Investigation of edible coatings on the physical, chemical and microbiological characteristics of processed melons during storage

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Abstract. The possible use of chitosan coating on fresh-cut melons (*Cucumis melo* var. *Cantalupensis*) was investigated in this research topic. Manually sliced melons were treated with solutions of 10 g/kg chitosan and 10 g/kg chitosan with calcium lactate and then stored at 4°C for 8 days. Physical, physicochemical, microbiological and sensory properties of the samples were monitored during the storage period. It was found that chitosan coatings inhibited the growth of microorganisms and affected significantly and positively the storage time of the products. Changes in the sensory qualities of taste were evaluated. A chitosan coating retarded water loss and the drop in sensory quality, increasing the soluble solid content and titratable acidity. The data revealed that applying a chitosan coating preserved effectively the quality and extended the shelf life of fresh-cut melons.

1 Introduction

Edible films and coatings have many advantages over other methods when products are stored at the appropriate temperature, which depends on your product. They can act as barriers against the penetration of moisture and gases, control microbial growth, preserve the color, texture and moisture of the product, and effectively extend the shelf life of the product. The two most important advantages of this technology are the reduction of synthetic packaging waste and the possibility of incorporating functional ingredients into biodegradable materials obtained from natural sources [1].

The use of edible films and coatings is expanding for a wide range of food products, including fresh and minimally processed fruits and vegetables. The reasons for their use are to extend product shelf life [2], to control oxidation and respiration reactions [3], to preserve/improve texture and sensory characteristics, and to be environmentally friendly pure [4].

Chitosan-based edible films have been applied to many fresh fruits and vegetables. The most frequently discussed impacts of coating include lowering respiration rate, maintaining fruit color and firmness, and extending product shelf life.

The incorporation of fatty acids and essential oils in minimal concentrations in the chitosan matrix can improve the barrier properties of the coating and reduce weight loss. [5, 6, 7].

2 Material and methods

2.1 Materials

Melons Cantaloupe variety (*Cucumis melo* var. *Cantalupensis*) were bought from local market. Low molecular weight chitosan (M_w = 400 Da) with 85% N-deacetylation was obtained from Sigma. All other chemicals were obtained from commercial sources and were of analytical grade.

2.2 Sample preparation

The melon was rinsed gently with tap water by hand and dried naturally. Then they were peeled, cored and chopped for cubes (20 × 20 × 10 mm). The cubes were dipped into chitosan solutions and Chitosan+CaL_2 (0, Chitosan 10 g/kg, Chitosan+CaL_2 10 g/kg) for 1 min and air-dried for 15 min.

Uncoated {C}, coated with chitosan {Ch}, and coated with Chitosan+CaL_2 {CH + CaL} were packed by aluminium folio and stored refrigerated (4°C) for 8 d [5].

2.3 Methods

2.3.1 Physical methods

Texture (elasticity modulus): The elasticity modulus was measured on the fresh-cut melon cubes by Stable Micro Systems XT2Aplus texture analyzer, with Ø25 mm cylindrical measure probe and slowly deformation speed.

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Elasticity modulus were calculated based on the area of the ruptured cube-side.

Dielectric parameters: impedance of the fresh-cut melon cubes was investigated by GW IN STEK 8110G precision LCR meter in frequency range between 20Hz–10MHz with pin electrodes (gap 10 mm). Similar method and calculations were used earlier for monitoring the changing of the impedance during drying by Zsivanovits and Vozáry [8].

Visual parameters: For further analysis the fresh-cut melon cubes were captured by computer supported image analysing system. The browning of the fresh-cut melon cubes was monitored based on the pictures.

2.3.2 Sensory analysis

Sensory parameters were evaluated by hedonic ratings of sensory attributes. Seventh consumers joined to the panel every times.

2.3.3 Proximate composition

Chemical parameters were analyzed by standard methods for total soluble solids, total sugar content, titratable acidity and antioxidant activity.

2.3.4 Microbiological analysis

Microbiological parameters were analyzed by standard traditional methods based on ISO 4833:2003 standard for horizontal method for the enumeration of microorganisms – colony-count technique at 30°C for the Total Plate Count microorganisms (cfu/g) and ISO 21527-1 for the Total number of moulds (cfu/g) was used. Pathogens were analyzed based on ISO 4832-2006 (coliforms) and ISO 16649-2:2001 (*E. coli*).

