

Influence of agricultural cultivation methods on the physicochemical and colour parameters of Florina apples during storage in conventional refrigerated rooms

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Abstract

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The scientific experiment was conducted at the Department of Food Technology at IFPQ-Plovdiv with the aim of assessing the impact of three-month refrigerated (1-4°C) storage on the physicochemical and colour properties of biologically and conventionally cultivated Florina apples.

The assessment is made on the basis of dry substance content, %; antioxidant activity measured in $\mu\text{molTE}/100\text{ g}$; total polyphenols content in $\text{mg GAE}/100\text{g}$ and colour properties – brightness and intensity of the red/green and yellow hues – as measured by the CieLab system.

Keywords: apples, colour parameters, storage

Introduction

Storage process is designed to preserve fruits for as long as possible after harvest without significant alternations in their physicochemical, biochemical and biological parameters. Fruit preservation of different varieties and sorts is genetically determined; however, it may be affected by the applied storage method (Lichev et al., 2020).

Physiological, technological, ecological and agro technical factors generally influence the quality of stored fruits.

Stored fruit have to be picked from a tree when they are technically ripe. Careful harvesting and handling are important as is eliminating the deceased, damaged and deformed fruits (Fikiin et al., 1975).

At the post-harvest stage, the respiratory, transpiration and ethylene release processes are of particular importance. Breathing leads to dissolving the organic substances to carbon dioxide and water, while transpiration leads

to water loss (Fikiin & Kalinov, 1974; Hardenburg et al., 1994).

Both processes reduce mass and degrade quality and appearance. They are influenced above all by temperature and relative humidity in the air.

Temperature is of crucial importance for extending the length of storage. Lowering the temperature suppresses breathing but it is important to avoid freezing. Critical temperatures vary among different fruit varieties and sorts (for apples, temperatures can vary from -1.5 to -3.0°C). In order to avoid freezing due to uneven air circulation, temperature should not fall below -1.0°C (Yanuk & Bondarev, 1984).

Air humidity of 100% prevents water loss but increases the risk of developing fungal disease. Therefore, humidity of 95-98% is generally recommended. Thin-layer varieties, such as Golden Delicious, are predisposed to wilting and require higher humidity. Other varieties, such as Jonathan, suffer from increased risk of browning in the hypodermic

layer due to high humidity. For fruits of the Florina variety, relative air humidity of 85 to 90% is recommended. There is a negative correlation between temperature and relative air humidity.

A number of ecological factors influence fruit preservability and quality. While it is difficult to assess the weight of each factor individually, either one of them, including soil type, latitude, altitude and temperature fluctuations, etc., may be of significant importance.

Fruit quality and preservability are influenced by the shape of tree crowns, pruning strength and the ratio of leaves to fruits. High fertilization rates and notably one-sided fertilization degrade the fruit preservability and quality.

The current paper aims to determine the influence of a three-month refrigerated storage at 1-4°C on the physicochemical and color properties of Florina apples which are biologically and conventionally cultivated.

Materials and Methods

Fruit cultivation and harvesting methods.

Apples of the Florina variety were biologically and conventionally cultivated on the trial fields of AU – Plovdiv.

Traditional technology is applied in the case of conventionally-cultivated plants. This includes crop-protection interventions (three prophylactic winter interventions against the hibernating forms of the economically-significant diseases, pests, as well as various vegetation ailments), nourishing fertilization, localized drip irrigation and several tillages. The treatments were conducted with specialized equipment for deep and shallow treatment of orchards (Borovinova & Djuvinov, 2013; Todorov et al., 1974). The inter-row spacing was maintained in two ways – through the “black fallow” system and grassing. In connection with the first method, several shallow treatments are performed in the inter-row space which would lead to higher saturation of nutrients, as well as proper air and water exchange. These activities are also instrumental in removing weeds.

Another deep treatment was conducted during the autumn in the inter-row space at a depth of 18-20cm. The machinery used included a plow, a disc harrow, a cultivator and a milling machine. Under the second set of machinery, the inter-row space was grassed over and then periodically mowed. The mulcher used shreds the mowed material and spreads it between the rows. Grasses and their roots contribute to soil loosening; thereby, leading to a favorable air and water regime within. As a result, it is possible to stimulate the microbiological activity and enrich the soil with organic substances. The other agricultural practices conducted at the orchards include fertilization and plant-protection interventions. Fertilization

was done with a centrifugal fertilizer spreader equipped with deflectors in order to spread the fertilizer closer to the trees. A trailed fan sprayer was used in order to apply the liquid fertilizers together with the plant-protecting agents. The plants in the organic orchard were cultivated by a technique which excluded the application of mineral fertilizers and synthetic pesticides. Only organic fertilizers and plant-protection agents were used. The inter-row soil surface was maintained according to the above-mentioned two systems. Localized drip irrigation was precisely dosed.

