POSTHARVEST QUALITY AND SAFETY OF FRESH-CUT MELON FRUITS COATED WITH WATER SOLUBLE CHITOSAN FILMS

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Abstract The research presents the effect of novel edible coatings based on low molecular weight chitosan on some properties of fresh-cut melon fruits – weight loss, total soluble solids, total acidity, mechanical strength and bacteria growths. Three different compositions were used as coatings – pure chitosan, chitosan and Ca lactate and alginate/chitosan multilayers. It was sown that the additional alginate layer substantially improves the protective properties of pure chitosan coating, resulting in preservation of cell structure. Negligible negative effect on the antibacterial activity of pure chitosan is demonstrated.

Key words*: *Melo sativus fruits, edible films, chitosan, alginate, cell structure, texture, antibacterial activity, weight loss, sugar/acid ratio

Introduction

In the recent years a growing interest to fresh-cut fruit and vegetables has been observed, which is mostly due to their advantages such as freshness, low caloric content, commodity to be used and an active promotion of fruits and vegetables as basic components of a healthy diet (Raybaudi-Massilia et al., 2008). As consumers become aware of this importance in their eating habits and have less time for food preparation, the production of fresh-cut fruits is increasingly more relevant from the food processor perspective (Olivas & Barbosa-Canovas, 2005).

Fresh-cut melons are among the most commercially important fresh-cut fruit products representing about 22 % of the market (Cook, 2014). Melons are popular with consumers because of their unique flavor and nutritional value. They are naturally low in fat and sodium, have no cholesterol, and provide many essential nutrients such as potassium, vitamin A, and vitamin C. Melons were recommended as essential diet ingredient to ensure adequate nutrition, promote individual health, and reduce one's risk of chronic diseases (Lester, 1997).

Despite their advantages, minimally processed products are more perishable due to tissue injuries during peeling, slicing, and cutting operations. Wounding of fruit tissue induces a number of physiological disorders that need to be minimized in order to obtain fresh-cut products with high quality and nutritional value. The intensification of metabolic activity results in the increase of respiration rate and ethylene production, which accelerates the senescence process, promoting changes in fruit quality parameters and reducing product shelf life (Soliva-Fortuny & Martín-Belloso, 2003).

One of the most promising methods to increase the shelf-life of fresh-cut melons is application of edible coatings. Edible coatings protect food products from mechanical, physical, chemical, and

microbial damage and can extend their shelf life (Baldwin et al., 2011). They attract much interest and practical research since they are based on natural, biodegradable, and edible components that satisfy environmental concerns and respond to customer demands for safe and healthy food (Han & Gennadios, 2005). To be of practical application, edible coating needs to have perfect adhesion abilities, highly effective microbial protection, appropriate gas and moisture exchange properties, a good esthetic appearance, and to be totally tasteless, all with a reasonable cost.

An interesting biopolymer used in edible coatings is chitosan. Chitosan is a linear polysaccharide consisting of β-(1→4)-linked 2-amino-2- deoxy-D-glucose residues, originating from deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature after cellulose (Coma et al., 2002). It is non-toxic, biodegradable, biofunctional, and biocompatible (Coma et al., 2002). Because of its positive charge, chitosan has strong antimicrobial and antifungal activities that could effectively control fruit decay (Dutta, 2012). It possesses very good adhesive abilities and can easily form coating on fruit and vegetable, and the respiration rate of fruit and vegetable was reduced by adjusting the permeability of carbon dioxide and oxygen (Casariego, 2007)].

There are already some attempt for application of chitosan coatings on fresh-cut melons (Zsivanovits at al., 2012; Poverenov, 2014; Chong, 2015). It was found that the chitosan coatings caused to decrease F. oxysporum growth and delayed changes in their external color (Reyhan, 2013). Typically chitosan is combined with some other substances like calcium ions to strengthen the fruit cell wall and increase the effect of preservation. Poverenov and coauthor introduced for the first time alginate-chitosan bilayers and demonstrated grate effect in conservation of fresh-cut melon quality (Poverenov, 2014).

A major disadvantage of the acidic soluble, high molecular weight chitosan coatings is their poor acceptance by consumers because of the acidic aftertaste due to the acids used for the chitosan solutions preparation (Zhelyazkov et al., 2014). Based on our knowledge there are no attempt to use water soluble, low molecular weight chitosan as edible coatings. Therefore the aim of the present research is investigation the effect of edible coatings based on water-soluble chitosan on the quality of fresh-cut melon fruits.

