Environmental and Experimental Biology (2011) 9: 53–59 *Original Paper*

Evaluation of microbiological contamination of apple fruit stored in a modified atmosphere

Karina Juhneviča*, Gita Skudra , Liga Skudra

Latvia State Institute of Fruit-Growing, Graudu 1, Dobele LV-3701, Latvia

*Corresponding author, E-mail: karina.juhnevica@lvai.lv

Abstract

The quality of fruit during storage depends on the storage environment and fruit microorganisms, since the activity of microorganisms can cause fruit decay. Properly chosen composition of the gas mixture in the storage environment prolongs storage time by reducing development of microorganisms, ensuring their microbiological safety. The aim of the study was to evaluate five apple cultivars during and after storage for five months in controlled modified atmospheric conditions. The highest microbial diversity on the apple fruit surface was found when stored in a cooler in a conventional atmosphere, suggesting that these conditions were not suitable for apple fruit storgae. A modified atmosphere reduced the development of bacteria, yeasts and moulds. The total amount of bacteria was reduced by 50% in the used modified atmosphere, in comparison to samples stored in a cooler. The proportion of moulds increased three-fold in control samples, but their number in the modified atmosphere did not change significantly. Storage of apple fruits in a Ultra Low Oxygen 2 chamber with 1.5% O_2 and 2.5% CO_2 appeared to be most prospective for several commercial apple cultivars. In particular, cultivars 'Auksis' and 'Orlik' showed the least proportion of damaged fruit after storage in these conditions.

Key words: apple fruit, controlled modified atmosphere, microbiological contamination.
Abbreviations: CFU, colony-forming units; LSIFG, Latvia State Institute of Fruit-Growing; ULO, Ultra Low Oxygen.

Introduction

One of the factors favourably influencing human health and lifespan is use of fresh and safe high-quality food. Quality of fruit during storage to a great extent depends on the storage environment and fruit microorganisms, since the activity of microorganisms can cause fruit decay, as a result reducing their quality.

Postharvest physiology of apple fruit has received much attention during the last decades (Song, Bangerth 1996; Argenta et al. 2000; DeEll et al. 2002; Zude et al. 2006). It is essential to maintain not only high apple fruit quality, but also to ensure their microbiological safety during storage. If too many pathogenic microorganisms develop on the apple surface during storage, they can promote decay of apples and cause serious metabolic disorders to humans through the action of microorganism-derived toxins (Stinson et al. 1981; Beretta et al. 2000). Such fruits after storage can become a source of contamination for other products. Therefore, production technologies regarding storage need to be developed, to allow to maintain fruit nutritional value while reducing the development of microorganisms.

The most prospective method for apple fruit storage a controlled modified atmosphere with a properly chosen gas mixture. This method is increasingly used in practice as it prolongs fruit storage by up to one year and reduces development of microorganisms. A modified controlled atmosphere for apple cultivars 'Elstar', 'Gala' 'Golden Delicious' and 'Jonagold' prolonged fruit storage for even up to one year with minimal weight loss, damage and with a minimum reduction in biochemical characteristics (Mohamed et al. 2000; Akbudak et al. 2002). Many microbial species (*Penicillium*, *Cladosporium* etc.) are aerobic and their growth is inhibited in a modified environment with a low concentration of oxygen (Nikolajeva 2007).

The aim of the present study was to determine the optimal storage conditions for fruit of commercial apple cultivars grown in Latvia. To maintain microbiological safety, development of microorganisms on the surface of fruit in controlled modified atmospheric conditions during storage was evaluated.

Materials and methods

Cultivars and scheme of investigation

The study was conducted at the Experimental Processing Department of the Latvia State Institute of Fruit-Growing (LSIFG) in Dobele and at the Laboratory of Microbiology of the Food Technology Faculty of the Latvian University of Agriculture. The duration of the investigation was five months. Five different apple commercial cultivars ('Gita', 'Saltanat', 'Orlik', 'Auksis', 'Korichnoe Novoe') grown in the orchard of LSIFG were used. To determine changes in quality of apple fruit during and after storage in different

Table 1. Modified atmosphere gas composition in the storage chambers

Gaseous environment	O_2 (%)	CO_2 (%)	N_2 (%)	Temperature (°C)	Relative humidity (%)
Modified atmosphere (ULO 1)	1.00	2.00	97.00	2 ± 0.2	85 – 90
Modified atmosphere (ULO 2)	1.50	2.50	96.00	2 ± 0.2	85 – 90
Cooling chamber	20.96	0.03	78.06	2 ± 0.2	85 – 90

storage environments, controlled modified Ultra Low Oxygen (ULO) chambers were used with different gas mixtures (Table 1). Fruit samples stored in a cooler was used as a control. The gas mixture used, temperature and relative air humidity used for fruit storage in the present investigation was similar to that utilized in other studies (Lau 1998; Kaack 2011).

