Grapes are rich in easily digestible carbohydrates, mineral compounds, vitamins, phenolic compounds. and other vital components. It is known that fresh grapes can be used from September to December. To prolong the terms of consumption of this valuable raw material, the most appropriate varieties and conditions for storing grapes have been determined. White, pink, and red grape varieties were taken as the object of research. The changes in the activity of the pectinesterase enzyme were determined depending on the degree of ripening of table grape varieties, the change in the pectinesterase enzyme during storage of table grape varieties in various variants was investigated. Statistical processing and calculation of variations in the indicators of changes in the activity of the enzyme pectinesterase were performed, depending on the degree of ripening of grapes of the Shamakhi Marandi variety.

During the study, the pectinesterase enzyme remained more stable in mature varieties. This means that in ripe table grape varieties, the absorption of nutrients in the respiratory process is significantly slowed down. However, as they mature, the activity of the pectinesterase enzyme gradually increases. Therefore, for long-term storage in refrigerated chambers, fully ripe varieties of table grapes were used; to this end, grapes of the white Ganja table variety, the pink Shamakhi Marandi variety, and the red Black Asma variety are more suitable.

The comparison of the investigated variants showed that table grape varieties, when stored in a refrigerated chamber in a controlled atmosphere, at 3–4 % CO_2 and 2–3 % O_2, retain better quality than other variants. When storing table grape varieties of various variants in the refrigerator, the enzyme activity decreases but is not completely suppressed

Keywords: grape varieties, Shamakhi Marandi, enzyme pectinesterase activity, controlled gas environment

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DETERMINING THE PECTINESTERASE ENZYME ACTIVITY WHEN STORING TABLE GRAPE VARIETIES DEPENDING ON THE DEGREE OF RIPENING

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1. Introduction

Fresh grapes are a high-calorie food product with valuable, taste, dietary, and medicinal properties [1]. It is rich in simple sugars, organic acids, phenolic compounds, vitamins, macro-and microelements, and other vital components necessary for the normal development of the human body [2]. However, until now, it could be used for fresh consumption for 1.2–2.5 months. Long-term storage of grapes makes it possible to significantly increase the period of their consumption.

Simple sugars, mainly glucose and fructose, are easily absorbed by the human body. They improve people's moods. Glucose and fructose in grapes do not undergo enzymatic hydrolysis. They enter the bloodstream and are used to nourish cells, filling the body with energy [3].

Organic and aromatic acids of grapes affect the transparency of the blood, normalize blood pressure, and regulate the level of cholesterol in the body. The presence of aliphatic and aromatic organic acids in people's daily diet is very important for their health. Grapes are rich in natural organic acids [4, 5].

Grapes are rich in phenolic compounds that are important for the normal functioning of the human body. These compounds are much more in the skin than in grape juice. Phenolic compounds have high antioxidant, antimicrobial, antiviral properties. These compounds, in addition to slowing down the activity of viral microorganisms, also regulate the process of blood circulation, strengthen memory, and relieve fatigue. Phenolic compounds even reduce the activity of malignant and benign tumors [6].

Mineral compounds of grapes play an important role in the regulation of metabolic processes in the human body. Mineral compounds are used in the synthesis of proteins, enzymes, hormones, vitamins, and other biological substances necessary for the human body. Since grapes are a quality food product, it is recommended to consume them all year round [7]. Therefore, providing people with high-quality products is a relevant task of modern viticulture, the effective solution of which can be implemented on the basis of advanced technologies.

Providing the population with environmentally friendly products is the main task of each state. The issues of ensuring the safety of table grapes, improving the conditions of their storage, and reducing their losses are of great importance. In this regard, the study of the theoretical foundations and practical issues of grape storage is a relevant task.

2. Literature review and problem statement

Grapes amaze by their diversity. Grape varieties are different in terms of use, in the size of bunches and berries, the presence or absence of seeds, abound in the palette of colors of berries, and a variety of flavors. Sweet and juicy grapes are a favorite among fruit lovers around the world [8].

One effective way to store table grape varieties is to store them in a controlled gaseous environment.

Researchers from different countries conduct numerous studies to study the effect of high doses of carbon dioxide on the safety of grapes. During the research, it was established [9] that the storage conditions of grapes include low temperature, high relative humidity, and a modified composition of the regulated gas environment.

Storage of grapes in refrigeration chambers with a controlled gaseous environment (CGE) is a quite rare technique because it requires a separate refrigerator and special treatment. To extend the shelf life and reduce the loss of grapes in the refrigerator, certain conditions are created that implies up to 5 % of oxygen, up to 8 % of carbon dioxide, and up to 92 % of nitrogen [10].

In order to further improve the efficiency of production processes, carry out technical and technological modernization, the feasibility of investment in the construction of grape storage facilities with a controlled gas environment (CGE) has been analyzed. Innovation contributes to the growth of both product quality and shelf life of wine materials. The use of storage rooms with CGE could make it possible to sell grapes in the period from December to March. At ZAO "Primorskoye", the shelf life of grapes in a conventional refrigerated environment is 90 days. The introduction of a controlled gaseous environment would prolong the shelf life. The area of the planned storage facility with a controlled gas environment is 407.34 m^2 [11].