3 Results and discussion

An undesirable consequence of cutting is softening; tissue stress results in a loss of firmness, principally owing to enzymatic hydrolysis of cell wall starch substances and the action of pectinolytic enzymes, the decrease in cellulose crystallinity and the thinning of cell walls [12]. The firmness in our experiments was characterized by the elasticity module in compression mode static experiments. The elasticity modulus of the control (melon cubes without chitosan coating) decreased significantly (about 60%) during the 8 d of storage (Fig. 1).

Coating with chitosan effectively retarded the tissue softening in the samples. The hardest samples at the end of the storage period were these coated with 10 g/kg chitosan solutions and CaL_2. Coating with 10 g/kg chitosan solution did not show significant effect. The results are in agreement with many previous reported research papers [5].

The color changes in the minimally processed melons examined on the basis of a comparison of the photographs taken during storage (optical sensor) are presented in Fig.1.

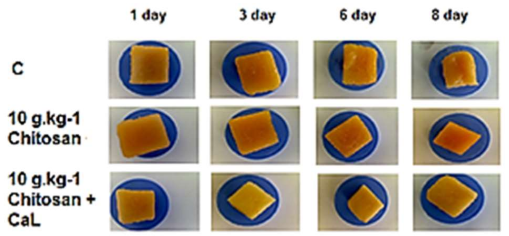

Fig. 1. Visual changes of chilled minimally processed melons during storage

A minor change in color in the direction of darkening was found for the coated samples. Darkening occurs most quickly in the control. Therefore, coatings prepared with chitosan and a combination of chitosan and calcium lactate can retain the color of the samples for 8 d.

The increase in the values of the red component of the color at a constant yellow fraction during storage of the melon samples determined the differences in the values of the color tone (Fig. 2) compared to the non-stored product and confirmed the darkening detected by an optical sensor. Browning was more slowly for coated melon cubes in comparison with the results reported in the literature [5, 6].

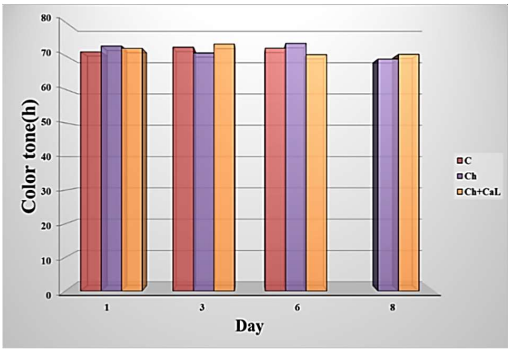

Fig. 2. Color tone change of chilled minimally processed melons during storage

The comparative analysis shows that the color tone of the samples with chitosan and calcium lactate embedded in the coating retains the color better, as their color tone at the end of storage has a value higher than that of melons with chitosan in the coatings (Fig. 3).

No changes in storage were observed in chitosan-coated melons. C values increase in untreated melons and in those with added calcium lactate in the coating.

The CIELAB (L*, a*, b*) parameters of the samples were examined by Colorgard 2000 type colorimeter on the 1, 3, 6 and 8 d. The color difference vector (ΔE^*), between the sample and the white etalon, was calculated for further statistical analysis [9]:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta ba^{*2} + \Delta b^{*2}} \quad (1)$$

The ΔE index also shows color differences depending on the applied coatings. Smaller differences in the values of the total color difference during storage for minimally treated melons with chitosan coating indicate smaller changes in their color (Fig. 4).

The firmness (texture) of the fruits was measured by a Stable Micro Systems, UK TA-XT2 plus texture analyzer. The stress-deformation curves were obtained by a cylindrical measure probe (d = 5 mm) at low deformation speed (0.1 mm/s). Elastic modulus was used for further analysis.

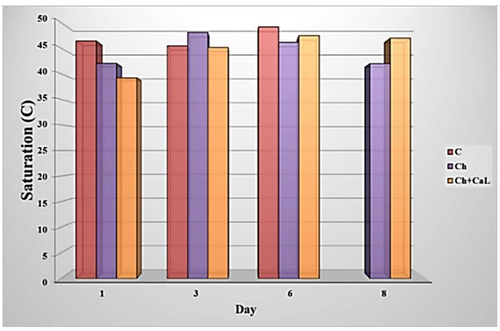

Fig. 3. Change in saturation of chilled minimally processed melons during storage

