

CHANGES IN THE FATTY ACID COMPOSITION OF AVOCADO FRUIT TREATED WITH PREPARATIONS DURING STORAGE

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Abstract

This article presents data on the fatty acid composition of avocado fruit and the dynamics of the free fatty acid content during storage of fruit treated with preparations “Agrohit”, “KHAN-8”, and “Extrasol-90”, which have antimicrobial properties. Avocado fruit treated with preparations by spraying and without treatment were placed in polymer containers and stored at a temperature of $(4\pm 1)^{\circ}\text{C}$ and relative humidity of 90–95% for 45 days. During the storage of avocado fruit, the fat content was determined by the Soxhlet method and free fatty acids were determined on a Shimadzu 20-AD chromatograph. It has been established that palmitic, palmitoleic, oleic, and linolenic acids predominate in Fuerto avocado fruit. It has been shown that during storage of avocado fruit treated with preparations, the hydrolysis of fats (triacylglycerides) is slowed down, which reduces the accumulation of free fatty acids. It was determined that treatment of fruit with the preparation “KHAN-8” slows down the oxidation of arachidonic acid, which is a resistance inducer of the fruit to phytopathogens.

Keywords: *chitosan, bacteria-antagonists, fatty acid composition, free fatty acids, avocado fruit, storage*

Received: 28.02.2019

Accepted: 06.06.2019

1. Introduction

Currently, in countries with a tropical and subtropical climate, more than 20 varieties of avocado fruit are grown, of which Fuerte, Hass, Nabal, and Fortunia are promising and widespread.

The chemical composition of fruit significantly depends on the variety and soil and climatic conditions of cultivation. Avocado fruit contains 1–4% protein; 2–10% carbohydrates; 13–35% fat; 65–80% water; vitamins A, B1, B2, C, PP, D, E, and K; and mineral substances iron, calcium, and phosphorus. Avocados are useful in the diet at diabetes, increased stomach acidity, acute digestive disorders, and cardiovascular diseases, primarily atherosclerosis. It should be noted that most varieties of avocado fruit are not resistant to phytopathogens and are not suitable for long-term storage at low positive temperatures [1–4].

To reduce the losses of fruit during storage caused by phytopathogens and to increase the duration of their storage, domestic and foreign researchers offer various physical, chemical, and biological remedies [5–12]. A promising area of research is the use of chitin derivatives, as well as the waste products of bacteria-antagonists of various genera and strains. The influence of physiologically active substances (elicitors) can activate both the mechanisms of the natural resistance of a plant cell to pathogens and inhibit the growth of microorganisms.

It should be noted that treatment of avocado fruit with preparations that reduce microbiological spoilage can influence physiological and biochemical processes and changes in chemical composition. Avocado fruit are characterized by high fat content, which is unstable when stored at low positive temperatures.

The aim of this study was to investigate the fatty acid composition of avocado fruit and the effect of treatment with preparations on the dynamics of free fatty acids during storage at a temperature of $(4\pm 1)^{\circ}\text{C}$.

2. Materials and Methods

The Fuerte variety mainly enters the Russian market directly from Israel, Spain, South Africa, and Kenya or re-exports from these countries through Holland. The object of this study was the variety of Fuerte avocado that is a Guatemala-Mexican hybrid and belongs to climacteric fruits. For this research, Fuerte avocado fruit with a mass of (320 ± 10) g, grown in South Africa and harvested in October at the physiological degree of maturity (mature green), were used. Deliveries of fruit to St. Petersburg were carried out by sea refrigerated transport at a temperature of $5\text{--}6^{\circ}\text{C}$ for 10–12 days. The investigated avocado variety complied with the requirements of the UN/ECE Standard FFV-42 for avocado fruit entering international trade. To increase the storage time and reduce losses from micro-biological spoilage, additional (to cold storage) agents are used, in particular, bacteria-antagonists and their metabolites and chitin derivatives.

In study by Ndonho a Botoro H.O. [13], it was shown that treatment of fruit of this variety with the preparations “Agrohit”, “KHAN-8”, and “Extrasol-90” inhibits growth of the main causative agents of infectious diseases of avocado fruit during storage, causing the storage duration to increase 1.5–2 times.

