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AN ATTEMPT TO APPLY A PULLULAN AND PULLULAN-PROTEIN COATINGS TO PROLONG APPLES SHELF-LIFE STABILITY

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Abstract. The aim of the research was to study the influence of the pullulan and pullulan-protein edible coatings on the reduction of the apples mass loss during the storage. The research was carried out on pullulan received from *A. pullulans* white mutant B-1 in a batch cultivation process and the 'Malinova' and 'Champion' apples. In the first stage of the research the apples were covered with 15% and 20% pullulan water solution and storage at 4°C and 22°C during 39 days. In the second stage of the research the apples were covered with pullulan-protein coating obtained from the mixture of pullulan and soy protein. Apples covered with the pullulan-protein coatings were stored at the temperature of 2°C during 10 weeks. Mass losses of apples, durability of the pullulan and pullulan-protein coatings as well as changes in the appearance of the surface coating covered fruit in comparison with the one uncovered were evaluated. Pullulan edible coating significantly limited apples mass losses. Apples covered with coatings showed lower mass losses than the ones uncovered. The smallest mass losses were observed in apples covered with the coatings where the pullulan to protein ratios were: 6:4 and 5:5. It was observed that by adding protein to pullulan the coating stuck better to apples surface. During the storage process the protein-containing layer was less susceptible to crumbling and to peeling off.

Key words: pullulan, edible coating, apples shelf-life

INTRODUCTION

Both in UE and in Poland, apples are undoubtedly the fruits that are the most frequently consumed, and their production constitutes approximately 70% of the total fruit production. As a rule, all fruits and vegetables are harvested when they are completely ripe. The main problem with climacteric fruits (like apples) is, that they continue to ripen while they are being stored.

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Applying edible coatings might be one of the methods preventing fruits from water loss. The influence that edible coatings have on fruits and vegetables durability after crops lies in the fact, that it limits gas exchange between fruit intercellular spaces and environment. Transpiration is an essential reason of deterioration of fruits and vegetables quality after the crop, because it causes irreversible wilt and commercial value loss. Covering fruits with edible coatings is the effective method of food storage in the room temperature. This method causes decrease in microorganisms growth on the fruits surface, slows down gases exchange, controls respiration process, limits mass loss and R.H. transmission [Gontard et al. 1996, Xu et al. 2001, Tharanathan 2003].

Applying edible coatings modifies internal and external atmosphere. That makes possible gases exchange among fruits and environment.

Low oxygen content and high carbon dioxide CO_2 concentration decrease enzymes activity and therefore help to preserve fruit and vegetables hardness [Tasdelen and Bayindirli 1998].

Pullulan is extracellular polysaccharyde produced by *Aureobasidium pullulans*. It has got the permissin to be applied as a food additive [Kimoto et al. 1997, U.S. Food... 2002, European... 2004]. Pullulan coatings and films are colourless, odourless, tasteless and have a high mechanical resistance. They are edible and can be easily removed by washing the covered food in water [Yuen 1974]. A tendency to apply edible coatings is being continuously observed worldwide. In Poland, this exact method of preserving food during its storage is still rather seldom in use, but hopefully it will be introduced in the foreseeable future.

MATERIALS AND METHODS

In order to obtain pullulan the *Aureobasidium pullulans* B-1 strain was used. It was taken from the Collection of the Pure Cultures of Food Biotechnology and Microbiology Division of Warsaw Agricultural University, Poland. It was white mutant, received as a result of associated mutagenization. This mutant does not produce melanine compounds and at the same time produces more pullulan than the parent strain. In order to cultivate the fungus and get pullulan, the liquid base of the following composition was used (g/L): saccharose – 60.0, K_2HPO_4 – 7.5, NaCl – 1.5, $(NH_4)_2SO_4$ – 0.72, $MgSO_4{\cdot}H_2O$ – 0.4 (all from PPH, Gliwice, Poland), yeast extract – 0.4 (BioMerieux, France). The medium pH was 6.0 and was adjusted with 1 mol/L HCl before sterilization [Gniewosz et al. 1999].

