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APPLICATION OF MODIFIED ATMOSPHERE PACKAGING TO EXTEND SHELF-LIFE OF MINIMALLY PROCESSED SPINACH

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Key words: modified atmosphere, spinach, minimal processing, sensory quality, microbiological quality

The study assessed the effect of modified atmosphere packaging with varied contents of oxygen and carbon dioxide on sensory and microbiological quality, physicochemical properties and physiological activity of minimally processed spinach. The following variants of modified atmosphere composition were applied: (% O_2/% CO_2/% N_2) 10/10/80; 20/5/75; 20/25/55, as well as air atmosphere. On the basis of experiments it was found that modified atmosphere packaging of spinach made it possible to obtain a product maintaining good sensory quality for 6 days. Prolonged storage resulted in unacceptable changes in sensory attributes. The application of atmosphere with the composition of 10% O_2/10% CO_2/80% N_2 had the most advantageous effect on the sensory quality of minimally processed spinach. The level of microbial contamination of fresh spinach was slight (10^4 CFU/g). Counts of mesophilic and psychrophilic bacteria were higher in the raw material than in minimally processed samples; however, the counts of microorganisms in most samples increased during 12-day storage, irrespective of the modified atmosphere variant applied in packaging. In all spinach samples, irrespective of the initial composition of atmosphere, oxygen content dropped rapidly; in most samples after 6-day storage anaerobic conditions were generated, which indicates too low permeability of the applied packaging material.

INTRODUCTION

Modified atmosphere packaging (MAP) of foodstuffs, generally speaking, consists in the application of an appropriate gas composition inside the packaging, different than the natural atmosphere. The objective of modified atmosphere packaging is to create inside the packaging an appropriately balanced gas composition, which would enabled the greatest possible reduction in the physiological activity of the product. Moreover, adopted levels of oxygen and carbon dioxide in the packaging may not have a negative effect on the product [Pretel *et al*., 2000]. The effectiveness of modified atmosphere packaging depends on many factors: freshness and degree of product processing, its properties, including the character of metabolism and microbiological quality, appropriate composition of a gas mixture, barrier potential of packaging material and its dependence on the temperature and intensity of product respiration [Hertog *et al*., 1998; Fonseca *et al*., 2002].

Spinach (*Spinacia oleracea* L.) is a vegetable with a high nutritional value due to considerable contents of minerals (up to 24% d.m.), carotenoids and fiber [Jaworska & Kmiecik, 1999; Ismail, 2004]. Spinach is available on the fresh market for a long time due to its cultivation both in the autumn and spring season; however, this raw material after being harvested may be stored only for a very short time [Grevsen & Kaack, 1996]. The application of minimal processing in combination with modified atmosphere packaging, at the selection of its appropriate composition and packaging material, may extend the shelf-life of spinach after harvest and thus its availability.

The study assessed the effect of modified atmosphere packaging at varied contents of oxygen and carbon dioxide on sensory and microbiological quality, physicochemical properties and physiological activity of minimally processed spinach.

MATERIAL AND METHODS

Raw material. Experiments were conducted on leaves of spinach cv. Sporter. Raw material for analyses was purchased in retail.

Technological process. Spinach leaves, after sorting and removal of leaf stalks, were washed in cold running water. After being drained they were immersed in a mixture of ascorbic acid (0.5%) and citric acid (0.5%) (solutions temperature 6°C), for approx. 10 min, then drained and dried on blotting paper. Next, the leaves were chopped into fragments of approx. 2 cm x 2 cm, packaged and stored at 4°C (in a refrigerator) for 12 days.