Table grapes must be harvested when they are ripe enough, have acquired their valuable taste and nutritional qualities; however, it is important to have time before the berries become dense and begin to fall. Fruits are considered ripe when they reach their varietal characteristics: size, color, aroma.

For long-term storage of table grapes, it is best to use the technology of a controlled gaseous environment that makes it possible to preserve their marketable qualities without the use of chemicals. Under this technological process of storage, it becomes possible to prolong the shelf life of grapes as there is a conservation of ripening processes. That is achieved by reducing the amount of oxygen; it is reduced from 21 % to 3–5 % while the concentration of carbon dioxide is 5 % to 8 %. Such storage in a controlled gas environment makes it possible to prolong the allowable shelf life of grapes, without loss of commercial qualities of products. Given this storage technology, a kind of cocktail of nitrogen, carbon dioxide, and oxygen is formed [12].

The long-term storage of grapes has its own characteristics. Preliminary manual sorting of grapes is carried out in the field, during harvesting. Of great importance is the packaging for transportation to the warehouse for long-term storage. This is achieved through the correct selection and use of containers. Boxes with grapes are formed into bags for subsequent loading on a vehicle. In this case, it is necessary to observe certain rules of packing.

In addition to maintaining a controlled atmosphere in the chamber, the grapes are periodically treated with sulfur dioxide SO_2 to suppress phytopathogenic microflora. The sensitivity of different grape varieties to the effects of SO_2 requires a very precise dosage, which varies according to a certain algorithm (a larger amount of SO_2 at the beginning of storage and its reduction during storage). The treatment time with sulfur dioxide is quite short (20–30 minutes); after treatment, it should be quickly removed from the chamber. To remove sulfur dioxide from the chamber, SO_2 absorbers are used [13].

The most common way to store fruits and vegetables is to store them in refrigerators. The duration of storage is determined by a number of factors, ranging from the influence of soil and climatic conditions of crop cultivation, varietal characteristics, rational use of fertilizers, agricultural techniques, irrigation, protection system against pests, diseases, and weeds, terms and methods of harvesting, commodity processing, and, of course, techniques and conditions of storage. Fruits and vegetables intended for long-term storage must be healthy and free from mechanical damage [14, 15].

Enzymatic processes occur in all living organisms, including grapes. The vital activity of living organisms is closely related to enzymes, and even photosynthesis, respiration, fermentation, and assimilation of food without their participation are not possible. All fruits and berries, as well as grapes, are formed, ripened, and break down nutrients in the presence of enzymes [16]. To this end, the storage of grape quality depends on the adjustment of enzymatic processes, including pectinesterase. This enzyme belongs to the class of hydrolases and catalyzes the breakdown of pectin into polygalacturonic acid and methyl alcohol [17].

Therefore, in order to prolong the shelf life, the dynamics of the decrease in the enzyme pectinesterase among the widespread varieties of table grapes during storage in refrigeration chambers were investigated.

Numerous studies are being conducted on the storage of grapes in refrigerating chambers with a controlled gaseous environment (CGE) [12, 13]. However, no research into the activity of the enzyme pectinesterase during the storage of table grapes is conducted.

Paper [12] reports the results of studies of grape storage involving CGE. CGE is known in science; this method is used not only in the storage of grapes but also other fruits and berries. However, the need for an enzyme system to maintain the quality of the grapes was not fully realized in [12]. The reason for this may be objective difficulties associated with the conditions of the technological regime.

It is known that during the storage of fruits and berries, the metabolic process continues. The metabolic process occurs in the presence of enzymes. However, the activity of the enzyme system in the storage of grapes, fruits, and berries (fumigation and under CGE conditions) consisting of hydrolytic enzymes, especially pectinesterase, during storage has not been studied [13, 14].

In addition, work [13] reports the results of studies of grape storage by fumigation. However, it has not been shown that the technological regime affects the inhibition of an enzyme such as pectinesterase. A variant to overcome the relevant difficulties may be to identify the activity of this enzyme in three ways.

An unsolved part of the problem is to provide the population with fresh grapes not only for one or two months but all year round. The main issue in the storage of grapes is their softening; the nutritional value of softened berries is very low. This is mainly due to the fact that the activity of the enzyme pectinesterase softens the grapes while deteriorating

their appearance and quality. One can prevent this problem by reducing the activity of the enzyme pectinesterase.

All this suggests that it is advisable to study the role of the enzyme pectinesterase in the storage of grapes depending on the degree of ripening since this enzyme is important for storage.

3. The aim and objectives of the study

The purpose of this work is to study the activity of the enzyme pectinesterase during the storage of table grape varieties of varying degrees of maturity in a refrigerator with CGE. This will make it possible to suppress or significantly reduce the activity of the enzyme, prevent softening of the grapes, and preserve their original appearance and nutritional value.

