Встановлено, що досліджувані антиоксидантні композиції пригнічували розвиток епіфітних мікроорганізмів на поверхні плодів протягом зберігання. Було зафіксовано підвищення їх стрес-толерантності. Результати експерименту доводять зменшення рівня щодобових втрат від мікробіологічних захворювань у 2...3,5 разів. Найбільший позитивний ефект при зберіганні усіх видів плодів був отриманий при обробці композицією на основі дистинолу і лецитину

Ключові слова: плоди яблуні, плоди груші, плоди сливи, антиоксиданти, післязбиральна обробка, мікроорганізми, грибні гнилі

Установлено, что исследуемые антиоксидантные композиции подавляли развитие эпифитных микроорганизмов на поверхности плодов при хранении. Было зафиксировано повышение их стресс-толерантности. Результаты эксперимента доказывают снижение уровня ежесуточных потерь от микробиологических заболеваний в 2...3,5 раза. Наибольший положительный эффект при хранении всех видов плодов был получен при обработке композицией на основе дистинола и лецитина

Ключевые слова: плоды яблони, плоды груши, плоды сливы, антиоксиданты, послеуборочная обработка, микроорганизмы, грибные гнили

UDC 664.8.037.1
DOI: 10.15587/1729-4061.2017.103858

DEVELOPMENT OF FRUIT DISEASES OF MICROBIAL ORIGIN DURING STORAGE AT TREATMENT WITH ANTIOXIDANT COMPOSITIONS

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1. Introduction

Fresh fruit products is considered to be the most important component of the functional nutrition of humans. Special physiological value of fruits is in providing human body with a large amount of natural antioxidants, biologically active substances, carbohydrates, essential vitamins and mineral elements. Dietary action is predetermined by especially favourable combination of these substances with organic acids [1].

Given this, consumption of fresh fruit production should be uniform throughout the whole year. Unfortunately, fruits directly from the garden can be obtained for only 3...4 months. Therefore, of special priority is the issue of arranging their long-term storage.

The main problem that occurs during storage of fruit raw materials is a loss of consumer properties due to infection with fungal rots. Existing storing technologies are not sufficiently effective and do not provide complete protection of fruits from fungal diseases. In this case, the losses of products depending on the season range from 6.2 to 23.2 % on average [2].

Therefore, a task of development of modern, efficient storage technologies that would contribute to the reduction of losses and to the preservation of high biological value of fruit products is relevant and requires immediate solution.

2. Literature review and problem statement

Microbial contamination of fruit raw materials is considered to be the most dangerous problem in the production, storage and processing of fruit products [3, 4].

Juicy fresh fruits are considered an excellent environment for the growth of development of pathogenic microflora. Their infection happens in garden during cultivation, transportation and storage [5].

Low positive temperatures and high relative air humidity in the cooling chambers contribute to the growth of spores of psychrophilic microorganisms. Such regime parameters stimulate their active development. As a result, the neighboring healthy fruits are infected. The disease quickly spreads throughout the entire mass of products [6].

About 150 types of fungal flora that infect fruit products dusring cultivation and storage are characterized, but the most widely spread and dangerous are 10–12 species [7].

Among them, the most often observed are fungal flora of genera Penicillium, Gloeosporium, Alternaria, Botrytis, Monila.

Fungi of the genus Alternaria during storage of pome fruit crops cause surface and core rot. At least nine species of Alternaria are mentioned as Alternaria pathogens on apples and pears. However, we can say with full confidence about the proliferation of non-specialized semisatrotrophic species of Altemaria that belong to three generic groups: A. arborescens, A. infectoria i A. tenuissima. Infection of the fruit happens in garden, but the signs of disease manifest themselves during storage, especially over the last period when fruits begin to overripen [8].

Gray rot caused by Botrytis cinerea Pers is considered to be one of the most common infectionss of fruits after harvesting [9]. The pathogen is related to fungi of broad specialization, infests many fruit plants, penetrate the fruits through punctures, breakouts and other damage to the rind and leads to the loss of comsumer properties. The fruits display brown, slightly deepened, stains of rot. Next forms fluffy «wadded» mycelium of the fungus. Mold quickly proceeds to healthy fruit forming nests. The pulp of the fruit is softened, gets brown and acquires musty and sour smell [10].