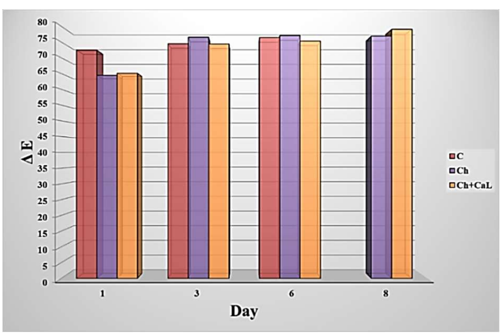

Fig. 4. Total color difference of chilled minimally processed melons during storage

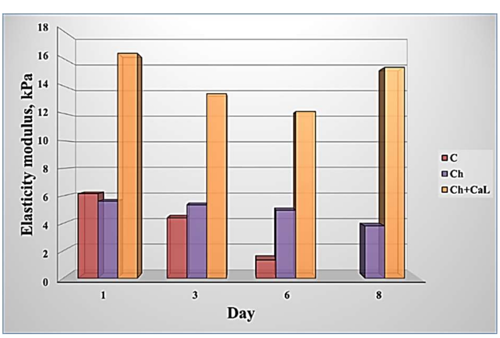

Fig. 5. Modulus of elasticity of chilled minimally processed melons during storage

The texture of the melon samples was determined by the modulus of elasticity in compression mode. The modulus of elasticity of the control (without chitosan coating) decreased significantly (about 80%) during the 6 d storage period (Fig. 5). During the same period, it decreased by about 35% in the pieces of melon with chitosan coating and about 18% in those with chitosan and calcium lactate coating.

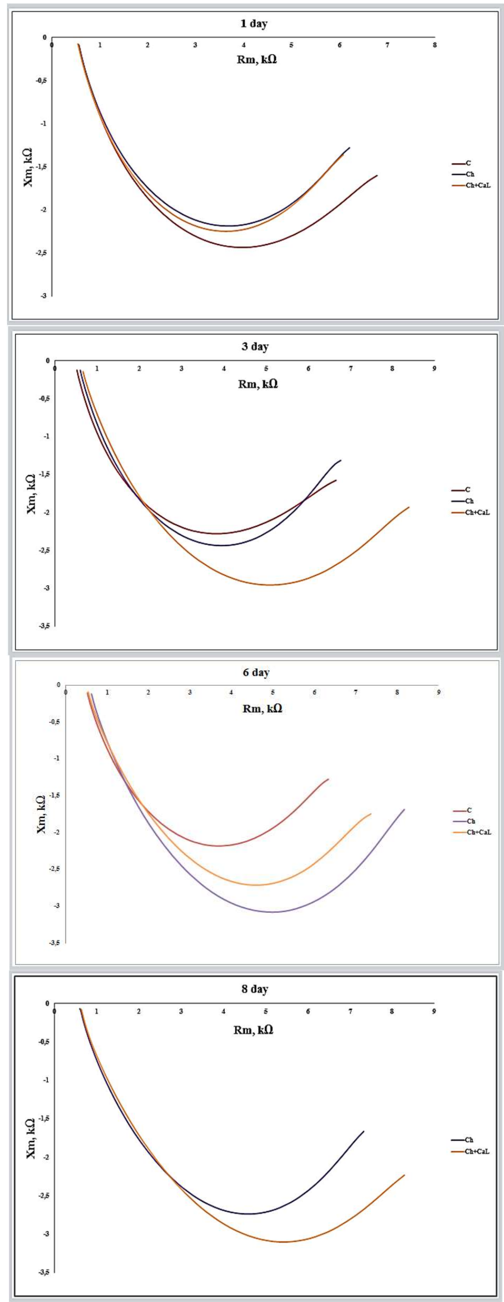

Fig. 6. Dielectric impedance curves of chilled minimally processed melons during storage

Samples with higher fruit hardness at the end of the storage period (8 d) of edible coated melons are those coated with a solution of chitosan and calcium lactate.

Dielectric parameters: On 10 pieces of a randomized sample for each series of minimally processed fruits during storage, the dielectric parameters were measured: impedance and phase angle with a precision LCR meter GW INSTEK 8110G in the frequency range between 20 Hz - 10 MHz with needle-shaped electrodes with a distance between them of 10 mm. The electrical impedance and the phase angle (ϴm) are measured, on the basis of which the real part (Rm) and the imaginary part (Xm) of the measured impedance are calculated:
The complex impedance Zm can be represented in the form:

$$Z_m = R_m + jX_m \tag{2}$$

where:
R_m is real part on Z_m

$$R_m = |Z_m| \cdot |\cos\theta_m| \tag{3}$$

and X_m is imaginer part on Z_m

$$X_m = |Z_m| \cdot |\sin\theta_m| \tag{4}$$

$$j = \sqrt{-1} \tag{5}$$

The experimental dependences, of |Zm| and ϴm of the frequency are averaged over the measurements of the ten pieces of fruit of the same series. Measurements were repeated during the storage period of the series.

