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Influence of 1-MCP treatment and storage conditions on the development of microorganisms on the surface of apples grown in Latvia

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Abstract

The quality of fruits during storage depends on the storage environment and fruit microorganisms, since the activity of microorganisms can cause the decay of fruits. A correctly selected composition of gas mixture in a storage chamber extends shelf-life by reducing development of microorganisms, accordingly ensuring microbiological safety of fruits. The aim of the study was to evaluate the dynamics of microflora on the surface of apples within six months of cold storage that prior to storage had been treated with 1-methylcyclopropene (1-MCP), and those stored in ultra low oxygen (ULO) under different controlled atmosphere conditions. The highest microbial diversity and amount on the apple fruit surface was found when stored in cold storage. At the beginning of storage, 70% of the total surface microflora of fruits consisted of the following microscopic fungi: *Penicillium* spp., *Alternaria* spp., *Botrytis* spp., *Aspergillus* spp., *Mucor* genus and *Cladosporium* spp., bacteria from *Bacillus* genus, and yeasts like *Candida curvata*, *C. fomata*, *Pichia etchellsii* and *P. carsonii*. After three and six months of apple storage, partial microorganism inhibition was observed when comparing cold storage and cold storage + 1-MCP treatments, in turn, the most positive significant result was achieved when apples were stored under controlled atmosphere conditions.

After six months of apple storage, the lowest amount of colony forming units (CFU) of bacteria, mould and yeast was estimated on the surface of apple fruits that had been stored using controlled atmosphere technique. Microorganisms, including *Penicillium*, *Bacillus* spp., *Candida curvata*, *Pichia etchellsii* and *C. fomata* were identified on the surface of ULO1 samples, while on ULO2 samples only *Penicillium* spp., *Bacillus* spp., *C. curvata* and *C. fomata*. After storage in controlled atmosphere, 90% of all microscopic fungi present on the surface of apples were yeasts.

Key words: controlled atmosphere, microorganisms, storage technologies, surface.

Introduction

Fruit quality during storage mainly depends on the fruit microflora and microbial activity. Microorganisms in appropriate conditions can cause fruit decay, leading to reduction of fruit quality (Barth et al., 2009).

Microflora of apples is very diverse, since fruits present nearly ideal conditions for the survival and growth of many types of microorganisms. Typical apple microflora is various microorganism species of microscopic fungi: (*Penicillium* spp., *Botrytis* spp., *Alternaria* spp., *Aspergilus* spp., *Cladosporium* spp., *Geotrichum* spp., *Fusarium* spp., *Eurotium* spp., *Wallemia* spp. and *Trichoderma* spp.), yeasts (*Candida*, *Pischia*, *Saccharomyces*, *Zygosaccharomyces*, *Hanseniaspora* and *Debaryomyces*), as well as bacteria (*Bacillus* spp.) (Barta, 2006). These microorganisms predominantly occur on the apple surface, but the frequency and share of colonisation can vary. Fresh apple skin with active substances, natural wax coating and its firmness act as a barrier and microbes are restrained to remain outside of fruit flesh as long as the skin is healthy and intact (Barta, 2006; Rodrigo et al., 2012).

The first apple contamination with the microscopic fungi *Botrytis* spp., *Fusarium* spp., *Trichothecium* spp., *Alternaria* spp., *Penicillium* spp., *Monilinia* spp. and others may occur during crop growth in the field. Most of the mentioned microscopic fungi belong to the normal fruit microflora, but often in suitable environment conditions (high level of relative humidity and temperature) at specific fruit ripening stage cause apple spoilage. Contamination occurs predominantly during the growing season and the main contamination sources are apple residues, damaged apples, infected branches and shoots. For instance, if the tree is infected with *Neonectria galligena*, then after harvesting there is a high risk that apples will be also contaminated with these fungi, which leads to apple spoilage (Beresford, Kim, 2011). The second phase for risk of contamination is apple harvesting time, when they come in contact with

soil, during transportation and further processing and handling (Oranusi, Wesley, 2012).

