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CHANGES IN LIPASE ACTIVITY DURING GERMINATION OF OIL SEEDS

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Abstract

The process of germination of oilseeds is accompanied by enzymatic hydrolysis of lipids under the action of lipase, which catalyzes the hydrolytic cleavage of triacylglycerols to glycerol and fatty acids. This article studied the activity of lipase in germinated oilseeds. The aim of the study was to determine the activity of oilseed lipase, analyze the effect and identify the dependence of lipase on the fatty acid composition at different exposure times.

The objects of study are oilseeds of the 2021 harvest: spring rapeseed variety "Gulsary"; sunflower grade "Rauan"; flax oil grade "Kostanay"; safflower variety "Nika 80". Determination of lipase activity (dependence of the enzymatic activity of lipase on the duration of the germination of oilseed grains, dynamics of the enzymatic process, which is characterized by changes in the quality of the objects of study, depending on the timing of germination, and change in the acid number of fat in sunflower, flax and safflower seeds depending on the timing of germination) was carried out by the conventional titrimetric method on two types of buffer solutions (alkaline phosphate buffer with pH 8 and acid acetate buffer with pH 4.7).

Analysis of the obtained data when studied the dependence of the enzymatic activity of lipase on the duration of the germination of oilseed grains, showed that an increase in the germination of oilseed grains, as a result of active hydrolysis of the lipid bonds of the fatty composition, leads to an acceleration of the fermentation process - lipase activity, rapidly splitting triacylglycerols to glycerol and fatty acids. Regarding the dynamics of the enzymatic process, which is characterized by changes in the quality of the objects of study, depending on the timing of germination, the analysis of the presented data showed that an increase in the germination of spring rape seeds significantly affects the change in the acid number of fat, which confirms the active course of the enzymatic process due to the breakdown of vegetable oil molecules by lipase into free fatty acids. When studied the change in the acid number of fat in sunflower, flax and safflower seeds depending on the timing of germination, dependence analysis showed that with an increase in the terms of germination of seeds of sunflower, flax and safflower, an increase in the values of the acid number of fat is observed.

As a result of studying the process of fermentation of oilseeds with lipase during germination, it was found that the timing of germination actively affects the quality indicator - the acid number of fat. The acid number indicates the amount of free fatty acids, which leads to the oxidation of the final product and oxidative spoilage. An increase in acid number leads to a reduction in the shelf life of the finished product. The increased content of free fatty acids occurs due to the fact that during the germination of grain, the lipase enzyme is activated, which breaks down the oil molecules in oilseeds into free fatty crops. The longer the grain germination process, the higher the lipase activity becomes. High activity of lipases leads to an increase in the acid number of fat.

***Key words**: Enzymes, Lipolytic activity, Lipase, Functional drinks, Safflower, Sunflower, Linen rape.*

1. Introduction

The most promising direction in solving the problem of eliminating the deficiency of macro- and microelements is the enrichment with natural biologically active substances of food products of daily mass consumption, which will allow for the correction of the diet of the general population [1 - 4]. It is a scientific fact that liquid foods or drinks make it possible to most effectively fill the deficit and maintain the necessary balance of biologically active substances in the body [5]. It is these drinks enriched with natural nutrients (biologically active substances, micro and macro elements, vitamins, amino acids, etc.) that differ from traditional ones in increased nutritional and biological value.

In this regard, the development of technology for the enrichment of food with microelements to impart a therapeutic and prophylactic principle of action based on germinated grain is an urgent and timely direction in the field of healthy and rational nutrition. It is these grain products, compiled according to a scientifically based recipe and enriched with components that will meet modern requirements for food products [6 - 8]. However, before developing the technology, it is necessary to study the biochemical processes occurring in oilseeds during germination, as well as to study the qualitative indicators that affect the decrease in consumer properties, which is what this article is aimed at.

The aim of the study was to determine the activity of oilseed lipase, analyze the effect and identify the dependence of lipase on the fatty acid composition at different exposure times.