On the first day, the impedance (Rm-Xm) curves are close to each other and the effect of the treatments on the impedance is not yet clear (Fig. 6). On the third day, the control has a smaller Xm maximum, which may be due to structural changes. Treatment with chitosan and calcium lactate shows a better preservative effect. On the sixth day, the differences in the effect of the applied coating are already observed: the control shows the largest structural changes and the effect of the added chitosan and calcium lactate is observed. The formation of chitosan film slows down the structural changes, but does not prevent them. Their effect could be due to changes in respiratory activity and a decrease in the activity of enzymes that convert insoluble pectin into its soluble form. The addition of chitosan with calcium lactate stops the destruction and preserves the structure due to the formation of a strong polymer-ion network. Calcium ions react with pectin in the cell walls, forming calcium pectate, which strengthens the cellular structure of the fruit [10, 11].

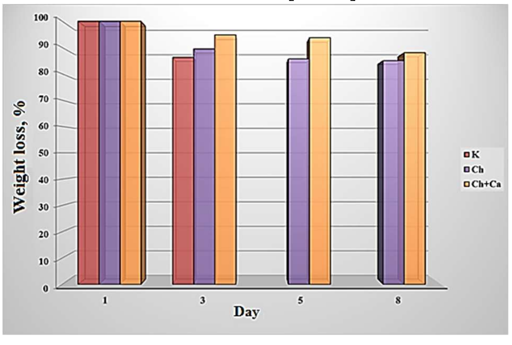

Fig. 7. Mass losses of chilled minimally processed melons during storage

There is a negligible effect of the coating on the weight loss. The highest loss was determined for the control sample, and the lowest for the samples treated with chitosan and calcium lactate. The difference between the coated samples was not significant (Fig. 7).

The results of the chemical analyses express the trend and dynamics in the changes that occur in the main chemical components of the minimally processed melons during storage.

Soluble solids (Brix) of the samples were measured by an Abbe type refractometer with temperature correction. For further analysis the sugar/acid ratio was calculated [9]:

$$\frac{\text{Sugar}}{\text{acid}}\, ratio = \frac{Brix.10}{\frac{g}{l}\text{acid}} \tag{5}$$

During the experimental period, a tendency to decrease in the content of soluble dry matter was established during storage. According to this indicator, the control shows instability and a greater decrease in soluble dry matter (4%) during storage (Fig. 8). The change in soluble dry matter in melons coated with chitosan is identical to those coated with chitosan and calcium lactate and is minimal (0.5%).

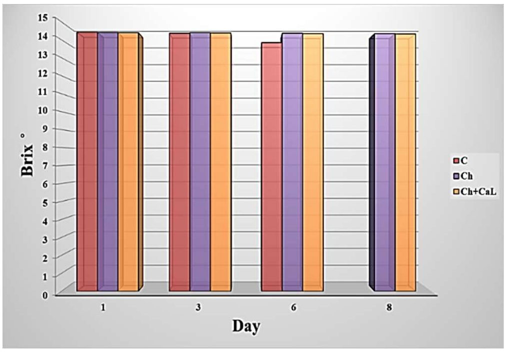

Fig. 8. Changes in soluble solids content in chilled minimally processed melons during storage

The change in total sugars in the storage process follows the trend outlined by the soluble dry matter in the coated minimally processed melons and the control sample (Fig. 9).

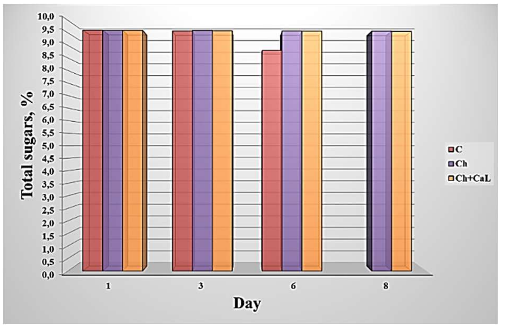

Fig. 9. Changes in total sugar content in chilled minimally processed melons during storage

The insignificant change from the beginning in the total sugars in the coated samples shows that the applied treatments reduce the respiratory activity. An exception to this trend is the control sample, for which a more significant decrease in the total sugar content was reported for a period of six days of storage. There is no difference between minimally treated melons with chitosan coating and with chitosan and calcium lactate coating.