If apple are collected with soiled hands or after harvesting washed with water whose quality does not conform to hygiene requirements, the fruits can be contaminated with *Enterobacter*, *Shigella*, *Salmonella* spp., *Vibrio cholerae*, *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora caytanensis*, *Toxiplasma gondii*, *E. coli* 0157:H7, etc., that can cause both fruit spoilage and human poisoning (Barta, 2006). Apples, which have fallen to the ground, can be contaminated with bacteria *Listeria monocytogenes* and *Sarcina* spp. In both phases (growing and harvesting), apple surface can be contaminated with soil particles that contain a wide range of soil-born aerobic and anaerobic forms of endospores of *Bacillus* spp. and *Clostridium* spp., as well as various yeast species.

Active acidity (pH) of cell juice, water activity, relative air humidity, temperature, storage environment, and osmotic pressure determine the intensity of microorganism development. The pH value of fresh apples varies from 2.9 to 3.3 (Lampel et al., 2012), while for most bacteria such pH is not suitable. The optimum pH for growth of most bacteria is close to neutral (6–8). However, yeasts and moulds are usually acid-tolerant and therefore associated with the spoilage of acidic foods. Microscopic fungi grow in slightly acidic pH 5.0, while yeasts can grow in a pH range of 4.5 to 5.5. The genera of lactic acid bacteria include *Lactobacillus*, *Leuconostoc*, *Streptococcus* and some gram-positive pathogenic bacteria such as *Clostridium* also called as acidophiles and alkaliphiles bacteria that can grow at extreme pH media. For instance, *Bacillus* genera can be attributed to alkaliphiles bacteria that usually occur on apples (Juhneviča et al., 2011).

It is well known that water is important for cells, microorganism existence and development; furthermore, the amount of water depends on the specific microorganism genera (Tapia et al., 2007). Pure water activity is 1.0, but in fresh fruits water activity is slightly lower and corresponds to 0.99. These characteristics make fruit more suitable for the growth of most microorganisms both Gram positive and Gram-negative bacteria. Gram-positive bacteria are very sensitive to changes in water activity. If water activity is too low, for instance 0.75–0.61, halophilic bacteria, xerophilic microscopic fungi and osmophilic yeasts multiplication can occur (Barta, 2006).

Properly chosen relative humidity in store-rooms extends the shelf-life of fruit due to prevention of water losses throughout storage. During fruit storage, adequate control of relative humidity reduces the rate of water loss, maintains water activity at a high level, thereby negatively affecting microorganism development. Advisable relative humidity in storage chambers is 85–95%, depending on the type of commodities. During storage, relative humidity in storage chambers should be controlled. When the humidity is insufficient, a water loss is more pronounced and it leads to softening process, resulting in the decline in fruit quality. In turn, when water activity is too high, the risk of microorganism development increases (Henney et al., 2010).

Storage temperature is another major extrinsic factor that strongly influences the microorganism development. Advisable storage temperature for most of apples is from +2 to +4°C depending on the cultivar. Additionally, the content of oxygen in storage chambers has also great influence on the development of microorganisms. For instance, aerobic bacteria well grow in the presence of oxygen, especially when the concentration of oxygen is close to atmospheric 21%.

