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QUALITY OF SAVOY CABBAGE STORED UNDER CONTROLLED ATMOSPHERE WITH THE ADDITION OF ESSENTIAL OILS

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Key words: savoy cabbage, controlled atmosphere, essential oils, vitamin C, chlorophyll pigments, microbiological quality

The objective of this research was to determine the influence of controlled atmosphere and controlled atmosphere with the addition of tea tree or cardamom essential oil on the kinetics of changes in vitamin C content, chlorophyll pigments and on the total number of microorganisms and anaerobic titre during storage of two cultivars of savoy cabbage: 'OWASA' and 'WIROSA'.

The cabbage was stored at 0–1°C under controlled atmosphere (CA) composed of 4% CO_2, 3% O_2 and 93% N_2, in CA with the addition of tea tree oil (oil 1) or cardamom essential oil (oil 2) at the concentration of 15 ppm, and in the air (K). It was proved that, irrespective of the presence of an essential oil, CA slowed down the degradation of vitamin C and chlorophyll pigments in the stored vegetables. The controlled atmosphere prolonged the period when vegetables maintained their high biological value during cold storage. The tea tree essential oil added to CA improved the general microbiological quality of the stored cabbage, causing a reduction in the total number of microorganisms by two logarithmic units after 1–2 months of storage, while the cardamom essential oil decreased it by one logarithmic unit related to CA free from essential oil.

INTRODUCTION

Numerous studies confirmed the efficiency of a vegetable-rich diet in the prevention of ageing processes and many civilisation-related diseases, mainly cardiovascular and neoplastic diseases and many other chronic illnesses, including rheumatoid arthritis, lung diseases, cataract, Parkinson's or Alzheimer's disease. Vegetables have such properties owing to a significant content of various antioxidants [Mitek & Kalisz, 2003; Guo *et al*., 2001; Gil *et al*., 2002]. Of great importance for the protection of antioxidative properties of fresh vegetables are their storage conditions, including optimum temperature. Storage in the controlled atmosphere is evaluated the highest in this respect [Bastrash *et al*., 1993; Adamicki, 1998, 1999; Gajewski, 2001; Krala & Witkowska, 2002, 2004]. In literature there is no detailed information on the influence of CA on the transformation of main nutrient components in savoy cabbage during storage. Results published so far have been limited mainly to sensory properties of this cabbage [Gajewski, 2001].

From the point of view of food hygiene, the microbiological quality of vegetables is very important because they are consumed fresh in high quantities. Beside lactic bacteria and yeast typical of plant environment, the microflora of cabbage contains sporulating aerobic bacteria *of Bacillus* and anaerobes of *Clostridium* species, as well as filamentous fungi. The level of polluting microflora is different, depending on climatic conditions, methods of harvesting, transport and storage of vegetables. In general, it is assumed that the total number of bacteria living on cabbage leaves ranges from 10^5 to 10^8 cfu/g [Burbianka *et al*., 1983]. When cabbage is stored under proper conditions, filamentous fungi usually do not cause any problems. Characteristic symptoms of microbiological deterioration are black stains on the surface of leaves induced by *Pseudomonas, Xanthomonus* and *Corynebacterium* bacteria. Vegetables get soiled inevitably and in such cases a real hazard is putrid microflora whose presence is indicated by anaerobic bacteria [Burbianka *et al*., 1983; Benwart, 1989; Oberman *et al*., 1997]. Under conditions of limited oxygen availability, *i.e.* when cabbage is stored in the controlled atmosphere, *Clostridium sp.* bacteria can develop.

An increasing applicability of essential oils or their components in food preservation [Holley & Patel, 2005] indicates that they can also be used as components of the modified atmosphere for vegetable storage [Kalemba & Kunicka, 2003]. According to authors knowledge this problem has not been a subject of research so far. In the experiments, tea tree essential oil was used because of its reported antibacterial and antioxidative properties [Kalemba & Kunicka, 2003; Ponce *et al*., 2004]. Another tested oil, the cardamom oil, reveals a strong inhibitory action against many bacteria of *Enterobacteriaceae* family and faecal streptococci which can originate from the soil, and can reduce the development of sporulating aerobic bacteria *Bacillus subtilis* [Kalemba & Kunicka, 2003].

The basic aim of the research was to test the influence

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of controlled atmosphere and controlled atmosphere supplemented with either tea tree or cardamom essential oil on the kinetics of changes in vitamin C content, chlorophyll pigments and on the total number of microorganisms and anaerobic titre during storage of two cultivars of savoy cabbage: OWASA and WIROSA.