2. Materials and Methods

The following oilseeds of the 2021 harvest were identified as the objects of study: spring rapeseed variety "Gulsary"; sunflower grade "Rauan"; flax oil grade "Kostanay"; and safflower variety "Nika 80". The presented varieties are the latest breeding achievements of the leading teams of research and production centers in the field of crop production.

According to the literature, there are many ways to determine lipolytic activity using various physical and physico-chemical methods using various substrates. The determination of lipase activity was carried out by the generally accepted titrimetric method according to Ermakov on two types of buffer solutions (alkaline phosphate buffer pH 8.0 and acid acetate buffer pH 4.7) [9].

Samples of purified kernels of oil seeds weighing 2.0 ± 0.01 g were ground in a mortar and 1 mL of vegetable oil was added. After that, it was stirred and 5 mL of alkaline phosphate buffer with pH 8.0 was added, thoroughly mixed, placed in a laboratory conical flask with a capacity of 250 mL, while washing away traces of the crushed mass from the mortar and pestle with 5 mL of distilled water. Two drops of toluene were added, closed with a cork, and placed in a shaker-incubator for 2 hours at a temperature of 30 ^{0}C. Then, 50 mL of an alcohol-ether mixture was added to the experimental samples, allowed to settle, and titrated with a 0.2 M alcohol solution of KOH in the presence of phenolphthalein [9].

Control samples, taking into account the background content of fatty acids in sunflower seeds, were prepared in the same way as the experimental ones, but they were titrated immediately without incubation in a thermostat.

The results of the studies were recorded in a laboratory journal. After that, the enzymatic activity of lipase was calculated by the formula [9]:

$$A = \frac{(a-b)\cdot k\cdot 10}{n}$$

Where: A is the activity of lipase, cm^3 of 0.1 M KOH solution per 10 g of seeds in 2 hours; a is the amount of 0.2 M KOH solution used for titration of the test sample, cm^3; *b* is the amount of 0.2 M KOH solution used for titration of the initial sample, cm^3; k - correction factor to 0.1 M KOH solution (k-2); n is the weight of the sample of seeds, g; 10 - recalculation of lyapse activity per 10 g of seeds.

3. Results and Discussion

The enzymatic activity of lipase is of great importance during the germination of oilseeds. With an increase in humidity and the timing of germination of oilseeds, lipases actively break down vegetable fats into free fatty acids, which leads to an increase in acidity and a decrease in the quality of products. Based on the experimental data, a graph was plotted between the enzymatic activity of lipase A (mE/g) and the duration of germination t (days) of oilseed grains. The presented diagram (Figure 1) characterizes the activity of fermentation of the fatty acid composition of oilseeds by lipase.

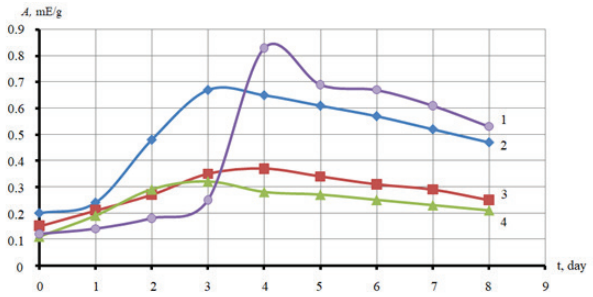

Figure 1. Lipase activity during germination of oilseeds 1. Rrape, 2. Sunflower, 3. Linen, 4. Safflower

Figure 1 indicates that an increase in the germination time of oilseed grains, as a result of active hydrolysis of the lipid bonds of the fatty composition, leads to an acceleration of the fermentation process - lipase activity, rapidly splitting triacylglycerols to glycerol and fatty acids.

