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Microbiological parameters during storage of minimally processed melons with and without edible coating

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The aim of the study was to analyze the microbiological parameters of minimally processed melons with and without edible coating during storage in the refrigerated state and to determine their shelf life. The use of chitosan coating with solutions of diferent concentrations on peeled and sliced melons stored at 4±1 °C was studied. It was found that all edible chitosan coatings inhibit the growth of microorganisms. We used a variant of edible coating of 1% chitosan and 1% calcium lactate as an alternative to prolong the shelf life of minimally processed melons according to their quality characteristics. The storage period was determined according to the analyzed microbiological indicators - 7 days for minimally processed melons with the edible coating compared to 5 days for the control.

Keywords: melon, edible coatings, chitosan, microorganisms

INTRODUCTION

Minimally processed fruits

Fresh-cut fruits and vegetables are defined as fresh fruits or vegetables, or any combination thereof, which has undergone physical changes from their original form but without treatment with heat, negative temperatures or chemical preservatives and remain fresh [1, 2]. Freshly chopped plant products include peeled, cleaned, washed, core-cut, sliced but still raw fruits and vegetables [3, 4]. They are categorized as ready-to-use, partially processed or minimally processed products [5-7].

Changes in whole and minimally processed plant products

By minimal processing of fruits and vegetables are obtained products convenient to use, but their shelf life is reduced. A current challenge for the rapidly expanding sector of minimally processed foods is to ensure their safety and quality [8].

Minimally processed products are very perishable, the main reasons for this are the removal of the bark (the natural protective layer) and the physical stress they go through during the processes of peeling, slicing, etc. [7-9]. As a result of the mechanical effects described above, the synthesis of ethylene, the water activity of the surface, the weight loss and the rate of respiration are increased [7-10]. These changes also lead to cell wall damage (which leads to enzymatic side effects), loss of cellular components, loss of moisture [10], which is the reason for the reduction of their shelf life [3, 4, 7, 8, 10, 11]. If these changes are not controlled, they can lead to rapid deterioration and aging of the product [10]. Therefore, minimally processed products should be stored at temperatures below those recommended for whole fruits and vegetables [7]. But even during refrigerated storage, fresh fruits and vegetables are characterized by active metabolism [12, 13] reports that some of the factors that affect the intensity of decay are the type of plant product, the variety, the degree of maturity, the temperature, the concentration of oxygen and carbon dioxide, and the water vapor pressure. Studies of all these parameters are necessary to ensure that healthy and high-quality products are offered to consumers [7].

The minimum processing of fresh fruit aims to preserve the freshness of the fruit, with minimal loss of nutritional quality and to ensure a shelf life sufficient to allow distribution in the region of consumption. The microbiological, sensory and nutritional shelf life of the minimally processed fruit should be at least 4-7 days, depending on the market. During peeling and other technological operations of minimal processing, many of the cells of the product are disrupted, resulting in oxidative processes. The quality of minimally processed products deteriorates due to physiological aging, biochemical changes, and microbial spoilage, which can lead to degradation of color, texture, and aroma [14, 15].

Microbiological changes

As minimally processed fruits are not subjected to heat treatment, regardless of the presence of preservatives or the type of packaging, they have to be processed and stored at temperatures of 5 °C or

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lower in order to achieve an optimally long shelf life and microbiological safety. Some pathogenic microorganisms that can be transmitted by plant products, such as *Escherichia coli* O157: H7, *Listeria monocytogenes, Yersinia enterocolitica, Salmonella spp.* and *Aeromonas hydrophila* can survive and thrive at the said low temperatures [16, 17].

Most of the microorganisms that are in the soil and air get into the fruit by insects, animals and humans but are not able to grow at pH that is typical for fruits. These conditions are favorable only for the growth and development of molds, yeasts and some lactic acid bacteria. Therefore, with few exceptions, the representatives of these three groups of microorganisms are the dominant microflora, causing the spoilage of fresh fruit. It has been shown that spoilage may occur much faster in minimally processed fruits than in unprocessed ones, as mechanical disruption of fruit integrity provides access to microorganisms to an environment with optimal concentrations of sugars, salts and water activity (aw).

In the case of mechanically injured fruits, in which adequate sorting has not been carried out before storage, the mold species *Penicillium expansum* and *Botrytis cinerea* have been identified. They can cause a significant loss of raw material, destroying the places of injury, creating lesions, causing cross-contamination of neighboring fruits. *P. expansum* [18] and *B. cinerea* [19] are pathogenic to apples, pears and a number of other pectin-rich fruits. Temperatures below 5 °C slow the growth of molds and yeasts and lead to an extension of the shelf life of the product [20, 21].

