

Effects of Ethanol-based Zein Coatings on Physiological Responses and Quality of Fresh-cut Carrots

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Abstract

Fresh-cut carrots are highly susceptible to physiological deterioration and microbial spoilage after processing. This study evaluated the effects of ethanol-based zein coatings applied alone or enriched with olibanum extract on the quality of fresh-cut carrot cubes during refrigerated storage. Carrot cubes of two cultivars ('Brava' and 'Warmia') were coated with zein (Z) or zein with olibanum extract (ZO) and stored at 3°C for 9 days. Weight loss, respiration activity, carotenoid retention, colour, microbial growth and sensory quality were assessed. Contrary to expectations, zein-based coatings increased weight loss and respiration intensity compared with uncoated controls, suggesting enhanced stress-related metabolism induced by the ethanol-based solvent system. CO₂ production in coated 'Brava' samples increased from 94.0 to 218.9 ml·kg⁻¹·h⁻¹ during storage. Zein coating alone moderately reduced bacterial and yeast growth, whereas olibanum enrichment did not consistently improve microbiological stability. Coatings significantly affected colour parameters, particularly L* and b*(lightness and yellow-blue axis), producing perceptible colour changes ($\Delta E > 6$). Sensory analysis indicated that zein-coated samples retained favourable texture attributes, while olibanum negatively affected flavour acceptability. The results demonstrate that the effectiveness of zein coatings in fresh-cut vegetables strongly depends on solvent composition and tissue characteristics and cannot be directly extrapolated from studies on intact produce.

The findings highlight that coating performance in fresh-cut products cannot be directly extrapolated from studies on intact horticultural commodities.

Highlights

- Zein coatings increased respiration intensity in fresh-cut carrots.
- Olibanum extract altered colour and sensory quality of carrot cubes.
- Zein coating reduced bacteria and yeast proliferation during refrigerated storage of carrots cv. Warmia.
- Carotenoid degradation correlated with tissue water loss.
- Coating performance differed substantially between carrot cultivars.

Introduction

Carrot (*Daucus carota* L.) is a widely consumed root vegetable belonging to the *Apiaceae* family and valued for its sensory and nutritional properties. Its edible roots are a source of carbohydrates, dietary fibre, minerals, and vitamins, particularly β -carotene, a precursor of vitamin A essential for vision, immune response, and skin health. The high carotenoid content characteristic of carrots determines their vivid orange colour, enhancing the visual appeal of fresh, minimally processed, and processed food products [1, 2].

The demand for minimally processed and ready-to-eat vegetables has increased considerably in recent years due to changing consumer preferences and the growing market for convenient fresh products. Fresh-cut carrots are among the most popular components of ready-to-eat salads and snack products. However, tissue disruption during peeling and cutting accelerates respiration, moisture loss, enzymatic reactions and microbial growth, resulting in rapid quality deterioration and shelf-life reduction. Fresh-cut carrot tissue is also susceptible to physiological disorders such as white blush formation [3, 4], lignification and sensory deterioration associated with wound-induced metabolism [5]. In addition, the absence of thermal stabilization increases susceptibility to contamination by bacteria, moulds and yeasts, including human pathogens such as *Salmonella spp.*, *Escherichia coli* and *Listeria monocytogenes* [6].

Current preservation strategies for fresh-cut vegetables combine refrigeration, modified atmosphere packaging (MAP), sanitation treatments, edible coatings and non-thermal technologies [7]. Among these technologies, edible coatings have gained considerable attention due to their ability to reduce moisture loss, regulate gas exchange and act as carriers of antimicrobial and antioxidant compounds. Recent reviews emphasize that edible coatings, particularly when combined with complementary preservation strategies such as MAP, represent one of the most promising approaches for extending the shelf life of minimally processed produce [8, 9].

Various edible coatings have been investigated for carrot preservation, including polysaccharide-, lipid- and protein-based systems [5]. Among protein-based materials, zein has attracted particular interest due to its film-forming properties, hydrophobic character, relatively low cost and wide availability. Owing to its ability to form cohesive films and coatings, zein is considered a promising biodegradable biopolymer for food-packaging applications [10, 11]. Zein-based coatings have demonstrated effectiveness in maintaining postharvest quality by reducing microbial growth, delaying colour changes and limiting moisture loss in horticultural products [12, 13].

Zein exhibits amphiphilic self-assembly behaviour in aqueous ethanol systems, where solvent composition strongly influences microphase formation, molecular organization and the barrier properties of the resulting films and coatings [14]. Since zein requires aqueous ethanol solutions for dissolution, solvent composition substantially affects coating microstructure and barrier properties [15, 16]. Moreover, zein may serve as an effective carrier matrix for bioactive compounds and plant extracts through hydrophobic and hydrogen-bond interactions [17].

Among natural bioactive additives, frankincense (olibanum) resin derived from *Boswellia serrata* has recently attracted attention due to its antimicrobial and antioxidant properties. Olibanum contains essential oils, terpenoids and boswellic acids with documented biological activity [18, 19]. Previous studies demonstrated that olibanum-based films and coatings may improve antimicrobial activity, barrier properties and oxidative stability of food packaging systems [20–22].

Despite the promising properties of zein-based coatings, their effectiveness in fresh-cut root vegetables remains insufficiently explored, particularly in systems involving alcohol-based solvents and bioactive

plant extracts. Therefore, this study investigated the effects of zein coatings applied alone or enriched with olibanum extract on the physicochemical properties, respiration activity, microbiological stability, sensory quality and carotenoid retention of fresh-cut carrot cubes during refrigerated storage. To the best of our knowledge, this is the first study addressing the physiological and sensory responses of fresh-cut carrot tissue to ethanol-based zein coatings enriched with olibanum extract.