The analysis of the presented curve showed that as a result of the hydrolysis of the fatty composition of oilseeds, the activity of lipase slightly increases already on the first day. Noticeable changes were observed in oil flax seeds, the values of lipase activity, which increased sharply from 0.15 to 0.21 mE/g. Also, the activity of lipase in the first days of observations in germinated safflower seeds, the changes ranged from 0.11 mE/g in the original seeds before germination to 0.19 mE/g germinated during the day. On the second day, a sharp increase in A values was observed in sunflower seeds, lipase activity was 0.48 mE/g, reaching the maximum value for the entire observation period. A slight increase in lipase was found in flax seeds up to 0.27 mE/g, rapeseed - 0.18 mE/g and 0.29 mE/g in germinated safflower seeds, which also corresponded to the maximum values.

On the third day, a similar picture was observed, a significant increase in lipase in sunflower seeds, and a slow increase in the content of lipase in flax and rape seeds. The fourth day of observation of germinated oilseeds showed an explosive increase in lipase activity in rape seeds, sharply increasing the values to 0.83 mE/g, which corresponded to the maximum values of lipase activity for the entire observation period. Starting from the fifth day of observations, a gradual attenuation of lipase activity in germinated oilseeds was established, which was characterized by a decrease in A.

Next, we studied the dynamics of the enzymatic process, which is characterized by changes in the quality of the objects of study, depending on the timing of germination. Based on the results of chemical analysis, diagrams were constructed describing the change in the acid number of fat from the timing of germination. The tests were carried out in accordance with GOST 31700-2012.

Figure 2 shows a diagram of changes in the fatty acid composition of germinated seeds of rapeseed variety "Gulsary".

The analysis of the presented data showed that an increase in the germination time of spring rape seeds significantly affects the change in the acid number of fat, which confirms the active course of the enzymatic process due to the breakdown of vegetable oil molecules by lipase into free fatty acids. So, for

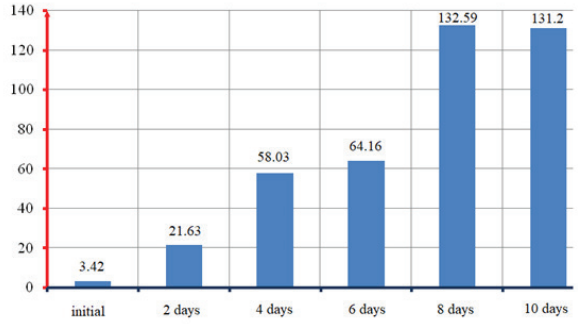

Figure 2. Acid number of fat of sprouted seeds of spring rapeseed variety "Gulsary"

example, when germinating for one day, the acid number of fat was 3.42 mg x KOH/g, a further increase in the germination time increases the indicator by 6.23 times. On the fourth and sixth days, we observe a slowdown in the fermentation process due to the accumulation of lipase activity. The eighth day of germination is characterized by an explosive increase in the acid number of fat to 132.59 mg x KOH/g. On the tenth day, with the rapid growth of rapeseed sprouts, a decrease in the values of the acid number of fat was observed to 131.2 mg x KOH/g. Further oxidation of rape seeds leads to the appearance of defects in taste and smell, which characterizes the unsuitability of the use of germinated seeds for food purposes.

Figure 3 shows a diagram of the change in the acid number of fat in sunflower seeds of the "Rauan" variety, depending on the timing of germination.

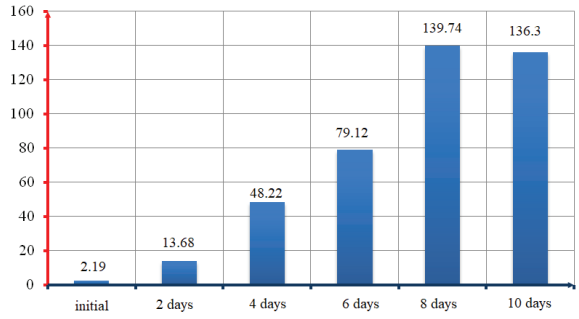

Figure 3. Acid number of fat of sprouted sunflower seeds of the "Rauan" variety

An analysis of the presented graph showed a similar situation with the oxidation of free fatty acids in sunflower seeds of the Rauan variety. An increase in the germination period leads to an increase in the acid number of fat from 2.19 mg KOH/g on the first day of germination to 139.74 mg x KOH/g on the eighth day. An increase in the germination period up to 10 days leads to a slow decrease in the values of the acid number of fat to 136.3 mg x KOH/g. At the same time, a sharp jump in values occurs between the sixth and

eighth days of germination of sunflower seeds of the Rauan variety, where the increase in values was 1.76 times.