Pathogens of gleosporis bitter rot are fungi Gloeosporium fructigenum Berk., G. album Osterw., G. perennans Zeller. Fruit infection occurs in garden, from the infected branches, trunks, herbaceous plants, mummified infected fruits. However, active development of disease begins at the last stage of storage. Several closely located round, clearly limited brown stains appear on the surface of the fruit. Gradually, the stains deepen and display a bed of conidial sporification in the form of micro pillows. At high relative air humidity in the storing chambers stains coalesce, rot develops inside, and infected flesh acquires bitter taste [11, 12].

The cause of bluish mold-like rot is the development of fungi Penicillium digitatum (Pers.) Sacc. and Penicillium expansum (Lk.) Thom. Infection of fruits of pome cultures occurs during storage. The source of sporification may include walls of the chambers, packaging and the fruits themselves. Microbe penetrates inside the fruit through damaged rind, sometimes through the stem of the fruit. White mycelium of the fungus develops on the surface of the fruit, followed by bluish-green pads of its sporification. Diseased fruits become soft, slightly wrinkled, richly secrete juice, acquire unpleasant smell and taste [13].

Pathogens of moniliary rot on the fruits of pome cultures are the fungi Monilia fructigena Pers, and on stone fruit – Monilia cinerea Bon. Infection penetrates the fruit in garden. Development of the disease when storing apples and pears begins with the emergence of a brown stain, which gradually grows and can cover the entire fruit. Characteristic concentric circles of yellowish-gray pads form on the surface of rotten area. The surface of pome cultures is covered with small, very dense gray pads. The pulp of the fruit becomes brown, softens, becomes spongy and acquires sweet-wine taste [14, 15].

The degree of infection and speed of development of pathogenic microflora on the surface of fruits is determined by many factors. The main among them are the level of infection load and species and varietal immunity. Important factors are technologies of cultivation and transportation [16]. Significant impact is exerted by weather conditions during cultivation and harvesting [17]. Over the extended storage, the dominant factor is the method and mode of storage [18, 19].

To inhibit the development of microbiological diseases and reduce losses of fruit products during storage, there are many different technological measures. The most common is storing in regulated and modified gas atmosphere [20, 21]. Among physical methods, ozonization, ionization, radiation treatment are applied [22]. Common measure is the use of various food coatings [23].

However, the developed techniques to protect fruit products from fungal diseases have not been widely applied in the industry, due to their complexity, high cost and questionable ecological safety.

Therefore, the most important task of reducing the losses of fruit products from the development of microbiological diseases during storage remains relevant and requires immediate solution by undertaking additional research.

3. Research goal and objectives

The goal of conducted study was to scientifically substantiate expediency of conducting after-harvest treatment with antioxidant compositions to prevent development of pathogenic microflora on the surface of fruits over long-term storage.

To accomplish the set goal, the following tasks had to be solved:

– to determine quantitative and qualitative composition of pathogenic microflora on the surface of fruits over the stages of storing and to establish the impact of antioxidant compositions on the kinetics of its development;

– to explore the impact of antioxidant compositions on the development of microbiological diseases during storage of fruit raw materials;

– to determine, using a multifactor analysis, the factor that exerts a dominant influence on the level of daily losses from the diseases of microbial origin during storage at their treatment with antioxidant compositions.

4. Materials and methods for examining the influence of antioxidant compositions on the development of diseases of microbial origin during storage

Experimental studies have been conducted under conditions of laboratory of the technology of preliminary treatment and storage of crop products at the NDI of Agriculture and Ecology of Tavria State Agritechnological University (Melitopol, Ukraine). The examined objects are apples of the varieties Idared, Golden Delicious, Simirenko Renet, pears of the varieties Victoria, Cure and Izyuminka Crimea, plums of the varieties of Voloshka, Stanley, and Ugorka Italian.

More details on the procedure of examining the influence of antioxidant compositions on the development of diseases of microbial origin during storage of fruits can be found in article [24].