Changes in the content of titratable organic acids during storage of melon samples show that they are consumed in the course of metabolic processes (Fig. 10).

A comparison of the results obtained for the titratable organic acids shows a more favourable effect of the coatings on delaying their reduction during storage.

For the samples coated with chitosan and chitosan and calcium lactate, a decrease in their consumption was observed, without a difference between the two coatings. A more active participation of titratable organic acids in the biochemical transformations in the control samples was established.

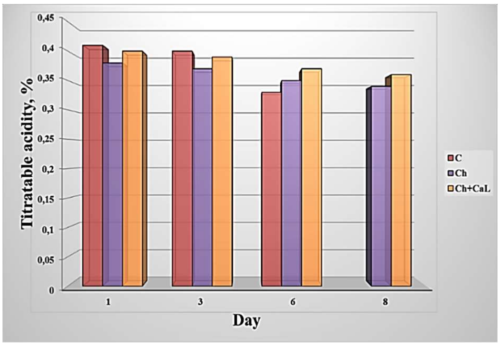

Fig. 10. Changes in the content of titratable organic acids in chilled minimally processed melons during storage

The more active consumption of titratable organic acids during storage of minimally processed melons is accompanied by an increase in the ratio of sugars to titratable organic acids (Fig. 11). The established more active participation of titratable organic acids in the biochemical transformations in the control is evident from the more significantly increased values of sugars to titratable organic acids at a shorter storage period.

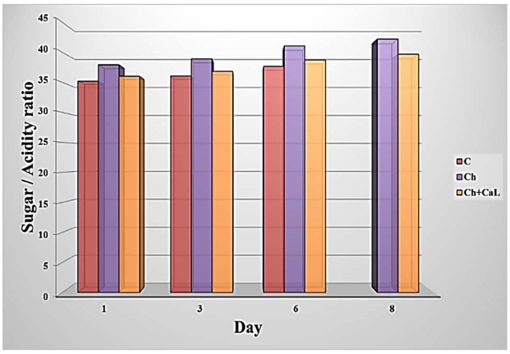

Fig. 11. Total sugar/titratable organic acid ratio of chilled minimally processed melons during storage

At the end of the period, edible coated melons retain their advantage over the control in terms of the amount of total sugars and titratable organic acids during storage, without taking into account the influence of the type of coating.

Studies on the carotenoid component determining the yellow color of ripe melons illustrate the amount in the individual samples and the dynamics of its change (Fig. 12). The graphical expression of the data shows a decrease in the content of β-carotene during storage.

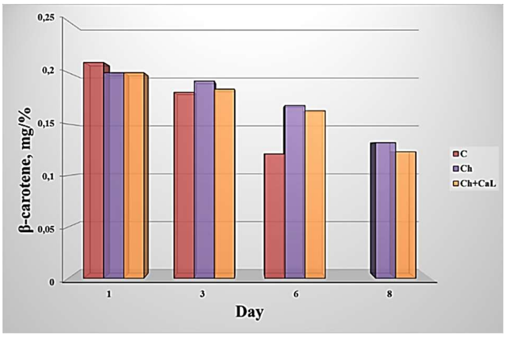

Fig. 12. Changes in β-carotene content in chilled minimally processed melons during storage

The changes in this indicator again show the beneficial effect of the coatings, due to the fact that minimally treated melons coated with chitosan and chitosan and calcium lactate have close to control values for the amount of β-carotene at prolonged exposure under storage conditions.

Extinction of the solution was measured in a 1cm cuvette against a petroleum ether blank at λ= 451 nm.

- β-carotene, mg/100g - spectrophotometric method - the method for determining total carotenoids gives the total content of the three isomers, from which β-carotene is then calculated according to the following eq. (6):

$$\beta\text{-carotene}=\frac{E.V1.V3}{0{,}25.V2.g}, \text{ mg/g} \tag{6}$$

E – measured extinction
V1 – initial volume of extract in ml
V2 – amount of extract to be chromatographed in ml
V3 – amount of eluate in ml
0.25 – conversion factor to ß-carotene
g – sample weight in g

After 8 d of storage, the losses of β-carotene for melons with both types of coating are about 36%, while already 40% for the control on the sixth day of storage.