Microbiological analyses of the air

Microbiological analyses of air in the ULO chambers and in the cooler were performed before and after storage of apples according to the standard LVS EN 13098:2001 to determine the total amount of microorganisms and presence of yeasts and moulds. Determination of the total amount of microorganisms – mesophylic aerobic and facultative anaerobic (bacteria) – was conducted according to the standard LVS EN 4833:2003. Determination of yeasts and moulds was performed according to the standard LVS ISO 21527:2003 (Fig. 1). The total amount of bacteria was calculated by using the formula (McLancdsborough 2005)

$$X = (a \times r \times 10 / S) \times 100.$$

X is the total amount of bacteria in 1 m^3 of air; a – number of colonies in Petri dishes; r – opening time of Petri dishes (min); 10 – volume of the air which flows out of the surface of a Petri dish during opening (5 min); S – area of Petri dish (cm^2); 100 – coefficient for recalculation to m^3.

The API test ID 32 C was used only for identification of yeasts; identification of other microrganism species (bacteria, moulds) was by morphological properties.

Microbiological evaluation of apple surface

Microbiological evaluation of the apple fruit surface was performed before and after storage according to the standard LVS EN ISO 7218:2007 to determine the total amount of microorganisms and presence of yeasts and moulds. Determination of the total microorganisms, mesophylic aerobic and facultative anaerobic microorganisms (bacteria) was performed accordingly to the standard LVS EN 4833:2003; yeasts and moulds were detected according to ISO 21527-1:2008 and *Pseudomonas* spp. according to LVS EN ISO 12780:2003 (Fig. 2).

Detection of microscopic fungi

Detection of microorganisms was performed using the moist chamber method. Samples of damaged apple tissues were placed in Petri dishes, moisturized and grown in a chamber for seven days at 25 °C. Developed fungi were identified by their morphological features according to previously described methods (Malcolm, Shurtleff 1995; Sinclair, Dhingra 1995).

Data analysis

The results obtained in the experiments were analyzed using the MS Excel program. Groups were summarized using descriptive statistics, and the significance level was P = 0.05. The obtained groups of data were compared using mean values. Standard deviations were used for characterization of the variability of the obtained results. The proportion of decayed fruits was expressed in %.

Results

Microbiological analysis of air microflora

Air microbiological analyses in the ULO chamber and cooler were performed before apple fruit storage to determine the total amount of microorganisms, and

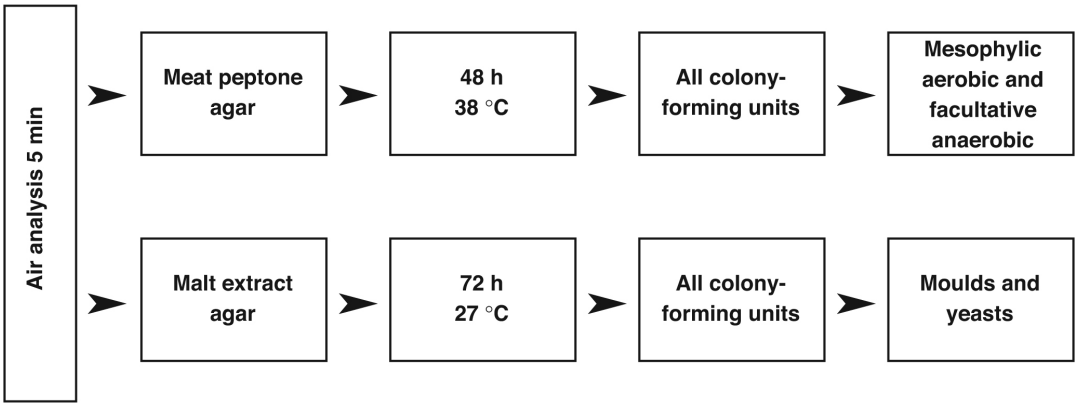

Fig. 1. A scheme of microbiological air testing.

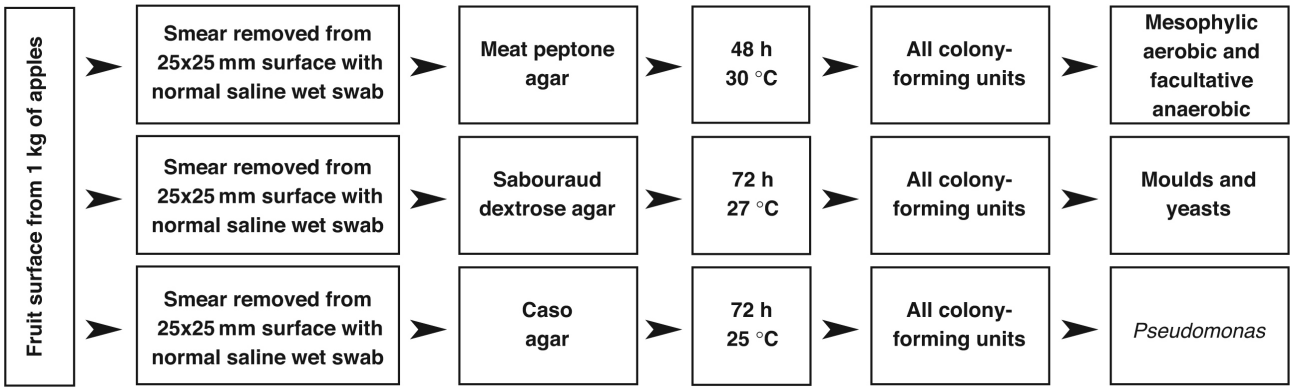

Fig. 2. A scheme of microbiological testing of apple fruit surface.

presence of yeasts and moulds. The results showed that the air contained a relatively small amount of microorganisms, which was achieved by disinfection of storage chambers (Fig. 3).

