

The object of the study is the oxidative stability of the lipid component of linseed treated with a citric acid and sodium chloride solution. The rational composition of chemical reagents for inactivating linseed lipoxygenases was determined in the work. The obtained results make it possible to develop an effective linseed treatment method for increasing stability to oxidative spoilage. The proposed composition of the linseed treatment solution (citric acid – 1.0...1.3 %; sodium chloride – 0.6...0.8 %) significantly reduces the peroxide and anisidine numbers of the lipid component. This helps reduce oxidative spoilage during accelerated oxidation and storage under normal conditions. Rational treatment conditions were determined based on the approximate dependency of these indicators on the concentrations of chemical reagents. The data obtained in the work are explained by chemical interactions between the solution components and the enzyme complex of linseed, leading to enzyme denaturation and, accordingly, increased oxidative stability of the lipid component. A feature of the obtained results is the competitiveness of treated linseed, characterized by increased nutritional value due to improved technological properties. The results of the study allow minimizing the loss of nutritional value and increasing the shelf life of linseed products. The results are important for developing new oilseed processing technologies. This makes it possible not only to increase the stability of products against oxidative spoilage, but also to preserve their high nutritional value. Further research in this area will contribute to improving oilseed processing technologies, in particular linseed, which is an important contribution to the development of the food industry

Keywords: oxidative stability, endogenous lipoxygenase, lipid component, linseed, chemical reagents

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DEVELOPMENT OF A METHOD FOR INACTIVATING LIPOXYGENASES IN LINSEED USING CHEMICAL REAGENTS

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1. Introduction

Linseed has many attractive aspects for consumers, making it popular and desirable in the food industry [1, 2]. Linseed processing technology provides unlimited opportunities for expanding the range of food products [3]. This allows satisfying the individual tastes and preferences of consumers. Innovations in linseed processing technology can help producers attract the attention of consumers who are looking for new flavors and

health products. As a food component, linseed is attractive for its high nutritional value. In particular, it has a high content of ω -3 polyunsaturated fatty acids, proteins, cellulose, vitamins and minerals. Linseed is rich in vitamins B and E, β -carotene, macro- and microelements, lignans, flavonoids, essential oils, sterols, organic acids. Adding linseed to foods significantly increases their nutritional value. Moreover, linseed gives a unique taste and texture to food products, making the product more interesting for consumers [2, 3].

The process of oilseed storage, in particular, linseed, is an important technological technique that ensures the quality of products from it during further processing. From a physico-chemical point of view, oilseed is a complex multicomponent structure: the nucleus consists of protein globules, spherosomes containing oil and a large number of pores and capillaries through which water and nutrients are transported. Spherosomes, in turn, are surrounded by a membrane that acts as a biological protection of the oil from various external influences. Control over the content of reactive oxygen species in oilseed is carried out with antioxidants and some enzymes, in particular, superoxide dismutase, catalase, peroxidase, lipoxigenase, etc. [4]. During the storage and processing of linseed, the most important from a technological point of view are endogenous hydrolytic (lipases) and redox (lipoxigenases) enzymes. The enzyme complex of seeds, in particular lipoxigenases, catalyzes the rapid hydrolysis of triacylglycerols and the oxidation of free fatty acids of the lipid component. The resulting fatty acid hydroperoxides have high oxidative capacity due to the presence of peroxide oxygen and can further oxidize lipids labile to oxidation – unsaturated fatty acids, carotenoids. This leads to increased acid and peroxide numbers of the lipid component in the seed and deterioration of its quality in general [2, 5]. In addition, during oilseed storage under the influence of various external factors, its shell and kernel may be damaged. The oil in the seed changes its localization, flowing out into the intercellular space under the influence of capillary forces. Losing its biosecurity, it easily undergoes hydrolytic and oxidative changes due to the free access of seed enzymes and air oxygen [6, 7].

The search for ways to regulate the activity of this enzyme complex is becoming an increasingly urgent task for preserving the oxidative stability of seed lipids and, therefore, expanding the range of products based on it [8]. It should also be noted that, despite the rather well-studied linseed composition, scientific and technical sources lack comprehensive information on the chemical composition of the seed, and data on its enzyme complex activity are limited. Given the high oil content in linseed prone to enzymatic oxidation [4], it is of interest to develop methods for reducing the activity of native lipoxigenases. Such treatment will help inhibit the hydrolytic and oxidative destruction of the seed lipid complex.

So, research aimed at finding and substantiating factors affecting the oxidation intensity of oilseed lipids in general and linseed in particular can reveal the dependence of shelf life on the features of technological processing. The data obtained will rationalize the technology of food production with oilseed addition, in particular linseed. The obtained scientific results in this area are relevant for food production, as there is a need to increase the shelf life of linseed as a raw component and products based on it. This will expand the range of health food products with, in addition to biological value, increased resistance to oxidative spoilage.

