

ESTIMATION OF OXIDATIVE STABILITY OF THE LIPID COMPLEX OF CREAMY-SHAKEN CANDIES WITH CHIA SEEDS AT STORAGE

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Abstract

One of ways of developing the confectionary industry is a search for raw material resources with a high content of nutrients, useful for the human organism. A promising type of such raw materials is non-traditional oil seeds, especially chia seeds. The aim of the study was to estimate the oxidative stability of the lipid complex of creamy-shaken candies with chia seeds at storage. It is noted, that lipase of chia seeds doesn't manifest activity in an acid medium. That is why chia seeds introduction to creamy-shaken candies (with pH<6,0) doesn't accelerate hydrolytic disintegration of their lipid component at storage. A type of jelly-former, used at candy production (agar-agar, pectin, modified starch), doesn't influence the accumulation dynamics of free fatty acids in candies. It is noted, that the lipid component of creamy-shaken candies, produced with agar-agar and pectin, is characterized with the equal dynamics of a peroxide value. The accumulation of peroxide compounds in starch products takes place slower. It has been established, that despite the high content of polyunsaturated fatty acids, the lipid component of candies with chia seeds comparing with control is characterized by less inclination to oxidation. It is noted, that at the 30th day of storage relative changes of peroxide value indices of products with supplements are 1.00 and 0.81 against 1.85 and 1.49 in control samples on agar-agar (pectin) and starch respectively. The braking effect of chia seeds on oxidizing processes is conditioned by the high content of tocopherols and phenol compounds (quercetin, coffee, chlorogenic acids, rutin, polyphenol compounds) with antioxidant properties in them.

It has been established, that during the studied storage term products didn't gain a rancid smack, testifying to the accumulation of secondary oxidation processes. The structure and color of the studied samples of candies didn't also change. That is during the normative storage term (60 days) creamy-shaken candies with chia seeds correspond to normative requirements by organoleptic parameters and quality ones of the lipid complex.

Keywords: chia seeds, hydrolysis of lipids, oxidation of lipids, lipase, lipoxygenase, creamy-shaken candies, storage.

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1. Introduction

Modern world tendencies of the confectionary industry development are mainly oriented on creation of products, healthy for humans. Taking it into account, today producers accent attention on using raw materials, including physiologically healthy nutrients: polyunsaturated fatty acids, mineral substances, vitamins, phenol compounds, soluble and insoluble dietary fiber and so on at making confectionary products [1]. An essential amount of such substances is contained in vegetable raw materials, especially in oil seeds.

Main types of oil seeds, used today in confectionary technologies, are sunflower, flax, sesame, amaranth, rape and so on. Seeds are introduced whole and comminuted as flour (fatty or fatless), by-products of oil production (meals, oilcake).

These raw materials are most widely used in technologies of floury confectionary products. Rape meal (25 % of the flour mass) [2, 3], flax and sesame meal (12...20 % of the flour mass) [4, 5], whole seeds of sunflower or flax (10...13 % to the flour mass) [6, 7], sunflower flour (up to 17 % of the flour mass) [8] and so on are known for using for this product. Oils seeds are also used in confectionary technologies. There is offered a base for fat-containing candy masses, including comminuted sunflower (12 %), sesame (18 %) and flax (30 %) seeds [9]. Recommendations about using amaranth flour at producing pomade masses (10 % of the sugar mass) are given [10].

The given information testifies that the introduction of oil seeds and their processing products to these technologies is realized in rather high concentrations. That is the enrichment of products with physiologically healthy nutrients will be essential. Especially, the use of fatless products of oil seeds (meals, oilcakes, flour) allows to enrich products mainly by proteins (in 1.3...2.4 times) and dietary fiber (in 4 times and more) [4, 5, 8, 10]. At using native whole or comminuted seeds, the content of polyunsaturated fatty acids in products additionally rises (in 2 times and more [6, 7, 9]). It is known, that these nutrients strengthen protective functions of the human organism, favor normalization of cholesterol metabolism, prevent the development of cardiovascular diseases and so on. But fats, containing the essential amount of acyls of unsaturated acids, are easily oxidized under the influence of technological factors and in the process of storage, creating peroxide radicals. These compounds disintegrate, creating secondary oxidation products (aldehydes, ketones), giving fat a rancid smack. The presence of lipase and lipoxygenase that activate processes of hydrolytic and oxidizing spoilage of fats is typical for all types of oil seeds. So, at using native oil seeds in confectionary technologies, it is necessary to control the oxidative stability of the lipid component of a product at storage.