Development of moulds during storage was significant in the cooler, as their amount increased by nearly two times from 8 CFU m^{-3} before storage to 15 CFU m^{-3} after storage. However, the amount of yeasts and moulds was reduced in chambers with a modified atmosphere. Similar results were obtained for mesophylic aerobic and facultative anaerobic microorganisms: reduced by 25% in the chamber ULO 1 and ULO 2. Consequently, a controlled modified atmosphere significantly reduced the development of microorganisms. The most substantial decrease of the total amount of microorganisms was observed in the ULO 2 chamber with a gas content of 1.5% and 2.5%, for O_2 and CO_2, respectively.

Total amount of microorganisms on apple fruit surface

The development of bacteria was most intensive on apple fruits when stored in a cooler, where the total counts increased by up to ten-fold (Fig. 4). The total amount of bacteria on apples stored at a modified atmosphere was significantly less (nearly by half), compared to the number of bacteria on fruit stored in a cooler. The lowest increase of bacteria was observed on apple samples stored in the ULO 2 chamber. The number of bacteria increased three times an average during storage in the cooler; their number in the ULO 1 chamber was only slightly higher and did not differ significantly.

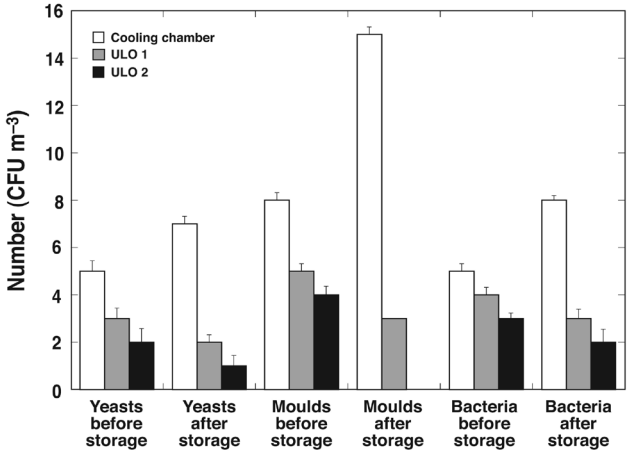

Fig. 3. Microbiological test results of air samples from storage chambers before and after apple fruit storage. Data are means from three replicates ±SD.

Similar results were obtained for presence of yeasts on apples, their development occurred most intensively for apples stored in the cooler (Fig. 5). The number of CFU of yeasts on apples stored in the cooler increased seven times on average during storage. A lower number of yeasts was observed on apples stored in the ULO 2 chamber; their number increased about five times. The highest number of yeasts on apples was found for cultivars 'Korichnoe Novoe' and 'Orlik'.

The total amount of moulds on apples stored in the cooler increased significantly during storage. The number of colony forming units even doubled, and the number of moulds on cultivar 'Gita' increased by more than four times from 92 CFU cm^{-2} to 402 CFU cm^{-2} (Fig. 6). Development of moulds on apples stored in the ULO 1 chamber was significantly less than on apples stored in the cooler. Few moulds were found on apples stored in the ULO 2 chamber; the amount was 50% less than in the ULO 1 chamber. The gas mixture in the ULO 2 chamber had a higher concentration of CO_2 (2.5 %), which depressed the development of moulds. The largest number of moulds on apples at the end of storage was found for cultivars 'Korichnoe Novoe' and 'Orlik'.

Microorganisms on apples

Units of colony forming microorganisms developed from samples taken before and after storage were identified. The dominating microscopic fungi before storage were *Penicillium*, *Alternaria*, *Botrytis*, *Aspergillus* and *Cladosporium macrocarpum*. *Penicillium italicum* was the most abundant species from the genus *Penicillium*, and *Pseudomonas fluorescens* and *Bacillus cereus* were

