Keeping ability of apple fruits of new cultivars under different storage technologies

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Abstract. The fruits of new apple cultivars bred by the FSBSI "Federal Scientific Center named after IV Michurin", adapted to the stressful weather conditions of the Central Black Earth Region of Russia, were used. During storage of control and 1-MCP-treated fruits of 5 studied apple cultivars in air (regular atmosphere: CO₂ = 0.03%, O₂ = 21%), modified atmosphere (O₂ = 16-19%, CO₂ = 1.5-5.0%) and controlled atmosphere with ultra-low oxygen content (O₂ = 1.2-1.5%, CO₂ = 1.2-1.5%), the following main diseases were identified: for cv. Uspenskoye – scald, bitter pit, breakdown; for cv. Fregat – bitter pit, breakdown, CO₂-skin injury. Development of several diseases at the same time is also possible (CO₂-skin injury + bitter pit, bitter pit + breakdown); for cv. Flagman - scald, bitter pit, breakdown, scald + bitter pit; for cv. Bylina - CO₂-skin injury, bitter pit, breakdown, soft scald; for cv. Vympel - CO₂-skin injury. Maximum storage efficiency was shown by fruits cv. Vympel stored under CA + 1-MCP technology. Data on the keeping ability of apple fruits of the studied cultivars allow developing high-precision varietal storage technologies.

1. Introduction

In recent years, the development of horticulture in Russia has been ensured by the active establishing of intensive apple orchards, which effectiveness is largely defined by the planting productivity and fruit quality. At the same time, the set of zoned apple cultivars adapted to the stressful weather conditions of the Central Black Earth Region of Russia and used in industrial horticulture is significantly inferior in quality to the world sales leaders. A possible solution is the introduction of cultivars or production of new domestic cultivars that can compete in quality with foreign ones.

Plant breeders of the FSBSI "Federal Scientific Center named after IV Michurin" have created new apple cultivars (Uspenskoye, Fregat, Flagman, Bylina, Vympel) with relatively high or high winter hardiness and monogenic sustainability to scab (controlled by the Vf gene) [1]. However, the technologies for their production and storage have not been developed, and its efficiency has not been identified that limits the possibilities of their use.

It is known that the genotypic characteristics of the cultivar are among the main factors determining fruit susceptibility to physiological diseases [2, 3]. However, storage factors (temperature, content of O₂, CO₂, etc.), the algorithm of their influence, technological methods (1-MCP-treatment and others) are able to cause, enhance, inhibit, or have no significant effect on their development [4-8].
Studies of the susceptibility of apple fruits cv. Uspenskoye, Fregat, Flagman, Bylina, Vympel to physiological diseases when stored in a regular, modified and controlled atmosphere with a characteristic effect on the state of the fruits have not been carried out. That confirms their relevance.

The aim of this research was to study the storability of fruits of new apple cultivars Uspenskoye, Fregat, Flagman, Bylina, Vympel under the influence of various postharvest factors for the development of effective varietal storage technologies.

2. Materials and methods

The experiments were carried out at the Federal State Budgetary Scientific Institution "Federal Scientific Center named after IV Michurin" (Tambov region, Russia). The fruits of new apple cultivars bred by the Federal Scientific Center were used: Uspenskoye – with an autumn ripening period, Fregat, Flagman, Bylina, Vympel – with a winter ripening period. Fruits cv. Uspenskoye were stored only under RA conditions.

Treatment with 1-MCP (ethylene biosynthesis inhibitor Fitomag®, Russia) was applied on the half of the total experiment fruit on the harvest day. Losses from scald and other diseases were evaluated visually after 4 months of storage plus 7 days of shelf life at T = +20 °C, and results were expressed as percentages of the total number of fruit.

Internal ethylene concentration was determined by gas chromatography (GC-2014, SHIMADZU, Japan) and expressed in ppm [9].

The content of α-farnesene and its oxidation products (CT_{281}) in fruit skin was determined by spectrophotometry (UV-1800, SHIMADZU, Japan) and expressed in nmol/cm² [10].

Fruit firmness was measured with an 11-mm tip of the penetrometer (FT-327) and expressed in kg/cm².