Chitosan is a regulator of various physiological reactions of plant cells and induces protective reactions, including the synthesis of phytoalexins, protective proteins, callose, and lignification [14].

The protective action mechanism of chitosan is to induce enhanced synthesis of its own hydrolases (glucanases, chitinases, and chitosanases) by plants after processing. Under the action of these enzymes, the destruction of the cell walls of bacteria and phytopathogenic fungi occurs, which significantly increases the resistance of plants to

disease. On the other hand, an increase in plant defence reactions is observed due to the blocking of a number of enzymes in pathogenic microorganisms by plant lectin and the synthesis of inhibitors of pathogenicity factors of pathogenic agents, which leads to disruption of the normal growth of pathogens [15].

At the moment, there is no scientific information on the effect of these preparations on the kinetics of fat hydrolysis reactions (triacylglycerides) since the main components of the chemical composition of avocado fruit.

The preparation "Agrohit", 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 with a molecular mass of 10 kDa in a 1% solution of lactic acid. Chitosan for the preparation "Agrohit" is obtained from the chitin of Far Eastern crabs of domestic production.

The preparation "KHAN-8" is a light-yellow powder, made of chitosan with a molecular mass of 15 kDa and a degree of deacetylation of 68%.

The basis of the preparation "Extrasol-90" are rhizospheric, nitrogen-fixing bacteria-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 *Arthrobactermysorens* 7, *Flavobacterium* sp. L-30, *Agrobacteriumradiobacter* 10, *Agrobacteriumradiobacter* 204, *Azomonasagilis* 12, *Bacillus subtilis* H-13, *Pseudomonasfluorescens* 2137, and *Azospirillum lipoferum* 137.

Preparation of solutions was carried out as follows: the preparation "Agrohit" was used for spraying without prior pre-treatment. One mL of the preparation "KHAN-8" was dissolved in 99 mL of a 2% solution of acetic acid with vigorous stirring. Forty mL of the preparation "Extrasol-90" was dissolved in 1 litre of water with vigorous stirring as well. Ready-made solutions for spraying the fruit were used immediately after preparation.

Upon admission to storage, the avocado fruit were sprayed with solutions containing the preparations "Agrohit" (sample No. 1), "KHAN-8" (sample No. 2), and "Extrasol-90" (sample No. 3). Fruit not treated with preparations served as control samples (control).

Test (control) and experimental (treated) samples weighing 320 ± 10 g were packed in one row in 4 kg polymer containers and stored at a temperature of $(4 \pm 1)^\circ\text{C}$ and relative humidity of 9–95% for 45 days.

During storage of the avocado fruit, the fat content (triacylglycerides) was determined by the Soxhlet Extraction method, and the free fatty acids were determined by the gas chromatography method [16].

The release of methyl ethers is based on the extraction of fat by hexane with the addition of a methanol solution of potassium hydroxide. After vigorous mixing and settling, the glycerin is separated. Then, the upper layer is decanted, containing fatty acid methyl ethers, which were determined with a Shimadzu 20-AD gas chromatograph. Tridecanoic acid was used as an internal standard [16].

The data were processed by mathematical statistics methods, finding the confidence interval with a probability of 95% using MS Excel.

3. Results and Discussion

The mechanism of the chemical and biochemical reactions of the transformation of triacylglycerides is complex and involves the formation of both labile and highly reactive intermediates and stable end-products, such as carbonyl compounds. When studying the reaction mechanisms based on the chemical–kinetic approach, the sequence of transformation of triacylglycerides into intermediate products, including hydrolysis

products (free fatty acids) and oxidation, affecting the quality and safety of the fat during storage, is important.

At the same time, the formation of triacylglycerides of saturated and unsaturated fatty acids during hydrolysis, which are involved in the oxidation reaction and the formation of unstable peroxide and hydroperoxide compounds, and then aldehydes and ketones, which can impart a rancid taste to the fat, is of importance.

