DYNAMICS OF PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES IN AVOCADO FRUIT TREATED WITH PREPARATIONS DURING STORAGE

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Abstract

This article presents data on the effect of processing avocado fruit on the dynamics of physiological and biochemical processes during low-temperature storage. The aim was to explore the effect of processing Fuerte avocado fruit with 'Agrokhit', 'KHAN-8' and 'Extrasol-90' preparations on the dynamics of physiological and biochemical processes during storage. The effectiveness of the preparations on the change in physiological and biochemical processes during storage of avocado fruit was evaluated by the release of carbon dioxide during their respiration, by the activity of terminal oxidases and the hydrolysis of pectin substances. The respiration rate, the activity of terminal oxidases – catalase, peroxidase, phenol oxidase, tyrosinase – and the kinetics of the hydrolysis of protopectin and pectin depended on the type of preparation and the duration of storage. Processing avocado fruit with the examined preparations did not violate metabolism, which was regulated by change in the activity of terminal oxidases of the plant cells, a reduced respiration rate and the rate of hydrolysis of protopectin and pectin. As a result, there was an increase in the storage period and fruit ripening. To slow down the physiological and biochemical processes – to increase the duration of ripening and storage of avocado fruit – it is recommended that they be treated with chitin derivatives Agrokhit and KHAN-8.

Keywords: *avocado fruit, chitosan, storage, respiration rate, terminal oxidases, pectin substances*

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1. Introduction

Avocados occupy a prominent place among a wide variety of tropical and subtropical fruit. Avocado fruit are a valuable food in the human diet because they contain 1–4% protein, 2–10% carbohydrates, 13–35% fat, 65–80% water, vitamins – A, B1, B2, C, PP, D, E, K – and minerals – iron, calcium, phosphorus [1].

The main causes of deterioration in the quality of avocado fruit during storage are infectious diseases and a decrease in nutrient and biologically active substances as a result of physiological and biochemical processes in plant cells with the participation of various hydrolases, oxidoreductases, pyridine and flavin dehydrogenases [2]. To reduce losses caused by phytopathogens, to preserve the nutritional and biological value of plant products as much as possible and to increase the storage duration, various modified and regulated gaseous media and physicochemical and biological agents are used [3–5]. In recent years, metabolites of bacterial antagonists and chitin derivatives have been used in technologies for growing and storing food raw materials and food products [5, 7].

An analysis of the results of studies that have examined the use of chitin and its derivatives revealed that these products are most widely used in various fields of human activity. It is believed that in the future, due to their unique properties, these compounds will displace synthetic analogs and will be regarded as a 'polymer of the 21st century' [8]. In agriculture, chitin and its derivatives are used to treat plants as a regulator of various physiological reactions, as an additive to fertilisers, which is based on their biological activity, manifested in the ability to induce at the molecular level disease resistance of living plant cells to phytopathogens and adverse environmental factors [7, 9].

The paramount importance in the regulatory mechanisms of the biological oxidation of plant cell metabolites is the respiration rate, which is a combination of redox reactions that take place with the participation of many enzymes and determines the biochemical adaptation of the plant organism to adverse external factors and phytopathogens. Many researchers have attempted to link the resistance of fruit and vegetables to infectious diseases with the level of respiration of their tissues. It had been assumed that fruit with a low level of respiratory gas exchange should have higher stability, because with increased respiration, the consumption of nutrients increases, and this should lead to faster depletion of plant tissues [2, 10–12].

After fruit ripens, storage conditions that favour the maximum possible preservation of quality, biologically active substances and protection against phytopathogens must maintain respiratory gas exchange in tissues at a certain level, eliminating any disturbances in the coordinated work of individual links in the entire biological oxidation chain, which is regulated by enzyme activity. The preservation of the structure of plant tissue is also important; it depends on the conversion of pectin substances and the activity of pectolytic enzymes [13].

When adapting to low storage temperatures for avocado fruit treated with various preparations, it is necessary to consider alternative mechanisms of oxidation of respiration substrates with the participation of terminal oxidases that contain iron and copper ions with variable valence. The activity of terminal oxidases changes under the influence of external factors. This indicator can serve as one of the criteria for the effectiveness of fruit processing with preparations based on bacterial metabolites - antagonists and chitosan [4, 12, 14, 15].

The aim of this study was to explore the effect of treating avocado fruit with 'Agrokhit', 'KHAN-8', and 'Extrasol-90' preparations on the physiological and biochemical processes that occur during storage at 4 ±1°C.

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2. Materials and Methods

The object of the study was the Fuerte avocado fruit, which is a Guatemalan–Mexican hybrid climacteric fruit. For research, we used Fuerte avocado fruit weighing 320 ± 10 g, grown in South Africa and harvested in October at a technical degree of maturity (mature green). The fruit were delivered to St. Petersburg by sea refrigerated transport (5–6°C for 10–12 days). The studied avocados met the requirements of the UN/ECE FFV-42 standard for avocado fruit coming into international trade.