Subsequently, aerobic bacteria divide into: obligate aerobes that require oxygen for growth, facultative aerobes that grow well in partial presence of oxygen, as well as without it and microaerophilic – members of this group can grow under reduced oxygen (5% to 10%) and increased carbon dioxide presence (8% to 10%). Anaerobe microorganisms that do not require oxygen for growth can be divided into: facultative anaerobes that do not use oxygen, but they can grow both in aerobe as well as anaerobe conditions and obligate anaerobe. Obligate anaerobe microorganisms are oxygen sensitive, grow only under reduced oxygen conditions. Hence, storage in controlled atmosphere (with reduced oxygen) has a great advantage because it substantially reduces the possible development of spores as well as limits the probable infection of healthy apples (Juhneviča et al., 2011). In turn, it is well known that treatment of climacteric fruits with ethylene inhibitor 1-MCP allows significantly delay the ripening processes, which are associated with biochemical and physiological changes. Since unambiguous researches about the impact of 1-MCP on microbial growth have not yet been observed, it is important to ascertain the impact of 1-MCP on microbial growth. Therefore, the aim of the study was to evaluate the dynamics of microflora on the surface of apples, pre-storage treated with 1-methylcyclopropene (1-MCP), within six months of cold storage and on those stored in ultra low oxygen (ULO) under different controlled atmosphere conditions.

Material and methods

The studies took place in 2012 and 2013 at the Experimental Processing Department of the Latvia State Institute of Fruit-Growing (currently – Institute of Horticulture, Latvia University of Agriculture) in Dobele and at the Laboratory of Microbiology of the Faculty of Food Technology in the Latvia University of Agriculture. In each harvesting year, the duration of experiment was six months. Analyses were carried out before apple storage, then after three and six months of storage. One apple commercial cultivar 'Auksis', grown in the orchard of the Institute, was used.

Shortly after harvesting, apples were air-cooled for 24 hours in a cooling chamber at up to +4 ± 0.5°C temperature. From the selected cultivar 'Auksis' 40 fruits were sampled, their average mass was ~6 kg. For each storage technology, the same weight of apples was prepared. Then the samples were placed in a polypropylene boxes with perforated walls. The total number of boxes was twelve. The cooled down apple samples were divided into four groups for post-harvest storage: 1) cold storage – control, apples were stored under traditional conditions at air temperature +2 ± 1°C and relative air 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 in Fruit Control Equipment (Italy) by selecting two different gas compositions in the mixture of controlled atmosphere.

The treatment with ethylene inhibitor 1-MCP was performed in a air-tight fruit processing container. The ethylene action inhibitor 1-methylcyclopropene 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 ml L^{-1}. This ratio was selected on the well-founded scientific research of Polish researchers affirming 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 (RandH, Italy) recommendations in 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 traditional conditions. The apples treated with 1-MCP and untreated (control) were stored at the same conditions but on different pallets.

The microbiological analysis of air in ULO chambers and cooled store-room was carried out in accordance with LVS EN 13098:2001 standard Workplace atmospheres – Guidelines for measurement of airborne micro-organisms and endotoxin.

Microflora of apple surface was determined in accordance with LVS ISO 18593:2007 standard Microbiology of food and animal feeding stuffs – Horizontal methods for sampling techniques from surfaces using contact plates and swabs. The microbiological analyses were carried out in accordance with the following reference methods: for detection of mesophilic aerobic and anaerobic micro-organisms were used standard LVS EN ISO 4833-2:2014 Microbiology of food chain – Horizontal method for the enumeration of microorganisms. Part 2: Colony count at 30 degrees C by the surface plating technique (ISO 4833-2:2013). Yeasts and moulds were determined in accordance with standard LVS ISO 21527-1:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0.95. *Pseudomonas* spp. was determined in accordance with standard LVS EN ISO 16266:2008 Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration. Microscopic fungi were identified in accordance with the method provided by the group of scientists (Juhnevič et al., 2011).

Identification of yeasts was performed using strips of biochemical test-system API – yeasts with API 20 C AUX and ID32 C, but bacteria with API 50 CHB.

The processing of data was carried out by the methods of mathematical statistics, where arithmetic average value of results, standard deviation and standard error were calculated using software *Microsoft 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

Air microflora in a storage room is crucial because it is influenced by the microflora and quality of the products. Therefore, in the current study the analysis of initial air microflora in ULO chambers, as well as cooled store-room was carried out and colony forming units of bacteria, yeast and mould were determined (Table). The air contains organic and inorganic mixture of particles, which is combined with dust. Due to pollution, the air can be considered as a source of pathogenic microorganisms, which can cause allergic reactions, respiratory problems and generally adversely affect the human health (Lignell et al., 2007; Karvala et al., 2008; Łukaszuk et al., 2015).