Studies have shown that in the Fuerte avocado fruit fat content in storage is $19.6 \pm 0.5\%$, and the fatty acid composition includes the following fatty acids: myristic, 0.1%; palmitic, 9.1%; stearin, 0.5%; palmitoleic acid, 22.0%; oleic, 55.4%; linoleic, 11.6%; linolenic, 1.1%; and arachidonic, 0.2%.

Figure 1 and Figure 2 show chromatograms of the fatty acid composition of the Fuerte avocado fruit without treatment (control) upon storage and after 45 days of storage at $t=(4 \pm 1)^\circ\text{C}$. Figures 3–5 shows the fruit treated with preparations (samples No. 1–3) after 45 days of storage at $t=(4 \pm 1)^\circ\text{C}$

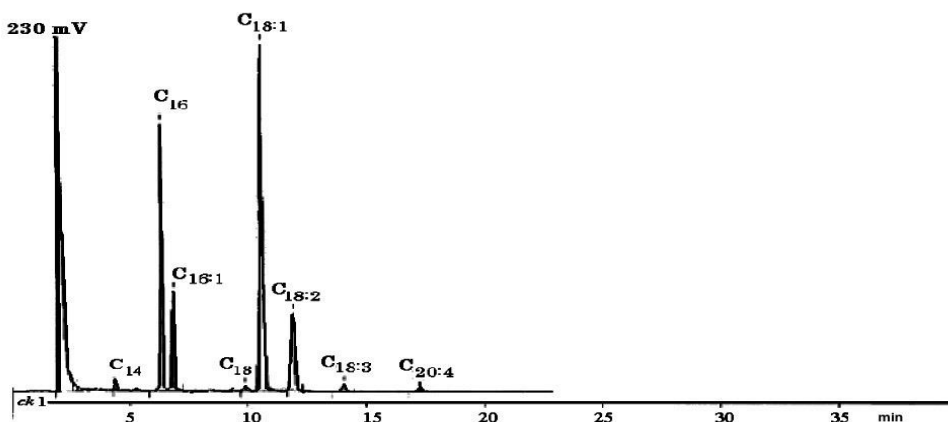


Figure 1. Chromatogram of fatty acid composition of Fuerte avocado fruit (control) upon admission to storage at $t=(4 \pm 1)^\circ\text{C}$

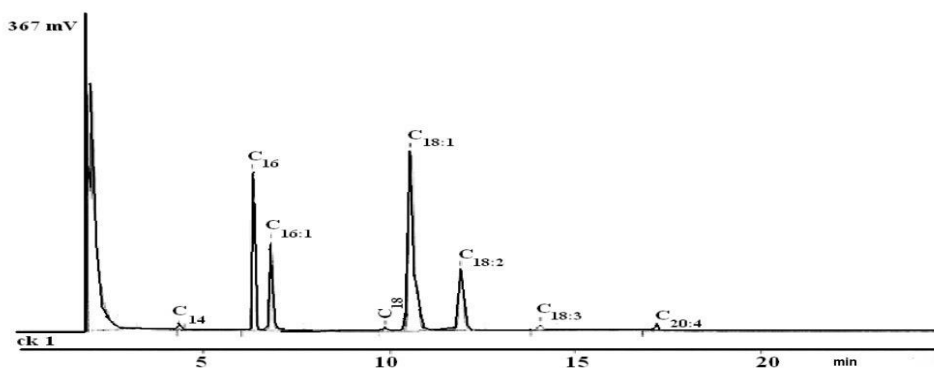


Figure 2. Chromatogram of fatty acid composition of Fuerte avocado fruit (control) after 45 days of storage at $t=(4 \pm 1)^\circ\text{C}$