Materials and Methods

Materials: The fruits of ***Melo sativus*** **variety "honeydew"** were bought from a local marketplace in fully matured stage based on colour and outside appearance, without physical and microbiological damages. Fruits were washed with tap water and then manually peeled and cut into 20-mm cubes with a sharp knife. All the utensils used in the processing were previously sanitized with alcohol solution.

Materials for coating solution: The edible coatings were prepared with three different solutions: 1g/100 ml water soluble low molecular weight chitosan in distilled water (Ch); 1g/100 ml water soluble low molecular chitosan and 1g/100 ml calcium lactate in distilled water (Ch + Ca); the third edible coating contained two layers, prepared by 1g/100 ml solution of sodium alginate in distilled water and 1g/100 ml solution of water soluble low molecular weight chitosan in distilled water (Ch +Al). All the solutions were prepared by stirring the polymers with magnetic stirrer overnight until they were completely dissolved.

Manufacture of coatings: The fruits, coated with chitosan or chitosan and calcium lactate were immersed into the corresponding solution for two minutes and air dried at room temperature (25°C) for 30 minutes. After that they were placed into plastic containers (10 pieces into each) with punched cups and stored at temperature 4±1°C.

The layer-by-layer polyelectrolyte deposition process was used to prepare double-layer coating. First the melon cubes were dipped into alginate solution for two minutes, then they were dried at room temperature for 60 minutes, and finally were immersed in chitosan solution for another two minutes. After second drying the fruits were packed into plastic containers and stored temperature 4±1°C and humidity (RH% 60±5). These settings were controlled by datalogger in every minute.

Storage quality evaluation: *Weight Loss*. 10 melon cubes were weighed after the processing (day 1) and along storage (at days 5, 8, 11, 15 and 18). The results were expressed as percentage loss of initial weight.

pH measurement: The pH of the homogenized samples was determined by Microsyst MS2011 portable pH-meter, with temperature compensator.

Total acidity **(**TA) was examined with a slowly titration the NaOH into the juice/water solution towards the end point of the titration up to reach pH 8.1. The result is expressed in g/litre: (OECD Standards, 2005):

$$\frac{g}{l}\ acid = \frac{Titre \cdot Acid\ factor \cdot 100 \cdot 10}{10\ (ml\ juice)} \quad (1)$$

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 (OECD Standards, 2005):

$$\frac{Sugar}{acid} ratio = \frac{Brix \cdot 10}{\frac{g}{l}\ acid} \quad (2)$$

Firmness: 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=25 mm) at low deformation speed (0.1 mm/s). Elastic modulus (slope of the first linear section) was used for further analysis.

Structural Changes: The structural changes of melon tissue were evaluated through light microscopy analysis at days 1, 5, 8, and 14. Samples were examined using light microscope Magnum T, trinokular, equipped with camera Si 3000, 3 megapixel USB 2.0. For each treatment, two samples from different fruits were used for the microscopic evaluation.

Microbiologycal Analysis: Samples were analyzed in respect to aerobic bacteria, yeast and mold growth, coliforms, Escherichia coli and Salmonella spp. Viable counts were expressed as colony-forming units (CFU) per gram of fruits. The samples were serially diluted in 0.9% NaCl and aerobic plate counts were determined by surface inoculation of plate count agar (Oxoid, UK). The plates were incubated at 30ºC for 48 h. Mold and yeast counts were determined by surface inoculation of Sabouraudo dextrose agar supplemented with chloramphenicol (Oxoid, UK). The plates were incubated at 25ºC for 5 days. Total coliforms and *E. coli* were determined according to ISO 4832:2006 and ISO 16649:2001. Salmonella spp were determined according to ISO 6579:2002.

Statistical Analysis: The results were statistically evaluated by analysis of variance, using the software Statgraph, in order to determine significant differences among the samples. Mean separation was performed with the Multiple Range Test at $p \leq 0.05$.

Results and discussion

Weight loss. (Fig. 1.) The increase of weight loss is predominantly due to water loss that resulted from surface water evaporation respiration and transpiration. No substantial differences could be observed in the non-treated and treated samples up to 8th day of storage. At the end of storage period (after the 15th day) samples, coated with Chitosan + Ca lactate and multilayers from Chitosan and Alginate show slower increase of the weight loss. According to (Ferrary et al., 2013) Ca salts maintaining the strength of the cell wall due to formation of ionic binding between the Ca cations and pectin from the cell wall. It is well known that chitosan and alginate interact to form of water insoluble polyelectrolyte complexes (Tapia, 2004). The appearance of cross-linked pectin network or insoluble coating delays the process of water evaporation and reduces the weight loss.

