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AN ATTEMPT TO APPLY A PULLULAN COATING TO REDUCE OXIDATIVE CHANGES AND MASS LOSS IN NUTS DURING STORAGE

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Key words*: Aureobasidium pullulans, pullulan,* edible coating, nuts, oxidative changes, mass loss

Samples of peanuts, hazelnuts and walnuts were covered with a pullulan coating prepared from 10% anhydrous solution of pullulan. The pullulan was obtained from a batch culture of a white mutant of *Aureobasidium pullulans* B-1. Over 90 days of nuts storage at a room temperature, analyses were carried out for changes in the acid and peroxide numbers of fat extracted from the nuts, as well as for changes in nut mass loss. The pullulan coating applied was found to exert a positive impact on the reduction of physicochemical changes occurring in the stored nuts. It inhibited processes of hydrolytic rancidity and oxidation of fat of the nuts. It had especially beneficial effect on walnuts, in which it inhibited the negative changes in lipids over the entire storage period. It was also observed that mass losses of the coated nuts were smaller and occurred in substantially shorter time span as compared to the uncoated nuts.

INTRODUCTION

Nuts are single-seed fruits with a lignified pericarp, belonging to various botanical families. Due to climatic conditions, only walnuts and hazelnuts are cultivated in Poland, though peanuts are of high interest too. Nuts contain over 50% of fat, moreover they are a rich source of vitamin B_1 and C as well as of phosphorus, potassium, magnesium, iron, calcium and sodium. They are applied in diets for children and patients as they are characterised by a high content of easily available components [Sikorski, 2002]. Recent researches have proved that nuts can also be a source of substances playing a key role in the protection of the cardiac muscle and the entire cardiovascular system, hence are recommended constituents of an everyday diet [Kornsteiner *et al.,* 2006].

The shelf life of deshelled nuts stored at a room temperature reaches a few months. The major reason of unfavourable sensory changes occurring in deshelled nuts stored for a longer period of time are changes in the profile of their unsaturated fatty acids [Sikorski, 2002].

Nowadays, no methods are applied that would extend the shelf life of nuts. Coating the nuts with edible films is likely to prevent undesirable processes or to inhibit their effects.

An ideal edible coating should form a thin layer on the surface of the coated food product and constitute a barrier to external factors, such as: water vapour, moisture or temperature. In addition, it should be capable for restricted absorption of oxygen and constitute a selective barrier to gases, mainly carbon dioxide [Guilbert *et al*., 1996; Tharanathan, 2003]. Since penetration of water into nuts accelerates oxidation processes, components of edible coatings should be tailored so as to provide a barrier also to moisture [Alderman *et al.,* 2000].

The efficiency of edible coatings on nut is determined by storage temperature. Investigations carried out by Baker *et al*. [1994] demonstrated that nuts stored at a temperature of 23-27°C were the most stable, whereas at a temperature of 38°C the process of fat rancidity proceeded at the same rate in both coated and uncoated nuts.

Pullulan is a polysaccharide produced by the fungus *Aureobasidium pullulans.* Purified pullulan has a form of tasteless and odourless white powder. It is well soluble in water [Milind *et al*., 1992]. It has no toxic properties nor negative effects on a human body, which affords the opportunity of applying it as a constituent of edible coatings [Kimoto *et al.*, 1997]. In 2004, the European Food Safety Authority (EFSA) expressed a positive opinion on the possibility of applying pullulan as a new food additive [EFSA Journal, 2004].

Aqueous solutions of pullulan have the capacity to form coatings, owing to which coatings from 5-10% aqueous solutions of pullulan can be produced directly on food products [Yuen, 1974]. The coatings produced are extremely thin, have very good mechanical properties, are transparent and have a low coefficient of oxygen permeability [Leathers, 2003]. Studies carried out on pullulan as a constituent of edible coatings have confirmed its advantages. The edible coating applied delayed the aging process of kiwi fruits [Xu *et al*., 2001] and reduced mass losses of strawberries [Diab *et al*., 2001]. Therefore, an attempt was undertaken to apply pullulan for coating nuts and to determine physicochemical changes proceeding in nuts during storage.

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MATERIAL AND METHODS

Three types of nuts: peanuts, hazelnuts and walnuts, purchased in one of the Warsaw supermarkets, were used in the study. Experiments were carried out on selected deshelled, undamaged and well developed nuts sorted according to size.

