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Microbiological changes and severity of decay in apples stored for a long-term under different storage conditions

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Abstract

Apples occupy most of the Latvian vegetable market, nevertheless there is a lack of good quality locally grown apples. Using advanced storage technologies, such as storage in controlled atmosphere in ultra low oxygen (O_2) (ULO) and elevated carbon dioxide (CO_2) conditions, as well as pre-treatment of fruits with 1-Methylcyclopropene (1-MCP) will substantially improve the quality of fruits and globally assist apple growers to distribute more qualitative and safe fruits on the local market. The aim of the study was to ascertain the causes of apple decay that occurred during storage under different conditions. Two apple storage technologies were tested in this study: cold storage under conventional conditions + 1-Methylcyclopropene treatment and controlled atmosphere – 2.0% CO_2, 1.0% O_2 (ULO1) and 2.5% CO_2, 1.5% O_2 (ULO2) conditions. After apple storage for six months the following microscopic fungi were isolated: *Penicillium expansum* (30.44%), *Monilinia fructigena* (26.08%), *Neofabraea alba* (21.73%), *Colletotrichum acutatum* (13.04%), *Botrytis cinerea* (8.69%) in cold storage, while *P. expansum* (35.26%), *Colletotrichum acutatum* (23.57%), *Neofabraea alba* (11.76%), *B. cinerea* (11.76%), *M. fructigena* (11.76%) and *Fusarium avenaceum* (5.89%) were isolated from fruits that prior to storage were pre-treated with 1-MCP. Apples that had been stored under ULO1 conditions predominantly were contaminated with: *N. alba* (33.34%), *M. fructigena* (26.69%), *B. cinerea* (13.33%), *P. expansum* (6.66%), *F. avenaceum* (6.66%), *Phomopsis/ Diaporthe eres* (6.66%) and *Mucor circinelloides* (6.66%), while under ULO2: *N. alba* (33.33%), *M. fructigena* (33.33%), *F. avenaceum* (16.67%) and *P./D. eres* (16.67%). Diversity of microscopic fungi, which were isolated from differently stored and decayed apple samples, was quite similar, though with insignificant difference. For instance, microscopic fungus *F. avenaceum* was not found only in cold storage kept fruits, while microscopic fungus of the genus *P./D. eres* was identified when apples were stored under ultra low oxygen conditions.

Key words: apple fruit, controlled atmosphere, cultivar, 1-Methylcyclopropene, microbiological contamination.

Introduction

A short storage life of apples could be explained by the various reasons, the main one is microscopic fungi that retain their biological activity even after apple harvesting, thus limiting the storage time. Depending on the species of microscopic fungi they can start to develop during apple vegetation or after harvesting. Presence of such microscopic fungi is difficult to identify prior to storage as the first most common symptoms of infection appear only during storage.

Most common microscopic fungi that can be present on the surface of apples cause certain types of spoilage. Typical spoilage that can take place during apple storage is rot and moulds. The literature describes several rot agents that have been isolated from spoiled apples (Juhneviča et al., 2011; Juhnevica-Radenkova et al., 2016). They can be as a single source of infection or can create a complex of infections.

Colletotrichum gloeosporioides causes apple decay, wounds and dead skin fragments. Initial signs of infected fruits are circular rot spots with concentric different in size brown shades. Spore masses are bright orange or yellow (Ellis, 2008).

Neofabraea spp. earlier was known as *Gloeosporium* spp., *Cryptosporiopsis* spp. and *Plychtaena* spp. *Neofabraea* spp. predominantly exists as a saprophyte on dead and defective bark. Infection occurs during vegetation period, as well as in post-harvest storage from the pathogen spores. Usually, lesions are circular or oval in shape, pale brown spots with a darker outer ring. The rotten tissues usually are relatively firm, acervulus are frequently present in old lesions under humid conditions (Neri et al., 2009).

Monilinia laxa, *M. fructigena* and *M. fructicola* cause brown rot damage on fruits, the fungus can also infect leaves, blossoms, as well as shoots. Infection most commonly remains over winter on mummified fruits, on the ground, and in cankers. Infected fruits turn brown and soft (Tahir, 2006).