To increase the duration of storage, additional cold agents are used, in particular, bacterial antagonists and their metabolites and chitin derivatives. The preparation Agrokhit, developed at the Bioengineering Center of the Russian Academy of Sciences, includes the active substance chitosan lactate in the form of a 4% solution of a low-molecular-weight chitosan (10 kDa) in 1% lactic acid. Chitosan for the preparation Agrokhit is obtained from the chitin of Far Eastern crabs of domestic production. KHAN-8 is a light yellow powder made on the basis of chitosan with a molecular weight of 15 kDa and a degree of deacetylation of 68%. The basis for Extrasol-90 are rhizospheric, nitrogen-fixing bacterial antagonists and their metabolites. The preparation was developed at the All-Russian Research Institute for Agricultural Microbiology and is a mixture of pure cultures of producing bacteria, namely *Arthrobacter mysorens* 7, *Flavobacterium* sp. L-30, *Agrobacterium radiobacter* 10, *A. radiobacter* 204, *Azomonas agilis* 12, *Bacillus subtilis* H-13, *Pseudomonas fluorescens* 2137, and *Azospirillum lipoferum* 137.

The preparations we used in the form of solutions prepared as follows. Agrokhit was used for spraying without prior pre-treatment. One millilitre of KHAN-8 was dissolved in 99 mL of a 2% solution of acetic acid with vigorous stirring. Forty millilitres of Extrasol-90 was dissolved in 1 L of water with vigorous stirring. Ready-made solutions for spraying the fruit were used immediately after preparation. Upon admission to storage, the avocado fruit were sprayed with the appropriate solution. The fruit not treated with preparations served as control samples (control).

Test (control) and experimental (treated) samples (320 ± 10 g) were packed in a row in 4 kg polymer containers and stored at 4 ± 1°C and relative humidity of 90–95% for 45 days. During storage, the respiration rate was determined by the release of carbon dioxide, pectin substances were evaluated by the carbosol method and the activity of terminal oxidases – catalase, peroxidase, phenol oxidase and tyrosinase – were recorded using previously described methods [16]. The rate constants (pseudo first order) of the hydrolysis of protopectin (K_{pr}) and pectin (K_p) were calculated.

The data were processed using Microsoft Excel to determine the 95% confidence intervals.

3. Results and Discussion

Fig. 1 shows the dependence of the changes in the respiration rate of the Fuerte avocado fruit treated with the examined preparations on the duration of storage. The respiration rate depended on the type of preparation and the duration of the storage. Thus, the KHAN-8 treatment significantly reduced the respiration rate compared with the control sample. The respiration rate of avocado fruit immediately increased after treatment with Extrasol-90 and Agrokhit, and with subsequent storage it decreased compared with the control sample.

Avocado are climacteric fruit, which is characterized by an increase in respiration while ripening in storage. Some authors believe that the climacteric rise in respiration means the culmination of the maturation processes, followed by overripening. The rise in respiration is only an external manifestation of a qualitative restructuring of the

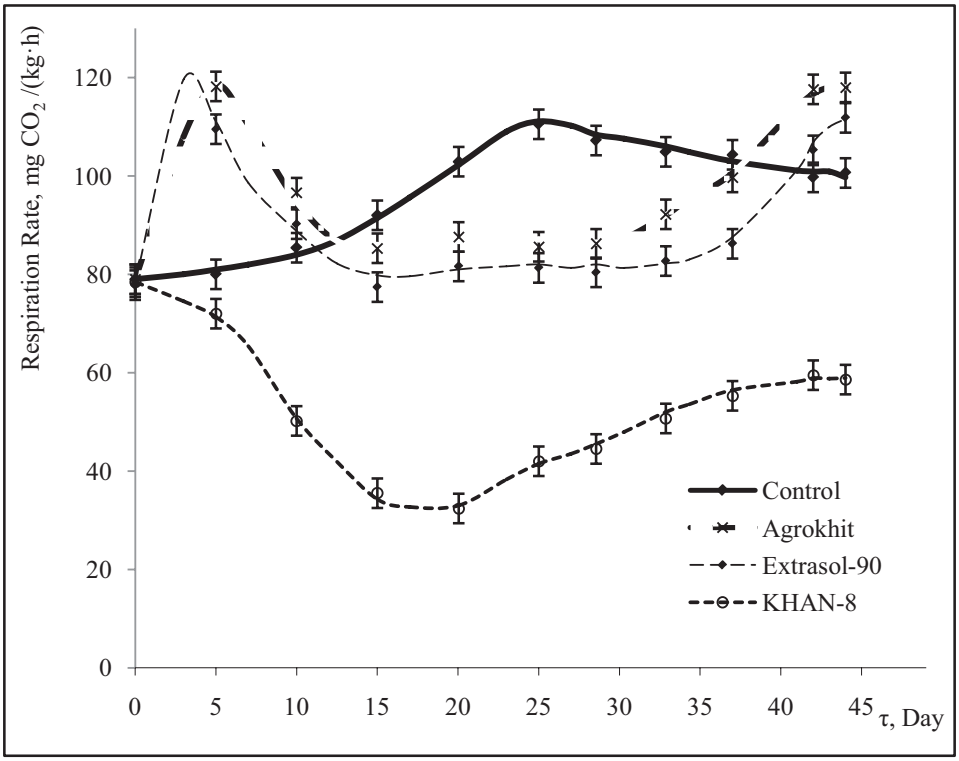

Figure 1. Changes in the respiration rate during storage of the avocado fruit.