2. Literature review and problem statement

The work [9] presents the results of research on promising methods for obtaining linseed oil and increasing its yield while maintaining quality in terms of oxidative spoilage. It is shown that one of the key methods of preparing seeds for pressing, which increases the oil yield, is to heat them by a

conductive method. However, during seed heating, problems may arise related to changes in the gas composition of the cell structure, affecting the oxidative stability of lipids.

The studies [10] proved that during oilseed heating, its respiration is intensified, and during further storage, the volume of voids in the cell structure filled with oxygen is replaced by about 60 % carbon dioxide. As a result, the gas composition of the cell structure changes, and the extracted oil is less oxidized, which positively affects the peroxide number reduction. However, issues remain regarding the influence of other seed preparation methods on lipid oxidative stability, in particular vacuum treatment. This aspect was investigated in [11], namely the effect of vacuum during linseed preheating before cold pressing. Seed preheating was found to increase the oil yield compared to conventional first cold pressing without preheating. The acid and peroxide numbers of the studied oil exceeded those of the oil obtained by one-time cold pressing without seed preheating.

Issues related to the influence of UV irradiation during oilseed preparation on the oxidative stability of lipids are of interest, which was discussed in [12]. Grain irradiation with UV light was found to increase the peroxide oxidation level of lipids and antioxidant content. Prolonged UV exposure, probably due to the activation of compensatory mechanisms, manifested in increased antioxidant content, helps to reduce the peroxide oxidation level of lipids. Thus, during soaking seeds after preliminary UV irradiation, the greatest reactivity of the antioxidant system is manifested after 24 hours in the endosperm. This is due to accelerated hydrolytic processes in the endosperm, promoting the release of reserved functionally active compounds with antioxidant properties. Oxidation of antioxidants is carried out by peroxidase, the activity of which increases sharply during seed germination [6]. Thus, the influence of oilseed endogenous enzymes on the lipid composition, as well as the mechanisms of antioxidant action during storage and processing, remains an unresolved issue. At the same time, the studies [13] proved that sunflower and sesame lipolytic enzymes differ in optimum pH, temperature, and substrate nature. One of the critical conditions for lipase activity was found to be excessive moisture content. In particular, seeds with a moisture content of up to 12 % can be stored with little lipase activation, but with increased moisture content, there is a noticeable increase in lipase activity.

In [14], it was determined that dry oilseed lipase is resistant to temperature, however, when the seeds are moistened, the enzyme is rapidly inactivated. The study [15] identified water-soluble and water-insoluble lipases in different types of oilseeds. In particular, water-insoluble lipase is found in castor seed and has an optimum action at pH 3.6, while soluble lipase is contained in most oilseeds with an optimum of pH 8.0. The optimum pH of lipase action varies depending on the physiological state of seeds: for example, it is 5 in resting soybeans, and 7 in germinating beans. This pattern is also true for seeds of other oil crops (hemp, cotton, flax, etc.). However, critical conditions for increasing lipase activity have not been found. In [16], this question was somewhat answered, and lipase activity in certain types of oilseeds was shown to be affected by their maturity degree. In addition, lipase activity is influenced differently by the technological processes of seed processing. It is worth noting that these studies did not find clear conditions for significant changes in lipase activity. The reasons for this are the variety of oil crops studied, as well as the difficulty of controlling moisture content and temperature during seed storage and the vari-

ability of the enzyme composition depending on the physiological state of seeds. Technological aspects related to the influence of other seed preparation methods on the oxidative stability of lipids, in particular ultrahigh-frequency (microwave) radiation, are of interest; this was considered in [17–20].

The results of the studies [17] showed that linseed oil obtained by first cold pressing of linseed with preliminary microwave treatment meets the requirements of regulatory documents for edible oil. However, the values of the acid and peroxide numbers are slightly higher than those in the first cold pressed oil. Thus, the studies confirm the effectiveness of microwave treatment to increase oil yield, but questions remain about the effect of this method on lipid oxidative stability. There is no data on the mechanisms of microwave treatment effect on the composition and stability of lipids, making it difficult to fully understand the process and optimize it. Further research is needed to determine microwave treatment conditions that would reduce the intensity of oxidative processes in lipids, as well as to study the effect of microwave treatment on other bioactive components.

Research on the inactivation of the oilseed enzyme complex during technological processing is considered in [18]. The influence of the time of microwave treatment of a ground mixture of flax, sunflower and sesame seeds and its initial moisture content on the degree of further enzymatic hydrolysis of protein was determined. A rational range of the described treatment factors was defined to maximize the biological value of the protein-fat base. The rational time of microwave pretreatment of the protein-fat base is 250–350 s, the initial moisture content of the raw material is 12–14 %. There are results of studies on the inactivation of inhibitors of proteolytic enzymes of ground sesame seeds using a similar treatment [19]. Rational treatment conditions for sesame seeds for the inactivation of anti-alimentary factors are substantiated almost in the initial ranges: moisture up to 10–13 %, microwave treatment time 220–240 s. However, the issue related to the influence of the pH value of the aqueous solution for ground oilseed treatment before microwave treatment remained unresolved. This question is important because the pH of the medium can significantly affect the enzyme activity and inactivation degree. The reason for the unresolved problem is, perhaps, limited resources, as well as insufficient understanding of the mechanisms of interaction between the pH of the medium and enzymatic activity under microwave exposure.