Materials and Methods

Raw material characteristic

As experimental raw material, two carrot cultivars were selected: (1) 'Brava' cv., an orange Berlikumer-type carrot cultivar characterized by uniform cylindrical roots with a smooth surface, suitable both for the fresh market and for processing, with good storage potential; and (2) 'Warmia' cv., an orange Flakkee-type carrot cultivar producing medium-long tapered roots with a blunt tip, characterized by high processing suitability and recommended for long-term storage. Both cultivars were obtained from the experimental field of the Warsaw University of Life Sciences (SGGW) in Żelazna (Skierniewice district, Poland).

Experimental material preparation

The overall experiment design is given in Fig. 1. After digging up, the roots were pre-selected, damaged or diseased roots were discarded. The carrots were washed in running water from the remains of soil, rinsed and then peeled. Roots were then shredded using an industrial cutting machine, obtaining uniform cubes ($8 \times 8 \times 8$ mm). For the initial decontamination of the roots, a commercial food-grade disinfectant BioActiW 2000 Food (hypochlorous acid) was used in the concentration recommended by the manufacturer (200 ppm). Then cubes were kept in ambient temperature on sieves for 30 minutes to let them dry.

Two coating-forming solutions were prepared. The first one (Z) was prepared from zein alone ($\sim 9,2\%$ w/v) by dissolving 146g of zein (Pol-Aura, Olsztyn, Poland) in 1580 ml of aqueous ethanol solution (60% v/v, prepared from 95% pure ethanol) previously heated to 50°C using an electrically heated stirrer (Thermomix 31 – 1, Vorwerk Deutschland Stiftung & Co. KG, Berlin, Germany) and mixed at 400 rpm for 15 min. The second coating (ZO) was prepared by enriching zein solution with olibanum extract (a 73g tincture of frankincense resin (*Boswellia serrata* Roxb. ex Colebr)) previously prepared by maceration of a mill-crushed olibanum resin (frankincense) poured with ethanol (60% v/v) in a ratio of 1:5 (w/v) and extracted for 14 days at $\sim 22^{\circ}\text{C}$ without access to light.

Carrot cubes were immersed once in the coating solutions for 10 min at $22 \pm 1^{\circ}\text{C}$ with gentle manual stirring to ensure uniform coating distribution. After draining the cubes were distributed in monolayer on sieves and left to drain for 30 min in room temperature, and then transferred in portion of about 90–100 grams to perforated plastic containers ($142 \times 96 \times 49$ mm). Samples were stored for 9 days at 3°C and 80–85% relative humidity. As the control samples, carrot cubes only decontaminated with BioActiW

2000 Food, not subjected to coating treatment were taken. For each combination 25 boxes were measured.

Weight loss

To determine weight (water) losses, each box with diced carrots was weighed before and after 9 days of storage, using WPS2100/C/2 laboratory balance (Radwag, Poland) with accuracy ± 0.01 [g].

Respiration rate and ethylene production

Respiration activity was determined by measuring carbon dioxide production and oxygen concentration in the headspace of sealed containers using a CheckMate 3 gas analyzer (O_2/CO_2 ; Dansensor, Denmark). For respiration and ethylene measurements, samples of carrot cubes were enclosed in gas-tight containers and incubated for 1 h at 18°C. Although carrot is considered a non-climacteric vegetable, ethylene production may serve as an indicator of wound-induced stress metabolism in fresh-cut tissues.

After incubation, a 1 mL gas sample was collected from the headspace and injected into a gas chromatograph (HP 5890 II, Hewlett Packard, USA) equipped with a packed glass column and flame ionization detector (FID) for ethylene determination. Carbon dioxide concentration was determined using the AMETEK® MOCON® Dansensor® CheckMate 3 gas analyser (MOCON Europe A/S, Denmark). Measurements were performed in triplicate immediately after cutting (day 0) and after 9 days of refrigerated storage for all experimental combinations. Results were expressed as $\mu l \cdot kg^{-1} \cdot h^{-1}$ for ethylene production and $ml \cdot kg^{-1} \cdot h^{-1}$ for CO_2 production.

Carotene content

The total carotenoid content was determined by high-performance liquid chromatography (HPLC) according to the method described by Bohoyo-Gil et al. [23]. Analyses were carried out using a liquid chromatograph equipped with a diode array detector (DAD).

The results were initially expressed as mg per 100 g of fresh mass (fm). Due to changes in sample mass during storage, the final total carotenoid content was recalculated on a dry matter basis and expressed as mg per 100 g of dry matter (dm).

Colour measurement

The colour of the carrot cubes was measured using the CIE Lab system with a spectrophotometer (Konica Minolta Sensing Inc., Japan), model CR-2600d. Measurements were performed on samples consisting of 10 carrot cubes for each treatment. The parameters L^* , a^* , and b^* were recorded as colour indicators, where:

L* – lightness (luminance), ranging from 0 (black) to 100 (white),

a* – red/green coordinate, with positive values indicating red and negative values indicating green,

b* – yellow/blue coordinate, with positive values indicating yellow and negative values indicating blue.

The effect of the edible coating on colour change relative to the control sample was calculated using the total colour difference (ΔE), according to the formula:

$$\Delta E = \sqrt{\{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2\}}$$

where:

- L, a, b – values for the coated sample,
- L_0, a_0, b_0 , values for the control sample.

Microbiological analyses

Microbiological analyses were performed to determine the total viable aerobic bacteria as well as the abundance of yeasts and moulds in fresh-cut carrot samples. A 10 g portion of plant material was homogenized with 90 mL of sterile peptone water for 10 min using a BagMixer 400P (Donserv, Poland). The obtained homogenate was subjected to serial ten-fold decimal dilutions. Appropriate dilutions were plated in triplicate using the spread plate method (0.1 mL) onto selective microbiological media: Plate Count Agar (PCA; Merck, Germany) for total aerobic bacteria (10^3 – 10^5 dilutions) and Yeast Extract Glucose Chloramphenicol Agar (YGC; Merck, Germany) for yeasts and moulds (10^1 – 10^3 dilutions). After incubation under appropriate conditions (PCA: 30°C, 72 h; YGC: 24°C, 96 h), colonies were counted and expressed as colony-forming units per gram of fresh weight (CFU·g⁻¹). Microbial counts were calculated according to the standard formula:

$$\text{CFU} \cdot \text{g}^{-1} = N \times D \times V^{-1},$$

where N is the mean number of colonies counted per plate (from three plates), D is the dilution factor, and V is the volume of inoculum (mL). All analyses were performed in triplicate. The analyses were performed on the initial day of the experiment and after 2, 4 and 9 days of storage at 3°C.