MATERIAL AND METHODS

Experimental material. The investigated material was savoy cabbage. Raw material was harvested from ground cultivations in autumn and was purchased from a qualified plantation in the Sieradz region. Two cultivars were examined in the study: (1) savoy cabbage *(Brassica oleracea var. sabauda)* OWASA cultivar, was taken directly from the plantation; the time from harvesting to starting experiments was two days; and (2) savoy cabbage *(Brassica oleracea var. sabauda)* WIROSA cultivar. Before the experiments started the cabbage had been stored in a refrigerator at the plantation, at a temperature of 0–1°C for about a month. This is a very late cultivar, with medium size heads and strong, intensive green colour. Before storage, loose outer leaves were removed from cabbage heads.

Experimental methods. After weighing, the cabbage was divided into three experimental groups, 20 heads in each group. One of them was stored in the controlled atmosphere (CA) composed of 4% CO_2, 3% O_2 and 93% N_2, determined on the basis of the existing knowledge and the authors' own experience [Krala & Witkowska, 2004]. The second group was stored in CA with the addition of tea tree essential oil - (oil 1, savoy cabbage OWASA cultivar) or cardamom oil (oil 2, savoy cabbage WIROSA cultivar) at the concentration of 15 ppm. The third part of cabbage was stored in closed chambers filled with air. This group was treated as a control group (K). Relative humidity of CA and air was kept at the level of 95±2%, by putting flat plates filled with water inside the hermetic chambers. The temperature was maintained at 0–1°C. The composition of CA was systematically controlled with the use of a gas analyser and adjusted to previous composition.

A basis for quality evaluation of the cabbage were results of determinations made once a week. For each analysis two cabbage heads were taken from each variant of storage conditions. The analyses were repeated four times. The following assays were carried out: (i) vitamin C content – by Tillmans' method, after a previous reduction of dehydroascorbic acid to ascorbic acid by Pijanowski's method [PN-90/A-75101/11]; (ii) the content of chlorophyll pigments – by Vernon's method [Vernon, 1960]; and (iii) the total number of microorganisms and anaerobic bacteria titre were determined as follows: 10 g of cabbage (wet mass) was homogenised with 90 cm^3 of peptone water. A series of successive decimal dilutions of this homogenate was prepared and plated on PCA medium (Plate Count Agar, BioMerieux). The plates were incubated at 30°C for 72 h. Results were expressed in cfu/g. The titre of anaerobic bacteria was determined on Wrzosek medium [Burbianka *et al*., 1983]. The plates were incubated at 30°C for 72 h. Additionally, the presence of anaerobic bacteria was confirmed by the microscopic method [Burbianka *et al*., 1983]. The results of microbiological analyses were completed with observations of changes in the appearance, colour and aroma of the cabbage. This comparison was done at every analytical period.

Statistical analysis. Results of the studies were analysed statistically using one-way analysis of variance at the confidence level of 0.05 with Snedecor test. Changes factor was the storage condition. Calculations were made with the use of Microsoft EXCEL with addition of Analysis ToolPac and Solver software.

RESULTS AND DISCUSSION

Changes in vitamin C content in the savoy cabbage depended on cultivar and storage conditions. The controlled atmosphere considerably reduced the losses of vitamin C in all tested vegetables.

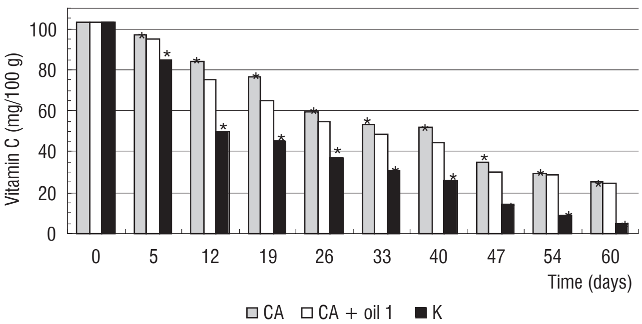

FIGURE 1. Vitamin C content in savoy cabbage (OWASA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with tea tree essential oil (CA+ oil 1) and in the air (K), * differences between the compared storage conditions are significant ($p<0.05$).