This was considered in [20], where the method of this seed treatment for inactivating hydrolytic and redox enzymes by treating the seeds with an aqueous solution of edible acid and subsequent microwave treatment was substantiated. The available research results leave unresolved the question of the influence of other electrolytes, in particular salts, on the inactivation of the enzyme complex of oilseeds with intact shells to preserve raw materials for further use. Thus, finding out the effect of chemical reagents on the inactivation efficiency of the enzyme complex of linseed and other oilseeds during microwave treatment is an urgent task. An especially important aspect is the inactivation of lipoxygenases, being the main oxidation catalysts for polyunsaturated fatty acids in linseed. Their inactivation with chemical reagents is a necessary step to improve product stability. Preventing the oxidation of the lipid component will preserve the quality of linseed oil and other linseed products, which is an urgent task for the food industry. The results of the study would increase the shelf life of seeds as a raw ma-

terial component, which would expand the range of health products with increased resistance to oxidative spoilage and contribute to revealing the potential biological activity of the raw material resource.

3. The aim and objectives of the study

The aim of the study is to develop an effective method for inactivating lipoxygenases in linseed using chemical reagents. This will expand linseed application in the food industry, as well as increase the stability and shelf life of linseed products.

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

- to determine the chemical composition of linseed;
- to determine the dependence of the oxidative stability of the linseed lipid component on the chemical composition of the lipoxygenase inactivation solution;
- to analyze the dynamics of changes in the peroxide and anisidine numbers of the linseed lipid component over storage time.

4. Materials and methods

4.1. Object and hypothesis of the study

The object of the study is the oxidative stability of the linseed lipid component, expressed in analytical numbers (peroxide and anisidine). The main hypothesis of the study is that using chemical reagents, in particular adjusting the solution pH and adding sodium chloride, can effectively inactivate lipoxygenases in linseed. This will increase the oxidative stability of the lipid component and preserve the quality of the seeds during storage.

The study assumes that linseed from the same batch has a homogeneous chemical composition, which allows generalizing the results of the study. In addition, it is assumed that the selected concentrations of chemical reagents (citric acid, sodium chloride) and solution pH are sufficient to achieve a noticeable effect of lipoxygenase inactivation. It is also assumed that there is a linear or predictable relationship between the concentration of chemical reagents, the solution pH and the degree of lipoxygenase inactivation.

The study adopted the following simplification: the entire batch of linseed has the same chemical composition and lipoxygenase activity, simplifying the analysis and interpretation of the results.

4.2. Experimental materials

The following materials were used in the study:

- linseed (produced in Ukraine), according to DSTU 4967/CAS 8001-26-1;
- citric acid (produced in Ukraine), according to DSTU 908/CAS 77-92-9;
- sodium chloride (produced in Ukraine), according to DSTU ISO 2479/CAS 7647-14-5.

4.3. Methods for determining the chemical composition of linseed

The moisture content of linseed was determined by the gravimetric method. The protein content was determined by the Kjeldahl method. The lipid component of linseed was determined by the Soxhlet method. The total carbohydrate content was determined by the difference between the total

sample mass and the masses of proteins, fats and moisture. Additionally, colorimetric methods with the Lane-Eynon reagent were used to determine the content of mono- and disaccharides. The cellulose content was determined by enzymatic gravimetry. The seed samples were treated with enzymes to hydrolyze non-cellulosic polysaccharides, after which the residue was weighed. The fatty acid composition of fatty raw materials was determined by gas-liquid chromatography according to DSTU ISO 5508 on a Shimadzu chromatograph (Japan).

4. 4. Method of linseed treatment with a lipoxygenase inactivation solution

Solutions with different concentrations of citric acid and sodium chloride were prepared for the study. Linseed samples were soaked in these solutions at a 1:10 seed-solution ratio for 2 hours at room temperature. After treatment, the seed samples were treated in a microwave field at 2,450 MHz for 240 seconds according to [18–20]. The treated seeds were then dried at 105 ± 1 °C to the initial moisture content (7.20 ± 0.29 %).

After drying, the linseed samples were subjected to organoleptic testing for smell, taste, texture and appearance. To compare the organoleptic parameters, a control linseed group was used, which was not treated with chemical reagents, but underwent all other treatment stages (soaking in distilled water, drying, exposure and grinding). In case of a negative impact of chemical reagents on the organoleptic indicators, the concentrations of citric acid and sodium chloride in the solution were adjusted to minimize this effect.

4. 5. Method of accelerated linseed oxidation

Accelerated oxidation of linseed was carried out at a temperature of 30 ± 1 °C for 7 days. During accelerated oxidation, primary (peroxides and hydroperoxides) and secondary (aldehydes and ketones) oxidation products accumulated in triacylglycerols. The seeds were then ground, and the lipid component was extracted. The primary and secondary oxidation products were identified by determining the peroxide and anisidine numbers, respectively.