Sensory evaluation

Sensory analysis of fresh-cut carrots was conducted after 9 days of storage at 3°C. The evaluation was performed using the Quantitative Descriptive Analysis (QDA). The following attributes were assessed: aroma (carrot, sweet, foreign), colour, texture (crispness, hardness, crunchiness, juiciness), taste (carrot, sweet, bitter, foreign), and overall quality.

The selection of sensory descriptors was based on preliminary focus group sessions with trained assessors. The intensity of each attribute was evaluated using an unstructured graphical scale with defined boundary descriptors. The results were converted into numerical values ranging from 0 to 10 arbitrary units (AU), where 0 indicated no intensity and 10 indicated very high intensity.

The analysis was carried out in a sensory laboratory of National Institute of Horticulture Research (Skierniewice, Poland), compliant with ISO 8589:2010 [24]. The panel consisted of 10 trained assessors with verified sensory sensitivity, qualified according to ISO 8586:2023 [25]. Each sample was evaluated in duplicate and presented in a randomized order. Samples were served in coded plastic containers (250 mL) with lids. Water was provided as a palate cleanser between samples.

Preliminary data analysis was performed using ANALSENS software, and the results were visualized using principal component analysis (PCA).

Statistical analysis

Statistical analysis was performed using Statistica 13.3 PL software (TIBCO Software Inc., USA). Three-way ANOVA was applied to evaluate the effect of treatments, storage time and the cultivars. Duncan's multiple range test was used to determine significant differences between mean values at a significance level of $\alpha = 0.05$.

Results and discussion

Physiological responses

As previous studies on coated carrots demonstrated improved storability and reduced dehydration [2], the weight loss observed during 9 days of refrigerated storage (Fig. 2) revealed unexpected results. Contrary to expectations, carrot cubes coated with zein (Z) and zein enriched with olibanum (ZO) exhibited significantly higher weight loss than the control samples (K) in both cultivars. Although control samples of both cultivars showed comparable mass losses, their responses to coating application differed considerably. The 'Brava' cultivar exhibited higher water losses than 'Warmia' following coating treatment.

The observed effect may be associated with the solvent system used for coating preparation. Unlike many previously described protein coatings prepared in water-based systems, the zein coating in the present study required aqueous ethanol for dissolution. Due to its high diffusivity and affinity for water, ethanol may promote moisture migration from disrupted carrot tissues by facilitating concentration equilibration through simple diffusion processes. Consequently, in fresh-cut carrot tissue lacking its natural protective barrier, the ethanol-based zein coating system may enhance rather than reduce water loss during refrigerated storage.

Similarly, respiration activity, expressed as CO₂ and ethylene production was not reduced in fresh-cut carrot cubes treated with zein-based edible coatings (Table 1).

Table 1

Ethylene and CO₂ production as indicators of respiration rate in freshly cut carrot cubes (day 0) and after 9 days of storage. K, control; Z, zein coating; ZO, zein + olibanum coating.

		Ethylene production ($\mu\text{l}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)		CO ₂ production ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$)	
		‘Brava’	‘Warmia’	‘Brava’	‘Warmia’
Freshly cut	0	0.226 a*	0.212 a	69.1 a	61.7 a
Control (K)	9	0.114 a	0.066 b	94.0 b	56.8 a
Zein (Z)	9	0.334 a	0.459 c	174.6 c	130.9 b
Zein+ Olib (ZO)	9	0.633 b	0.223 a	218.9 d	132.9 b

*Means followed by the same letter within each column do not differ significantly ($p = 0.05$, $n = 3$).

Immediately after cutting (day 0), both cultivars exhibited low ethylene production and moderate CO₂ emission, reflecting baseline metabolic activity associated with tissue wounding. After 9 days of storage, clear differences between treatments were observed. In the control samples, ethylene production decreased in the ‘Warmia’ cultivar but remained relatively stable in ‘Brava’. At the same time, CO₂ production increased, particularly in ‘Brava’, indicating intensified respiration processes during storage. This behaviour is typical for fresh-cut vegetables, where tissue disruption leads to increased metabolic activity and accelerated senescence.

The application of zein coating (Z) resulted in increased ethylene and CO₂ production compared with the control, especially in the ‘Warmia’ cultivar. This suggests that the coating, despite forming a physical layer on the tissue surface, did not sufficiently restrict gas exchange and may have induced stress-related metabolic responses. The zein coating enriched with olibanum extract (ZO) further intensified respiration activity, particularly in the ‘Brava’ cultivar, where the highest ethylene and CO₂ production values were recorded. Bioactive compounds present in olibanum may act as elicitors, stimulating wound-related physiological pathways and enhancing respiratory metabolism. While coatings are generally expected to reduce respiration by limiting oxygen availability and moisture loss, in some cases they may instead modify the internal atmosphere in a way that stimulates metabolic activity.

The observed cultivar-dependent response indicates that the effectiveness of edible coatings is strongly influenced by the physiological characteristics of plant tissue. Although zein-based films and coatings are generally reported to reduce moisture loss and respiration intensity [26, 27], most previous studies were conducted using dried films or intact horticultural products. Similar beneficial effects of zein

coatings have been reported for mango, tomato, grapes, apple and garlic, where reduced transpiration and delayed deterioration were observed [13, 28–31].

In contrast, the present study demonstrated that direct contact of ethanol-based zein coatings with wounded fresh-cut carrot tissue resulted in increased water loss and enhanced respiration intensity. These findings suggest that coating performance in fresh-cut systems cannot be directly extrapolated from studies performed on intact fruits or pre-formed films. The lack of a natural protective barrier in cut tissue may facilitate solvent penetration, partially disrupt membrane integrity and increase tissue permeability, thereby enhancing water diffusion and intensifying stress-related metabolic activity instead of storage stabilization[32].

