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CHANGES IN CONTENTS OF NITRATES(V) AND NITRATES(III) IN SMALL RADISH PACKAGED AND STORED IN MODIFIED ATMOSPHERE

ZMIANY ZAWARTOŚCI AZOTANÓW(V) I AZOTANÓW(III) W RZODKIEWCE ZAPAKOWANEJ I PRZECHOWYWANEJ W ATMOSFERZE MODYFIKOWANEJ

Abstract: The aim of the study was to analyze changes in contents of nitrates(V) and nitrates(III) in small radish packaged in modified atmosphere using film with different oxygen permeability (3000 $cm^3 \cdot m^{-2} \cdot 24 h^{-1} \cdot bar^{-1}$ and 200 $cm^3 \cdot m^{-2} \cdot 24 h^{-1} \cdot bar^{-1}$). In the packaging process air atmosphere was applied as well as modified atmosphere with the two following composition: 10 % O_2, 10 % CO_2, 80 % N_2 and 20 % O_2, 25 % CO_2, 55 % N_2. Samples were stored for 12 days at 4 C.

In fresh small radish nitrate(V) content was 1608 $mg \cdot kg^{-1}$. During storage of analyzed samples a statistically significant ($p \le 0.05$) increase was observed in the contents of the above-mentioned compounds, irrespective of the applied atmosphere composition and the type of packaging film. The presence of nitrates(III) was detected only after 12 days of storage in small radish packaged in modified atmosphere using film with oxygen permeability of 200 $cm^3 \cdot m^{-2} \cdot 24 h^{-1} \cdot bar^{-1}$. It results from conducted analyses that the application of film with high oxygen permeability in the packaging of small radish, both in case of air atmosphere and atmosphere with the percentage composition of $O_2/CO_2/N_2$: 10/10/80 and 20/25/55, makes it possible to maintain aerobic conditions inside the packaging and thus prevent the accumulation of nitrates(III).

Keywords: small radish, nitrates(V) and nitrates(III), modified atmosphere, films with different oxygen permeability

Vegetables are valuable sources of vitamins, minerals and bioactive compounds in human diet, but at the same time they may contain substances harmful for the human organism, such as nitrates(V) and nitrates(III) as well as heavy metals, which they absorb from the soil and atmosphere. Nitrates(V) are accumulated first of all in root vegetables, vegetables with a short vegetation period as well as early vegetable cultivars

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[1]. One needs to focus here on greenhouse-grown vegetables, which due to the intensive nitrate(V) fertilization, insufficient lighting and limited vegetation period, are to a considerable degree at risk of nitrate(V) accumulation [2]. The accumulation of nitrates(V) in plants is affected by botanic factors, such as species, cultivar or maturation stage, and environmental factors, eg nitrogen rate and date of fertilizer application, temperature, insolation, mineral resources and moisture content of soil [3]. The adopted methods of transport and storage after harvest also have an effect on the metabolism of nitrates(V) and nitrates(III) in vegetables.

Small radish as a vegetable with a very short vegetation period, accumulating reserve substances in the thickening of the stem section below the cotyledon, frequently grown in greenhouses for early spring harvest, tends to accumulate nitrates(V), which may next be reduced to nitrates(III). Literature data confirm that the activity of nitrate(V) reductase is connected with the availability of sugars and light. Thus insufficient lighting in case of greenhouse or early spring growing creates conditions reducing the course of photosynthesis (formation of carbohydrates) and inhibiting the activity of nitrate(V) reductase responsible for nitrate metabolism [4]. In case of small radish excess nitrates(V) in the stem thickening result not only from intensive fertilization, but also conditions and duration of transport and storage, as well as those under which the vegetable is stored by consumers at home, which frequently leads to wilting and scalding of vegetables. Plants with yellow, rotting leaves constitute a potential source of harmful nitrates(III) and N-nitroso compounds [5].