The multiple comparison highlighted the minimally treated melons with the two coatings according to the studied chemical parameters (Table 1).

During storage of minimally processed melons, a statistical difference of soluble dry matter compared to their initial amount was proved. According to this indicator, the control shows instability during storage. As early as the third day of storage, the amount of soluble dry matter was the same as in the samples with chitosan coating on the eighth day and in those with chitosan and calcium lactate coating on the sixth day. There were no statistically significant differences between the soluble solids in the minimally treated melons with the two coatings.

The reduced consumption of total sugars in the coated samples leads to an indistinguishability from the control, identical to the reduction of soluble solids. There was no statistical difference in total sugar content between melons coated with chitosan and those coated with chitosan and calcium lactate compared to the initial amount and at the end of storage.

Table 1. Content of basic chemical components in chilled minimally processed melons without and with chitosan coatings*

Storage days	Soluble dry matter (%)	Total sugars (%)	Titratable organic acids (%)	Sugar / acid ratio	β-carotene mg%
C					
1	14.42a	9.61a	0.41a	23.44f	0.210a
3	14.37cd	9.58ab	0.40ab	23.95ef	0.181bcd
6	13.85f	8.81c	0.33g	26.70abc	0.121e
Ch					
1	14.41ab	9.61a	0.38bcd	25.29cde	0.200ab
3	14.40abc	9.60a	0.37cde	25.95bcd	0.192b
6	14.37cd	9.58ab	0.35efg	27.37ab	0.168cd
8	14.35de	9.57ab	0.34fg	28.15a	0.132e
Ch + CaL					
1	14.40abc	9.60ab	0.40ab	24.00ef	0.200ab
3	14.38bcd	9.59ab	0.39abc	24.59def	0.184bc
6	14.35de	9.57ab	0.37cde	25.86bcd	0.163d
8	14.33e	9.55b	0.36def	26.53abc	0.123e

* Different letters after the values within one column show statistically significant differences (Tukey's test, $p<0.05$)

In the amount of organic acids, a statistical difference was found in the control and edible-coated melons at the end of their storage compared to the beginning. However, no statistical difference was found in the amount of organic acids between the control on the sixth day of storage and after two more days of the coated samples, with minimal advantage of the chitosan and calcium lactate coating.

The changes in the sugar-acid complex respectively led to an increase in the sugar-acid coefficient for each treatment, due to the significant reduction in the amount of organic acids. The obtained results show that at the end of the period the melons with coatings retain their advantage in terms of the amount of total sugars and organic acids. This can be explained by a more even consumption of substrates (sugars and acids) during storage. It was found that in the ratio of total sugars and organic acids throughout the storage period, regardless of the applied coating, there is no statistical difference.

The decrease in the amount of β-carotene was more intense in the control sample. In melons coated with chitosan and calcium lactate, the reduction on the eighth day was statistically indistinguishable from the content in the control on the sixth day. In the samples treated with chitosan and chitosan with calcium lactate, no significant difference was found between them and at the end of their storage.

For the overall acceptability of minimally treated melons without and with chitosan coating during storage, a positive correlation was found with the analyzed soluble dry matter, total sugars and organic acids and a negative correlation for sugars/acids ratio (Table 2).

The overall sensory score is highly correlated ($r > 0.700$) with soluble solids, total sugars ($p < 0.01$) and organic acids ($p < 0.05$) during storage control. Chitosan coating significantly affected ($p < 0.05$) the dependence total sensory evaluation-total sugars and organic acids during storage of melons with high correlation coefficients, respectively $r = 0.920$ and $r = 0.962$.

The high correlation between the total sensory evaluation and the soluble solids indicates a link between the total sugars, titratable organic acids and other components entering the soluble solids in terms of the overall acceptability of the quality of the minimum processed melons during storage. For melons coated with chitosan and calcium lactate, there is a relationship, but it is statistically significant between the overall sensory evaluation and organic acids.

The data from the analysis indicators determining the microbiological quality of the product are presented in Table 3.