Carotenoid content

Table 2
Characteristics of carotenoids in ‘Warmia’ and ‘Brava’ carrot cubes before storage.

Carotenoids (mg·100g ⁻¹)	lutein	α-carotene	β-carotene	cis-β-carotene	total carotenoids
‘Brava’	0.180 a*	3.963 a	6.583 a	0.347 a	11.07 a
‘Warmia’	0.243 a	5.180 b	8.120 b	0.373 a	13.91 b

* Different letters in columns indicate significant differences at $p < 0.05$.

Carotenoids are among the most important nutritional components of carrots. The analysed cultivars differed significantly in the content of individual carotenoid fractions (Table 2). The ‘Warmia’ cultivar contained significantly higher levels of α-carotene and β-carotene compared to ‘Brava’, resulting in a higher total carotenoid content.

Changes in carotenoid content during storage of fresh-cut material did not follow a clear, consistent trend. However, when carotenoid retention was analysed in relation to tissue dehydration expressed as weight loss, a clear relationship became evident (Fig. 3). Increased mass loss was associated with lower carotenoid content, indicating that tissue dehydration contributed to carotenoid degradation during storage.

Interestingly, the cultivars differed in the rate of carotenoid depletion. Despite lower respiration intensity (Table 1) and relatively smaller mass losses, the ‘Warmia’ cultivar exhibited a faster decline in carotenoid content than ‘Brava’. This suggests that carotenoid stability in fresh-cut carrots may depend not only on respiration rate and water loss but also on cultivar-specific structural and biochemical characteristics of the tissue.

The type of coating applied did not appear to significantly influence carotenoid degradation. Instead, the observed changes were more closely associated with tissue disruption and the absence of natural protective barriers in fresh-cut carrot cubes, which likely intensified oxidative processes.

In contrast to the present findings, studies conducted on intact fruit tissues, such as mango, reported increased carotenoid content during storage as a result of ongoing metabolic activity and carotenoid biosynthesis. For example, Gol and Rao [29] observed increased carotenoid levels in mangoes during refrigerated storage regardless of coating treatment. The opposite trend observed in the present study may therefore result from the extensive tissue damage associated with fresh-cut processing, which accelerates oxidation and pigment degradation.

Microbiological stability

Microbiological analysis revealed a significant effect of coating treatment on the growth dynamics of microorganisms during refrigerated storage (Table 3). In all combinations, microbial counts increased progressively throughout storage; however, the intensity of growth depended on both cultivar and coating type.

In the control samples, intensive growth of bacteria as well as yeasts and moulds was observed during storage. The zein coating (Z) exhibited a moderate bacteriostatic effect in both cultivars, resulting in lower microbial counts compared with the control. This effect was particularly evident in the 'Warmia' cultivar, where both bacterial and yeast growth were significantly reduced throughout storage. In contrast, the zein coating enriched with olibanum extract (ZO) did not consistently improve microbiological stability and, depending on the cultivar, showed microbial counts comparable to or higher than the control samples.

The observed antimicrobial activity of zein coatings is consistent with previous studies reporting that zein may act as a carrier matrix for antimicrobial compounds and simultaneously form a physical barrier limiting microbial proliferation. Zhou and Wang [27] demonstrated that zein-based composite films enriched with thyme oil exhibited enhanced antimicrobial properties due to reduced volatility and prolonged retention of active compounds within the film matrix. Similarly, Ansarifar and Moradinezhad [33] reported that zein nanofibers containing thyme essential oil effectively inhibited the growth of *Bacillus cereus*, *Escherichia coli* and *Aspergillus fumigatus*.

Previous studies also indicated that zein coatings may enhance the effectiveness of incorporated antimicrobial agents by protecting them from environmental degradation and enabling their gradual release. Lungu and Johnson [34] observed that zein coatings enriched with nisin and organic acid salts effectively reduced *Listeria monocytogenes* growth on turkey frankfurters, while Carlin et al. [35] reported partial bacteriostatic effects of zein coatings against *L. monocytogenes* on sweet corn. However, these authors emphasized that zein alone may not provide sufficient microbiological protection and recommended combining it with additional antimicrobial compounds.

The present results only partially support this concept. Although zein coating alone reduced microbial growth, the addition of olibanum extract did not consistently enhance antimicrobial effectiveness. This may indicate that the concentration of active compounds released from the coating matrix was insufficient to exert a stable antimicrobial effect in fresh-cut carrot tissue. Moreover, interactions

between ethanol, plant tissue and bioactive compounds may have altered coating structure and functionality during storage.

Comparable inhibitory effects of zein coatings on aerobic bacteria, yeasts and moulds were previously observed in apples by Belay et al. [12], who reported reduced microbial proliferation throughout refrigerated storage. In contrast, the weaker antimicrobial performance observed in the present study may result from the specific characteristics of fresh-cut carrot tissue, including extensive surface damage, high moisture availability and intensified wound-induced metabolism, which together create favorable conditions for microbial growth.

Table 3

Changes in bacterial and yeast counts in coated carrot cubes during refrigerated storage. Values are expressed as log CFU·g⁻¹.