4. 6. Research planning and results processing

In studies to develop a method for inactivating linseed lipoxygenases using chemical reagents, two-factor and one-factor experiments were used. Each experiment was repeated three times. Statistical models of the relationship between the oxidative stability of the linseed lipid component and the concentration of chemical reagents in the lipoxygenase inactivation solution were calculated by approximating experimental data by constructing a trend surface. Statistical models of peroxide and anisidine number dynamics for the treated and control samples during 12 months of storage were calculated by approximating experimental data by constructing trend lines. Processing of the obtained data and construction of graphical dependencies were performed using Stat Soft Statistica v 6.0 (USA) and Microsoft Excel (USA) packages.

5. Results of studies on linseed lipoxygenase inactivation using chemical reagents

5. 1. Determination of the linseed chemical composition

To develop an effective method of lipoxygenase inactivation, it is necessary to study the chemical composition

of linseed in detail. This will allow us to determine the effect of lipoxygenase inactivation reagents on the overall product quality. The analysis of the linseed chemical composition was carried out by the following parameters: the contents of moisture, proteins, fats, carbohydrates, and cellulose (Table 1).

Table 1

Chemical composition of linseed

Indicator	Value
Moisture and volatile content, %	7.20 ± 0.29
Protein content, %	20.80 ± 0.83
Lipid content, %	35.50 ± 1.42
Carbohydrate content, %, including mono- and disaccharides:	26.10 ± 1.02
Glucose	0.80 ± 0.03
Fructose	0.50 ± 0.02
Saccharose	1.30 ± 0.05
Maltose	0.20 ± 0.01
Cellulose, %	10.40 ± 0.40

The content of polyunsaturated fatty acids in the linseed lipid component was determined (Table 2).

Table 2

Fatty acid content in the linseed lipid component

Fatty acid	Content, % of total
Myristic (C _{14:0})	0.050 ± 0.002
Palmitic (C _{16:0})	5.800 ± 0.232
Palmitoleic (C _{16:1})	0.150 ± 0.006
Stearic (C _{18:0})	4.550 ± 0.182
Oleic (C _{18:1})	20.300 ± 0.812
Linoleic (C _{18:2})	14.100 ± 0.564
Linolenic (C _{18:3})	54.750 ± 2.190
Arachidonic (C _{20:0})	0.100 ± 0.004
Gondoic (C _{20:1})	0.100 ± 0.004
Behenic (C _{22:0})	0.050 ± 0.002
Lignoceric (C _{24:0})	0.050 ± 0.002
Total	100.000

The high content of polyunsaturated fatty acids, in particular, α -linolenic and linoleic, in linseed emphasizes the importance of preventing their oxidation to preserve the quality of linseed oil, as well as linseed products. Lipoxygenases are the main catalysts of this process, so their inactivation with chemical reagents is a necessary step to increase the linseed stability. This also helps preserve the nutritional value and improve the organoleptic properties of linseed and products on its basis.

5. 2. Determination of the dependence of the oxidative stability of linseed lipids on the chemical composition of the lipoxygenase inactivation solution

The results of the study showed that linseed treatment with a citric acid and sodium chloride solution significantly affects the oxidative stability of the lipid component.

Approximate dependencies of the peroxide and anisidine numbers of the linseed lipid component on the citric acid and sodium chloride concentration in the lipoxygenase inactivation solution are presented by equations (1) and (2):

$$PN_{lins.lip.}(c_{CA}, c_{NaCl}) = 11.3936 - 16.7125 \cdot c_{CA} - 6.845 \cdot c_{NaCl} + 10.4613 \cdot c_{CA}^2 + 2.21 \cdot c_{CA} \cdot c_{NaCl} + 2.3595 \cdot c_{NaCl}^2; \tag{1}$$

$$AN_{lins.lip.}(c_{CA}, c_{NaCl}) = 5.2753 - 8.8089 \cdot c_{CA} - 2.9148 \cdot c_{NaCl} + 5.6063 \cdot c_{CA}^2 + 0.7786 \cdot c_{CA} \cdot c_{NaCl} + 0.9229 \cdot c_{NaCl}^2; \tag{2}$$

where $PN_{lins.lip.}$ – peroxide number of the linseed lipid component, mmol ½ O/kg;

$AN_{lins.lip.}$ – anisidine number of the linseed lipid component, units;

c_{CA} – citric acid concentration, %;

c_{NaCl} – sodium chloride concentration, %.

The significance of the equation of approximate dependencies (1), (2) is determined by calculating the Fisher's test (F), based on the assumption (null hypothesis) that the equation is statistically insignificant. The calculated Fisher's test values were:

– for approximate dependency (1): $F(2.8)=16.482$;

– for approximate dependency (2): $F(2.8)=12.465$.