biochemical processes occurring in the fruit. As can be seen from Fig. 1, the ripening process of control avocado fruit occurred after 20–25 days of storage, and then the intensity of respiration decreased. At the same time, due to dissociation of oxidative phosphorylation and deprivation of the necessary energy of the cell, there was a change in the permeability of cell membranes, and a violation of the structure of the cell's organelles are possible, which leads to the overripening of avocado fruit [13, 14].

A decrease in the respiration rate after KHAN-8 treatment is associated with a slowdown in gas exchange with the environment due to the formation of a polysaccharide film on the skin of the fruit. The increase in respiration rate immediately after processing the fruit with Agrokhit and Extrasol-90 can be explained by the release of additional energy by plant cells; this response is a necessary adaptation to the effects of the metabolic products of bacterial antagonists. This is also associated with a change in the activity of redox enzymes.

The minimum respiration rate during ripening is characteristic of treated avocado fruit, which can be explained by the suppression of accumulated acids (oxaloacetic, citric, isolimonic and α-ketoglutaric) and decarboxylating activity of succinate dehydrogenase (malic-enzyme), the main enzyme that characterizes climacteric respiration rise [13]. At the end of the storage period, there was an increase in the respiration rate of mature fruit. This phenomenon is obviously due to an increase in the content of malic acid, which is a substrate of respiration.

It should be noted that the treatment of avocado fruit before putting them into storage does not disturb the biological oxidation chain and at the same time slightly reduces the

respiration rate during ripening in storage. Changes in the respiration rate depend on the activity of the enzymes dehydrogenases and oxidases [17].

During the fruit ripening, various enzymes are activated, which leads to the formation of peroxides. Peroxidation products are highly toxic: they accelerate the polymerization of proteins, destroy sulfhydryl groups of enzymes – thereby inactivating them – change the permeability of biological membranes due to the modification of the lipid layer, etc. To inactivate the toxic effects of hydrogen peroxides, cells functionalize catalases that break down hydrogen peroxide and peroxidases that break down organic peroxides [15, 18]. Studies have shown that chitosan produces reactive oxygen species in plants and, above all, treatment of plants with chitosan causes the accumulation of hydrogen peroxide in them. Given that catalase is involved in the breakdown of hydrogen peroxide, the activity of this enzyme can be used to judge its accumulation in the plant cell in response to treatment [14, 15, 19].

Fig. 2 shows the dependence of the changes in catalase activity in Fuerte avocado fruit on the storage duration. The avocado fruit treated with KHAN-8 during storage and ripening had lower catalase activity than the control fruit (Fig. 2). This can be explained by an increase in the concentration of salicylic acid in response to the action of the elicitor. Salicylic acid inhibits the activity of catalase and leads to the accumulation of hydrogen peroxide [20, 21]. Catalase activity was greater for the avocado fruit treated with Agrokhit and Extrasol-90 compared with the control. In the course of ripening during storage in experimental and control samples, catalase was activated. The need to neutralize a large amount of accumulated hydrogen peroxide likely activates the enzyme. The increased catalase activity in the avocado fruit treated with bacterial antagonists in Extrasol-90 during ripening and storage can be explained by the presence of substances among the metabolic products, which, acting as elicitors, cause the activation of NADP·N-oxidase, resulting in