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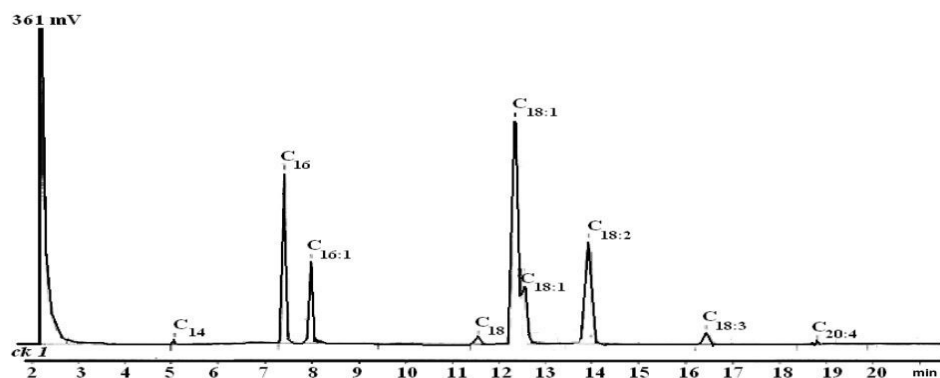


Figure 3. Chromatogram of fatty acid composition of Fuerte avocado fruit treated with "Agrokhit" (sample No. 1) after 45 days of storage at $t=(4\pm 1)^\circ\text{C}$.

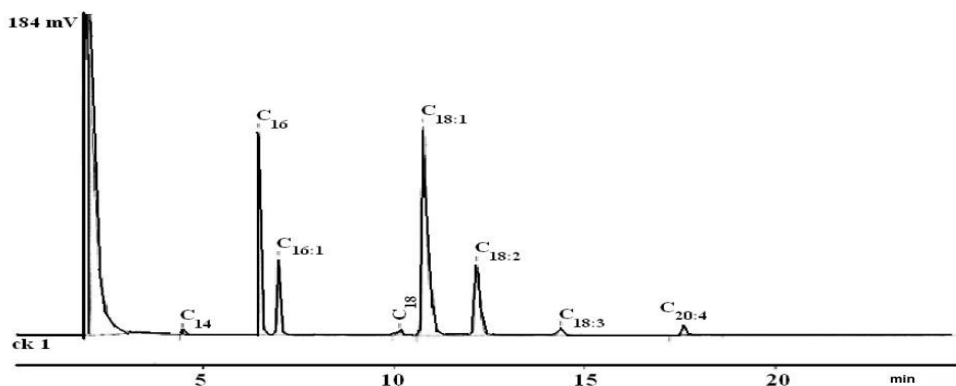


Figure 4. Chromatogram of fatty acid composition of Fuerte avocado fruit treated with "KHAN-8" (sample No. 2) after 45 days of storage at $t=(4\pm 1)^\circ\text{C}$.

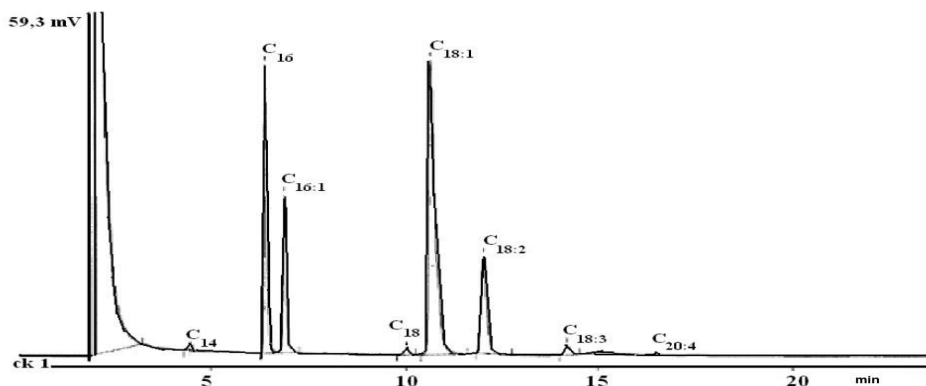


Figure 5. Chromatogram of fatty acid composition of Fuerte avocado fruit treated with "Extrasol-90" (sample No. 3) after 45 days of storage at $t=(4\pm 1)^\circ\text{C}$.

Figures 6–10 show the dependence of the changes in free fatty acid content in avocado fruit on the storage time at $t=(4\pm 1)^{\circ}\text{C}$.