Pullulan was produced with the use of B-1 mutants of *A. pullulans* strain originating from the Pure Cultures Collection of the Department of Food Biotechnology and Microbiology, Warsaw University of Life Sciences. The white B-1 mutant was selected after associated mutagenesis of a natural, black strain *A.pullulans* A.p.-3. As compared with the parent strain, the B-1 mutant was characterised by a higher yield of pullulan production and a lack of production of melanin compounds contaminating the crude pullulan preparation [Gniewosz *et al*., 1999; Gniewosz, 2003].

Production of pullulan

In the culture of fungus and production of pullulan use was made of a liquid medium with the following composition (g/L): saccharose 60, K_2HPO_4 7.5, NaCl 1.5, $(NH_4)_2SO_4$ 0.72, $MgSO_4 \times H_2O$ 0.4, yeast extract 0.4, pH 6.5 [Gniewosz *et al*., 1997]. The culture of *A.pullulans* B-1 was run at a temperature of 28°C for 96 h on a reciprocating shaker (SM-30/C, Otto Gmbh, Germany) at 200 rpm. After that time, the culture was centrifuged for 20 min 18,800 × *g*, at a temperature of 4°C (Centrifuge 5804 R, Eppendorf, Germany) to separate cell biomass from the supernatant. Crude pullulan was precipitated from the supernatant with 96% ethanol added at a ratio of 1:1 (v/v). The precipitated pullulan was purified according to Roukas & Biliaderis [1995].

Coating and storage condition of nuts

Single nuts were immersed in a 10% aqueous solution of pullulan for 20 s and left to dry for 24 h. Dried, coated nuts (experimental samples) and uncoated nuts (control samples) were stored in dark place at an ambient temperature (R.H. 53%) for 90 days.

Extraction of fat

Pullulan-coated and uncoated samples of nuts with a mass of *ca*. 100 g were comminuted in a mortar, transferred to Erlenmeyer flasks, poured over with 96% hexane and left for 24 h. The next day, the flasks with a mixture were fixed in a reciprocating shaker (SM-30 Control, Otto Gmbh, Germany) for 2 h at 150 rpm for mixing and better penetration of the solvent. Next, the mixture was filtrated through filter paper. The filtrate obtained was fixed in a vacuum evaporator (Rotavapor R-200, Büchi, Switzerland) at a temperature of 45°C, at reduced pressure, in order to evaporate hexane. So prepared fat was used for further analyses. Extraction of fat was carried out after each month of storage.

Determination of the acid number and peroxide number of fat

The acid number of the fat extracted from nuts was determined according to PN-EN ISO 660:2005, whereas the peroxide number – according to PN-EN ISO 3960:2005.

Determination of nuts mass

The mass of nuts was controlled with the use of an analytical scale (Radwag WP120/K, Poland). Mass measurements were performed both for the pullulan film-coated and uncoated nuts. Examinations were carried out for three months and the samples were weighed every week.

Statistical analysis

The results were analysed statistically to establish the mean value, standard deviation and differences between the means (two-way analysis of variance at p=0.05) with a computer statistical software Statgraphics Plus ver. 4.1.

RESULTS

Effect of the pullulan coating on changes proceeding in nut lipids

Changes in the acid number of fat

The mean acid number of fat in fresh walnuts was low and accounted for 0.93 mg KOH/g fat (Table 1). In the uncoated nuts, significant changes in the acid number were observed to occur in the subsequent months of storage. In contrast, in the walnuts covered with the pullulan coating the acid number was observed to remain constant.

Initially, the acid number of the uncoated hazelnuts reached 1.25 mg KOH/g fat and increased as soon as after the first month of storage, reaching 3.59 mg KOH/g fat (Table 1). In the next month of storage it was observed to subsequently increase. In the samples of hazelnuts with the pullulan coating, the acid number also increased significantly after the first month of storage, yet – as in the case of walnuts – changes in

TABLE 1. Changes in the acid number of fat in nuts during storage.

Storage period (days)	Acid number (mg KOH/g fat), (Mean ± SD*)					
	Walnuts		Hazelnuts		Peanuts	
	coated	uncoated	coated	uncoated	coated	uncoated
0	0.93±0.05[b]		1.25±0.05[a]		0.48±0.07[a]	
30	0.88±0.22[b]	0.88±0.22[b]	2.91±0.06[c]	3.59±0.12[d]	0.58±0.06[b]	0.81±0.03[e]
60	0.80±0.01[b]	0.80±0.01[b]	2.13±0.06[b]	3.91±0.04[e]	0.63±0.01[bc]	0.82±0.04[e]
90	0.88±0.02[b]	0.88±0.02[b]	2.36±0.04[b]	3.61±0.06[d]	0.75±0.02[d]	1.17±0.06[f]

*SD – standard deviation; a, b, c,….- the same letters indicate a lack of a significant difference between results the acid number of coated nuts were significantly lower than those reported in the uncoated samples.