To accomplish the aim, the following tasks have been set:

– to identify the activity of the enzyme pectinesterase of table grape varieties, depending on the degree of ripening;

– to identify the activity of the enzyme pectinesterase during storage of table grape varieties for various variants (under conditions of controlled gas environment – 3–4 % CO_2, 2–3 % O_2; 1–2 % CO_2, 2–3 % O_2; by burning sulfur (fumigation) every 10 days before the end of storage);

– to carry out statistical processing and calculate variances in the indicators of change in the activity of the enzyme pectinesterase depending on the degree of maturation of the grape variety Shamakhi Marandi.

4. The study materials and methods

4. 1. Examined materials

The object of this study is the white table grape varieties widely used in Azerbaijan – Ganja table and Garaburnu, the pink grape varieties – Shamakhi Marandi, Typhi pink, as well as the red grape varieties – Black Asma and Pobeda. Table grape varieties were collected at the vineyards of the production company "Amin", operating in the village of Garayeri, Samukh region. Storage of individual table grape varieties was carried out in the refrigerating chambers at NAA Agrotara, operating near the city of Ganja.

4. 2. Research methods

The enzyme pectinesterase was investigated because, due to its activity, softening of the pulp of grapes occurs. In order to avoid softening, it is necessary to inhibit or significantly reduce the activity of this enzyme.

The enzyme pectinesterase is known to catalyze the conversion of pectin to polygalacturonic acid and methyl alcohol [6]. The issue related to the storage of grapes is the softening of the berry, the main cause of which is the formation of methyl alcohol in the berry due to the activation of pectinesterase at the expense of the methoxyl group OCH_3. The resulting methyl alcohol destroys the cellular structure of grapes, causing them to soften and degrade the quality. One can prevent this problem by reducing the activity of the enzyme pectinesterase.

The study was conducted over three years (2018–2020).

At the beginning of ripening, glucose predominates in grape berries. By the time of physiological maturity, the content of fructose and glucose is equalized. Therefore, a more accurate indicator of the onset of physiological maturity is the chemical composition of the berries.

The physiological maturity of grapes is determined by glucose-asidometric indices, that is the ratio of the value of sugar content to acidity (the value should be greater than 2.5 and less than 3.5). The ripening process in grapes is characterized by an increase in the sugar content and a decrease in the amount of acids. According to [1, 3], the ripening index of several varieties was determined: in the immature one, it was in the range of 2.2–2.4; in the mature one, it was 2.5–3.2; in the overripe one, it was 3.5–5.

The grapes were sorted and packaged at the end of September. Grape varieties were first sorted separately, cleaned of damaged berries, and then packed in special boxes with a capacity of 7–8 kg. Packed grapes are immediately placed in refrigeration chambers pre-disinfected with sulfur dioxide. Storage lasted for more than 5 months. Pre-cooling was not carried out. Grapes were placed in refrigeration chambers in the afternoon (between 16:00–17:00 hours), that is, the temperature at that time dropped.

To reduce the activity of the enzyme pectinesterase, a controlled gaseous environment was created (3–4 % CO_2; 2–3 % O_2; 1–2 % CO_2; 2–3 % O_2). The high activity of this enzyme negatively affects the quality of grapes.

The grape varieties stored in refrigeration chambers were studied in three variants:

– variant I – storing table grape varieties in refrigeration chambers under the conditions of controlled gaseous environment – 3–4 % CO_2, 2–3 % O_2;

– variant II – storing table grape varieties in refrigeration chambers under the conditions of controlled gaseous environment – 1–2 % CO_2, 2–3 % O_2;

The enterprise has a special installation; a controlled gaseous environment was created in advance. In addition, we took into consideration that CO_2 is formed during breathing. After the grapes were placed in the chamber, it was sealed hermetically.

Variant III (control) – storage in the refrigerator chamber, burning sulfur every 10 days, that is, fumigated with sulfur dioxide [18]. Directly in the chambers, 1.5 g/ m^3 of sulfur was ignited; subsequently, sulfur dioxide was formed.

In our study, the temperature in the refrigerator chamber was 0...+2 °C, and the humidity was 85–92 %. The temperature and humidity were set at the enterprise during storage. The optimal storage temperature of most grape varieties is −1 °C, +3 °C, the relative humidity of the air is 85–95 %. The activity of the enzyme pectinesterase was determined monthly for all variants and all varieties.

For table grape varieties, at the beginning and end of storage, the activity of the enzyme pectinesterase was determined by the potentiometric method for all variants [19].

Statistical treatment was carried out by standard methods [21] using the software Microsoft Excel [22].

To calculate the indicators of variance, we chose a variational type of the series, indicating the amount of initial data.

5. Results of detecting the enzyme pectinesterase during the storage of table grape varieties depending on the degree of ripening

5. 1. Detecting the activity of the enzyme pectinesterase of table grape varieties depending on the degree of ripening

The change in the enzyme pectinesterase activity (EPA) was determined depending on the degree of ripening of table grape varieties.

Our study considers the storage lasting a little more than 150 days (more than 5 months) but one can even prolong the shelf life to 180–210 days.

The dynamics of changes in the enzyme pectinesterase depending on the degree of maturation of table grape varieties are given in Table 1.

