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# INFLUENCE OF THE ETHYLENE SYNTHESIS INHIBITOR FITOMAG ON THE DEVELOPMENT OF THE PROCESSES OF RIPENING-SENESCENCE OF APPLE FRUITS

**164.02. PLANT PHYSIOLOGY**

**Summary of the doctoral thesis in biological sciences**

**CHIŞINĂU, 2023**

The thesis was elaborated within the Physiology of Fruit Plants and Fruit Maturation Laboratory of the Institute of Genetics, Physiology and Plant Protection of the Moldova State University

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The thesis defense will take place on 17 October 2023, at 14:00 in the meeting of the Commission for the Public Defense of the doctoral thesis within the Doctoral School of Biological, Geonomic, Chemical and Technological Sciences, Moldova State University (http://www.usm.md), at the address: Chisinau municipality, 20 Pădurii str., Institute of Genetics, Physiology and Plant Protection of the State University of Moldova, meeting room.

The thesis and the abstract can be consulted at the National Library of the Republic of Moldova, the Central Scientific Library „Andrei Lupan" (Institute), the Central Library of the Moldova State University (MD 2009, Chisinau municipality, 60 Alexei Mateevici str.), as well as on ANACEC web page (http://www.cnaa.md/) and the Moldova State University web page (http://www.usm.md).

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## CONCEPTUAL GUIDELINES OF THE RESEARCH

**The relevance and importance of the addressed issue.** The apple is a species with high nutritional value, the fruits contain significant amounts of sugars, organic acids, mineral salts, vitamins etc. [7].

Physiological-biochemical changes that occur during storage lead to the deterioration of the quality of agricultural products. The intensity with which these processes are carried out after harvesting is the main factor that determines the duration of their quality maintenance [2].

Currently, worldwide, the most widespread storage technologies – normal atmosphere (NA), modified atmosphere (MA) and controlled atmosphere (CA) have their advantages and disadvantages, they differ in terms of the costs of their implementation, they do not fully guarantee the protection of fruits from fungal diseases and physiological disorders, as well as the preservation of the initial quality of the fruits [6]. In addition to the key factors (temperature, humidity, content of $O_2$ and $CO_2$), which determine the ability to keep fruits [33], a decrease in the quality of fruits kept under conditions with NA and CA is also caused by processes associated with ripening – senescence, accompanied by a high level of synthesis and accumulation of endogenous and exogenous ethylene [3; 26]. In the Republic of Moldova, the most widespread method is storage in conditions with NA (cold storage with artificial cooling), precisely because it does not require significant expenses, such as keeping in cold storage with CA, which is quite expensive. Disadvantages of NA technology include: a short storage period, rapid fruit ripening, damage caused by physiological diseases etc. [32].

One of the storage technologies based on the principle of inhibiting ethylene biosynthesis based on 1-methylcyclopropene (1–MCP), is the post-harvest treatment of climacteric fruits and vegetables with the Fitomag preparation, which largely solves the problem of maintaining fruit quality and reducing losses produced by the scald [33].

Over the years in the Republic of Moldova, few studies have been carried out regarding the influence of the Fitomag preparation on the quality of apple fruits, grown in the pedoclimatic conditions of our country. The results obtained are fragmentary and do not provide a complex approach at different levels of the physiological process of fruit resistance to various deteriorations during storage under the postharvest influence of this preparation. Resulting from this, including the fact that the preparation is widely applied in more countries, with successes being recorded in the matter of maintaining quality and extending the storage period, we assumed the responsibility of carrying out complex research on the action of the preparation during the period of storage on the intensity of metabolic processes in apple fruits, cultivated in the conditions of our country. Starting from the described premises, the **aim** of the work was to evaluate the mechanisms of action of the Fitomag preparation on the ripening-senescence processes in the fruits of some late apple varieties, as a scientific basis for the optimization of fruit storage technologies in the Republic of Moldova.

**The objectives of the research consisted in:**

1. The scientific-practical argumentation of the necessity and opportunity of applying the Fitomag preparation, as a modern alternative technology for storing the

fruits of late apple varieties, grown in the pedoclimatic conditions of the Republic of Moldova;

2. Establishing the degree of influence of the Fitomag preparation on the intensity of the respiration process and the emission of ethylene, as indicators of appreciation of the degree of ripening, the speed of biodegradation of the reserve substances, the quality and the storage period of the apple fruits;

3. Evaluation of the influence of the storage method on the activity of antioxidant processes in apple fruits, by assessing the content of phenolic substances and the activity of the enzyme polyphenoloxidase, responsible for the immunity of the fruit;

4. Determination of the influence of the postharvest application of the Fitomag preparation on the intensity of the transformation and biodegradation processes of the main biochemical compounds (carbohydrates, titratable acids, ascorbic acid), as indicators for assessing the storage capacity and quality of apple fruits;

5. Evaluation of the influence of the biological particularities of the variety and the method of storage on the quality and degree of fruit damage by pathogens and physiological disorders.

***Research hypothesis:*** Fruit storage technologies in conditions with NA and CA have their advantages and disadvantages, they differ in terms of the costs of their implementation, they do not always guarantee the protection of fruits from fungal diseases and physiological disorders, as well as the preservation of the initial quality of fruits (freshness, firmness, nutritional value, etc.). Storing of fruits by applying the Fitomag preparation could serve as an alternative and less expensive technology to slow down the ripening-senescence processes in apple fruits for many producers and exporters of fruits in the Republic of Moldova, who cannot afford the construction of refrigerators equipped with modern equipment or transporting the fruit to long distances to other households to be preserved.

**Synthesis of research methodology and justification of chosen research methods.** To achieve the proposed goal and objectives, conventional research methods specific to the field of fruit storing, described in the specialized literature, have been used. In order to obtain some complementary data regarding the action mechanisms of the Fitomag preparation on the ripening-senescence processes in the fruits of some late varieties of apple, physiological and biochemical methods have been used (intensity of the respiration process and ethylene emission, titratable acidity, total sugar content, the content of dry substances, ascorbic acid and phenolic substances, the activity of polyphenoloxidase), biophysical (the degree of dehydration of the tissues; structural-textural firmness of the fruits) and statistical-mathematical comparative analysis. By means of these methods we can evaluate the influence of different storage technologies on the intensity of metabolic processes in fruits during storage.

**Scientific novelty and originality.** A complex evaluation of the influence of *Fitomag* preparation on the metabolism of apple fruits grown in the conditions of the Republic of Moldova was performed, based on physiological-biochemical and biophysical parameters, as indicators for assessing the degree of ripeness, quality, and shelf life of fruits.

**The important scientific problem solved.** Scientifically substantiated and experimentally proved the post-harvest effect of the ethylene biosynthesis inhibitor

*Fitomag* on the processes of ripening-senescence in the fruits of 4 late apple varieties, which made it possible to identify the positive effect of the preparation and evaluate the prospects for its use in the storage of fruits grown in the conditions of the Republic of Moldova.

**Theoretical significance.** The study contributes to supplementing knowledge about the features of the processes of ripening and senescence of apple fruits during storage, depending on the variety, weather conditions during the growing season and storage technology, in particular with the postharvest use of Fitomag preparation.

**The applicative value of the paper.** The postharvest treatment of apple fruits with the *Fitomag* preparation contributes to the reduction of losses caused by fungal diseases and physiological disorders, prolonging the storage period of apple fruits and preserving their quality (firmness, freshness, juiciness, etc.). Having a pronounced effect on the development of apple fruit ripening processes, this technology can compete favorably with the currently widely used, but rather expensive method of storage in a controlled atmosphere. For the success of fruit storage, it is recommended to harvest fruits at the optimal time, characteristic of each variety.

**Implementation of scientific results.** The results obtained during the study were implemented based on the storage complex of ,,Fortina-Labis" Ltd, Floreni village, Ungheni district. The results presented in the dissertation can be used as scientific and methodological material in teaching the courses of Plant Physiology and Storage of Agricultural Products.

## SUMMARY OF THESIS COMPARTMENTS

In the **Introduction**, the actuality and importance of the problem addressed, the inclusion of the theme in the national and international concerns are argued, emphasizing the importance of using post-harvest treatments to maintain the quality of apple fruits during cold storage. The purpose, the objectives, and the research hypothesis are formulated, the synthesis of the research methodology is presented with the justification of the chosen analysis methods and the summary of the thesis chapters.

## 1. THE QUALITY AND FRUIT STORAGE CAPACITY DEPENDING ON THE SPECIFIC CHARACTERISTICS OF THE VARIETY, CULTIVATION CONDITIONS AND STORAGE TECHNOLOGY (literature review)

The chapter includes the analysis of data from the specialized literature in relation to the addressed research topic. Particular attention has been paid to highlighting the main physiological, biochemical and biophysical changes in apple fruits during storage. The main biosynthesis and biodegradation processes in the harvested fruits have been described, as well as the extent to which they influence the quality of the fruits during cold storage. As a result of the critical analysis of the bibliography, a synthesis has been made of the main achievements in the field of research and the current state of research regarding the application of the Fitomag preparation both in our country and abroad. The advantages and disadvantages of this cold storage technology have been presented. The opportunity of applying the Fitomag preparation has been argued, as an alternative and less expensive method of slowing

down the ripening-senescence processes in apple fruits grown in the pedoclimatic conditions of the Republic of Moldova.

## 2. OBJECTS, CONDITIONS FOR CARRYING OUT INVESTIGATIONS AND RESEARCH METHODS

The fruits of the late apple varieties: Golden Delicious, Florina, Idared and Renet Simirenko, grown in the farm of the agricultural company "Lefcons-Agro SRL", Floreni commune, Ungheni district, have served as objects of study.

**2.1. Climatic conditions of the growing season during the research years.** In 2016, the vegetation period (May-September) was characterized by a higher thermal regime, the average temperature in this period being 20.9°C, exceeding by 1.5°C the average value of the temperature in 2014, respectively by 3.3°C in 2015, which negatively influenced the formation, development, growth and preservation of fruits. The highest amount of precipitation was recorded in 2016, constituting values of 16–203.0 mm. In 2015, the amount of atmospheric precipitation during the vegetation period was 1–23 mm. The amount of atmospheric precipitation during the vegetation period in 2014 varied within the limits of 14–126 mm. The vegetation period of 2015 was characterized by the lowest level of relative air humidity (59 %), and the vegetation period of 2016 (65 %) by the highest.

The meteorological conditions provided differences in the physiological, biochemical and storage capacity of apple fruits.