Bacteria cause a decay of minimally processed fruits with a neutral pH [22]. The most commonly isolated bacteria are members of the genera: *Erwinia, Xanthomonas, Pseudomonas, and Cytophaga*. They cause the so-called soft rot. The species that causes the most damage to a large group of vegetables and some fruits is *Erwinia carotovora* [23]. Relatively rarely, soft fruit rot is due to fluorescent bacteria from the *Pseudomonas river*. Bacterial growth is inhibited by storing the fruit in a controlled atmosphere (3% O_2 and 15 or 20% CO_2) [24].

There is no evidence that low levels of microbial contamination immediately after processing will provide an extension of shelf life in freshly chopped vegetables. However, with minimally processed fruit, the low concentration of microorganisms, especially yeast and mold, results in a longer shelf life.

Edible chitosan films on sliced melons

There is very little information in the scientific literature about edible coatings of chitosan on sliced melons [25]. These authors obtain two-layer polyelectrolyte coatings of alginate and chitosan, combining the good adhesion properties of alginate on sliced fruit and the proven antibacterial activity of chitosan. The melon slices covered in this way were stored at 6 °C for 21 days. The authors report a negligible effect of the coating on weight loss, while the texture of the packaged melons is preserved to a greater extent than that of the control. The addition of chitosan significantly reduces the rate of development of microorganisms on the surface. The authors conclude that physiological processes and microbiological spoilage of the fetus are probably the main factors influencing the texture.

MATERIALS AND METHODS OF ANALYSIS

Melons selected according to specified and summarized requirements after preliminary studies of the raw materials, were used as starting material for the experimental work.

Raw materials

Cantaloupe melon variety (*Cucumis melo* var. Cantalupensis) – Cantaloupe are musk-type melons. The fruit is round to oval, juicy and sweet with orange-colored fruit flesh. The peel is thin, with dark gray - gray-brown color, the surface is rough with green stripes The variety is suitable for desserts, fruit salads, purees and drinks. Melons were purchased from a local producer in consumer maturity, without physical and microbiological damage.

Materials

For the preparation of solutions intended for application as edible coatings were used: high molecular weight chitosan soluble in organic acids (M_w= 600 kDa) with 95% degree of N-deacetylation (DD); water-soluble chitosan with low molecular weight (M_w= 600 Da – 1.5 kDa) with 75-85% degree of N-deacetylation (DD). They were purchased and accompanied by a quality certificate. Drinking water meeting the requirements of Ordinance № 9/2001 on the quality of water intended for drinking and household purposes; lactic acid ($C_3H_6O_3$); acetic acid ($C_2H_4O_2$); calcium lactate ($C_6H_{10}CaO_6$) - all ingredients in the coatings were obtained from commercial sources of analytical purity and their application complies with Ordinance №8 / 2002 on the requirements for the use of food additives. Samples of fruit with edible coatings were stored in polypropylene boxes, complying with Ordinance №

2/2008 for materials and articles of plastics intended for contact with food.

Experimental staging

Preliminary preparation of the solutions

Solutions of high molecular weight chitosan used for application as edible coatings. All studied combinations of high molecular weight chitosan solutions were prepared as follows: quantities of 0.1%, 0.25%, 0.5%, and 1% high molecular weight chitosan were weighed on a technical scale, then added under constant stirring to 100 ml of 2% acetic acid solution at 25 °C to obtain a homogeneous solution. High molecular weight chitosan (0.5%) and 0.5%, 1%, 1.5% calcium lactate, respectively, were weighed on a technical scale, then added under constant stirring to 100 ml of lactic acid solution with concentrations of 1%, 1%, 1%, 1%, 0,5%, respectively, at a temperature of 25 °C to obtain a homogeneous solution.

Low molecular weight chitosan solutions used for application as edible coatings. A 1% solution of low molecular weight water-soluble chitosan and a solution of 1% low molecular weight water-soluble chitosan and 1% calcium lactate were prepared as follows: the dry ingredients were weighed on a technical scale and then added under constant stirring to 100 ml of water with a temperature of 45 °C.

Technological processing

Preliminary preparation of raw materials. Harvesting, delivery and handling of melon fruits, before refrigerated storage, was performed in two consecutive days to ensure uniform starting conditions.

Melons. Pretreatment of melons included washing with running water and removal of all physical impurities and injuries. The dried fruits were peeled, cored and chopped in pieces of 10 × 10 × 20 mm.