Table 3. Conditionally pathogenic and pathogenic microorganisms in chilled minimally processed melons without and with edible coating

Product Indicator	Day	C	Ch	Ch+CaL
L. monocytogenes cfu/25g (it is not allowed)	1	n. e.	n. e.	n. e.
	3	n. e.	n. e.	n. e.
	5	n. e.	n. e.	n. e.
	7	n. e.	n. e.	n. e.
Salmonella cfu/25g (it is not allowed)	1	n. e.	n. e.	n. e.
	3	n. e.	n. e.	n. e.
	5	n. e.	n. e.	n. e.
	7	n. e.	n. e.	n. e.
Coagulase positive staphylococci cfu (10-100)	1	< 1	< 1	< 1
	3	< 1	< 1	< 1
	5	< 1	< 1	< 1
	7	-	< 1	< 1
Coliforms cfu/g (100 - 1000)	1	< 10	< 10	< 10
	3	< 10	< 10	< 10
	5	< 10	< 10	< 10
	7	-	< 10	< 10
E. coli cfu/g (100 - 1000)	1	< 10	< 1	< 1
	3	< 10	< 1	< 1
	5	< 10	< 1	< 1

From the microbiological testing of the product, it was found that the main group of microorganisms causing spoilage in the product are yeasts. In the tested samples, the concentration of yeast cells was recorded in the order of 11.10^3 in melon pieces with an edible film of chitosan and lactate to 65.10^3 cfu/g in untreated melon pieces in Table 4. The microbiological analysis for mold detection did not report a high concentration of mold to cause spoilage of the products.

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Table 2. Correlations between sensory evaluation and chemical parameters of chilled minimally processed melons

		Control					Chitosan					Chitosan + Calcium lactate				
		Dry matter (Bx)	Total sugars	Titratable organic acids	S/A ratio	General sensory evaluation	Dry matter (Bx)	Total sugars	Titratable organic acids	S/A ratio	General sensory evaluation	Dry matter (Bx)	Total sugars	Titratable organic acids	S/A ratio	General sensory
Control	Dry matter (Re)	•														
	Total sugars	0.999**	•													
	Titratable organic acids	0.986*	0.983*	•												
	S/A ratio	-0.967*	-0.960*	-0.994**	•											
	General sensory evaluation	0.998**	0.999**	0.979*	-0.953*	•										
Chitosan	Dry matter (Re)	0.966*	0.956*	0.990**	-0.998**	0.949	•									
	Total sugars	0.919	0.905	0.963*	-0.986*	0.894	0.990*	•								
	Titratable organic acids	0.963*	0.951*	0.978*	-0.986*	0.943	0.994**	0.984*	•							
	S/A ratio	-0.957*	-0.943	-0.969*	0.978*	-0.935	-0.989*	-0.980*	-0.999**	•						
	General sensory evaluation	0.999**	0.999**	0.988*	-0.968*	0.998**	0.966*	0.920*	0.962*	-0.955*	•					
Chitosan + Calcium lactate	Dry matter (Re)	0.823	0.821	0.727	-0.682	0.825	0.703	0.624	0.751	-0.764	0.815	•				
	Total sugars	0.859	0.838	0.905	-0.941	0.823	0.956*	0.983*	0.965*	-0.968*	0.857	0.608	•			
	Titratable organic acids	0.919	0.905	0.963*	-0.986*	0.894	0.990*	0.999**	0.984*	-0.980*	0.920	0.624	0.983*	•		
	S/A ratio	-0.922	-0.908	-0.961*	0.984*	-0.897	-0.990**	-0.999**	-0.989*	0.986*	-0.922	-0.647	-0.987*	-0.999**	•	
	General sensory evaluation	0.993**	0.988*	0.996**	-0.989*	0.984*	0.989*	0.959*	0.987*	-0.981*	0.993**	0.781	0.911	0.959*	-0.961*	•

The results of the test of the according the indicator of total number of microorganisms show an increase in the number of mesophilic microorganisms during the storage period. The higher level of microbial contamination of melon products is due to the fact that microorganisms have direct contact with the carbohydrates and other biologically valuable substances of the cell due to the cutting of the product. It provides a larger contact surface and access to oxygen for microorganisms. At the beginning of storage in the minimally processed melons, the amount of microorganisms reached 450 cfu/g, and on the fifth day of their cold storage they reached an amount of 32.10^6 cfu/g. When using edible coatings of chitosan and chitosan with calcium lactate, a slower increase in the amount of mesophilic microorganisms was observed.

On the seventh day of storage of the samples of melons with edible coatings of Ch and ChL a total number of microorganisms above 10^5 cfu/g was reported, which is already an indication of deteriorated microbiological quality and creation of conditions for microbial spoilage.