5. Results of examining the influence of antioxidant compositions on the development of diseases of microbial origin during storage

Over the period of preparing the fruits for storing, the largest average number of epiphytic microflora was registered on the surface of fruits of plum and fruits of pear from a group of varieties of medium term of ripening (Fig. 1–3).

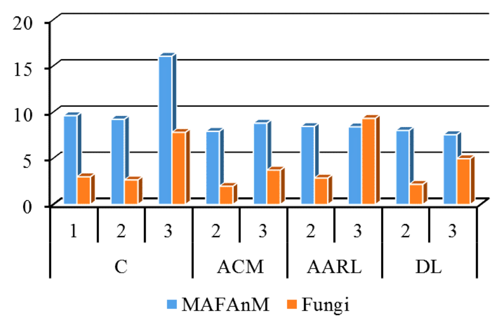


Fig. 1. Mean quantitative and qualitative composition of epiphytic microflora on the surface of apple by the stages of storage, $\times 10^3$ cfu/g: 1 – preparation for storage; 2 – pre-cooling and treatment with AOC; 3 – end of storage

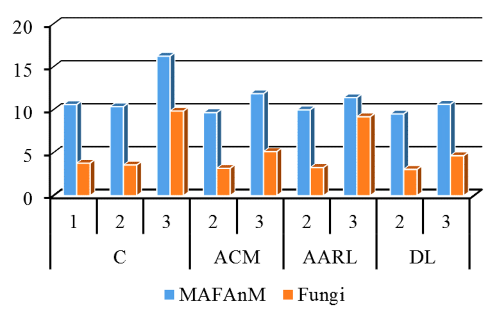


Fig. 2. Mean quantitative and qualitative composition of epiphytic microflora on the surface of pear by the stages of storage, $\times 10^3$ cfu/g: 1 – preparation for storage; 2 – pre-cooling and treatment with AOC; 3 – end of storage

The species composition of epiphytic microflora was dominated by spores of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM). At the surface of apple, their mean quantity ranged from $9.6 \cdot 10^3$ cfu/g. At the surface of plum, it reached almost $18 \cdot 10^3$ cfu/g.

The mean quantity of fungal flora ranged from $3 \cdot 10^3$ cfu/g on apple to $4.8 \cdot 10^3$ cfu/g on the surface of plum.

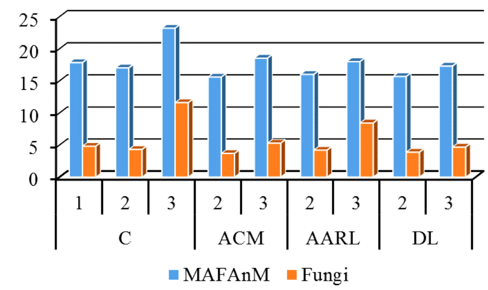


Fig. 3. Mean quantitative and qualitative composition of epiphytic microflora on the surface of plum by the stages of storage, $\times 10^3$ cfu/g: 1 – preparation for storage; 2 – pre-cooling and treatment with AOC; 3 – end of storage

After pre-cooling, quantity of epiphytic microflora on the surface of control fruits decreased by almost 4 %. The obtained data confirm results of other authors about a positive effect of low temperatures on the quantity of epiphytic microflora on the surface of fruits [25].

Combining a pre-cooling of fruits and their treatment with solutions of AOC by immersion proved more efficient and reduced the mean number of epiphytic microflora by almost 12 %.

During storage, we observed an increase in the population of epiphytic microflora both on the surface of the control and of the majority of examined fruits. However, the rate of growth was different (Table 1).

Calculated rate constants indicate that during storage of all kinds of fruit, both control and examined, the rate of growth in the population of fungal microflora was significantly higher compared with the rate of growth in the total quantity of MAFAnM. The most intensive growth of micromycetes was observed on the control fruits of plum. In this case, the rate constants of growth of microorganisms were 2.5 times larger compared with the fruits of pome cultures.

