical conditions under which lipase activity increases.

Such studies were carried out in [11], where it was found that one of the critical storage conditions for oilseeds in terms of lipase activity is excessive moisture content. It has been proven that seeds with a moisture content of up to 12 % can be stored with little lipase activation. An increase in moisture content leads to a noticeable increase in lipase activity. In addition, the work [12] revealed that the lipase of dry oilseeds is resistant to temperature, in wetted seeds the enzyme is quickly inactivated. The activity is also affected by the fatty acid composition of triacylglycerols. In particular, castor lipase cleaves triacylglycerols containing residues of unsaturated fatty acids faster than saturated ones. It would be appropriate to further study the influence of other factors on the activity of lipases and lipoxygenases of oilseeds, such as the composition of the gas environment during storage and the influence of technological processes during seed processing.

Similar studies are presented in [13], where it is proved that the gas environment has a noticeable effect on the lipase activity of mandarin seeds. When the oxygen content in the

gas environment surrounding the seeds decreases to 1–2 %, the catalytic activity of lipolytic and oxidative enzymes decreases. In [14], it was determined that lipase activity in some types of oilseeds is influenced by the degree of their maturity. It has been proven that the highest lipase activity is found in small, immature seeds, and the lowest lipase activity is observed in mature oilseeds. It was determined that the technological processes of oilseed processing have different effects on the activity of lipases. In particular, the collapse of seeds reduces the catalytic activity of the enzyme complex of seeds, and due to moisture-heat treatment, the enzyme activity increases. In [15], significant lipase activity in the production of castor oil and its rather high heat resistance were found. In addition, in [16], when studying the lipolytic and lipoxxygenase enzymes of oilseeds, it was found that in the process of direct extraction of soybean meal, the lipoxxygenase activity of the meal decreases four times. Lipoxxygenase is also inactivated by mechanical effects on the meal. In [17], it was proved that during the processing of sunflower seeds according to the prepressing – extraction scheme, the lipoxxygenase activity of the meal is reduced to almost zero, but soybean meal under the same conditions retains a significant lipoxxygenase activity. In the described studies, the issue related to the inactivation of lipases and lipoxxygenases in native oilseeds remains unresolved.

The issue of inactivation of the enzyme complex of oilseeds during technological processing in order to improve the quality of products from them is disclosed in [18]. The influence of the time of treatment of a crushed mixture of flax, sunflower and sesame seeds with ultrahigh-frequency (microwave) radiation and its initial moisture content on the degree of further enzymatic hydrolysis of protein was investigated. The optimum range of values of the described technological processing factors for the maximum possible increase in the biological value of the protein-fat base has been determined. The optimum time of pre-treatment of the protein-fat base with ultrahigh-frequency radiation is 250–350 seconds, the initial moisture content of the raw material is 12–14 %. The same authors investigated the inactivation of inhibitors of proteolytic enzymes of crushed sesame seeds using a similar treatment [19]. Rational treatment conditions for sesame seeds for the inactivation of anti-alimentary factors are justified almost in the initial ranges: wetting up to 10–13 %, time of microwave radiation treatment at 220–240 °C. However, the described studies did not take into account the influence of the pH value of the aqueous solution used to treat crushed seeds before microwave treatment. The possibility of processing seeds with intact shells is also not taken into account, which is especially relevant for preserving the quality indicators of the lipid component of milk thistle seeds during storage.

As shown by the results of research [15–19], the possibility of implementing the inactivation of the enzyme complex for seeds of some types of oil crops to increase the shelf life of food products based on them has been proven. But there is not enough scientific data on the influence of implementing such an effect on reducing the activity of the enzyme complex of thistle seeds. In addition, research on expanding the range of parameters of milk thistle seed treatment to inactivate lipases and lipoxxygenases is expedient. Considering the results of the works described in [18, 19], it is of interest to study the effect of changing the pH of thistle seed wetting solutions. Reasonable research would allow increasing the shelf life of milk thistle seeds

as a raw material. This fact will expand the range of health products, in particular, chocolate mass using thistle seeds, which has increased resistance to oxidative damage, thereby contributing to the disclosure of the biological activity potential of this raw resource.