Modified atmosphere packaging is a method used with increasing frequency to extend the shelf life and storage time for minimally processed vegetables. However, the composition of atmosphere inside the packaging, changing during vegetable storage, may contribute to a considerable deterioration of raw material quality or even pose a health hazard. Thus the application of a packaging material adequately selected to suit a given raw material is a significant element determining the quality of the stored product, including also a limitation of nitrate(III) accumulation and the application of biologically active compounds.

The aim of the study was to investigate changes in contents of nitrates(V) and nitrates(III) as well as physicochemical characteristics and sensory attributes of small radish packaged in modified atmosphere, using film with different oxygen permeability.

Materials and methods

The analyses were conducted on greenhouse-grown small radish, harvested in February and purchased in a retail outlet.

Tested raw material was washed, inedible parts were removed and then it was again washed and dried. Next radish was packaged in batches of 100 g into 15 × 21 cm plastic bags of elastic film with oxygen permeability of 3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ (My Films Standard, Cryovac, Polska) and 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ (PA/PE 75 MY, Multivac, Polska). Bags were sealed in air atmosphere and in modified atmosphere with the two following compositions: 10 % O_2, 10 % CO_2, 80 % N_2 and 20 % O_2, 25 % CO_2, 55 % N_2 (an A 300 Multivac sealing machine). The product was stored for 12 days at 4 °C.

Analyses of nitrate(V) and nitrate(III) contents, sensory examination as well as measurements of active acidity, soluble solids contents and changes in O_2 and CO_2 contents in the atmosphere inside the packaging were conducted after 1, 6 and 12 days of storage.

Contents of nitrates(V) and nitrates(III) were determined by colorimetry using Griess reagents (wavelength λ = 538 nm), with the use of direct reduction of nitrates(V) to nitrates(III) by metallic cadmium, in accordance with Polish Standard PN-92/A-75112, being an equivalent of ISO 6635:1984 [6]. In the preliminary stage of determination samples were shaken with active carbon in order to remove the colouring originating from chlorophyll.

Sensory examination was performed immediately after the opening of bags with radishes. Examinations were conducted using a 5-point scale, in three replications for each sample. Contents of oxygen and carbon dioxide in the atmosphere inside the packaging were determined in three replications using an OXYBABY®V wireless gas analyzer by Witt-Gasetechnik. Soluble solids contents were determined according to Polish Standard PN-90/A 75101/02 [7], while active acidity – according to PN-90/A 75101/06 [8].

Statistical analysis of results was conducted based on the univariate analysis of variance and LSD Fisher's test. Statistically significant differences were described at the significance level $p \leq 0.05$. The analysis was performed using Statistica ver. 7.0 software (StatSoft, Poland).

Results and discussion

In the raw material the content of nitrates(V) was 1608 mg $NaNO_3 \cdot kg^{-1}$ d.m., while nitrates(III) were not detected. Nitrate(V) content in samples after 1-day storage was similar to their level in the raw material, ranging from 1352 to 1884 mg $\cdot$ kg^{-1} d.m. (Fig. 1). In the course of storage of analyzed samples, a statistically significant ($p \leq 0.05$) increase was recorded in the contents of the above-mentioned compounds, irrespective of the applied composition of atmosphere and the type of packaging film. The increase in the contents of nitrates(V) in analyzed samples ranged from approx. 8 % in samples packaged in film with oxygen permeability of 3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ to approx. 25 % in samples packaged in film with permeability of 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$. Such an increase in the level of nitrates in plant origin materials may result from physiological changes in nitrogen compounds [9]. The contents of analyzed compounds in all samples were no more than 2000 mg $\cdot$ kg^{-1}.

Admissible daily intake of nitrates(V), according to FAO/WHO [10], is 350 mg $NaNO_3 \cdot day^{-1}$ (for an individual weighing 70 kg). In case of fresh radish a portion of approx. 220 g covers ADI (*Acceptable Daily Intake*, mg/kg)) for an individual weighing 70 kg, while in case of stored small radishes the admissible daily intake of nitrates(V) would be supplied in a portion of approx. 170 g. The above examples concern adults, while in case of children the consumption of several radishes