Chia seed is today a popular super-product that is conditioned by the wide spectrum of its healthy properties. It is proved, that regular consumption of these seeds prevents the development of autoimmune and cardiovascular diseases, has the anti-inflammatory, hepatoprotective and anti-diabetic effect [12]. A special feature of its chemical composition is the high content of polyunsaturated fatty acids – near 80 % of all lipids [13], easily subjected to oxidation under the effect of technological factors and enzymes as it was noted above. It is known, that inactivation of enzymes takes place at temperature higher 65 °C [14]. Stages of the technological process of producing creamy-shaken candies, at which chia seeds are introduced, don't provide such temperature regimes. That is enzymes of the supplement remain active.

Taking it into account, the aim of the study was to estimate the oxidative stability of the lipid complex of creamy-shaken candies with chia seeds at storage. It allows to determine the correspondence of new products to the regulated storage terms.

It was necessary to carry out the complex of interconnected tasks for attaining the set aim:

- to study the activity of lipase and lipoxygenase in chia seeds;
- to estimate changes of acid and peroxide values of the fat fraction of creamy-shaken candies with chia seeds at storage;
- to estimate organoleptic characteristics of candies with chia seeds at storage.

2. Materials and methods

2. 1. Research materials

The research subject was chosen as creamy-shaken candies. As raw materials for producing candies, there were used: white sugar (SSU 4623:2006); starch treacle (SSU 4498:2005); food agar-

agar (SSU 16280-88); lemon acid (SSU 908-2006); dry egg albumin (SSU 2212:2003); apple pectin SWEJ-1 (SSU 6088: 2009), modified oxidized jellying starch OPV-1 (SSU 4380:2005), food aromatic essences (SSU 4716:2007); drinking water (SSU 2874-82); fatty condensed milk with sugar (SSU 4274:2003); margarine (SSU 4498:2005); chia seeds (TC U 15.8-31062161-010:2008). Used chia seeds were from harvest 2018, imported by PE “SPC “Sigma” (Ukraine), country of origin – Bolivia. Seeds were introduced whole and comminuted (**Fig. 1**).

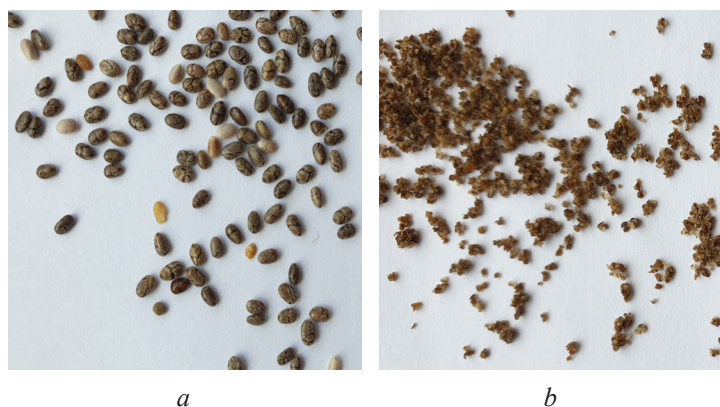


Fig. 1. Photo image of chia seeds: *a* – whole; *b* – comminuted

The study included samples of creamy-shaken candies, produced using different jelly-formers:

- creamy-shaken candies without a supplement on agar-agar;
- creamy-shaken candies with chia seeds on agar-agar;
- creamy-shaken candies without a supplement on pectin;
- creamy-shaken candies with chia seeds on pectin;
- creamy-shaken candies without a supplement on modified starch;
- creamy-shaken candies with chia seeds on modified starch.