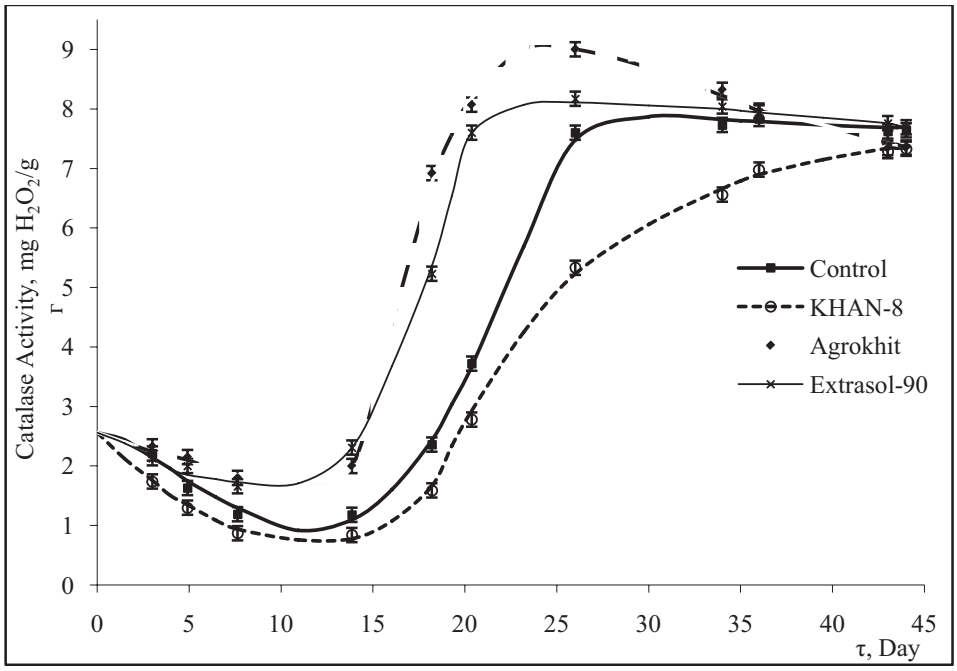

Figure 2. Changes in catalase activity during storage of the avocado fruit.

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hydrogen peroxide accumulation.

According to researchers, peroxidase is an indicator of a plant's immunity. It participates in the processes of metabolism, regulation of the maturation and protective reactions of plant tissues. Peroxidase – a poly-functional enzyme that catalyses the dehydrogenation of phenols, amines, flavones and amino acids – plays an important role in the respiration of plant objects, participating in electron transport [13].

When processing the fruit with the studied preparations, the peroxidase activity gradually decreased until the end of storage (Fig. 3). However, a comparative analysis between exogenous immunity inducers showed that during storage, peroxidase activity in samples treated with KHAN-8 decreased less actively than in samples treated with Agrokhit and Extrasol-90. Hence, more organic peroxides accumulated in the avocado fruit treated with Agrokhit and Extrasol-90.

The decrease in peroxidase activity is associated with the activation of the toxic effect of peroxides. The increased peroxidase activity in the avocado fruit treated with KHAN-8 compared with the other groups is probably due to the fact that KHAN-8 contributes more to the accumulation of phenols, flavones, amino acids and the active form of oxygen (hydrogen peroxide in response to the action of elicitors). Activation of physiological and biochemical processes leads to the changes in the activity of catalase and peroxidase in the avocado fruit during the storage.

Phenoloxidase acts on o-diphenols, polyphenols, tannins and monophenols. Researchers have speculated that phenoloxidase in the tissues of a healthy plant does not function or is weakly active, and does not play the role of the main oxidase at the final stage

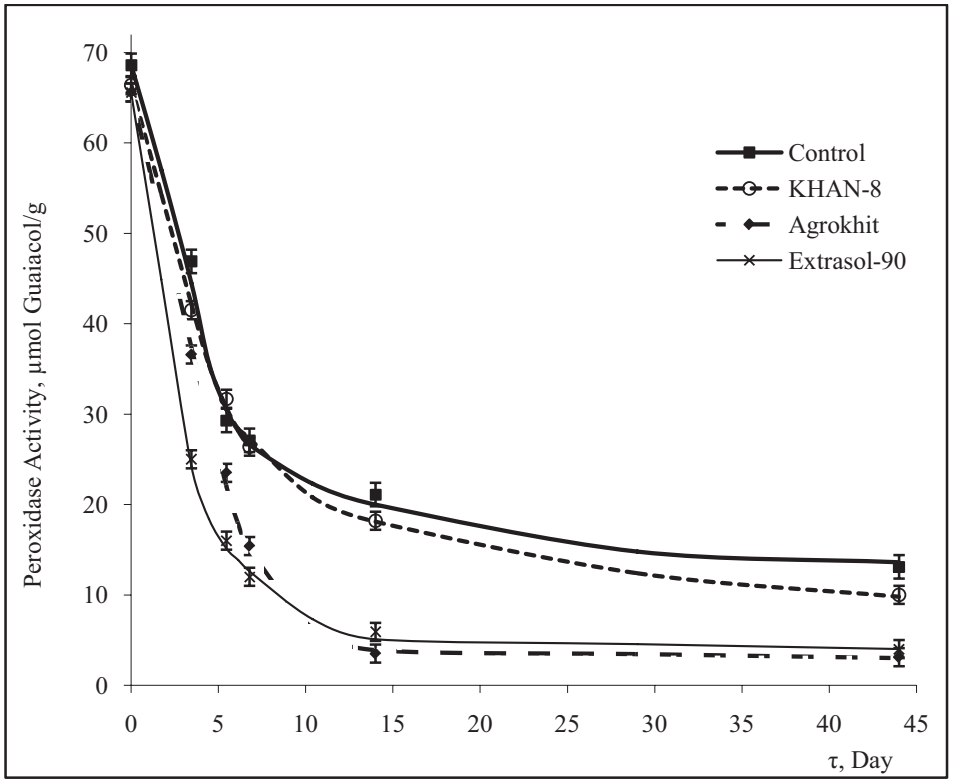

Figure 3. Changes in the peroxidase activity during storage of the avocado fruit.