Treating all kinds of fruit with AOC significantly reduced the speed of growth of both MAFAnM and micromycetes. Thus, during storage of fruits treated with ACM composition, the speed of growth of MAFAnM was by 2.4...6.8 times, and micromycetes by 1.8...4.3 times, lower compared to the fruits of control variants. When applying DL composition, the speed reduced, respectively, by 3.7... 13.5 and 1.7...11 times, depending on the species characterisitcs of fruits. It should be noted that the total population of epiphytic microflora on the surface all kinds of fruits after storage at treatment with compositions of ACM and DL did not exceed indicators established by the standards for this type of product.

The data presented allow us to state that fruits of the given variants are safe for the human organism by microbiological indicators.

Application of the composition AARL significantly decreased speed of growth of MAFAnM, however, it did not influence, and in some years even prompted, development of micromycetes. This may be linked to the fact that the composition AOC contains lecithin, which is considered a nutrient medium for the cultivation of certain kinds of microorganisms. At the end of storage, population of fungal microflora on the fruits of pome cultures treated with the composition AARL exceeded the established standard indicators by1.8 times. In this case, the growth of micromycetes was observed only over the last month of storing when active processes of overripening of fruits started and their immune properties significantly decreased. At the end of storing the fruits of plum of this variant, the population of micromycetes did not exceed the established standard indicators.

It should be noted that during storage of fruits of control variants, the growth of population of epiphytic microflora started after first 20...60 days of storage.

Table 1

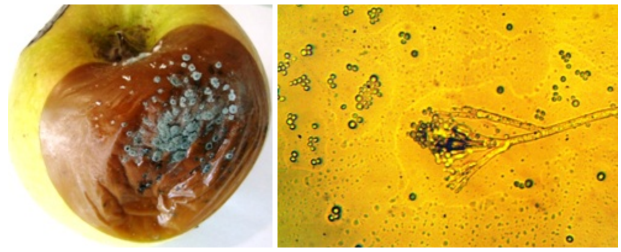
Rate constants of change in the population of epiphytic microflora on fruit treated with AOC during storage

Pomological variety	Rate constants of change in the population of epiphytic microflora on fruits at different types of treatment, k·10^{-2}, days^{-1}							
	C		ACM		AARL		DL	
	1*	2*	1*	2*	1*	2*	1*	2*
Apple								
Idared	0.24	0.48	0.11	0.28	0.05	0.30	0.03	0.39
Golden Delicious	0.37	0.61	0.06	0.34	–0.01	0.56	–0.01	0.32
Simirenko Renet	0.26	0.59	0.04	0.31	–0.02	0.54	–0.02	0.32
Florina	0.22	0.44	–0.04	0.22	–0.04	0.46	–0.09	0.21
Mean for varieties	0.27	0.53	0.04	0.29	–0.01	0.47	–0.02	0.31
HIP_{05}	0.067							
Pear								
Victoria	0.26	0.47	0.09	0.13	0.03	0.41	0.04	0.11
Conference	0.07	0.38	–0.04	0.08	–0.05	0.31	–0.06	0.01
Cure	0.40	0.94	0.23	0.49	0.19	0.67	0.18	0.47
Izyuminka Crimea	0.31	0.54	0.15	0.26	0.13	0.46	0.10	0.23
Mean for varieties	0.26	0.58	0.11	0.24	0.07	0.46	0.07	0.21
HIP_{05}	0.078							
Plum								
Voloshka	0.49	1.45	0.16	0.37	0.08	0.54	0.08	0.16
Stanley	0.34	1.25	0.1	0.27	0.05	0.45	0.05	0.06
Ugorka Italian	0.51	1.55	0.21	0.35	0.12	0.49	0.08	0.18
Mean for varieties	0.45	1.42	0.16	0.33	0.08	0.49	0.07	0.13
HIP_{05}	0.049							

Note: 1 – population of MAFAnM; 2* – population of fungal microflora*

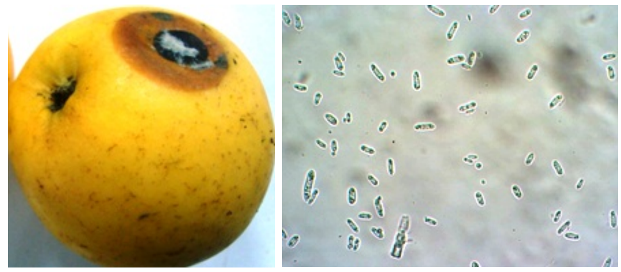
When carrying out a qualitative analysis of epiphytic microflora, we identified dominating fungal microflora. Most often on the fruits of pomes cultures we observed fungal flora of genera *Penicillium, Alternaria, Gloeosporium, Botrytis*. On the fruits of plum – of genera *Monili* and *Penicillium*.