3. The aim and objectives of the study

The aim of the study is to determine the influence of the moisture content of milk thistle seeds and the pH of the seed wetting solution on the activity of native lipases and lipoxxygenases in them. This will make it possible to develop a method for processing thistle seeds to increase the hydrolysis and oxidation stability of its lipid component. In turn, the data obtained will be useful for using crushed thistle seeds as a health-promoting component in chocolate mass.

To achieve the aim, the following objectives must be accomplished:

- to determine the composition indicators of thistle seeds;
- to investigate the dependence of the acid and peroxide values of the lipid component of milk thistle seeds, which indirectly indicate the activity of lipolytic enzymes, on the pH of the wetting solution and the degree of wetting;
- to justify rational values of thistle seed treatment parameters for the inactivation of lipases and lipoxxygenases;
- to investigate the oxidation stability of the lipid component of chocolate mass using thistle seeds of a modified composition.

4. Research materials and methods

4. 1. Research object and hypothesis

The object of the study is the analytical values of lipids of milk thistle seeds of a modified composition, namely acid and peroxide values, as well as the induction period of lipids of chocolate mass with the addition of milk thistle seeds. The main hypothesis of the study is a decrease in the activity of the enzyme complex of lipases and lipoxxygenases of milk thistle seeds through the technological processing of seeds, as well as the possibility of increasing the oxidative stability of chocolate mass with the addition of such seeds.

The study assumes that the treatment of milk thistle seeds with an aqueous solution of food acid and subsequent treatment in a microwave radiation field should reduce the activity of the enzyme complex that catalyzes the hydrolysis and oxidation of seed lipids. It is assumed that wetting thistle seeds with a solution having an acidic pH should be effective, since the optimum action of plant lipases and lipoxxygenases is in a weakly alkaline environment [20]. At the same time, the limiting pH value of the thistle seed wetting solution should be its negative effect on the organoleptic characteristics of seeds. It is advisable to test this assumption by determining the analytical values of seed lipids, which characterize:

- the content of free fatty acids in lipids, i.e. the degree of their hydrolytic cleavage (acid value);
- the content of primary products of lipid oxidation, i.e. degree of their oxidation (peroxide value).

It is also assumed that lipids of chocolate mass with the addition of milk thistle seeds of a modified composition will have higher oxidative stability due to the inactivation of milk thistle enzymes and, accordingly, the absence of enzymatic hydrolysis and oxidation processes.

The following simplification was adopted in the study:

- milk thistle seeds of different manufacturers and different batches have quality characteristics similar to those of the studied seeds, in particular, the weight of 1000 seeds, waste impurities, moisture content, mass fraction of crude protein, fiber, lipids, acid and peroxide values of the lipid component. The described simplification should prove the repeatability of the obtained experimental results, namely the dependence of the influence of the treatment conditions of thistle seeds on the acid and peroxide values of their lipids;
- the process of accelerated aging of thistle seeds imitates the corresponding processes that occur during seed storage under normal conditions (relative humidity 40...50 %, air temperature 5...10 °C, no direct sunlight, storage time about 2 years).

4. 2. Researched materials used in the experiment

During the research, thistle seeds (produced in Ukraine) were used, according to DSTU 7666/CAS 84604-20-6.

4. 3. Methods of determining the composition indicators of thistle seeds

The weight of 1,000 thistle seeds was determined according to DSTU 2949. Pest infestation of seeds was determined according to DSTU 8838. The content of waste impurities in the seed mass was determined according to DSTU 8837. The content of moisture, crude protein, lipids and fiber in seeds was determined according to DSTU 7491. The acid value of lipids extracted from milk thistle seeds was determined according to DSTU 8839, peroxide value – according to DSTU 8659.

4. 4. Method of determining the fatty acid composition of thistle seed lipids

The content of fatty acids in thistle seed lipids was determined according to ISO 5508 on a Shimadzu chromatograph (Japan).