During the storage in the controlled atmosphere, vitamin C content of savoy cabbage of OWASA cultivar was statistically significantly higher throughout the storage period as compared to the control samples kept in the air (Figure 1). A decrease in vitamin C content in the savoy cabbage of OWASA cultivar reached 95% in the material stored in the air, 75% in the cabbage kept in the controlled atmosphere and 76% in the samples stored in CA with the addition of tea tree essential oil (CA+ oil 1) (Figure 1). No significant effect of the tea tree oil was observed. The analysis of vitamin C degradation kinetics in the tested vegetables showed that storage under controlled atmosphere increased almost twice the half-life periods of this vitamin (Table 1).

The content of vitamin C in the savoy cabbage of WIROSA cultivar was decreasing faster than in the cabbage of OWASA cultivar. Probably it was correlated not only with the cultivar of savoy cabbage but also with the different time which passed from its harvesting to the starting of our experiments. Verification of this observation needs further research.

Despite that, after the whole storage period in the controlled atmosphere, in this cabbage vitamin C content was by 22% higher than in the control samples. Hence, the controlled atmosphere contributed to a much better preservation of vitamin C than the traditional cold storage.

Savoy cabbage of WIROSA cultivar stored in the control-

TABLE 1. Kinetic parameters for vitamin C decomposition, chlorophyll a, b in savoy cabbage stored in CA, in CA+ oil (1 or 2), K.

Tested factor	Savoy cabbage	Storage conditions	Constant speed k_0 (1/days)	Half-life period $\tau_{1/2}$ (days)
Vitamin C	OWASA	CA	1.2944	38
		CA+ oil 1	1.3247	37
		K	1.4607	18
	WIROSA	CA	1.1531	36
		CA+ oil 2	1.1122	38
		K	1.3316	21
Chlorophyll a	OWASA	CA	0.0221	55
		CA+ oil 1	0.0248	49
		K	0.0389	31
	WIROSA	CA	0.034	45
		CA+ oil 2	0.0376	42
		K	0.0543	25
Chlorophyll b	OWASA	CA	0.0126	59
		CA+ oil 1	0.0162	44
		K	0.0224	31
	WIROSA	CA	0.0252	48
		CA+ oil 2	0.0263	45
		K	0.0328	27

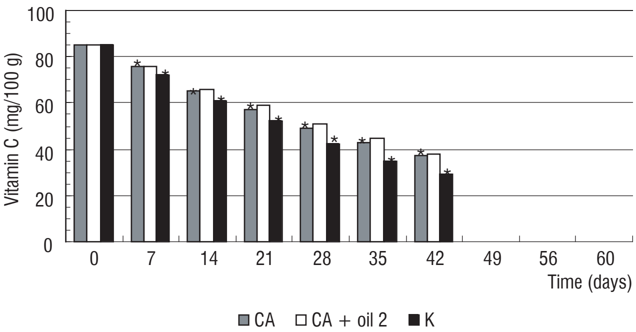

FIGURE 2. Vitamin C content in savoy cabbage (WIROSA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with the addition of cardamom oil (CA + oil 2) and in the air (K), * differences between the compared storage conditions are significant ($p<0.05$).

led atmosphere with the addition of cardamom essential oil (CA + oil 2) after two weeks contained the same amount of vitamin C as that kept in the controlled atmosphere without the essential oil added (Figure 2). On completion of the storage the content of vitamin C in the samples stored in CA supplemented with cardamom essential oil (oil 2) was approximately 3% higher than in the samples stored in CA without essential oil added (Figure 2). The statistical analysis showed that this difference was not significant. Hence, it was found that cardamom essential oil had a very weak beneficial effect on vitamin C maintenance.

Taking into account results of studies presented in our earlier work [Krala & Witkowska, 2004], it can be hypothesised that much better retention of vitamin C in the vegetables stored in the controlled atmosphere can result from the limited intensity of their transpiration relative to vegetables stored in the air. This effect was influenced by a decreased content of oxygen and an increased concentration of carbon dioxide in CA related to air (K).

Keeping OWASA savoy cabbage in the controlled atmosphere brought about smaller losses of chlorophyll pigments as compared to the cabbage stored in the air (Figures 3 and 4).