**2.2. Fruit storage conditions.** The second day after harvesting, some of the fruits were treated with the Fitomag preparation. The fruits were stored in experimental cold rooms (KNT-1M) within the "Carpotron" experimental complex of the Institute of Genetics, Physiology and Plant Protection, in conditions with NA and CA with different content of $O_2$ and $CO_2$, being subsequently storing for a period of 150 days, according to the scheme (fig. 2.1):

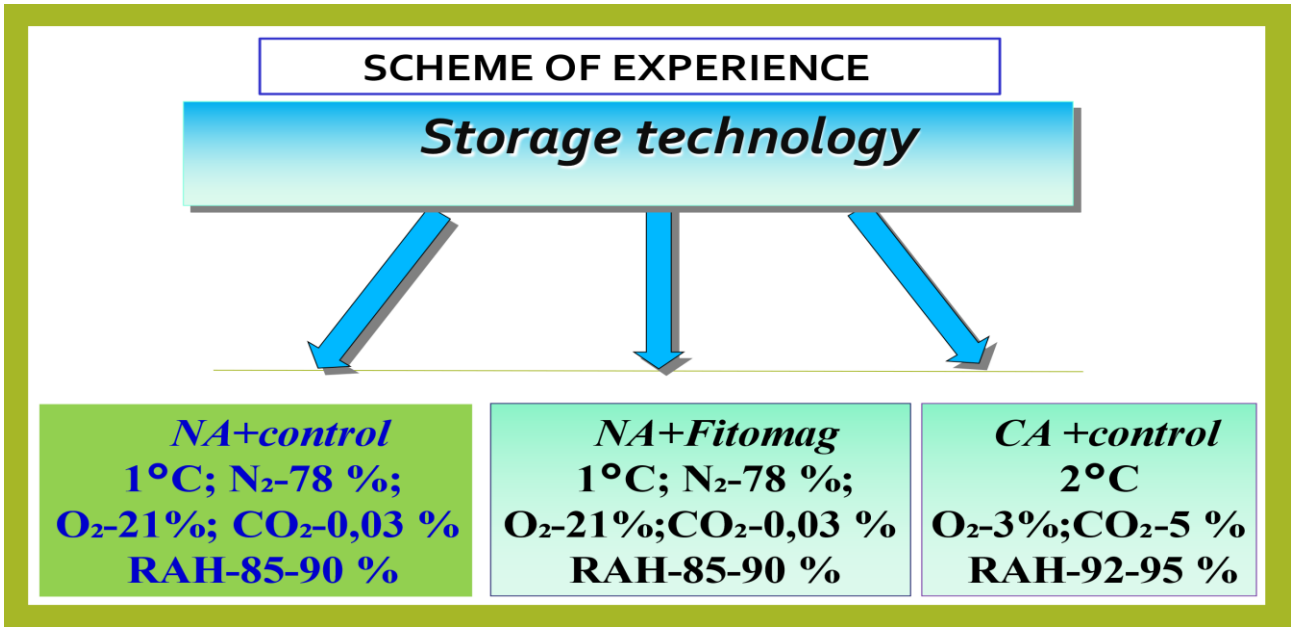


**Figure 2.1. Scheme of the experience**

**2.3. Research methods.** The observations during the vegetation period were aimed at determining the degree of ripeness and the optimal harvest term, by assessing the starch content [40], the structural-textural firmness (kg/cm$^2$) by the penetrometric method, the content of soluble dry substances by the refractometric method (% on the Brix scale), the Streif index (the ratio between firmness and the content of soluble dry substances). Penetrometrically, the firmness of the fruits was also investigated in the dynamics of the storage period.

*Laboratory determinations.* The investigations have been carried out in the Physiology of Fruit Plants and Fruit Maturation Laboratory of the Institute of Genetics, Physiology and Plant Protection. Standard protocols, promoted by the specialized literature, have been used. The assessment of the effect of the post-harvest application of the Fitomag preparation on the concentration and intensity of the endogenous ethylene emission was determined using the ICA56 ethylene analyzer. The intensity of fruit respiration was determined according to the method proposed by Починок Х. [38]. The ripening and senescence of the fruits, depending on the storage technology, was judged by the values of some biochemical indices of fruit quality: titratable acidity, content of ascorbic acid and total sugar, using the methods proposed by Ермаков А. and others [34]. The total content of phenolic substances in fruits was determined according to the method [24] using the Folin-Ciocalteu reagent, and the activity of the polyphenoloxidase enzyme was investigated using the photocolorimetric method [4]. The degree of dehydration of the fruit tissues was determined by weighing. Fungal diseases and physiological disorders were determined visually, using atlases and recognized expert guides [25; 29]. The degree of disease damage was expressed as a percentage of the total number of fruits. *The processing and statistical interpretation* of the obtained results was carried out through dispersion analyzes (bifactorial and trifactorial), based on the ANOVA test, with the help of the STATGRAPHICS Plus2 software package. The data results of 3–5 replicates obtained were expressed by calculating the arithmetic mean and standard deviation. The graphic presentation was executed using Microsoft Excel Office 2019 software.

## 3. THE POST-HARVEST EFFECT OF THE FITOMAG PREPARATION ON SOME PHYSIOLOGICAL INDICES OF APPLE FRUIT QUALITY

### 3.1. The influence of the Fitomag preparation on the intensity of fruit ethylene emission

The researched varieties from the variant with treated fruits have shown non-essential increases in ethylene production, its emission remaining practically at the same level during storage, compared to the fruits from the control variant, which showed a characteristic increase and decrease with the passage of the period of storage [19] (fig. 3.1). This fact indicates that treating fruits with the Fitomag preparation suddenly slows down its biosynthesis and accumulation. At the initial stage of storage, the level of ethylene emission in the fruits increased non-essentially, with a certain excess in the control fruits, after which it decreased, remaining at this level during the following months, this period being largely determined by the biological particularities of variety and storage technology (fig. 3.1).

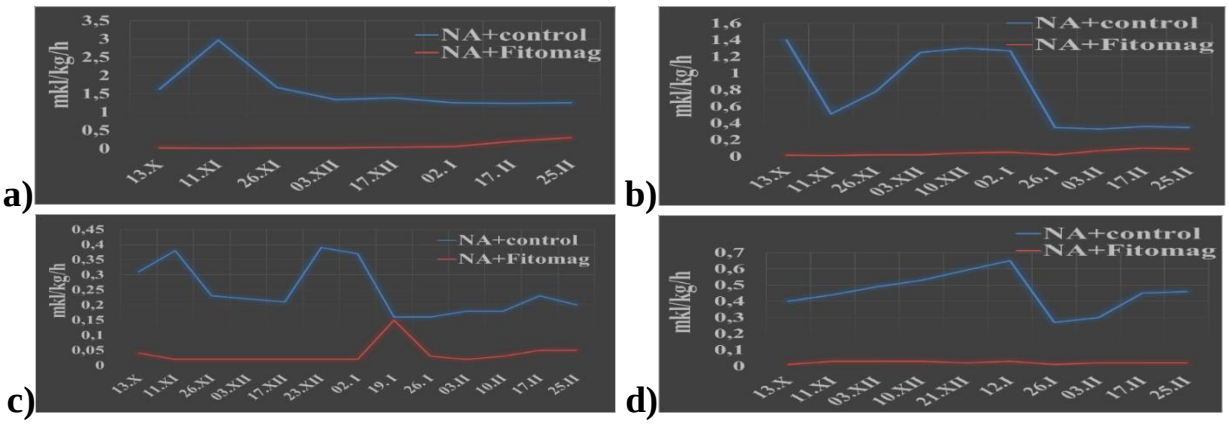


**Figure 3.1. Dynamics of ethylene elimination during storage of apple fruits: (a) – Golden Delicious variety; (b) – Florina variety; (c) – the Idared variety; (d) – Renet Simirenko variety**

The intensification of ethylene emission coincided with the climacteric period of the fruits, after which, reaching the maximum level, its quantity in the fruits decreased suddenly, the maximum of its elimination coinciding with the climacteric (respiratory) maximum phase [19; 23]. The treated apple varieties were distinguished by the lower rate of ethylene production, in relation to the control fruits, kept under the same conditions. Among the untreated fruits, those of the Golden Delicious variety stood out due to the more intense emission of ethylene during storage – 1.24–2.97 mkl/kg/h, and the lowest - the fruits of the Idared variety – 0.16–0.39 mkl/kg/h, they also show increased disease resistance during storage. The legality given was partially valid in the case of fruits treated with the Fitomag preparation, the lowest ethylene activity being noted in the Renet Simirenko variety – 0.01–0.03 mkl/kg/h [19]. As a result of the treatment of the varieties characterized by an increased intensity of ethylene emission (Golden Delicious and Florina), the development of physiological disorders and fungal diseases such as: *scald*, *withering and gray rot* was reduced or excluded, ensuring the preservation of quality initials. The effect of using the Fitomag preparation was most clearly manifested in the Renet Simirenko variety, at the end of 150 days of storage, the level of ethylene emitted by the treated fruits showed values of 0.02 mkl/kg/hour, and in the untreated ones – 0.46 mkl/kg/hour (a 23-fold decrease). Due to a less visible decrease (3.88 times), the fruits of the Florina apple variety stood out. Therefore, the post-harvest application of the Fitomag preparation allows the effective inhibition of ethylene biosynthesis and, as a result, of the fruit ripening process, ensuring quality preservation and their complex protection from many diseases [19].

### 3.2. The intensity of fruit respiration, depending on the storage technology

The physiological changes that occur in the fruit refer primarily to the variation in intensity breathing, this being an important indicator for appreciating the complexity of the transformations that take place in them [7]. The intensity of the respiration process of the fruits treated with the Fitomag preparation was significantly slowed down, the climacteric period occurring late, which made it possible for the respiratory maximum to appear for a later period, compared to the fruits of the control variant [23]

(fig. 3.2). In the storage dynamics, the highest degree of respiration intensity was recorded in the control fruits of the Golden Delicious variety – 1.80–5.40 mg $CO_2$/kg/hour, but lower in the treated ones of the Renet Simirenko variety – 0.60–2.80 mg $CO_2$/kg/hour [23].

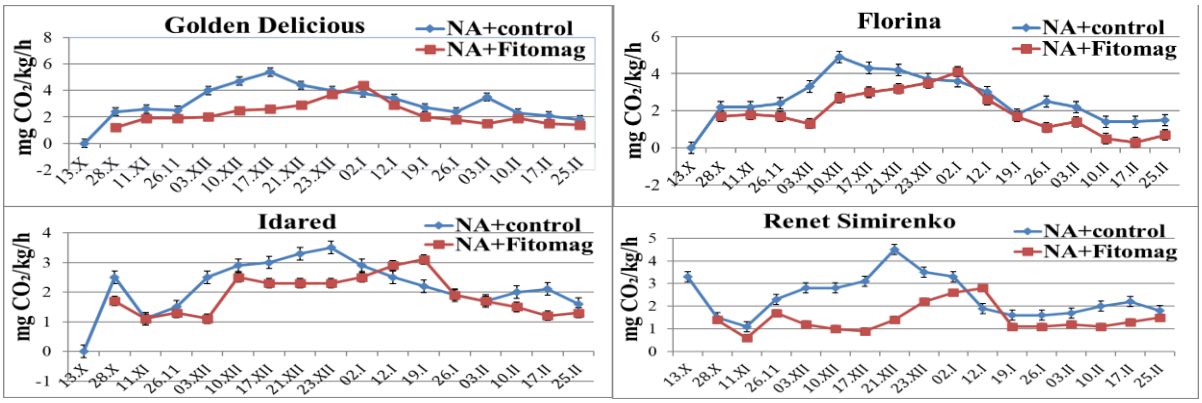


**Figure 3.2. The intensity of fruit respiration, depending on the storage technology**

The period of the climacteric maximum is mainly characterized by the strong emission of $CO_2$, during which essential changes in metabolism take place, the fruits are ripened, possessing the taste, aroma and color characteristic of the variety [1]. In the fruits of the Idared variety, the climacteric maximum was recorded on December 23, while in the fruits of the same variety treated with the Fitomag preparation, it occurred on January 19, so 27 days later. In the investigated fruits of the Florina variety, the climacteric maximum in the control variant was detected on December 10, and in the treated fruits it occurred 23 days later (January 2), in the same period as in the case of the Golden Delicious variety. In the control fruits of the Renet Simirenko variety, the onset of the climacteric maximum occurred on December 21, and in the treated fruits it occurred on January 12, so 22 days later than the untreated fruits. Next, after achieving a climacteric maximum, a sudden decrease in the intensity of breathing was attested. Towards the end of the storage period, however, a short-term slight increase was recorded, indicating that major changes occur in fruit metabolism, prevailing the processes of destruction (biodegradation), which is characteristic of the senescence period (fig. 3.2). During this period, the resistance of the fruits to physiological disorders and fungal diseases decreased, the weight loss and the consumption of biochemical substances increased, processes that essentially reduced the taste qualities of the fruits. The obtained results allow us to state that the post-harvest treatment of fruits with the Fitomag preparation contributes to a decrease in the intensity of respiration, less $CO_2$ is released [23], from which it follows that the consumption of plastic substances (sugars etc.) used as a substrate during the maintenance of this process it was reduced [13; 23].

### 3.3. Change in the content of dry matter in fruits, depending on the storage technology

Depending on the investigated apple variety, the content of dry matter in the variant with treated fruits at the time of release from storage was higher than that of the

fruits in the variant with control fruits – by 0.48–1.33 % and by 0.13–1.41 % in relation to the fruits kept under AC conditions. The increased content of dry matter in the treated fruits compared to the control fruits, found at the end of the 150 day storage period, confirms that the Fitomag preparation slowed down the biodegradation of plastic substances involved in the ripening-senescence processes of the fruits. As a result of the statistical processing of the data (ANOVA test), it was determined that the content of dry substances recorded in the apple fruits at the time of release from storage is primarily determined by the variety, followed by the year-variety interaction and the storage method with percentage contributions as follows: 60.87 %; 15.60 % and 6.77 % with a significant impact for P≤0.001.