2. 2. Method of preparing candy samples for studying

Dry egg albumin was combined with water (1:7) and left for hydration for 10 min. The albumin solution was introduced with whole chia seeds in amount 40 % of the albumin mass and left for 10 min for swelling. The obtained mixture was shaken during 20 min. The prepared protein mass was shaken during 12 ± 2 min with syrup, cooled to temperature 65 ± 2 °C. Syrup was made by boiling sugar, treacle and jelly-former (agar-agar, pectin or modified starch). Margarine with condensed milk and comminuted chia seeds were shaken separately. The dosage of comminuted seeds was 40 % of the margarine mass. The shaken protein mass was combined with the fat mixture and formed by smearing. The structure was created at temperature 19...21 °C during 60 min. The structured candy mass was cut in pieces and glazed by chocolate.

The candy samples were stored under standard conditions (temperature 20 ± 2 °C) in a polyethylene film during the normative term – 60 days. Samples for the analysis were taken each 10 days.

2. 3. Methods of studying the activity of lipases and lipoxygenases in chia seeds

Reagents for the study were prepared according to [15].

2. 3. 1. Method of studying lipase activity

The activity of acid and alkaline lipases was studied. Acid lipase manifests activity in the medium with pH 4.7...5.0, alkaline – in the medium with pH 8.0...8.4. So, the method of their determination is based on titration of fatty acids, formed under the effect of enzyme on fat in the light-acid or alkaline medium by the alkaline solution.

Batches of comminuted seeds of 2.50 ± 0.01 g were accurately rubbed in a pestle with 1 cm^3 of refined sunflower oil. The rubbed mass was put in conic flasks of 100 cm^3 , its residues from the pestle were washed away by 5 cm^3 of distilled water. 5 drops of toluol were added, accurately mixed, corked and thermostated during 20 hours at temperature $30 \text{ }^\circ\text{C}$. After ending of thermostating the flasks were poured by 50 cm^3 of the mixture of ethyl alcohol and ether (4:1). The mixture was precipitated and titrated by the solution of potassium hydroxide (0.1 mol/dm^3) with 0.5 cm^3 of thymolphthalein. For control samples, there were also taken 2 batches of comminuted seeds (of 2.50 ± 0.01 g) and the same actions, excluding thermostating, were conducted with them.

The lipase enzyme activity (AL, cm^3/g) was calculated by the formula

$$AL = \frac{(a-b)k}{m}, \quad (1)$$

where a – amount of 0.1 mol/dm^3 of the potassium hydroxide solution, spent for titrating the studied sample, cm^3 ; b – the same for the control sample; k – correcting coefficient to 0.1 mol/dm^3 of the potassium hydroxide solution, $k=1$; m – fat batch, g.

2. 3. 2. Method for studying the lipoxygenase activity

The method for determining the lipoxygenase activity is based on determining a peroxide value, changing in the process of absorbing molecular oxygen by the substrate and is accompanied by hydroperoxides formation under the effect of the enzyme. Sunflower oil was used as a substrate. The reactionary mixture was formed of the solution of the enzyme, phosphate-citrate buffer (pH 7.0) and substrate. Distilled water was used in the control sample instead of the enzyme. The peroxide value was determined in the experimental and control samples. The research method is presented in detail in [16].

2. 4. Method of removing fat from candies

Fat was removed from candies by the extraction method. The batch of candies (300...350 g), was released from glaze, comminuted and put to a conic flask with microsections. The comminuted mass was poured with chloroform (1:2), infused for 24 hours without a light access and filtered. All further analyzes were conducted with this extract. The amount of fat, transferred to the extract, was determined by the difference of the mass of 1 ml of the extract before and after drying. Drying was conducted in a drying chamber at temperature $105 \text{ }^\circ\text{C}$ up to the constant mass.

2. 5. Methods of studying the acid and peroxide value of fat

For determining the acid and peroxide values, there was taken the extract amount with the necessary fat amount. Then PV (Peroxide value) and AV (Acid Value) were determined by standard methods [17]. PV was expressed in $\text{mmol } \frac{1}{2}\text{O/kg}$, and AV – in mg KOH/g of the sample.

The dynamics of peroxides accumulation in the process of storage was estimated by the index of the peroxide value relative change δPV , calculated according to [18] by the formula

$$\delta PV = \frac{PV_t - PV_0}{PV_0}, \quad (2)$$

where PV_t , PV_0 – peroxide values of the samples, corresponding to the storage term t days and initial condition.