The following variants were formed in the case of biologically-cultivated fruits:

Variant 1 – Applied agricultural techniques:

1. Winter and vegetation plant-protection initiatives using organic agents;
2. Bio-fertilization;
3. Black fallow tillage;
4. Irrigation.

Variant 2 – Applied agricultural techniques:

1. Winter and plant-protection initiatives with bio-agents
2. Bio-fertilization
3. Mowing of the grassed inter-row spaces
4. Irrigation

The following variants were formed in relation to the conventional fruit cultivation:

Variant 1 – Used agricultural techniques:

- Winter and crop-protection initiatives using conventional agents;
- Mineral fertilization
 - Black fallow tillage
 - Irrigation

Variant 2 – Used agricultural techniques:

- Winter and crop-protection initiatives using conventional agents
- Mineral fertilization
 - Mowing the grassed inter-row spaces
 - Irrigation

Object of the study

The selected fruits of the Florina sort have the following pomological characteristics: fruits of average size – 110-150 g, with an oval conic shape. The main color is yellow while the cover color is red, with a wax coating and a purple tinge. The covering layer is of average thickness, characterized by density and elasticity. The apple content

is yellow-colored. The latter is also thick, tender, juicy and crunchy. The taste is sweet with a refined sourness and very good taste properties (Lichev et al., 2012). The fruits are only suitable for fresh consumption. According to the tasting scale, the fruits score good (4.1-4.4).

The ripening period falls at the end of September – beginning of October. This variety enters into fruit-bearing relatively early. It is resistant to winter cold and late-spring frosts. It is not sensitive to scabs. Part of the fruits' technical characteristics postulate that they take well to manipulation and transport, while taste quality does not degrade in ordinary refrigerated storage of up to three months.

Trial setting

Method of storage

The fruits were stored at the Food Technologies department at the Institute of Food Preservation and Quality – Plovdiv.

A classic storage method was used involving above-zero temperatures of 1-4°C as well as high air humidity of 85-90% in a Liebherr refrigerator for a period of three months.

Methods of analysis

The following physicochemical indicators were run at the Food Testing laboratory:

- Dry substance, refractometric, %- BSS EN 120143-00;
- Determination of total polyphenols. The content of total polyphenols is determined according to the Singleton and Rossi (1965) method in its following adaption: in a 10-milliliter measuring tube, 0,1ml of sample extract, ~7 ml of distilled water, 0,5ml of Folin-Ciocalteu reagent (diluted at 1:4 with distilled water) and 1.5ml of 7.5% (w/v) aqueous sodium carbonate solution were dosed in the outlined sequence. Distilled water was topped up to the marking. After letting it rest for 2 hours at a temperature of 20 – 25°C, the absorbance of the reacting mixture is measured at 750 nm. Similarly, a blank solution was prepared using distilled water instead of the extract. The achieved results are presented as gallic acid equivalents (GAE) per 100g of extract.

- Determining of the total antioxidant capacity

The total antioxidant capacity was assessed by determining the radical scavenging ability with a DPPH test. Trolox, which is a water-soluble analogue to vitamin E, was used as a standard and the results are expressed as equivalents of Trolox (TE) in μmol per 100g sample.

- DPPH test

The procedure is based on the method by Brand-Williams et al. (1995), applied in its modification as follows: 2250 μl of methanolic solution of DPPH (6 x 10⁻⁵ M) were mixed

with 250 μl of sample extract (diluted with distilled water in a 1:3 ratio, v/v); absorbance at 515nm was measured after a 15-minute rest of the reaction mixture in a closed cuvette in a dark room at a temperature of 20-25°C. Analogically, a blank was prepared by using methanol instead of extract.

- The color was measured instrumentally at the Food Testing Laboratory using a Colorgard 2000 colorimeter produced by BYK-Gardner Inc. USA.

The parameters were reported according to the CIE Lab system.

Three color coordinates were taken during the measurement: L, a and b;

L – color brightness (L = 0 – black, L = 100 – white)

a – the positive values of the parameter indicate the amount of red, while the negative indicate green values;

b – the positive values characterize yellow, while negative characterize blue.