Bacteria – ‘Brava’					
Days	0	2	4	9	mean
Combination					
BK	4.59 bA*	6.43 cB	7.22 bC	8.09 bD	6.58 b
BZ	4.04 aA	5.04 aB	7.11 abC	7.48 aD	5.92 a
BZO	5.35 cA	5.66 bA	6.94 aB	8.30 cC	6.56 b
	4.66 A	5.71 B	7.09 C	7.96 D	
Yeast – ‘Brava’					
Days	0	2	4	9	mean
Combination					
BK	3.55 aA	5.02 bB	6.22 cC	7.38 cD	5.54 b
BZ	3.20 aA	4.10 aB	5.08 aC	7.16 bD	4.89 a
BZO	4.52 bA	6.61 abA	5.84 bB	7.00 aC	5.49 b
	3.76 A	4.57 B	5.72 C	7.18 D	
Bacteria – ‘Warmia’					
Days	0	2	4	9	mean
Combination					
WK	4.63 aA	5.47 cB	6.59 bC	7.52 cD	6.05 b
WZ	4.21 aA	4.45 bA	5.77 aB	7.10 aC	5.38 a
WZO	4.55 aB	4.04 aA	5.71 aC	7.41 bD	5.43 a
	4.46 A	4.65 A	6.02 B	7.35 C	
Yeast – ‘Warmia’					
Days	0	2	4	9	mean
Combination					
WK	3.24 aA	4.53 cB	5.79 bC	6.48 bD	5.01 b
WZ	2.82 aA	3.05 bA	4.50 aB	5.83 aC	4.05 a
WZO	2.69 aA	2.63 aA	4.51 aB	6.42 bC	4.07 a

Bacteria – ‘Brava’			
2.92 A	3.40 B	4.93 C	6.24 D

*Different lowercase letters indicate significant differences between treatments within the same storage day, whereas uppercase letters indicate significant differences during storage within the same treatment ($p < 0.05$).

Sensory quality and colour changes of coated carrot cubes

The PCA projection (Fig. 4) explained 70.96% of the total variability (PC1 = 47.52%, PC2 = 23.44%) and revealed clear differences among treatments. The control samples and carrots coated with zein alone were located on the positive side of PC1 and were associated with desirable sensory attributes, including sweet taste, sweet aroma, juiciness and overall quality score. The highest sensory quality was observed for the control sample of the ‘Warmia’ cultivar (WK), which was positioned closest to the vectors representing sweet taste and overall quality.

Samples coated with zein alone (WZ and BZ) showed a sensory profile similar to the controls and remained associated with positive sensory attributes. Moreover, their proximity to texture-related descriptors, including crispness, hardness, crunchiness and juiciness, suggests that zein contributed to the preservation of desirable textural characteristics during storage.

In contrast, samples coated with zein enriched with olibanum extract showed a distinctly different distribution. The WZO sample was strongly associated with bitter taste and clearly separated from attributes related to high sensory quality. The BZO sample occupied an intermediate position, suggesting that the ‘Brava’ cultivar was less sensitive to sensory changes induced by olibanum than ‘Warmia’. These results indicate that olibanum reduced sensory acceptability, mainly through increased bitterness and the perception of foreign flavour notes.

The favourable sensory profile of zein-coated samples is consistent with previous studies reporting improved sensory quality of coated fresh-cut papaya [36]. Similar positive effects of zein-containing coatings have also been observed in cheese and poultry products[21, 37–38]. However, the present results demonstrate that the sensory response depends strongly on coating composition. While zein alone preserved desirable texture and flavour attributes, the addition of olibanum negatively affected consumer-relevant sensory characteristics. This suggests that optimisation of bioactive additives is essential when designing zein-based coatings for fresh-cut vegetables.

While sensory analysis primarily highlighted the impact of coating composition on flavour perception and texture attributes, visual appearance also represents an important quality factor influencing consumer acceptance. Therefore, colour changes induced by the coatings were further evaluated using instrumental CIELab measurements (Table 4).

Table 4
Colour parameters (CIELab) of fresh-cut carrot cubes after 9 days of storage.

Cultivar	Treatment	L*	a*	b*	ΔE
'Brava'	Control (K)	46.2 $\pm$ 4.8 a*	25.8 $\pm$ 3.9 a	33.1 $\pm$ 3.5 a	-
	Zein (Z)	48.9 $\pm$ 3.7 a	27.4 $\pm$ 2.6 a	34.8 $\pm$ 2.9 a	3.58
	Zein + Olibanum (ZO)	52.7 $\pm$ 5.2 b	24.1 $\pm$ 3.8 a	36.5 $\pm$ 4.1 b	7.07
'Warmia'	Control (K)	44.8 $\pm$ 5.5 a	24.9 $\pm$ 4.2 a	32.6 $\pm$ 3.9 a	
	Zein (Z)	47.5 $\pm$ 3.9 a	26.8 $\pm$ 3.1 a	33.9 $\pm$ 3.2 a	3.22
	Zein + Olibanum (ZO)	50.6 $\pm$ 4.6 b	23.7 $\pm$ 3.5 a	35.8 $\pm$ 4.4 b	6.26

*Values are expressed as mean $\pm$ standard deviation (n = 10). Different letters within the same row (within each cultivar) indicate significant differences at $p < 0.05$.

The statistical analysis confirmed that the application of zein coating enriched with olibanum extract (ZO) significantly affected the colour parameters of fresh-cut carrots. In both cultivars, ZO treatment resulted in a significant increase in L* and b* values compared to control and zein-only samples, indicating a shift towards brighter and more yellow colour tones. This effect was further confirmed by the total colour difference (ΔE), which reached values of approximately 6.3–7.1 for ZO-treated samples, indicating a clearly perceptible change in colour. In contrast, zein coating alone caused only moderate differences ($\Delta E \approx 3.2$ –3.6). Colour changes in fresh-cut carrots may also be associated with wound-induced physiological responses, including lignification and the development of white blush on cut surfaces, which represent natural defence mechanisms of damaged tissues [39].

In contrast, no significant differences were observed for the a parameter, suggesting that the red-orange component associated with carotenoid content remained stable regardless of the treatment. These findings indicate that the observed colour changes are more likely related to optical effects caused by the coating layer, such as light scattering and surface modification, rather than degradation or transformation of carotenoid pigments.

Conclusions

Zein-based coatings influenced the physiological, microbiological and sensory properties of fresh-cut carrot cubes during refrigerated storage. Contrary to expectations, ethanol-based zein coatings increased mass loss and respiration intensity, suggesting that the solvent system promoted physiological stress and moisture migration in wounded carrot tissue.