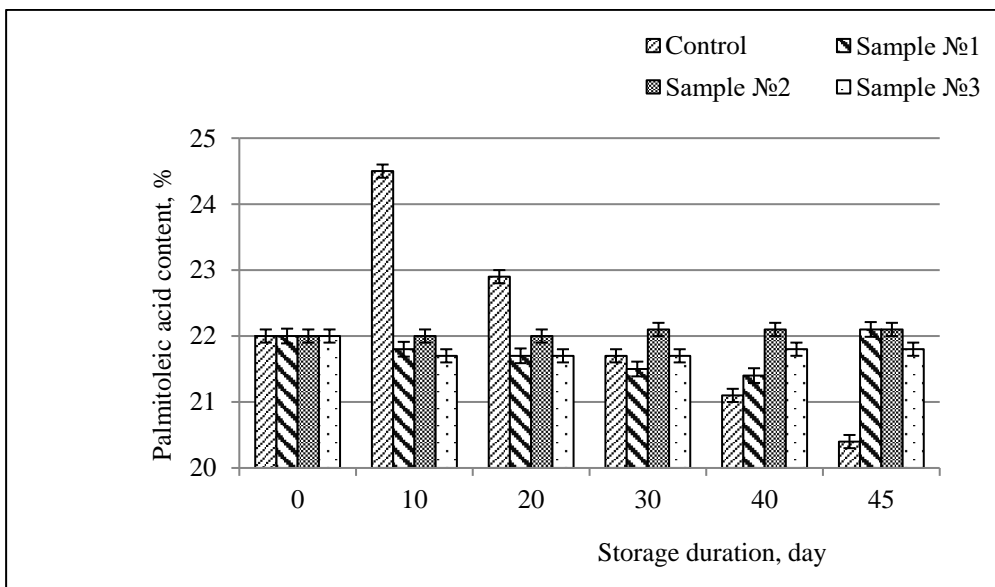


Figure 6. Changes in the content of palmitoleic acid during the storage of Fuerto avocado fruit at $t=(4\pm 1)^{\circ}\text{C}$.

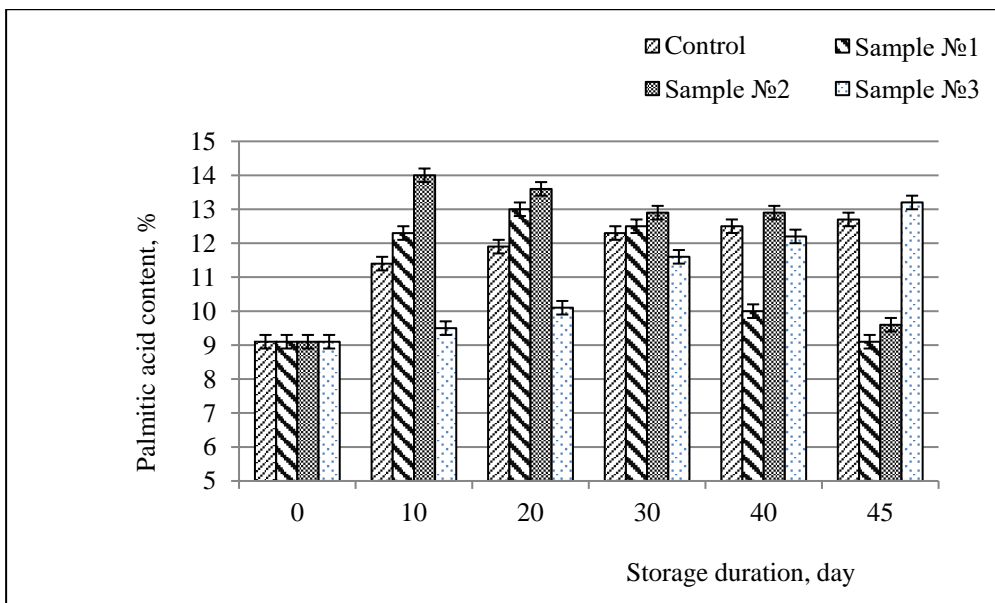


Figure 7. Changes in the content of palmitic acid during storage of Fuerto avocado fruit at $t=(4\pm 1)^{\circ}\text{C}$.