2. 6. Method of estimating organoleptic characteristics of candies

Organoleptic parameters of candies were controlled immediately after preparing and after storage ending (in 60 days). The estimation was conducted according to SSU 4683:2006 by the parameters: color, structure, smell and taste.

2. 7. Statistical processing of the results

The statistical processing of the experimental results was carried out using standard packages of programs Microsoft Office for a series of parallel measurements ($n=4-5$, $p<0,05$).

3. Research results of the activity of chia seed enzymes and the condition of the lipid complex of candies with it

3. 1. Study of the lipase and lipoxygenase activity in chia seeds

The lipase and lipoxygenase activity in chia seeds was analyzed comparing with one in other types of oil seeds (Table 1).

Table 1

The lipase and lipoxygenase activity in chia seeds comparing with one in other types of oil seeds ($p<0,05$, $n=4$, $\sigma=3,5...4,0$ %)

Enzyme	Enzyme activity		
	Chia seeds	Sunflower seeds [19]	Sesame seeds [20]
Lipase, cm ³ /g			
acid	inactive	1.45...2.28	inactive
alkaline	1.70	0.33...0.40	0.56
Lipoxygenase, mmol ¹ / ₂ O/kg	2.06	2.0...3.3	1.42

It is noted, that acid lipase of both chia seeds and sesame ones doesn't manifest activity. Whereas, its activity is rather high for sunflower seeds. However chia is characterized with the higher activity of alkaline lipase comparing with other seeds. That is it may be foreseen, that introduction of chia seeds to food systems with an acid medium must not initiate hydrolytic disintegration of lipids. The absence of hydrolysis products in a fat system in its turn prevents the lipoxygenase effect. It is conditioned by the fact that free fatty acids, formed at the expense of lipase effect, are faster oxidized by lipoxygenase than ones, bound with an acyl glycerin molecule. It is noted, that the activity of chia seed lipoxygenase is at the level of other types of oil seeds.

3. 2. Study of acid and peroxide values of fat

An oxidation degree of the lipid component of creamy-shaken candies with chia seeds was estimated by the acid (Table 2) and peroxide (Fig. 1) values.

Table 2

Acid value AV, mg KOH of lipids in the studied samples ($p<0,05$, $n=5$, $\sigma=3,5...4,0$ %)

Sample		Storage duration, days						
		0	10	20	30	40	50	60
On agar-agar	Control	1.11	1.17	1.26	1.37	1.5	1.61	1.69
	With chia seeds	1.16	1.22	1.29	1.41	1.55	1.67	1.75
On pectin	Control	1.13	1.19	1.27	1.38	1.52	1.63	1.71
	With chia seeds	1.18	1.23	1.31	1.43	1.58	1.7	1.8
On modified starch	Control	1.10	1.15	1.24	1.35	1.49	1.6	1.68
	With chia seeds	1.14	1.21	1.28	1.39	1.53	1.64	1.73

The index of peroxide value characterizes the presence of free fatty acids, formed as a result of hydrolysis of acyl glycerins, accelerated with a temperature increase and under the

effect of enzymes, in lipids. It is known [14], that hydrolytic enzymes of oil seeds are activated at comminution, medium humidity increase higher 12 % and in temperature interval 30...50 °C. According to the developed technology, there are used whole and comminuted chia seeds. Whole chia seeds are introduced at the stage of protein mass shaking, further boiled by syrup with temperature 65±2 °C. This temperature is not enough for inactivating enzymes. At the same time the obtained semi-product is characterized by high humidity (22.0±2.0 %), and is also favorable for the enzymatic activity. Introduction of comminuted seeds is realized at the stage of plasticized margarine shaking (temperature 37±2 °C) with condensed milk (temperature 20±2 °C). The temperature, comminution and humidity (16.0±2.0 %) also cause enzymes activation. Taking it into account, the samples of creamy-shaken candies after preparation are characterized by a bit higher acid value comparing with the control samples (**Table 2**). But in the process of storage the speed of hydrolytic processes in the control and experimental samples is almost equal. Especially, the increase of *AV* in the lipid complex of candies without a supplement in 60 days of storage is 1.51...1.53 times and in ones with chia seeds – 1.50...1.52 times. It may be explained by the fact that lemon acid is introduced at the final stage of preparing the creamy-shaken candy mass. As a result, the obtained mass has an acid medium (pH<6.0). As it is testified by the research results of the activity of hydrolytic enzyme of chia seeds (**Table 1**), its lipase in the acid medium doesn't manifest activity. Taking it into account, enzymatic hydrolysis of the lipid component of candies with chia seeds doesn't take place at storage. It is noted, that during the whole storage period the studied samples of candies correspond to the requirements of normative documents by the acid value – don't exceed 2 mg KOH/g.