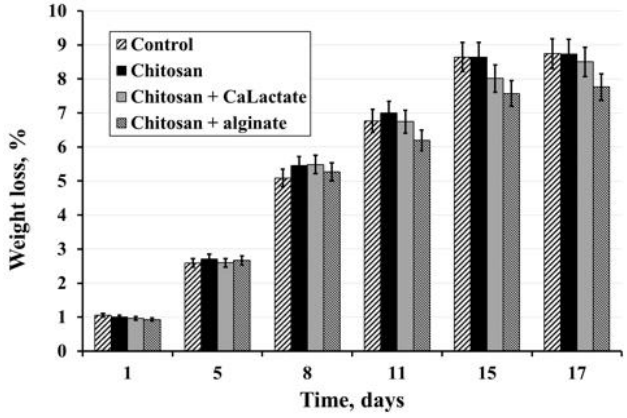

Fig. 1. Weight loss of fresh-cut melon during storage at 4 °C

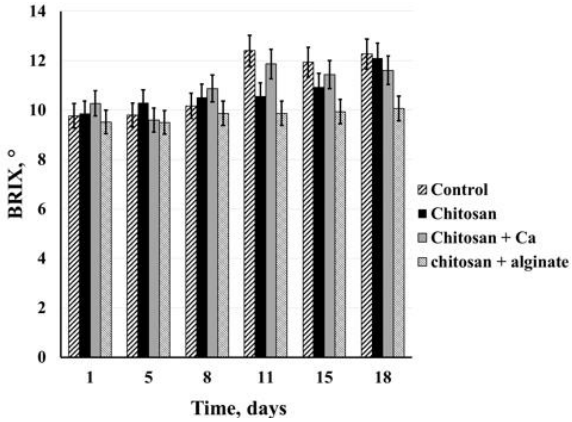

Fig. 2. BRIX of fresh-cut melon cubes during storage at 4 °C

Total soluble solids (BRIX). (Fig. 2.) Brix values of all the samples increased during storage period. This fact could be explained by a rise in the sugar concentration due to dehydration observed during storage. The control presented the highest Brix values and Chitosan + Alginate the lowest. The coating led to a lower increase in the Brix, which indicates that the control fruit presented a more obvious ripening development than the coated melons, and this could be related to the higher respiration rate observed in the uncoated samples. In addition, the process of coating could modify the internal atmosphere, showing similar effects to fresh-cut melons under modified atmosphere packaging conditions. These results agree with the findings of Martinez-Romero et al. (2006), and Aday and Caner (2010).

Total acidity (TA) and sugar/acidity ratio. TA decreased during the storage period for all investigated samples – Fig. 3. TA of the coated melon cubes was always higher than the average TA of the uncoated ones. The TA of melons coated with chitosan and chitosan/alginate was significantly higher than that of non-treated samples and these coated with chitosan+ Ca lactate. No statistical differences were determined between the values of TA during the storage period for

all coated samples. Therefore it could be assumed that the edible coatings act as a barrier and reduces the use of organic acids in respiration and mass transfer.