Botrytis cinerea predominantly occurs in fruits mechanically injured at harvest. *B. cinerea* commonly colonises shoots, leaves and fruit peel. Secondary infection can also occur during long-term storage of fruits through fruit-to-fruit contact and can cause considerable losses. It is caused by high relative humidity in storage chambers.

Penicillium expansum and *P. digitatum* predominantly occur in fruits that had been mechanically injured during harvesting or in fruit tissue that had been previously injured by different fungi. There is evidence that apples injured (peel and internal injuries) during harvesting will be susceptible to colonisation by *P. expansum* and *P. digitatum* pathogenic fungi (Tahir, 2006).

The action of microscopic fungi can be suppressed using fungicides and ethylene inhibitors such as 1-Methylcyclopropene (1-MCP). However, application of 1-MCP in organic farms is prohibited. Since organic growers stay away from synthetic fertilization and

fungicide treatment, they have more intensive fruit decay that causes fruit yield losses (Tahir, 2006). The data found in the scientific literature suggest that Swedish organic orchard had 20% more apples damaged by microscopic fungi compared to conventional orchard (Jönssson et al., 2010). Another important storage technique that has great advantages is ultra low oxygen storage (Juhnevica-Radenkova et al., 2016). This type of storage is essentially based on delaying the ripening processes by controlling the level of gases around fresh fruits and vegetables (Tahir, 2006). Ultra low oxygen storage may substantially reduce the possible development of microorganisms and limit the possible infection of healthy fruits (Juhneviča et al., 2011). Along with delaying ripening processes, respiration rate of fruits also can be suppressed; as a result fruit storability can be improved and quality maintained. Due to high carbon dioxide (CO_2) level in a storage room, antimicrobial properties of this compound can also be observed (Tahir, 2006).

It is well known that apple decay can also be associated with physiological disorders. Nowadays, there are eight types of physiological disorders, which have different causes. For instance, bitter pit is a physiological disorder of apples that is related to calcium deficiency in fruit. Water core (particularly apple cultivars 'Tiina' and 'Zarja Alatau') is a physiological disorder that is associated with a high sorbitol concentration in fruits. There are cultivars that are susceptible to flesh browning, usually associated with water core at harvest, brown heart and also core breakdown. Another important physiological disorder is apple rust which is particularly associated with meteorological conditions during apple ripening. Superficial scald is a physiological disorder causing brown or black patches on apple fruit skin that appears during storage. Superficial scald is primarily associated with insufficient storage conditions and particularly with ventilation intensity.

Types of storage, incorrect storage temperature and concentration of protective gases in storage chambers have a great influence on the development of physiological disorders. Flesh browning is the most frequent physiological disorder that causes significant losses during apple storage (associated with low temperature during storage) and superficial scald (associated with low CO_2 concentration during storage). Flesh browning symptoms during cold storage appear in the cortex (diffuse) or vascular tissue (radial) like patches around the flesh. During apple storage, progressing diseases and premature apple harvesting can contribute to the faster development of this disorder. Flesh browning disorders of apples often are cultivar specific. For instance, apple cultivars 'Ligita' and 'Liivika' are very susceptible to this disorder. Superficial scald is a worldwide distributed physiological disorder that has a great effect on external quality of apples. Apples harvested at earlier stages have more prevalent superficial scald incidence. Susceptibility of cultivars 'Sinap Orlovkij' and 'Edite' apples is high. Scientific papers indicate that at least partial control of the disorder can be achieved from application of antioxidants, especially the commonly used diphenylamine (DPA) or using technologies that provide low oxygen concentration during storage (Calvo, 2010). Therefore, control of temperature and concentration of gases in storage chambers is important not only during storage but also shelf-life period (Juhnevica-Radenkova et al., 2014).

The aim of study was to ascertain the causes of apple decay that occurred during storage under different conditions.

Material and methods

Research time and place. The research was carried out during the period from 2012 to 2014. The studies were conducted at the Experimental Processing Department of the Latvia State Institute of Fruit-Growing (at present – Institute of Horticulture, Latvia University of Agriculture) in Dobele and at the Laboratory of Microbiology, Faculty of Food Technology, Latvia University of Agriculture. The number of damaged fruits for each cultivar during storage was counted and apple rotting agents analysed according to the morphological features and DNA sequences (Juhneviča et al., 2011; Volkova et al., 2013). Sequencing analyses were performed at Latvian Plant Protection Research Centre.