The value of the color hue or the dominating wavelength is presented by the a/b ratio, while saturation, darkening coefficient, yellow index, TC index and the color differences were calculated using the following formulas:

$$C^* = \sqrt{a^2+b^2} - \text{color saturation}; \quad (1)$$

$$BI = x - 0,31.100 - \text{darkening index}; \quad (2)$$

$$0,172 \quad (3)$$

$$YI = 142,86.b - \text{yellow index}; \quad (3)$$

$$L \quad (4)$$

$$TC = 2000.a \quad (4)$$

$$L.\sqrt{a^2+b^2} \quad (5)$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} - \text{generalized color difference} \quad (5)$$

$$\bullet \Delta L = L - L_0 \quad (6)$$

$$\bullet \Delta a = a - a_0 \quad (7)$$

$$\bullet \Delta b = b - b_0 \quad (8)$$

where L₀, a₀ and b₀ are the values for the fruits on the day of harvest while L, a and b are the values as measured in the fruits during storage;

- Statistical analysis – the presented results are the arithmetic mean values taken from at least three parallel extrapolations, while keeping the variation coefficients under 5%. Statistical data processing was performed by ANOVA and Microsoft Excel applications.

Results and discussion

Refractometrically-measured dry substance is an important quality indicator which correlates with the structure and composition of the fruits. The data from the investigated variants of apple storage and cultivation are presented in figures 1 and 2.

In both cultivation methods, apples of the Florina variety demonstrate statistically insignificant differences across variants with their corresponding storage period lengths ($p > 0,05$).

The storage period does not influence the content of soluble dry substances ($p > 0,05$).

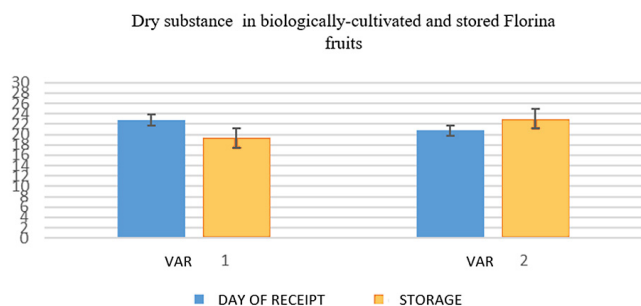


Figure 1. Dry substance in biologically-cultivated and stored Florina fruits.

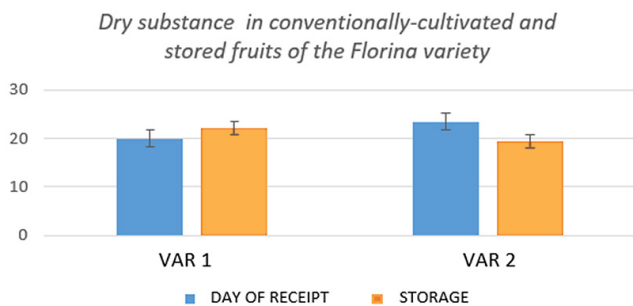


Figure 2. Dry substance in conventionally-cultivated and stored fruits of the Florina variety.

The determined antioxidant activity and total polyphenols content of the stored apple fruits are shown in figures 3, 4, 5 and 6.

The data taken are compared against the accessed data on the harvesting day. In relation to the variants of biologically-cultivated fruits, the antioxidant activity decreases significantly during refrigerated storage – as much as 17 times for the stored fruits of variant 1 and 11 times with the second variant. The fruits, which manage to retain the most of their antioxidant activity through storage, are the ones of the second variant at 413,26 $\mu\text{mol TE}/100\text{g}$. At the harvesting day of biologically-cultivated apples, the antioxidant activity is not influenced by the applied agricultural technique ($p > 0,05$) and values exceed 4400 $\mu\text{mol TE}/100\text{g}$. The applied method of storage does not significantly influence the investigated indicator ($p < 0,05$).

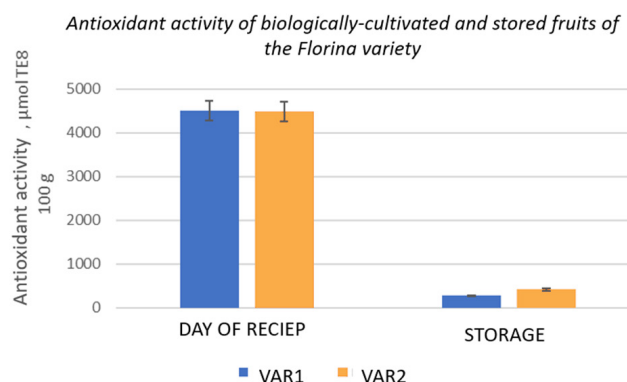


Figure 3. Antioxidant activity of biologically-cultivated and stored fruits of the Florina variety.