As a result of development of the specified microflora, we found the following microbiological diseases on the surface of apple and pear during storage: penicillosis, anthracosis, alternaria, moniliary rot, botrytis (Fig. 4–8).



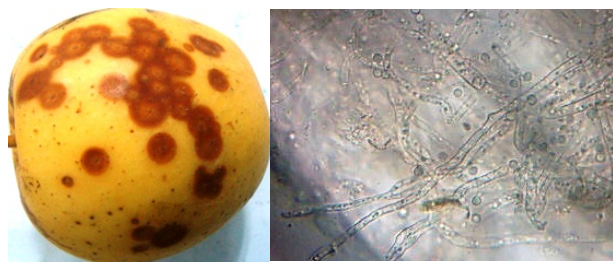
a *b*

Fig. 4. Development of penicillosis (bluish mold-like rot) on the surface of apple: *a* – infected fruit; *b* – microstructure of fungus *Penicillium expansum (Lk.) Thom*



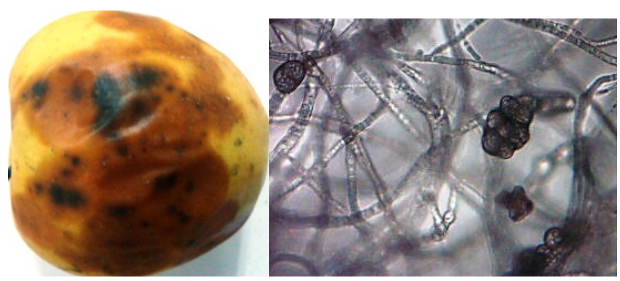
a *b*

Fig. 5. Development of anthracnose (gleosporious bitter rot) on the surface of apple: *a* – infected fruit; *b* – microstructure of fungus *Gloeosporium fructigenum Berk*



a *b*

Fig. 6. Development of anthracnose (gleosporious bitter rot) on the surface of apple: *a* – infected fruit; *b* – microstructure of fungus *Gloeosporium perennans Zeller*



a *b*

Fig. 7. Development of alternaria (olive mold-like rot) on the surface of apple: *a* – infected fruit, *b* – microstructure of fungus *Alternaria tenuis Nees*

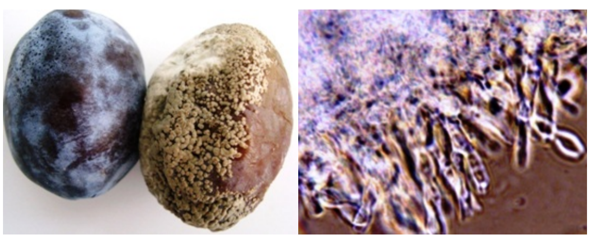
Treatment with AOC, which includes lecithin, stimulated development of penicillosis and botrytis and inhibited all other microbiological diseases.

First fruits with signs of microbiological diseases during storage of control apples of the varieties Idared and Golden Delicious were detected on day 150 of storage, of the varieties Simirenko Renet and Florina on day 180. During storage of pears of the varieties Victoria and Conference, the first fungal diseases appeared on day 60, of the variety Izyuminka Crimea – on day 90, of the variety Cure – on day 120 of storage. The first fungal rot was discovered on plums of the varieties Voloshka and Ugorka Italian on day 20, of the variety Stanley – on day 40 of storage.

a *b*

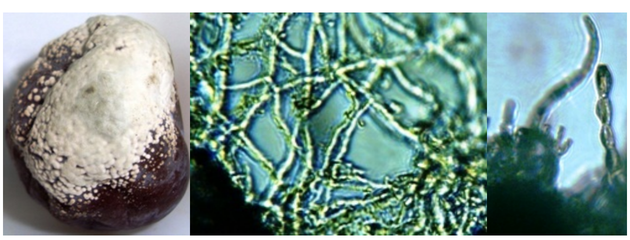
Fig. 8. Development of botrytis (grey mold-like rot) on the surface of pear: *a* —infected fruit; *b* — microstructure of fungus *Botrytis cinerea Pers*

We observed most often on the fruits of plum gray fruit rot whose pathogen is fungal flora *Monilia cinerea Bonord*, moniliary rot, caused by fungal flora *Monilia fructigena pers* and bluish mold-like rot – the pathogen is *Penicillium expansum (Lk.) Thom* (Fig. 9–11).