The calculated Fisher's test values are greater than its critical table value $F_{table}(2.8)=4.46$ at a significance level $p=0.05$. This result allows us to reject the null hypothesis and, with a 95 % probability, recognize the values of the coefficients of determination as significant, and the equations of approximate dependencies (1) and (2) – significant. The values of the coefficients of determination:

– $R^2=0.947$ for dependency (1);

– $R^2=0.952$ for dependency (2).

Fig. 1, *a, b* show graphical dependencies of the peroxide and anisidine numbers of the linseed lipid component on the concentrations of chemical reagents (citric acid and sodium chloride) in the treatment solution.

Based on the results of experimental research, the rational composition of the chemical solution for linseed lipoxygenase inactivation was substantiated, providing the greatest inactivation efficiency of lipoxygenase enzymes. This is indirectly evidenced by a decrease in the oxidative spoilage parameters of the seed lipid component, such as peroxide and anisidine numbers.

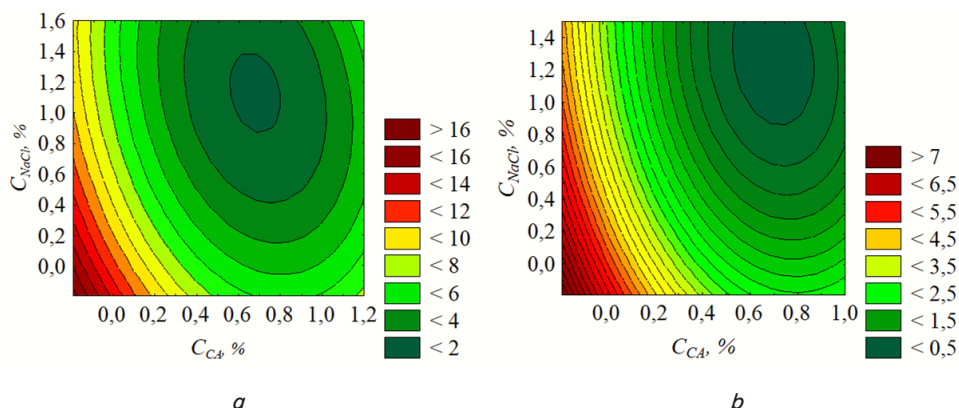


Fig. 1. Dependence of the oxidative stability of the linseed lipid component on the citric acid and sodium chloride concentration in the lipoxygenase inactivation solution:

a – dynamics of changes in the peroxide number;

b – dynamics of changes in the anisidine number

5. 3. Analysis of the dynamics of changes in the peroxide and anisidine numbers of the linseed lipid component over storage time

Analysis of the dynamics of the oxidation parameters of the seed lipid component – peroxide and anisidine numbers, allows evaluating the effectiveness of the lipoxygenase inactivation methods applied and the stability of the linseed lipid component during storage. The oxidation parameters of the lipid component of two linseed samples were investigated:

– treated sample – seeds treated with a lipoxygenase inactivation solution containing citric acid and sodium chloride, with a reasonable concentration;

– control sample – seeds that were not subjected to treatment.

Graphical dependencies of the dynamics of changes in peroxide and anisidine numbers for the treated and control samples during 12 months of storage are shown in Fig. 2, *a, b*.

Approximate dependencies of the peroxide number of the linseed lipid component samples during 12 months of storage are presented by equations (3) and (4):

$$PNS_{lins.lip.In.}(\tau) = 0.4655 \cdot \tau; \tag{3}$$

$$PNS_{lins.lip.Or.}(\tau) = 0.5559 \cdot \tau^2 - 0.1032 \cdot \tau; \tag{4}$$

where $PNS_{lins.lip.In.}$ – peroxide number of the linseed sample treated with chemical reagents during storage, mmol ½ O/kg;

$PNS_{lins.lip.Or.}$ – peroxide number of the control linseed sample during storage, mmol ½ O/kg;

τ – storage time, months.

Approximate dependencies of the anisidine number of the linseed lipid component samples during 12 months of storage are presented by equations (5) and (6)^

$$ANS_{lins.lip.In.}(\tau) = 0.4873 \cdot \tau; \tag{5}$$

$$ANS_{lins.lip.Or.}(\tau) = 0.9745 \cdot \tau; \tag{6}$$

where $ANS_{lins.lip.In.}$ – anisidine number of the linseed sample treated with chemical reagents during storage, units;

$ANS_{lins.lip.Or.}$ – anisidine number of the control linseed sample during storage, units;

τ – storage time, months.