Application of edible coatings. After preliminary preparation of the fruit, the pieces of melon were immersed in the solutions for 1 min. After removal, they were drained and dried under natural air circulation for 30 min. Of all the fruits, one control sample remained for comparison.

Packing. The products - pieces of melon with edible coatings were placed in polypropylene packaging with perforated lids.

Sample storage. The storage of the finished products, the pieces of melon with edible coatings based on chitosan, was carried out in a refrigerated state in a controlled-storage chamber HotCold at a temperature T = 4 ± 0.5 °C and relative humidity φ = 60 ± 5%.

Methods of analysis

Antimicrobial activity of chitosan and microbiological parameters of the products. Each of the series during storage was analyzed for the presence of:

- *Listeria monocytogenes* - EN ISO 11290-2: 2017 Microbiology of food and feed. Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Part 2: Enumeration method (ISO 11290-2: 1998);
- *Salmonella* - EN ISO 6579:2017 Microbiology of food and feed. Horizontal method for the detection of *Salmonella spp*. (ISO 6579: 2002);
- Coagulase-positive staphylococci *(Staphylococcus aureus)* - EN ISO 6888-1: 2005 / A1: 2005 Microbiology of food and feed. Horizontal method for enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1: Technique using Baird-Parker agar. Amendment 1: Includes precision data (ISO 6888-1: 1999 / Amd 1: 2003);
- Coliforms - according to ISO 4832: 2006 Microbiology. Basic guide to listing coliforms. Colony-counting technique;
- *E. coli* - ISO 16649: 2014 Microbiology of food and feed. Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli*. Colony counting technique at 440C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide;
- Total number of microorganisms - EN ISO 4833-1: 2013 Microbiology of the food chain. Horizontal method for enumeration of microorganisms. Part 1: Counting of colonies at 300C by crop flooding technique (ISO 4833-1: 2013)
- Molds and yeasts - according to BDS ISO 21527-1: 2011 Microbiology of food and feed. Horizontal method for counting yeasts and molds. Part 1: Colony - counting technique in products with a water activity greater than 0.95.

RESULTS AND DISCUSSION

Antimicrobial activity of chitosan and microbiological indicators of minimally processed fruits during storage

In-vitro determination of the minimum inhibitory concentration (MIC) of chitosan against indicator and pathogenic species of microorganisms by the method of serial dilutions in liquid medium. One of the main requirements of edible coatings is that they do not support the development of microorganisms

that cause spoilage of the product or are pathogenic to humans. Chitosan is a polymer that has antimicrobial activity, highly dependent on its molecular weight, degree of acetylation, strain of the respective test microorganism and others. MIC is defined as the lowest concentration of test substance that inhibits the growth of microorganisms after incubation. Many studies have been performed to determine the MIC of chitin, chitosan, their derivatives or a combination thereof against a wide range of microorganisms. Quantitatively significant differences in the results for different microorganisms were obtained. Due to the fact that there are a number of non-standardized procedures for determining the MIC, it is difficult to compare the results of different teams.

The minimum inhibitory concentration (MIC) of low molecular weight chitosan used in the experiments was determined for the following reference strains by the National Bank for Industrial Microorganisms and Cell Cultures (NBMPMK) - *Aspergillus niger*, *Candida tropicalis*, *Escherichia coli*, *Bacilluscous cereus*. The results of MIC determination show that in Mueller - Hinton broth with a chitosan concentration higher than 50 ppm the species *Candida tropicalis*, *Escherichia coli*, *Entherococcus faecalis* stop their development (Table 1).

Table 1. Determination of the minimum inhibitory concentration of chitosan against some conditionally pathogenic and indicator microorganisms

Microorganism	Chitosan concentration (ppm)							
	230	200	150	100	50	10	5	2
Aspergillus niger NBIMCC 3564	R	R	R	R	R	R	R	R
Candida tropicalis NBIMCC 8614	S	S	S	S	**S**	BS	R	R
Escherichia coli NBIMCC	S	S	S	S	**S**	BS	R	R
Bacillus cereus NBIMCC 1085	BS	S	S	S	**S**	S	R	R
Entherococcus faecalis NBIMCC 3915	S	S	S	S	**S**	BS	R	R
Salmonella choleraesuis NBIMCC 251	**S**	BS	R	R	R	R	R	R

Our results regarding the low MIC of chitosan relative to *E. coli* confirm the studies of Liu [26].