In respect to the conventionally-cultivated and stored apple fruits, the trend remains the same with antioxidant activity decreasing 5 and 13 times for variants 1 and 2, respectively. At the harvesting day, the highest antioxidant activity of the conventionally-cultivated fruits is observed in the representatives of the second variant (4027,97 $\mu\text{mol TE}/100\text{g}$), while the fruits of the first variant have higher values at 390,4526 $\mu\text{mol TE}/100\text{g}$ after storage. With conventionally-cultivated fruits, the antioxidant activity is influenced by the applied agricultural technique and the method of fruit storage ($p < 0,05$), fig. 4.

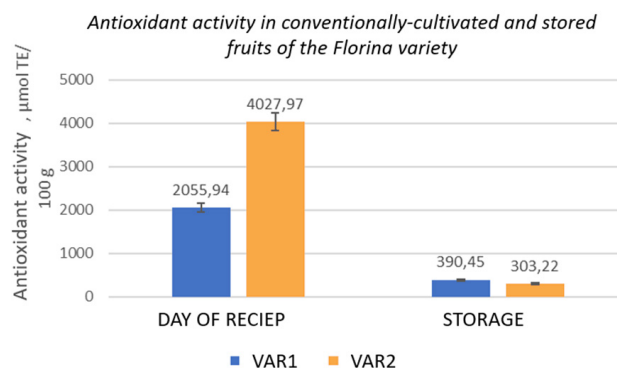


Figure 4. Antioxidant activity in conventionally-cultivated and stored fruits of the Florina variety.

At the harvesting day, the total polyphenols content of biologically-cultivated apple fruits exhibit the highest value for variant 2 (116mg GAE/100g). There is no significant difference in value of total polyphenols after storing the fruits of this variant ($p > 0,05$).

Results show that the fruits of variant 1 exhibit increasing total polyphenols content during storage equal to 1,7 times. The applied agricultural technique does influence the value of total polyphenols on the day of harvesting ($p < 0.05$), while the storage method only influences the fruits of variant 1 ($p < 0.05$). The values of total polyphenols across the variants of biologically-cultivated apples exhibit statistically-insignificant differences ($p > 0.05$), fig. 5.

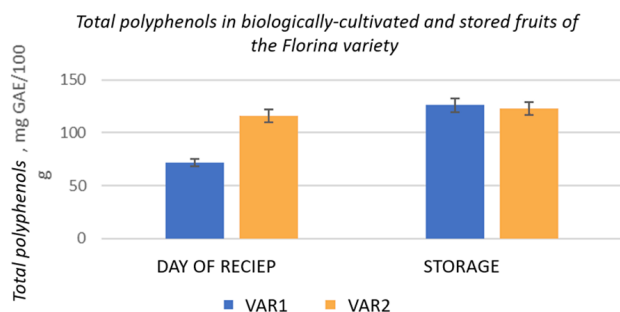


Figure 5. Total polyphenols in biologically-cultivated and stored fruits of the Florina variety.

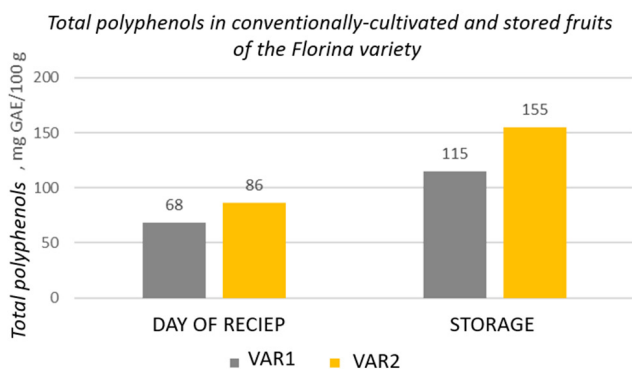


Figure 6. Total polyphenols in conventionally-cultivated and stored fruits of the Florina variety.

