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APPLICATION OF PHYSICOCHEMICALLY MODIFIED POLYMERIC FOIL TO DEVELOP ANTIOXIDANT PACKAGING

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The method of propyl gallate immobilization on the surface of polymeric film has been developed. Surface of polyethylene foil was modified by electric corona and coated with a chitosan layer prepared from 1% solution of polysaccharide dissolved in 1% lactic acid. Then, it was gelated in a mixture of ethanol and NaOH solution. A portion of gallate ranging from 2.87 to 5.67 $\mu g/cm^2$ was immobilized in the coating prepared with the sorption method. The film obtained was characterised by antioxidant activity measured by scavenging DPPH$^{\bullet}$ radicals and its mean value was equal to 9×10^{-3} TE/cm^2. Preliminary measurements showed that the obtained polyethylene film coated with the chitosan layer containing immobilized propyl gallate lost half of its activity after about 5 months of storage at room temperature.

INTRODUCTION

Since the late nineties the concept of active packaging (functional packaging) has been present in literature concerning food technology. The term refers to the packaging which actively interacts with the product or atmosphere inside the packaging. It has a positive effect on the product's quality or extends the time of safe storage besides a common function consisting in insulation of food products from outside. Active packaging can serve various purposes such as: elimination of oxygen, ethylene, carbon dioxide, undesirable odours or excess of water, liberation of aromas, carbon dioxide, ethanol or display antibacterial and antifungal action [Appendini & Hotchkiss, 2002; de Kruijf *et al*., 2002; Cagri *et al*., 2004; Ozdemir & Floros, 2004; Suppakul *et al*., 2003]. So far, most attention has undoubtedly been focused on packaging characterised by the antimicrobial action [Cha & Chinnan, 2004; Veremeiren *et al*., 2002]. There is relatively less elaborations concerning packaging which improve the quality of food products or make them more healthy. The examples of these searches are studies on packaging containing immobilized naringinase intended to reduce bitterness in citrus juices [Del Nobile *et al*., 2003] or packaging allowing enrichment of beverages with minerals and vitamin E [Park & Zhao, 2004].

While designing the active packaging, special attention was focused on possibilities to apply chitosan. This glucosamine polymer, prepared by deacetylation of chitin, shows useful physicochemical features [Sieliwanowicz *et al*., 1995] and distinct antiseptic properties [Roades & Roller, 2000; Wu *et al*., 2002]. This polysaccharide is widely applied in medicine as a carrier which allows controlled liberation of immobilized drugs [Barreiro-Iglesias *et al*., 2005; Dini *et al*., 2003; Leffer & Muller *et al*., 2000]. Moreover, it was applied to design the edible coatings and active packaging for food [Coma *et al*., 2003; Moller *et al*., 2004; Ouattar *et al*., 2000; Park & Zhao, 2004].

Changes proceeding during storage and transportation of food very often cause quality deterioration and food spoilage resulting from oxidation processes. Therefore, packages characterised by the antioxidative action belong to an important group of active packaging. This group includes also packaging binding oxygen being an alternative for expensive vacuum packaging or packaging in which air is replaced by other neutral gas [Charles *et al*., 2005; Fernandez-Alvarez, 2000; Maloba *et al*., 1996; Suppakul *et al*., 2003] or finally, packaging with coatings containing an immobilized antioxidant [Garde *et al*., 2001; Hodgson *et al*., 2002; Oussalah *et al*., 2004; Tovar *et al*., 2005]. This packaging is first of all intended for storage of fats [Maloba *et al*., 1996; Shin & Lee, 2003] but it is also suitable to pack various cereal products. The above-mentioned packaging, which allow enriching juice in vitamin E, is also included in the group of antioxidant packaging [Park & Zhao, 2004].

The aim of our studies was to develop the method for preparation of polymeric film characterised by antioxidative properties. To this end, modified polyethylene foil with chitosan coating containing immobilized antioxidant has been applied.