Figure 4 shows a diagram of the change in the values of the acid number of fat during the germination of flax seeds of the oilseed variety "Kostanay".

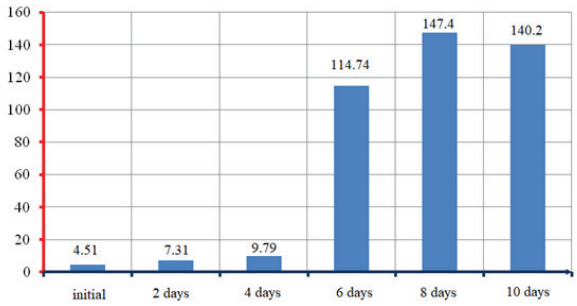

Figure 4. Acid number of fat of germinated flax seeds of the oilseed variety "Kostanay"

The presented diagram also indicates that with an increase in the germination of oil flax seeds, an increase in the values of the acid number of fat is observed. At the same time, in the first four days, we observe a slow increase in the values of the acid number of fat from 4.51 mg·KOH/g on the first day of germination to 9.79 mg·KOH/g on the fourth day of germination. On the sixth day of observations, there is a sharp increase in the values of the acid number of fat to 114.74 mg x KOH/g, which indicates the active splitting of linseed oil molecules into fatty acids. The eighth day of germination slightly increased the acid number of fat to 147.4 mg x KOH/g. After that, a decrease in the experimental values of the acid number of fat was observed.

An analysis of the presented diagram showed that, similarly to previous experiments, with an increase in the germination of safflower seeds of the Nika 80 variety, an increase in the values of the acid number of fat is observed. At the same time, the dynamics of changes in the experimental values of the acid number of fat has a uniform dynamic character without obvious sharp drops. At the same time, the minimum values of the acid number of fat fall on the first day of germination - 9.19 mg x KOH/g.

Figure 5 shows a diagram of the change in the values of the acid number of fat, depending on the timing of the germination of seeds of safflower variety "Nika 80".

The maximum values fall on the eighth day of germination, which amounted to 49.81 mg x KOH/g. As a result of the experiment, we can say with confidence that the molecules of safflower seed oil

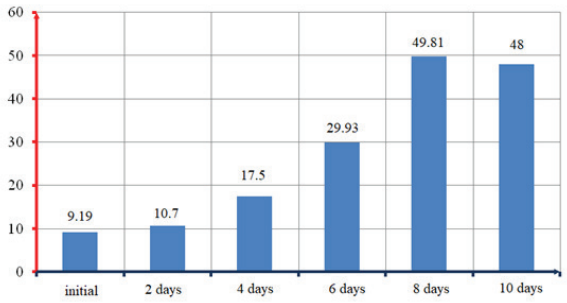

Figure 5. Acid number of fat germinated safflower seeds variety "Nika 80"

are more resistant to oxidation, which gives them high technological properties and value in the development of oxystable compositions of vegetable oils.

Acknowledgement

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4. Conclusions

- As a result of the scientific work done, the process of fermentation of oilseeds with lipase during germination was studied. At the same time, the timing of germination actively affects the quality indicator - the acid number of fat. The acid number indicates the amount of free fatty acids, which leads to the oxidation of the finished product and oxidative spoilage. An increase in acid number leads to a reduction in the shelf life of the finished product.
- The increased content of free fatty acids is explained by the fact that during the germination of grain, the lipase enzyme is activated, which breaks down the oil molecules in oilseeds into free fatty crops. The longer the duration of grain germination, the higher the activity of lipases becomes, which increases the acid number of fat.

5. References

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