Packaging. The minimally processed product was packaged in batches of 30 g in packages made of plastic laminate: oriented polyamide/polyethylene with layer thickness of 50/40 μg, of 15 x 21 cm and gas permeability (in cm3/m2/24 h at 23°C): carbon dioxide – 200, oxygen – 45 (water vapour: 2-3 g/m^2/24 h), and sealed using an A 300

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vacuum packaging machine by Multivac. In the packaging of spinach atmosphere variants with the following composition were used: (1) air; (2) 10% oxygen/10% carbon dioxide/80% nitrogen; (3) 20% oxygen/5% carbon dioxide/75% nitrogen; and (4) 20% oxygen/25% carbon dioxide/55% nitrogen.

Physicochemical analysis. Determination of active acidity (pH) was performed according to the standard PN-90/A-75101/06 using a 710A pH–meter (Orion). Determination of extract was performed according to the standard PN-90/A-75101/02. Determination of total acidity was performed according to the standard PN-90/A-75101/04 and the results were given in terms of oxalic acid. Spinach samples were homogenized prior to physicochemical analyses (homogenizer IKA T25). Three replications were performed for each sample.

Measurement of oxygen and carbon dioxide contents in packages with spinach. Assays were performed using a Gaspace 2 Systech Instruments BV. Three replications (measurements in three packages) were performed for each sample.

Sensory analysis. Sensory examinations were performed using a 5–point scale with a specially developed sensory evaluation chart (Table 1) [Baryłko-Pikielna, 1975]. Products were evaluated by a 5–member panel, immediately after the package was opened after 1, 3, 6, 9 and 12 days of storage. The assessed attributes included colour, taste, aroma, consistency and the presence of drip in the package. Samples of fresh (unprocessed) spinach were analysed as well.

Microbiological analysis. Microbiological analysis conducted after 1, 6 and 12 days of storage included the determination of total counts of mesophilic and psychrophilic bacteria, total counts of moulds and mesophilic and psychrophilic yeasts, as well as the total count of lactic acid bacteria.

Preparation of samples. Spinach (10 g), directly after the package was opened, was transferred to sterile flasks with 90 mL physiological solution (8.5 g/L NaCl, 1 g/L peptone tryptone, Difco). Next, the entire volume was shaken in a shaker for 15 min at a room temperature. Decimal solutions were prepared from samples within the range of 10^{-1}–10^{-7}.

Conditions of microbiological analysis. In the determinations of all the above mentioned groups of microorganisms quantitative culture was applied using the Koch platelet method [Burbianka *et al*., 1983]. Medium composition for mesophilic and psychrophilic bacteria was prepared according to Burbianka *et al*. [1983]. Incubation time for mesophilic bacteria was 48-72 h at 30°C, while for psychrophilic bacteria it was 72-120 h at 4°C. For the determination of mould and yeast counts medium with the following composition was applied: yeast extract 5 g/L, glucose 20 g/L, chloramphenicol 0.1 g/L, bacterial agar 15 g/L, and pH 6.6 (BTL, Łódź, Poland). Incubation time for moulds and yeasts was 72-96 h, at 30°C – for mesophilic moulds and yeasts and 15°C – for psychrophilic moulds and yeasts. For assays of counts of lactic acid bacteria agar MRS (BTL, Łódź, Poland) was used as medium, incubation time in this case was 72 h at 30°C.

Statistical analysis of results. Results were analysed by the analysis of variance (ANOVA) and Fisher(s least significant difference (LSD) multicomparison test. Statistically

TABLE 1. A sensory examination chart.

Quality attribute	Weighting coefficient	Point scores				
		5	4	3	2	1
Colour – type	3	typical green	green (typical) with scarce dark-green or light-green spots	green with numerous spots	yellow-green or mostly dark-green	dark-green or yellow
– equalization	3	very equalized	equalized	medium equalized	non-equalized	mosaic
Odour – character (+description)	3	very intrinsic	intrinsic	medium intrinsic	rather extrinsic	off-odour
– intensity	1	very intensive	intensive	medium intensive	weakly intensive	undetectable
Taste – palatability	3	very intrinsic, desirable	intrinsic, desirable	medium intrinsic, rather desirable	not intrinsic	off-flavour
Consistency – leaf tenderness	3	very crisp, firm	crisp, firm	medium firm, slightly soft	not firm, soft, slightly gummy	gummy or sticky
– moisture	3	dry leaves	slightly moist leaves	moist leaves	moist, slightly sticky leaves	very wet, strongly stuck leaves
Drip	1	no drip	slight drip (up to 1 mL)	visible drip (up to 3 mL)	considerable drip (over 3 mL)	abundant drip (condemning)