Materials used for research. Duration of the experiment was six months. Apples were analysed before storage and after six months of storage. The following apple cultivars were chosen for experiments: autumn cultivars 'Auksis', 'Orļik' and 'Gita', harvested twice in 2012 (06 09 and 11 09) and in 2013 (10 09 and 14 09), and winter cultivars 'Antej', 'Belorusskoje Maļinovoje', 'Sinap Orlovskij' and 'Zarja Alatau', harvested twice in 2012–2013 (28 09 and 03 10) and in 2013–2014 (14 09 and 21 09). Apples were harvested at the technical stage of maturity, assessed by starch content using Starch iodine test (Drudze, 2005) that characterizes the degree of fruit ripeness.

Structure of the research. Shortly after harvesting apples were air-cooled for 24 hours in a cooling chamber at a temperature of up to +4 ± 0.5°C. From each cultivar 40 fruits were selected, their average weight was ~6 kg. For each storage technology, the same amount of apples was prepared. Then the samples were placed in polypropylene boxes with perforated walls. The cooled down apple samples were divided into four groups for post-harvest storage: 1) cold storage (control) – apple storage was implemented under traditional conditions at an air temperature of +2 ± 1°C and relative humidity of 85%; 2) cold storage + 1-Methylcyclopropene (1-MCP) treatment; 3) ultra low oxygen (ULO1) – O_2 1.00%, CO_2 2.00%; 4) ULO2 – O_2 1.50%, CO_2 2.50%. Storage in ULO was implemented by "Fruit Control Equipment" (Italy), selecting two different gas compositions in the mixture of controlled atmosphere.

Fruit treatment with 1-Methylcyclopropene (1-MCP). The treatment with ethylene inhibitor 1-MCP was performed in an air-tight fruit processing container. The ethylene action inhibitor 1-MCP was purchased from "RandH" (Rohm and Haas Company, Italy). The material consists of a homogeneous mixture of 1-MCP at a concentration of 3.3% together with related manufacturing impurities, in the form of a complex with alpha-cyclodextrin, together with any other necessary co-formulants. 1-MCP powdery substance was dissolved in warm water +50 ± 2°C by ratio of 1-MCP to water as 1:30, the concentration of the obtained solution was 0.625 µl L^{-1}. This ratio was chosen based on the well-founded scientific research of Polish researchers affirming the above-mentioned concentration as more suitable for apple treatment with 1-MCP (Wawrzynczak et al., 2007). The solution was prepared in an Erlenmeyer flask, which was subsequently placed in an air-tight processing container with apples intended for storage. Based on 1-MCP manufacturer's recommendations in a room capacity of 0.5 m^3 the amount of 1-MCP preparation could be 0.5 g. The treatment with 1-MCP was performed at a temperature of 18 ± 1°C in an air-tight fruit processing container for 24 h. After treatment, fruit samples were stored in cold storage under conventional conditions. The apples treated with 1-MCP and untreated (control) were stored in the same conditions but on different pallets.

Detection of apple deterioration agents – microscopic fungi. Detection of apple lesions was performed using the method of moist chamber. Damaged apples separately from each other were placed in a plastic box with a lid. A pad of filter paper soaked with distilled water was placed in the box base under apples. The

samples were stored for 14 days at a temperature of +25 ± 1°C in variable daily light and darkness mode (natural conditions – 12 h of dark and 12 h of light). After typical sporulation of microscopic fungi on damaged areas of apple surface, pure cultures of microscopic fungi were developed for molecular analysis. The rDNA (ribosomal deoxyribonucleic acid) was isolated from pure culture to determine taxonomic status. Internal transcribed space (ITS) region using a polymerase chain reaction (PCR) was analyzed by combination of universal primers ITS 1F and ITS4 (standard for Ascomycota), sequencing and following sequence comparison with the NCBI GeneBank database (Volkova et al., 2013).