## 4. THE POST-HARVEST EFFECT OF THE FITOMAG PREPARATION ON SOME BIOCHEMICAL INDICES OF APPLE FRUIT QUALITY

After harvesting the fruits, the biosynthesis processes are much less intense, predominating those of biodegradation [2]. In order to maintain the quality of horticultural products, the need to apply storage technologies is obvious, the ones which inhibit the pace of these processes.

### 4.1. The effect of the Fitomag preparation on the change in total sugar content during fruit storage

In 2016, when the sum of the active temperatures during the vegetation period was higher than in 2014 and 2015, a higher content of sugar was detected at the time of harvesting (fig. 4.1), the starch being almost completely transformed into sugar, and the content of titratable acids presented low values. This process contributed to the increase of the sugar-acid ratio, which had a negative impact on the storage capacity of the fruits of the Renet Simirenko and Golden Delicious varieties, as physiological disorders were detected in the form of scald [16].

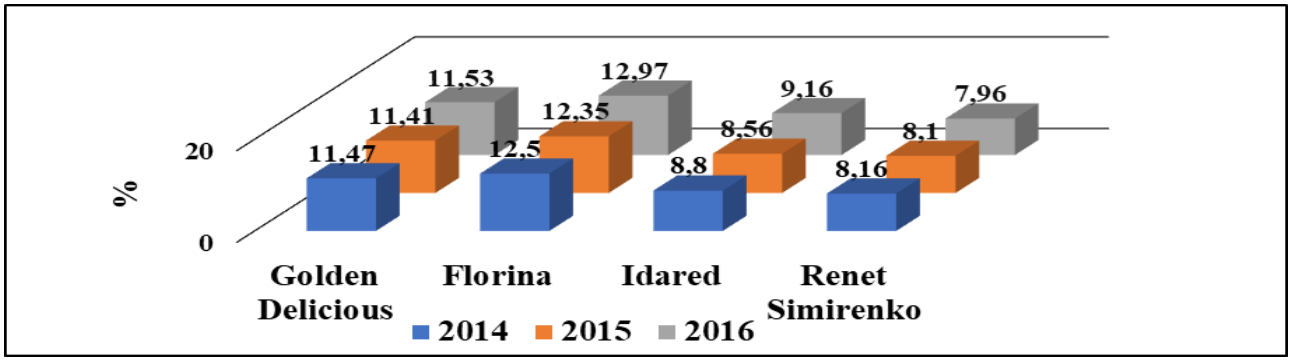


**Figure 4.1. The total content of sugar in apple fruits at the harvest stage, depending on the variety and the weather conditions of the year**

As a result of the statistical processing of the data, it has been laid down that the total sugar content recorded in the apple fruits at the harvest stage is primarily determined by the *variety*, the percentage contribution being 98.87 %, followed by the interaction of the factors *year-variety* and *year* with shares of 0.57 % and 0.42 % respectively. All sources of variation analyzed have a significant impact for P ≤ 0.001 (tab. 4.1).

**Table 4.1. Analysis of variance of total sugar content at stage of harvesting (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| Year (A) | 0,531539 | 2 | 0,265769 | 36,17*** |
| Variety (B) | 124,267 | 3 | 41,4224 | 5637,83*** |
| Interaction AB | 0,711239 | 6 | 0,11854 | 16,13*** |
| Total | 125,686 | 35 | | |

*Note:* ***- significant differences for P≤0.001

In the case of the treated fruits, the metabolic processes took place more slowly, the intensity of the respiration process was reduced, thus slowing down the biodegradation processes of sugars [14]. The investigated biochemical index recorded higher values at the end of storage compared to the untreated fruits, stored under the same conditions, proof of their increased vitality and storage capacity (tab. 4.2). In the fruits of the Florina and Idared varieties, both during storage and towards the end of storage, no significant differences have been detected in the content of total sugars, the control fruits showing even higher values during the first 3 months of storage. Most likely, in the case of the given varieties, the preparation led to a more pronounced slowing down of the intensity of the ripening processes. This was confirmed by the slower increase in the total content of sugar in the treated fruits, compared to the higher rates of increase in the content of sugars in the control version, due to the more advanced degree of starch hydrolysis, simultaneously with the decrease in the content of titratable acids. Later, after 4 months of storage, the fruits of the Idared variety showed an equalization of the total content of sugar. This legality was practically manifested until the end of the storage period, the treated fruits presenting at the time of release from storage an insignificant advantage compared to the control variant, by only 0.13 %. In the fruits kept under CA conditions, no essential changes have been detected compared to the initial content. The fruits of the Idared and Renet Simirenko varieties at the end of storage showed higher values compared to the initial content, most likely because their source of accumulation was some polysaccharides [14]. In the specialized literature [28; 35; 39] there are conflicting opinions regarding the correlation of the keeping ability of the fruits of many apple varieties with the consumption of sucrose in them during cold storage (the lower the consumption of sucrose, the more losses during storage are insignificant). In the case of our research, some varieties with higher values in the sucrose content showed a higher amount of affected fruits towards the end of storage, while others with a lower content were highlighted by a higher storage capacity (tab. 4.2), a fact that allows us not to consider sucrose as an internal factor for maintaining the quality of apple fruits.

**Table 4.2. The dynamics of changes in the total content of sugar, depending on the technology of storage fruits of different apple varieties, %**

| Variety | The technology of storage | X | | | XI | | | XII | | | I | | | II | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | mono- | sucrose | **sum** | mono- | sucrose | **sum** | mono- | sucrose | **sum** | mono- | sucrose | **sum** | mono- | sucrose | **sum** |
| **Golden Delicious** | **NA+control** | 8,05 | 3,42 | 11,47±0,07 | 8,39 | 3,63 | 12,02±0,02 | 9,02 | 3,71 | 13,20±0,19 | 9,30 | 2,93 | 12,53±0,02 | 10,15 | 2,09 | 12,24±0,25 |
| | **NA+Fitomag** | | | | 8,39 | 3,65 | 12,04±0,40 | 9,87 | 3,86 | 14,31±0,03 | 10,24 | 3,07 | 13,83±0,11 | 10,16 | 2,39 | 12,55±0,09 |
| | **CA+control** | | | | | | | | | | | | | | | 11,41±0,15 |
| DL, 5% | | | | | | | | | | | | | | 8,07 | 3,34 | 0,16 |
| **Florina** | **NA+control** | 8,57 | 4,40 | 12,61±0,06 | 8,43 | 3,54 | 11,97±0,09 | 8,55 | 3,72 | 12,27±0,13 | 9,30 | 2,96 | 12,26±0,21 | 9,81 | 1,90 | 11.71±0,17 |
| | **NA+Fitomag** | | | | 8,12 | 3,52 | 11,64±0,16 | 8,30 | 3,90 | 12,29±0,03 | 9,27 | 3,03 | 12,30±0,19 | 10,14 | 1,98 | 12,12±0,25 |
| | **CA+control** | | | | | | | | | | | | | 8,69 | 3,14 | 11,83±0,06 |
| DL, 5% | | | | | | | | | | | | | | | | 0.05 |
| **Idared** | **NA+control** | 6,59 | 2,25 | 8,84±0,07 | 6,91 | 2,37 | 9,28±0,18 | 7,30 | 2,64 | 9,94±0,05 | 7,54 | 2,15 | 9,69±0,23 | 8,50 | 1,62 | 10,12±0,07 |
| | **NA+Fitomag** | | | | 7,25 | 2,68 | 9,93±0,15 | 7,06 | 2,62 | 9,68±0,01 | 7,47 | 2,22 | 9,69±0,13 | 8,50 | 1,75 | 10,25±0,14 |
| | **CA+control** | | | | | | | | | | | | | 7,92 | 1,86 | 9,78±0,33 |
| DL, 5% | | | | | | | | | | | | | | | | 0,18 |
| **Renet Simirenko** | **NA+control** | 6,16 | 1,91 | 8,07±0,03 | 7,79 | 2,11 | 9,90±0,05 | 7,89 | 2,17 | 10,06±0,19 | 7,88 | 1,68 | 9,56±0,31 | 9,08 | 0,88 | 9,96±0,10 |
| | **NA+Fitomag** | | | | 7,73 | 2,11 | 9,84±0,13 | 8,12 | 2,18 | 10,30±0,22 | 8,06 | 1,77 | 9,83±0,10 | 9,10 | 1,08 | 10,18±0,07 |
| | **CA+control** | | | | | | | | | | | | | 7,86 | 1,72 | 9,58±0,10 |
| DL, 5% | | | | | | | | | | | | | | | | 0,10 |

It was found that at the time of removal from storage, the treated fruits contain a greater reserve of sugar, their total content exceeding by 0.22–0.41 % the values recorded in the control variant and by 0.29–1.14 % the values recorded in those kept under CA conditions. With the highest degree of decrease in the content of sugar, the fruits of the Florina variety in the control variant were highlighted, registering a decrease of 7.14 % compared to the initial content [14]. As a result of the statistical processing of the data through three-factor analysis, it has been determined that the change in the total content of sugar in the fruit is primarily determined by the *variety*, followed by the *year-variety* interaction and the *storage technology* with percentage contributions as follows: 76.27 %; 9.53 % and 5.01 % with a significant impact for P≤0.001 (tab. 4.3).

**Table 4.3. Analysis of the variance of the total sugar content at the removal from storage stage (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| Year (A) | 0,194013 | 2 | 0,0970065 | 13,56*** |
| Variety (B) | 108,51 | 3 | 36,17 | 5055,47*** |
| Storage technology (C) | 7,13115 | 2 | 3,56557 | 498,36*** |
| Interaction AB | 13,5651 | 6 | 2,26085 | 316,00*** |
| Interaction AC | 3,89337 | 4 | 0,973343 | 136,04*** |
| Interaction BC | 2,70602 | 6 | 0,451003 | 63,04*** |
| Interaction ABC | 5,76171 | 12 | 0,480143 | 67,11*** |
| Total | 142,277 | 107 | | |

*Note:* ***- significant differences for P≤0.001.

According to the obtained data, we can conclude that the fruit quality reflected by the sugar content was dependent on the biological particularities of the variety and the storage technology.

## 4.2. The influence of the Fitomag preparation on the titratable acidity of fruits

The taste properties of the fruits of several crops and varieties, especially the sweet taste, are due to differences not only in the sugar content, but also in other substances and, first of all, organic acids [34]. At the harvest stage, the lowest value of titratable acidity was recorded in the fruits of the Golden Delicious apple variety, especially in 2016 (fig. 4.2), as a result of unfavorable weather conditions during the vegetation period (high temperatures etc.). This served as one of the causes of more pronounced impairment during storage with physiological disorders.

As a result of the statistical processing of the data, it has been established that the titratable acidity of the apple fruits recorded at the time of harvesting is primarily determined by the *variety*, followed by the *year* and the *year-variety* interaction with percentage contributions as follows: 77.09%; 17.69 % and 4.88 % with a significant impact for P≤0.001 (tab. 4.4).