Table 1

Change in the activity of the enzyme pectinesterase depending on the degree of ripening of table grape varieties (per relative unit of 1 mg of acetone), (μmol/sec)

Grape variety	Not ripe			Ripe			Overripe		
	Day								
	1	5	10	1	5	10	1	5	10
Ganja, table variety	20.6	21.4	23.4	23.6	24.0	26.4	28.7	32.4	38.8
Garaburnu	18.3	20.2	21.9	22.1	22.4	23.5	27.6	30.2	37.9
Shamakhi Marandi	8.3	10.1	11.8	12.2	14.5	12.8	15.8	16.2	16.8
Typhy pink	9.4	10.2	12.4	15.6	15.7	15.8	22.6	28.9	39.2
Black Asma	10.5	12.8	15.4	20.1	21.3	22.4	30.2	34.3	36.6
Pobeda	16.2	20.7	22.1	25.6	27.3	30.6	38.9	44.2	52.4
HCP_{05}	4.7	4.96	4.79	4.63	4.49	6.04	5.01	8.23	10.44

Table 1 gives the results regarding the studied samples on day 1, day 5, day 10. For the ripened grape varieties, the activity of the enzyme was almost stable. The values show that the activity of the enzyme increases as the grapes ripen.

The significance of the differences between the activity values of the enzyme pectinesterase according to HCP for all grape varieties by variants was determined. For the second variant, at 5 days, HCP accepts the minimum value, 4.49. Therefore, the second variant (ripe) is the most acceptable. Thus, the ripened grapes were taken for storage.

5. 2. Detecting the activity of the enzyme pectinesterase when storing table grape varieties in various variants

The activity of the enzyme pectinesterase when storing table grape varieties in various variants (under the conditions of CGE and fumigation every 10 days) was investigated.

The dynamics of reducing the enzyme pectinesterase when storing table grape varieties in various variants are given in Table 2.

During the study, it was found that the activity of the enzyme pectinesterase gradually decreases under conditions of a controlled gas environment compared to the control.

In the study, SO_2 not only slows down the activity of microorganisms (the control variant) but also significantly reduces the activity of the enzyme pectinesterase. In addition, the grape varieties studied by us do not react equally to the types of CGE, as well as to the degree of ripening.

Our study determined the significance of the differences between the activity values of the enzyme pectinesterase in terms of HCP for all variants. The confidence intervals of pectinesterase activity in variants I, II, and control overlap and have identical regions, therefore, under CGE conditions, 3–4 % CO_2, 2–3 % O_2, the actual difference between variants is greater than HCP, therefore, the differences between the variants are significant.

In addition, conducting a study, the value was found monthly; we calculated the mean arithmetic (in November, December, January, February, March). Thus, for Shamakhi Marandi, in the control variant, it was 79.1 %; for variant I, the value increased by 95.4 %, while the activity of the enzyme pectinesterase significantly decreased, almost inhibited, than that in variant II. Similarly, this was also observed for other grape varieties.

Table 2

Dynamics of decreasing activity of the enzyme pectinesterase when storing table grape varieties, (%)

No.	Grape variety	CGE conditions		Burning sulfur every 10 days (control)
		3–4 % CO_2 2–3 % O_2	1–2 % CO_2 2–3 % O_2	
1	Ganja, table variety	78.4	70.4	61.7
2	Garaburnu	74.9	67.0	54.3
3	Shamakhi Marandi	95.4	92.1	79.1
4	Typhy pink	59.1	54.8	48.4
5	Black Asma	85.6	81.3	73.9
6	Pobeda	59.7	49.8	46.2
	HCP	13.1	14.5	12.4

5. 3. Statistical treatment and calculation of variance in the activity of the enzyme pectinesterase

Statistical treatment was carried out; variances in the indicators of changes in the activity of the enzyme pectinesterase were calculated depending on the degree of maturation, using an example of the table grape variety Shamakhi Marandi.

Table 3 gives the calculation of variance in the activity of the enzyme pectinesterase depending on the degree of maturation of the table grape variety Shamakhi Marandi.

To assess the indicators of change depending on the degree of ripening of the grapes of Shamakhi Marandi variety, indicators that are the center of distribution were found. The calculation of the indicators of variance is based on the procedure from [21]. We have calculated the following:

– mode, median;

– absolute indices of variance (weighted average, variance, corrected variance, standard deviation, range of variance;

– quartiles;

– relative indices of variance (linear coefficient of variance, coefficient of variance, oscillation coefficient);

– indicators of the shape of the distribution (central moment of the third order, moment coefficient of asymmetry, mean square error of the asymmetry coefficient, the central moment of the fourth order, structural coefficient of asymmetry, coefficient of excess, mean square error of the coefficient of excess);

– confidence interval for the general share (average error of the EPA share, the lower limit of the share of indicators, the upper limit of the share of indicators);

The mode (*Mo*) of indicators of changes in the activity of the enzyme pectinesterase (EPA), which is most common among the series of data, was found. The maximum value at x=5 days (f=14.5). Therefore, in this case, the mode is 5, that is, this is the level around which the indicators of the change in the activity of the enzyme pectinesterase are concentrated. This means that the distribution of these indicators of changes in EPA is symmetrical from the center.