Statistical analysis. The processing of data was carried out by the methods of mathematical statistics, where arithmetic average value, standard deviation and standard error were calculated using the software *Excel 2007*. Significant differences between the samples were calculated and analysed by the *SPSS 20.0*, using a two-factor analysis of variance (*ANOVA*), LSD test and Tukey's test. The significance of differences was determined at $p < 0.05$.

Results and discussion

Results shown that significantly ($p < 0.05$) higher occurrence of disorders caused by apple decay was when apples were kept under cold storage conditions, from 9.76% to 88.10% (1st harvesting) and from 6.50% to 100% (2nd harvesting) (Table, research year 2012–2013). Whereas, severity of decay caused by pathological and physiological diseases in apples treated with 1-MCP was statistically less pronounced compared with cold storage: from 4.08% to 30.95% (1st harvesting) and from 2.08% to 100% (2nd harvesting). Decay of apple samples, which had been stored under controlled atmosphere conditions in ULO1, accounted for from 2.35% to 5.51% (1st harvesting) and from 3.21% to 100% (2nd harvesting).

Apple samples those stored under ULO2 conditions were spoiled from 1.43% to 4.65% (1st harvesting) and from 0% to 100% (2nd harvesting).

Analysis of the results obtained within research year 2013–2014 showed that spoilage of apple fruits stored in cold storage was from 12.23% to 100% (1st harvesting) and from 5.51 to 100% (2nd harvesting). In turn, deterioration severity significantly ($p < 0.05$) decreased when apple samples prior to long-term storage were treated with 1-MCP. Apple decay accounted for from 5.32% to 100% (1st harvesting) and from 3.22% to 100% (2nd harvesting). Decay of apple samples, which had been stored under controlled atmosphere conditions in ULO1, accounted for from 3.15% to 5.65% (1st harvesting) and from 1.32 to 100% (2nd harvesting), while under ULO2 conditions decay of apples ranged from 1.54% to 5.01% (1st harvesting) and from 1.95% to 100% (2nd harvesting). The lowest deterioration severity of apples stored under controlled atmosphere conditions in ULO was associated with the reduced oxygen and increased carbon dioxide CO_2 content that adversely affected the microbial growth and development. Similar observation has been made by the group of researchers from Brazil, pointing out that the best ultralow oxygen conditions for the storage of cv. 'Royal Gala' apples is 1.0 kPa O_2 combined with 2.0 kPa of CO_2 at 1.0°C (Weber et al., 2011). A group of researchers from Latvia stated that significantly lowest ($p < 0.05$) amount of microorganisms was found on apples that had been stored under controlled atmosphere (Juhneviča et al., 2011; Juhnevica-Radenkova et al., 2016).

Spoilage of apples stored in cold storage (control) was 48.96% (average data for all apple cultivars), while the lowest ($p < 0.05$) spoilage of apples was found when apple samples prior to cold storage had been treated with 1-MCP – up to 21.62%. In turn, deterioration severity of samples stored in ULO1 and ULO2 was 34.70% and 9.91%, respectively. As can be seen the highest deterioration intensity of apples was in cold storage, while the lowest in ULO2. The high intensity of apple decay in ULO1 can be associated with the physiological disorders, particularly with elevated concentration of carbon dioxide CO_2. Describing the damage, which was caused by elevated concentration of CO_2, scientists indicate that the main cause of this disorder is cultivar susceptibility (apples are very sensitive to high concentration of CO_2). Disorder is associated with high relative humidity in storage chambers (humidity should be reduced gradually), high initial concentration of CO_2 as well as delayed cooling after harvesting (Watkins, Lui, 2010).

During six months of cold storage (control) apples of cvs. 'Gita', 'Orlik', 'Zarja Alatau', 'Auksis' and 'Belorusskoje Maļinovoje' were removed from the research due to non conformity to requirements.

The poor quality of cvs. 'Orlik' and 'Gita' apples was related to over ripening, but fruits of cvs. 'Auksis' and 'Zarja Alatau' had pronounced softening. Signs of scab were also observed on the surface of cv. 'Belorusskoje Maļinovoje' fruits.

Physiological disorders of cvs. 'Orlik' and 'Gita' apples stored under cold storage + 1-MCP treatment were associated with the over ripening, while apples stored under controlled atmosphere conditions (ULO1) had obvious signs of CO_2 injury.