The cultures were shaken (200 rpm) at 28°C for 24 hours. 1 ml cultures were then transferred into the fresh medium and cultivated in the same conditions as above for 96 h. The fungus biomass was centrifuged (11 000 rpm, 20 min at 4°C) in order to separate cellular biomass from supernatant liquid. Pullulan was precipitated from the supernatant with 96% ethanol and the precipitate was centrifuged (11 000 rpm for 20 min). Then pullulan was purified following Roukas and Biliaderis procedure [1995]. After the purifying process pullulan was dried up in 80°C. The dry pullulan was then grinded in laboratory mill.

The investigations were carried out on apples cv. 'Malinova' and 'Champion'. Apples of the equal masses, different no more than 5 g, were selected.

In the first stage of the research dried pullulan was dissolved in a distilled water so as to get 15%, and 20% water solutions. The mixture was then heated up to 80°C in a water bath and continously stirred to dissolve. The solution obtained in that way was thereafter sterilized in 117°C for 10 min and stored in cooling temperature.

Apples were washed, dried up, weighed and then covered with formely prepared pullulan solutions using a sterile little paint brush. As soon as the pullulan solution dried and the edible coating was formed, the apples were weighed again.

'Malinova' apples were covered with 15% and 20 % solutions and stored at 4°C and relative humidity (R.H.) was 75% (refrigerator AMW with MSB 120T02F) and at room temperature (22°C) and R.H. 58%. Measurements and testings were carried out for 39 days.

In the second stage of the research 'Champion' apples were covered with 10% pullulan water solutions mixed together with a gelling soy-bean protein FXP-15 (Soley Company, USA) in the weight ratios: 10:0, 9:1, 8:2, 7:3, 6:4, 5:5. 'Champion' apples covered with pullulan and pullulan-protein coatings were stored at 2°C and at R.H. > 95% in the Coolex (Poland) refrigerator. Measurements and testings were carried out for 10 weeks.

The macroscopic assessment included checking the durability of the pullulan and pullulan-protein coatings. At the same time the surfaces of the fruits both with and without coats were observed and compared.

Weight of apples was also controlled as well as changes that occurred during storage process. The research was repeated three times. Then, mass losses per 100 g of apples were calculated. The results were analysed statistically to establish the mean value, standard deviation and differences between the means (two-factors variance analysis at $p = 0.05$) with a computer statistical program Statgraphics Plus ver. 4.1 software.

RESULTS

Table 1 shows mass losses of 'Malinova' apples stored at 4°C and R.H. 75%, covered with the coatings produced from 15% and 20% pullulan water solutions, set together with the mass losses of control apples (uncovered). For 15% pullulan coat the biggest mass loss occurred during the first two day of storage and then between 18th and 22nd day. For these covered with 20% pullulan coating also the biggest mass loss was observed in the first two days and the next considerable fall between 13th and 18th day of storage.

On the following days apples mass losses tended to decrease independently from the pullulan solution concentration used for producing edible layer. Their values changed from 0.49%-0.58% after the first day of storage up to 0.11%-0.13% after the very last day. For the comparative apples (without pullulan coatings) the mass loss was evidently bigger than for these covered and was 0.9% after first day of storage and 0.2% after the last day.

Table 2 presents mass losses for the apples covered and uncovered with coatings produced from 15% and 20% pullulan water solutions stored at the room temperature of 22°C and R.H. 58%.

In this case, during the 39-day storage period the mass losses observed for the apples covered with the pullulan coating were less than for the control apples (uncovered).

Table 1. 'Malinova' apples covered and uncovered with pullulan coating stored at 4°C for 39 days. Mass losses per 100 g of apples
Tabela 1. Jabłka 'Malinowa' pokryte i niepokryte powłoką pullulanową przechowywane w 4°C przez 39 dni. Ubytki masy w przeliczeniu na 100 g jabłek