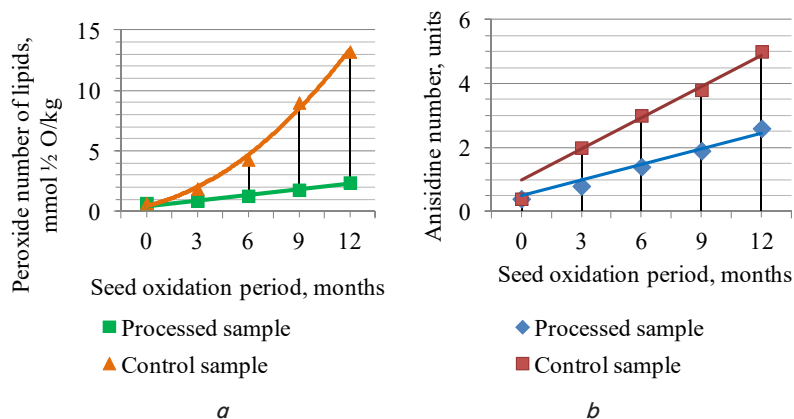


Fig. 2. Dynamics of changes in the oxidation parameters of the lipid component of the linseed sample treated with chemical reagents and the control one during 12 months of storage: *a* – changes in the peroxide number; *b* – changes in the acid number

The adequacy of the regression equations (3)–(6) was verified by the coefficients of determination R^2 0.983, 0.980, 0.995, and 0.991, respectively. The obtained values of the coefficients of determination for dependencies (3)–(6) indicate a high influence of storage time variations on variations in the peroxide and anisidine numbers of the lipid component of the seed samples.

The linseed sample treated with chemical reagents has a linear dependence of the peroxide number on the storage time (equation (3)). The control, untreated linseed sample shows a quadratic dependence of the peroxide number on the storage time (equation (4)). The dynamics of changes in the anisidine number during the storage of both linseed samples has a linear dependence on the storage time (equations (5) and (6)). However, ANS of the control sample increased about twice as fast (0.9745 compared to 0.4873) per month as that of the chemical-treated one.

6. Discussion of the results of developing a method for linseed lipoxygenase inactivation

The results of analyzing the linseed chemical composition (Table 1) indicate its high nutritional potential. This raw material is a significant source of lipids (35.5%), proteins (20.8%), as well as dietary fiber, in particular cellulose (10.4%). The carbohydrate content is 26.1%, of which the bulk is mono- and disaccharides. The content of polyunsaturated fatty acids in the linseed lipid component (Table 2) is quite attractive in terms of nutritional and biological value. Linolenic ($C_{18:3}$) and linoleic ($C_{18:2}$) fatty acids make up about 54.75% and 14.10%, respectively. But the high content of these polyunsaturated fatty acids causes extremely low linseed stability to oxidative spoilage, leading to a loss of nutritional value and critical deterioration in the organoleptic properties of products based on it during storage.

The results of the study showed that linseed treatment with a citric acid and sodium chloride solution significantly affects the oxidative stability of the lipid component. Based on the obtained experimental data (Fig. 1, *a*, *b*), equations (1), (2)), the rational composition of the chemical solution for linseed lipoxygenase inactivation was substantiated. Using this composition provides the highest inactivation efficiency of lipoxygenase enzymes, con-

firmed by a decrease in the oxidative spoilage parameters of the seed lipid component, such as peroxide and anisidine numbers. The proposed composition of chemical reagents in an aqueous solution for lipoxygenase inactivation is: citric acid – 1.0...1.3%; sodium chloride – 0.6...0.8%. Thus, approximate dependencies (1), (2) will be useful for developing a linseed treatment technology, which helps reduce the oxidative spoilage of lipids. Using reasonable ratios of the specified chemical components, it is expedient to obtain food products with increased oxidative stability, differing in certain technological characteristics and having a longer shelf life. The proposed linseed treatment solutions can increase its nutritional value and reduce the impact of oxidative spoilage, which is an important aspect for the food industry.

The linear dependence of the peroxide number of the sample treated with chemical reagents (equation (3)) indicates a constant, controlled level of oxidation throughout the entire storage period. The quadratic dependence of the peroxide number of the control sample (equation (4)) indicates an increasing level of oxidation over time, which can lead to the accumulation of oxidation products at a higher rate in later storage periods. The linear dependence of the anisidine number of the sample treated with chemical reagents (equation (5)) indicates a stable formation of secondary oxidation products, which is controlled throughout the entire storage period. In turn, the control sample (equation (6)) shows a much steeper linear dependence, indicating a faster accumulation of secondary oxidation products during storage.

Without chemical treatment, linseed accumulates secondary oxidation products much faster, leading to poorer quality and lower stability of the lipid component during storage. Chemical treatment slows down this process, improving linseed stability and quality during storage. The obtained research results confirm the effectiveness of chemical treatment in reducing the oxidation level of the linseed lipid component. This improves the stability and quality of the lipid component during storage.