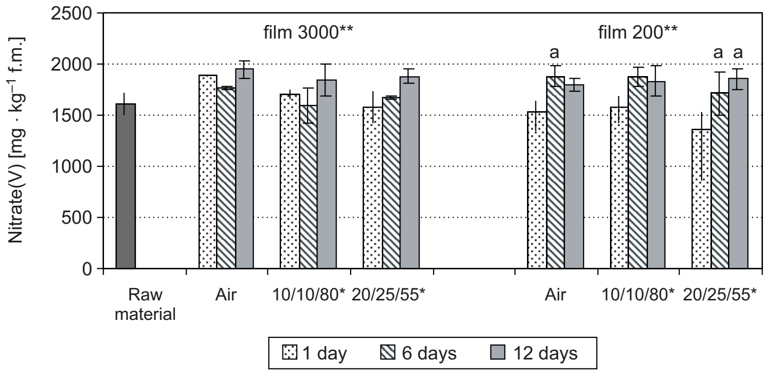

Fig. 1. Changes in nitrates(V) contents in stored small radish packaged in modified atmosphere using film with different oxygen permeability: * – composition of modified atmosphere $\%O_2/\%CO_2/\%N$; ** – film with oxygen permeability in $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$; a – statistically significant differences ($p \leq 0.05$) between: the contents nitrates(V) after 6, 12 days of storage and after 1 day storage (within the same variant)

would result in exceeded ADI for nitrates(V). Thus it is advisable to suggest a reduced consumption of large amounts of small radishes, especially in the autumn and winter period. In that time this vegetable comes from greenhouse cultivation, with intensive fertilization and poor lighting under short day conditions. This results in the reduction of photosynthesis and the accumulation of nitrates(V) absorbed from the substrate [5]. Light is the primary factor regulating the activity of nitrate(V) and nitrate(III) reductases, contributing to the assimilation of reduced forms of nitrogen [11, 12].

After 12-day storage in radish packaged in modified atmosphere in film with oxygen permeability of 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ the presence of nitrates(III) was detected (Fig. 2). In these bags anaerobic conditions were generated as early as after 6 days of storage. In contrast, in analogous samples packaged in film with oxygen permeability of 3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$, nitrates(III) were not detected and the concentration of

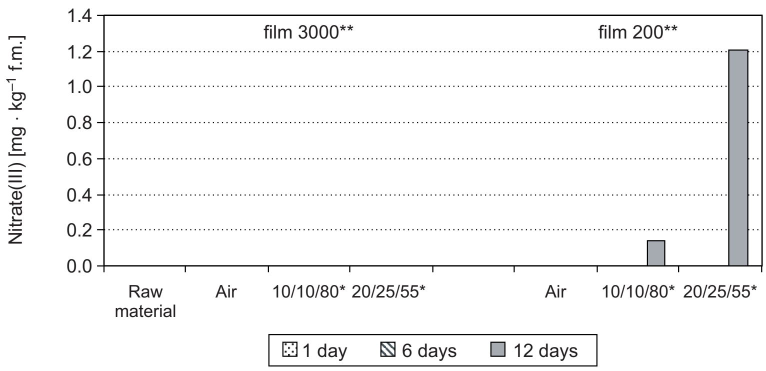

Fig. 2. Changes in nitrates(III) contents in stored small radish packaged in modified atmosphere using film with different oxygen permeability (explanations as in Fig. 1)

oxygen in the packagings was at least 10 %. Thus it may be concluded that the application of packagings with high oxygen permeability during storage of radishes in air or modified atmosphere with the two following compositions: 10 % O_2/10 % CO_2/80 % N_2 and 20 % O_2/25 % CO_2/55 % N_2, prevents the generation of anaerobic conditions in the packaging, which would be conducive of the accumulation of nitrates(III) in the stored product. Bakowski et al [13] recorded an increase in the contents of nitrates(III) to approx. 100 mg $\cdot$ kg^{-1} in spinach packaged in impermeable plastic bags as early as after 4 days of storage.

Radish, as a vegetable of the turnip family, is characterized by high respiration intensity, ie 40–70 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ [14]. This raw material after harvest wilts fast and becomes scalded, which makes it suitable only for immediate consumption [15]. However, small radishes are available both in wholesale and retail sold on trays and sealed with solid film, which – as it results from the analyses presented above – may constitute a health hazard at their considerable consumption.