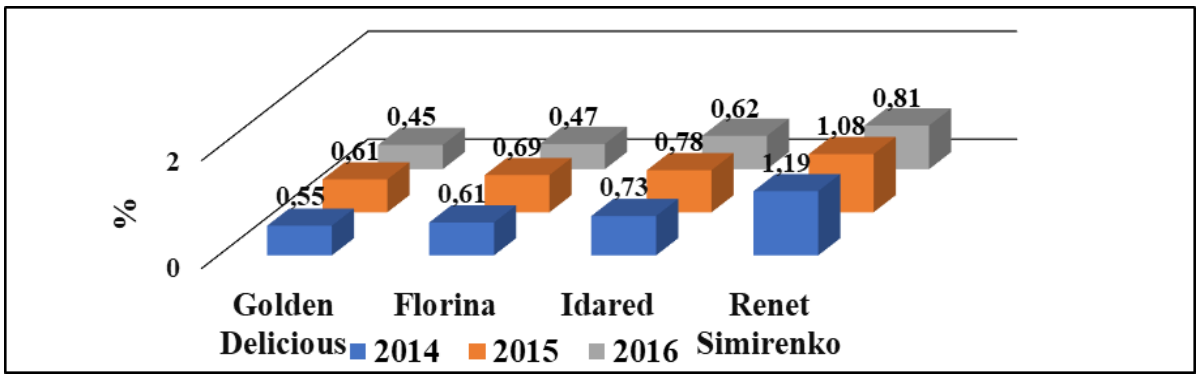


**Figure 4.2. Titratable acidity of apple fruits at the harvest stage, depending on the variety and the weather conditions of the year**

**Table 4.4. Analysis of variance of titratable acidity (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| *Stage: Harvesting* | | | | |
| Year (A) | 0,297772 | 2 | 0,148886 | 623,24*** |
| Variety (B) | 1,29783 | 3 | 0,43261 | 1810,93*** |
| Interaction AB | 0,08222278 | 6 | 0,0137046 | 57,37*** |
| Total | 1,68356 | 35 | | |
| *Stage: Removal from storage* | | | | |
| Year (A) | 0,674022 | 2 | 0,337011 | 1126,85*** |
| Variety (B) | 4,06611 | 3 | 1,35537 | 4531,88*** |
| Storage technology (C) | 0,658489 | 2 | 0,329244 | 1100,88*** |
| Interaction AB | 0,319741 | 6 | 0,0532901 | 178,18*** |
| Interaction AC | 0,0214722 | 4 | 0,00536806 | 17,95*** |
| Interaction BC | 0,0577185 | 6 | 0,00961975 | 32,17*** |
| Interaction ABC | 0,0507426 | 12 | 0,000299074 | 14,14*** |
| Total | 5,86983 | 107 | | |

*Note:* ***- significant differences for P≤0.001.

Both in the untreated fruits, kept in conditions with NA, and in the case of those treated with the Fitomag preparation, kept in the same conditions, there has been a tendency to decrease titratable acidity values (fig. 4.3). According to the content of titratable acids, the control fruits of the Golden Delicious variety were characterized by their intense biodegradation during the storage period, which explains to a large extent the appearance of physiological disorders in the form of *scald* [21]. The minimum level of titratable acidity, recorded in this variety at the time of release from storage, reached the value of 0.22 % (year 2017). At this critical level, the fruits were strongly affected by physiological disorders. The fruits of the Renet Simirenko variety at the time of harvest were distinguished by the highest value of titratable acidity, varying in the range of 0.81–1.19 % during the years of research. However, during storage, acidity decreased the most. In 2017, with a content of titratable acids at the end of storage of 0.52 %, the fruits of this variety were affected by *scald* in a proportion of 2.35 %, and in 2016, with a content of titratable acids of 0.93 % , affected within the limit of 1%. The untreated fruits of the Idared variety were characterized by moderate biodegradation of organic acids, which explains the absence of physiological disorders in them [21]. When the treated fruits were released from storage, a higher content of

titratable acids was recorded compared to the control variant, constituting an advantage of 0.03 %–0.16 %, depending on the variety. In the control variant, a greater amount of titratable acids were consumed in the fruits of the Florina and Renet Simirenko variety, which explains the appearance of the browning of the fruit tissues during storage [21].

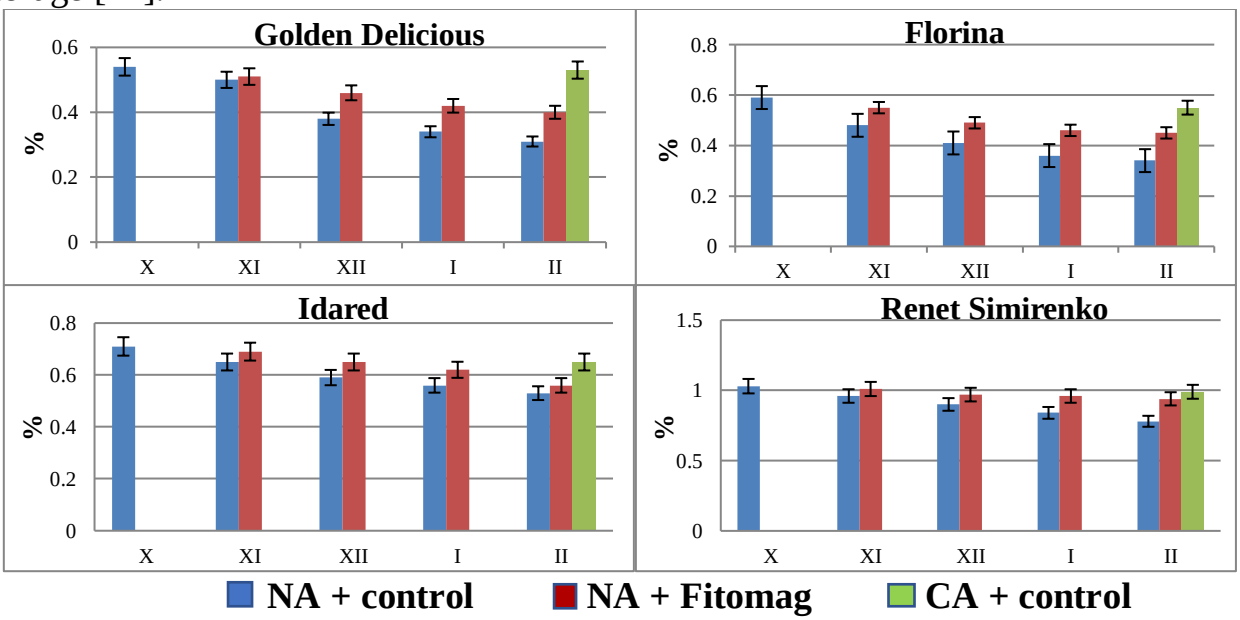


**Figure 4.3. The dynamics of the change in titratable acidity of apple fruits depending on the storage technology, % (expressed in malic acid)**

Depending on the variety, the content of titratable acids in the fruits stored under CA conditions exceeded by 0.05–0.13 % the values recorded in the variant with treated fruits and by 0.20-0.22 % the values recorded in the control fruits [21].

The results of the statistical processing of the data have shown that all the sources of variation analyzed have a significant impact for $P \leq 0.001$ (tab. 4.4), showing that the priority role in changing the titratable acidity of the fruits of the apple varieties taken in the study is held by the *variety* with a percentage contribution of 69.27 %, the legality also being valid at the time of fruit harvesting. Next comes the *year* (conditioned by the weather conditions during the vegetation period) and the *storage technology* with rates of 11.48 % and 11.22 % respectively. Analyzing titratable acidity values in fruits during the storage period, it can be noted that this is a genetic indicator of the variety, but which can vary from year to year depending on the biological particularities of the variety and storage technology [21].

Fruit taste is determined not so much by the absolute content of organic acids and sugars, but by the ratio of these substances in the fruit [30; 37], which finally leads to the realization of the charac-teristic taste for the respective variety [5]. During these researches, the sugar-acid ratio was considered as a diagnostic indicator of fruit quality in the storage process. It is considered that the optimal value of the sugar-acid ratio, which determines the harmonious sweet-sour taste, is 15–20 un. [41]. The highest value of the researched index at the time of removal from storage, depending on the variety, was detected in the control fruits, stored in conditions with NA (12.77–39.48), being higher than that of the fruits of the variant treated with 0.79–8.11 units, respectively with 3.09–17.95 in the case of fruits kept in CA conditions, which indicates that the

ripening processes in the control fruits proceeded with a greater intensity [16] . A level higher than 20 units at the time of removal from storage was registered to the Golden Delicious variety, storing by applying the 3 storage technologies. At this level, the fruits were characterized by a sweeter taste, but at the same time also by the lack of a specific taste, for which reason they were also given lower notes at the tasting. Through a lower value of the gluco-acid index of 15 units the control and treated fruits of the Renet Simirenko variety, kept in conditions with NA, were characterized, being characteristic of a weak-sour taste, which determines the sour taste of the fruits of this variety. The fruits of the same variety, kept in CA conditions (9.68 units) were characterized by a sour taste (gluco-acid index<10). The fruits of the Idared apple variety corresponded to the level between 15–20, in the case of all 3 storage technologies applied, achieving a pleasant-balanced taste, characteristic of the variety [18].

**4.3. The dynamics of changes in the content of ascorbic acid in fruits.**

The dependence of the ascorbic acid content on weather conditions, especially on the thermal regime during the growing season, is significant and is expressed by the decrease in its content in 2016 compared to 2014 for the Florina variety by 6.51 %, for the Idared variety by 4.41 %, for the Renet Simirenko variety with 4.36 % and respectively for the Golden Delicious variety with 3.64 % (fig. 4.4).

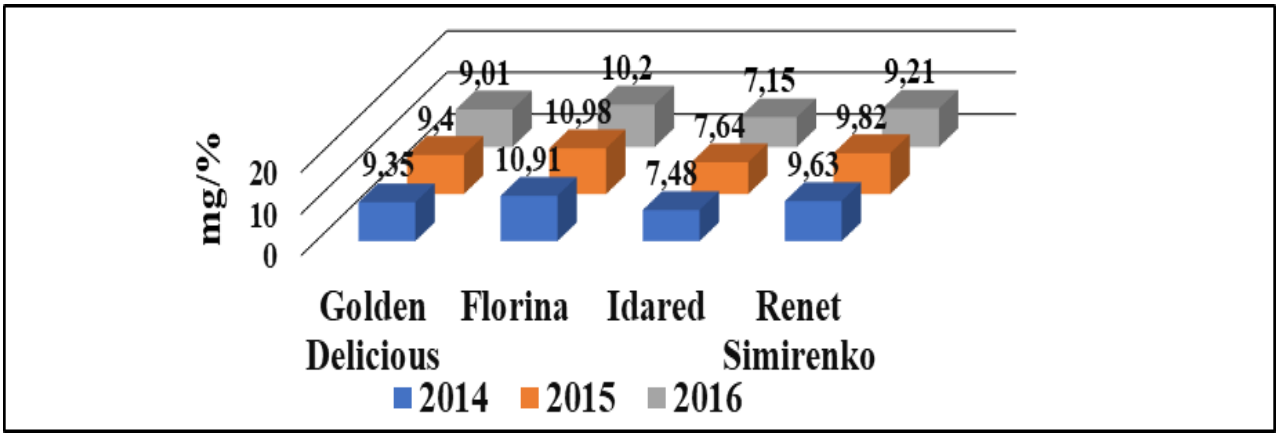


**Figure 4.4. The content of ascorbic acid in apple fruits at the time of harvest, depending on the variety and the weather conditions of the year**

In the years (2014; 2016) with high temperatures and lack of humidity during the growing season, the accumulation of vitamin C and phenolic substances was significantly lower, at the time of fruit harvesting low values of the ascorbic acid content were recorded, which which negatively influenced their storage capacity [17; 36]. As a result of the statistical processing of the data, it was determined that the ascorbic acid content recorded in the fruits at the harvest stage is primarily determined by the *variety*, the percentage contribution being 95.10 %, followed by the *year* with a share of 4.09 % with a significant impact for $P \leq 0.001$ (tab. 4.5). It should be noted that at a great distance in the direction of decreasing contribution (only 0.39 %, for $P \leq 0.01$) the impact of the *year-variety* interaction is highlighted.

