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THE EFFECT OF OSMOTIC DEHYDRATION AND STARCH COATING ON THE MICROBIOLOGICAL STABILITY OF APPLES

EFEKAT OSMOTSKE DEHIDRATACIJE I SKROBNOG PREMAZA NA MIKROBIOLOŠKU STABILNOST JABUKA

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ABSTRAKT

This paper examines the effect of starch coating and the osmotic dehydration in sugar beet molasses on the microbiological stability of apples. One-half of the osmotically treated/untreated apples were protected by starch coating, resulting in four sample groups (namely the K, P, OD and OD+P sample groups). E. coli, Salmonella spp. and Listeria monocytogenes were not detected in any of the samples. Enterobacteria were present in the K and P samples in the first four days (indicating a downward trend), but were not subsequently detectable. The total number of microorganisms (TVC) was found to be uniform in each sample group. However, the TVC values were significantly higher in the K and P sample groups than those of the OD and OD + P samples. Yeasts and molds were detected in the K and P samples, whereas the presence of yeasts and molds in the OD and OD+P samples was confirmed only after four days of storage. The results obtained indicate that osmotic dehydration is a suitable method for maintaining microbial stability, whereas starch coating did not justify its purpose.

Key words: *apple, osmotic dehydration, molasses, starch coating, microbial profile.*

REZIME

U ovom radu ispitan je uticaj osmotske dehidratacije u melasi šećerne repe i skrobnog premaza na mikrobiološku stabilnost jabuka. Polovina osmotski tretiranih/netretiranih uzoraka dodatno je zaštićena skrobnim premazom. Uzorci su upakovani u polipropilenske kesice u atmosferskim uslovima i skladišteni 10 dana na sobnoj temperaturi. Uzorcima je određena a_w vrednost i mikrobiološki profil. Rezultati su pokazali da E. coli, Salmonella spp. i L. monocytogenes nisu detektovane ni u jednoj grupi uzoraka. Enterobacteria nisu kvantifikovane u uzorcima OD i OD+P tokom celog perioda skladištenja, dok su prisutne u uzorcima K i P u prva četiri dana sa opadajućim trendom, a kasnije se nisu mogle detektovati. Ukupan broj bakterija je ujednačen u okviru svake grupe uzoraka, a vrednosti su značajno veće kod K i P uzoraka. Kvasci i plesni su prisutni u K i P uzorcima tokom celog perioda skladištenja sa maksimalnom vrednošću 170.000 cfu/g za K uzorak u desetom danu skladištenja. Kod OD i OD+P uzorka kvasci i plesni su detektovani tek nakon četvrtog dana i dostigli su veću vrednost kod OD+P uzorka (120.000 cfu/g) nego kod OD uzorka (26.000 cfu/g). Uočena pojava kvasaca i plesni je posledica pogodne a_w vrednosti za njihov razvoj. Kod uzorka OD+P veći broj kvasaca i plesni je posledica prisutva skrobnog premaza polisaharidne prirode koji predstavlja pogodan supstrat za razvoj inicijalno prisutnih kvasaca i plesni. Rezultati su pokazali da je OD pogodna metoda za očuvanje mikrobiološke stabilnosti, dok skrobni premaz nije opravdao svoju namenu.

Ključne reči: *jabuka, osmotska dehidratacija, melasa, skrobni premaz, mikrobiološki profil.*

INTRODUCTION

Osmotic dehydration (OD) is a very old but still prevalent method of food preservation, mostly used as a complementary step in the integrated food processing chain (*Rastogi et al., 2002*). Owing to its low energy requirements, OD is a cost-effective method, which has been gaining wide acceptance over traditional drying methods (*Khin et al., 2005*). Osmotic dehydration is the process of immersing substrate (food) into a hypertonic solution (salt, sugar, or an active physiological component) at a defined temperature and time. The driving force for osmotic removal of water is the concentration gradient established on opposite sides of the cell membrane (*Rastogi and Raghavarao, 2004*). Due to the cell membrane permeability, a concentration gradient develops, facilitating the water loss (WL) and the solid gain (SG) through osmotic and diffusion mechanisms (*Jiménez-Hernández et al., 2017; Rascón et al., 2018*). The proper dehydration solution choice is a key factor for successful dehydration, and this decision is influenced by the relationship between water loss and solid gain (WL/SG) (*Sacchetti et al., 2001*). The osmotic solution must be safe for health, having a low value of water activity (a_w) and an acceptable sensory grade. Suitable solutions for OD are sucrose solutions, NaCl solutions, corn syrups, etc. Sugar beet molasses is an excellent medium for OD because of its high dry matter content and specific nutritional composition. From a nutritional perspective, sugar beet molasses significantly enriches the material being dehydrated relative to minerals and vitamins (*Lončar et al., 2015; Nićetin et al., 2015*).