significant differences were reported at p(0.05. Statistical analysis was performed using computer software Statistica ver. 7.0 (StatSoft, Kraków Poland).

RESULTS AND DISCUSSION

The value of pH in raw material was 6.54, while initial pH values of modified atmosphere packaged samples ranged from 6.43 to 6.98. During 12-day storage, pH was observed to increase in spinach samples (Table 2). The lowest pH values were found for samples with higher CO_2 concentrations in the package (10 and 25%). In turn, a 20% O_2 concentration in packages resulted in an increase of pH, both in relation to the raw material and the sample after 1-day storage. A reduction of pH in samples with high contents of carbon dioxide may have been caused by the dissolution of CO_2 and the formation of carbonic acid. Similar results were reported by Babic & Watada [1996] for spinach packaged in controlled atmosphere. The value of pH for spinach stored at 5°C increased from 6.4 to 7.0.

An increase in pH during storage was not equivalent to a decrease in total acidity of spinach (Table 2). Even a considerable elevation of pH, to 7.34, did not result in a decrease of total acidity, which may be explained by the considerable buffer capacity of this vegetable.

Extract content in fresh spinach was 6%. During storage of spinach samples, packaged both in modified atmosphere and in air, the content of extract decreased and after 12 days of the experiment it reached a value of 3.3% to 4.3%. A statistically significant effect of storage time on extract content was recorded in all MAP samples. Such a considerable decrease of extract content in samples could have been caused by physiological processes occurring in the product, as well as by the action of developing microorganisms. Changes in extract content of spinach in the course of 12-day storage are presented in Table 2.

TABLE 2. Changes in pH and extract content in samples of spinach packaged in modified atmosphere and in air during 12-day storage.

Sample packaged in atmosphere	Days of storage	pH	Extract (%)	Total acidity (g oxalic acid/100 g of product)
Raw material		6.54±0.06	6.0±0.0	0.06±0.0
Air	1	6.43±0.03	6.3±0.3	0.07±0.0[a]
	6	6.64±0.02	5.5±0.5	0.06±0.0
	12	6.97±0.10[a, b]	4.0±0.0[a b]	0.06±0.0[b]
10% O_2/10% CO_2	1	6.71±0.03[a]	4.3±0.3[a]	0.07±0.0[a]
	6	6.60±0.10	4.7±0.6[a]	0.06±0.0
	12	6.98±0.07[a]	3.3±0.6[a, b]	0.07±0.0[a]
20% O_2/5% CO_2	1	6.67±0.06[a]	5.8±0.0	0.06±0.0
	6	6.92±0.04[a, b]	4.3±0.1[a]	0.06±0.0[b]
	12	7.34±0.09[a]	3.5±0.5[a]	0.06±0.0
20% O_2/25% CO_2	1	6.59±0.01	5.5±0.0	0.06±0.01
	6	6.67±0.04[a]	4.5±0.8[a]	0.06±0.0
	12	6.83±0.18[a]	3.6±0.6[a, b]	0.05±0.01[a]

a – statistically significant difference between selected physicochemical factors of samples packaged in air and in MA of different composition, and the value of the analysed factor of fresh sample (raw material); b – statistically significant difference between selected physicochemical factors of samples after 6 and 12 days of storage, and the value of the analysed factor of samples after 1 day storage