After two-month storage of this cabbage in the controlled atmosphere the content of chlorophyll *a* decreased by 55% (Figure 3), while that of chlorophyll *b* by 50% (Figure 4). This content in the samples stored in the air decreased by 93% and 80%, respectively. An advantageous effect of the controlled atmosphere on the content of chlorophyll *a* and *b* is confirmed also by the calculated half-life periods ($\tau_{1/2}$) of these pigments. In the controlled atmosphere, $\tau_{1/2}$ is significantly higher than in the air (Table 1). A visual evaluation showed that the controlled atmosphere had a favourable influence on colour preservation in the stored savoy cabbage. The addition of tea tree essential oil to the controlled atmosphere had a negative effect on the content of chlorophyll *a* and *b* in the analysed savoy cabbage. From this point of view, the tea tree essential oil decreased the positive effect of CA on the preservation quality of the stored savoy cabbage, OWASA cultivar.

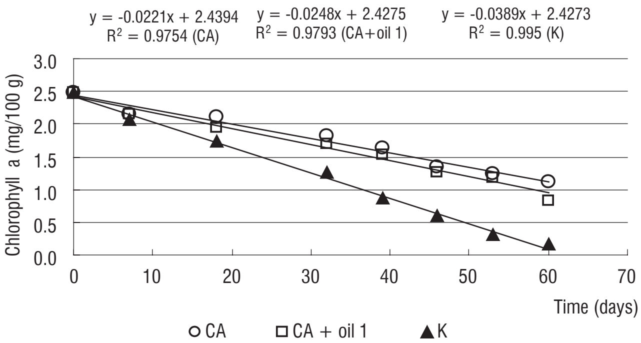

FIGURE 3. Chlorophyll a content in the savoy cabbage (OWASA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with the tea tree essential oil (CA+ oil 1) and in the air (K).

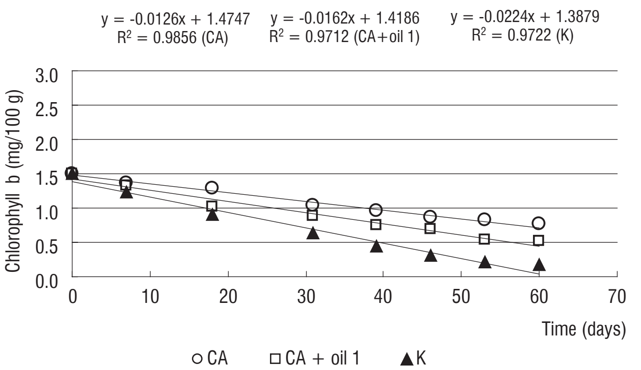

FIGURE 4. Chlorophyll b content in the savoy cabbage (OWASA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with the tea tree essential oil (CA+ oil 1) and in the air (K).

Storage conditions of savoy cabbage of WIROSA cultivar were found to affect the rate of degradation of chlorophyll *a* and *b* (Figure 5). The content of chlorophyll pigments in the analysed cabbage stored in both variants of CA was higher than in the control samples (Figures 5, 6). This difference was increasing with the storage time. The half-life peri-

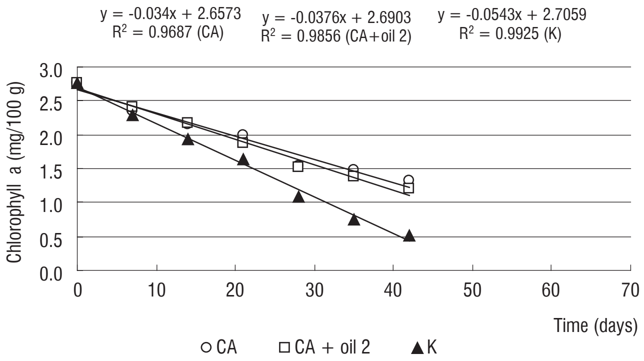

FIGURE 5. Chlorophyll a content in the savoy cabbage (WIROSA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with the addition of cardamom essential oil (CA+ oil 2) and in the air (K).

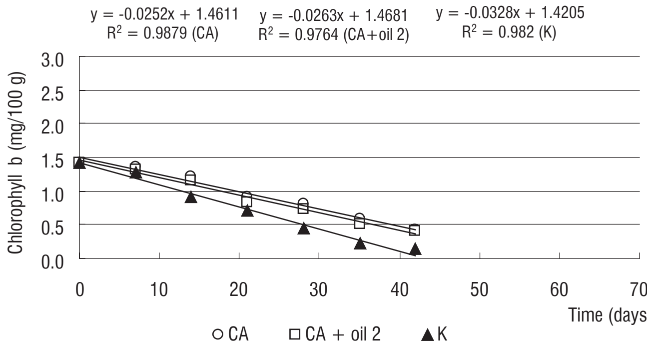

FIGURE 6. Chlorophyll b content in the savoy cabbage (WIROSA cultivar) stored in the controlled atmosphere (CA), in the controlled atmosphere with the addition of cardamom essential oil (CA+ oil 2) and in the air (K).

ods of chlorophyll *a* and *b* in the cabbage of WIROSA cultivar stored in CA did not depend on the presence of cardamon oil and were about twice as long as in the control samples (Table 1). The controlled atmosphere was more beneficial to the preservation of natural colour of the savoy cabbage than traditional storage conditions.