4. 5. Method of processing thistle seeds to reduce the activity of the enzyme complex

The enzyme complex of thistle seeds refers to enzymes whose substrates are lipids with an ester group, in particular triglycerides (lipase) and free fatty acids (lipoxygenase).

The essence of processing thistle seeds to reduce the activity of the enzyme complex consists in the denaturation of these enzymes and loss of their catalytic properties for the hydrolytic splitting of complex fatty acid esters and oxidation of fatty acids.

The stages of thistle seed processing to reduce the activity of the enzyme complex are as follows:

- wetting seeds to effective moisture content;
- mixing wetted seeds;
- further exposure of wetted seeds for 2 hours;
- processing in a microwave radiation field with a frequency of 2,450 MHz for 240 seconds according to [18, 19];
- drying treated seeds at 105 ± 2 °C to initial moisture content (5.6 ± 0.2 %).

4. 6. Method of lipid extraction for determining the degree of reduction in enzyme complex activity after treatment

The lipid fraction from milk thistle seeds was extracted using a Soxhlet extractor. The extractant is hexane. The extraction temperature is 70 ± 1 °C, the extraction time is 2.0 ± 0.1 hours.

4. 7. Method of determining the effectiveness of processing thistle seeds to reduce the activity of the enzyme complex

The effectiveness of processing thistle seeds to reduce the activity of the enzyme complex is estimated by the analytical values of seed lipids (acid and peroxide values) after accelerated aging. Accelerated aging of thistle seeds is the exposure of wetted seeds at elevated temperatures (30.0 ± 0.6 °C) for 5 days, provided that their moisture content is maintained at 10 %. During accelerated aging, the lipids of milk thistle seeds undergo the processes of enzymatic hydrolysis and oxidation.

The acid and peroxide values are determined in the lipid fraction extracted by hexane extraction from seeds subjected to accelerated aging and then dried to a moisture content of 5.6 ± 0.2 %, as well as the comparison sample.

The acid and peroxide values respectively characterize the content of the products of enzymatic reactions of lipases and lipoxygenases, namely free fatty acids and primary oxidation products. Thus, the acid and peroxide values indirectly characterize the degree of inactivation of the enzyme complex (lipases and lipoxygenases) of milk thistle seeds by wetting them and processing in a microwave radiation field with a frequency of 2,450 MHz.

4. 8. Method of obtaining chocolate mass using thistle seeds

Prepared milk thistle seeds are crushed to a particle diameter of 500...800 µm on a laboratory homogenizer (speed up to 3000 rpm). The next stage is fine grinding on a vertical knife grinder, which is capable of grinding particles up to a diameter of 150...250 µm (speed 8000...10,000 rpm).

Cocoa butter is melted. Cocoa powder, sugar, milk powder, and lecithin are added to the melted mass to create the actual chocolate mass. Then crushed thistle seeds in an amount of 10 % are added to the melted chocolate, taking into account the results of organoleptic studies of taste, aroma and texture. This amount allows the seeds to be felt in each piece of chocolate mass, without overloading the product with a specific nutty flavor and aroma. Crushed thistle seeds are homogenized so that they are evenly distributed throughout the chocolate mass. The resulting chocolate mass is tempered and then poured into special molds for further cooling and hardening. The cooled and hardened chocolate mass is packed in an airtight package for storage and further research.

4. 9. Method of accelerated aging of model samples of chocolate mass with thistle seeds

Accelerated aging of model chocolate mass samples to compare the oxidative stability of their lipid component is carried out under controlled conditions. In this study, the task was to determine in which of the studied samples the processes of oxidation of the lipid component, including those that are enzymatically catalyzed, occur more intensively. Samples of chocolate mass taken for research must be of the same weight and texture. Each sample is individually packed in wrapping paper. Packaged samples of chocolate mass are stored at 20–25 °C and humidity of about 60–65 % in a dark, light-protected box to prevent oxidation from exposure to light. The content of the primary oxidation products of the lipid component of the samples is regularly (every 2 weeks) controlled by determining the lipid peroxide value. The degree of oxidation and, accordingly, oxidation stability of model chocolate mass samples are compared based on the results of analyzing the induction period of lipid component oxidation.