**Table 4.5. Analysis of variance of ascorbic acid content (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| *Stage: Harvesting* | | | | |
| Year (A) | 2,13536 | 2 | 1,06768 | 116,86*** |
| Variety (B) | 49,6304 | 3 | 16,5435 | 1810,78*** |
| Interaction AB | 0,201 | 6 | 0,0335 | 3,67** |
| Total | 52,219267 | 35 | | |
| *Stage: Removal from storage* | | | | |
| Year (A) | 0,369369 | 2 | 0,184684 | 352,40*** |
| Variety (B) | 23,2693 | 3 | 7,75644 | 14800,27*** |
| Storage technology (C) | 84,7638 | 2 | 42,3819 | 80870,08*** |
| Interaction AB | 0,334061 | 6 | 0,0556769 | 106,24*** |
| Interaction AC | 2,14122 | 4 | 0,535305 | 1021,43*** |
| Interaction BC | 4,62322 | 6 | 0,770537 | 1470,28*** |
| Interaction ABC | 6,79504 | 12 | 0,566253 | 1080,48*** |
| Total | 122,334 | 107 | | |

*Note:* ** - significant differences for P≤ 0.01; ***- significant differences for P≤0.001.

In the case of the treated varieties, the significant decrease in the intensity of respiration, enzyme activity and other metabolic processes, contributed to the reduction of vitamin C losses, compared to the untreated ones [18; 23; 36] (fig. 4.5).

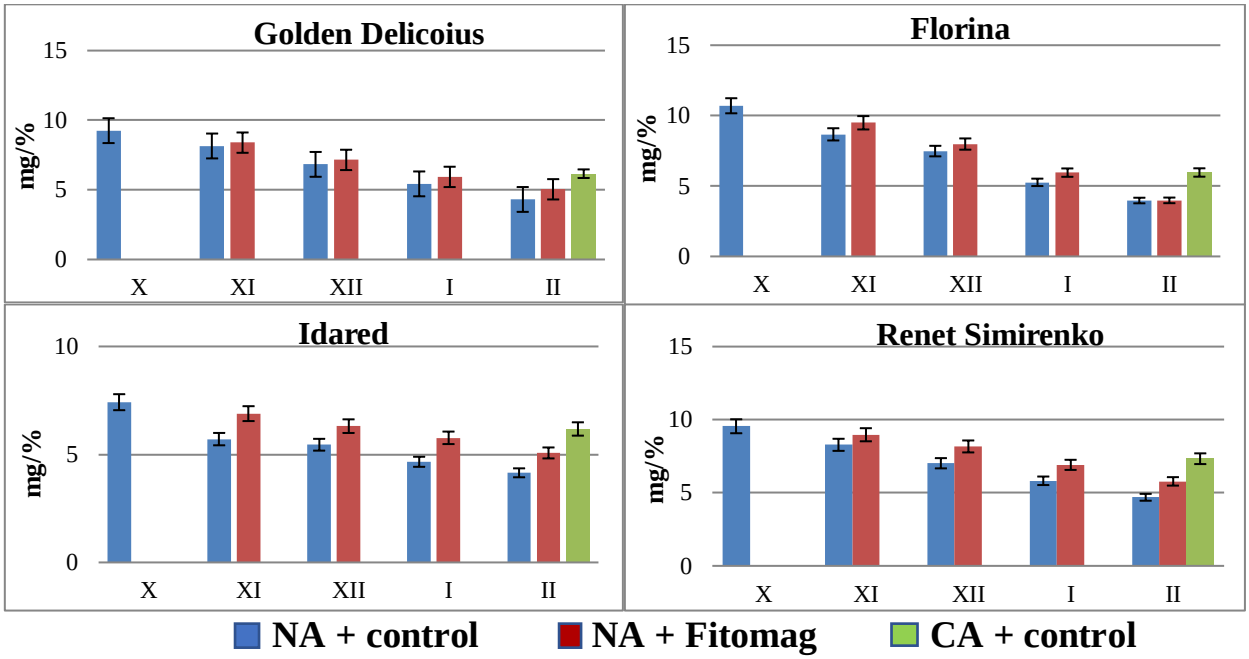


**Figure 4.5. The dynamics of changes in the content of ascorbic acid in apple fruits, depending on the storage technology, mg/%**

At the time of the release of the fruits from storage, depending on the researched variety, a higher content of ascorbic acid - from 5.96 to 7.33 mg/% was detected in the

fruits stored under CA conditions [36], followed by those treated with the Fitomag preparation, the ascorbic acid content showing higher values by 0.01–1.09 mg/% compared to the fruits of the control variant [13]. In the case of all three storage technologies, a slower biodegradation of vitamin C was attested in the fruits of the Idared variety, showing a decrease in the limits of 16.71 to 44.07 % compared to the initial content during the 150 days of storage , they also recorded the lowest breathing intensity during the storage period. A higher consumption of ascorbic acid was detected in the case of control fruits of the Florina variety, storing in conditions with NA – a decrease of 62.90 % compared to the initial content. Even if the fruits of the Florina apple variety did not show the highest intensity of respiration throughout storage, they nevertheless recorded the highest degree of biodegradation of vitamin C. One reason could be the fact that the fruits of the given variety recorded during the first 3 months of storage, the highest activity of the enzyme polyphenoloxidase [18], this probably manifesting a more pronounced action on the oxidation of the vitamin, because vitamin C is oxidized primarily by the enzymes polyphenoloxidase and ascorbinoxidase [2; 39].

As a result of the statistical processing of the data, it has been determined that the change in the content of ascorbic acid in apple fruits is primarily determined by the *storage technology*, followed by the *variety* and the *year-variety-storage technology* interaction with percentage contributions as follows: 69.29 % ; 19.02 % and 5.55 % with a significant impact for $P \leq 0.001$ (tab. 4.5). Therefore, the storage of fruits by applying the Fitomag preparation after harvest slows down the ripening-senescence processes of the fruits, which contributes to a lower consumption of vitamin C.

### 4.4. The influence of the polyphenols-polyphenoloxidase system on the immunity and quality of fruits in the storage process

*The polyphenols-polyphenoloxidase system* induces the beginning of the hypersensitive reaction, when the products of polyphenol oxidation localize the focus of the infection and further protect the fruit tissue from pathogens [1]. During the first 8 weeks of storage, as a result of the slowing down of metabolic processes, a decrease in the activity of the enzyme polyphenoloxidase (PFO) was attested, this process being characteristic of all the varieties studied (fig. 4.6). Next, as a result of the intensification of the oxidation-reduction processes, the activity of the researched enzyme gradually increased, this tempo being maintained until the end of storage, with more pronounced decreases, starting from February in the case of the Golden Delicous and Florina varieties, when the fruits were fully ripen. The highest activity of the enzyme was recorded during the climacteric period of the respiration process and during fruit senescence (January–February), but in these intervals, with the decrease in the amount of phenolic substances, the fruits were more sensitive to being affected by various diseases. It should be noted that during the senescence phase of fruits, ethylene determines the intensificaation of the activity of several enzymes [5].

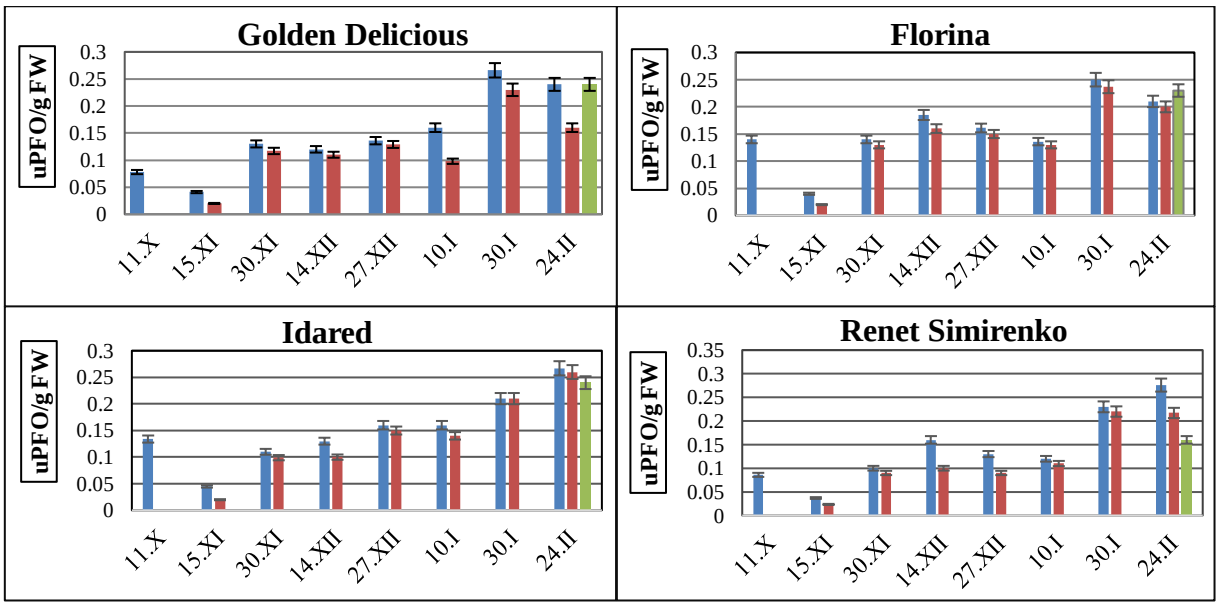


**Figure 4.6. Polyphenoloxidase activity in apple fruits during the postharvest period, depending on the applied storage technology**

It was highlighted that in the treated fruits the intensity of the oxidation processes is significantly reduced, registering a lower level of PFO activity, compared to the control fruits [18]. During the period of intensification of PFO activity, the smallest losses of polyphenols were found, as they were less exposed to fungal diseases and physiological disorders [17]. Storage for 5 months led to an increase in polyphenol oxidase activity in the control fruits by 1.5–3.21 times, respectively in the treated ones by 1.43–2.52 times, depending on the variety.

The unfavorable conditions during the growing season of 2016, when high temperatures were recorded, had a significant influence on the quality of the fruits during storage, which had a negative impact on the total polyphenol content, subsequently also recording heavy rains a month before harvest, as well as during harvest. Consequently, during storage, the fruits showed more visible signs of fungal disease and physiological disorders, especially scalding. The maximum amount of polyphenolic substances in fruits at the time of harvest was detected in 2015, when a relatively increased amount of precipitation was recorded against the background of a low amount of active temperatures.

It is known that the process of fruit ripening stimulates the synthesis of phenolic substances. At the beginning of the storage period, a maximum accumulation of polyphenols was recorded in the control fruits, stored in conditions with NA, as a result of the increased intensity of the ripening processes, so that the maximum accumulation of polyphenols occurred earlier [17]. The higher level of accumulation of phenolic substances in the first 6 weeks in the fruits of the control variant can be explained by the fact that there were no factors (ethylene inhibitor, increased $CO_2$ content, etc.) to inhibit their synthesis. The ripening processes took place more intensively, i.e. a greater amount of phenolic substances was accumulated, while the synthesis of polyphenols was inhibited in the treated fruits. However, even at this level the control fruits were not protected from the *scald*.

At the end of the storage period, the highest amounts of phenolic substances were recorded in the fruits of 3 varieties, stored in CA conditions (fig. 4.7). The exception was the fruits of the Golden Delicios variety, which also showed a higher consumption of phenolic substances, in relation to the treated fruits and even the untreated ones, registering a decrease of 47.40 % compared to the initial content [17]. The situation is justified by the fact that in controlled atmosphere conditions the increased level of $CO_2$ (5 %) and low $O_2$ (3 %) is a factor that significantly inhibits the accumulation of antioxidants in the fruits of this variety, and phenolic substances compared to other preparations are more visibly subject to changes during storage [31].