Concluding this part of investigation, we can state that the addition of cardamom oil (oil 2) to controlled atmosphere had no significant effect on the content of chlorophyll pigments in the stored cabbage.

The advantageous influence of the controlled atmosphere on the preservation of chlorophyll pigments in broccoli, Chinese cabbage and Brussels sprouts was also reported by Adamicki & Czerko [2002] and Bastrash *et al*. [1993].

The addition of tea tree essential oil to the controlled atmosphere caused a gradual decrease in the contaminating microflora, by two logarithmic units, found after 32 days of storage (Table 2). The cardamom essential oil caused a decrease of aerobic microflora by one logarithmic unit related to CA free from any essential oil and to the air (K), starting from the 7th day of storage (Table 3). After that time, the total number of microorganisms did not undergo sta-

TABLE 2. Microflora of savoy cabbage, OWASA cultivar, stored in the controlled atmosphere (CA), in the controlled atmosphere with the addition of tea tree essential oil (CA + oil 1) and in the air (K).

Time (days)	CA		CA + oil 1		K	
	Total number of microorganisms (cfu/g)	Anaerobe titre (g)	Total number of microorganisms (cfu/g)	Anaerobe titre (g)	Total number of microorganisms (cfu/g)	Anaerobe titre (g)
0	2.4×10^7	10^{-2}	2.4×10^7	10^{-2}	2.4×10^7	10^{-2}
7	6.2×10^6	10^{-3}	2.0×10^6	10^{-3}	1.3×10^5	10^{-3}
18	3.6×10^6	10^{-4}	1.1×10^5	10^{-4}	1.6×10^5	10^{-3}
32	3.0×10^6	10^{-5}	4.7×10^4	10^{-5}	1.2×10^6	10^{-3}
39	3.0×10^6	10^{-5}	2.5×10^4	10^{-5}	1.8×10^6	10^{-2}
46	4.2×10^6	10^{-5}	2.2×10^4	10^{-5}	2.0×10^6	10^{-2}
53	4.3×10^6	10^{-5}	1.2×10^4	10^{-5}	2.9×10^6	10^{-2}
60	5.1×10^6	10^{-5}	1.2×10^4	10^{-5}	1.8×10^6	10^{-2}

TABLE 3. Microflora of savoy cabbage, WIROSA cultivar, stored in the controlled atmosphere (CA), in the controlled atmosphere with the addition of cardamom essential oil (CA + oil 2) and in the air (K).

Time (days)	CA		CA + oil 1		K	
	Total number of microorganisms (cfu/g)	Anaerobe titre (g)	Total number of microorganisms (cfu/g)	Anaerobe titre (g)	Total number of microorganisms (cfu/g)	Anaerobe titre (g)
0	1.5×10^7	10^{0}	1.5×10^7	10^{0}	1.5×10^7	10^{0}
7	1.0×10^6	10^{-1}	3.0×10^5	10^{0}	1.3×10^6	10^{0}
14	1.6×10^6	10^{-2}	3.1×10^5	10^{-3}	3.9×10^6	10^{-2}
21	7.6×10^6	10^{-3}	6.9×10^5	10^{-3}	3.8×10^6	10^{-2}
28	3.4×10^6	10^{-3}	4.9×10^5	10^{-3}	3.3×10^6	10^{-2}
35	3.6×10^6	10^{-3}	1.0×10^5	10^{-3}	1.6×10^6	10^{-3}
42	4.5×10^6	10^{-3}	1.0×10^5	10^{-3}	1.7×10^6	10^{-4}

tistically significant changes, and was kept at the level lower by two logarithmic units as compared to the initial contamination (Table 3). Microbiological analysis of the cabbage stored in the air and in the controlled atmosphere free from any essential oil shows a similar dynamics of microorganism destruction. The number of microorganisms in these cases was maintained at the level of 10^6 cfu/g, *i.e.* by one logarithmic unit higher than in the cabbage kept in CA supplemented with cardamom essential oil (Table 3) and by two logarithmic units higher as compared to the cabbage kept in CA containing the tea tree essential oil (Table 2).