4. 10. Research planning and results processing

Two-factor experiments were used in research on the development of a method for reducing the activity of the enzyme complex of milk thistle seeds:

- determination of the dependence of the acid value of the lipid fraction on the amount of seed wetting and the pH of the wetting solution;
- determination of the dependence of the peroxide value of the lipid fraction on the amount of seed wetting and the pH of the wetting solution.

Each experiment was repeated three times. Statistical models of the dependences of the acid and peroxide values of the lipid fraction on the amount of seed wetting and time of treatment in a microwave radiation field were calculated by approximating experimental data by constructing trend surfaces. Processing of experimental data and construction of graphical dependencies were performed using Stat Soft Statistica v 6.0 packages (USA).

5. Results of research on the development of a method for reducing the activity of the enzyme complex of milk thistle seeds

5. 1. Determination of the composition indicators of thistle seeds

The composition indicators of milk thistle seeds, which are planned to be used in chocolate mass technology, have been determined, in particular, quality indicators (Table 1) and the fatty acid composition of the lipid component (Table 2).

Table 1

Quality indicators of thistle seeds

Indicator	Value
Weight of 1,000 seeds, g	24.0±0.7
Pest infestation	no
Waste impurities, %	0.30±0.01
Moisture content, %	5.6±0.2
Mass fraction of crude protein in terms of dry matter, %	18.3±0.5
Mass fraction of lipids in terms of dry matter, %	36.5±1.1
Mass fraction of fiber in terms of dry matter, %	29.5±0.8
Acid value of lipids, mg KOH/g	1.80±0.05
Peroxide value of lipids, mmol ½ O/kg	1.40±0.07

Table 2

Fatty acid composition of milk thistle lipids

Fatty acid	Content, % of the total amount
C _{14:0}	0.07
C _{16:0}	7.90
C _{16:1}	0.15
C _{18:0}	3.80
C _{18:1}	24.35
C _{18:2}	57.10
C _{18:3}	0.47
C _{20:0}	2.06
C _{20:1}	1.10
C _{22:0}	2.20
C _{24:0}	0.80
Total	100.00

The research results show that thistle seed samples meet the requirements of the relevant regulatory documentation (DSTU 7666/CAS 84604-20-6).

5. 2. Determination of the dependence of the acid and peroxide values of milk thistle lipids on the pH of the wetting solution and the degree of wetting

Research has been conducted aimed at increasing the shelf life of milk thistle seeds by reducing the activity of their enzyme complex (lipases and lipoxygenases), which is relevant for expanding the use of seeds in chocolate mass technology. The dependence of the acid and peroxide values of thistle seed lipids on the technological processing conditions of seeds was determined: the pH of the wetting solution and the degree of wetting. According to the research results [18, 19], as well as our own screening studies, the duration of treatment in a microwave radiation field with a frequency of 2,450 MHz for 240 seconds is rational. The factors of the experiment are the degree of wetting and the characteristics of the wetting solution:

- $w_{t.s.}$ – moisture content of milk thistle seeds after wetting, %;
- pH_s – pH of the thistle seed wetting solution, units;
- the response functions are the acid ($AV_{t.s.f.}$) and peroxide ($PV_{t.s.f.}$) values of thistle seed lipids.

The seeds were wetted to a moisture content in the range of 9...14 % with a step of 1 %. An acidic solution for wetting thistle seeds is a citric acid solution of various concentrations. The pH of the solution was varied in the range of 5...7 with a step of 1. The obtained values:

- the acid value of thistle seed lipids was within 2.0...8.3 mg KOH/g;
- the peroxide value of thistle seed lipids was within 1.5...14.1 mmol ½ O/kg.