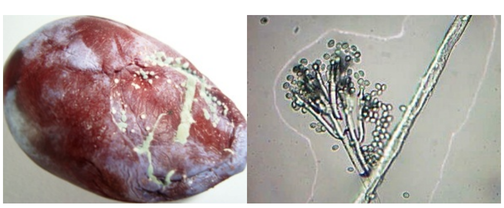
a *b*

Fig. 9. Development of monoliosis (grey fruit rot) on the surface of plum: *a* — infected fruit; *b* — microstructure of fungus *Monilia cinerea Bonord*



a *b* *c*

Fig. 10. Development of monoliosis (fruit rot) on the surface of plum: *a* — infected fruit; *b, c* — microstructure of fungus *Monilia fructigena pers*



a *b*

Fig. 11. Development of penicillosis (bluish mold-like rot) on the surface of plum: *a* — infected fruit; *b* — microstructure of fungus *Penicillium expansum (Lk.) Thom*

Treating fruits with AOC compositions prolonged internal mechanisms of fruit resistance and delayed the onset of development of microbiological diseases for 30...120 days depending on the kind of fruit and the variant of treatment. And during storage of apples of the varieties Simirenko Renet treated with ACM composition and of the variety Florina treated with ACM and DL compositions, over three examined years, we did not detect any microbiological diseases at all.

Thus, the lowest mean level of daily losses from microbiological diseases was registered during storage of apples treated wth ACM composition, of pears and plums treated with DL composition.

The highest level of daily losses was demonstrated by control apples, pears and plums of all pomological varieties.

In this case, quantitative value of the examined indicator was 2...3.5 times higher compared with fruits that were stored treated with ACM and DL compositions. It should also be noted that during storage of apples treated with AARL composition, the level of daily losses did not statistically differ from control. Instead, when storing other kinds of fruit, it was significantly lower than control variant.

For the purpose of determining the factor, which has a dominant influence on the level of daily losses from microbiological diseases during storage when treated with antioxidant compositions, we performed a multi-factor dispersion analysis. In the course of a 4-factor study 3×3×3×4, repeated 5 times, we studied the effect of three gradations of factors A (kind of fruits: fruits of apple, pear, plum), B (weather conditions over the three years of study), C (variety of fruits: 3 varieties of each kind) and four gradations of factor D (treatment with AOC) on the level of daily losses from the development of microbiological diseases. Results of the analysis are given in Table 2 and in Fig. 12.

Table 2

Results of dispersion analysis of a four-factor experiment on determining a dominant factor on the level of daily losses from microbiological diseases during storage of fruits

Source of variation	Sum of squares	Degrees of freedom	Mean square	Fisher criterion F	
				actual	theoretical
Total (Cy)	1841.219	539	–	–	–
Repetitions (Cp)	0.015	4	–	–	–
Factor A	447.896	2	223.948	6018.41	3.017
Factor B	131.077	2	65.539	1761.29	3.017
Interaction AB	56.807	4	14.202	381.66	2.393
Factor C	77.547	2	38.773	1042.00	3.017
Interaction AC	144.121	4	36.030	968.28	2.393
Interaction BC	99.786	4	24.946	670.41	2.393
Interaction ABC	113.842	8	14.230	382.43	1.960
Factor D	385.970	3	128.657	3457.54	2.626
Interaction AD	91.871	6	15.312	411.49	2.120
Interaction BD	71.462	6	11.910	320.08	2.120
Interaction CD	27.290	6	4.548	122.23	2.120
Interaction ABD	37.032	12	3.086	82.93	1.775
Interaction ACD	51.528	12	4.294	115.40	1.775
Interaction BCD	44.944	12	3.745	100.65	1.775
Interaction ABCD	60.046	24	2.502	67.24	1.543
Rest (C_Z)	15.926	428	0.037	–	–