Time, days Czas, dni	Mass losses per 100 g of apples, % ±SD* – Ubytek masy na 100 g jabłek, % ±SD*		
	uncovered apples jabłka niepokryte	apples covered with pullulan coating jabłka pokryte powłoką pullulanową	
		15% pullulan	20% pullulan
1	0.90 ±0.09^{i}	0.49 ±0.08^{cdefg}	0.58 ±0.09^{efghi}
2	0.78 ±0.03^{hi}	0.41 ±0.08^{abcdef}	0.51 ±0.07^{cdefg}
3	0.77 ±0.05^{ghi}	0.37 ±0.07^{abcdef}	0.47 ±0.07^{bcdef}
5	0.71 ±0.05^{fghi}	0.34 ±0.05^{abcde}	0.43 ±0.08^{abcdef}
11	0.53 ±0.04^{defg}	0.29 ±0.06^{abcde}	0.36 ±0.04^{abcde}
13	0.48 ±0.05^{cdefg}	0.26 ±0.06^{abcde}	0.31 ±0.06^{abcde}
18	0.38 ±0.04^{abcdef}	0.20 ±0.07^{abcd}	0.22 ±0.07^{abcd}
22	0.28 ±0.03^{abcde}	0.13 ±0.02^{ab}	0.17 ±0.03^{abc}
31	0.19 ±0.03^{dfg}	0.12 ±0.03^{a}	0.14 ±0.04^{ab}
39	0.20 ±0.05^{abcd}	0.11 ±0.02^{a}	0.13 ±0.04^{ab}

*SD – standard deviation.
Means in the same column with different superscripts are significantly different at $p \leq 0.05$.
*SD – odchylenie standardowe.
Średnie w kolumnie oznaczone różnymi literami różnią się istotnie przy $p \leq 0,05$.

Table.2. 'Malinova' apples covered and uncovered with pullulan coating stored at 22°C for 39 days. Mass losses per 100 g of apples
Tabela 2. Jabłka 'Malinowa' pokryte i niepokryte powłoką pullulanową przechowywane w 22°C przez 39 dni. Ubytki masy w przeliczeniu na 100 g jabłek

Time, days Czas, dni	Mass losses per 100 g of apples, % ±SD* – Ubytek masy na 100 g jabłek, % ±SD*		
	uncovered apples jabłka niepokryte	apples covered with pullulan coating jabłka pokryte powłoką pullulanową	
		15% pullulan	20% pullulan
1	1.24 ±0.11^{l}	0.69 ±0.09^{hi}	0.82 ±0.10^{j}
2	1.35 ±0.10^{m}	0.66 ±0.08^{hg}	0.74 ±0.09^{i}
3	1.19 ±0.08^{l}	0.62 ±0.09^{g}	0.71 ±0.07^{hi}
5	1.05 ±0.08^{k}	0.62 ±0.08^{g}	0.69 ±0.08^{hi}
11	0.74 ±0.09^{i}	0.44 ±0.05^{e}	0.51 ±0.06^{f}
13	0.73 ±0.06^{i}	0.37 ±0.06^{cd}	0.50 ±0.06^{f}
18	0.62 ±0.09^{g}	0.31 ±0.08^{abc}	0.34 ±0.07^{de}
22	0.53 ±0.06^{f}	0.28 ±0.05^{ab}	0.34 ±0.05^{cd}
31	0.55 ±0.08^{f}	0.27 ±0.07^{ab}	0.33 ±0.07^{bc}
39	0.54 ±0.05^{f}	0.26 ±0.03^{a}	0.32 ±0.07^{bcd}

*SD – standard deviation.
Means in the same column with different superscripts are significantly different at $p \leq 0.05$.
*SD – odchylenie standardowe.
Średnie w kolumnie oznaczone różnymi literami różnią się istotnie przy $p \leq 0,05$.

The biggest mass losses for the control apples were observed during the first 11 days of storage (and they were 1.35-0.74%). At the same time the mass losses of the apples covered with the protecting pullulan coatings were only 0.82-0.51%. The coatings produced from either 15% or 20% pullulan solutions had better physical properties in 4°C than in 22°C. In the lower temperature it was thin, shiny and smooth. At 22°C starting from the 5th day of storage, it became wrinkled and the fruit surface started to go off.

In the second stage of the research ‘Champion’ apples were covered with pullulan-protein coating.

Table 3 shows mass losses for the ‘Champion’ apples covered with 10% pullulan and pullulan-protein coatings, stored in the 10-week period. The most considerable mass losses were observed during the first 3 weeks. In the following weeks the mass losses tended to decrease gradually. The most mass losses were measured for the comparative apples and the apples covered with pure pullulan coating. Much lower mass loss was for the apples covered with the two-component coatings. The smallest mass losses were measured for the apples covered with pullulan-protein coatings where pullulan to protein ratios were: 6:4 and 5:5 respectively.