The acid number of fat extracted from fresh peanuts accounted for 0.48 mg KOH/g fat and over 3 months of storage increased up to 1.17 mg KOH/g fat of the uncoated nuts (Table 1). In the case of nuts coated with pullulan, the acid number was also found to increase, still in each month those changes were smaller than in the control samples.

Changes in the peroxide number of fat

In a sample of fresh walnuts, the peroxide number reached 0.23 O_2 milliequivalent/kg (Table 2) and was not subject to any significant changes during the first 30 days of storage. In the subsequent months, changes in the peroxide number were significantly greater. In the walnuts covered with the pullulan coating, the peroxide number was not observed to change over the first 2 months of storage (Table 2). It was not until day 90 when its increase was observed, which was however substantially smaller than in the control samples.

Fresh walnuts were characterised by a lower peroxide number of fat. It accounted for 0.12 O_2 milliequivalent/kg (Table 2) and after three months of storage appeared to increase significantly. Lower values of the peroxide number of fat were reported in the case of samples covered with the pullulan coating.

The peroxide number of fat extracted from fresh peanuts reached 3.35 O_2 milliequivalent/kg (Table 2) and was the highest of all the nuts examined. Over 3 months of storage of those uncoated nuts, the greatest changes were observed in the peroxide number as well. In the case of peanuts covered with the pullulan coating, the peroxide number appeared to increase slightly over the initial value.

Effect of the pullulan coating on mass loss of the nuts during storage

The pullulan coating was applied over the whole, non-infested hazelnuts and halves of walnuts and peanuts. During 90 days of nuts storage, the pullulan coating was observed to adhere very well to the surface of the nuts. It granted them sheen, which had a beneficial impact on the improvement of their appearance. Over the entire storage period, neither scaling nor wrinkling or cracking of the coating were observed. In contrast, over the storage period the coating appeared to become harder.

Mass losses of the nuts, both those coated and uncoated with pullulan, were controlled in weekly intervals. Mean mass losses of the nuts over the 90-day storage period were presented in Figures 1, 2 and 3. It was observed that over 3 months of storage, losses in the mass of nuts occurred only in the first month, in the subsequent months the mass of nuts was changing only to a little extent or was remaining at a constant level.

The greatest losses of the mass were reported for the uncoated walnuts within the first 3 weeks of storage (Figure 1). After two weeks, in the coated nuts the mass losses appeared to be 8 times smaller than those in the control fruits. That tendency was observed to maintain in the subsequent weeks. The application of the pullulan coating resulted in a twofold

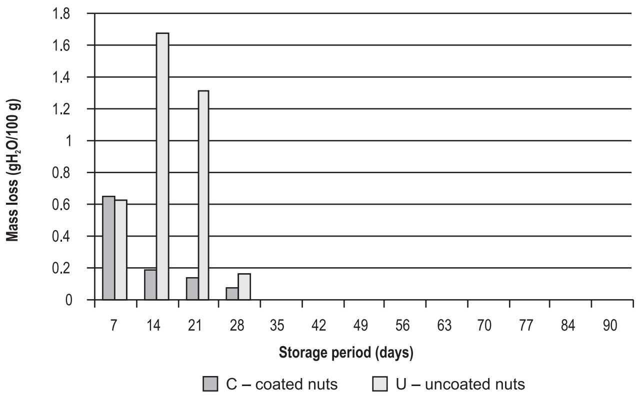

FIGURE 1. Mean losses of the mass of walnuts over 3-month storage period.

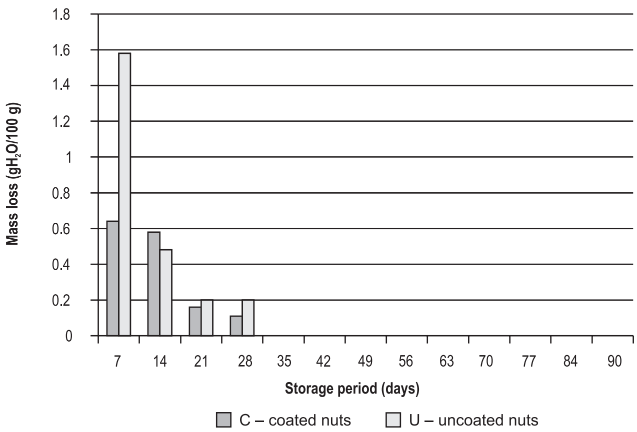

FIGURE 2. Mean losses of the mass of hazelnuts over 3-month storage period.