Table 3

Calculation of variance in the activity of the enzyme pectinesterase depending on the degree of maturation of the grape variety Shamakhi Marandi

x_i	EPA indicator, f_i	$x_i f_i$	Weighted average $\bar{x} = \frac{\sum x_i \cdot f_i}{\sum f_i}$	The frequency of EPA indicator occurrence, S	$\lvert x - \bar{x} \rvert \cdot f_i$	$(x_i - \bar{x})^2 \cdot f_i$	The relative frequency of the occurrence EPA of indicator, f_i/f
1	12.2	12.2	5,385	12.2	53.495	234.564	0.309
5	14.5	72.5		26.7	5.58	2.147	0.367
10	12.8	128		39.5	59.074	272.64	0.324
Σ	39.5	212.7		78.4	118.149	509.351	1

To assess the intensity of variance, as well as to compare it with data series in different sets of indicators of change in EPA, we determine the relative indicators of variance.

Table 4 shows that the relative spread of the values of the aggregate of indicators of change in EPA is 66.69 %.

The median (*Me*) of the EPA change indicators was found, which falls in the middle of the ranked data series. Let us find x_i, at which the occurrence S of the EPA index would be greater than $\Sigma f/2=20$. The value of x_i would equal 5. Based on this, the median is also equal to 5, that is, this is the level around which the indicators of changes in the activity of the enzyme pectinesterase are concentrated. This means that the distribution of these indicators of changes in EPA is symmetrical from the center.

With a symmetrical distribution of the data series, the values of the mode and the median of the indicators of change in EPA coincide with the average value $\bar{x}$, that is, $\bar{x} = Mo = Me$. With a moderately asymmetric distribution of the data series, the values of the mode and median indicators of changes in EPA follow the ratio:

$$3 \cdot (\bar{x} - Me) \approx \bar{x} - Mo.$$

We find the coefficients of variance and the shapes of distribution (Table 4).

The value of the average linear deviation in Table 4 shows that each of the values of a number of indicators of EPA change differs from each other by an average of 2.991.

The value of the standard deviation in Table 4 shows that each value of a number of measures of EPA change differs from the average of 5.385 (Table 3) by an average of 3.591. And the RMS estimate is 3.637.

Table 4

Calculation of the coefficients of variance and the shape of the distribution of values of EPA change indicators

Calculation of absolute indicators of variation		
The scope of the variation	$R = x_{max} - x_{min} = 10 - 1 = 9$	
Average linear deviation	$d = \frac{\sum \lvert x_i - \bar{x} \rvert \cdot f_i}{\sum f_i} = \frac{118.149}{39.5} = 2.991$	
variance	$D = \frac{\sum (x_i - \bar{x})^2 \cdot f_i}{\sum f_i} = \frac{509.351}{39.5} = 12.895$	
Corrected variance	$S^2 = \frac{\sum (x_i - \bar{x})^2 \cdot f_i}{\sum f_i - 1} = \frac{509.351}{38.5} = 13.23$	
Root mean square deviation	$\sigma = \sqrt{D} = \sqrt{12.895} = 3.591$	
Estimation of the standard deviation	$s = \sqrt{S^2} = \sqrt{13.23} = 3.637$	
Calculation of the relative coefficients of variation		
The coefficient of variation	$\upsilon = \frac{\sigma}{\bar{x}} = \frac{3.591}{5.385} \cdot 100\,\% = 66.69\,\%$	
Linear coefficient of variation	$K_d = \frac{d}{\bar{x}} = \frac{2.991}{5.385} \cdot 100\,\% = 55.55\,\%$	
Oscillation coefficient	$K_T = \frac{R}{\bar{x}} = \frac{9}{5.385} \cdot 100\,\% = 167.14\,\%$	
Calculation of central moments		
x_i	$(x - \bar{x})^3 \cdot f_i$	$(x - \bar{x})^4 \cdot f_i$
1	–1,028.65	4,510.641
5	–0.82747	0.319
10	1,258.129	5,806.264
∑	228.65	10,317.224
Calculation of distribution shape		
The central moment of the third order	$M_3 = \frac{\sum (x_i - \bar{x})^3 \cdot f_i}{\sum f_i} = \frac{228.65}{39.5} = 5.8.$	
Moment asymmetry coefficient	$A_S = \frac{M_3}{\sigma^3} = \frac{5,8}{3.591^3} = 0.125$	
The root-mean-square error of the skewness coefficient	$S_{A_S} = \sqrt{\frac{6 \cdot (n-2)}{(n+1) \cdot (n+3)}} = \sqrt{\frac{6 \cdot (3-2)}{(3+1) \cdot (3+3)}} = 0.5$	
The central moment of the fourth order	$M_4 = \frac{\sum (x_i - \bar{x})^4 \cdot f_i}{\sum f_i} = \frac{10,317.224}{39.5} = 261.2$	
Structural asymmetry coefficient	$A_{SP} = \frac{\bar{x} - M_0}{\sigma} = \frac{5.385 - 5}{3.591} = 0.11$	
Kurtosis coefficient	$E_x = \frac{M_4}{\sigma^4} - 3 = \frac{261.2}{3.591^4} - 3 = 1.5708 - 3 = -1.43$	
The root-mean-square error of the kurtosis coefficient	$S_{E_X} = \sqrt{\frac{24n \cdot (n-2) \cdot (n-3)}{(n+1)^2 \cdot (n+3) \cdot (n+5)}} = \sqrt{\frac{24 \cdot 3 \cdot (3-2) \cdot (3-3)}{(3+1)^2 \cdot (3+3) \cdot (3+5)}} = 0$	