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of oxidation [22, 23]. It is well known that phenolic compounds play an important role in the formation of immunity. The protective effect is determined not so much by their direct toxicity to phytopathogenic microorganisms as by the transformations that they undergo under the action of phenol oxidase. For example, as a result of the action of this enzyme, o-diphenols turn into o-quinone, which is a highly toxic substance [22, 23].

Fig. 4 shows the dependence of phenoloxidase activity on the storage duration. During the course of maturation in storage, phenoloxidase activity was lower for the treated compared with the control avocado (Fig. 4). However, during storage, the phenoloxidase activity in the samples treated with KHAN-8 increased, and the activity of phenol oxidase in the samples treated with Agrokhit and Extrasol-90 decreased. A decrease in the activity of phenoloxidase in experimental samples compared with the control during the storage can be associated with an increase in tyrosinase activity during this period.

Fig. 5 shows the dependence of the changes in tyrosinase activity in the Fuerte avocado fruit during the storage duration. The tyrosinase activity was higher in all treated avocado compared with the control fruit (Fig. 5). It can be assumed that tyrosinase activity is associated with the functioning of the lipoxygenase signalling system and increases in response to the action of the elicitor. The decrease in tyrosinase activity compared with the control may be associated with a decrease in the number of oxidation substrates. As can be seen from Figs. 4 and 5, the treatment of the avocado fruit before putting them into storage increased tyrosinase activity, which leads to an activation of the protective response of the plant cell in response to the elicitor.

The effectiveness of the preparations on the ripening process of the avocado fruit during storage was evaluated by changing the content of pectin substances – polymer com-

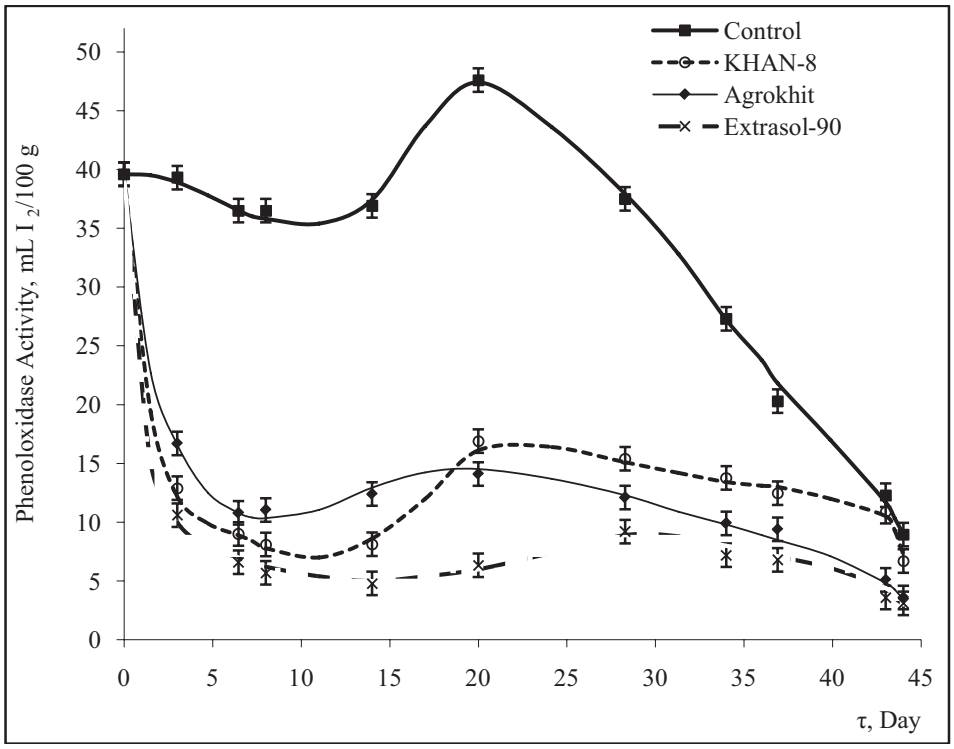

Figure 4. Changes in phenoloxidase activity during storage of the avocado fruit.