During the experiment microscopic fungi were found on the surface of apples that were stored in cold storage (control). Those fruits had visible signs of spoilage (Fig. A) caused by *P. expansum* (30.44%). Most frequently blue mold caused by the *P. expansum* was detected on the surface of apples with mechanically injured tissue.

In accordance with the report provided by the group of researchers it is evident that the fungus, *P. expansum*, not only causes fruit decay but also produces the carcinogenic fungal metabolite called patulin. This fungal metabolite may exibit a number of toxic effects both in humans and animals and its presence in food products is undesirable (Barreira et al., 2010).

During storage of apples for six months, 26.08% of all the microorganisms located on the surface of apples were *Monilinia fructigena.* Brown rot caused by this fungus is a major post-harvest disease of pome and stone fruits worldwide (Janisiewicz et al., 2010). Infection by the microscopic fungus occurs during blooming, then for a long period of vegetation remains latent and after fruit harvesting, during 24–48 hours begins the stage of development (Spadoni et al., 2013). In the current research, most frequently it was isolated from the apples of cvs. 'Antej' and 'Auksis'. Scientists believe that development of *M. fructigena* can be suppressed by use of fruit storage under controlled atmosphere (Karabulut, Baykal, 2004), as well as hot water treatment at 48°C temperature for 12 minutes (Jemric et al., 2011). Liu et al. (2012) observed a statistically positive effect, achieved when fruits were subjected to heat-treatment at 40°C temperature for 10 minutes.

Neofabraea alba is routinely described as an important cause of apple spoilage in various European countries. Fruit infection occurs in the orchard, but the disease symptoms appear only several months after harvest (Neri et al., 2009). Figure (A) shows that 21.73% of apples were damaged directly by *N. alba* and more pronounced lesions were found in cvs. 'Antej', 'Zarja Alatau', 'Belorusskoje Maļinovoje' and 'Gita' apples. As it was mentioned above, the infection occurs already in the orchard but some symptoms appear during 3–4 months of cold storage, when numerous lesions can develop on a single fruit. Fungi generally live as saprophytes on dead bark, pruning snags and leaves of pome fruits. The main disease symptoms appear only when the fruit is ready to eat (Neri et al., 2009). The

Table. Percentage (%) of damaged apples after six months of storage

Cultivars	Storage conditions	Research year and harvesting time			
		2012–2013		2013–2014	
		1st harvesting	2nd harvesting	1st harvesting	2nd harvesting
'Auksis'	cold storage	30.00 aA	14.42 aB	100.00 aA	9.90 aB
	cold storage + 1-MCP	22.22 bA	2.08 bB	10.76 bA	6.31 bB
	ULO1	2.35 cA	100.00 cB	3.38 cA	4.05 cA
	ULO2	1.43 cA	1.87 bA	1.54 dA	2.35 dB
'Orlik'	cold storage	88.10 aA	100.00 aB	100.00 aA	100.00 aA
	cold storage + 1-MCP	30.95 bA	25.93 bB	100.00 aA	100.00 aA
	ULO1	5.51 cA	100.00 aB	5.16 bA	6.01 bB
	ULO2	2.33 dA	3.96 cB	3.57 cA	3.12 cA
'Gita'	cold storage	100.00 aA	100.00 aA	100.00 aA	100.00 aA
	cold storage + 1-MCP	21.95 bA	100.00 aB	21.21 bA	18.38 bB
	ULO1	7.89 cA	100.00 aB	5.43 cA	6.26 cB
	ULO2	3.28 dA	5.85 bB	4.14 dA	5.32 dB
'Antej'	cold storage	24.24 aA	13.89 aB	19.23 aA	10.68 aB
	cold storage + 1-MCP	11.76 bA	5.56 bB	15.45 bA	6.36 bB
	ULO1	2.23 cA	3.21 cB	3.29 cA	3.91 cA
	ULO2	1.45 dA	2.58 dB	1.89 dA	3.04 cB
'Belorusskoje Maļinovoje'	cold storage	39.02 aA	14.29 aB	28.35 aA	100.00 aB
	cold storage + 1-MCP	12.50 bA	9.30 bB	12.31 bA	13.05 bA
	ULO1	2.98 cA	100.00 cB	3.15 cA	100.00 cB
	ULO2	3.21 cA	6.18 dB	2.19 dA	100.00 cB
'Sinap Orlovskij'	cold storage	9.76 aA	6.50 aB	12.23 aA	5.51 aA
	cold storage + 1-MCP	4.08 bA	0.00 bB	5.32 bA	3.22 bB
	ULO1	0.00 cA	100.00 cB	0.00 cA	1.32 cB
	ULO2	0.00 cA	0.00 bA	0.00 cA	1.95 cB
'Zarja Alatau'	cold storage	15.38 aA	15.33 aA	14.21 aA	100.00 aB
	cold storage + 1-MCP	15.79 aA	11.11 bB	9.82 bA	10.04 bA
	ULO1	100.00 bA	100.00 cA	5.65 cA	100.00 aB
	ULO2	4.65 cA	100.00 cB	5.01 cA	6.79 cB