The experiment was composed of the following storage conditions:

1. Control fruit (without 1-MCP-treatment) stored under regular air (T = +1 °C, CO₂ = 0.03%, O₂ = 21%) - RA-C;
2. 1-MCP-treated fruit stored under regular air (T = +1 °C, CO₂ = 0.03%, O₂ = 21%) - RA-T;
3. Control fruit stored under modified atmosphere (T = +1 °C, CO₂ = 1.5-5.0%, O₂ = 16-19%) - MA-C;
4. 1-MCP-treated fruit stored under modified atmosphere (T = +1 °C, CO₂ = 1.5-5.0%, O₂ = 16-19%) - MA-T;
5. Control fruit stored under controlled atmosphere with ultra-low oxygen content (T = +1 °C, CO₂ = 1.2-1.5%, O₂ = 1.2-1.5%) - CA-C;
6. 1-MCP-treated fruit stored under controlled atmosphere with ultra-low oxygen content (T = +1°C, CO₂ = 1.2-1.5%, O₂ = 1.2-1.5%) - CA-T.

To ensure RA conditions, cold storage rooms with a capacity of 5 tons were used. Packages Xtend ("StePac", Israel) were used to establish MA storage conditions. For this, the packages were placed in RA cold storage rooms. The modified atmosphere with O₂ 16-19% and CO₂ 1.5-5% was obtained by the product respiration and the selective permeability of the packages. Industrial storage rooms of 150 tons capacity were used for CA storage. Storage conditions (O₂ = 1.2-1.5%, CO₂ = 1.2-1.5%) were established by a nitrogen generator and a CO₂-scrubber. There were 4 boxes with 10-11 kg of fruits for each storage option.

3. Results and Discussion

Differences in response of the fruits of studied cultivars to the influence of RA, MA and CA storage conditions with or without postharvest 1-MCP-treatment occurred in the content of biochemical and other indicators (internal ethylene concentration, flesh firmness, α-farnesene and its oxidation products - CT_{281}) and in fruit susceptibility to specific diseases and injuries during storage.

Internal ethylene concentration. Ethylene is a maturation hormone that has a direct or indirect effect on many physiological and biochemical processes, and reflects the state of the fruits [2, 6, 9, 11].
After 4 months of storage, the highest internal ethylene concentration was observed in control fruits of studied cultivars stored under RA-C conditions (there are some exceptions). The lowest concentration among non-treated fruits was measured in ones stored under CA-C conditions (table 1). The highest ethylene content in control fruits stored under RA conditions for 4 months was measured in fruits cv. Fregat (738.8 ppm), then Flagman (448.2 ppm), Vympel (418.5 ppm), Bylina (162.1 ppm). It should be pointed out that the relatively low level of ethylene content in fruits cv. Uspenskoye (248 ppm) is evidently due to the late harvest date and the postclimacteric ethylene decrease.

**Table 1.** Effects of storage conditions and postharvest 1-MCP-treatment on the internal ethylene concentration, flesh firmness, content of α-farnesene and CT\(_{281}\) in fruits of various apple cultivars. Data of 2019-2020

| Cultivar/indicator          | Storage conditions | RA-C | RA-T | MA-C | MA-T | CA-C | CA-T |
|-----------------------------|--------------------|------|------|------|------|------|------|
| Internal ethylene concentration, ppm |                    |      |      |      |      |      |      |
| 1. Uspenskoye               |                    | 248.0| 60.0 | -    | -    | -    | -    |
| 2. Fregat                   |                    | 738.8| 107.8| 554.2| 12.0 | 426.4| 4.2  |
| 3. Flagman                  |                    | 448.2| 128.9| 232.3| 4.7  | 136.3| 2.8  |
| 4. Bylina                   |                    | 162.1| 74.6 | 226.8| 5.7  | 230.4| 2.8  |
| 5. Vympel                   |                    | 418.5| 15.5 | 346.0| 7.1  | 180.2| 3.9  |
| LSD\(_{0.05}\)              |                    | 46.2 | 12.1 | 53.7 | 2.7  | 19.8 | 0.6  |
| Flesh firmness, кг/см\(^2\) |                    |      |      |      |      |      |      |
| 1. Uspenskoye               |                    | 3.8  | 3.4  | -    | -    | -    | -    |
| 2. Fregat                   |                    | 4.6  | 6.4  | 5.1  | 8.0  | 4.8  | 6.8  |
| 3. Flagman                  |                    | 4.9  | 4.8  | 4.6  | 9.3  | 5.4  | 8.5  |
| 4. Bylina                   |                    | 4.6  | 4.7  | 4.8  | 7.7  | 4.9  | 7.7  |
| 5. Vympel                   |                    | 5.4  | 9.4  | 6.6  | 9.4  | 7.0  | 10.0 |
| LSD\(_{0.05}\)              |                    | 0.4  | 0.4  | 0.5  | 0.6  | 0.4  | 0.6  |
| α-farnesene, nmol/cm\(^2\) |                    |      |      |      |      |      |      |
| 1. Uspenskoye               |                    | 49.4 | 33.2 | -    | -    | -    | -    |
| 2. Fregat                   |                    | 58.9 | 35.0 | 68.3 | 6.0  | 22.2 | 4.3  |
| 3. Flagman                  |                    | 32.5 | 23.3 | 40.7 | 10.5 | 38.3 | 6.0  |
| 4. Bylina                   |                    | 49.6 | 27.1 | 24.6 | 2.5  | 12.6 | 2.5  |
| 5. Vympel                   |                    | 28.4 | 19.4 | 23.3 | 5.6  | 21.3 | 5.6  |
| LSD\(_{0.05}\)              |                    | 0.4  | 0.4  | 0.5  | 0.6  | 0.4  | 0.6  |
| CT\(_{281}\), nmol/cm\(^2\) |                    |      |      |      |      |      |      |
| 1. Uspenskoye               |                    | 25.4 | 1.8  | -    | -    | -    | -    |
| 2. Fregat                   |                    | 6.2  | 1.1  | 11.1 | 0.5  | 3.9  | 0.4  |
| 3. Flagman                  |                    | 2.3  | 1.7  | 8.8  | 0.8  | 2.8  | 0.7  |
| 4. Bylina                   |                    | 1.8  | 1.1  | 1.6  | 0.2  | 0.8  | 0.3  |
| 5. Vympel                   |                    | 3.1  | 1.9  | 2.6  | 1.2  | 3.3  | 1.0  |
| LSD\(_{0.05}\)              |                    | 1.4  | 0.3  | 0.7  | 0.07 | 0.2  | 0.05 |