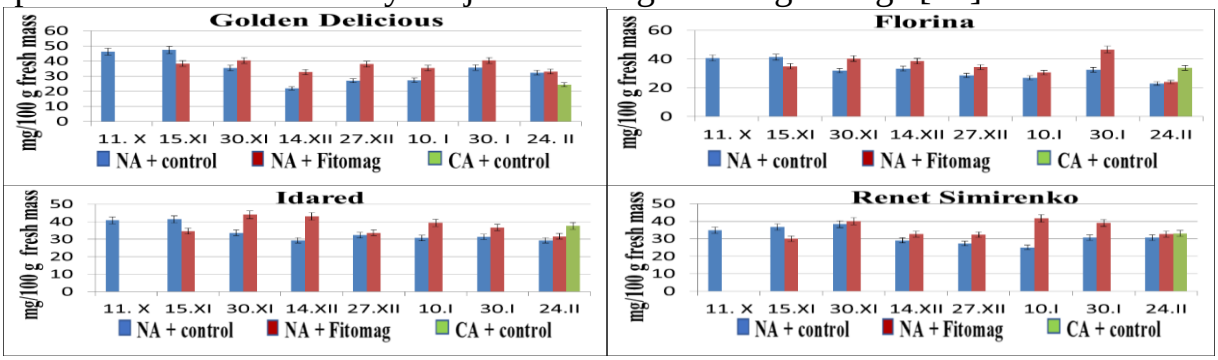


**Figure 4.7. The dynamics of changes in the total content of phenolic substances in apple fruits, depending on the storage technology**

In the case of the fruits treated with the Fitomag preparation, as a result of the slowing down of the ripening processes, the inhibition of the synthesis of ethylene and phenols, the preservation of polyphenols was ensured, but to a lesser extent than in conditions with CA. During the first 8 weeks of storage, there was a slight tendency to accumulate phenolic substances, which denotes the ability of the Fitomag preparation to preserve the antioxidant complex, followed finally by an insignificant decrease during the storage process. In the second half of the storage period, with the intensification of fruit senescence processes, the amount of phenolic substances increased. After this period, in general, a decrease was attested, more pronounced towards the end of storage, in the phase when the senescence period occurred (fig. 4.7). At the time of removal from storage, the total content of phenolic substances in the version with the application of the preparation exceeded by 0.67–2.33 mg/100 g fresh mass the values recorded in the control version, depending on the variety [17]. The received data indicate that through the influence of exogenous factors, such as ethylene biosynthesis inhibitory substances, the intensity of the oxidation processes occurring in the fruit can be modulated. In the fruits treated with the Fitomag preparation, the oxidation-reduction processes took place more slowly, which had a positive influence on their quality and resistance to various deteriorations.

## 5. FRUIT QUALITY AND THEIR RESISTANCE TO LOSSES DEPENDING ON STORAGE TECHNOLOGY

Losses in the storage process are grouped into the following categories [39]: 1) weight loss, due to the normal development of metabolic processes (respiration, transpiration); 2) qualitative impairments, caused by mechanical damage, fungal diseases and physiological disorders.

### 5.1. The degree of dehydration of apple fruit tissues

The lowest degree of tissue dehydration was found in the fruits of the Florina variety (0.54 %), stored in CA conditions, and the highest values were found in the control fruits of the Golden Delicious variety, reaching the level of 6.44 % [12] (fig. 5.1).

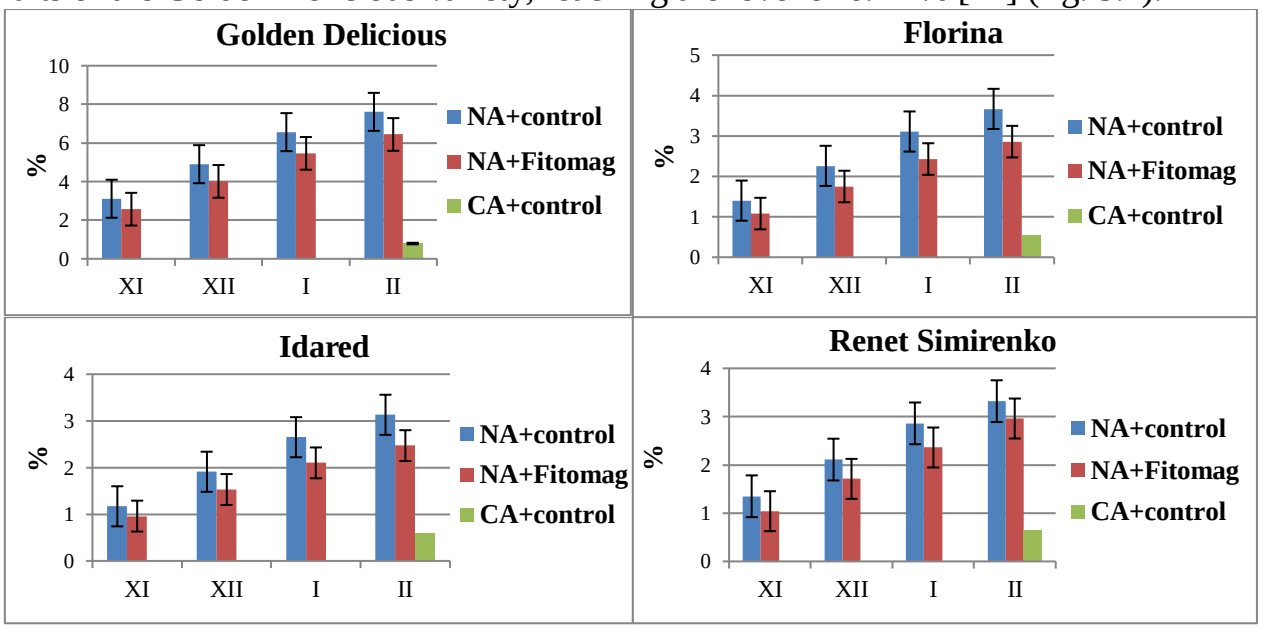


**Figure 5.1. The dynamics of the degree of dehydration of the fruit tissues, depending on the storage technology**

The increased intensity of ripening processes in the control fruits correlated with the degree of dehydration of the tissues, with higher values being recorded by 0.36–1.17 % in relation to the fruits treated with the Fitomag preparation and by 2.55–6.82 % compared to those kept under CA conditions. Analyzing the obtained results, we can state that the process of removing water from apple fruits, in addition to the climatic conditions during the vegetation period and the biological particularities of the variety, is largely conditioned by the storage technology applied.

### 5.2. Quality deterioration of fruits during storage

***Fungal diseases and physiological disorders in apple fruits, depending on genotype and storage technology.*** In the fruits of the Idared variety, no physiological disorders have been detected, they present an increased potential for preserving quality during the post-harvest period. A more significant damage with physiological disorders was recorded of the Rennet Simirenko variety, which were more pronounced than the fungal diseases. Predominant in the case of the given variety was *bitter pit*, detected in the case of all 3 storage technologies studied [20]. Losses caused by this disease have been recorded only in the case of this variety. As a result of treating the fruits with the Fitomag preparation, the losses caused by this disease were reduced by 88.02 %, compared to the untreated fruits. In the fruits of the Renet Simirenko variety, the highest degree of *scald* damage was also attested – 1.11 %, detected only in the control fruits [20]. In 2016, already after 50 days of storage, the Golden Delicious and Renet Simirenko varieties stood out for this physiological condition. The development of this disease during storage was most likely also facilitated by the stressful weather

conditions during the vegetation period, the sum of the active temperatures being higher compared to the first two years of research, and abundant rains were subsequently recorded with a month of days until harvest as well as during harvest. Because of this, the harvest was delayed by a few days, which ultimately led to an increase in the turgidity of the cells, the Fitomag preparation in this case presenting a lower effectiveness [20]. These aspects must be taken into account by the engineers - technologists within the refrigeration complexes, when the fruit treatment and realization scheme is planned. Also, in the dynamics of the research, it was established that the total content of phenolic substances decreases significantly with the appearance of the *scald* on the surface of the fruits and its increase in intensity.

The treated fruits, stored under the same conditions as the control variant, were also affected by *withering*, but to a lesser extent, the intensity of the withering process decreasing by 4.35 times [20] (fig. 5.2).

**a)**  **b)** 


**Figure 5.2. Withered apples, Golden Delicious variety: (a) - fruit from the control variant; (b) - fruit treated with the Fitomag preparation**

Losses caused by fungal diseases have been recorded in all investigated varieties. The most aggressive fungal disease whose pathogens affected fruits was gray mold (*Botrytis cinerea Pers*.) [10; 20]. Insignificant losses caused by pathogenic diseases to the fruits of the Idared apple variety, and Renet Simirenko was characterized as a less resistant variety [10].

***Standard fruits.*** The study carried out by three-factor dispersion analysis at the stage of fruit removal from storage, highlights the impact of the analyzed factors and their interactions on the share of standard (healthy) fruit (tab. 5.1). In the control version, the amount of standard fruits was reduced by 0.8–12.48 % compared to the version with the application of the Fitomag preparation, respectively by 0.81–4.66 % compared to the version stored under CA conditions [20]. As a result of the statistical processing of the data, it was established that the share of standard fruits is primarily determined by the *storage technology* applied, followed by the *variety* and the interaction of the *variety-storage technology* with percentage contributions as follows: 36.97 %; 27.18 % and 25.41 % with a significant impact for $P \leq 0.001$ (tab. 5.1). At a great distance in the direction of decreasing contribution (only 1.33%, for $P \leq 0.01$), the impact of the year is highlighted, conditioned by the weather conditions preceding the moment of fruit harvesting, while the other interactions do not show statistical significance.

**Table 5.1. Analysis of variance of standard fruit share at the stage of removal from storage (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| Year (A) | 39,7283 | 2 | 19,8642 | 6,76** |
| Variety (B) | 812,742 | 3 | 270,914 | 92,22*** |
| Storage technology (C) | 1105,48 | 2 | 552,738 | 188,14*** |
| Interaction AB | 24,0347 | 6 | 4,00578 | 1,36$^{ns}$ |
| Interaction AC | 18,7246 | 4 | 4,68116 | 1,59$^{ns}$ |
| Interaction BC | 760,014 | 6 | 126,669 | 43,12*** |
| Interaction ABC | 18,2296 | 12 | 1,51914 | 0,52$^{ns}$ |
| Total | 2990,47 | 107 | | |

*Note:* **- significant differences for P≤ 0.01; ***- significant differences for P≤ 0.001;
ns- not significant value

***The structural-textural firmness of the fruits.*** In the conditions of the experiments, the average temperature between May and September of 2016 was higher by 1.5°C compared to the same period of 2014, and according to specialized literature, the high temperatures during fruit conditions the reduction of the level of fruit firmness [27], as a result of the metabolic changes that lead to the loss of the integrity of the membranes [8]. It was established that the firmness of the apple fruits recor-ded at the time of harvest is primarily determined by the interaction of the *year-variety* factors, the percentage contribution being 54.61%, followed by the *variety* and the *year* with shares of 25.03 % and 5.95 % respectively. The sources of variation analyzed have a significant impact for P ≤ 0.001 (tab. 5.2).

**Table 5.2. Analysis of variance of fruit firmness (ANOVA test)**

| Source of variation | Sum of Squares | Degrees of Freedom | Mean Squares | F-ratio |
|---|---|---|---|---|
| *Stage: Harvesting* | | | | |
| Year (A) | 5,61267 | 2 | 2,80633 | 22,33*** |
| Variety (B) | 23,5953 | 3 | 7,86511 | 62,59*** |
| Interaction AB | 51,4747 | 6 | 8,57911 | 68,27*** |
| Total | 94,2547 | 119 | | |
| *Stage: Removal from storage* | | | | |
| Year (A) | 113,426 | 2 | 56,7129 | 1074,75*** |
| Variety (B) | 51,2305 | 3 | 17,0768 | 323,62*** |
| Storage technology (C) | 74,4921 | 2 | 37,246 | 705,84*** |
| Interaction AB | 42,2047 | 6 | 7,03412 | 133,30*** |
| Interaction AC | 7,69711 | 4 | 1,92428 | 36,47*** |
| Interaction BC | 44,0751 | 6 | 7,34584 | 139,21*** |
| Interaction ABC | 18,0064 | 12 | 1,50054 | 28,44*** |
| Total | 368,229 | 359 | | |

*Note:* ***- significant differences for P≤ 0.001.

The effectiveness of the post-harvest treatment with the Fitomag preparation on fruit firmness was manifested due to the modification of the hydrolytic processes of the polysaccharides in the researched fruits, maintaining their firmness at a higher level towards the end of storage by 0.22–2.20 kg/cm$^2$, compared to the control. After 150 days of storage, the fruits of the Florina variety treated with the Fitomag preparation registered a higher firmness (6.93 kg/cm$^2$) even compared to those stored under CA

conditions (5.90 kg/cm$^2$), which denotes the role of Fitomag in slowing down the ripening-senescence processes (fig. 4.3). Relatively increased firmness (4.73–5.22 kg/cm$^2$) characterized the fruits of the Idared, Renet Simirenko and Golden Delicious varieties, kept in CA conditions, and the lowest (4.08 kg/cm$^2$) fruits witness of the Idared variety, stored in NA.