MATERIALS AND METHODS

The polyethylene foil prepared in the Institute for Plastics Processing "Metalchem" in Toruń by the research group

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administered by prof. M. Żenkiewicz [2000] was used in our studies. The film was activated by electric corona generated in transistorized activator of AGT-2 type produced by Metalchem Toruń. As a result of activation, the roughness of its surface (R_a) increased from about 3 nm (non-activated foil) to about 20 nm (activated foil). Roughness was determined by an Atomic Force Microscope (AFM).

In our studies, use was made of chitosan purchased from Fluka and characterised by the mean molecular weight of 150,000.

Preparation of film coated with a chitosan layer containing immobilized propyl gallate. The film was degreased with ethanol and coated with 1% chitosan solution in 1% lactic acid (7–20 mL of a solution per 100 cm^2 of foil). Then, the film was immersed in a mixture of equal volumes of ethanol and 5% NaOH water solution for 60 min. The film with chitosan coating was fixed in hydroxide, the excess of NaOH was rinsed with distilled water and then, it was inserted into the sorption solution containing 0.5–3 mg of propyl gallate per 1 mL. The sorption solution was prepared by diluting the concentrated solution of propyl gallate containing 0.2 g of ester per 1 mL of ethanol with distilled water. During the sorption process lasting for 18 h, the solution was gently mixed. Then, the film was rinsed with water in order to remove the unbounded gallate and then dried at room temperature.

Determination of propyl gallate immobilized in chitosan coating on the surface of polyethylene foil. Content of propyl gallate was determined with the spectrophotometric method after acidic hydrolysis of chitosan. Samples of the tested film of a known surface area were flooded with 1 molar HCl solution and inserted into boiling water bath for 60 min. Gallate content was calculated on the basis of absorbance of hydrolysates at a wavelength of $\lambda = 270$ nm. Film samples coated with chitosan coating without propyl gallate were used as control.

Determination of antioxidant activity. Antioxidant activity was determined on the basis of scavenging the synthetic $DPPH^{\bullet}$ radicals (1,1 diphenyl-2-picrylhydrazyl). Antioxidant activity was determined in reaction with $DPPH^{\bullet}$ on the basis of scavenging the synthetic radicals from 0.1 mmol/L solution in 80% methanol [Kim *et al*., 2002]. The determination consisted in spectrophotometric measurement of absorbance drop ($\lambda = 520$ nm) in radical solution under the influence of incubation (20 min at 25°C) with the material tested.

Antioxidant activity was expressed in TE (Trolox equivalent) defined as the activity corresponding to the action of 1 μmole of Trolox, water soluble analogue of vitamin E.

RESULTS AND DISCUSSION

Films characterised by the antioxidant activity were obtained by immobilization of propyl gallate in the chitosan layer spread on polyethylene foil with modified surface. Polyethylene foil underwent significant modifications under the action of electric coronas generated in an linear accelerator of LAE 13/9 film. The pits and voids were formed on the film surface and moreover, new charges occurred periodically [Żenkiewicz, 2000]. It is common knowledge that the film modified by a stream of high-energy electrons is a better carrier for overprint than the non-modified film. According to our hypothesis, the film bombarded by a stream of electrons should be also a better carrier for immobilization of various compounds owing to both the new charges spaced on the surface and wider diversification of surface.

Chitosan dissolved in an acid solution was applied on degreased film surface and formed uniform coating after drying, however, the coatings were dissolved in water after 2–3 h. Therefore, they were additionally subjected to fixation. To this end, a mixture of 5% NaOH with ethanol was used. This treatment, resulting in chitosan gelation, was applied in our previous works during producing a carrier for immobilization of enzymes [Krakowiak *et al*., 2003; Trzcińska, 1998]. Chitosan coating was insoluble in water when fixed in the NaOH solution. In experiments with foil modified by electric discharges, chitosan formed the uniform film after drying. Chitosan, applied by the analogous method on the unmodified foil, formed a coating which was easily separated from the base. We can assume that modification of the foil surface by electric discharges, appearance of extra "roughness" and extra electric charges facilitated chitosan bonding with foil surface. Chitosan gel was additionally treated with a polyphosphate solution in polyphosphoric acid in our papers cited above. Chitosan phosphate derivatives were characterised both by better mechanical properties and stability and moreover, they proved to be a better carrier for immobilization of enzymatic proteins. Some studies have shown that chitosan cross-linked with glutaric aldehyde had a favourable effect on bond and allowed controlling liberation of the compound immobilized in chitosan [Barreiro- Iglesias *et al*., 2005; Leffler & Muller, 2000]. Our tests proved that the formation of chitosan phosphate derivative was unfavourable once chitosan layer was coated on polyethylene foil. The formation of chitosan phosphate was accompanied by a distinct contraction of gel structures and, therefore, the foil was curling and chitosan coating was cracking during drying. A similar effect was observed when glutaric aldehyde was used for cross-linking. As a result of observations described above, we abandoned applying chitosan phosphate derivatives and cross-linking with the use of glutaric aldehyde.