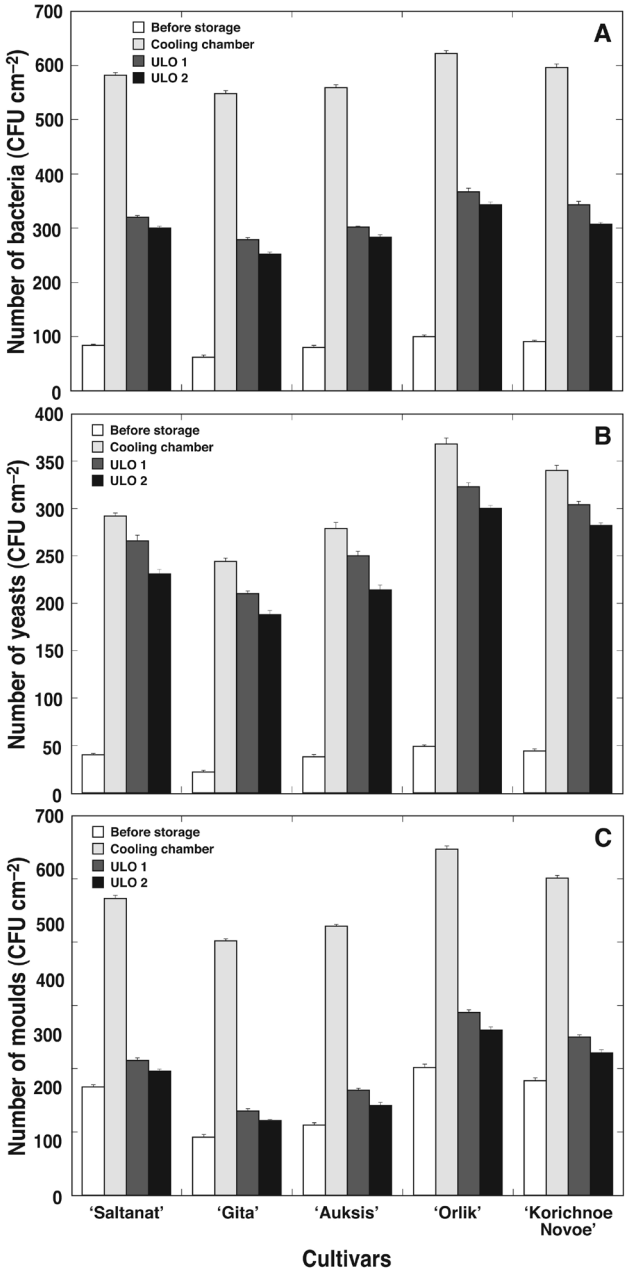

Fig. 4. Number of bacteria (A), yeasts (B) and moulds (C) on apples before and after fruit storage in different conditions. Data are means from three replicates ±SD.

the dominant bacteria and *Candida curvata*, *Candida famata*, *Pichia carsonii*, *Pichia etchellsii* were the dominant yeasts. Microscopic fungi, including yeasts and moulds, constituated 80% of the total microorganisms at the beginning of storage.

Microorganisms more often found on apple fruit surface were from the genera *Penicillium, Alternaria, Aspergillus, Cladosporium, Candida* and *Bacillus*. Microorganisms from the other genera were observed more seldom. Species composition on the fruit surface changed during storage. The microorganisms *Botrytis, Penicillium* spp.,

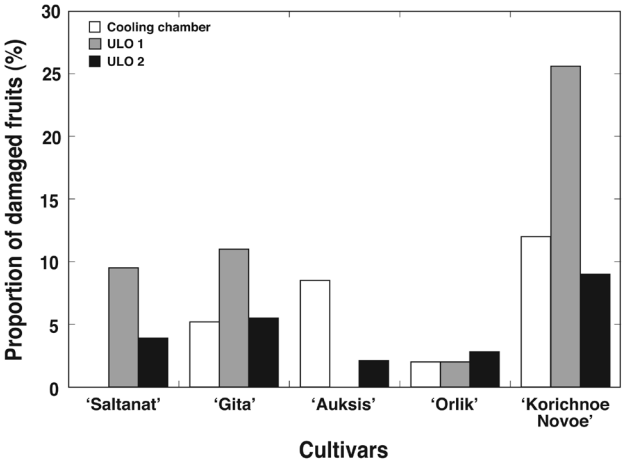

Fig. 5. Proprotion of damaged apples for various cultivars after storage in different conditions..

Penicillium italicum, Pseudomonas fluorescens, Bacillus cereus, Candida curvata, Candida famata, Pichia etchellsii, and *Pichia carsonii* were found on apples stored in the cooler. The diversity of microorganisms on apples stored in the cooler at the level of genera and species was extremely high, showing that storage in a cooler was not useful. The microorganisms *Penicillium italicum, Bacillus cereus, Pseudomonas fluorescens, Candida curvata, Pichia etchellsii,* and *Candida famata* were identified on fruits stored in the ULO 1 chamber. Microorganism species such as *Penicillium* spp., *Candida curvata, Candida famata* were identified also in the ULO 2 chamber, but the species diversity was lower. Yeasts, as facultative anaerobic microorganisms, were observed as dominating microorganisms on apples after storage in the modified atmosphere and accounted for 70% of all microscopic fungi. *Penicillium* constituted 80% of all mould and yeast colony forming units and genus *Botrytis* – 5 % on fruit after storage in cooler. The occurrence of yeasts on apples was much less pronounced in the cooler when compared to the modified atmosphere. The microorganism genera *Alternaria, Aspergillus* and *Cladosporium* did not develop on apples stored in the cooler.