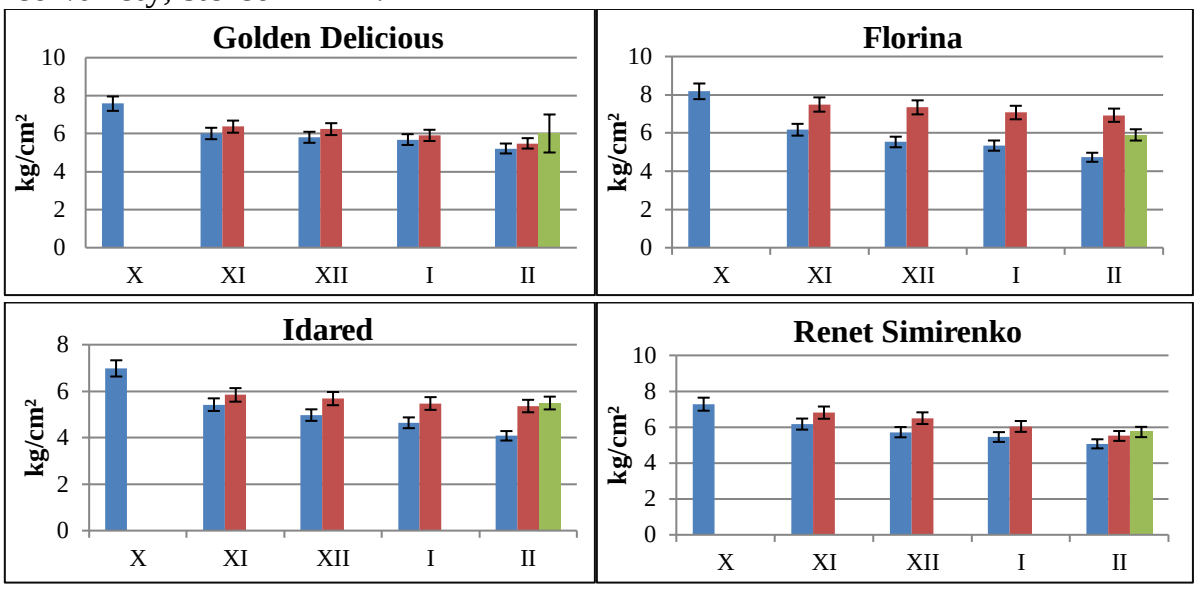


**Figure 5.3. Change in fruit firmness value according to variety and storage technology**

The results of the statistical processing of the data (ANOVA test) have shown that all the analyzed sources of variation have a significant impact for P≤0.001 (tab. 5.2), highlighting that the priority role in changing the fruit firmness index is held by the *year* (conditioned by the weather conditions during the vegetation) with a percentage contribution of 30.8 %, followed by *storage technology* and *variety* with shares of 20.23 % and 13.91 % respectively.

**5.3. Implementation of scientific results**

The researches were carried out in the cold storages of the agricultural company "Fortina-Labis SRL" (Floreni, Ungheni district), the volume of the studied production constituting 8 tons of fruit (4 t. - treated with Fitomag at the beginning of storage; 4 t. untreated fruit). Treated and untreated fruits were stored for 150 days under conditions with NA at a temperature of 1°C and RAH (relative air humidity) – 85–90 %. At the time of removal from storage, the amount of weight loss in the apple fruits taken in the study varied depending on the storage technology and the biological particularities of the variety, from 1.97 % to 7.95 %. The increased intensity of the ripening processes in the control fruits (untreated) correlated with the degree of tissue dehydration, which was increased by 0.19–4.29 % compared to the fruits treated at the beginning of storage with the Fitomag preparation. In the variant with untreated fruits, the amount of standard fruits was reduced by 0.83–11.71 % compared to the variant with the application of the Fitomag preparation (tab. 5.3).

**Table 5.3. The effectiveness of Fitomag technology in the process of keeping fruits in cold storages with increased capacity** (SRL „Fortina-Labis", Floreni commune, Ungheni district)

| Indicators | Golden Delicious | | Florina | | Idared | | Renet Simirenko | |
|---|---|---|---|---|---|---|---|---|
| | un-treated | treated | un-treated | trea-ted | un-treated | treated | un-treated | treated |
| **Stored for safekeeping, kg** | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| **Weight loss, %** | 7.95 | 6.30 | 3.42 | 3.24 | 2.32 | 1.97 | 2.20 | 2.01 |
| **Standard fruits, %** | 91.67 | 94.17 | 93.34 | 95.10 | 94.17 | 95.00 | 79.96 | 91.67 |

For a cold room with treated apples, 30 % less electricity was spent on average. This situation can be explained by the fact that, as a result of treating the fruits with the Fitomag preparation, the ripening-senescence processes are slowed down, the intensity of the ethylene emission and the respiration process is decreased, respectively the amount of heat released in the respiration process is significantly decreased. It is known that part of the energy released in the breathing process is eliminated outside in the form of heat. This directly produces temperature fluctuations in the cold room, which is inadmissible in the storage process. In order to eliminate the heat released as a result of the breathing process, cold stores are equipped with ventilation systems. In the case of treated fruit, these ventilation systems and other equipment used to maintain optimal storage conditions do not operate at full capacity, which also reduces electricity consumption.

## GENERAL CONCLUSIONS

1. The complex researches carried out in order to implement an effective system for storing the fruits of 4 late apple varieties (Golden Delicious, Florina, Idared, Renet Simirenko) have highlighted the fact that the storage technology significantly influences the intensity of the ripening-senescence processes in the fruits of apple in the postharvest period.

2. The positive effect of the Fitomag preparation on the suppression of endogenous ethylene emission activity by apple fruits during storage was established. The action of the preparation was most clearly manifested in the Renet Simirenko variety (Paragraph 3.1). The post-harvest application of the Fitomag preparation allows the effective inhibition of ethylene biosynthesis and, as a result, of the fruit ripening process, ensuring quality preservation and their complex protection from many diseases [19].

3. The increased content of plastic substances (total carbohydrates, titratable acids, ascorbic acid) in the fruits treated with the Fitomag preparation, compared to the characteristic one for the untreated fruits, determined at the end of the storage period, suggests that the Fitomag preparation inhibits the metabolism of the biochemical preparations involved in fruit ripening-senescence processes (Chapter 4) [13; 15; 22].

4. It has been demonstrated that the storage method in CA conditions, as well as the one by applying the Fitomag preparation, lead to a decrease in the consumption of phenolic substances, a fact that protects the fruits from disease. In the treated fruits, the intensity of oxidation processes decreased significantly, registering a reduced level of PFO activity (Paragraph 4.4) [17; 18].

5. The main diseases of the fruit of the apple varieties studied were determined, depending on the storage technology: *Scald* (Renet Simirenko and Golden Delicious); *Bitter pit* (Renet Simirenko); *Internal breakdown* (Florina and Renet Simirenko); *Withering* (Golden Delicious); fungal diseases (all varieties studied) (Paragraph 5.2).

6. Harvesting the fruits of the varieties studied at the optimal harvest time ensures the preservation of commercial qualities (firmness, color., etc.) and the reduction of losses caused by physiological disorders and fungal diseases in conditions with NA and CA, in relation to those harvested late. In the case of late harvesting of the fruits of the Renet Simirenko variety, a sudden increase in the losses produced by the process during storage is possible (Paragraph 5.2).

7. At the time of removal from storage, the fruits treated with the Fitomag preparation differ from those of the control variant by a lower level of tissue dehydration, fungal diseases, physiological disorders; more attractive appearance, skin color, crunchiness, juiciness, aroma, freshness and more pronounced taste, phenomena due to which the storage period of the fruits could be extended (Chapter 5) [9; 10–13; 15; 18; 20; 22].

8. Treating apple fruits with the Fitomag preparation contributes to a better preservation of their structural-textural firmness, which ensures safe transportation over long distances, as well as extending the terms of consumption of fresh apples (Paragraph 5.2).

9. The storage technology by applying the Fitomag preparation can compete with that of storage in CA conditions, given the fact that it has a number of advantages: minimal investments for the procurement of equipment, simplicity in application and low consumption of electricity (Paragraph 5.3) [10; 12; 13; 20–22].

10. In general, the obtained data suggest that the storage period of apple fruits can be extended using procedures that ensure the formation of the specific content of metabolites and reserve substances both during cultivation and during the maintenance of the fruit in a dormant state, due to treatment in the post-harvest period with substances that inhibit respiration processes.

## RECOMMENDATIONS

1. In order to increase the effectiveness of the Fitomag preparation, the fruit must be harvested in the optimal period characteristic of the variety.

2. In the fruit storing process, it is necessary to take into account the biological characteristics of the variety, as well as the weather conditions of the year, especially the last month before harvesting.

3. Based on the fact that most of the producers and exporters of fruits in the Republic of Moldova cannot afford the construction of cold storages equipped with modern equipment, it is recommended to apply the storage technology by applying the Fitomag preparation, as an effective and less expensive alternative method.

4. The presented results are recommended to specialists and agricultural producers specializing in long-term storage of fruits, as well as to be implemented in university curricula for higher undergraduate and master studies in the disciplines: *Plant Physiology, Biochemistry and Storage of Agricultural Product*.

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# PUBLICATIONS ON THE TOPIC OF THE THESIS

**Articles in scientific journals (overseas)**

1**. NICUTA, A**. The effect of the postharvest application of Fitomag technology on apple quality. In:*''Scientific Studies and Researches. Biology series*.'', 2020, vol. 29, nr.1, p. 9-13. ISSN 1224-919X (print). ISSN 2457-5178 (online).

2. **NICUTA, A.,** BUJOREANU, N., HAREA, I. Intensity of the apples respiration process depending on the storage technology applied. In:*''Scientific Studies and Researches. Biology series*.'', 2021, vol. 30, nr.1, p. 9-13. ISSN 1224-919X (print). ISSN 2457-5178 (online).

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**Articles in various journals of the National Register of profile journals (category B)**

1. **NICUŢA, A**. Influența preparatului „Fitomag" asupra modificării valorilor unor indici biochimici la fructele de măr pe parcursul perioadei de păstrare îndelungată. In: *Studia Universitatis (Seria Ştiinţe Reale şi ale Naturii)*. 2018, nr. 1(111), pp. 82-85. ISSN 1814-3237.

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1. NICUȚĂ, A. Influenţa preparatului "Fitomag" asupra gradului de afectare a fructelor de măr cu boli fungice şi dereglări funcţionale pe durata perioadei postrecoltare. In*: Ştiinţa în Nordul Republicii Moldova: realizări, probleme, perspective*. Conferința științifică națională cu participare internațională, Ediţia a II-a, 29-30 septembrie 2016. Bălţi: Tipogr. "Foxtrot", 2016, pp. 109-111. ISBN 978-9975-89-029-8.

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**Materials/Theses at scientific forums**

1**.** NICUȚĂ, A. Influenţa preparatului "Fitomag" asupra fructelor de măr pe durata perioadei postrecolte. In: *Ştiinţele vieţii în dialogul generaţiilor: Conexiuni dintre mediul academic, universitar şi de afaceri: mater. conf. intern*., 25 martie 2016, Chişinău: Tipografia „Biotehdesign, p.147. ISBN 978-9975-933-78-0.

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## ADNOTARE

**Nicuţă Alexandru, „*Influența inhibitorului sintezei etilenei Fitomag asupra derulării proceselor de maturare-senescență la fructele de măr*”, teză de doctor în științe biologice, Chişinău, 2023.**

Teza include introducere, 5 capitole, concluzii şi recomandări, bibliografie din 375 titluri, 7 anexe, 132 pagini de text de bază, 12 tabele, 43 figuri. Rezultatele obţinute sunt publicate în 16 lucrări științifice.