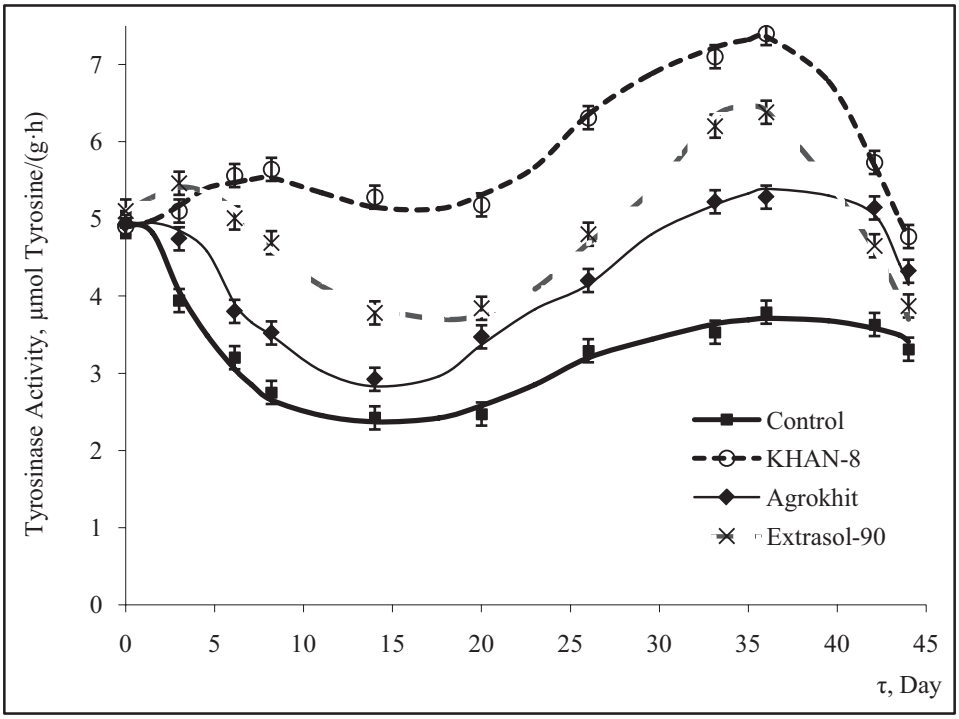

Figure 5. Changes in tyrosinase activity during storage of the avocado fruit.

pounds with a high molecular weight, consisting mainly of galacturonic acid residues bound by a α-1-4-glycosidic bond. Depending on the degree of methoxylation, they differ in solubility in water and are localized in various organelles of the plant cell. Water-insoluble protopectin is part of cell membranes; the median plates are largely composed of it. This compound is a cementing material that combines plant cells and creates a tissue structure that softens during maturation as a result of protopectin hydrolysis [13]. Water-soluble pectin is in vacuole juice and intercellular layers of tissue of mature fruit; it affects the water-holding ability. Changes in pectin substances in the untreated and treated avocado fruit are shown in Figs. 6–9.

During storage, the amount of protopectin decreased over the period of 20 days for all samples: control (3.2 fold), Agrokhit treated (2.5 fold), KHAN-8 treated (1.1 fold) and Extrasol-90 treated (1.6 fold). During the same time, the pectin content increased by 2.3 fold (control), 1.3 fold (Agrokhit treated), 1.3 fold (KHAN-8 treated) and 1.1 fold (Extrasol-90).

Protopectin hydrolysis reaction rate (K_{pr}, 1×10^{-2}, day^{-1}) constants in control and samples treated with Agrokhit, KHAN-8 and Extrasol-90 preparations were -6.1, -4.2, -2.5 and 3.4, respectively. Pectin hydrolysis reaction rate constants (K_p, 1×10^{-2}, day^{-1}) in control and samples treated with Agrokhit, KHAN-8 and Extrasol-90 were 3.1, 1.4, 1.1 and 1.8, respectively. It should be noted that in the experimental samples during the storage period from 20 to 35 days, the amount of protopectin and pectin did not change. However, in the control samples, the amount of pectin significantly decreased during storage from 20 to 35 days, and in experimental samples pectin decreased after 35 days.

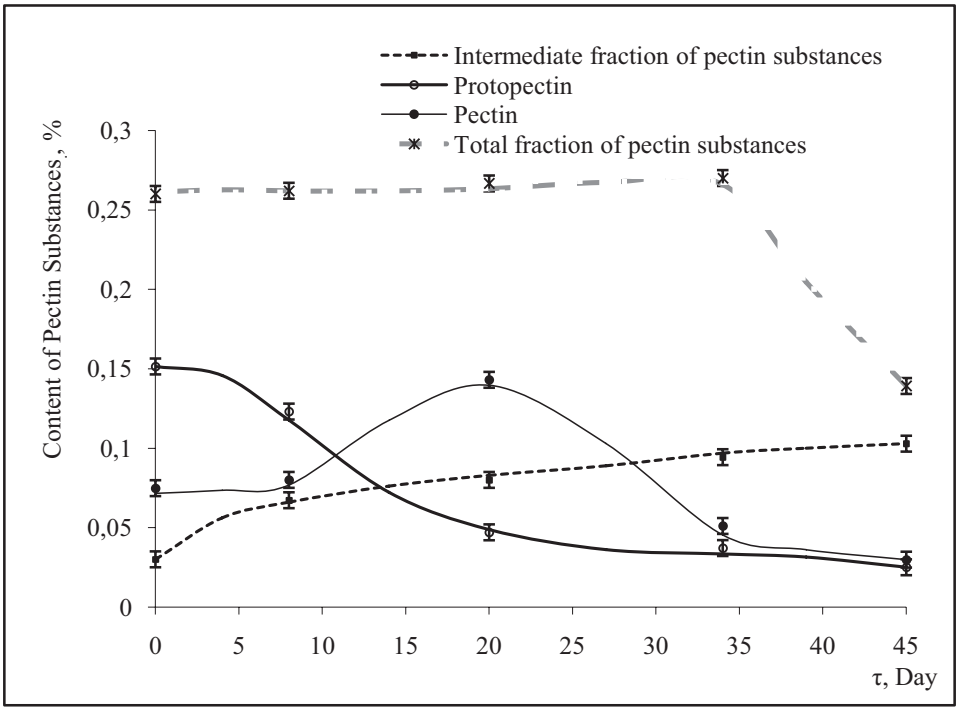

Figure 6. Changes in pectin substances during storage of the control avocado fruit.