Raw material used for analyses exhibited good quality and received overall sensory examination score of 5.0, which means that all attributes received the highest possible notes. After 1-day storage, high notes in overall sensory evaluation were given for all samples of spinach packaged in modified atmosphere and in air, and the values ranged from 4.4 to 5.0. During storage, the values of overall sensory evaluation decreased. After 3-day storage they remained at the level from 4.0 to 4.7. However, after 12-day storage the quality of the product deteriorated considerably. The highest scores were given for spinach leaves stored in atmosphere with the composition of 10% O_2/10% CO_2/80% N_2 and those air-packaged, notes for overall sensory evaluation of these samples after 12-day storage were 2.2–2.3 points. A statistically significant effect of storage time on the value of overall sensory evaluation of the samples was found in all samples (Table 3).

A reduction of scores for overall sensory evaluation of samples was connected mainly with deterioration of scores given for aroma and taste. Already after 3-day storage of MAP spinach a significant decrease was recorded in the intrinsic character of aroma. After 6-day storage a strong aroma was detected, defined as the smell of decaying, wet grass and given the score of 1.0–2.3 points (Table 3). According to literature sources [Babic & Watada, 1996] the concentration of CO_2 above 13% in packages with spinach causes unacceptable changes in the aroma after 7-day storage. Jacxsens *et al*. [1999] investigated the effect of packaging with high permeability for O_2 and CO_2 on the quality of selected minimally processed vegetables, including spinach, stored under EMA (equilibrium modified atmosphere) conditions. They found that EMA storage of minimally processed vegetables, at an appropriately low temperature and the application of packaging of high gas permeability (towards O_2 and CO_2), had an advantageous effect on the quality of sensory attributes, including aroma.

TABLE 3. Results of sensory evaluation of aroma, taste and overall sensory evaluation of spinach packaged in modified atmosphere and in air, after 1, 6 and 12 days of storage at 4°C.

Sample packaged in atmosphere	Days of storage	Sensory evaluation of aroma	Sensory evaluation of taste	Overall sensory evaluation
Raw material		5.0±0.0	5.0±0.0	5.0±0.0
Air	1	4.2±0.3[a]	5.0±0.0	4.8±0.1[a]
	6	1.3±0.6[a b]	3.0±0.0[a b]	3.5±0.2[a b]
	12	1.0±0.0[a]	2.0±0.0[a b]	2.2±0.1[a b]
10% O_2/10% CO_2	1	4.2±0.3[a]	5.0±0.0	4.7±0.1[a]
	6	1.8±0.3[a b]	3.2±0.3[a b]	3.5±0.0[a b]
	12	1.0±0.0[a]	2.0±0.0[a b]	2.2±0.0[a b]
20% O_2/5% CO_2	1	5.0±0.0	5.0±0.0	5.0±0.0
	6	2.3±0.3[a b]	4.0±0.0[a b]	3.3±0.1[a b]
	12	1.0±0.0[a b]	1.3±0.6[a b]	1.7±0.1[a b]
20% O_2/25% CO_2	1	5.0±0.0	4.7±0.3	4.8±0.1[a]
	6	2.0±0.0[a b]	2.8±0.3[a b]	2.9±0.1[a b]
	12	1.0±0.0[a b]	1.0±0.0[a b]	1.9±0.1[a b]

a – statistically significant difference between the scores of sensory evaluation (respectively: aroma, taste and overall sensory evaluation) of samples packaged in air and in MA of different composition, and the scores of fresh sample (raw material); b – statistically significant difference between the scores of sensory evaluation of samples after 6 and 12 days of storage, and the scores of samples after 1 day storage

The score of overall sensory evaluation was also affected by notes given for taste. After 3-day storage in all MAP samples taste was evaluated as good, receiving scores between 3.8 and 4.5 points. However, already after 6-day storage in most samples the taste became less intrinsic and became bitter, after 12 days it was given scores of 1.0 to 2.0 points and was characterised as bitter, leaving the raw, grassy after-taste (Table 3).