The 1-MCP treatment inhibited ethylene accumulation compared with control fruit. The lowest efficiency of treatment was observed in RA-T fruit, while the highest efficiency was noted in CA-T fruit for all studied cultivars. The lowest internal ethylene concentration was observed in fruits cv. Vympel (CA-T - 3.9 ppm), which ensures the possibility of quality maintaining during further storage and low risks of senescent breakdown.

Low efficiency of 1-MCP treatment for fruits cv. Uspenskoe, Flagman, Bylina under RA conditions is probably connected with high rates of ethylene accumulation and formation of new binding site for ethylene [3, 11].
Flesh firmness is one of the main indicators of the internal fruit quality, which determines the storage duration and affects the selling price of the product (it is not allowed for sale when the indicator decreases to 5.6 kg/cm$^2$).

After 4 months of storage, the fruits of studied cultivars stored under CA conditions had a higher firmness compared with MA and RA fruits. For all studied cultivars the 1-MCP application resulted in a higher firmness retention compared with control fruits (table 1). The maximum flesh firmness retention under of RA, MA and CA storage was observed in apple fruits cv. Vympel compared with other cultivars. Postharvest 1-MCP treatment ensured the highest level of firmness maintenance under CA-T conditions, which indicates a high potential for fruit quality preservation during further storage.

α-farnesene is an unsaturated hydrocarbon localized in the cuticle of the fruit skin. Among its oxidation products there are conjugated trienes (CT$_{281}$), which cause scald development, while the synthesis of α-farnesene is initiated by the maturation hormone ethylene [10-12].

After 4 months of storage the maximum content of α-farnesene among control fruits was found in fruits of studied cultivars stored under RA-C and MA-C, a lower level of α-farnesene was noted in CA-C, which is probably due to the low oxygen content in the storage atmosphere (table 1). The highest level of α-farnesene accumulation was observed in fruits cv. Vympel, its content under RA, MA and CA conditions was 28.4, 23.3 and 21.3 nmol/cm$^2$, respectively, which indicates low risks of scald development.

The 1-MCP treatment inhibited the accumulation of α-farnesene in fruits of all studied cultivars compared with non-treated fruits. The highest inhibition efficiency was found under CA-T storage. The low level of α-farnesene in the cuticle of the fruit skin (within 2.5-6.0 nmol/cm$^2$) indicates the absence of risks of scald development.

Conjugated trienes (CT$_{281}$) are oxidation products of α-farnesene, causing scald development in apple fruits [5, 10-12]. The maximum level of CT$_{281}$ accumulation after 4 months of storage was noted in the cuticle of the fruit skin under cold storage in RA and MA conditions.