The results of the study differ from those of [9–11] by the fact that a linseed treatment method was developed not only for oil extraction by pressing, but also for use in confectionery and other branches of the food industry. The studies described in this paper focused on the use of enzyme complex inactivators – citric acid and sodium chloride for linseed treatment, which is a new approach compared to UV irradiation considered in [12]. In [13, 14], the effect of pH and moisture content on lipase activity was investigated, but the specifics of the effect of different concentrations of chemical reagents on the activity of endogenous lipoxygenases were not considered. Unlike the work [17], emphasizing the effect of microwave treatment on seed oil yield, this paper focuses on lipoxygenase inactivation. The main objective of the study is to reduce the oxidative spoilage of lipids by chemical treatment and subsequent microwave treatment. This improves the oxidative stability of the final product. As for the works [18, 19], their goal was to determine the effect of microwave radiation and initial moisture content on the degree of protein enzymatic hydrolysis and inactivation of inhibitors of proteolytic enzymes in seeds without taking

into account other chemical reagents. The studies [19, 20] did not take into account the effect of the pH of the aqueous solution for oilseed treatment before microwave radiation. This work provides an answer to the stated question, offering a reasonable composition of the chemical solution, ensuring effective lipoxygenase inactivation, as confirmed by a decrease in the peroxide and anisidine numbers. In addition, previous works focused on the inactivation of endogenous proteolytic enzymes and antialimentary factors of oilseeds, rather than lipoxygenases, which initiate oxidative spoilage of the lipid component.

The limitation of using the obtained results (equations (1)–(6)) is that linseed with certain physicochemical characteristics was used in the experimental studies. Therefore, when using other linseed varieties or other plant seeds, it is necessary to take into account the specifics of their chemical composition, in particular the fatty acid composition. The initial levels of peroxide and anisidine numbers should also be considered to adjust the ratio of chemical reagents for lipoxygenase inactivation. Differences in the fatty acid composition and antioxidant content can significantly affect the oxidation dynamics of the seed lipid component, and, accordingly, its stability during storage.

The shortcoming of the study is the lack of data on the effect of individual antioxidants on the oxidative stability of the linseed lipid component. Such antioxidants can be applied to the surface of seeds treated with chemical reagents to increase the oxidative stability of their lipid component.

Based on the results obtained, promising areas of research on stabilizing the linseed lipid component against oxidative spoilage can be outlined. This is primarily exploring the use of natural antioxidants, such as tocopherols, carotenoids or flavonoids, for additional protection of the seed lipid component from oxidation during storage. Thus, seed treatment with chemical reagents and natural antioxidants may be appropriate to create a combined technology for stabilizing the lipid component of valuable linseed during storage.

7. Conclusions

1. The chemical composition of linseed was determined. The high content of linoleic (54.750 ± 2.190 %) and linoleic (14.100 ± 0.564 %) polyunsaturated fatty acids proves the

need to prevent its oxidation in order to preserve the quality of linseed oil and linseed products.

2. The dependence of the oxidative stability of the linseed lipid component on the chemical composition of the lipoxygenase inactivation solution was determined. The rational composition of the chemical solution for linseed lipoxygenase inactivation was substantiated. Using a solution containing 1.0–1.3 % citric acid and 0.6–0.8 % sodium chloride provides the maximum efficiency of lipoxygenase inactivation. This leads to a significant decrease in the peroxide and anisidine numbers of the lipid component during accelerated seed oxidation, indicating the reduction in the oxidative spoilage of the seed lipid component.

3. The dynamics of changes in the peroxide and anisidine numbers of the linseed lipid component over storage time were analyzed. Using a citric acid and sodium chloride solution for linseed treatment significantly increases the oxidative stability of its lipid component, reducing oxidative spoilage during storage for 12 months. This allows us to recommend linseed treatment with this solution to improve its quality and shelf life in the food industry.

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.

References

- Rashid, F., Ahmed, Z., Hussain, S., Huang, J.-Y., Ahmad, A. (2019). *Linum usitatissimum* L. seeds: Flax gum extraction, physicochemical and functional characterization. *Carbohydrate Polymers*, 215, 29–38. <https://doi.org/10.1016/j.carbpol.2019.03.054>
- Belinska, A., Bochkarev, S., Varankina, O., Rudniev, V., Zviahintseva, O., Rudnieva, K. et al. (2019). Research on oxidative stability of protein-fat mixture based on sesame and flax seeds for use in halva technology. *Eastern-European Journal of Enterprise Technologies*, 5 (11 (101)), 6–14. <https://doi.org/10.15587/1729-4061.2019.178908>
- Kajla, P., Sharma, A., Sood, D. R. (2014). Flaxseed – a potential functional food source. *Journal of Food Science and Technology*, 52 (4), 1857–1871. <https://doi.org/10.1007/s13197-014-1293-y>
- Dunford, N. T. (2022). Enzyme-aided oil and oilseed processing: opportunities and challenges. *Current Opinion in Food Science*, 48, 100943. <https://doi.org/10.1016/j.cofs.2022.100943>
- Rozhmina, T., Bankin, M., Samsonova, A., Kanapin, A., Samsonova, M. (2021). A comprehensive dataset of flax (*Linum usitatissimum* L.) phenotypes. *Data in Brief*, 37, 107224. <https://doi.org/10.1016/j.dib.2021.107224>
- Jandali, R., Agha, M. I. H. (2022). Lipoxygenase Inhibitory Activity of Some Extracts Prepared from Flaxseed (*Linum usitatissimum* L.). *Current Bioactive Compounds*, 18 (3). <https://doi.org/10.2174/1574893616666211012091140>