Consistency of MAP spinach leaves also affected the overall evaluation score, especially in the last days of the experiment. After 9 and 12 days of storage the samples were characterised by a considerable loss of firmness and strong wetness, evaluated at 1.5-2 points. Colour and drip generated during storage affected overall sensory evaluation score only to a slight extent.

During 12-day storage the analysis of gas contents (O_2 and CO_2) in packages with the product showed a very fast decrease of oxygen content, at the simultaneous increase in the concentration of carbon dioxide. After 3-day storage oxygen was only found in samples with a 20% initial content of this gas. In the case of samples packaged both in MA and in air, anaerobic conditions were produced in packages after 6-day storage (Table 4). It was observed that a 25% CO_2 concentration in samples with a 20% initial content of O_2, resulted in oxygen being retained in the package for a longer period. On the basis of conducted experiments it may also be found that the applied type of packaging had a disadvantageous effect on most quality attributes of minimally processed spinach. Its gas permeability (oxygen 45 $cm^3/m^2/24$ h, carbon dioxide 200 $cm^3/m^2/24$ h) proved to be too low. In further studies it would be necessary to apply packaging materials with higher gas permeability.

TABLE 4. Contents of oxygen and carbon dioxide in packages with spinach stored for 12 days in modified atmosphere and in air.

Sample packaged in atmosphere	Days of storage	Oxygen contents (%)	Carbon dioxide contents (%)
Air	1	4.6±0.6	16.1±0.6
	3	1.7±1.4	19.4±1.3
	6	0.0±0.0	25.7±0.4
	9	0.0±0.0	26.2±0.3
	12	0.0±0.0	27.1±0.8
10% O_2/10% CO_2	1	0.3±0.5[a]	20.9±0.1[a]
	3	0.0±0.0[a]	23.3±0.5[a]
	6	0.0±0.0	24.5±0.7[a]
	9	0.0±0.0	25.0±0.1[a]
	12	0.0±0.0	26.5±1.6
20% O_2/5% CO_2	1	11.2±0.5[a]	14.3±0.7[a]
	3	2.9±0.9[a]	21.8±0.9[a]
	6	0.0±0.0	28.1±0.7[a]
	9	0.0±0.0	29.1±0.5[a]
	12	0.0±0.0	29.5±0.3[a]
20% O_2/25% CO_2	1	15.1±0.1[a]	29.0±0.3[a]
	3	9.9±0.5[a]	33.7±0.6[a]
	6	0.3±0.5	45.7±1.3[a]
	9	0.0±0.0	47.7±0.9[a]
	12	0.0±0.0	47.0±0.5[a]

a – statistically significant difference between gas contents (O_2 and CO_2) in packaging for samples stored in MA of different composition, and analysed gas contents for samples stored in air atmosphere, after the same time of storage

Fresh spinach used in the experiments exhibited a low contamination level. The presence of mesophilic bacteria at $3.5x10^4$ CFU/g and psychrophilic bacteria at $2.3x10^4$ CFU/g was recorded. According to literature sources [Babic & Watada, 1996], the level of spinach contamination with these microorganisms for mesophilic and psychrophilic bacteria was 10^7–10^8 CFU/g. In most MAP samples the counts of colonies, after 12-day storage, exceeded the level of contamination for raw material, amounting to 10^4–10^5 CFU/g. Babic & Watada [1996] stated that low oxygen concentrations in combination with a high content of carbon dioxide in MAP reduces the counts of aerobic mesophilic and psychrophilic bacteria ten or even a hundred times at 5°C, but is ineffective at 10°C. This effect was not recorded in this study.

No lactic acid bacteria were recorded in fresh spinach, similarly as it was the case in a study by Babic & Watada [1996]. However, after 12-day storage lactic acid bacteria were found at a level ranging from $0.3x10^2$ CFU/g to $1.7x10^2$ CFU/g. In a study by Babic *et al*. [1996], after identical storage time, the count of 10^3–10^4 CFU/g lactic acid bacteria was recorded.