Zein coating alone exhibited moderate bacteriostatic effects and reduced yeast growth, particularly in the 'Warmia' cultivar. However, enrichment with olibanum extract did not consistently improve

microbiological stability and negatively affected sensory quality due to increased bitterness and foreign aroma perception.

The coatings significantly modified colour parameters, especially L^* and b^* , producing perceptible visual changes ($\Delta E > 6$) without substantially affecting carotenoid-associated orange-red tones. Interestingly, despite increased water loss and respiration intensity, zein-coated samples maintained favourable texture-related attributes such as crispness and hardness, suggesting that zein may contribute positively to structural stability of fresh-cut carrot tissue.

The results indicate that coating performance in fresh-cut systems strongly depends on tissue characteristics and solvent composition and cannot be directly extrapolated from studies on intact produce. Further optimization of ethanol concentration and bioactive additive composition is necessary to improve the balance between storage stability, microbial safety and sensory acceptability.

Declarations

Conflicts of Interest:

The authors declare that they have no competing interests, either financial or non-financial, that could be perceived as influencing the results or interpretation of this work. All authors have been informed about the submission of this manuscript, have approved its final version, and agree to its publication.

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Clinical trial number

not applicable

Ethics approval:

Ethical approval for sensory testing of fruits, vegetables and their products is not required at scientific institutions in Poland. The tests must be conducted in accordance with PN-EN ISO 8589:2010 which specifies the requirements for equipment and appropriate conditions in the laboratory. At our institute, a team of the expert panel (panellists) has been trained by the professional company Hamilton to conduct this type of testing. Prior to each test, panellists are informed about the products being tested and the possible risks associated with their evaluation (e.g. allergies to a particular substance present in the product). There is no compulsion to participate; panellists give voluntary verbal consent to participate in the study. They may have the right to withdraw from the study at any time. Anonymity is maintained; the personal information of panellists is not disclosed.

Human Ethics and Consent to Participate declarations

not applicable

Author Contribution

D.K. and J.A.Z. conceived the experimental design. J.A.Z. and K.P.R. coordinated the research activities. J.A.Z. conducted the laboratory work related to storage conditions and experimental execution. A.W. performed the sensory analyses and prepared Fig. 4, including the statistical analysis. B.K. carried out the microbiological investigations. D.K. provided supervision. Figures 1–3 were prepared by D.K. and J.A.Z. All authors reviewed and approved the final manuscript. AI tools were used solely for language editing of the manuscript and for enhancing the visual appearance of Fig. 1 based on the original schematic.

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Figures

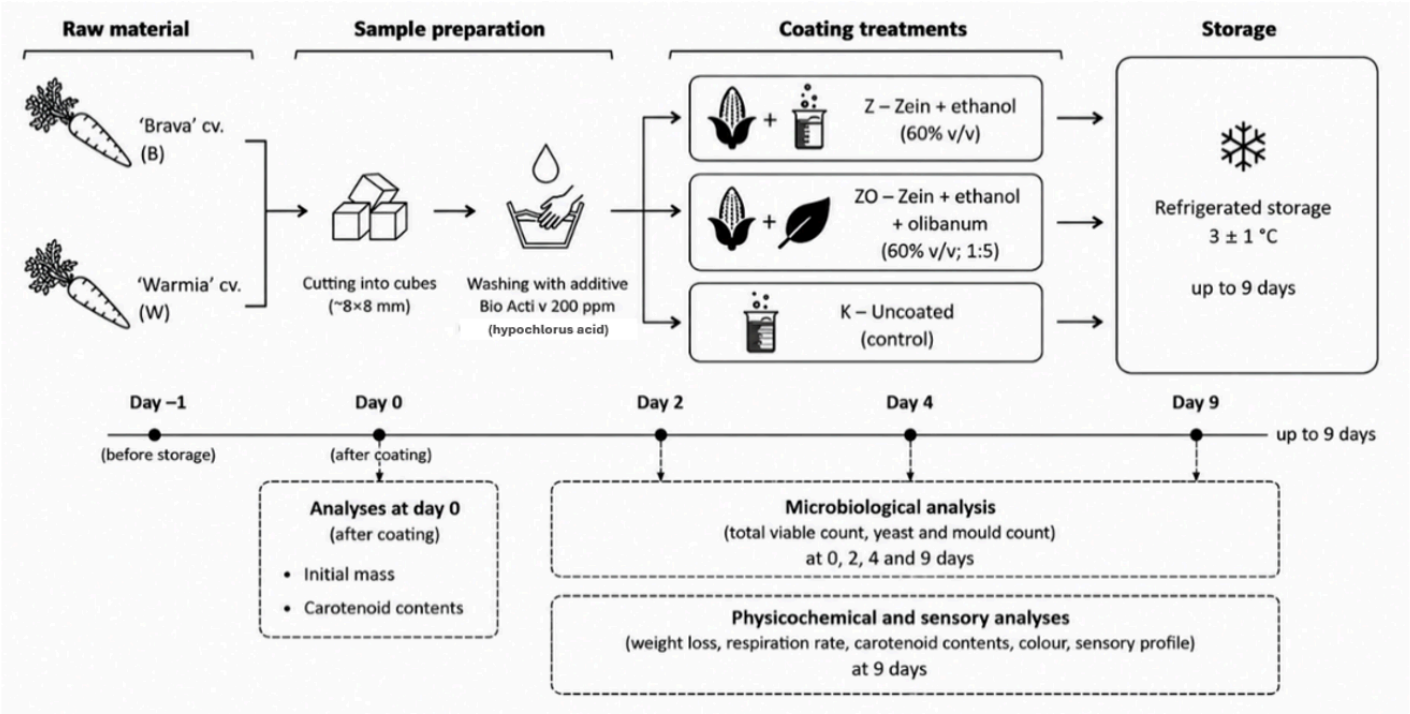


Figure 1

Schematic representation of the experimental design.