Notes. Means (percent of rotten apples) followed by different small letters within the same column and cultivar, are significantly different at the 0.05 level (differences between storage types). Mean (percent of rotten apples) followed by different capital letters within the same row and year, are significantly different at the 0.05 level (differences between harvesting time).

N. alba population seems to be sensitive to post-harvest treatment with thiabendazole (TBZ) fungicide (Spotts et al., 2009). However, due to toxicity of this compound, use of TBZ has been prohibited in the Baltic countries and in other countries of North Europe.

Recently, great interest has been shown by several scientists to hot water treatment as natural means to control fruit post-harvest decay. Evidence points that hot water treatment reduces the development of the following microscopic fungi: *Botrytis cinerea*, *Penicillium* spp., *Mucor piriformis* and *Monilinia* spp. (Neri et al., 2007; Sholberg, Randall, 2007; Mari et al., 2008), while significantly positive effect on the development of *N. alba* has not been achieved (Maxin et al., 2005).

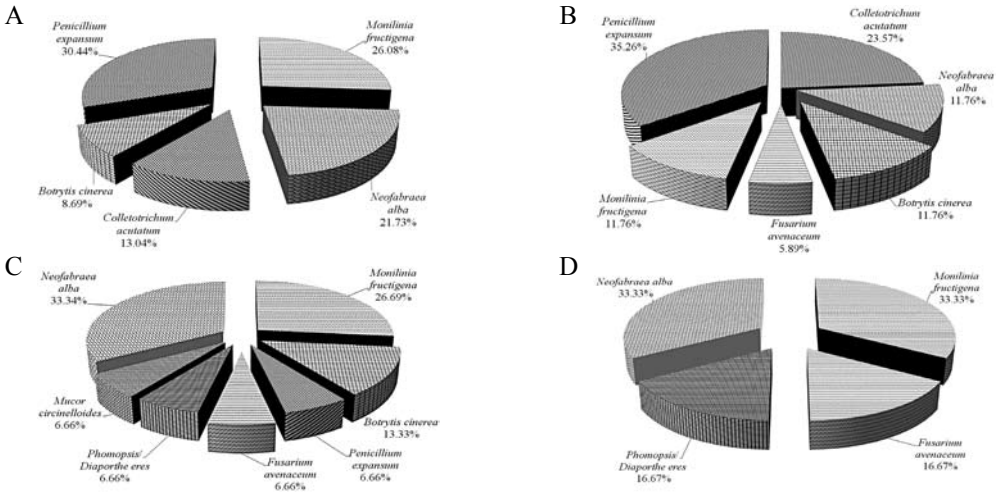
During the experiment in 13.04% cases microscopic fungus *Colletotrichum acutatum* was isolated from damaged apples in cold storage (Fig. A).