When determining the MIC of chitosan against strain 251 of *Salmonella choleraesuis*, no growth was observed at chitosan concentrations in the culture medium above 230 ppm. The results of our MIC determination are comparable to the data presented by Rejane [27], which also indicate that regardless of the different types of *Salmonella* bacteria and the MIC determination methods, *Salmonella* spp strains are suppressed by higher concentrations of chitosan, compared to those observed in *Candida tropicalis*, *Escherichia coli* and *Entherococcus faecalis.*

In our study, none of the concentrations of chitosan used had an inhibitory effect on the fungus *Aspergillus niger* which is the cause of the spoilage of a number of fruits and vegetables. Studies by Tsai [28] also show that species of the genus Aspergillus have very high resistance to chitosan (> 2000 ppm) and its MIC is higher than that for other tested microorganisms (*Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*).

Microbiological parameters of chilled minimally processed fruit without and with edible coatings of water-soluble chitosan

Microbiological analysis of the components used and the edible coatings. The safety, quality and shelf life of ready-to-eat foods are largely determined by the species composition and quantity of pathogenic microorganisms, as well as those that cause spoilage of the product [29]. Consumption of fruits, vegetables and minimally processed products is usually defined as a risk factor for possible infection with pathogenic and toxicogenic microorganisms. Strains of *Listeria monocytogenes*, *Salmonella spp*, *E. coli* O157: H1, coliforms, *Yersinia enterocolitica*, *Staphylococcus ssp*, strains of *Enterobacteriaceae* were isolated from foods of plant origin. The sources of contamination of plant products with the listed

bacteria are diverse and include organic fertilizer, contaminated irrigation water, direct contamination by animals and poor hygiene of post-harvest technological processes (transportation, packaging, storage, distribution).

To ensure the microbiological stability and safety of the products (chilled minimally processed melons without and with edible coatings of chitosan and chitosan with calcium lactate), microbiological control of the incoming raw materials was initially performed. The microbiological criteria specified in their certificates are completely adequate and allow the production of a safe product that preserves its microbiological quality and stability. The results of the performed microbiological analysis are presented in Table 2.

Table 2. Microbiological characteristics of components and edible coatings

Object of test / Microbiological criterion	Chitosan	Chitosan coating	Calcium lactate	Chitosan and calcium lactate coating
Total number of microorganisms cfu/g (1000)	<100	< 10	<100	< 10
Molds cfu/g (1000 - 10 000)	<100	<10	<10	<10
Yeast cfu/g (1000- 10 000)	<100	<10	<100	<10
L. monocytogenes cfu/25g (it is not allowed)	not established	not established	not established	not established
Salmonella cfu/25g (it is not allowed)	not established	not established	not established	not established
Coagulase-positive *Staphylococci* cfu/g (it is not allowed)	not established	not established	not established	not established
Coliforms cfu/g (it is not allowed)	not established	not established	not established	not established
E. coli cfu/g (it is not allowed)	not established	not established	not established	not established
Enterococcus sp. cfu/g	< 1	< 1	< 1	< 1

No strains of the pathogenic microorganisms *L. monocytogenes*, *Salmonella spp.*, *E. coli* and Coagulase-positive staphylococci were identified in the study of chitosan, calcium lactate and edible coating solutions prepared therefrom. As a result of the conducted microbiological monitoring, compliance of the results obtained by us with those declared by the manufacturer was established. The absence of the mentioned pathogenic microorganisms in the raw materials for edible coatings is a proof of compliance with the Good Hygiene and Manufacturing Practices (GHMP) by the manufacturer. Absence of pathogenic microorganisms above the permissible quantities in the used raw materials is a major factor - a prerequisite for subsequent production of a safe product. The absence of ecterococci (faecal forms) and the low level of microbial contamination with a total number of microorganisms, yeasts and molds below 100 and below 10 cfu/g, respectively, is an indicator of good hygiene of technological operations and the absence of secondary contamination from the environment. Said groups of microorganisms affect the quality of the product in which they will be ingredients, as they are the main causes of spoilage.

Microbiological analysis of chilled minimally processed fruit with edible coatings

To prove the microbiological safety of chilled minimally treated melons without and with edible coatings based on water-soluble chitosan, they were tested on basic microbiological safety indicators (Table 3). According to Regulation (EC) № 1441/2007, in ready-to-eat foods that do not promote the growth of *L. monocytogenes*, the bacterium must not be found in 25 g of the product. No strains of *L. monocytogenes* were found in any of the products tested during the shelf life. As a result of studies by Sivapalasingam [30], *Salmonella* bacteria (≈60%)