Proportion of decayed apples during storage

The proportion of decayed apples was relatively high for the cultivar 'Korichnoe Novoe' in all storage regimes, reaching 12% in the cooler, 9% in the ULO 2 chamber and 26% in the ULO 1 chamber (Fig. 7). Increased number of decayed fruits during storage caused by microscopic fungi was observed for cultivars 'Gita' and 'Korichnoe Novoe', especially in the cooler. A large amount of decayed apples of the cultivar 'Korichnoe Novoe' in ULO chambers and of the cultivar 'Gita' in the ULO 1 chamber was observed. Relatively large number of decayed apples was noted for the cultivar 'Auksis', reaching 8.5% in the cooler. The gas content in the ULO 1 chamber was more suitable for storage since decayed apples were not found for the cultivar 'Auksis'. However, in

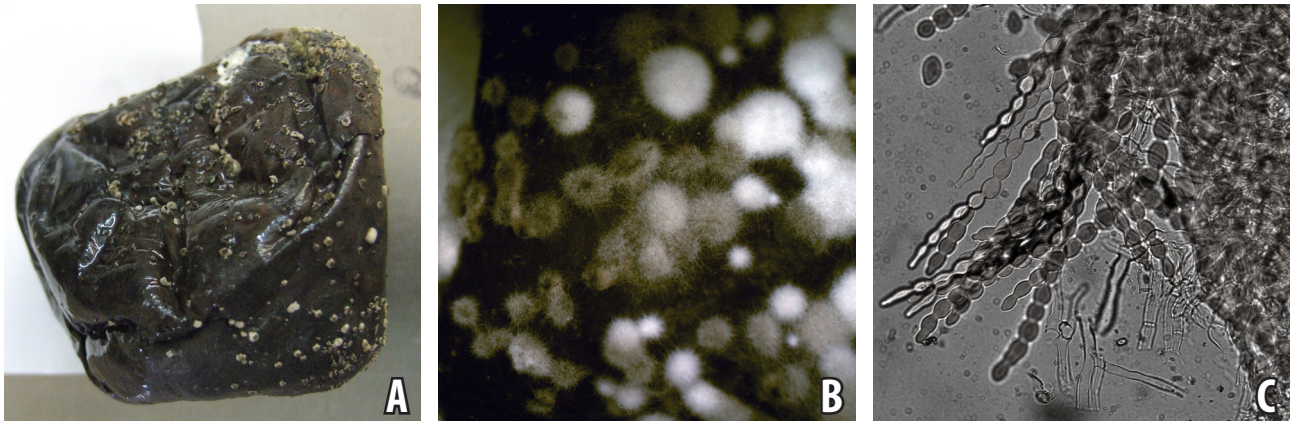

Fig. 6. Identification of the morphological characteristics of brown rotting agent fungi *Monilia* spp. A, general view of damaged apple, fruit; B, close-up view of damaged surface; C, isolated fungal structures.

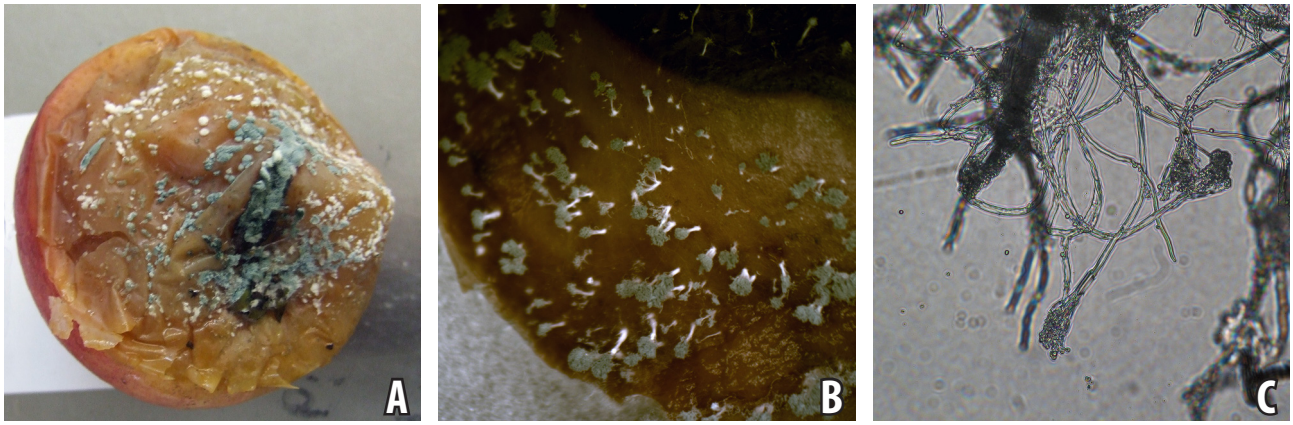

Fig. 7. Identification of the morphological characteristics of green rotting agent fungi *Penicillium italicum*. A, general view of damaged apple, fruit; B, close-up view of damaged surface; C, isolated fungal structures.