The following microbiological agents can be present in the air: *Alternaria* spp., *Cladosporium* spp., *Penicillium* spp. and *Aspergillus* spp., bacteria *Bacillus* spp., *Clostridium* spp., *Streptomycineae* spp., *Micrococcus* spp. and *Corynebacterium* spp., but yeasts *Candida* were general representatives (Aithal, 2009). Some of these microorganism species such as *Aspergillus*,

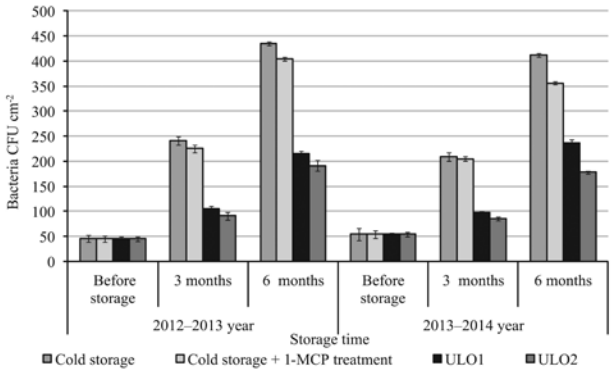
Table. The dynamics of microflora (CFU m^{-3}) in the apple storage environment during the study period

Storage technology	2012–2013			2013–2014		
	before storage	during 3 months of storage	during 6 months of storage	before storage	during 3 months of storage	during 6 months of storage
Yeasts						
Cold storage	9 a	12 a	13 a	8 a	10 a	15 a
ULO1	4 b	6 b	5 b	3 b	7 b	5 b
ULO2	3 b	4 b	5 b	3 b	5 b	3 b
Moulds						
Cold storage	12 a	22 a	31 a	11 a	19 a	33 a
ULO1	6 b	4 b	5 b	4 b	3 b	5 b
ULO2	5 b	2 b	3 b	2 b	2 b	4 b
Mesophilic aerobic bacteria						
Cold storage	10 a	13 a	15 a	9 a	12 a	18 a
ULO1	5 b	5 b	4 b	4 b	6 b	4 b
ULO2	4 b	3 b	4 b	3 b	4 b	2 b

Notes. The different letters in the same column represent significant differences between values by the least significant difference. The mean difference is significant at the 0.05 level. CFU – colony forming unit, ULO – ultra low oxygen.

Penicillium and *Alternaria* attack fruits during growth, harvesting or further processing. Mycotoxin production mainly depends on the microorganism growth intensity and storage conditions. If conditions are favourable for fungal growth during fruit storage, the concentration of mycotoxins will be increased. Mycotoxins produced in the host tissues may be aflatoxin, ochratoxin A, patulin and *Alternaria* toxins. Some of these mycotoxins are known to be carcinogenic – those are very stable during storage and processing and, therefore, can reach the consumer. If the fruits are visually rotten, consumers refuse to buy such fruits; however, processed fruit products may still contain virulent agents that can pose a serious threat to human and animal health (Battilani et al., 2008).

The development of bacteria was most intensive on the surface of 'Auksis' apples, which were stored in cold storage for six months (Fig. 1) The total plate count of bacteria was 435 CFU cm^{-2} (storage year 2012–2013) and 412 CFU cm^{-2} (storage year 2013–2014). The data obtained after storage of apples for six months in cold storage + 1-MCP treatment evidenced that the total plate count was 404 CFU cm^{-2} in storage year 2012–2013, while in storage year 2013–2014 it was 356 CFU cm^{-2}. There were significant differences in CFU between the years (2012–2013 and 2013–2014).