and *E. coli* were found to be the main etiological agents in foodborne diseases caused by the consumption of foods of plant origin. In the present test, in chilled minimally treated melons without and with edible coatings based on water-soluble chitosan strains of *Salmonella spp* in 25 g of the tested samples were not isolated. Based on an analysis of the microbiological risk of minimally processed fruits and vegetables by the Institute of Environmental Science and Research - New Zealand, the main raw materials of melons and products developed from them were tested for the presence of coliforms and coagulase-positive staphylococci. The results of the microbiological analysis (Table 3) show that the amount of strains of the respective species in all tested samples is within the permissible limits of the indicated indicators. Based on the obtained results, it can be assumed that the raw materials used are not a source of coliforms and coagulase-positive staphylococci and all hygienic norms are observed during the technological processing. According to the national microbiological criteria [31], ready-to-eat fruits are considered safe when the amount of *E. coli* in all samples is below 100 cfu/g and does not exceed 1000 cfu/g. Microbiological analysis of the raw material and finished products revealed an amount of *E. coli* below 10 cfu/g, which proves the safety of the used batches of melons and their products.

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

Product / Indicator	Day	Melon (washed, sliced, chilled)	Chilled pieces of melon coated with chitosan	Chilled pieces of melon coated with chitosan and calcium lactate
L. monocytogenes cfu/25g (it is not allowed)	1	not established	not established	not established.
	3	not established	not established	not established
	5	not established	not established	not established
	7	not established	not established	not established
Salmonella cfu/25g (it is not allowed)	1	not established	not established	not established
	3	not established	not established	not established
	5	not established	not established	not established
	7	not established	not established	not established
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

The fruits are characterized by a high content of carbohydrates and low values of active acidity (pH), which favors the growth of specific types of bacteria (lactic acid, *Pseudomonas spp*., *Erwinia spp. Xanthomonas spp*. *Acidovorax spp*.), yeast (*Candida spp*., *Torulopsis spp*., *Rhodotorula spp*.) and molds (*Penicillium, Fusarium, Botrytis, Mucor, Rhizopus, Phthyophthora*). In general, the amount

of microbial associations on the fruit varies from 103 to 106 cfu/g. Molds, yeasts and bacteria cause various types of decay. The natural microflora, especially yeast, causes fermentation of the plant mass [32]. Based on the literature data for the purposes of the present study, all chilled fruits without and with edible coatings of chitosan and chitosan and calcium lactate were tested on the following indicators: total number of microorganisms, molds and yeasts.

In the present study, a microbiological test was performed on minimally treated melons without and with edible coatings of chitosan and chitosan with calcium lactate according to indicators determining the microbiological quality of the product. The data from the analysis are presented in Table 4. The results of the testing of the two products on the indicator 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 compared to those of cherries is due to the fact that microorganisms have direct contact with carbohydrates and other biologically valuable substances of the cell due to the cutting of the product This 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 reaches 450 cfu/g, and on the fifth day of their refrigerated storage they reach a quantity of 32.106 cfu/g. Due to the created conditions for decay (fermentation) and change in the organoleptic, physicochemical and rheological parameters, their storage has been suspended. When using edible coatings of chitosan and chitosan with calcium lactate, a slower increase in the amount of mesophilic microorganisms is observed.

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

Product Indicator	Day	Melon (washed, sliced, chilled)	Chilled pieces of melon coated with chitosan	Chilled pieces of melon coated with chitosan and calcium lactate
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	-	-	
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

On the seventh day of storage of the samples of melons with edible coatings of chitosan and chitosan with calcium lactate, a total number of microorganisms over 105 cfu/g was reported, which is already an indication of deteriorating microbiological quality and creating conditions for microbial spoilage. From the conducted microbiological testing of the products (pieces of melon without and with edible coatings of chitosan) it was established that the main group of microorganisms causing spoilage in the product are yeasts. In the tested samples, a concentration of yeast cells in the range of 11.10^3 in pieces of melon with an edible film of chitosan and lactate to 65.10^3 cfu/g in untreated pieces of melon was registered. The microbiological analysis for the detection of molds did not show a high concentration of molds, which would cause the spoilage of the products.

CONCLUSION

The conducted microbiological testing of the minimally processed melons proves that the applied chitosan coatings have a strong effect against the microbial population (mesophilic microorganisms), the cause of the spoilage of the products. During the

technological stages used to obtain minimally processed fruits with edible coatings, no contamination of the product with the studied pathogenic and opportunistic microorganisms was found.

An edible coating of 1% chitosan and 1% calcium lactate is an alternative for storing minimally processed melons, with quality characteristics for a longer period and the requirement to extend the shelf life for consumption is met. The storage period was determined according to the analyzed microbiological indicators - 7 days for minimally processed melons with the edible coating compared to 5 days for the control.

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