The calculation of the asymmetry indices only in the central part of the distribution reveals that the average quadratic error of the excess coefficient is less than 3, that is $E_x / S_{E_x} = -1,43 / 0 = 0 < 3,$ then the deviation from the normal distribution of EPA indicators would not be significant (Table 4).

To conduct an interval assessment of the center of the general population of indicators of changes in the activity of the enzyme pectinesterase, it is necessary to determine the confidence interval for the general share.

In this case, $2\Phi(t_{kp})=\gamma$. Then

$$\Phi(t_{kp}) = \gamma / 2 = 0.954 / 2 = 0.477.$$

Using the Laplace function, we find at what t_{kp} the value is $\Phi(t_{kp})=0.477$. Consequently, $t_{kp}(\gamma) = (0.477) = 2$.

The EPA indices shares are located within the given intervals with a probability of 0.954 [22].

For the general average value of the changes in the activity of the enzyme pectinesterase, the confidence interval is

$$\left(\overline{x} - t_{kp} \cdot \frac{s}{\sqrt{n}}; \overline{x} + t_{kp} \cdot \frac{s}{\sqrt{n}}\right).$$

The t_{kp} value is determined from the Student distribution table. According to this table, we find:

$$T_{table}\left(n-1;\alpha/2\right) = T_{table}\left(38.5;0.025\right) = 2.329.$$

The standard error of the obtained values of the activity index of the enzyme pectinesterase (EPA) for the average is calculated from the following formula:

$$s_C = \frac{s}{\sqrt{n}} = \frac{3.637}{\sqrt{39.5}} = 0.5787.$$

Knowing the standard error of the average, we determine how much the average activity of the enzyme pectinesterase of 5.385 differs from the average general population.

Define the marginal sampling error:

$$\varepsilon = t_{kp} \cdot \frac{s}{\sqrt{n}} = 2.329 \cdot \frac{3.637}{\sqrt{39.5}} = 1.348.$$

The confidence interval is

(5,385–1,348; 5,385+1,348)=(4,037; 6,733).

With a probability of 0.95, it is stated that the average value of EPA indicators for a larger sample would be located in the found interval.

6. Discussion of results of determining the enzyme pectinesterase when storing different varieties of table grapes of different degrees of ripening

Table 1 shows that the activity of the enzyme pectinesterase in the non-ripened variety of Ganja table grapes is 20.6...23.4 μmol/s, in the mature one – this indicator is 23.6...26.4 μmol/s, and in an overripe one – 28.7...38 μm/s. The lowest degree of activity of this enzyme is observed in Shamakhi Marandi. The activity of the enzyme pectinesterase in the immature grapes of Shamakhi Marandi was 8.3...11.8 μmol/s; in the ripened – 12.2...12.8 μmol/s, and in the overripe – 15.8...16.8 μmol/s.

In addition, judging by the values of the confidence intervals of pectinesterase activity and the average values of HCP (respectively, 4.82; 5.05; 7.89) for all variants, the most acceptable for all grape varieties is variant II (ripe) on day 5.

In addition, the confidence intervals of the activity of pectinesterase for variants I (unripe) and III (overripe) (for Shamakhi Marandi) do not overlap and do not have identical regions, therefore, in overripe grape fruits, the activity of pectinesterase is great, which is unacceptable. When comparing variants I and III, the HCP is 2.923042. The actual difference between variants I and III is 6.2, which is greater than HCP, that is, 6.2>2.923042. Thus, the differences between the variants are significant.

The confidence intervals of the activity of pectinesterase for variants II and III (in Shamakhi Marandi) overlap and have the smallest overlapping area. When comparing variants II and III, the HCP is 2.078297. The actual difference between variants I and III is 3.1, which is greater than HCP, that is, 3.1>2.078297. Thus, the differences between the variants are significant.

The confidence intervals of the activity of pectinesterase for variants I and II (for Shamakhi Marandi) overlap and have the largest overlapping area. When comparing variants I and II, the HCP is 3.399743. The actual difference between variants I and III is 3.1, which is less than HCP, that is 3.1<3.399743. Thus, the differences between the variants are not significant. Therefore, among the compared variants, the most successful is variant II, with a larger overlapping area of the confidence intervals and a smaller HCP for variants I and II, the enzyme activity in variant II, that is, in the ripened grape fruits it remains almost stable compared to variants I and III.