An oxidation degree of lipids is determined by the peroxide value. It is noted, that the lipid component of creamy-shaken candies, prepared on agar-agar and pectin, is characterized with the equal dynamics of this parameter change (**Fig. 2, a**). It is conditioned by the fact that their recipe composition differs only by the type of jelly-former. Creamy-shaken candies, based on modified starch, are characterized with the lower content of fat in the recipe; as a result the dosage of chia seeds in them is also lower. At the same time candies, based on starch, are characterized with more density–0.920...0.960 g/cm³ against 0.600...0.620 g/cm³ in candies on pectin or agar. Taking it into account, their structure is less aired that is a probability of contacts between fat molecules with oxygen decreases. That is why the accumulation of peroxide compounds in the lipid component of candies on starch is slower (**Fig. 2, b, Table 3**).

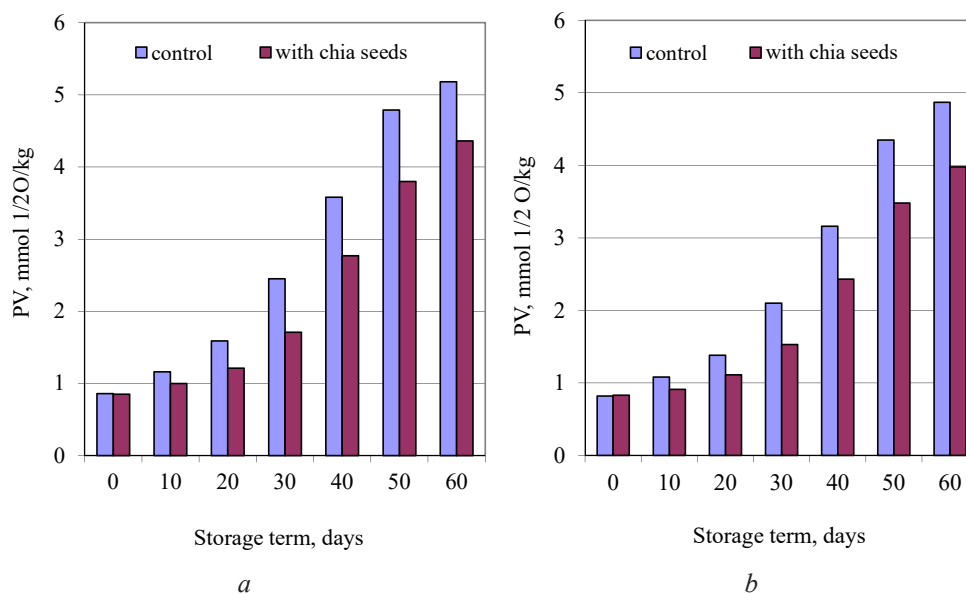


Fig. 2. Changes of peroxide values of the lipid component of the studied samples of creamy-shaken candies at storage: *a* – candies, prepared on agar-agar or pectin; *b* – candies, prepared on modified starch ($p < 0.05$, $n = 5$, $\sigma = 4.0...4.3$ %)