Based on the conducted measurements of gas contents in the atmosphere inside the packaging with stored radish it was found that in samples packaged in film with low oxygen permeability (200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$) anaerobic conditions are generated as early as after 6-day storage and at the same time high contents of carbon dioxide were recorded (from 25 to 40 %). In these samples, as it was described above, the presence of nitrates(III) and a higher increase in the contents of nitrates(V) were found. It results from literature data that the application of too high concentration of carbon dioxide in the atmosphere inside the packaging may cause in case of vegetables physiological changes and microbiological spoilage [16]. Sensitivity to high carbon dioxide concentrations is dependent on the species of the raw material, the degree of its comminution and storage temperature. In most studies concerning modified atmosphere packaging of vegetables a 20–30 % concentration of carbon dioxide is reported to be the threshold level, not causing physiological damage to packaged products [17–19]. In case of radish there is no available literature data defining the threshold concentration of carbon dioxide in the atmosphere, which would not cause any physiological changes.

Adverse changes caused by a high proportion of carbon dioxide in the atmosphere inside the packaging with stored radish were confirmed by sensory examination. Samples in which anaerobic conditions were formed after 12-day storage received the lowest scores in the overall sensory examination (2.6, 2.3, 2.1) (Table 1).

In samples packaged in film with high oxygen permeability (3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$), both in air and modified atmosphere, the presence of oxygen was recorded throughout their entire storage, at the simultaneous low levels of carbon dioxide in the packaging. These samples also received high scores in sensory examination throughout their storage, from 4.7 to 5.0 (Table 1).

Values of pH in analyzed products after 1-day storage ranged from 6.3 to 6.4. During storage a significant decrease in pH of samples was recorded and after 12-day storage these values ranged from 6.1 to 6.3 (Table 1). A similar dependence was reported in case of soluble solids contents in the analyzed product, which decreased significantly

Table 1

Changes of sensory and physicochemical properties and contents of oxygen and carbon dioxide in the atmosphere inside the package with small radish packaged in modified atmosphere, using film with different oxygen permeability

Composition of the atmosphere in package	Oxygen permeability of packaging fim	Time of storage	Parametr evaluated				
			Contents of CO_2 in atmosphere inside the package	Contents of O_2 in atmosphere inside the package	Overall sensory examinations	pH	Soluble solids
	[$cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$]	[days]	[%]		[-]	[-]	[%]
Raw material			—	—	5.0	6.1	2.4
Air	3000	1	2.3	16.0	5.0	6.3	2.3
		6	3.5	11.5 a	5.0	6.2	2.5 a
		12	3.0	16.0	5.0	6.1	2.1
10 % O_2/10 % CO_2/ 80 % N_2		1	4.6 b	12.1 b	5.0	6.3	2.1
		6	3.4	14.4 ab	4.7 ab	6.2	2.5 a
		12	2.2 a	18.7 ab	5.0	6.1	2.2
20 % O_2/25 % CO_2/ 55 % N_2		1	3.9	18.9 b	5.0	6.3	2.2
		6	1.8 a	18.7 b	4.9 a	6.2 a	2.5 a
		12	1.2 a	20.2 b	5.0	6.1 a	2.1

Table 1 contd.

Composition of the atmosphere in package	Oxygen permeability of packaging fim	Time of storage	Parametr evaluated				
			Contents of CO_2 in atmosphere inside the package	Contents of O_2 in atmosphere inside the package	Overall sensory examinations	pH	Soluble solids
	[$cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$]	[days]	[%]		[-]	[-]	[%]
Raw material			—	—	5.0	6.1	2.4
Air	200	1	3.8	13.3 c	5.0	6.4	2.3
		6	13.7 ac	0.0 ac	4.9	6.4 c	2.4
		12	24.5 ac	0.0 ac	2.6 ac	6.3 c	2.1 a
10 % O_2/10 % CO_2/ 80 % N_2		1	8.9 bc	4.8 bc	5.0	6.3	2.3
		6	17.0 abc	0.0 ac	4.4 abc	6.4 c	2.5 a
		12	21.2 abc	0.0 ac	2.3 abc	6.4 c	2.1 a
20 % O_2/25 % CO_2/ 55 % N_2		1	18.9 abc	16.4 bc	5.0	6.4	2.3
		6	29.5 abc	0.0 ac	3.9 abc	6.4 c	2.5 a
		12	39.4 abc	0.0 ac	2.1 abc	6.3 c	2.1 a