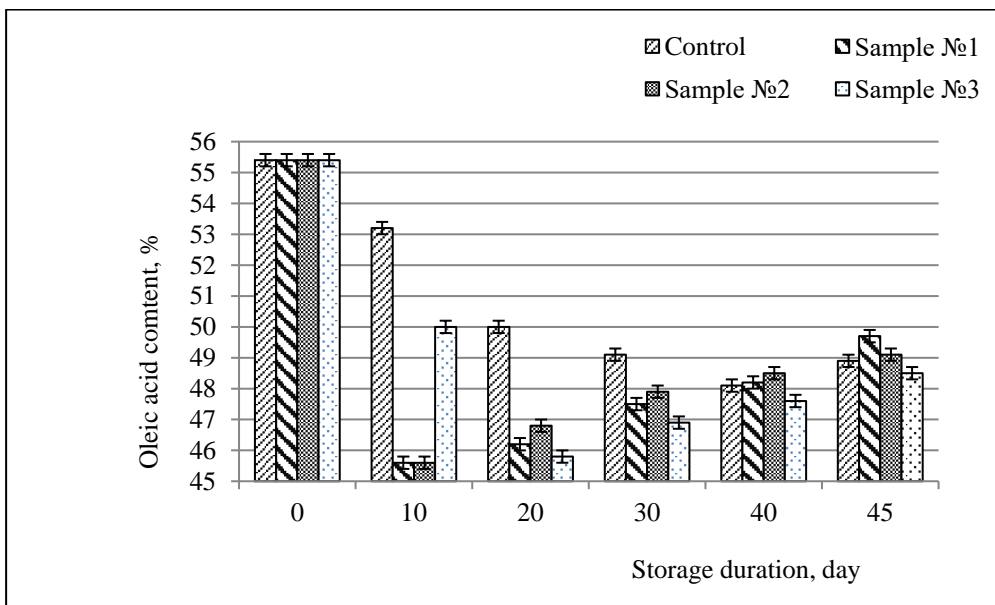


Figure 8. Changes in the content of oleic acid during storage of Fuerto avocado fruit at $t=(4\pm 1)^{\circ}\text{C}$

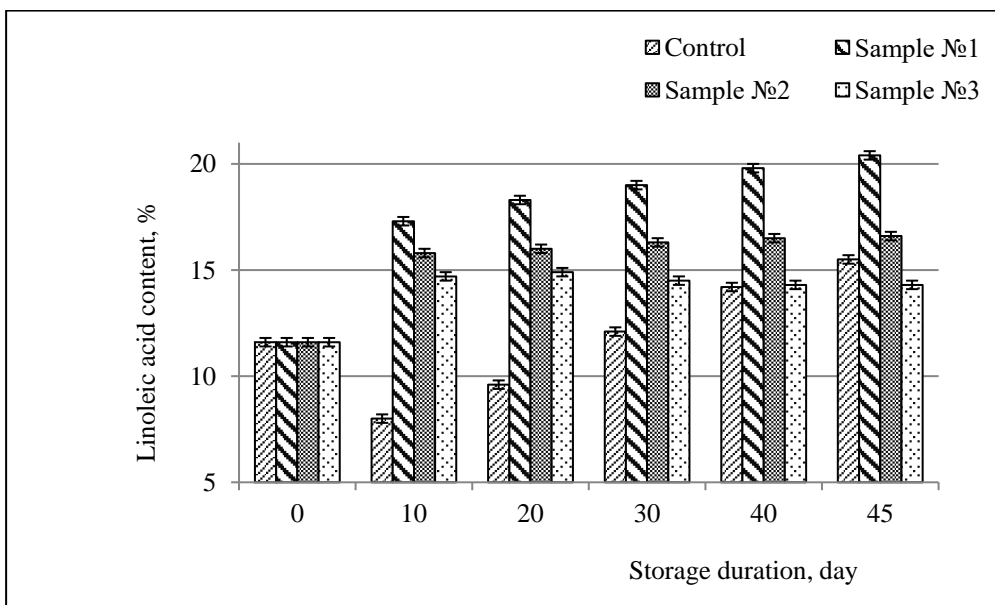


Figure 9. Changes in the content of linoleic acid during storage of Fuerto avocado fruit at $t=(4\pm 1)^{\circ}\text{C}$.