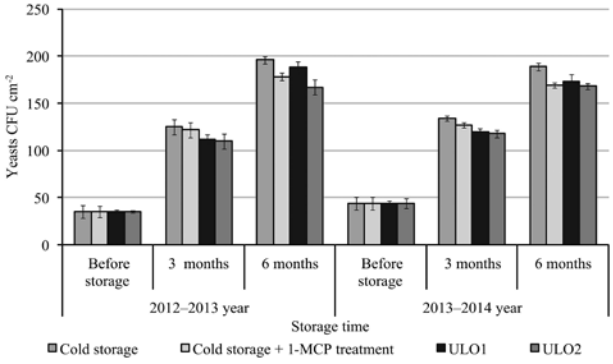
CFU – colony forming units, ULO – ultra low oxygen

Figure 1. The dynamics of total plate count of bacteria on the apple surface during storage

The highest amount of bacteria after a six-month storage was found on the apples, which had been stored in cold storage compared with those stored in ULO chambers. This phenomenon can be explained by the fact that apple fruits that had been stored using cold storage were contaminated repeatedly during storage. This means that during fruit storage, contamination of healthy fruits commonly occurs by spores through fruit-to-fruit contact, especially if fruits are injured during harvesting (Jones, Aldwinckle, 2004).

At the end of apple storage under controlled atmosphere, the total amount of bacteria on the surface of apples was significantly ($p < 0.05$) lower (twice) compared with the microflora of the apples stored under normal atmosphere. Hence, we may conclude that in ULO environment the positive effect was achieved by suppressing the growth of aerobic bacteria, while facultative aerobic bacteria continued to multiply.

The total amount of colony forming units of yeasts on the surface of apples did not differ significantly ($p < 0.05$) between the storage treatments and research years (Fig. 2). However, the highest amount of yeasts was found on the surface of apples stored in cold storage. Assessing the results obtained in the current research

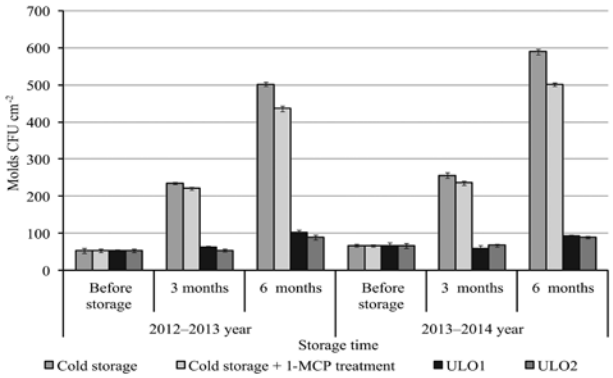


CFU – colony forming units, ULO – ultra low oxygen

Figure 2. The dynamics of total plate count of yeasts on the apple surface during storage

it can be concluded that statistically positive influence of 1-MCP treatment and ULO conditions on the yeast proliferation during the storage was not achieved. This phenomenon can be explained by the fact that yeasts are able to grow under both aerobe and anaerobe conditions. The total amount of colony forming units increased throughout the fruit storage in cold storage, as well as under controlled atmosphere in ULO (Fig. 2).

After a six months storage, the total amount of mould on apple surface was considerably ($p < 0.05$) increased and the most pronounced increasing was found on the surface of apples that had been stored in cold storage 502 CFU cm^{-2} (storage year 2012–2013) and 589 CFU cm^{-2} (storage year 2013–2014) (Fig. 3). No statistically significant differences in the number of colony forming units were observed between the cold storage and cold storage + 1-MCP treatments. However, the total plate count of mould on the surface of apples when stored under controlled atmosphere in ULO was significantly ($p < 0.05$) lower. This can be explained by the anaerobic conditions in controlled atmosphere, which delayed the development of moulds. The analysis of the data evidenced that the amount of colony forming units on apple surface under controlled atmosphere ULO1 was 103 CFU cm^{-2} (storage year 2012–2013), while in storage year 2013–2014 the amount of CFU was 92 CFU cm^{-2}. Somewhat lower, but not significantly different ($p < 0.05$) amount of colony forming units was detected on the surface of apples stored in ULO2. The amount of colony forming units equal to 89 CFU cm^{-2}.