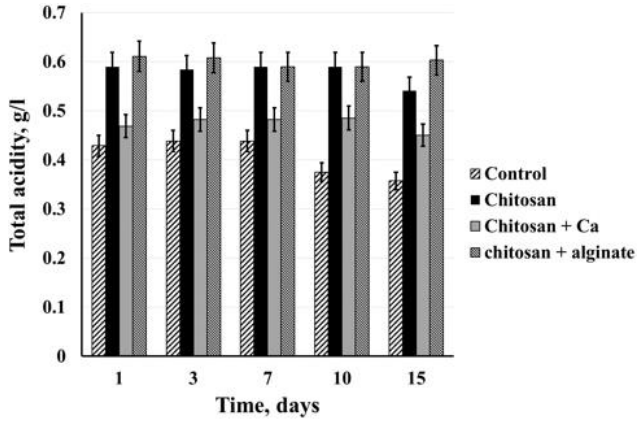

Fig. 3. Total acidity of fresh-cut melon cubes during storage at 4 °C

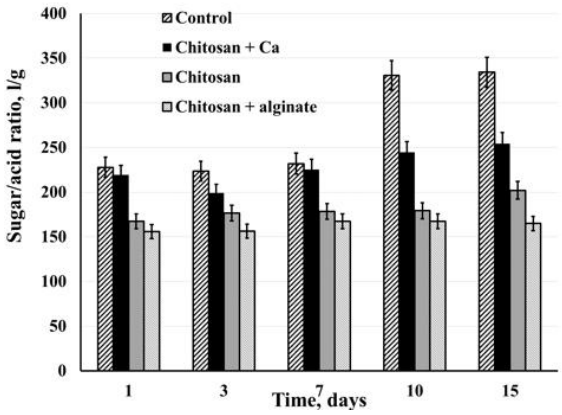

Fig. 4. Sugar/acid ratio of fresh-cut melon cubes during storage at 4 °C

Figure 4 shows the sugar/acidity ratio plotted as a function of storage time for the coated and uncoated samples. During the storage sugar content increased while TA decreased, resulting in an increase in sugar/acidity ratio.

Mechanical strength. The strength (Fig. 5) of the fresh-cut melon was characterized based on the values of normalized (based on the value for the firth day) elastic modulus during compression test. During 18 days of storage the elastic modulus of non-treated samples decreased dramatically to 40% of its initial value. Vary similar are the results in case of pure chitosan coating – the elastic modulus on the 18^{th} day is only 42% from the initial. Therefore it could be concluded that after the 15^{th} day of storage chitosan coating had no more beneficial effect on product texture. Considerably higher are the moduli in case of coatings chitosan + Ca and chitosan/alginate - 53 and 73 % respectively. Our results are in good agreement with previously reported data, based on which edible coatings physically enhance the structure of fresh-cut fruits and slow down their texture degradation (Baldwin et al. 2011). They also confirm the synergistic effect of chitosan/alginate multilayers, observed by Poverenov et al (2014). There are several hypothesis explaining the strengthening effect of edible coatings. Based on Chen et al. (2002) the bacteriostatic effect of chitosan based films could inhibit the production of microbial hydrolytic enzymes affecting the cell wall integrity of fresh-cut products. During the storage enzymatic hydrolysis of cell wall pectic substances and the action of pectinolytic enzymes decrease the cellulose crystallinity and the thinning of cell walls. The cross-linking of Ca ions from the coating with the pectin in the cell wall delays the process of cell wall degradation and therefore the chitosan Ca coatings retain the texture for a longer time (Qi, 2011). The best texture preservation is demonstrated from chitosan/alginate multilayers. One of the reasons for that might be that the combined LbL coating had advantages of both coating materials when internal alginate layer provided perfect adhesion and the external chitosan layer mitigated structure degradation caused by microbial enzymes (Poverenov, 2014). Another reason could be formation of hydrophobic polyelectrolyte complex, which delay the degeneration processes in the fruits.

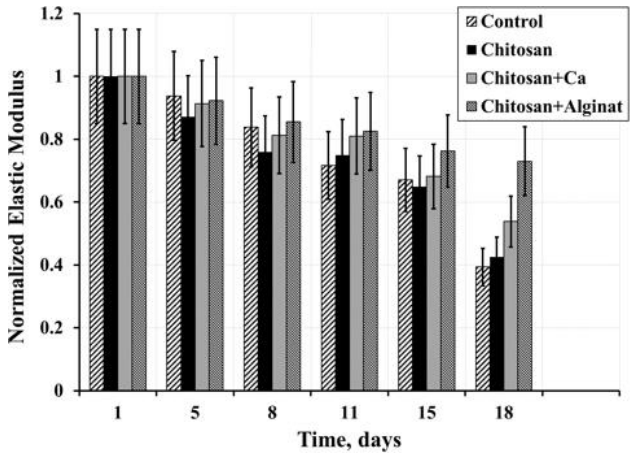

Fig. 5. Mechanical strength of fresh-cut melon cubes during storage at 4 °C

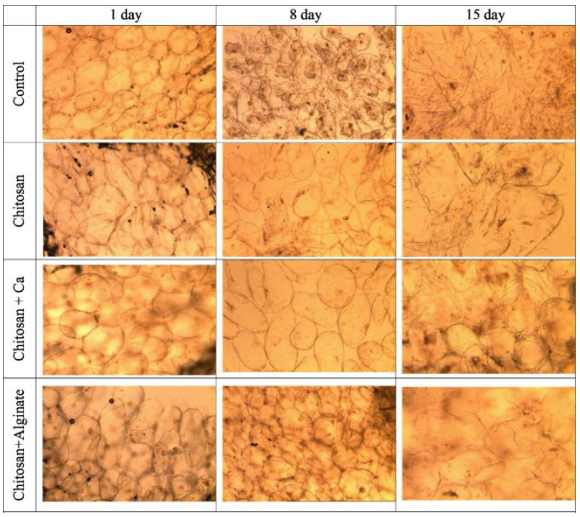

Fig. 6. Micrographs of melon fruit parenchyma tissue during storage at 4 °C

Microscopic investigation. Further investigations of structural changes of fresh-cut melon cubes have been made by the use of microscopic measurement (Fig. 6)