The highest content of CT$_{281}$ was observed in fruits cv. Uspenskoye - 25 nmol/cm$^2$ in RA-C. A high level of CT$_{281}$ accumulation was measured in fruits cv. Fregat and Flagman in MA-C - 11.1 and 8.8 nmol/cm$^2$ respectively, which indicates high risks of scald development. The assessment of fruit state of all studied cultivars showed that CA conditions ensured a low level of CT$_{281}$ in the cuticle of the fruit skin of control fruits (less than 4 nmol/cm$^2$) and, therefore, scald sustainability (table 1).

Postharvest 1-MCP treatment inhibits the synthesis of ethylene, α-farnesene and CT$_{281}$. The content of CT$_{281}$ in fruits stored in RA-T, MA-T, CA-T was less than 2 nmol/cm$^2$ for all studied cultivars, which corresponds to low risks of scald development during storage, transportation and sale period.

Physiological diseases of apple fruits.

Bitter pit (BP). Four of studied cultivars were affected by BP. The highest losses from the disease with a severe degree of its development was observed in fruits cv. Flagman, then - Fregat and Uspenskoye with the highest values under RA and MA conditions. A low level of losses from BP in fruits cv. Bylina (less than 1.5%) was observed only in RA (figure 1). CA conditions contributed to the reduction or elimination of losses from BP in fruits of all susceptible cultivars. Probably, a further decrease in the oxygen content in the storage atmosphere is a promising way to reduce losses from BP [13, 14]. The 1-MCP treatment did not have an unambiguous effect on BP development.

Senescent breakdown. The highest senescent breakdown after 4 months of storage was observed in fruits cv. Fregat stored under RA-C and CA-C - 18.8 and 17.7% respectively, while under MA-C losses were 7.6%. In fruits cv. Uspenskoye, Flagman and Bylina senescent breakdown did not exceed 2.4%. The 1-MCP treatment ensured a decrease or elimination of losses from senescent breakdown of fruits of all studied cultivars in RA, MA and CA (figure 1).

Scald. Among the studied cultivars, 3 of them were affected by scald (Uspenskoye, Flagman, Bylina). The highest scald incidence after 4 months of storage was observed in fruits cv. Uspenskoye (up to 46% in RA-C). Fruits cv. Flagman were affected by the disease only in MA-C (23.5%). Single
fruits cv. Bylina were affected by scald also only under MA conditions (3%). There were no losses from scald among control fruits of all studied apple cultivars stored under CA conditions that confirms the role of low oxygen storage in controlling the disease [7, 14]. The 1-MCP treatment ensured fruit protection from scald for all studied cultivars under RA, MA and CA conditions (figure 2).

**Figure 1.** Effects of storage conditions and postharvest 1-MCP treatment on bitter pit and senescent breakdown incidence in fruits of studied apple cultivars. Data of 2019-2020.

**Figure 2.** Effects of storage conditions and postharvest 1-MCP treatment on scald incidence and CO\textsubscript{2}-skin injury in fruits of studied apple cultivars. Data of 2019-2020.

\textit{CO\textsubscript{2}-skin injury}. The highest CO\textsubscript{2}-skin injury was observed in fruits cv. Fregat (39.8%) and Bylina (49.2%). There were isolated cases of the disease among fruits cv. Vympel. The increased carbon dioxide content in the storage atmosphere (MA 1.5-5%, CA 1.2-1.5%) in combination with postharvest 1-MCP treatment caused the CO\textsubscript{2}-skin injury appearance in fruits of susceptible cultivars, but there were no signs of the disease in control fruits (figure 2). It is likely that 1-MCP treatment inhibits the synthesis of antioxidants that in its turn inhibit the development of CO\textsubscript{2}-skin injury [4]. So 1-MCP application may be an additional stress factor that initiates the development of the disease [4].

\textit{Soft scald}. Only one of all studied cultivars (Bylina) was affected by soft scald under RA and MA conditions, and besides losses from the disease did not exceed 2%. There were no losses from soft scald in CA conditions. It is likely thata high oxygen content in the storage atmosphere (RA, MA - 16-21%) contributed to the appearance of the disease, while a low oxygen level (RA - 1.2%) retarded its development. The unambiguous effect of postharvest 1-MCP treatment on the development of soft scald has not been detected.

It was confirmed that RA conditions provide a low level of quality preservation for all studied cultivars, because low storage temperature which provokes scald appearance in susceptible cultivars [5] is the only factor inhibiting respiration rate and aging processes. Fruits cv. Vympel showed relatively high storability under RA conditions.