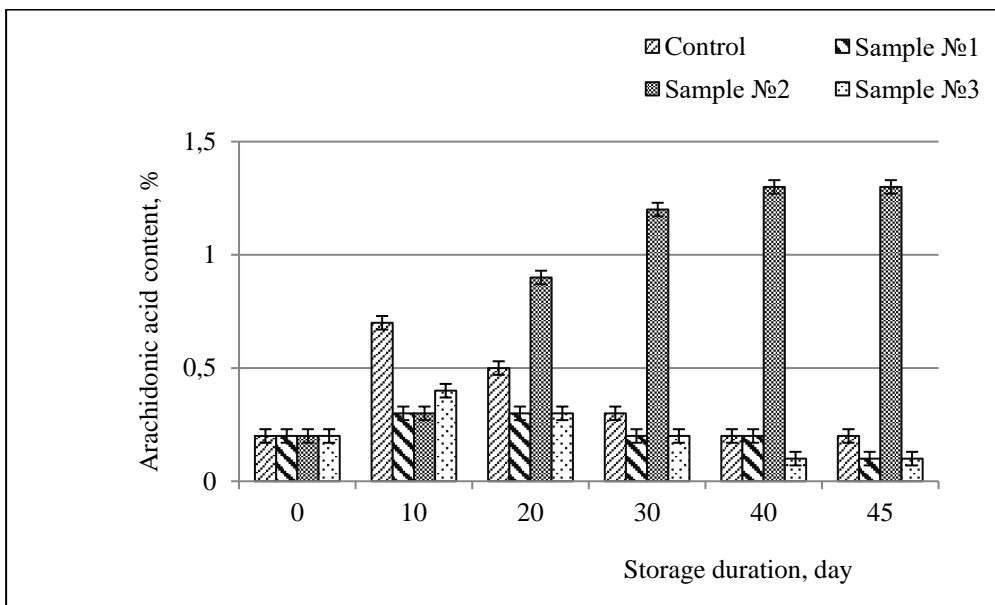


Figure 10. Changes in the content of arachidonic acid during storage of Fuerto avocado fruit at $t=(4\pm 1)^{\circ}\text{C}$.

As follows from Figures 6–10, by the end of the storage period of the control and treated samples, the content of saturated and unsaturated fatty acids, except for linoleic, linolenic, and arachidonic, decreased. It should also be noted that the amount of linoleic, linolenic, and arachidonic acids increased by the end of storage, which can be explained by slowing down the oxidation reaction of these compounds and the formation of secondary carbonyl compounds.

By the end of the storage period of avocado fruits, the amount of oleic and arachidonic acids, both in the controls and in samples treated with "Agrohit" and "Extrasol-90" preparations, decreased. However, the amount of arachidonic acid in the fruit treated with the preparation "KHAN-8" increased during storage. In the latter case, such a change can be explained by different rates of hydrolysis of triacylglycerides and the oxidation of fatty acids, which are an inducer of plant cell immunity.

Arachidonic acid is a C20-unsaturated fatty acid and an active principle of the lipoglycoprotein complex, which induces resistance to infectious diseases of fruit. Arachidonic acid is a precursor of biologically active eicosanoids that are involved in inducing systemic resistance [1].

Thus, it has been shown that palmitic, palmitoleic, oleic and linolenic acids predominate in Fuerte avocado fruit. It was established that during storage of avocado fruit treated with the studied preparations, the hydrolysis of triacylglycerides is slowed down and, as a result, the rate of accumulation of free fatty acids decreases, which can be explained by inhibition of the activity of lipase enzymes and phospholipase. It has been revealed that the treatment of fruit with the preparation "KHAN-8" slows down the oxidation of arachidonic acid, which is an inducer of fruit resistance to pathogens.

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