**Cuvinte cheie**: fructe de măr, păstrare, *Fitomag*, atmosferă obișnuită, atmosferă controlată, indici fiziologici, indici biochimici, indici biofizici, maturare-senescență, dereglări fiziologice, boli fungice, calitatea fructelor.

**Scopul lucrării:** Evaluarea mecanismelor de acţiune a preparatului *Fitomag* asupra proceselor de maturare-senescență la fructele unor soiuri tardive de măr, ca bază ştiinţifică de optimizare a tehnologiilor de păstrare a fructelor în Republica Moldova.

**Obiectivele cercetării**: 1. Argumentarea ştiinţifico-practică a necesităţii şi oportunităţii aplicării preparatului *Fitomag*, ca tehnologie alternativă modernă de păstrare a fructelor soiurilor tardive de măr, cultivate în condițiile pedoclimatice ale Republicii Moldova; 2. Stabilirea gradului de influenţă a preparatului *Fitomag* asupra intensităţii procesului de respiraţie şi emisiei etilenei, ca indicatori a gradului de maturare, vitezei de biodegradare a substanţelor de rezervă, calităţii şi duratei de păstrare a fructelor de măr; 3. Evaluarea influenței metodei de păstrare asupra activităţii proceselor antioxidante în fructele de măr, prin aprecierea conţinutului de substanțe fenolice şi activităţii enzimei polifenoloxidaza, responsabile de imunitatea fructului; 4. Determinarea influenței aplicării postrecoltă a preparatului *Fitomag* asupra intensităţii proceselor de transformare şi biodegradare a principalilor compuşi biochimici (glucidele, acizii titrabili, acidul ascorbic), ca indicatori de apreciere a capacităţii de păstrare și calității fructelor de măr; 5. Evaluarea influenţei particularităţilor biologice ale soiului şi metodei de păstrare asupra calităţii, gradului de afectare a fructelor de agenţii patogeni şi dereglări fiziologice.

**Noutatea şi originalitatea ştiinţifică**: în baza aprecierii valorii unor parametri fiziologici, biochimici şi biofizici, ca indicatori a gradului de maturare, calităţii şi termenului de păstrare a fructelor, a fost efectuată evaluarea complexă a influenței preparatului Fitomag asupra metabolismului fructelor de măr cultivate în condiţiile R. Moldova.

**Problema științifică importantă soluționată:** a fost argumentat științific și demonstrat experimental efectul postrecoltă al inhibitorului de biosinteză a etilenei *Fitoma*g asupra proceselor de maturare-senescenţă la fructele a 4 soiuri tardive de măr, *ceea ce a contribuit* la elucidarea acțiunii preparatului, *permițând* aprecierea perspectivelor utilizării inhibitorului în tehnologiile de păstrare a fructelor cultivate în condițiile Republicii Moldova.

**Semnificaţia teoretică:** studiul realizat contribuie la completarea cunoștințelor despre particularitățile proceselor de maturare-senescență a fructelor de măr pe durata păstrării în funcție de soi, condițiile meteorologice în perioada de vegetație și tehnologia de păstrare, în particular cu aplicarea postrecoltă a preparatului *Fitomag*.

**Valoarea aplicativă a lucrării**: Tratarea postrecoltă a fructelor de măr cu preparatul Fitomag contribuie la reducerea pierderilor produse de boli fungice şi dereglările fiziologice, la prelungirea perioadei de păstrare și menținerea indicilor de calitate a lor (fermitatea, prospețimea, suculența etc.). Având o acţiune bine conturată asupra desfăşurării proceselor de maturare a fructelor, tehnologia propusă are avantaje economice față de metoda de păstrare în atmosferă controlată, actualmente larg utilizată, dar care este destul de costisitoare. În scopul sporirii eficacității păstrării fructelor, se recomandă de efectuat recoltarea în perioada optimă caracteristică fiecărui soi.

**Implementarea rezultatelor ştiinţifice**: Rezultatele obţinute în perioada anilor de cercetare au fost implementate la complexul de păstrare a SRL „Fortina-Labis” com. Floreni, r-nul Ungheni. Rezultatele expuse în teză pot fi utilizate în calitate de material ştiinţifico-didactic la predarea cursului de Fiziologie vegetală și Păstrarea producţiei agricole.

## ANNOTATION

**Nicuță Alexandru, „Influence of the ethylene synthesis inhibitor Fitomag on the development of the processes of ripening-senescence of apple fruits", PhD thesis in biological sciences, Chisinau, 2023.**

The thesis include introduction, five chapters, general conclusions, and recommendations, 375 bibliographic sources, 7 appendices, 132 basic text pages, 12 tables, and 43 figures. The results obtained are published in 16 scientific papers.

**Keywords**: apple fruits, storage, the ethylene biosynthesis inhibitor *Fitomag*, normal atmosphere, controlled atmosphere, physiological indicators, biochemical indicators, biophysical indicators, ripening-senescence, physiological disorders, fungal diseases, fruits quality.

**The aim of the study:** Evaluation of the mechanism of action of the *Fitomag* preparation on the processes of ripening-senescence of the fruits of some late apple varieties, as a scientific justification for optimizing fruit storage technologies in the Republic of Moldova.

**The objectives of the study:** 1. The scientific-practical argumentation of the necessity and opportunity of applying the Fitomag preparation, as a modern alternative technology for storing the fruits of late apple varieties, grown in the pedoclimatic conditions of the Republic of Moldova; 2. Establishing the degree of influence of the Fitomag preparation on the intensity of the respiration process and the emission of ethylene, as indicators of appreciation of the degree of ripening, the speed of biodegradation of the reserve substances, the quality and the storage period of the apple fruits; 3. Evaluation of the influence of the storage method on the activity of antioxidant processes in apple fruits, by assessing the content of phenolic substances and the activity of the enzyme polyphenoloxidase, responsible for the immunity of the fruit; 4. Determination of the influence of the postharvest application of the Fitomag preparation on the intensity of the transformation and biodegradation processes of the main biochemical compounds (carbohydrates, titratable acids, ascorbic acid), as indicators for assessing the storage capacity and quality of apple fruits; 5. Evaluation of the influence of the biological particularities of the variety and the method of storage on the quality and degree of fruit damage by pathogens and physiological disorders.

**Scientific novelty and originality:** a complex evaluation of the influence of *Fitomag* preparation on the metabolism of apple fruits grown in the conditions of the Republic of Moldova was performed, based on physiological-biochemical and biophysical parameters, as indicators for assessing the degree of ripeness, quality, and shelf life of fruits.

**The important scientific problem solved:** scientifically substantiated and experimentally proved the post-harvest effect of the ethylene biosynthesis inhibitor *Fitomag* on the processes of ripening-senescence in the fruits of 4 late apple varieties, which made it possible to identify the positive effect of the preparation and evaluate the prospects for its use in the storage of fruits grown in the conditions of the Republic of Moldova.

**Theoretical significance** the study contributes to supplementing knowledge about the features of the processes of ripening and senescence of apple fruits during storage, depending on the variety, weather conditions during the growing season and storage technology, in particular with the postharvest use of Fitomag preparation.

**The applicative value of the paper:** the postharvest treatment of apple fruits with the *Fitomag* preparation contributes to the reduction of losses caused by fungal diseases and physiological disorders, prolonging the storage period of apple fruits and preserving their quality (firmness, freshness, juiciness, etc.). Having a pronounced effect on the development of apple fruit ripening processes, this technology can compete favorably with the currently widely used, but rather expensive method of storage in a controlled atmosphere. For the success of fruit storage, it is recommended to harvest fruits at the optimal time, characteristic of each variety.

**Implementation of scientific results:** The results obtained during the study were implemented based on the storage complex of **„**Fortina-Labis**"** Ltd, Floreni village, Ungheni district. The results presented in the dissertation can be used as scientific and methodological material in teaching the courses of Plant Physiology and Storage of Agricultural Products.

## АННОТАЦИЯ

**Никуцэ А, «Влияние ингибитора синтеза этилена Фитомаг на развитие процессов созревания-старения плодов яблони», диссертация на соискание ученой степени доктора биологических наук, Кишинэу, 2023.**

Работа состоит из введения, 5 глав, общих выводов и рекомендаций, библиографии из 375 источников, 7 приложений, 132 страниц общего объема, 12 таблиц и 43 рисунков. Полученные результаты опубликованы в 16 научных публикациях.

**Ключевые слова**: плоды яблони, хранение, ингибитор биосинтеза этилена *Фитомаг,* обычная атмосфера, регулируемая атмосфера, физиологические показатели, биохимические показатели, биофизические показатели, созревание-старение, физиологические расстройства, грибковые заболевания, качество плодов.

**Целью данной работы является:** Изучить особенности действия препарата *Фитомаг* на процессы созревания-старения плодов некоторых поздних сортов яблони, в качестве научного обоснования оптимизации технологий хранения плодов в Республике Молдова.

**Задачи исследования**: 1. Научно-практическое обоснование необходимости и возможности применения препарата Фитомаг, как современной альтернативной технологии хранения плодов поздних сортов яблони, выращенных в педоклиматических условиях Республики Молдова; 2. Выявление степени влияния препарата *Фитомаг* на интенсивность процесса дыхания и выделения этилена, как показателей оценки степени созревания, скорости биодеградации запасных веществ, качества и сроков хранения плодов яблони; 3. Оценка влияния способа хранения на активность антиоксидантных процессов в плодах яблони путем определения содержания фенольных веществ и активности фермента полифенолоксидаза, ответственных за иммунитет плодов; 4. Определение влияния послеуборочного применения препарата *Фитомаг* на интенсивность трансформации и биодеградации основных биохимических соединений (углеводов, титруемых кислот, аскорбиновой кислоты), для оценки качества и лёжкоспособности плодов яблони; 5. Оценка влияния биологических особенностей сорта и способа хранения на качество и степень поражения плодов патогенами и физиологическими расстройствами.

**Новизна и научная оригинальность**: проведена комплексная оценка влияния препарата *Фитомаг* на метаболизм плодов яблони, выращенных в условиях Республики Молдова, на основе физиолого-биохимических и биофизических параметров, с целью определения степени созревания, качества и сроков хранения плодов.

**Решенная научная проблема**: научно обосновано и экспериментально доказано послеуборочное влияние ингибитора биосинтеза этилена *Фитомаг* на процессы созревания-старения в плодах 4 поздних сортов яблони, что позволило выявить положительное действие препарата и оценить перспективы его использования в технологии хранения плодов, выращенных в условиях Республики Молдова.

**Теоретическая значимость**: исследование способствует дополнению знаний о особенностях процессов созревания-старения плодов яблони при хранении в зависимости от сорта, погодных условий в период вегетации и технологии хранения, в частности с послеуборочным применением препарата Фитомаг.

**Прикладная ценность**: Послеуборочная обработка плодов яблони препаратом *Фитомаг* способствует снижению потерь от грибковых заболеваний и физиологических расстройств, продлению срока хранения яблок и сохранению их качества (твердость, свежесть, сочность и др.). Оказывая выраженное влияние на развитие процессов созревания плодов яблони, данная технология может конкурировать с широко применяемым в настоящее время, но дорогим методом хранения в контролируемой атмосфере. Для успеха хранения плодов рекомендуется проводить уборку плодов в оптимальные сроки, характерные для каждого сорта.

**Внедрение научных достижений**: Результаты, полученные в течение исследования, были внедрены на базе комплекса хранения ООО «Фортина-Лабис», село Флорень, Унгенского района. Результаты, представленные в диссертации, могут быть использованы в качестве научно-методического материала при преподавании курсов Физиологии растений и Хранения сельскохозяйственной продукции.

**NICUȚĂ ALEXANDRU**

# INFLUENCE OF THE ETHYLENE SYNTHESIS INHIBITOR FITOMAG ON THE DEVELOPMENT OF THE PROCESSES OF RIPENING-SENESCENCE OF APPLE FRUITS

**164.02. PLANT PHYSIOLOGY**

**Summary of the doctoral thesis in biological sciences**


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