Our study showed that of all the examined varieties, the enzyme pectinesterase remained more stable in the mature variety. This means that in ripe table grape varieties, the absorption of nutrients in the respiratory process is significantly slowed down. However, as it is re-matured, the activity of the enzyme pectinesterase gradually increases, and this leads to softening of the pulp. Therefore, for long-term storage in refrigeration chambers, fully ripened varieties of table grapes were used. Often, at enterprises, during storage, the degree of ripeness of grapes is not taken into consideration. Therefore, paying attention to the degree of ripeness of the grapes, we used fully ripe ones and received good results.

The values in Table 2 show that when storing table grape varieties in various variants in the refrigeration chamber, the activity of enzymes is significantly reduced but is not completely suppressed. The literature data (6) and our research have demonstrated that conditions must be created in the refrigeration chamber that regulate the processes of assimilation and dissimilation to significantly reduce or suppress the activity of enzymes, including pectinesterase.

In addition, Table 2 shows that the activity of the enzyme pectinesterase in the Shamakhi Marandi grape variety decreased between 92.1...95.4 % during the storage period in a controlled gas environment; this figure decreased by 79.1 % during storage due to the burning of sulfur (fumigation) every 10 days. Table 2 shows that

when stored in a controlled gas environment at 3–4 % CO_2 and 2–3 % O_2, the activity of the enzyme pectinesterase is significantly reduced in all varieties of table grapes, compared to other variants. In the first variant, when storing the grape variety Ganja table grapes, the activity of the enzyme pectinesterase decreased by 78.4 %; this figure for the second one was 70.4 %, for the third one – 61.7 %, and, for Karaburnu, 74.9, 67.0, 54.3 %, respectively. The comparison of white table grape varieties showed that the activity of the enzyme pectinesterase when soring Ganja table grapes, significantly reduced compared to the Garaburnu grape variety.

Our studies of changes in the activity of the enzyme pectinesterase in various grape varieties have shown that during storage in three variants, the activity of the enzyme pectinesterase in the pink grape variety Shamakhi Marandi significantly decreased compared to the grape variety Typhy pink. During storage, the activity of the enzyme pectinesterase in the grape variety Typhy pink decreased from 59.1...48.4 %; in Shamakhi Marandi, this figure was 95.4...79.1 %. The comparison of the red grape varieties showed that during storage, the activity of the enzyme pectinesterase decreased most in the Black Asma variety and amounted to 85.6...73.9 %, and in the Pobeda variety – 59.7...46.2 %. There is a difference between the studied variants: the higher the values, the lower the activity of the enzyme. Of all the varieties (white, pink, and red), the lowest activity of the enzyme pectinesterase was observed in Shamakhi Marandi, which was stored under the conditions of CGE (3–4 % CO_2 and 2–3 % O_2).

It is established (Table 2) that for long-term (more than 5 months) and high-quality storage in the refrigerator chamber, more suitable are: from white grape varieties, Ganja table variety; from pink varieties – Shamakhi Marandi; from red varieties – Black Asma. In the grape varieties Garaburnu, Typhy pink, Pobeda, due to the high activity of the enzyme pectinesterase, the quality was poor, that is, the fruits were softened.

The main disadvantage of storing grapes is that softening the grapes degrades their quality. From the source [6] it is known that the reason for this is associated with the breakdown of methoxylated polygalacturonic acid due to the increased activity of pectinesterase, a representative of pectin enzymes. This is due to the fact that due to the increased activity of the enzyme pectinesterase, it is formed after the breakdown of pectin of the methoxyl group. The resulting methyl alcohol destroys the cells of the grapes, as a result, the grapes become soft, and lose most of their natural color.

The confidence intervals of the activity of pectinesterase in variants I, II, and control overlap and have a common area, therefore, under the CGE conditions of 3–4 % CO_2, 2–3 % O_2, the actual difference between variants is greater than HCP, which means that the differences between variants are significant.

The comparison of the studied variants showed those table grape varieties when stored in a refrigerator chamber under a controlled gaseous environment, for variant I (3–4 % CO_2 and 2–3 % O_2), retain better qualities, that is, the pulp did not soften compared with the control because the enzyme pectinesterase was significantly retailed. As a result, the grapes retained their original state and naturalness compared to the control variant.

During the statistical processing, the results were given.

To assess the indicators of change depending on the degree of ripening of the grapes of the Shamakhi Marandi variety, indicators were found that are the center of the distribution.

The mode (*Mo*) of the indicators of changes in the activity of the enzyme pectinesterase (EPA), which is most common among the data series, was found. The mode is equal to 5, that is, this is the level around which the indicators of change in the activity of the enzyme pectinesterase are concentrated. This means that the distribution of these indicators of changes in EPA is symmetrical from the center. The median (*Me*) of the EPA change indicators was found, which falls in the middle of the ranked data series. The median is also 5, that is, this is the level around which the indicators of changes in the activity of the enzyme pectinesterase are concentrated. This means that the distribution of these indicators of changes in EPA is symmetrical from the center. With a symmetrical distribution of the data series, the values of the mode and the median of the indicators of change in EPA coincide with the average value $\bar{x}$, that is, $\bar{x} = Mo = Me$. With a moderately asymmetric distribution of the data series, the values of the mode and the median of the indicators of EPA change follow the ratio: $3 \cdot (\bar{x} - Me) \approx \bar{x} - Mo$. The values of the indicators of change in EPA in the ranked series of data distribution are represented by quartiles.