The conventionally-cultivated fruits increase their relative content of total polyphenols during storage by a factor of nearly 1.7. Data show that the fruits of the second variant exhibit the highest content thereof both on the day of harvesting and after three-month storage, with 86mg GAE/100g and 155mg GAE/100g, respectively. The applied agricultural techniques and storage method are thus shown to significantly influence total polyphenols content ($p < 0.05$), fig. 6.

During storage, the color of the external layer of apples usually changes from brighter to a darker tinge. One of the most common reasons refers to the little brown spots, which

are due to enzyme-related browning, remaining unnoticed during the first visual inspections after gathering.

Figures 7, 8 9 and 10 show the results of the color measurements conducted on the investigated variants of biologically- and conventionally-cultivated and stored apple fruits.

Color parameters of stored and biologically-cultivated fruits of the Florina variety, variant 1

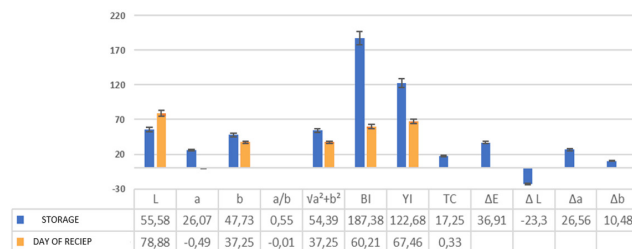


Figure 7. Color parameters of stored and biologically-cultivated fruits of the Florina variety, variant 1.

Data show that fruit brightness decreases 1.4 times during storage in comparison to the day of harvest. The fruits of this variant were the only samples with a green color tinge on the day of harvesting in contrast with the other variants; however, during storage the color parameter changes to red. During storage, the yellow color component increased by 1.2 times, while the yellow index increased by 1.8 times.

The qualitative indicators – color tone and saturation – have increased respectively 55 and 1.4 times respectively during storage. The TC index correlates with the visually-perceived color by increasing significantly during storage by 25.27 times. The browning index increased 3.11 times during apple storage in comparison to harvesting day, while the yellow index increased 1.8 times.

The color differences in the quantitative color indicators of the stored fruits measured on the day of harvesting are summarized in the value of the color difference $\Delta E = 36,91$; thereby, indicating significant alterations in the color of the fruits.

Color parameters of stored and biologically-cultivated fruits of the Florina variety, variant 2

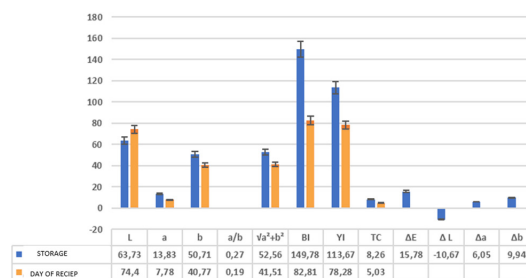


Figure 8. Colour parameters of stored and biologically-cultivated fruits of the Florina variety, variant 2.

The biologically-cultivated and stored under refrigerator fruits exhibit 1.4 times lower values of the indicator for color brightness. In relation to the red and yellow color components, these values increased when compared to the value on the harvest day by 1.78 and 1.24 times, respectively. The color tone remains almost unchanged, while the color saturation increased 1.26 times during storage. Similarly to the biologically-cultivated fruits, the browning index increased 1.8 times in this case, while the yellow index increased 1.45 times and the TC index grew less significantly by 1.6 times in comparison to the harvesting day. The grassed orchards are shown to influence these parameters when the method of farming is considered ($p < 0.05$). The total color difference amounts to ΔE e15,78.

The comparative analysis shows that the biological-cultivated and stored fruits with grassland exhibit more stable qualitative and quantitative color parameters throughout storage in comparison to the biologically-cultivated and stored fruits on cultivated land.

Color parameters of stored and conventionally-cultivated fruits of the Florina variety, variant 1.

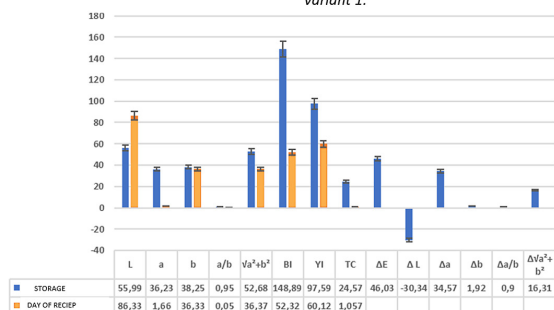


Figure 9. Colour parameters of stored and conventional-cultivated fruits of the Florina variety, variant 1.