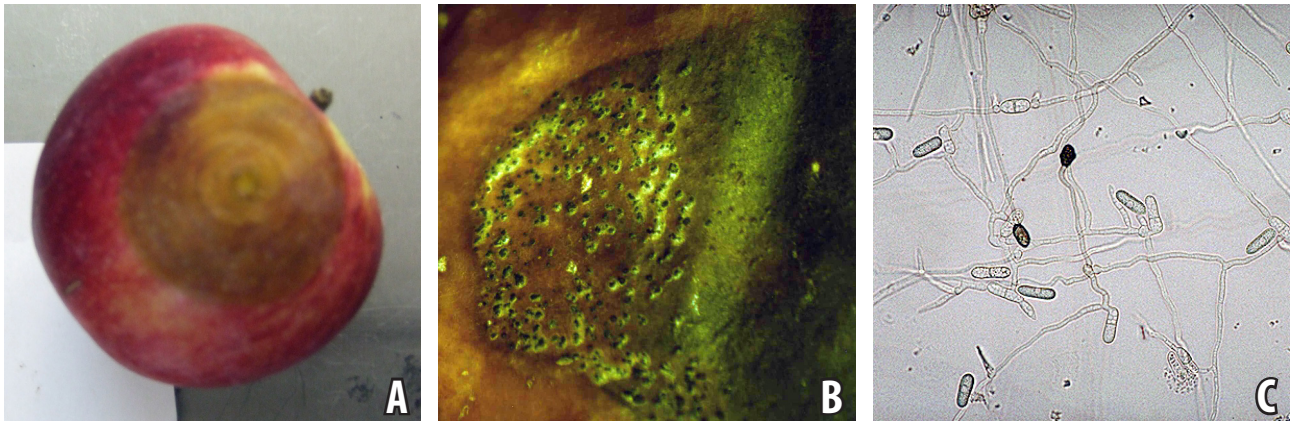

Fig. 8. Identification of the morphological characteristics of bitter rotting agent fungi *Colletotrichum* spp. A, general view of damaged apple, fruit; B, close-up view of damaged surface; C, isolated fungal structures.

the ULO 2 chamber, the number of decayed fruit was only 2.1%. The amount of decayed apples of the cultivar 'Orlik' was only 2% in the ULO 1 chamber, compared to 2.8% in the ULO 2 chamber. It can be concluded that the content of modified gases was most appropriate for the cultivars 'Auksis' and 'Orlik'.

Reasons of apple decay during storage

Apple decay was caused mainly by *Monilia* rot (Fig. 6), *Penicillium* rot (Fig. 7) and *Colletotrichum* rot (Fig. 8).

The cultivar 'Korichnoe Novoe' (Fig. 9A) was the most susceptible to the influence of microscopic fungi in the cooler and ULO 2 chamber, as it was infected by two

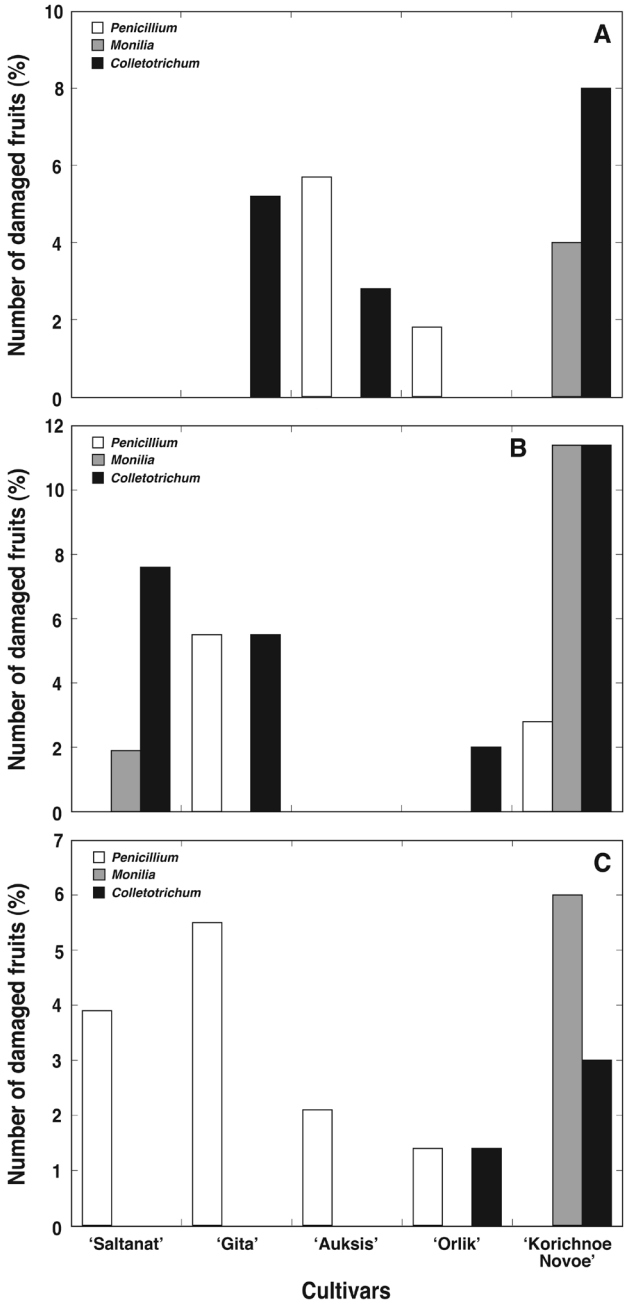

Fig. 9. Number of damaged fruits with particular microbiological agent after storage in the cooler (A), ULO 1 chamber (B), ULO 2 chamber (C).