In previous studies, properties of chitosan coating have been shown to depend on the kind of acid applied to dissolve polysaccharide. Chitosan solutions in hydrochloric or citric acid are characterised by considerably lower viscosity than analogous solutions prepared in formic, acetic or lactic acid. Coatings prepared with the use of lactic or citric acid are more soft and extensible than the coatings prepared with the use of hydrochloric, formic or acetic acid [Begin & Van Calsteren, 1999; Peh *et al*., 2000]. Peh *et al.* [2000] compared chitosan coatings prepared with the use of acetic and lactic acid. Determinations of mechanical properties showed that the coating obtained from chitosan dissolved in lactic acid was three times more extensible and adhered to substrate almost 2.5 times stronger than the coating prepared with the use of acetic acid. In the present study, we employed lactic acid to prepare chitosan coating due to the viscosity of solutions and higher elasticity of the coatings formed. The preliminary tests showed that coatings obtained from chitosan dissolved in lactic acid strongly adhered to the film and did not break during drying. In the case when acetic acid was applied to prepare coatings, the product obtained was less

elastic and fractured during drying, which proved insufficient flexibility of foil.

Taking into account the preliminary tests described above and the cited literature data, the following procedure was adopted: the activated film was coated with 1% chitosan solution in 1% lactic acid and then, the film obtained was fixed in a sodium hydroxide solution. In order to remove the excess of sodium hydroxide, the film was rinsed with distilled water and inserted into the solution of propyl gallate. After sorption for 18 h, the films were rinsed in order to remove unbounded gallate. Optimum concentration of propyl gallate in the sorption solution was selected experimentally by determining the amount of gallate bounded in chitosan layer while applying various sorption solutions (Table 1). However, it was found that the amount of immobilized gallate varied considerably depending on the amount of chitosan coated on foil (thickness of chitosan layer) and while applying sorption solution of 2 mg of gallate per 1 mL, it was within the range from 4.12 μg/cm^2. Therefore, the fragments of the same film prepared by a single procedure were used in each experiment with the aim to avoid differences resulting from various thickness of the chitosan layer.

TABLE 1. Effect of sorption solution concentration on the amount of propyl gallate immobilized in chitosan lactate forming coating on the surface of polyethylene foil (mean results from three independent experiments).

	Gallate concentrations in sorption solution in (mg/mL)			
	0.5	1.0	2.0	3.0
Gallate immobilized in chitosan on the surface of film (μg/cm^2 of film)	2.87	5.66	5.67	5.61

As a result of experiments presented, it was found that the optimum concentration of propyl gallate in the sorption solution was equal to 1 mg/mL. It is the concentration of sorption solution that allows immobilization of gallate in chitosan coating in the amount of about 5.5 μg/cm^2. A further increase in propyl gallate concentration in the sorption solution has no effect on the amount of compound bounded in coating.

The method based on scavenging free radicals from the medium was used to determine the antioxidant activity of film with chitosan coating containing propyl gallate. The synthetic DPPH$^\bullet$ radicals were applied in measurements.