TABLE 2. Changes in the peroxide number of fat of nuts during storage.

Storage period (days)	Peroxide number (milliequivalent of O_2/kg fat), (Mean ± SD*)					
	Walnuts		Hazelnuts		Peanuts	
	coated	uncoated	coated	uncoated	coated	uncoated
0	$0.23±0.00^{a}$		$0.12±0.01^{a}$		$3.35±0.16^{b}$	
30	$0.26±0.00^{a}$	$0.28±0.05^{ab}$	$0.23±0.01^{b}$	$0.27±0.00^{c}$	$2.53±0.07^{a}$	$3.62±0.04^{bc}$
60	$0.25±0.04^{a}$	$0.56±0.01^{c}$	$0.30±0.01^{d}$	$0.40±0.01^{e}$	$3.48±0.09^{b}$	$8.19±0.05^{d}$
90	$0.31±0.01^{b}$	$0.81±0.01^{d}$	$0.44±0.01^{f}$	$0.49±0.01^{g}$	$3.82±0.13^{c}$	$9.17±0.07^{e}$

*SD – standard deviation; a, b, c,….- the same letters indicate a lack of a significant difference between results

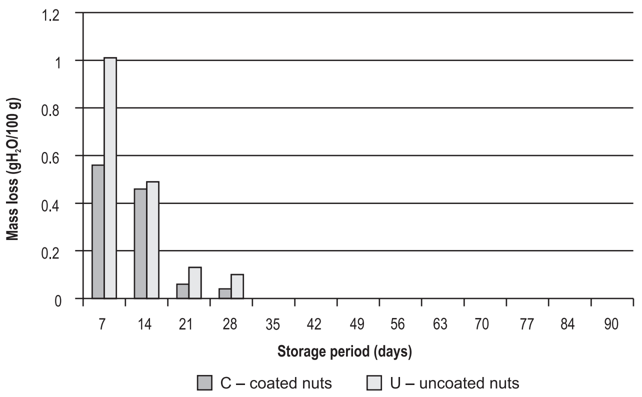

FIGURE 3. Mean losses of the mass of peanuts over 3-month storage period

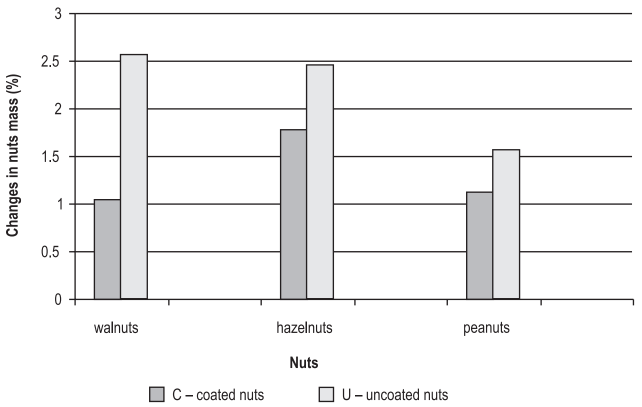

FIGURE 4. Changes in the mass of the nuts after 90 days of storage.

reduction in mass losses of those nuts. A change in the mass between day 1 and 90 of the storage period reached as little as 1.05% for the coated nuts and 2.56% for the uncoated nuts (Figure 4).

Equally beneficial effect of the pullulan coating applied was observed in the case of hazelnuts. Likewise for the walnuts, changes in their mass were observed only in the first 4 weeks of storage (Figure 2). After 7 days, over 2.5-fold lower loss of the mass was found in the coated nuts as compared to the control ones. In the other days of the storage period, the differences were however negligible.

The loss of mass of those nuts between day 1 and 90 of the storage period accounted for 1.78% and was lower by 0.68% than that reported for the uncoated nuts (Figure 4).

Peanuts were characterised by a lower mass loss out of all the nuts examined (Figure 3). Within the first 7 days of storage, mass losses of the coated nuts were 1.6-times lower than those of the control. In the other weeks of storage, the mass losses were remaining at a constant level. The coating was found to reduce the loss of mass between day 1 and 90 of the storage period by 0.44% in respect of the uncoated nuts (Figure 4).

DISCUSSION

The shelf life of nuts has not been stipulated by any norms, usually producers adopt the period of *ca*. 1-1.5 year. In that period, a variety of physicochemical changes are likely to proceed in nuts that result in deterioration of their quality.

Nuts contain over 50% of fat and its changes are the major cause of deteriorating quality of the nuts during storage. Undesirable sensory changes, linked with the generation of unpleasant taste and odour, result from hydrolysis and oxidation of lipids contained in the nuts. Those processes were controlled by introducing changes in the acid number and peroxide number of fat extracted from the nuts under study.