CFU – colony forming units, ULO – ultra low oxygen

Figure 3. The dynamics of total plate count of moulds on the apple surface during storage

Since fruit microflora depends mainly on the storage environment and conditions, the identification of microorganisms was carried out both before and after the fruit storage. The results of research indicate that before the storage, 70% of the total microflora on the fruit surface consisted of the following microscopic fungi: *Penicillium* spp., *Alternaria* spp., *Botrytis* spp., *Aspergillus* spp., *Mucor* genera and *Cladosporium* spp., while *Bacillus* spp., bacteria and yeasts like *Candida curvata*, *Candida fomata*, *Pichia etchellsii* and *Pichia carsonii*. Similar observations of apple surface microflora were obtained by other scientists (Scheper et al., 2007; Juhneviča et al., 2011).

Currently, scientists pay more attention to those problems that are associated with the human health and fruit microflora that affect human health. For instance, some trials have been conducted using heat-treatment technologies aimed to decrease *Escherichia coli* O157:H7 and *Salmonella muenchen* population, present on the surface of apples (Wang et al., 2012).

Our observations in the current research revealed that pathogenic microflora like *Escherichia* spp. and *Salmonella* spp., which can have harmful influence on the human organism, was not detected. On the surface of apples, which were stored under normal atmosphere in cold storage, significantly lower CFU of *Botrytis* spp., *Penicillium* spp., *Alternaria* spp., *Mucor* spp., *Pseudomonas fluorescens*, *Bacillus* spp., *Candida curvata*, *C. fomata*, *Pichia etchellsii* and *P. carsonii* was found. The highest amount of microorganisms was found on the surface of apples that had been stored in cold storage, compared with apples stored under controlled atmosphere.

After six months of apple storage under controlled atmosphere in ULO1, on the surface of apples the following microorganisms were identified: *Penicillium*, *Bacillus* spp., *Candida curvata*, *Pichia etchellsii* and *Candida*, *fomata*, while on the apples stored in ULO2 the following microorganism species and genera were identified: *Penicillium* spp., *Bacillus* spp., *Candida curvata* and *C. fomata*. Lower prevalence of microorganism species was found on the surface of apples stored in ULO2. During storage of apples for six months in ULO1 and ULO2, 90% of all the microorganisms located on the surface of apples were yeasts due to their facultative anaerobe properties. The results from the current study revealed that the genus of *Penicillium* was also detected on apples that had been stored under controlled atmosphere conditions. Based on the literature, it became clear that some *Penicillium* species can grow in low-oxygen tensions. *Penicillium expansum* and *Penicillium roqueforti* are able to grow normally in 2% of oxygen. In turn, no other representatives of microscopic fungi were detected.

Based on the results obtained in this research, it can be concluded that before storage microflora of apples consisted of *Penicillium* genera and gas composition that had been selected, significantly reduced the proliferation of microscopic fungi, excluding *Penicillium* genera. The same observation was established by the researcher from Latvia (Juhneviča et al., 2011). In turn, microflora of apple surface after cold storage consisted of 85% of *Penicillium* spp. Based on the results in this research, it can be concluded that microflora of apple surface consisted predominantly of *Penicillium* spp., which prevented the development of other microscopic fungi.

Conclusions

1. The highest number of colony forming units (CFU) of bacteria was observed on the surface of apples stored for six months in cold storage and those stored in cold storage + 1-methylcyclopropene (1-MCP) treatment, while the lowest on fruits stored in controlled atmosphere (ultra low oxygen, ULO).