After osmotic dehydration, both the water content of the substrate tissue and the a_w value decrease, thus slowing down the undesirable microbial and biochemical reactions. Consequently, a longer sustainability of products is achieved (*Santhurn et al., 2012*). In addition, the loss of aroma is reduced and unwanted textural changes and product calibration were neutralized (*Petrotos and Lazarides, 2001*). Osmotic dehydration does not provide a complete sustainability of the treated material as significant amounts of water remain in the material (up to 50 %). If the fresh appearance of a dehydrated product is required, the effect of dehydration must be 30 %, which makes such products moderately stable and they should be further dried, frozen or treated with additives.

There are many applicable post-harvest techniques in the fruit and vegetable industries (namely cooling, controlling relative humidity/gaseous atmosphere, the application of edible films and coatings, etc) aimed at assuring the quality of products, meeting the consumers' needs, etc. (*Popović et al., 2018*). Edible coatings are used in the fruit and vegetable industries to reduce the metabolic activity of fruits and vegetables, prevent their weight loss (by moisture retention), hinder the transfer of gasses, inhibit microbial growth, slow aerobic respiration and improve their appearance (i.e. decrease the color and flavor losses) (*Conforti and Zinck, 2002; Kerch, 2015*).

In the literature, a number of studies accentuate the effect of new edible emulsion coatings on the quality of fresh fruits and vegetables after processing and during storage (*Synowiec et al., 2014; Mohammadi et al., 2015; Azarakhsha et al., 2014; Cisse et al., 2015; Choi et al., 2016; Ghidelli et al., 2015*).

In addition to cellulose, starch is the most widespread carbohydrate in nature, which is considered a suitable material for the formation of edible films and coatings (*Falguera et al., 2011*). Starch can be easily translated into edible, environmentally friendly, low-cost, flexible and transparent films (*Bilbao-Sáinz et al. 2010; Müller et al. 2009*). Starch films are neutral, with no effect on the sensorial properties of products (*Chiumarelli and Hubinger, 2012; Wang et al. 2012*).

The purpose of this paper is to examine the effect of osmotic dehydration and starch coating on the microbiological stability of fresh-cut apples during the course of 10 days at room temperature.

MATERIAL AND METHOD

Material preparation

'Golden Delicious' apples were cut into cubes with dimensions of 1×1×1 cm^3. The initial dry matter content of the apples was 13.05 %. One-half of the initial amounts of the sample apples were untreated, whereas the remaining apples were osmotically dehydrated in a sugar beet molasses solution (80 %). The experiment was conducted under atmospheric pressure at 22 °C for 5 hours. The solution to sample ratio was 5:1 (w/w) in order to avoid a significant dilution of the medium by water removal. Every 15 min, the apple samples in osmotic solutions were stirred to provide a better homogenization of the osmotic solution. After OD, the apple samples were removed from the osmotic solution, rinsed with distilled water and gently blotted to remove excessive water from the surface. The dry matter content of the treated apple samples was 48.73 %.

One-half of the both sample groups, i.e. the OD treated and untreated sample groups, was immersed in a previously prepared starch solution (1.5 g of starch + 0.5 g of glycerol/100 ml of water) in order to provide additional protection in the form of biopolymer edible packaging. Accordingly, the following four sample groups were formed:

K – untreated apple samples (control),
P – apple samples coated with starch,
OD – osmotically dehydrated apple samples,
OD + P – apple samples osmotically dehydrated and subsequently coated with starch.

All the samples were packed in polypropylene bags under atmospheric conditions and stored for 10 days at room temperature. Sampling was performed on the first, second, fourth, seventh and tenth days of storage.

Methods

The water activity values (a_w) were determined using the TESTO 650 water activity tester (Testo, Inc., 40 White Lake Rd, Sparta, NJ, USA) with a special probe with an accuracy of ± 0.001 at 25 ºC. The determination of total bacterial counts was performed according to the standard SRPS EN ISO 4833:2014. The presence of *Enterobacteriaceae* was determined according to SRPS ISO 21528-2:2017. The determination of *E coli*, presence was performed on the basis of the standard SRPS ISO 16649-2:2008. The determination of *Salmonella spp*. was made according to the standard SRPS EN ISO 6579: 2017. The presence of *Listeria monocytogenes* was determined according to the standard SRPS EN ISO 11290-1: 2017. The number of yeasts and molds was determined according to the standard SRPS ISO 21527-2: 2011.