During storage of the walnuts, the acid number of fat was subject to significant changes only in the case of the control sample. In nuts covered with the pullulan coating, it was remaining at a constant level over the entire storage period, which may point to complete inhibition of the hydrolytic rancidity of fat. The pullulan coating appeared to exert a beneficial effect also on the value of the peroxide number. In the samples covered with the coating, a significant change in its value was observed already after 90 days of storage, whereas in the control samples significant changes in its value occurred as soon as after 60 days of storage. It was also observed that the nuts covered with the pullulan coating were characterised by lower values of the peroxide number in the entire storage period. This is likely to indicate a positive impact of the coating on the inhibition of oxidation processes of fat in those nuts.

In the case of hazelnuts, significant changes were observed for both the acid number and the peroxide number of fat. In the sample of the uncoated nuts, the value of the acid number increased nearly three times as soon as after the first month of storage. The pullulan coating applied failed to completely inhibit the process of hydrolytic rancidity, yet it reduced its rate. In the subsequent months of storage, the acid number in the sample coated with pullulan was significantly lower than that of the control ones. Similar tendencies were observed in the case of the peroxide number.

Fresh peanuts were characterised by the lowest value of the acid number of fat and, simultaneously, by the highest value of the peroxide number. Over the three-month storage period the acid number of fat extracted from nuts covered with the pullulan coating was subject to smaller changes, as compared to both the control samples (peanuts without the film) and to the other types of the nuts examined. The high initial value of the peroxide number may indicate a very high susceptibility of that type of nuts to the processes of lipid oxidation. In comparing the results of changes in the peroxide number of fat from peanuts it may be observed that the pullulan coating applied delayed the fat oxidation process by 2 months.

The application of the pullulan coating had a similarly positive impact on the reduction of water losses in the stored nuts. The greatest losses of mass were observed in the uncoated walnuts, whereas the smallest ones in the peanuts covered with the pullulan coating. In all three types of the nuts, a decrease of mass occurred within the first month of storage, both in the coated and control samples. Many authors also indicated the important role of edible coatings and films in reducing the mass losses of the fruits stored under different conditions [Han *et al.,* 2004; Yaman & Bayoindirli, 2002; Ayranci & Tunc, 2004; Xu *et al*., 2001; Tanada-Palmu & Gross, 2005]. Wu *et al.* [2000] also reported that the edible coatings based on wheat gluten, soy protein, carrageenan and chitosan were effective in reducing moisture loss in beef patties during storage. Additionally, coating with wheat gluten was the most effective in lipid oxidation.

So far, attempts of applying coatings to nuts have only been undertaken for peanuts. The protein coatings (from whey protein and zeins) applied with the addition of antioxidants have been reported to prevent the oxidation processes and to extend the shelf life of nuts. Their fault was, however, their low elasticity as a result of which the coated nuts lost their taste and crispness as soon as after 8 days of storage at a temperature of 45°C and relative air humidity of 90% [Alderman *et al*., 2000].

CONCLUSIONS

1. The pullulan coating produced delayed the processes of hydrolytic rancidity and oxidation of fat contained in the nuts, which had a positive impact on the elongation of the stability of nuts during 90 days of storage.

2. Mass losses of the nuts covered with the pullulan coating, for all the three types of nuts, were smaller as compared to the uncoated nuts.

3. Over the 3-month storage period the coating produced was observed to adhere well to the entire surface of nuts. In addition, it was not subject to either scaling or wrinkling.

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PRÓBA ZASTOSOWANIA POWŁOKI PULLULANOWEJ DO OGRANICZENIA ZMIAN OKSYDATYWNYCH I UBYTKÓW MASY ZACHODZĄCYCH W ORZECHACH PODCZAS PRZECHOWYWANIA

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Próbki orzechów arachidowych, laskowych i włoskich powleczono powłoką pullulanową sporządzoną z 10% wodnego roztworu pullulanu. Pullulan otrzymano z hodowli wgłębnej białego mutanta *Aureobasidium pullulans* B-1. Podczas 90 dni przechowywania orzechów w temperaturze pokojowej badano zmiany liczby kwasowej i nadtlenkowej w tłuszczu wyekstrahowanym z orzechów, a także zmiany ubytków masy orzechów. Stwierdzono, że zastosowana powłoka pullulanowa korzystnie wpłynęła na ograniczenie procesów utleniania tłuszczu w przechowywanych orzechach (szczególnie w orzechach włoskich). Ubytki masy orzechów powleczonych były mniejsze niż w orzechach niepowleczonych.