a – statistically significant differences ($p \le 0.05$) between: the value of researches parameter after 6, 12 days of storage and after 1 day storage (within the same variant); b – statistically significant differences ($p \le 0.05$) between: the value of researches parameter of samples packaged in modified atmosphere and samples packaged in air, after the same storage time; c – statistically significant differences ($p \le 0.05$) between: the value of researches parameter of samples packaged in film with oxygen permeability 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ and samples packaged in film with oxygen permeability 3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$, after the same storage time.

after 12-day storage in all samples, irrespective of the packaging material and the composition of atmosphere applied in packaging.

Conclusions

1. Contents of nitrates(V) in the raw material and in samples after 1-day storage ranged from 1352 to 1884 mg · kg^{-1} d.m.

2. In small radish packaged in modified atmosphere using film with oxygen permeability of 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ the presence of nitrates(III) was detected after 12-day storage.

3. In samples packaged in film with oxygen permeability of 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ after 6-day storage in anaerobic atmosphere was generated with high contents of carbon dioxide. These samples also received the lowest scores in the overall sensory examination.

4. It results from the conducted analyses that the application of film with high oxygen permeability to package small radish, both in air and modified atmosphere with the percentage composition of $O_2/CO_2/N_2$: 10/10/80 and 20/25/55, makes it possible to maintain aerobic conditions inside the packaging and thus prevent the accumulation of nitrates(III).

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ZMIANY ZAWARTOŚCI AZOTANÓW(V) I AZOTANÓW(III) W RZODKIEWCE ZAPAKOWANEJ I PRZECHOWYWANEJ W ATMOSFERZE MODYFIKOWANEJ

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Abstrakt: Celem pracy było zbadanie zmian zawartości azotanów(V) i (III) w rzodkiewce zapakowanej w atmosferze modyfikowanej, z zastosowaniem folii o różnej przepuszczalności tlenu (3000 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$ oraz 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$). Do pakowania zastosowano atmosferę powietrza oraz atmosferę modyfikowaną o następującym dwóch wariantach składu: 10 % O_2, 10 % CO_2, 80 % N_2 oraz 20 % O_2, 25 % CO_2, 55 % N_2. Próbki przechowywano przez 12 dni w temperaturze 4 °C.

W świeżej rzodkiewce zawartość azotanów(V) wynosiła 1608 $mg \cdot kg^{-1}$. W czasie przechowywania badanych próbek, odnotowano istotny statystycznie ($p \le 0.05$) wzrost zawartości azotanów(V), niezależnie od zastosowanego składu atmosfery oraz rodzaju folii opakowaniowej. Obecność azotanów(III) stwierdzono jedynie po 12 dniach przechowywania, w rzodkiewce zapakowanej w atmosferze modyfikowanej przy zastosowaniu folii o przepuszczalności tlenu 200 $cm^3 \cdot m^{-2} \cdot 24\ h^{-1} \cdot bar^{-1}$. Z przeprowadzonych badań wynika, że zastosowanie do pakowania rzodkiewki folii o dużej przepuszczalności tlenu zarówno przy zapakowaniu jej w atmosferze powietrza, jak i w atmosferze o procentowym udziale $O_2/CO_2/N_2$: 10/10/80 i 20/25/55 pozwala zachować warunki tlenowe wewnątrz opakowania, a tym samym uniknąć kumulacji azotanów(III).

Słowa kluczowe: rzodkiewka, azotany(V) i (III), atmosfera modyfikowana, folie o różnej przepuszczalności dla tlenu