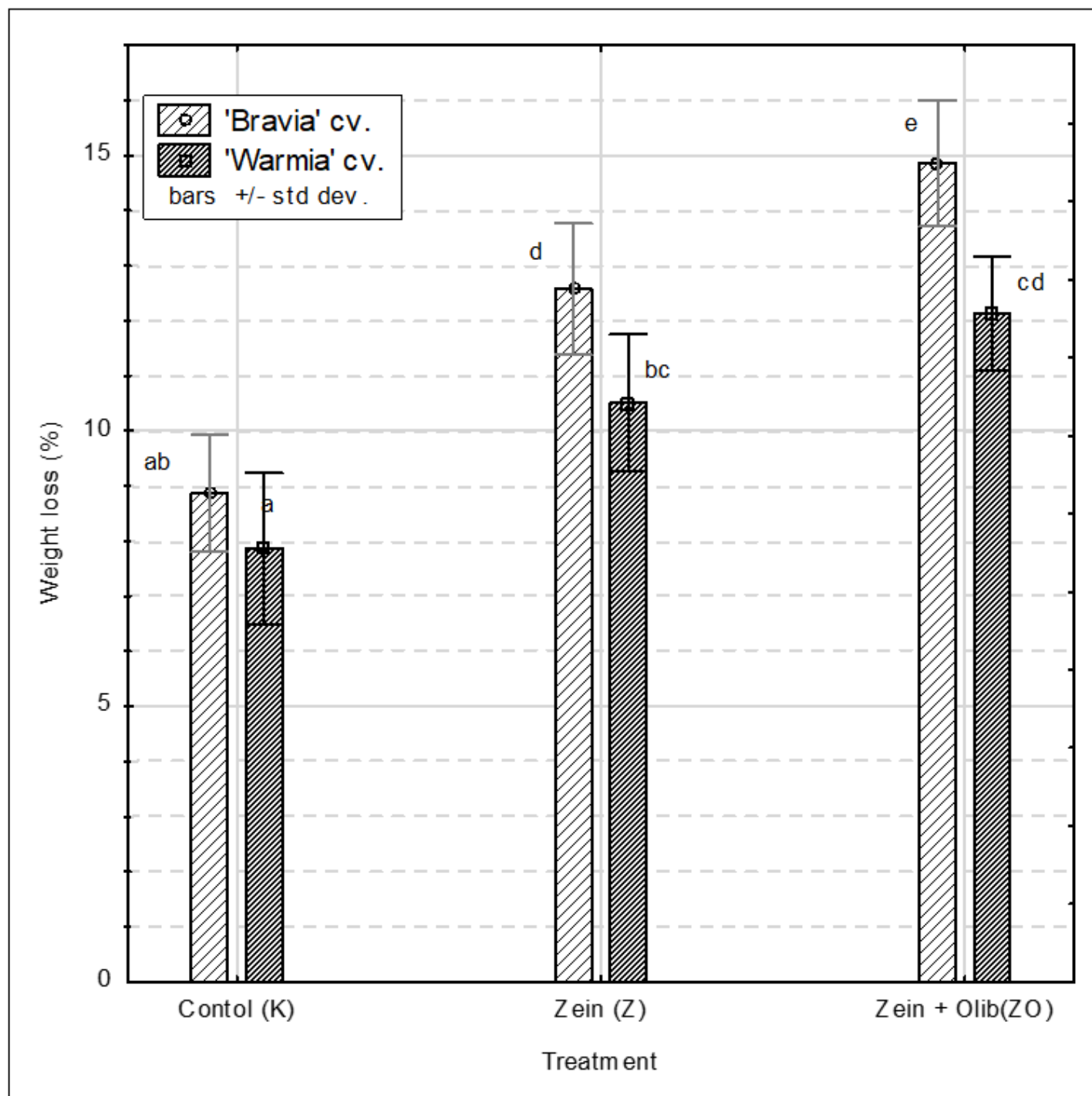


Figure 2

Weight loss of carrot cubes during 9 days of storage. Means followed by the same letter within a cultivar are not significantly different at $p = 0.05$ ($n = 25$).