The research results (**Fig. 2, Table 3**) testify that despite the high content of polyunsaturated fatty acids, the lipid component of candies with chia seeds is characterized with less inclination to oxidation comparing with the control. Especially, it is noted, that at 30th day of storage relative changes of the peroxide value of the products with the supplements are 1.00 and 0.81 against 1.85 and 1.49 in the control samples on agar-agar (or pectin) and starch respectively (**Table 3**). It is noted, that the peroxide accumulation speed in the control samples at the 60th day becomes a bit slower. Especially, the peroxide value in lipids of candies on agar-agar (or pectin) and starch in the period from the 40th to 50th day rises by 33.8 % and 37.6 % respectively. And for the period 50th–60th day the peroxide value of these samples rises only by 8.1 and 11.9 %. It testifies to the fact that in 50 days of storage peroxide compounds become to disintegrate, creating secondary oxidation products. This tendency is less expressed for the samples of candies with chia seeds. Especially, from 40th to 50th day the content of peroxides in the lipid fraction of candies with the supplement on agar-agar (or pectin) rises by 37.18 %, and in the following 10 days – by 14.7 %. The increase of the peroxide content in these periods for candies on starch is 43.2 % and 14.36 % respectively. The braking influence of chia seeds on oxidation processes of the lipid component of creamy-shaken candies may be explained by the high content of phenol compounds with antioxidant properties in them. It is known, that chia seeds contain quercetin, coffee, chlorogenic acids, rutin, polyphenol compounds [21]. The high antioxidant activity of phenol compounds of chia seeds is confirmed by researches [22]. The abovementioned antioxidants are water-soluble. Their effectiveness as to braking fat oxidation is conditioned by the following. Creamy-shaken candy masses are polydispersed systems, including an emulsion component. An antioxidant, solved in the dispersed medium, covers the emulsion phases' distribution surface. It provides its maximal contact with a lipid phase and allows to bind peroxide radicals. Chia seeds also contain the essential amount of tocopherols (1.48 mg/100 g [12]), solving in the fat medium and inhibiting creation of lipid radicals directly in the fat component.

Table 3

Relative change of the peroxide value δ PV of the lipid component of the studied samples at storage (relative to the first day)

Sample		Storage term, days					
		10	20	30	40	50	60
On agar-agar or pectin	Control	0.35	0.85	1.85	3.16	4.57	5.02
	With chia seeds	0.18	0.42	1.00	2.26	3.47	4.13
On modified starch	Control	0.32	0.68	1.49	2.85	4.30	4.94
	With chia seeds	0.10	0.34	0.81	1.93	3.19	3.80

The peroxide value index must not exceed 10 mmol $\frac{1}{2}$ O/kg for high-quality fatty systems. It has been established, that fat fractions of all studied samples of candies correspond to these requirements.

3. 3. Study of organoleptic characteristics of candies

It is known, that peroxide compounds disintegrate with creation of secondary oxidation products (aldehydes, ketons and so on) that cause a rancid smack in fat-containing products. But the analysis of quality characteristics and organoleptic parameters of the studied samples of candies demonstrated that in 60 days of storage none product get rancid signs. The structure of products and color also don't change at storage.

That is during the regulated storage term (60 days) creamy-shaken candies with chia seeds correspond to the normative requirements by quality parameters and organoleptic characteristics of the lipid complex.

4. Conclusions

It is noted, that lipase of chia seeds doesn't manifest activity in the acid medium. That is why their introduction to creamy-shaken candies doesn't accelerate hydrolytic disintegration of its lipid component at storage. A type of jelly-former, used at preparing candies (agar-agar, pectin, modified starch, doesn't influence the accumulation dynamics of free fatty acids in candies.

It is noted, that the lipid component of creamy-shaken candies, produced with agar-agar and pectin, is characterized with the equal dynamics of a peroxide value. The accumulation of peroxide compounds in starch products takes place slower (at the expense of less mass aeration). The braking effect of chia seeds on oxidizing processes is conditioned by the high content of tocopherols and phenol compounds with antioxidant properties in them.

It has been established, that during the studied storage term products didn't gain a rancid smack, testifying to the accumulation of secondary oxidation processes. The structure and color of the studies samples of candies didn't also change.

The research results allow to make a conclusion about the correspondence of creamy-shaken candies with chia seeds to the regulated storage terms by the quality parameters of the lipid complex and by the organoleptic characteristics. But the presented studies don't consider changes that take place at storage with other quality parameters of creamy-shaken candies with chia seeds (humidity, acidity, strength, microbiological safety) that is a weak side of the presented materials. The authors plan to continue the researches in this direction for developing the obtained results.

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