The value of the average linear deviation in Table 4 shows that each of the values from the series of indicators of EPA change differs from each other by an average of 2.991. The value of the standard deviation in Table 4 shows that each value of the series of indicators of EPA change differs from the average of 5.385 by an average of 3.591. And the RMS estimate is 3.637.

The coefficient of variance makes it possible to find out what proportion of the average value of this value is its average spread. Since the coefficient of variance is greater than 30 % but less than 70 %, the variance is considered moderate.

Table 4 shows that the linear coefficient of variance is 55.55 %, which is used to characterize the fraction of the average value of the EPA absolute deviation from the average.

The oscillation coefficient *KT*, used to reflect the relative variability of the extreme values of the EPA change indicators around the average, is 167.14 % (Table 4).

To analyze the shape of the distribution, the asymmetry coefficient is calculated. Table 4 shows that the ratio of the moment coefficient of asymmetry A_s to the root mean square error of the asymmetry coefficient SA_s is less than 3, that is, the condition $|A_s|/SA_s=0.125/0.5=0.25<3$ is met, so the asymmetry can be considered insignificant. Therefore, the indicators of change in EPA are distributed symmetrically in the general population.

To analyze the asymmetry only in the central part of the distribution, the indicators were calculated (Table 4).

With the help of the Laplace function, it was found at what t_{kp}, the value is $\Phi(t_{kp})=0.477$. Therefore $t_{kp}(\gamma) = (0.477) = 2$.

Knowing the standard error of the average, it is determined by how much the average activity of the enzyme pectinesterase of 5.385 differs from the average general population. A marginal sampling error has been defined. The confidence interval was (5,385–1,348; 5,385+1,348)=(4,037, 6,733).

With a probability of 0.95, it is stated that the average value of EPA indicators for a larger sample would be located in the found interval.

Statistical treatment was performed to establish the probability of the confidence interval, the average value of the

changes in the activity of the enzyme pectinesterase. Since the weighted average value of the changes in the activity of the pectinesterase enzyme is 5,385, with a probability of 95 %, it is stated that the average value of the changes in the activity of the pectinesterase enzyme with a larger set of values of these indicators would be located in the range from 4,037 to 6,733.

Statistical processing was carried out to find HCP data for all grape varieties; variants I (unripe), II (ripe), and III (over-ripened). Judging by the values of the confidence intervals of the pectinesterase activity and the average values of HCP for all variants, the most acceptable for all grape varieties is variant II (ripened) on day 5, the minimum value is 4.49.

In addition, when finding HCP for variants I (CGE 3–4 % CO_2, 2–3 % O_2), II (CGE 1–2 % CO_2, 2–3 % O_2), and III (control) for all grape varieties, the confidence intervals of pectinesterase activity for variants I (CGE 3–4 % CO_2, 2–3 % O_2), II (CGE 1–2 % CO_2, 2–3 % O_2), and control overlap and have identical regions. Therefore, under the conditions of CGE of 3–4 % CO_2, 2–3 % O_2, the actual difference between the variants is greater than HCP, which means that the differences between the variants are significant.

These results are used to determine the degree of accuracy of enzyme activity when storing under CGE conditions. For variant I (3–4 % CO_2 and 2–3 % O_2), the values reveal that the activity of the enzyme has decreased significantly.

7. Conclusions

1. Our studies have shown that pectin enzymes, especially pectinesterase, are constantly active during the ripening period of grapes. Pectin enzymes remain stable for a long time throughout the entire period of ripening of grapes. However, as they mature, the activity of the enzyme pectinesterase gradually increases. Therefore, for long-term storage in the refrigerators, fully ripened varieties of table grapes are used.

2. Our studies have demonstrated that for long-term and high-quality storage in the refrigerator chamber in three variants, more suitable are: among white grape varieties – Ganja table variety; among pink varieties – Shamakhi Marandi; and from red varieties – Black Asma.

The comparison of these variants has revealed that for variant I (3–4 % CO_2 and 2–3 % O_2), they retain better qualities compared to other variants because the enzyme pectinesterase was significantly retained, almost inhibited.

3. Variances in the activity of the enzyme pectinesterase were calculated depending on the degree of maturation, using an example of the table grape variety Shamakhi Marandi. The standard error of the obtained values for the activity index of the enzyme pectinesterase (EPA) for the average is calculated; it is equal to 0.5787. The standard average error shows how much the average of the obtained values of the activity index of the enzyme pectinesterase (EPA) differs from the average general population of indicators of EPA change and confirms the reliability of the values obtained during our experimental studies.

Under CGE conditions of 3–4 % CO_2, 2–3 % O_2, the actual difference between variants is greater than HCP, which means that the differences between the variants are significant. These results are used to determine the degree of accuracy of enzyme activity when storing grapes under CGE conditions.

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