It was found that the antioxidant activity of the designed systems clearly depended not only on the concentration of the sorption solution. Also antioxidative activities of coatings obtained by using the sorption solution of the same concentration were significantly different in the successive experiments. For example, when concentration of the sorption solution applied was 2 mg/mL, the activity towards DPPH$^\bullet$ of the obtained foil was within the range from 6.4×10^{-3} to 12.6×10^{-3} TE/cm^2 (mean 9.0×10^{-3} TE/cm^2). These variations, similarly as the differences in the amount of the immobilized propyl gallate, were undoubtedly connected with the differences in thickness of the chitosan layer. The antioxidant activity of films obtained as a result of sorption in propyl gallate solutions of various concentrations was determined by making use of DPPH$^\bullet$ radicals. In order to avoid the influence of thickness of chitosan coating on the result of experiment, fragments of the same sheet of film were applied in the described tests and chitosan was applied in the amount of 15 mL/100 cm^2 (Table 2).

TABLE 2. Antioxidant activity of films prepared on polyethylene foil with the use of various sorption solutions

Gallate concentration in sorption solution (mg/mL)	Antioxidant activity (TE/cm^{-2})
0.5	4.8×10^{-3}
1	8.2×10^{-3}
2	8.5×10^{-3}
3	8.7×10^{-3}

The preliminary measurements of the stability of the prepared films were carried out. It was demonstrated that the film coated with chitosan containing immobilized propyl gallate preserved the antioxidant activity for a long time although the decrease of activity was observed during storage (Table 3).

TABLE 3. Antioxidant activity of polyethylene foil coated with chitosan containing immobilized propyl gallate after various storage times.

Term of determination	Directly after preparation	After 5 months	After 7 months
Antioxidant activity (TE/cm^2)	7.4×10^{-3}	3.5×10^{-3}	2.5×10^{-3}

After 5 months of film storage at room temperature, its antioxidant activity was reduced to about 50%, whereas after 7 months it was only 35% of the initial antioxidant activity.

CONCLUSIONS

1. Polyethylene foil coated with chitosan can be used as a carrier for immobilization of propyl gallate.
2. Propyl gallate can be permanently immobilized in chitosan layer (chitosan dissolved in lactic acid and fixed in NaOH) on polypropylene foil modified by electric discharges.
3. Due to the favourable physicochemical properties, it is recommended to use a chitosan solution in lactic acid to prepare chitosan coating on the surface of polyethylene foil.
4. As a result of propyl gallate immobilization in chitosan coating on the surface of polyethylene foil, the film characterised by antioxidant activity of about 9×10^{-3} TE/cm^2 was obtained.
5. Polyethylene foil coated with chitosan containing immobilized propyl gallate loses half of its activity after about 5 months of storage.

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WYKORZYSTANIE MODYFIKOWANEJ FIZYKO-CHEMICZNIE FOLII POLIMEROWEJ DO OPRACOWANIA OPAKOWAŃ O WŁAŚCIWOŚCIACH PRZECIWUTLENIAJĄCYCH

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Opracowano metodę unieruchomienia galusanu propylu na powierzchni folii polietylenowa pokrytej powłoką chitozanową. W utrwalonej w etanolu i NaOH warstwie, utworzonej z rozpuszczonego w 1% kwasie mlekowym chitozanu, naniesionej na modyfikowaną wyładowaniami elektrycznymi powierzchnię folii polietylenowej, unieruchomiono galusan propylu. Ze względu na korzystne właściwości fizykochemiczne przy konstruowaniu powłoki chitozanowej na powierzchni folii polietylenowej użyto roztworu chitozanu w kwasie mlekowym. Metodą sorpcyjną, w zależności od wyjściowego stężenia roztworu, unieruchamiano od 2.87 do 5.67 μg galusanu propylu/cm^2 folii (tab. 1). Uzyskano folie wykazującą aktywność przeciwutleniającą, mierzoną w reakcji rodnikowej z DPPH, wynoszącą około 9×10^{-3} TE/cm^2 (tab. 2). Wstępne pomiary wykazały, że folia polietylenowa pokryta chitozanem z unieruchomionym galusanem propylu traciła połowę aktywności po około 5 miesięcznym okresie przechowywania w temperaturze pokojowej (tab. 3).