Fruits stored under MA conditions with limited air exchange at a high O\textsubscript{2} content (16-21%) and an increased CO\textsubscript{2} content (1.5-5%) showed insignificant inhibition or absence of inhibition of ethylene biosynthesis, a decrease in firmness, senescent breakdown, the highest CT\textsubscript{281} accumulation in the cuticle of fruit skin. This contributed to scald appearance and confirmed the low efficiency of the MA-C technology for all studied cultivars (with a relatively high storability of fruits cv. Vympel).

A low oxygen level (1.2-1.5%) under CA conditions contributed to inhibition of ethylene synthesis, accumulation of \(\alpha\)-farnesene and its oxidation products - CT\textsubscript{281} and restrained scald development [7] for all cultivars, including Flagman and Bylina. It could possibly suppress other oxidative processes
leading to BP development [13, 14], which is confirmed by the absence of losses from the disease compared with MA and, especially, RA for fruits cv. Fregat, Flagman, Bylina. So CA storage with low oxygen content is a promising way of fruit quality preservation.

Postharvest 1-MCP treatment under CA, MA, and in a less degree - RA conditions ensures a low level of accumulation of ethylene, α-farnesene and CT281 in fruits, a higher flesh firmness compared with non-treated fruits [6-7, 12-14]. The present research also confirmed that susceptibility and sustainability to specific physiological diseases in response to storage factors are controlled by cellular metabolism due to genetic information contained in each genotype [12].

4. Conclusion

The main commercially significant diseases of 5 studied apple cultivars under RA, MA, CA (which may increase during the sales period) were revealed. For cv. Uspenskoe they are scald, BP, senescent breakdown; for cv. Fregat - BP, breakdown, CO2-skin injury, or their combination (CO2-skin injury + BP, BP + breakdown); for cv. Flagman - scald, BP, breakdown, scald + BP; for cv. Bylina - CO2-skin injury, BP, breakdown, soft scald; for cv. Vympel - CO2-skin injury.

Postharvest 1-MCP treatment ensured protection from scald, protection or reduction of losses from breakdown, higher flesh firmness of fruits of susceptible cultivars in CA-T, MA-T and in a less degree - RA-T. But it could be an additional stress factor initiating the development of CO2-skin injury under storage conditions with an increased CO2 content (MA-T and CA-T) in fruits cv. Fregat, Bylina, in a less degree - Vympel.

Apple fruits cv. Vympel showed the highest keeping ability under RA, MA and CA storage conditions with or without 1-MCP.

Data on the keeping quality of fruits of studied apple cultivars allow us to search for new technological possibilities for their protection from diseases and to develop high-precision varietal storage technologies using active factors of influence on the fruit in the most verified and physiologically justified range for each cultivar.

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References

[1] Saveliev N I et al (eds) 2009 Catalog of cultivars of fruit and berry crops bred by All-Russian Research Institution of Genetics and Breeding of Fruit Plants named after I.V. Michurin (Michurinsk: GNU VNIIGiSPR n.a. I.V. Michurin) p 79
[2] de Freitas S T and Mitcham E J 2012 Hortic. Rev. 40 107–146
[3] Gudkovsky V A, Kozhina L V 2019 Food industry 12 58-62
[4] Argenta L C, Fan X T and Mattheis J P 2002 Postharvest Biol. Technol. 24 13–24
[5] Watkins C B, Bramlage W J and Gregoe B A 1995 J. Amer. Soc. Hort. Sci. 120 88–94
[6] Watkins C B, Nock J F and Whitaker B D 2000 Postharvest Biol. Technol. 19 17–32
[7] Zanella A 2003 Postharvest Biol. Technol. 27 69–78
[8] Gudkovsky V A, Kozhina L V and Gucheva R B 2017 Storage and processing of agricultural raw materials 7 20-25
[9] Rakitin V Yu and Rakitin L Yu 1986 Plant Physiology vol 33 (Moscow: Nauka) pp 403-413
[10] Morozova N P and Salkova E G 1980 Biochemical Methods (Moscow: Nauka) pp 107-112
[11] Lurie S and Watkins C B 2012 Postharvest Biol. Technol. 65 44–60
[12] de Freitas S T and Pareek S (eds) 2019 Postharvest Physiological Disorders in Fruits and Vegetables (CRC Press) p 824
[13] Mattheis J P, Rudell D R and Hanrahan I 2017 Hortscience 52(1) 132–137
[14] Pesis E, Ebeler S E, de Freitas S T, Padda M and Mitcham E J 2010 J. Sci. Food Agric 90(12) 2114–2123