In all samples, as well as the raw material, counts of mesophilic and psychrophilic moulds and yeasts were $1.7x10^1$–$1.1x10^3$ CFU/g, the count of these microorganisms increased with storage time to $0.3x10^3$–$1x10^5$ CFU/g (after 12 days).

Babic & Watada [1996] found that a low concentration of oxygen and a high concentration of carbon dioxide in packages with spinach reduced the number of pathogens, but also deteriorated product's quality. On the basis of microbiological analyses in this study it was found that in the case of minimally processed spinach the modified atmosphere packaging, with different atmosphere composition variants, had a diverse effect on the level of its contamination during storage. Some authors [Luo & Barbosa–Canovas, 1996] reported that citric and ascorbic acids, applied for pre-treatment, enhanced microbiological stability of the product.

CONCLUSIONS

On the basis of experiments it was found that modified atmosphere packaging of spinach made it possible to obtain a product maintaining good sensory quality for 6 days. Further storage resulted in unacceptable changes in sensory attributes. A reduction of the note for overall sensory evaluation of the sample was connected mainly with deterioration of notes given for aroma, next for taste and consistency of the product, while to a lesser extent for colour. Among the variants of atmosphere composition applied in packaging of spinach the most advantageous effect on its sensory quality was found for atmosphere with 10% O_2/10% CO_2/80% N_2. The level of microbiological contamination of fresh spinach was slight (10^4 CFU/g). Counts of mesophilic and psychrophilic bacteria were higher in the raw material than in minimally processed samples; however, the degree of microbial contamination in most samples increased during 12 days of storage, irrespective of the MAP variant. In all spinach samples, irrespective of the initial composition of atmosphere, oxygen content decreased rapidly; in most samples anaerobic conditions were generated after 6-day storage, which indicates too low permeability of the applied packaging material.

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ZASTOSOWANIE PAKOWANIA W ATMOSFERZE MODYFIKOWANEJ DO PRZEDŁUŻENIA TRWAŁOŚCI SZPINAKU O MAŁYM STOPNIU PRZETWORZENIA

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W pracy oceniano wpływ pakowania w atmosferze modyfikowanej o różnej zawartości tlenu i ditlenku węgla na jakość sensoryczną, mikrobiologiczną, właściwości fizykochemiczne oraz aktywność fizjologiczną szpinaku o małym stopniu przetworzenia. Zastosowano następujące składy atmosfery modyfikowanej: (% O_2/% CO_2/% N_2) 10/10/80; 20/5/75; 20/25/55 oraz atmosferę powietrza. Na podstawie przeprowadzonych doświadczeń stwierdzono, że pakowanie szpinaku w atmosferze modyfikowanej pozwoliło na uzyskanie produktu utrzymującego dobrą jakość sensoryczną przez 6 dni. Dalsze przechowywanie powodowało nieakceptowalne zmiany cech sensorycznych. Zastosowanie atmosfery o składzie: 10% O_2/10% CO_2/80% N_2 najkorzystniej wpływało na jakość sensoryczną szpinaku o małym stopniu przetworzenia. Poziom skażenia mikrobiologicznego świeżego szpinaku był niewielki (10^4 jtk/g). Liczba bakterii mezofilnych i psychrofilnych była wyższa w surowcu niż w próbach o małym stopniu przetworzenia, jednak podczas 12 dni przechowywania następował rozwój mikroflory w większości prób, niezależnie od wariantu MA zastosowanego do pakowania. We wszystkich próbach szpinaku, niezależnie od początkowego składu atmosfery, bardzo szybko spadała zawartość tlenu; w większości prób po 6 dniach przechowywania wytwarzały się warunki beztlenowe, co wskazuje na zbyt małą przepuszczalność zastosowanego materiału opakowaniowego.