microscopic fungi at the same time. Other authors had found that this cultivar is very susceptible to diseases during storage (Ikase 2006). Total losses from decayed apples for this cultivar was estimated at 12%, which is relatively high. The proportion of decayed apples was lower for the other cultivars. Cultivar 'Auksis' was also infected by two fungi (*Penicillium* and *Colletotrichum*) with total losses of 8%.

Apple decay in the ULO 1 chamber due to infection by microscopic fungi was stronger than in the cooling chamber (Fig. 9B). The modified atmosphere in this chamber was not suitable for storage of the cultivar 'Korichnoe Novoe', since the decay was caused by *Monilia* spp., *Penicillium* spp. and *Colletotrichum* spp. Losses from the decay of this cultivar reached 25.6%. The cultivar 'Saltanat' in the ULO 1 chamber also was infected mostly by *Colletotrichum* spp. and *Monilia* spp. with high total losses (9.5%).

Penicillium spp. (Fig. 9C) was the main cause of apple rot in the ULO 2 chamber. Total loss from decayed apples for all cultivars in the ULO 2 chamber was 23.3% which was the lowest proportion among treatments. As in the other chambers the largest proportion of decayed apples in this chamber was for the cultivar 'Korichnoe Novoe' (9%), which was less than half of that in the ULO 1 chamber.

Discussion

The significant reduction of microorganisms in the chambers with the modified atmosphere was due to the low content of oxygen, as under these conditions aerobic microorganisms, as well as moulds, do not develop (Jacxsens et al. 2001). The present study confirms results of other investigations (Yackel et al. 1971), where development of microorganisms was reduced by 50% in a controlled modified atmosphere, due to inhibition of growth of aerobic bacteria (Littlefield et al. 1966). However, development of facultative anaerobic microorganisms is not reduced in these conditions (Schelhorn et al. 1951). It was shown also that a controlled modified atmosphere did not fully inhibit the development of aerobic bacteria (Fortuny et al. 2004).

Since yeasts are anaerobic and can develop in an environment withot oxygen (Nikolaeva 2007), the amount of yeasts on apples stored in ULO chambers was relatively high. The air contamination level in the cooler was five times higher than that in a controlled modified atmosphere. In addition, the gas composition in the cooler was beneficial for the development of microorganisms, and it is possible that secondary contamination occurred.

Apple decay in the cooler was caused mainly by *Colletotrichum* spp. which is one of the widespread pathogenic micro-organisms during storage in chambers without modified atmosphere. This decay is sometimes called as "storage disease" (Osterloh 1994).

It has been observed that a choice of unsuitable gas mixture in chambers caused a high level fruit decay, such as flesh browning and unpleasent aroma caused by anaerobic processes in fruits (Wang 1990). Unproper choice of a gas mixture promoted apple decay of cultivar 'Antonovka', reaching 40% of all fruits (Babina et al. 2005). An unsuitable gas composition caused apple decay of cultivar 'Korichnoe Novoe' and 'Gita' (35 and 16% of fruits). Development of moulds was inhibited much more in chambers with an increased content of CO_2. Similar results have been obtained in other studies (Gurin et al. 2008). The aerobic *Penicillium* spp. was the only microorganism species that survived with increased CO_2, since this gas mixture is

not suitable for development of the other species (Carol 1996). In a similar study, *Penicillium, Botrytis, Alternaria, Aspergillus, Cladosporium macrocarpa* survived (Scheper et al. 2007).

The highest microbial diversity on apples was found in the cooler in conventional atmosphere suggesting that these conditions were not suitable for apple fruit storgae. Storage of apple fruits in a ULO 2 chamber with 1.5% O_2 and 2.5% CO_2 appeared to be most prospective for several commercial apple cultivars. In particular, the cultivars 'Auksis' and 'Orlik' showed the least proportion of damaged fruit after storage in these conditions.

Acknowledgements

The study was performed within the framework of the project co-funded by the EU „Scientific capacity building in fruit-growing, forestry and information technology sectors, providing research on environmentally friendly growing strategies, product development and introduction aided by computer technologies", No. 2009/0228/1DP/1.1.1.2.0/09/APIA/VIAA/035. Dr. Inga Morocko-Bicevska, leading researcher of Latvia State Institute of Fruit-Growing, kindly helped with the identification of microscopic fungi.