Table 4. Indicator microorganisms in chilled minimally processed melons without and with edible chitosan coating

Product Indicator	Day	C	Ch	Ch+CaL
Total number of microorganisms cfu/g	1	450	7	12
	3	95.10^3	32.10^3	13.10^2
	5	32.10^6	90.10^3	2.10^3
	7	-	76.10^5	58.10^5
Molds cfu/g	1	<10	<10	<10
	3	15	10	10
	5	26	15	10
	7	-	-	17
Yeast cfu/g	1	130	< 10	<10
	3	12.10^2	15.10^1	17
	5	65.10^3	58.10^2	25.10^2
	7	-	35.10^3	11.10^3

The sensory parameters were evaluated by consumer tests with 7 participants attending. The assessors were first asked to rank three coded samples (melon cubes) by color from the most preferred (= 6) to least preferred (= 1) and then for each one to evaluate the appearance, color, texture, flavor and sweetness on a 9-points hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). The tests were repeated later during the storage as well [12].

During the first day of the minimally processed melons (Fig. 13), the three samples were rated high in sensory attributes appearance, color, firmness, fruity taste, aroma, sweetness, juiciness, astringent taste and its intensity ($x \geq 7.0$). The control and coated samples were perceived as slightly liked ($x \geq 6.0$) for sweetness intensity, juiciness intensity, aftertaste intensity, with minimal preference for the control and chitosan-coated sample.

In the storage period, the quality of the control was greatly compromised, the ratings decreased, and on the sixth day it was rated from moderate to very disliked ($2.3 \leq x \leq 3.1$). A higher rating is reserved only for the astringent taste and its intensity.

At the end of storage with markedly better complex performance were those treated with edible coatings, with tasters giving a higher rating for most sensory attributes to the minimally processed melons coated with chitosan and calcium lactate, except for astringency intensity, juiciness intensity, aftertaste intensity. However, these ratings remain moderately liked.

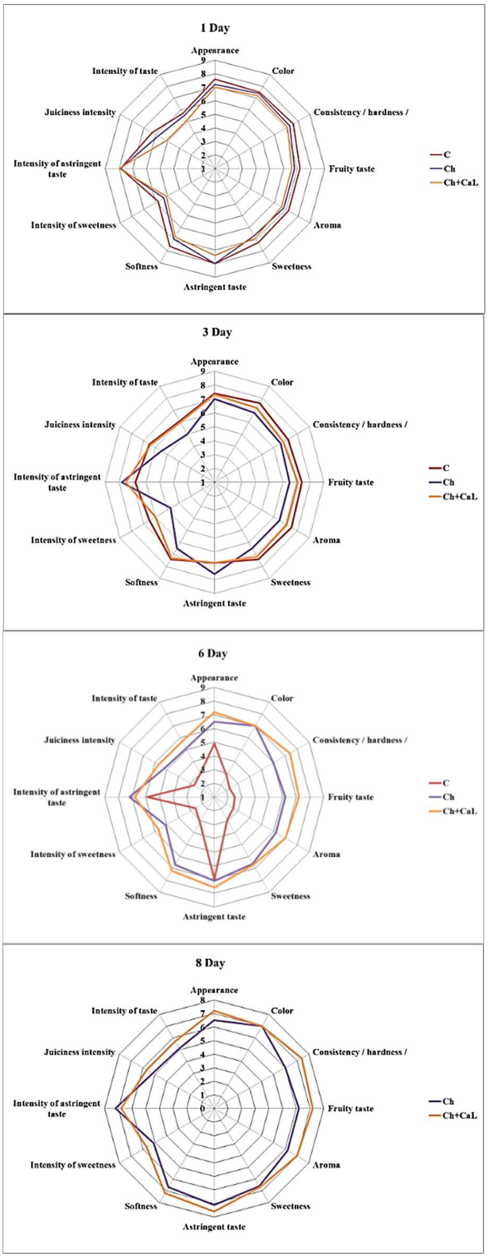

Fig. 13. Sensory parameters

4 Conclusion

In the present study the effect of different Ch based edible coatings on the postharvest parameters of fresh-cut melon fruits were investigated. It was demonstrated that low molecular weight Ch coatings improve all the investigated parameters. The addition of CaL_2 showed beneficial effect on weight loss, Brix, TA and texture of the melons and preserved the cellular structure of the parenchyma tissue of the melon fruit. The bacteriostatic activity of Ch decreased, but the coatings still can supply antibacterial protection against bacteria, yeasts and molds. Edible coating of 1% Ch and 1% CaL is an alternative for storing minimally processed melons, such as quality characteristics for a longer period, good consumer acceptance, and met the requirement of shelf life extension.

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