The approximate dependence of the acid ($AV_{t.s.f.}$) and peroxide ($PV_{t.s.f.}$) values of thistle seed lipids on the moisture content of the seeds after wetting ($w_{t.s.}$) and the pH of the thistle seed wetting solution (pH_s) are presented by equations (1) and (2):

$$AN_{t.s.f.} = 24.9616 - 3.6361 \cdot w_{t.s.} - 2.506 \cdot pH_s + 0.1682 \cdot w_{t.s.}^2 + 0.0393 \cdot w_{t.s.} \cdot pH_s + 0.3208 \cdot pH_s^2; \quad (1)$$

$$PN_{t.s.f.} = 76.1448 - 7.5386 \cdot w_{t.s.} - 14.6005 \cdot pH_s + 0.3071 \cdot w_{t.s.}^2 + 0.0971 \cdot w_{t.s.} \cdot pH_s + 1.55 \cdot pH_s^2. \quad (2)$$

The significance of the coefficients of the dependence equations (1) and (2) of the acid and peroxide values of thistle seed lipids on the moisture content of the seeds after wetting and the pH of the wetting solution was checked by the least squares method. The adequacy of the obtained equations (1) and (2) was verified by the coefficients of determination R^2 (equal to 0.970 and 0.953, respectively). The calculated coefficients of determination for dependencies (1) and (2) indicate the predominant influence of such variations as the moisture content of thistle seeds after wetting and the pH of the wetting solution on variations in the acid and peroxide values of thistle lipids.

The surfaces of the obtained dependencies are presented in Fig. 1, 2.

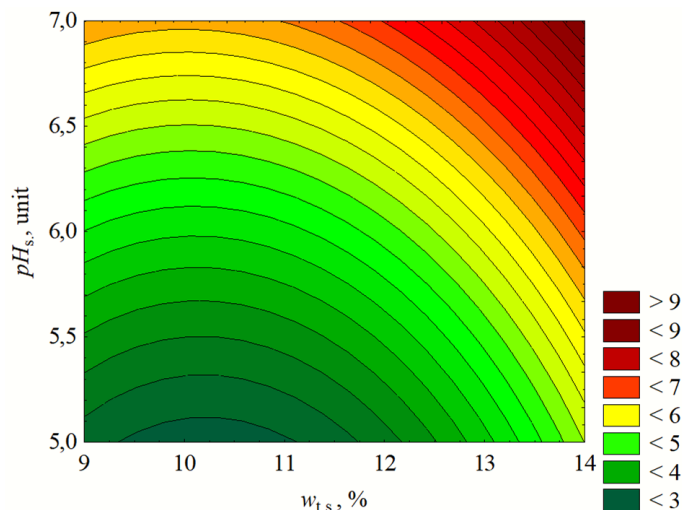


Fig. 1. Dependence of the acid value of thistle seed lipids after accelerated oxidation on seed processing conditions: pH of wetting solution and degree of wetting

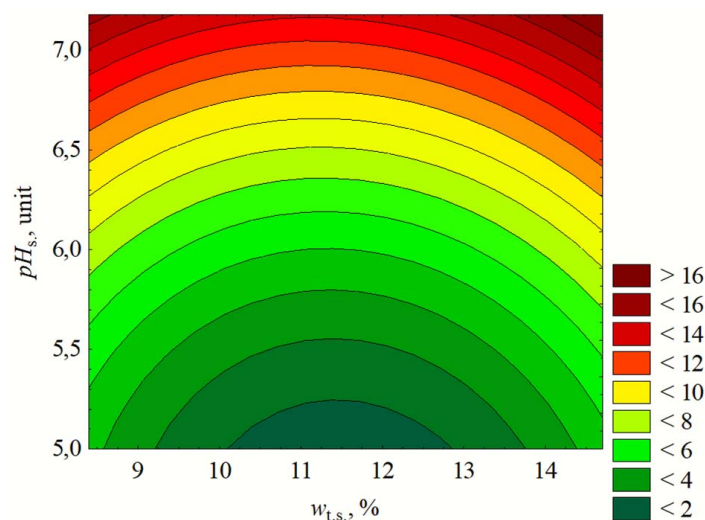


Fig. 2. Dependence of the peroxide value of thistle seed lipids after accelerated oxidation on seed processing conditions: pH of wetting solution and degree of wetting

5.3. Justification of rational values of thistle seed processing parameters for lipase and lipoxigenase inactivation

Analyzing the obtained experimental results, rational values of thistle seed processing parameters for the inactivation of lipases and lipoxigenases were substantiated. In order to clarify the obtained statistical data (Fig. 1, dependencies (1) and (2)), a number of experiments on seed processing were conducted, taking into account graphic and approximation dependencies. The results of these experiments, as well as their comparison with calculated data (according to dependencies (1) and (2)) are given in Table 3.