Table 3. ‘Champion’ apples covered and uncovered with pullulan-protein coating stored at 2°C for 10 weeks. Mass losses per 100 g of apples
Tabela 3. Jabłka ‘Champion’ pokryte i niepokryte powłoką pullulanowo-białkową przechowywane w 2°C przez 10 tygodni. Ubytki masy w przeliczeniu na 100 g jabłek

Time weeks Czas tygodnie	Mass losses per 100 g of apples, % ±SD* – Ubytek masy jabłek na 100 g jabłek, % ±SD*						
	uncovered apples jabłka niepokryte	apples covered with pullulan-protein coatings jabłka pokryte powłoką pullulanowo-białkową 10% pullulan; pullulan to protein ratios in coating stosunek pullulanu do białka w powłoce					
		10:0	9:1	8:2	7:3	6:4	5:5
1	0.68^{s} ±0.02	0.59^{prst} ± 0.03	0.61^{rs} ± 0.02	0.56^{nopr} ± 0.04	$0.60p^{rs}$ ±0.08	$0.50^{\text{łmnop}}$ ± 0.03	0.57^{opr} ± 0.07
2	0.61^{rs} ± 0.03	$0.48^{\text{łmno}}$ ± 0.03	$0.48^{\text{łmno}}$ ± 0.03	$0.47^{\text{lłmn}}$ ± 0.05	$0.50^{\text{łmno}}$ ± 0.10	$0.41^{\text{jkłł}}$ ± 0.03	$0.47^{\text{lłmno}}$ ± 0.04
3	$0.55^{\text{łmnopr}}$ ± 0.03	0.34^{ghj} ± 0.04	0.37^{hij} ± 0.05	0.37^{ijkl} ± 0.03	0.36^{hijk} ± 0.06	0.26^{cdefg} ± 0.04	0.29^{fghi} ± 0.06
4	$0.46^{\text{klłm}}$ ± 0.06	0.26^{cdefg} ± 0.05	0.27^{defgh} ± 0.05	0.29^{efghi} ± 0.03	0.25^{cdefg} ± 0.08	0.19^{abcde} ± 0.03	0.20^{abcdef} ± 0.04
6	$0.41^{\text{jkłł}}$ ± 0.06	0.21^{bcdef} ± 0.04	0.20^{abcdef} ± 0.03	0.22^{abcdef} ± 0.03	0.20^{abcdef} ± 0.07	0.14^{ab} ± 0.02	0.14^{ab} ± 0.02
10	0.35^{ghij} ± 0.04	0.18^{abcd} ± 0.03	0.17^{abc} ± 0.03	0.18^{abcd} ± 0.03	0.18^{abcd} ± 0.06	0.12^{a} ± 0.02	0.13^{ab} ± 0.02

*SD – standard deviation.
Means in the same column with different superscripts are significantly different at $p \leq 0.05$.
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Średnie w kolumnie oznaczone różnymi literami różnią się istotnie przy $p \leq 0{,}05$.

It was observed, that pullulan and pullulan-protein solutions were easier to spread out on the 'Champion' apples surfaces. It probably came from smaller content of the natural wax on these apples skin and the fact their surface is rougher.

The results of the macroscopic testing showed, that adding protein to the pullulan coating caused its better adhesion to the fruit surface as well as prevented its peeling off during the storage time. Apples covered with pullulan and pullulan-protein coatings had shiny and smooth surfaces. If the protein content in the layer was higher, the apple surface was matt and rough, but on the other hand also more elastic and less susceptible to cracking. The apples covered with coatings of higher protein content looked better for a longer period of time.

DISCUSSION

The temperature of 22°C is not good for a long period storage. The information from the available sources says, that applying edible coatings limits moisture loss occurring at this temperature [Diab et al. 2001]. The results received in the present experiment seem to confirm it. Apples covered with coatings showed much lower mass loss than control (uncovered) ones, independently from pullulan concentration in the coating. It refers as well to the apples stored at 22°C and the apples stored at 4°C.