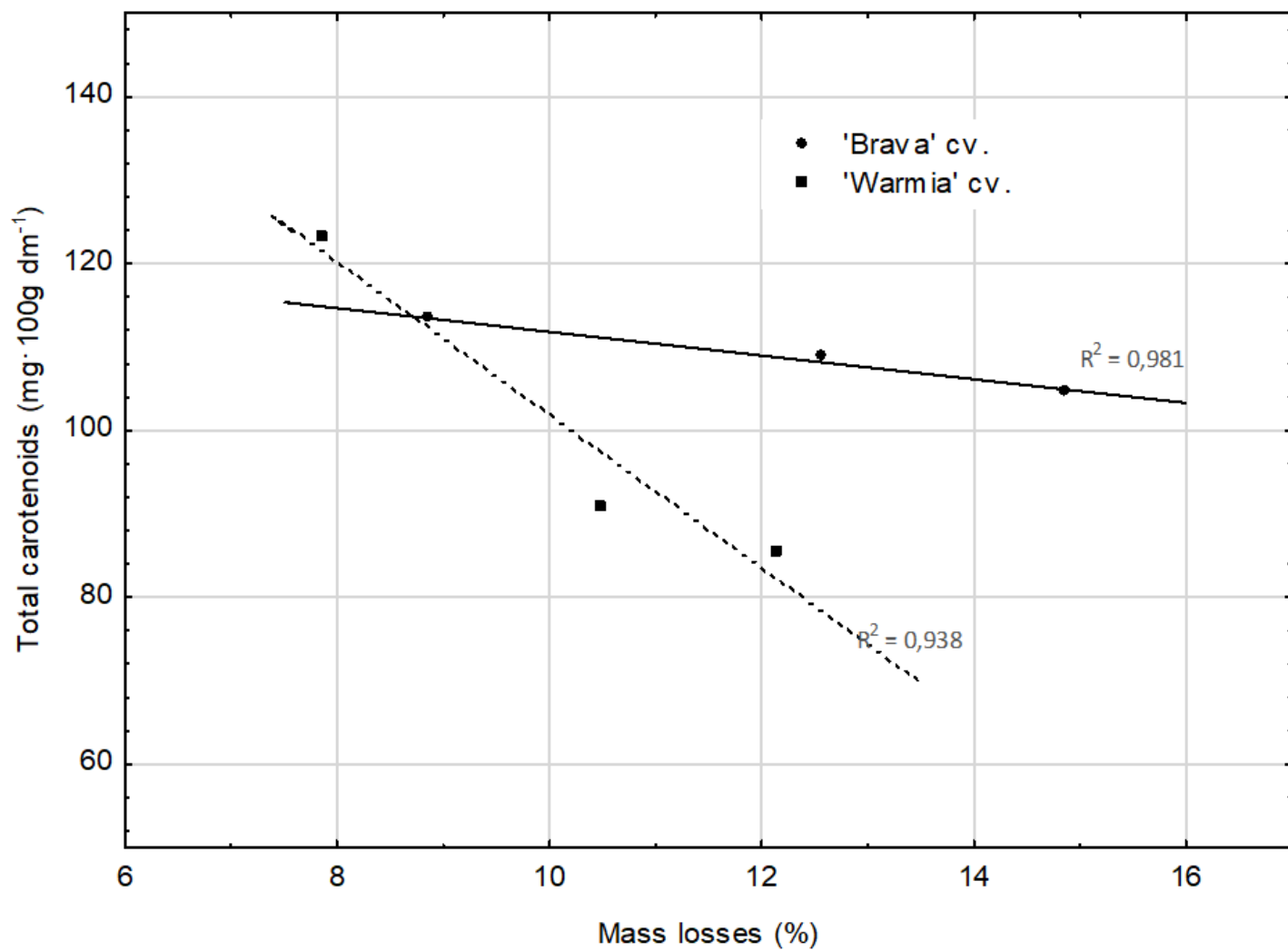


Figure 3

Relationship between weight loss and total carotenoid content in fresh-cut carrot cubes after 9 days of storage.

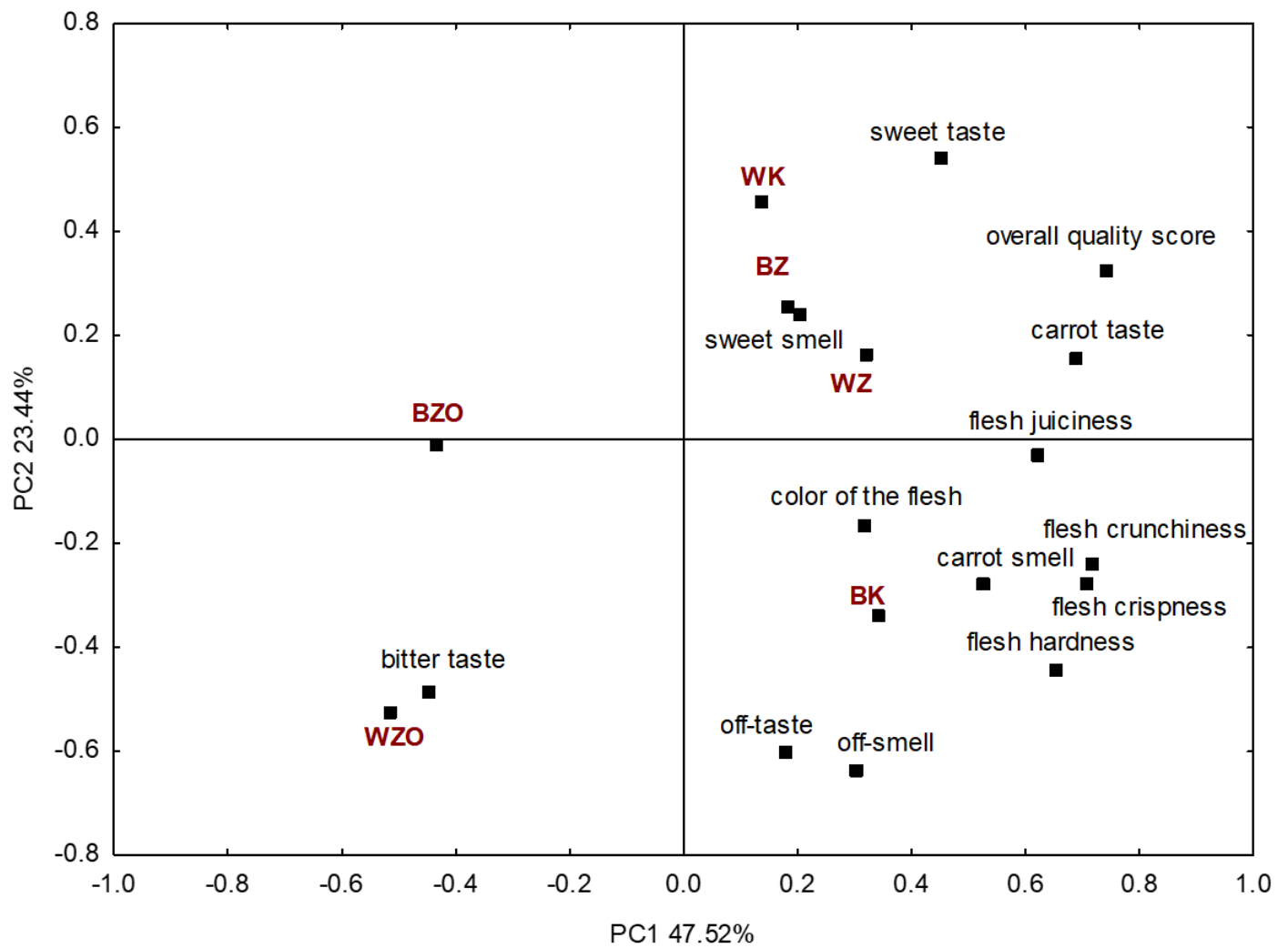


Figure 4

Principal component analysis (PCA) of sensory attributes carrot cubes treated with coatings (Z, ZO) and control (K) of 'Brava'(B) and 'Warmia' (W) cultivars