Based on the experimental results (Table 3), it is advisable to use the following parameters in the technological processing of thistle seeds to inactivate the lipase and lipoxigenase complex:

- pH of the seed wetting solution – 5.0...5.2 units;
- moisture content of milk thistle seeds after wetting – 11 %;
- treatment in a microwave radiation field with a frequency of 2,450 MHz for 240 s according to [18, 19].

Table 3

Influence of milk thistle seed processing conditions on the value of indicators characterizing the activity of the lipase and lipoxigenase complex

Experiment No.	pH of the seed wetting solution, units	Moisture content of seeds after wetting, %	Analytical values of seed lipids after accelerated aging			
			Acid value, mg KOH/g		Peroxide value, mmol $\frac{1}{2}$ O/kg	
			exp.	calc.	exp.	calc.
1	5.0	10.5	2.80±0.08	2.88	1.80±0.05	1.70
2	5.2	10.5	3.20±0.10	3.12	2.20±0.07	2.14
3	5.0	11.0	3.00±0.10	2.97	1.50±0.05	1.47
4	5.2	11.0	3.20±0.10	3.21	2.00±0.06	1.92
5	5.0	11.5	3.20±0.10	3.14	1.50±0.05	1.40
6	5.2	11.5	3.50±0.12	3.38	1.90±0.06	1.86

It should be noted that the use of a solution for the necessary wetting of thistle seeds with a pH below 5.0 leads to a change in the organoleptic indicators of the chocolate mass produced with the addition of seeds. This is unacceptable in terms of consumer properties of products. This fact is a limitation for using seed wetting solutions with a pH below 5.0 units.

5.4. Research on the lipid oxidation stability of chocolate mass using modified milk thistle seeds

A number of model samples of chocolate masses with the addition of thistle seeds were obtained. Screening studies have proven the rational concentration of crushed seeds in chocolate mass at the level of 10 %. It should be noted that the addition of crushed thistle seeds affects the “mouth feeling” or sensation when consuming chocolate:

- gives the chocolate mass a more crunchy texture due to the presence of particles;
- adds notes of nut or light bitterness, which is characteristic of thistle, to the taste of chocolate;
- adds notes of bitter chocolate to the aroma of chocolate.

The composition of model samples of chocolate mass with the addition of crushed thistle seeds, as well as a comparison sample, is given in Table 4.

Table 4

Recipe composition of model samples of chocolate mass

Component, %	Model samples			
	1	2	3	4
Cocoa butter	27	27	27	30
Powdered sugar	31.4	31.4	31.4	34.4
Grate cocoa	31	31	31	35
Soy phosphatide concentrate (lecithin)	0.5	0.5	0.5	0.5
Flavoring agent	0.1	0.1	0.1	0.1
Crushed native milk thistle seeds	–	10	5	–
Crushed milk thistle seeds of a modified composition	10	–	5	–
Total	100.00			

The induction periods of accelerated aging of model samples of chocolate mass without and with the addition of crushed thistle seeds (native and modified composition), the recipes of which are given in Table 3, are shown in Fig. 3.

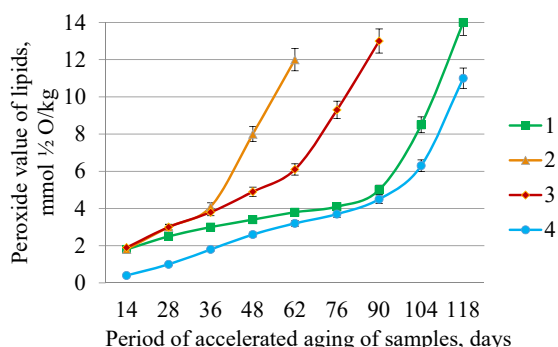


Fig. 3. Induction periods of oxidation of model samples of chocolate masses during accelerated aging: 1 – chocolate mass with the addition of 10 % crushed thistle seeds of a modified composition; 2 – chocolate mass with the addition of 10 % native thistle seeds; 3 – chocolate mass with the addition of 5 % crushed thistle seeds of a modified composition and 5 % native thistle seeds; 4 – chocolate mass without adding thistle seeds