The titre of anaerobic bacteria of cabbage stored in CA supplemented either with tea tree or cardamom essential oils increased 100 and 1000 times, respectively. That provided evidence of their stimulating effect on the growth of anaerobic microflora (Tables 2 and 3).

When experiments with savoy cabbage of OWASA cultivar started, the vegetable had a characteristic aroma and intensive green colour. After 47 days of storing this cabbage in the air, noticeable quality changes were observed. Dark stains caused by microbiological changes occurred on the outer leaves, which was not observed in the cabbage stored in the controlled atmosphere. After 60 days of storage, the control samples were disqualified because of significant colour changes, while quality of the samples stored in CA remained good. The influence of the tested essential oils on the quality of savoy cabbage during storage was different. The tea tree essential oil added to CA induced changes in flavour and aroma of the stored cabbage. Outer leaves of the cabbage stored in the CA supplemented with this essential oil wilted faster than the leaves of control samples. With respect to sensory properties, the effects of cardamom oil (oil 2) added to CA were beneficial. There were no changes in aroma, colour nor wilting of the outer leaves of the stored cabbage. Cardamon oil as an accepted additive of foods, comprising vegetable preserves [Decree of Ministry of Health of 23 April 2004], so there are no toxicological contraindications to its application for cabbage preservation. Tea oil is included neither in the list of the accepted food additives nor in the list of harmful substances and is frequently used in cosmetics, mainly for treatment of dermal bacterial infections.

CONCLUSIONS

1. The investigated varieties of savoy cabbage much differed in vitamin C content and dynamics of its depletion. A lower initial content of vitamin C in savoy cabbage of WIROSA cultivar could be related to its storage conditions before the experiments started.

2. Vitamin C content in savoy cabbage of both cultivars kept in the controlled atmosphere decreased about twice as slow as in the case of vegetables stored in the air. As a result, the controlled atmosphere contributed to a better preservation of the cabbage biological value during storage.

3. No significant influence of the tee tree and cardamom essential oils on vitamin C degradation in savoy cabbage was observed during its storage under controlled atmosphere.

4. The controlled atmosphere slowed down the decomposition of chlorophyll pigments and contributed to the preservation of green colour in the stored vegetables.

5. The comparative research of different analytical materials showed that the addition of the tea tree essential oil to the controlled atmosphere caused a decrease in the total number of aerobic microorganisms by two logarithmic units after 1–2 months of storage, while the addition of cardamom essential oil reduced it by one logarithmic unit.

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JAKOŚĆ KAPUSTY WŁOSKIEJ PRZECHOWYWANEJ W KONTROLOWANEJ ATMOSFERZE Z DODATKIEM OLEJKÓW ETERYCZNYCH

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Badano wpływ kontrolowanej atmosfery oraz kontrolowanej atmosfery z dodatkiem olejku herbacianego lub kardamonowego na kinetykę zmian zawartości witaminy C, barwników chlorofilowych oraz na wzrost bakterii podczas przechowywania dwóch odmian kapusty włoskiej: OWASA i WIROSA.

Warzywa przechowywano (t=0–1°C) w kontrolowanej atmosferze (CA) o składzie 4% CO_2, 3% O_2, 93% N_2, w kontrolowanej atmosferze CA z dodatkiem olejku herbacianego (olejek 1) lub kardamonowego (olejek 2) o stężeniu 15 ppm oraz w powietrzu (K). Wykazano, że CA niezależnie od obecności olejku eterycznego spowalnia degradację witaminy C (rys. 1, 2) oraz barwników chlorofilowych (rys. 3–6) w przechowywanych warzywach. Kontrolowana atmosfera przedłuża okres zachowania wysokiej wartości biologicznej badanych warzyw podczas chłodniczego przechowywania. Olejek herbaciany dodany do CA poprawia jakość mikrobiologiczną przechowywanej kapusty, powodując obniżenie ogólnej liczby drobnoustrojów o dwie jednostki logarytmiczne po 1–2 miesiącach przechowywania (tab. 2), natomiast olejek kardamonowy o jedną jednostkę (tab. 3).