Research results indicate that in 8.69% cases microscopic fungus *Botrytis cinerea* was isolated from damaged apples in cold storage as the only significant microorganism that potentially can cause the spoilage of fruits. Post-harvest losses caused by infection with *B. cinerea* can be reduced by minimising mechanical injury, maintaining fruits and vegetables in their early stages of ripening, storing them under optimal conditions, treating with antimicrobial agents. From the results obtained in the current research it is clear that apple samples that prior to storage had been treated with 1-MCP also were infected with microscopic fungi. The microscopic spectrum, isolated from the apples was not considerably different compared with the control fruits. For instance, microscopic fungus *P. expansum* was found in the same frequency as in the control – 35.26% and 30.44%, respectively (Fig. B and A). In this treatment also microscopic fungus *Fusarium avenaceum* was found (5.89%). To the best of our knowledge, this is the first report of *Fusarium* rot caused by *F. avenaceum* on apple fruit that prior to cold storage had been treated with 1-MCP in Latvia. Furthermore, compared with the cold storage the highest amount of microscopic fungus *Colletotrichum acutatum* (23.57%) was found in this type of storage. The same results were obtained by the group of researchers Janisiewicz et al. (2003) pointing out that 1-MCP treatment increased bitter (*C. acutatum*) rot and blue mold (*P. expansum*) decays. However, 1-MCP treatment was more effective compared with the application of antagonist and heat treatment (Janisiewicz et al., 2003). Figure (C and D) shows that in 33% cases microscopic fungus *N. alba* was isolated from infected tissue of apples that had been stored under controlled atmosphere conditions in ULO1 and ULO2.

It was estimated that in 26.69% (ULO1) and 33.33% (ULO2) cases microscopic fungus *Monilinia fructigena* was isolated from damaged apples. Furthermore, scientists (Karabulut, Baykal, 2004) argue that growth and development of this fungus can be maintained using fruit storage under optimal conditions in controlled atmosphere. Unfortunately, effectiveness of controlled atmosphere as a tool of reduction of microscopic fungus *M. fructigena* was not been proved.

One cause of the decay in apples stored under controlled atmosphere conditions in ULO was microscopic fungus *Phomopsis/Diaporthe eres*. Incidence of *Ph./D. eres* infection on apples stored in ULO1, was 6.66%, while in ULO2 16.67%. *Diaporthe* spp. are responsible for diseases on a wide range of plant hosts, causing root and fruit rots, dieback, cankers, leaf spots, blights, decay and wilt (Thompson et al., 2011). Infection of microscopic fungus manifested itself as typical spores, whose development occur on the surface and can be isolated from pure culture. This microscopic fungus is a typical apple causal agent (Davidzon et al., 2010). The initial systematic infection usually occurs during apple flowering due to the fact that *Phomopsis* spp., advantageously colonise dead limbs and trunks. The first symptoms can be observed when apple is ready for use (Prusky et al., 2009).

Figure (C) shows that in 6.66% cases microscopic fungus *Mucor circinelloides* was isolated from damaged apples that had been stored under controlled atmosphere conditions in ULO1. Based on the research report provided by Gherbawy and Hussein (2010) it is evident that despite the use of advanced storage technologies



Note. A – cold storage (control), B – cold storage + 1-MCP treatment, C – ULO1, D – ULO2; data are expressed as the average for two research years, 2013 and 2014.

Figure. The spectra of isolated microorganisms from spoiled apples

and methods, the losses due to various postharvest diseases are estimated to range from 10–30% per year. Notwithstanding the fact that several scientists consider a microscopic fungus *M. circinelloides* as the main cause of apple spoilage, more detailed analysis of scientific papers indicated that the main cause of apple quality deterioration is associated with the microscopic fungus *Mucor* spp. that causes significant loss of apple yield during a long-term storage.

Conclusions

1. The average decay of apples stored in cold storage (control) was 48.96%, while significant ($p < 0.05$) decrease was observed when apple samples prior to cold storage had been treated with 1-Methylcyclopropene (1-MCP) – up to 21.62%. In turn, the severity of decay of apples stored in ultra low oxygen ULO1 and ULO2 was 34.70% and 9.91%, respectively.

2. The highest microbial diversity and amount on apples were found in the cold storage suggesting that these conditions were not suitable for long-term storage of apples. Storage of apple fruits in ULO2 with 2.5% CO_2, 1.5% O_2 appeared to be more promising for several commercial apple cultivars. In particular, cvs. 'Auksis', 'Orlik', 'Gita', 'Antej' and 'Sinap Orlovskij' showed the lowest spoilage rate.