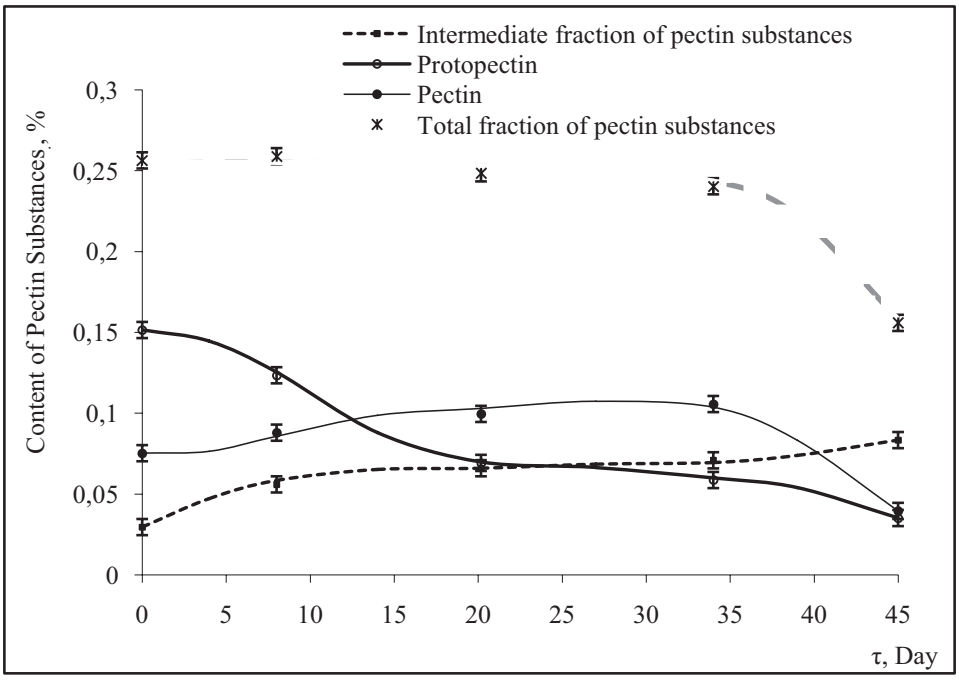

Figure 7. Changes in pectin substances during storage of the avocado fruit treated with Agrokhit.

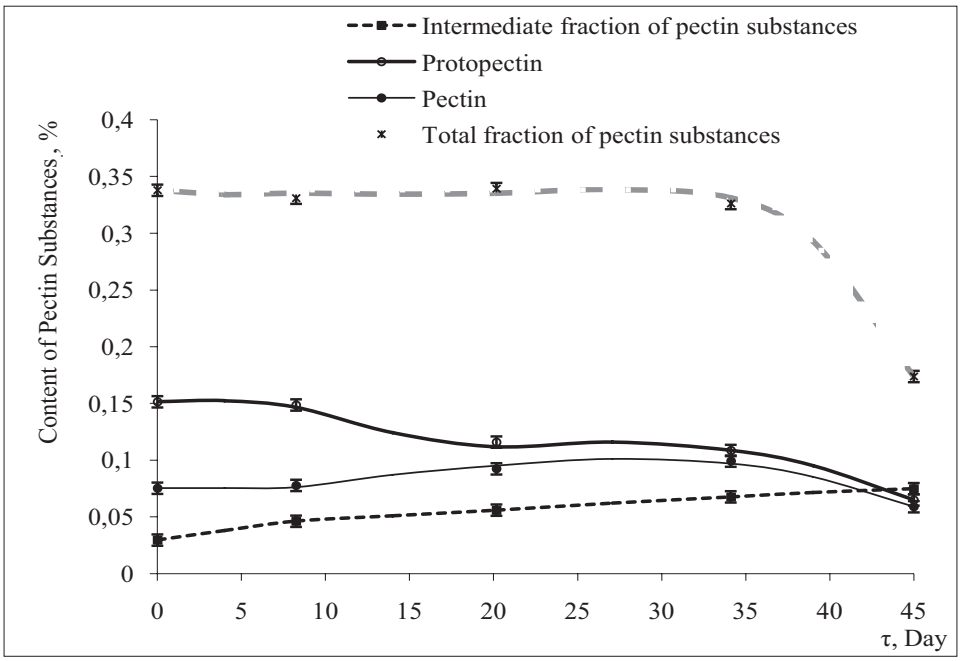

Figure 8. Changes in pectin substances during storage of the avocado fruit treated with KHAN-8.