It is obvious that the oxidation stability, and, therefore, the predicted shelf life of the studied samples of chocolate masses depend on the content of crushed thistle seeds in them and their processing. The induction period of chocolate mass with 10 % thistle seeds of a modified composition is 90 ± 3 days. This is 2.5 times longer than the induction period of a chocolate mass sample with 10 % of the same untreated seeds and 1.5 times longer than a sample containing modified and unmodified milk thistle seeds in a 50:50 ratio. In addition, the induction period of the chocolate mass with 10 % modified thistle seeds almost coincides with the induction period of the chocolate mass sample without the addition of thistle seeds, which is 102 ± 3 days.

6. Discussion of the results of the analysis of developing a method for reducing the activity of the enzyme complex of thistle seeds

A number of indicators of milk thistle seeds, which influence the quality of chocolate mass made with their addition, were studied, namely:

- quality indicators (weight of 1,000 seeds, pest infestation, waste impurities, moisture content, mass fractions of crude protein, lipids, fiber, as well as acid and peroxide values of lipids) (Table 1);
- fatty acid composition of lipids (Table 2).

It was determined that milk thistle seeds have a fairly small weight of 1000 seeds compared to such oil crops as sunflower and soybean, and are not infected with pests. This indicates the possibility of energy-saving grinding of seeds for use in chocolate mass technology. Compared to the mentioned crops, milk thistle seeds have a rather low moisture content and a low content of waste impurities, which consist mainly of spoiled seeds. This fact, in turn, confirms the technological feasibility of using this oil raw material in the technology of confectionery products, in particular, chocolate mass, due to the low probability of hydrolytic processes in the raw material and the finished product. Milk thistle seeds have a fairly high content of such components as crude protein and lipids, which makes them a valuable ingredient for enriching sugary confectionery. But the analytical values of lipids extracted from milk thistle seeds are quite high compared to sunflower and soybean seeds, which indirectly indicates the active flow of enzymatic hydrolysis and oxidation processes. This fact suggests the feasibility of inactivating seed enzymes that lead to hydrolytic and oxidative processes. It was determined that the lipid component of thistle seeds contains saturated fatty acids – 16.83 %, unsaturated – 25.6 %, and polyunsaturated – 57.10 % of the total amount of fatty acids. Thus, these seeds can be a significant source of polyunsaturated fatty acids in products with a saturated fatty acid composition, in particular, chocolate mass.

Rational values of thistle seed treatment parameters for the inactivation of lipases and lipoxygenases in order to inhibit the enzymatic oxidation of their lipid component have been determined. The obtained research results, namely graphical (Fig. 1, 2) and approximation dependences (1) and (2) allow us to predict the acid and peroxide values of seed lipids after accelerated oxidation, depending on the value of the processing parameters. Accordingly, the obtained dependencies (1) and (2) can be useful for developing a thistle seed processing technology in order to increase the hydrolysis and oxidation resistance of lipids.

Based on graphical (Fig. 1, 2) and approximate (1) and (2) dependencies, a number of experiments were conducted (Table 3), the results of which were used to substantiate rational values of thistle seed treatment parameters for the inactivation of lipases and lipoxygenases. Thistle seeds, processed according to the proposed method, are recommended to be used as a component of long-term health products, in particular, chocolate mass.