3. The most prevalent microorganisms (cold storage) that were isolated from spoiled apples were: *Penicillium expansum*, *Monilinia fructigena*, *Neofabraea alba*, *Colletotrichum acutatum* and *Botrytis cinerea*, while isolated spectrum of microorganisms from the samples treated with 1-MCP was as follows: *P. expansum*, *C. acutatum*, *N. alba*, *B. cinerea* and *M. fructigena*. Microorganisms isolated from the surface of apples stored under ULO1 and ULO2 conditions were as follows: *N. alba*, *M. fructigena*, *B. cinerea*, *P. expansum*, *Fusarium avenaceum*, *Phomopsis/Diaporthe eres* and *Mucor circinelloides*. Microscopic fungus *F. avenaceum* was not found only on the surface of apples that had been kept under cold storage conditions. In turn, microscopic fungus of the genus *Ph./D. eres* was identified when apples had been stored under ultra low oxygen conditions.

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Mikrobiologiniai pokyčiai ir obuolių puvinio intensyvumas ilgą laiką skirtingomis sąlygomis laikytuose obuoliuose

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Santrauka

Nors obuoliai užima didžiąją dalį Latvijos daržovių rinkos, šalyje trūksta geros kokybės vietoje užaugintų obuolių. Pažangių sandėliavimo technologijų taikymas, pavyzdžiui, laikymas kontroliuojamoje atmosferoje esant itin mažai deguonies (O_2) koncentracijai ir padidintai anglies dioksido (CO_2) koncentracijai, taip pat prieš laikymą obuolių apdorojimas 1-metilciklopropenu (1-MCP), gali smarkiai pagerinti vaisių kokybę ir padėti obuolių augintojams visame pasaulyje į rinką pateikti geresnės kokybės ir saugesnius vaisius. Tyrimo tikslas – nustatyti obuolių puvinio priežastis, juos laikant įvairiomis sąlygomis. Tirtos dvi obuolių laikymo technologijos: 1) laikymas šaltai esant įprastoms sąlygoms ir apdorous 1-metilciklopropenu, 2) laikymas kontroliuojamoje atmosferoje – 2,0% CO_2 1,0% O_2 (ULO1) bei 2,5% CO_2, 1,5% O_2 (ULO2). Po 6 mėnesių obuolius laikant šaltai buvo nustatyti šie mikroskopiniai grybai: *Penicillium expansum* (30,44 %), *Monilinia fructigena* (26,08 %), *Neofabraea alba* (21,73 %), *Colletotrichum acutatum* (13,04 %), *Botrytis cinerea* (8,69 %). *P. expansum* (35,26 %), *Colletotrichum acutatum* (23,57 %), *Neofabraea alba* (11,76 %), *B. cinerea* (11,76 %), *M. fructigena* (11,76 %) ir *Fusarium avenaceum* (5,89 %) buvo išskirti iš obuolių, kurie prieš laikymą buvo apdoroti 1-MCP. Obuoliai, laikyti ULO1 sąlygomis, buvo labiausiai užsikrėtę *N. alba* (33,34%), *M. fructigena* (26,69%), *B. cinerea* (13,33%), *P. expansum* (6,66%), *F. avenaceum* (6,66%), *Phomopsis/Diaporthe eres* (6,66%), *Mucor circinelloides* (6,66%), o laikyti ULO2 sąlygomis – *N. alba* (33,33%), *M. fructigena* (33,33%), *F. avenaceum* (16,67%) ir *P./D. eres* (16,67%). Mikroskopinių grybų, išskirtų iš nevienodomis sąlygomis laikytų puvinio pažeistų obuolių, įvairovė yra gana panaši. Tačiau mikroskopinis grybas *F. avenaceum* nebuvo rastas tik šaltai laikytuose obuoliuose, o mikroskopinis *P./D. eres* genties grybas buvo nustatytas, kai obuoliai laikyti esant itin mažai deguonies koncentracijai.

Reikšminiai žodžiai: kontroliuojama atmosfera, 1-metilciklopropenas, mikrobiologinis užterštumas, obuolių vaisiai, veislė.

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