References

Akbudak B., Ozer M., Erturk U. 2003. A research on controlled atmosphere (CA) storage of cv. "Elstar" on rootstock of MM 106. *Acta Hort.* 599: 657–663.

Argenta L., Fan X., Mattheis J. 2000. Delaying establishment of controlled atmosphere or CO_2 exposure reduces 'Fuji' apple CO_2 injury without excessive fruit quality loss. *Postharv. Biol. Technol.* 20: 221–229.

Awad M.A., de Jager A. 2000. Flavonoid and chlorogenic acid concentrations in skin of 'Jonagold' and 'Elstar' apples during and after regular and ultra low oxygen storage. *Postharv. Biol. Technol.* 20: 15–24.

Babina R.D., Gorb N.N., Turbin V.A., Untilova A.E. 2005. Factors influencing storage possibilities of apples. *Engineering Sciences* 23: 178–180. (In Russian)

Beretta B., Gaiaschi A., Galli C.L., Restani P. 2000. Patulin in apple based foods: Occurrence and safety evaluation. *Food Addit. Contam.* 17: 399–406.

Carol A. 1996. Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *Int. J. Food Sci. Tech.* 31: 463–479.

DeEll J.R., Murr D.P., Porteous M.D., Rupasinghe H.P.V. 2002. Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* 24: 349–353.

Fortuny S.C., Martínez E.E., Belloso M.O. 2004. Microbiological and biochemical stability of fresh-cut apples preserved by modified atmosphere packaging. *Innov. Food Sci. Emerg. Technol.* 5: 215–224.

Gurin A.V., Krivorot A.M. 2008. Changes of biochemical content of apples stored in different gas mixtures. RUE 'Institute plodovotstva' 249 p. (In Russian)

Ikase L. 2006. Factors affecting apple quality. *Agro Tops* 1: 64. (In Latvian)

Jacxsens L., Devlieghere F., Van der Steen C., Debevere J. 2001. Effect of high oxygen modified atmosphere packaging on microbial growth and sensorial qualities of fresh-cut produce. *Int. J. Food Microbiol.* 71: 197–210.

Kaack K., Pedersen H.L. 2011. Modelling of the optimum harvest time for long-term storage of 'Elstar' and 'Aroma' (Malus domestica) apples at low oxygen concentration. *Acta Agric. Scand. B Plant Soil Sci.* 3: 10–25.

Kachan V. 2006. Microbiological methods for isolation and identification of pathogens in bacterial food poisoning. Instruction 4.2., Minsk, 8: 10-15-21. (In Russian)

Lau O.L. 1998. Effect of growing season, harvest maturity, waxing, low O_2 and elevated CO_2 on flesh browning disorders in 'Braeburn' apples. *Postharvest Biol. Technol.* 14: 131–141.

Littlefield N.A., Wankier B.N., Salunkhe D.K., Mcgill J.N. 1966. Fungistatic effects of controlled atmospheres. *Appl. Env. Microbiol.* 4: 579–582.

Malcolm C., Shurtleff C.W. 1995. *The Plant Disease. Clinic and Field Diagnosis of Abiotic Diseases*. APPS Press, pp. 100–105.

McLandsborough L. 2005. *Food Microbiology Laboratory.* CRC Press, Boca Raton, 179 p.

Nikolajeva V. 2007. *Food Microbiology.* LU Akadēmiskais apgāds, Rīga, 130 p. (in Latvian).

Osterloh D. 1994. *Obstlagerung*. Deutscher Landwirtschaftsverlag, Berlin, pp. 320–342.

Schelhorn V.M. 1951. Control of microorganisms causing spoilage in fruit and vegetable products. *Adv. Food Res.* 3: 429–482.

Scheper A., Rogers D., Walker J., Manning M., Wood P. 2007. The incidence of storage rots after postharvest apple washing. *New Zealand Plant Protection* 60: 7–14.

Sinclair J.B., Dhingra O.D. 1995. *Basic Plant Pathology Methods*. CRC Press, pp. 273–275.

Song J., Bangerth F. 1996. The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. *Postharv. Biol. Technol.* 8: 259–269.

Stinson E.E., Osman S.F., Heisler E.G., Siciliano J., Bills D.D. 1981. Mycotoxin production in whole tomatoes, apples, oranges and lemons. *J. Agric. Food Chem.* 29: 790–792.

Wang C. Y. 1990. Physiological and biochemical effects of controlled atmosphere on fruits and vegetables. In: *Food Preservation by Modified Atmospheres.* CRC Press, Boca Raton, pp. 197–224.

Yackel V.C., Nelson A.I., Wei L.S., Steinberg M.P. 1971. Effect of controlled atmosphere on growth of mold on synthetic media and fruit. *Appl. Env. Microbiol.* 22: 513–516.

Zude M., Herold B., Roger J.-M., Bellon-Maurel V., Landahl S. 2006. Non-destructive tests on the predivtion of apple fruit flesh firmness and soluble solids on tree and in shelf life. *J. Food Eng.* 77: 254–260.

Received 30 January 2011; received in revised form 15 March 2011; accepted 26 June 2011