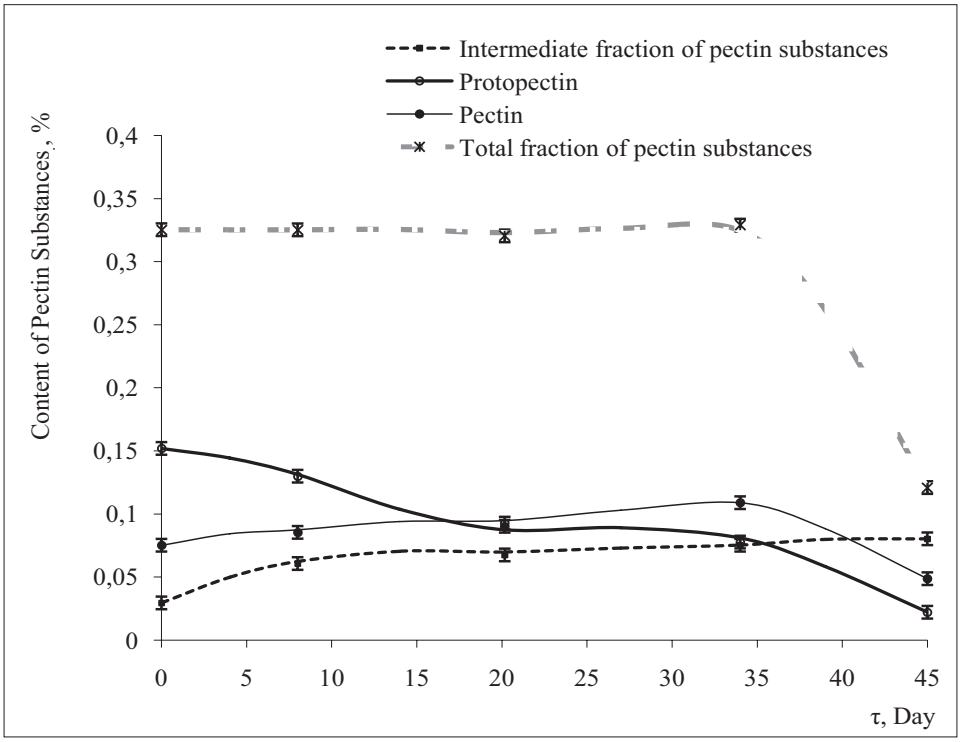

Figure 9. Changes in pectin substances during storage of the avocado fruit treated with Extrasol-90

Figs. 6–9 also show that regardless of the treatment conditions, during storage (up to 35 days), the total content of pectin substances (total fraction of pectin substances) did not change. With subsequent storage from 35 to 45 days, the amount of these compounds in control and samples treated with Agrokhit, KHAN- 8 and Extrasol-90 were reduced by 2.2, 1.4, 1.7 and 2.0 fold, respectively. These changes can be explained by different rates of hydrolysis of protopectin and pectin, as evidenced by the values of the rate constants. The fraction of pectin substances of intermediate solubility in water (intermediate fraction of pectin substances) increased throughout the entire storage period, regardless of the treatment.

Changes in the structure of pectin polysaccharides of avocado fruit obviously occur under the action of pectolytic enzymes, beginning with endo- and exopoligalacturonases, which catalyse the hydrolytic cleavage of α-(1→4)-glycosidic bonds between unesterified residues of galactopyranosyluronic acids [13]. It should be assumed that the treatment of avocado fruit with chitin derivatives and metabolites of bacterial antagonists inhibits the activity of pectolytic enzymes, resulting in a decrease in the rate of hydrolysis of protopectin and pectin, which affects the structure and water-holding capacity of the fruit and, as a result, the ripening process of the fruit slows down and the storage time increases. Thus, the dynamics of physiological and biochemical processes in avocado fruit depend on the type of preparation and the duration of storage. In such a way, KHAN-8 treatment significantly reduced the respiratory rate, while Extrasol-90 and Agrokhit preparations increased it immediately after processing and decreased it with respect to control during subsequent storage.

Treatment of the avocado fruit with the studied preparations slowed down the physiological and biochemical processes without disturbing metabolism, which is regulated by a change in the activity of terminal oxidases of the plant cell. Thus, peroxidase activity in the samples treated with the studied preparations decreases, tyrosinase increases, the maturation process slows down and the protective response of the plant cell is activated during storage relative to the control. In samples treated with the KHAN-8, catalase activity decreased; with Agrokhit and Extrasol-90 preparations, it increased relative to the control. The phenol oxidase activity in the samples treated with KHAN-8 increased, while Agrokhit and Extrasol-90 preparations decreased this activity relative to the control. Treatment of avocado fruit with preparations inhibited the rate of protopectin and pectin hydrolysis and increased the duration of their ripening and storage. A minimal decrease in the amount of protopectin and pectin is characteristic of fruit treated with KHAN-8.

Based on the collected data, to inhibit the physiological and biochemical processes and increase the duration of ripening and storage, it is recommended that avocado fruit be treated with chitin derivatives Agrokhit and KHAN-8.

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