The results of the study differ from the results of the works [13–17] in that a method of thistle seed processing that is quite simple in technological implementation has been developed, which is proposed to be implemented not in the process of seed treatment, but before storage. This allows food enterprises that use thistle seeds in technological processes to form certain stocks of this raw material. Also, the development differs from the studies [18, 19] in that a new parameter of influence on the enzyme complex of seeds is proposed – a solution with an acidic pH for wetting the raw material. In addition, this study, in contrast to the works [18, 19], proved the possibility of inactivating the enzyme complex in seeds without destroying their membranes. Thus, determining the influence of the pH of the thistle seed wetting solution, as well as the moisture content of thistle seeds after wetting, on the acid and peroxide values of the lipid fraction is interesting from both a scientific and a practical point of view. The results of the research (Fig. 1, 2 and approximate dependences (1) and (2)) allow us to visualize the effectiveness of seed treatment to inhibit the processes of enzymatic hydrolysis and oxidation of thistle seeds during storage.

It is worth noting that the limitation of using the obtained results (Fig. 1, 2 and dependences (1) and (2)) is that the effect of wetting thistle seeds with acidic solutions on the activity of the enzyme complex was investigated only up to and including pH 5. A further decrease in pH can be effective in terms of enzyme inactivation, but is unacceptable due to the negative impact on the organoleptic characteristics of products obtained using such seeds, in particular, chocolate mass.

In addition, the “mouth feeling” assessment after adding crushed thistle seeds to the chocolate mass will depend on the personal taste preferences of consumers, so it is advisable to conduct tasting tests and research the reaction of the mass consumer before the industrial production of the proposed products. That is, the personal taste preferences of consumers of chocolate mass can limit the practical use of the obtained research results.

The limitation of this study is the availability of data only on selected types of thistle seed processing parameters (moisture content of thistle seeds after wetting, pH of the thistle seed wetting solution) and their defined limits (moisture content of seeds: 9...14 %, pH of the wetting solution: 5...7 units). Also, data on the stability of the lipid component only under conditions of accelerated aging can be called a limitation of the work.

Thus, it is possible to outline promising areas for the development of chocolate mass technology using thistle seeds of a modified composition. This is, first of all, a study of the features of inhibition of enzymatic hydrolysis and lipid oxidation of milk thistle seeds of a modified composition under normal storage conditions and determination of dependencies:

- between the process of accelerated aging of thistle seeds (with a modified composition) and the process of storing the same seeds under normal conditions;
- between the process of accelerated aging of chocolate mass with thistle seeds and the process of storing the same samples under normal conditions.

7. Conclusions

1. The composition indicators of milk thistle seeds, namely quality indicators (weight of 1,000 seeds, pest infestation, waste impurities, moisture content, mass fractions of crude protein, lipids, fiber, as well as acid and peroxide values of lipids) were determined. Quite high acid and peroxide values of the lipid component of seeds indirectly testify to the flow of the processes of enzymatic hydrolysis and oxidation. The type of fatty acids of milk thistle lipids, as well as their ratio, was identified. The examined thistle seed samples meet the requirements established in the relevant regulatory documentation – DSTU 7666/CAS 6-20-84604.

2. The dependence of the acid and peroxide values of the lipid component of milk thistle seeds, which indirectly indicates the activity of lipolytic enzymes, on the parameters of inactivation of the seed enzyme complex was investigated. Approximate dependences of the acid and peroxide values of thistle seed lipids on the pH of the seed wetting solution and the degree of such wetting were obtained. It was found that reducing the pH of the solution used to wet seeds before treatment in a microwave radiation field to 5.0 is more effective for reducing the activity of the enzyme complex than pH 7. Effective wetting of seeds is 10.5 % for lipase inactivation and 11.5 % for lipoxygenase inactivation.

3. Rational values of thistle seed processing parameters for the inactivation of lipases and lipoxygenases are substantiated. They are: pH of the seed wetting solution – 5.0...5.2 units; the moisture content of thistle seeds after wetting – 11 %; treatment in a microwave radiation field with a frequency of 2,450 MHz for 240 seconds.

4. The oxidation stability of the lipid component of the chocolate mass using thistle seeds of a modified composition was investigated. It was determined that the induction period of the chocolate mass with 10 % milk thistle seeds of a modified composition is 2.5 times longer than the induction period of the chocolate mass sample with thistle seeds that were not subject to processing.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship, or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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