7. Belinska, A., Bliznjuk, O., Masalitina, N., Bielykh, I., Zviahintseva, O., Gontar, T. et al. (2023). Development of biotechnologically transesterified three-component fat systems stable to oxidation. *Eastern-European Journal of Enterprise Technologies*, 5 (6 (125)), 21–28. <https://doi.org/10.15587/1729-4061.2023.287326>
8. Chakraborty, S., Pulivarthi, M. K., Raj, A. S., Prakash, S. D., Bommina, H., Siliveru, K. (2024). Inactivation of lipase and lipoxygenase in whole wheat flour using atmospheric cold plasma and steam treatments: Kinetics, mechanism, and impact on its compositional properties. *Journal of Cereal Science*, 117, 103889. <https://doi.org/10.1016/j.jcs.2024.103889>
9. Farag, M. A., Elimam, D. M., Afifi, S. M. (2021). Outgoing and potential trends of the omega-3 rich linseed oil quality characteristics and rancidity management: A comprehensive review for maximizing its food and nutraceutical applications. *Trends in Food Science & Technology*, 114, 292–309. <https://doi.org/10.1016/j.tifs.2021.05.041>
10. Pointner, T., Rauh, K., Auñon-Lopez, A., Kostadinović Veličkovska, S., Mitrev, S., Arsov, E., Pignitter, M. (2024). Comprehensive analysis of oxidative stability and nutritional values of germinated linseed and sunflower seed oil. *Food Chemistry*, 454, 139790. <https://doi.org/10.1016/j.foodchem.2024.139790>
11. Sytnik, N., Korchak, M., Nekrasov, S., Herasymenko, V., Mylostyvyi, R., Ovsianikova, T. et al. (2023). Increasing the oxidative stability of linseed oil. *Eastern-European Journal of Enterprise Technologies*, 4 (6 (124)), 35–44. <https://doi.org/10.15587/1729-4061.2023.284314>
12. Dun, Q., Yao, L., Deng, Z., Li, H., Li, J., Fan, Y., Zhang, B. (2019). Effects of hot and cold-pressed processes on volatile compounds of peanut oil and corresponding analysis of characteristic flavor components. *LWT*, 112, 107648. <https://doi.org/10.1016/j.lwt.2018.11.084>
13. Al-Haidari, A. M. Dh., Alsaadawi, I. S., Khudhair, S. H. (2021). Determination the Optimum Conditions of the Activity and Stability of Lipase Extracted from Sunflower Germinated Seeds. *Iraqi Journal of Science*, 62 (2), 431–440. <https://doi.org/10.24996/ij.s.2021.62.2.8>
14. Zhang, Y., Zhang, Y. (2020). Effect of lipoxygenase-3 on storage characteristics of peanut seeds. *Journal of Stored Products Research*, 87, 101589. <https://doi.org/10.1016/j.jspr.2020.101589>
15. Machado, S. A., Da Rós, P. C. M., de Castro, H. E., Giordani, D. S. (2021). Hydrolysis of vegetable and microbial oils catalyzed by a solid preparation of castor bean lipase. *Biocatalysis and Agricultural Biotechnology*, 37, 102188. <https://doi.org/10.1016/j.bcab.2021.102188>
16. Zhang, Y., Li, X., Lu, X., Sun, H., Wang, F. (2021). Effect of oilseed roasting on the quality, flavor and safety of oil: A comprehensive review. *Food Research International*, 150, 110791. <https://doi.org/10.1016/j.foodres.2021.110791>
17. Szydłowska-Czerniak, A., Tymczewska, A., Momot, M., Włodarczyk, K. (2020). Optimization of the microwave treatment of linseed for cold-pressing linseed oil - Changes in its chemical and sensory qualities. *LWT*, 126, 109317. <https://doi.org/10.1016/j.lwt.2020.109317>
18. Bochkarev, S., Krichkovska, L., Petrova, I., Petrov, S., Varankina, O., Belinska, A. (2017). Research of influence of technological processing parameters of protein-fat base for supply of sportsmen on activity of protease inhibitors. *Technology Audit and Production Reserves*, 4 (3 (36)), 27–30. <https://doi.org/10.15587/2312-8372.2017.108376>
19. Belinska, A., Petik, I., Bliznjuk, O., Bochkarev, S., Khareba, O. (2022). Bioengineering studies of inactivation of sesame proteolytic enzyme inhibitors in sports nutrition. *Food Resources*, 10 (19), 38–46. <https://doi.org/10.31073/foodresources2022-19-04>
20. Stankevych, S., Zabrodina, I., Lutsenko, M., Derevianko, I., Zhukova, L., Filenko, O. et al. (2023). Use of thistle seeds of modified composition in chocolate mass technology. *Eastern-European Journal of Enterprise Technologies*, 6 (11 (126)), 83–91. <https://doi.org/10.15587/1729-4061.2023.291042>