RESULTS AND DISCUSSION

The samples obtained with/without osmotic dehydration and with/without starch coating are presented in Figure 1.

Fig. 1. Obtained apple samples
1 – untreated sample, control (K)
2 – undehydrated sample with starch coating (P)
3 – dehydrated sample (OD)
4 - dehydrated sample with starch coating (OD+P)

After osmotic dehydration in sugar beet molasses, the apple samples changed color to a distinctive molasses color. The application of starch coating did not cause changes in the sensory attributes of apples/osmotically dehydrated apples.

Table 1 shows the a_w values of the apple samples during the storage period.

Table 1. The a_w values of the osmotically untreated/treated apple samples with/without starch coating

Samples	Storage time (days)					
	0	1	2	4	7	10
K	0.911	0.907	0.903	0.905	0.911	0.912
P	0.923	0.905	0.908	0.907	0.919	0.918
OD	0.868	0.873	0.873	0.882	0.889	0.876
OD+P	0.899	0.897	0.834	0.891	0.894	0.895

K – untreated apple samples (control); P - apple samples coated with starch; OD - osmotically dehydrated apple samples; OD + P – apple samples osmotically dehydrated and subsequently coated with starch.

The results obtained indicate that the untreated K and P apple samples had higher a_w values (ranging from 0.903 to 0.923) than the osmotically treated apple samples (ranging from 0.834 to 0.899). Starch coating exerted no effect on the measured a_w values. According to *Doe (2002)*, the optimal a_w values for the active development of yeasts, molds and most bacteria causing food spoilage are 0.85, 0.80 and 0.91, respectively. Based on the

results obtained in the present study, a more intense growth of microorganisms can be expected in the samples which were not osmotically dehydrated in sugar beet molasses.

The results of microbiological tests show that *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* were not detected in any of the sample groups examined, which is in agreement with the findings of other authors (*Filipović et al., 2012; Lončar et al., 2014*). The total number of microorganisms (TVC) was found to be uniform in each sample group. However, the TVC values were significantly higher in the K and P sample groups than those of the OD and OD + P samples (Figure 2).

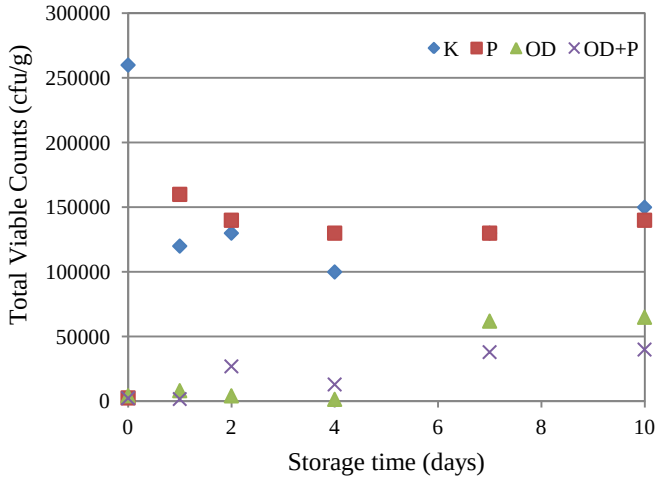

Fig. 2. Total Viable Counts for the osmotically untreated/treated apple samples with/without starch coating (K – untreated apple samples (control); P - apple samples coated with starch; OD - osmotically dehydrated apple samples; OD + P – apple samples osmotically dehydrated and subsequently coated with starch)

The TVC values for the K and P apple samples stayed uniform throughout the entire storage period. However, the OD and OD+P apple samples had significantly lower TVC values, which were constant until the 4th day of storage and slightly increased subsequently. At the end of the storage period, the following TVC values were recorded: 150,000 for the K sample, 140,000 for the P sample, 65,000 for the OD sample and the lowest value of 40,000 for the OD+P sample. *Enterobacteria* were not quantified in the OD and OD+P samples throughout the entire storage period. Osmodehydration treatments are known to have a highly reductive effect on most microorganisms (*Filipović et al., 2012). Enterobacteria* were present in the K and P apple samples in the first four days (indicating a downward trend), but could not be detected subsequently (in the last two sampling points) (<10 cfu/g) (Table 2). Lower values were observed for the P sample as a result of the starch coating presence, which provided additional protection (*Šuput et al., 2019*). Yeasts and molds were found to be present in the K and P samples throughout the entire storage period with a maximum value of 170,000 cfu/g for the control sample (K) on the 10th day of storage. The observed appearance of yeasts and molds in the K and P samples is a consequence of their a_w values which favored their development. Yeasts and molds were detected in the OD and OD+P samples only after four days of storage, with higher values in the OD+P sample (120,000 cfu/g) than in the OD apple sample (26,000 cfu/g) (Figure 3).