2. The highest amount of colony forming units of moulds after a six-month storage was found on apples that had been stored in cold storage. In turn, significantly lowest ($p < 0.05$) amount of CFU was found on apples that had been stored under controlled atmosphere. Anaerobic conditions that provide ULO storage significantly suppressed the development of oxygen-dependent microorganisms.

3. At the beginning of storage, 70% of the total surface microflora on the fruits consisted of such microscopic fungi as *Penicillium* spp., *Alternaria* spp., *Botrytis* spp., *Aspergillus* spp., *Mucor* genus, and *Cladosporium* spp., bacteria from *Bacillus* genus, and yeasts like *Candida curvata*, *C. fomata*, *Pichia etchellsii* and *P. carsonii*.

4. During six months of cold storage, on the surface of apples a vast majority of the above mentioned microorganisms along with *Pseudomonas fluorescens* were detected. At the same time, on the surface of fruits stored in controlled atmosphere considerably lower amount of microorganisms was found compared with the control samples. *Penicillium* spp., *Bacillus* spp., *Candida curvata*, *Pichia etchellsii* and *C. fomata* were identified on the surface of apples stored in ULO1, while on those apples stored in ULO2 samples only *Penicillium* spp., *Bacillus* spp., *Candida curvata* and *C. fomata* were detected.

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Apdorojimo 1-MCP ir laikymo sąlygų įtaka mikroorganizmų vystymuisi ant Latvijoje užaugintų obuolių paviršiaus

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Santrauka

Vaisių kokybė sandėliavimo metu priklauso nuo sandėlio aplinkos ir vaisių mikroorganizmų, nes jie gali sukelti vaisių puvinį. Tinkamai parinkta dujų mišinio obuolių laikymo kameroje sudėtis gali pailginti vaisių vartojimo laiką sumažindama mikroorganizmų vystymąsi ir užtikrindama vaisių mikrobiologinę saugą. Tyrimo tikslas – įvertinti mikrofloros ant obuolių paviršiaus dinamiką per 6 laikymo šaltai mėnesius, prieš tyrimą vaisius apdorojus 1-metilciklopropenu (1-MCP) ir ant obuolių, laikomų esant itin mažai deguonies koncentracijai skirtingomis kontroliuojamos atmosferos sąlygomis. Didžiausia mikrobų įvairovė ir kiekis ant obuolių paviršiaus buvo laikant šaltai. Laikymo pradžioje 70 % visos obuolių paviršiaus mikrofloros sudarė *Penicillium* spp., *Alternaria* spp., *Botrytis* spp., *Aspergillus* spp. bei *Mucor* genties mikroskopiniai grybai, *Cladosporium* spp. bei *Bacillus* genties bakterijos ir *Candida curvata*, *C. fomata*, *Pichia etchellsii* bei *P. carsonii* mielės. Po obuolių laikymo 3 ir 6 mėnesius buvo nustatytas mikroorganizmų slopinimas, lyginant jų laikymą šaltai ir laikymą šaltai + apdorojimą 1-MCP, o didžiausias teigiamas esminis rezultatas buvo gautas obuolius laikant kontroliuojamos atmosferos sąlygomis.
Po 6 mėnesių laikymo mažiausias kiekis bakterijų, pelėsių ir mielių kolonijas formuojančių vienetų buvo nustatytas ant paviršiaus obuolių, laikytų kontroliuojamos atmosferos sąlygomis. Mikroorganizmai *Penicillium*, *Bacillus* spp., *Candida curvata*, *Pichia etchellsii* ir *C. fomata* ant obuolių paviršiaus buvo nustatyti mažos deguonies koncentracijos 1 variante, o 2 variante buvo nustatyti tik *Penicillium* spp., *Bacillus* spp., *C. curvata* ir *C. fomata*. Po laikymo kontroliuojamos atmosferos sąlygomis 90 % visų mikroskopinių grybų, esančių ant obuolių paviršiaus, buvo mielės.

Reikšminiai žodžiai: kontroliuojama atmosfera, laikymo technologijos, mikroorganizmai, paviršius.

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