During the first two weeks of storage some little differences between the apples covered with the coatings and these uncovered were observed. However, they tended to increase as the time passed. After 6 and 10 weeks the mass losses of the apples covered with the pullulan coatings were measured to be about two times smaller than the uncovered. Similar changes occurring for the fruits covered with polysaccharyde coatings were observed and studied by other researchers too. Apricots covered with the methyl cellulose coating and stored at 25°C inhibited less mass drops than the fruits uncovered with the layer. The coatings produced from the same polysaccharide, laid on the strawberries and fresh bean caused significant reduction in the fruits' mass losses [Ayranci and Tunc 1997].

Adding protein to the pullulan coating had undoubtedly reduced apples mass loss. The best effects were observed for the apples covered with pullulan-protein coatings where pullulan to protein ratios were: 6:4 and 5:5 respective.

The coatings produced both from 15% water solution and from 20% solution were more effectively preserving fruits at the temperature of 4°C than at 22°C. In the case of 22°C as early as on the 5th day of storage some visible wrinkles started to appear and preserving pullulan coating started to come off.

Following some resources in multicomponent coatings the components interact with one another. The result is, that complex coatings have much better preserving properties [Diab et al. 2001, Debeaufort et al. 1998, Guilbert and Biquet 1986]. In this experiment, protein was added to pullulan to act as a plastid that was supposed to lower the brittleness of the pure-pullulan coating. The similar effect was observed in the research done on apples, pears and tomatoes covered with the coatings produced on the corn protein basis [Park et al. 1996]. These coatings reduced mass losses and retarded ripening process. Coatings with sorbitol and ester addition improved strawberries and kiwi fruits outlook as well as considerably extended their shelf-lives [Diab et al. 2001]. Applying multicomponent edible coats containing soy-bean protein, pullulan, stearine acid and glycerol effectively retarded kiwi fruits rippening process [Xu et al. 2001].

CONCLUSIONS

1. The results of this experiment show, that applying edible pullulan coatings significantly limites apples mass loss during definite time period.
2. Adding protein to pullulan, evidently limited mass losses more effectively and caused better adhesion to the fruit surface.
3. The concentration of pullulan in the solutions used for the research had no significant influence on the apples mass losses.

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PRÓBA ZASTOSOWANIA POWŁOKI PULLULANOWEJ I PULLULANOWO-BIAŁKOWEJ DO PRZEDŁUŻENIA OKRESU TRWAŁOŚCI PRZECHOWALNICZEJ JABŁEK

Streszczenie. Badano skuteczność jadalnej powłoki pullulanowej oraz pullulanowo-białkowej w hamowaniu ususzki przechowalniczej jabłek. W badaniach wykorzystano pullulan otrzymany z hodowli wgłębnej białego mutanta *Aureobasidium pullulans* B-1 oraz jabłka odmian 'Malinowa' i 'Champion'. W pierwszym etapie badań jabłka pokryto powłoką pullulanową uzyskaną z 15-procentowego i 20-procentowego wodnego roztworu pullulanu i przechowywano w temperaturze 4°C i 22°C przez 39 dni. W drugim etapie badań jabłka pokryto powłoką pullulanowo-białkową otrzymaną z pullulanu i żelującego białka sojowego FX-15 i przechowywano je w temperaturze 2°C przez 10 tygodni. Badanie obejmowało sprawdzenie ubytków masy jabłek, trwałości powłoki pullulanowej i pullulanowo-białkowej oraz wyglądu zewnętrznego całych owoców w porównaniu z owocami bez powłok. Jabłka pokryte powłokami wykazywały mniejsze ubytki mas niż jabłka niepokryte. Najmniejsze ubytki mas stwierdzono w jabłkach pokrytych powłoką pullulanową, w której stosunek zawartości pullulanu do białka wynosił 6:4 oraz 5:5. Zaobserwowano, że dodatek białka do powłoki poprawił jej przyleganie do powierzchni jabłek. Podczas przechowywania powłoka pullulanowo-białkowa była również mniej podatna na kruszenie i łuszczenie.

Słowa kluczowe: pullulan, powłoka jadalna, przechowywanie jabłek

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