At the 1st day, all the samples showed similar characteristics, that is, turgid and round-shaped cells with a well-defined and thin cell wall. At the 8th day, the control fruit showed dehydration and maceration, the cells are separated and isolated, as well as cell wall are damaged. The samples coated with pure chitosan were soft and its cells are macerated. For the samples, coated with chitosan and Ca lactate or chitosan+alginate the cell structure is relatively well preserved and the parenchymatous tissue is complete. After 14 days of storage, structural alterations in the parenchyma tissue are observed for all samples – cell wall damage was more intense, cells were deformed, contracted, and collapsed. Some crystals were accumulated. The most preserved tissues were observed for these samples, which were coated with chitosan and Ca lactate or chitosan+alginate. The presence of Ca bound the cellular pectin kept the cell structure for longer time. The tissue structural changes could be related to higher weight loss during storage (Fig. 1), causing loss of cell turgor pressure.

Microbial analysis. Microbial analysis of the fresh-cut melons was done based on total aerobic count, yeasts and molds and some pathogens like *Salmonella* spp., *Escherichia coli* and Coliforms. *Salmonella* ssp. was not found in both treated and non-treated samples, while total coliform and *Escherichia coli* were below the detection limit of the method (<10 CFU g^{-1}). These results indicate that the hygienic practices and the sanitization process were effective (Table 1).

Total aerobic counts on the surface of all melon fruit samples (non-treated and coated) steadily increased during storage. The faster growth is observed for non-treated melon cubes and for all days statistical differences were verified. In the presence of chitosan coating alone there was an initial reduction in total counts of about two times (Table 1) within the initial day of the experiment. This difference remained constant during the whole storage time.

Table 1. Effect of chitosan based coatings on total aerobic counts (log CFU g^{-1}) and on yeasts and molds (log CFU g^{-1}) of fresh-cut melons during storage at 4 °C

	1st day		5th day		10th day		15th day	
Treatment	Aerobic	Yeast	Aerobic	Yeast	Aerobic	Yeast	Aerobic	Yeast
Ch	220[a]	190[a]	5400[a]	820[a]	43666[a]	22000[a]	1.60E+06[a]	137000[a]
ChCa	290[b]	210[a]	7100[ab]	920[a]	58000[ab]	24700[b]	2.13E+06[b]	154000[a]
ChAl	300[b]	215[a]	7333[b]	950[a]	59666[b]	25300[b]	2.20E+06[b]	158000[a]
Control	470[c]	340[b]	11666[c]	1500[b]	95033[c]	40000[c]	3.50E+06[c]	250000[b]

Statistical difference from the other coatings presents in all measurements. Chitosan/Ca and alginate/chitosan coatings also demonstrated antimicrobial activities, but they insure less protection than the chitosan alone.

The effects of coatings on mold and yeast growth (Table 1) were similar to the described above for total aerobic counts. No statistical differences were observed between the different coatings and all of them reduced the yeasts and molds in two times in comparison of the control samples.

The antimicrobial effect of chitosan is well known and discussed by many authors. Several mechanisms were proposed for its activity. In one mechanism, the polycationic nature of chitosan interferes with the negatively charged residues of macromolecules at the surface. Chitosan interacts with the membrane of the cell to alter cell permeability. The other mechanism involves the binding of chitosan with DNA to inhibit RNA synthesis (Xiao, F.L. et al., 2001).

Based on our results one could assume that the addition of Ca lactate and alginate did not significantly affect the antibacterial properties of chitosan coatings.

Conclusion

In the present study the effect of different chitosan based edible coatings on the postharvest parameters of fresh-cut melon fruits were investigated. It was demonstrated that low molecular weight chitosan coatings improve all the investigated parameters. The addition of Ca lactate and alginate showed beneficial effect on weight loss, TSS, TA and texture of the melons and preserved the cellular structure of the parenchyma tissue of the melon fruit. In some way it decreased the bacteriostatic activity of chitosan, but the coatings still can supply antibacterial protection against bacteria, yeasts and molds.

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