At the end of the storage period, the lowest yeast and mold count was detected in the OD samples. A large number of yeasts and molds were found in the P and OD+P samples due to the presence of starch coating, which is polysaccharide in nature and, therefore, a suitable substrate for the development of yeasts and molds initially present in small numbers. However, the highest yeast and mold count was recorded in the control sample at the end of the storage period.

Table 2. Enterobacteria in the osmotically untreated/treated apple samples with/without starch coating

Storage time (days)	Samples			
	K	P	OD	OD+P
0	11000cfu/g	2000cfu/g	<10 cfu/g	<10 cfu/g
1	12000cfu/g	3800cfu/g	<10 cfu/g	<10 cfu/g
2	10000cfu/g	1900 cfu/g	<10 cfu/g	<10 cfu/g
4	5500cfu/g	2000cfu/g	<10 cfu/g	<10 cfu/g
7	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g
10	<10 cfu/g	<10 cfu/g	<10 cfu/g	<10 cfu/g

K – untreated apple samples (control); P - apple samples coated with starch; OD - osmotically dehydrated apple samples; OD + P – apple samples osmotically dehydrated and subsequently coated with starch.

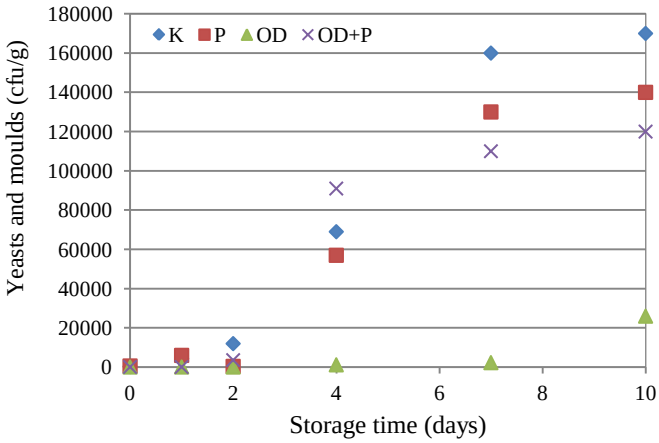

Fig. 3. Yeasts and molds in the osmotically untreated/treated apple samples with/without starch coating
K – untreated apple samples (control); P - apple samples coated with starch; OD - osmotically dehydrated apple samples; OD + P – apple samples osmotically dehydrated and then coated with starch.

CONCLUSION

The results obtained in the present study indicate that the untreated control apple sample had the worst microbial profile compared to other samples considered. The osmotic dehydration in sugar beet molasses was found to exert a positive effect on the microbial profile of osmotically dehydrated apples, resulting in healthy and safe semi-finished and finished products. Starch coating, as a means of additional protection for osmotically treated/untreated apples, did not justify its purpose.

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Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za određivanje broja ß-glukuronidaza pozitivne E. coli- Deo 2: Tehnika brojanja kolonija na 44 °C pomoću 5-bromo-4-hloro-3-indolil ß-D-glukuronida (SRPS ISO 16649-2:2008)

Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za otkrivanje i određivanje broja Enterobacteriaceae- Deo 2: Metoda brojanja kolonija (SRPS EN ISO 21528-2:2017). Temperatura inkubiranja je 37 °C

Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za određivanje broja kvasaca i plesni - Deo 2:Tehnika brojanja kolonija u proizvodima sa aktivnošću vode manjom od 0,95 ili jednakom 0,95 (SRP ISO 21527-2:2011)

Mikrobiologija hrane i hrane za životinje - Horizontalna metoda za otkrivanje, određivanje broja i serotipizacijuSalmonella spp. - Deo 1: Otkrivanje Salmonella spp. (SRPS EN ISO 6579-1:2017 izuzev Aneksa D)

Mikrobiologija hrane i hrane za životinje -Horizontalna metoda za otkrivanje i određivanje broja Listeriamonocytogenes i Listeria spp. - Deo 1: Metoda otkrivanja (SRPS EN ISO 11290-1:2017) Temperatura inkubiranja u delupotvrđivanje L. monocytogenes je 37°C

Mikrobiologija lanca hrane — Horizontalna metoda za određivanje broja mikroorganizama — Deo 1: Brojanjekolonija na 30 °C tehnikom nalivanja ploče (SRPS EN ISO 4833-1:2014

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