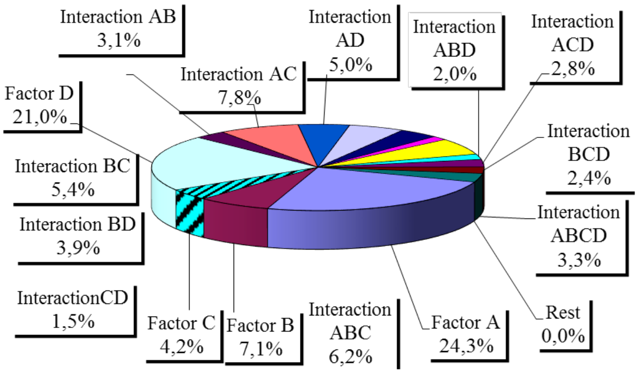


Fig. 12. Share of influence of factors on the level of microbiological diseases during storage of fruits treated with AOC: A – type of fruit; B – weather conditions during formation of fruit; C – variety of fruit; D – treatment with antioxidant compositions; AB, AC, BC, AD, DB, CD, ABC, ABCD – interaction of factors, rest – other factors

Since the received actual Fisher criteria significantly exceed theoretical values, then the action of analyzed factors and their interactions is considered reliable.

The data we received indicate dominance of two factors: species features of fruit raw materials (factor A) and treatment with antioxidant compositions (factor D). The shares of influence are, respectively, 24 and 21 %. The effect of other factors and their interactions was less substantial and did not exceed 8 %.

Significance of influence from the factors was confirmed by the computed lowest significant differences (HIP_{05}), which are equal for factors A, B, C – HIP_{05}=0.04, for factor D – HIP_{05}=0.05, for interaction ABCD – HIP_{05}=0.24. A relative error of the experiment is 1.97 that testifies to its high accuracy.

6. Discussion of results of examining the influence of antioxidant compositions on the development of microbiological diseases during storage

Studies that we conducted confirm efficiency of applying the antioxidant compositions ACM and DL to reduce daily losses as a result of development of fungal rots.

A significant positive consequence of the application of this technology for storage is considered to be an increase in the storability of fruit raw materials and maximal preservation of their qualitative indicators.

Despite the fact that the use of the antioxidant composition AARL stimulated development of penicillosis and botrytis, but the first signs of the disease appeared on the surface of fruit only at the last stage of storing. Along with this, development of microbiological diseases on control fruits started after 20 days of storage.

Introduction to the formulation of DL composition of dimethylsulfoxide, which possesses, in addition to antioxidant, the antibacterial properties, made it possible to neutralize the growth-stimulating effect of lecithin. And, as a consequence, the application of this composition yielded the largest positive effect.

Thus, obtained data and their scientific substantiation allow us to recommend to manufacturers to treat the fruits with antioxidant composition, which contains ionol, dimethylsulfoxide and lecithin, before their subsequent storage.

7. Conclusions

1. In the period to prepare the fruits for storing, the largest mean population of epiphytic microflora was registered on the surface of plums and pears with medium term of ripening. The species composition of epiphytic microflora was dominated by spores of mesophilic aerobic and facultative anaerobic microorganisms. Their mean population on the surface of apples was $9{,}6\cdot10^3$ cfu/g, on the surface of plums – $18\cdot10^3$ cfu/g.

Treating all kinds of fruit with AOC significantly decreased speed of the growth of both MAFAnM and micromycetes.

2. Applied antioxidant compositions reduced the level of daily losses of fruit from microbiological diseases over the entire period of storage by 2...3.5 times. The largest positive effect during storage of all kinds of fruits was obtained when applying the composition DL.

3. The level of daily losses from microbiological diseases during storage was affected by the dominant influence of factors of generic features of fruit raw materials (factor A) and treatment with antioxidant compositions (factor D). The shares of influence are, respectively, 24 and 21 %.

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