Conventionally-cultivated and stored apples of the first variant experience a decrease of color brightness equal to 1.4 times in comparison to the day of harvesting. All remaining qualitative and quantitative color parameters increase their values in comparison to the respective values during storage phase. The red values have significantly increased to 21.82 times the values on harvest day ($p < 0.05$). The yellow color component value remains unchanged. The yellow index of the fruits increases 1.6 times. The color saturation of the fruits increased 1.4 times, while the browning index increased 2.84 times during storage. The changes in the qualitative indicator of color tone are significant and equal to 19 times. This is also a prerequisite for the major increase in the TC index of 23 times, as well as the total color difference of $\Delta E = 46,03$.

Color parameters of stored and conventionally-cultivated fruits of the Florina variety, variant 2.

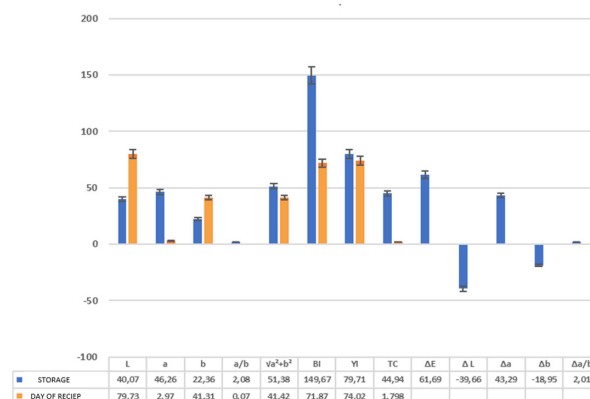


Figure 10. Color parameters of stored and conventional-cultivated fruits of the Florina variety, variant 2.

The fruits of the Florina variety of variant 2, which are conventionally-cultivated and stored, exhibit 1.9 times lower color brightness and yellow color tone in comparison to the values from the harvest day. The red color tone significantly increases during storage by 15.6 times. Color saturation is increased; however, the yellow index remains stable without significant difference resulting from storage. The values of the qualitative indicators are significant; with color tone and TC index measuring respectively 29.71 and 25.11, indicating color degradation.

Out of all the stored fruit variants, representatives of variant 2 exhibit the highest total color difference – $\Delta E = 61,69$.

The comparative analysis of the stored conventional-cultivated fruits shows that brightness decreases, while red color tone increases during storage. The increase is more significant in relation to the fruits of the first variant of the conventionally-cultivated apples. For the second variant of conventionally-cultivated fruits, the yellow color component decreases during storage, while an increase in the parameter is observed in relation to the first variant (fig. 9-10). The differences are statistically significant due to the applied agricultural technique ($p < 0.05$). The yellow index is stable for stored fruits of the second variant, while the representatives of the first variant exhibit an increase in the index. The darkening index for both variants increases at the same pace when fruits are stored. The TC index increases when storing fruits of either variant, 23.4 and 25.1 times, respectively. The visually assessed colour degradation is thus confirmed when taking into account the total colour difference which is statistically significant for both variants of stored fruits.

Conclusion

Following the three-month refrigerated storage period of biologically- and conventionally-cultivated Florina apple fruits at a temperature of 1-4°C, the following indicators were examined: quantitative color parameters – color brightness, red and yellow color tone, as well as mathematically determined qualitative color indicators of the fruits.

Research findings demonstrated decreased color brightness for all studied variants during storage. The red color tone remained stable for biologically-cultivated and stored fruits, while increasing significantly when storing conventionally-cultivated fruits. The yellow color component decreased only for fruits of the second variant of the conventional cultivation method, while the other variants experienced increased values at a unified pace. The yellow index increased during storage and exhibited high values for fruits of the first variant of the biologically- and conventionally-cultivated fruits. The color saturation for the investigated fruits increased, while the values for fruits of the second variant of biologically and conventionally-cultivated fruits remained equal. The fruits of the first variant of the biological and conventional methods exhibited equal values of color saturation. The color indices – the TC index and the darkening index – exhibited minimal values for fruits of the second variant of biologically-cultivated apples. All other variants exhibited high values thereof; thereby, indicating a notable degradation in color.

Out of all the fruits investigated, the biologically-cultivated apples in their second variant exhibited the most persistent colors through refrigeration. The applied agricultural technique is thus shown to positively influence the preservability of the qualitative and quantitative color parameters.

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