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The application of melanin modified gelatin coatings for packaging and the oxidative stability of pork lard

Łukasz Łopusiewicz*, Filip Jędra, Artur Bartkowiak

Center of Bioimmobilisation and Innovative Packaging Materials,
Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology Szczecin,
35 Janickiego Str., Szczecin 71-270, Poland

*E-mail address: lukasz.lopusiewicz@zut.edu.pl

ABSTRACT

The influence of gelatin coatings modified with fungal melanin on pork lard oxidative stability was studied. The lard was coated with gelatin coatings containing 0.1%; 0.5%; 1% of fungal melanin and control gelatin coating devoid of melanin. The peroxide values (POV), iodine values (IV) and acid values (AV) were studied after 7, 14 and 21 days storage in controlled conditions. Lard covered with modified coatings had lowered oxidative rancidity. Hence, modified coatings containing fungal melanin can be use effectively for the prevention of lard oxidation.

***Keywords*:** melanin, packaging, antioxidant, lard, gelatin, lipid oxidation

1. INTRODUCTION

In the last few decades, there has been a marked increase in the use of natural polymer-based film materials and coatings in packaging for the food industry, which protect food from external contamination, retarding its deterioration by extending shelf life and maintaining quality and safety [1]. In addition to consumer requirements and in order to substitute petroleum-based plastic packaging, a wide variety of biopolymers that come from agro-food industrial wastes and renewable low cost natural resources have emerged [1,2].

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Packaging is widely used for the protection of food quality, thereby ensuring hygiene and extending the shelf life of perishable items, especially those susceptible to oxidative and microbiological deterioration [3]. Gelatin based edible films and coatings have already been proposed to protect, maintain or extend the shelf-life of food products [1,4,5]. Factors that should be considered when designing this type of system include the chemical nature of food, the controlled release mechanism, food organoleptic characteristic and additive toxicity, storage and distribution, physical and the mechanical properties of packaging and regulations to be applied in this framework [1].

Nowadays, research in the field of active packaging is also focused on the development of novel food packaging materials with antioxidant agents from natural sources, such as plant and spices extracts rather than synthetic antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA), since synthetic antioxidants are suspected of raising some safety concerns and have been restricted in their use as food additives [4]. Antioxidant active packaging, an innovative concept, is defined as a packaging incorporated with certain antioxidants, such as natural antioxidants, in order to provide a sustained release of antioxidant during storage.

The antioxidant active packaging can retard lipid oxidation, which is one of main food deteriorations to extend the shelf-life of food products [6]. In this context, some studies have reported that natural antioxidants show sufficient capacity to control lipid oxidation inside the food packaging because the oxidative processes can cause the degradation of proteins, pigments and fats, limiting food shelf-life [1,7]. A great number of reports of gelatin modifications by antioxidants are known from literature. A wide spectrum of additives were used including: curcumin and its derivatives [8], essential oils [9-17], esculine [4], butylated hydroxytoluene and α-tocopherol [7], lignin [18,19], liquid smoke [20], tannin [21,22], carvacol [23], vanillin [24], riboflavin [25], gallic acid [6], aloe vera gel [26,27], tea polyphenols and green tea extracts loaded into chitosan nanoparticles [27], tomato pulp and tomato pomace oil extract [28,29], as well as other plant extracts [30].

In a previous study we developed modified gelatin/melanin films with antioxidant activity and improved the oxygen barrier properties [31]. The aim of this study was to investigate the influence of modified gelatin/melanin coatings based on a film forming formulation on the rancidity stability of coated pork lard.

2. MATERIAL AND METHODS

2. 1. Lard coating

Gelatin coatings were prepared based on formulation developed in the previous study [31]. Firstly, ammonia water was added to the distilled water and pH was adjusted to 10 (to enable a solubilisation of melanin). Next, fungal melanin was added to alkaline solutions to obtain a 0.1% (sample „1”), a 0.5% (sample „2”) and a 1% (sample „3”) (on gelatin dry basis), and the solutions were placed in a stirrer (120 rpm), at 50 °C for 24 h. The solution devoid of melanin addition served as a control coating solution (sample „0”). When the melanin was dissolved, the solutions were filtered through Whatman filter paper under vacuum. 8 g of gelatin was added to 100 mL of filtered alkaline solutions and kept for 2 h at 60 °C under continuous stirring (120 rpm) to obtain a homogenous solution. Then, 10 % w/w (based on gelatin dry mass) of glycerol was added (to be used as a plasticizer).

The solutions were allowed to stand until their temperature reached 40 °C, then the lard samples (2 cm × 2 cm × 2 cm) were immersed in the coating solution, immediately removed and inserted under an air fan to solidify the coatings. The procedure was repeated twice for each lard sample. The samples were conditioned in a chamber at 25 °C and 50% RH. All measurements were conducted at the beginning of experiment (time 0) and after 7, 14 and 21 days.

2. 2. Peroxide values (POV) measurements

The POV values of lard were measured according to Bao *et al*. based on the IUPAC method [32]. The sample (1–2 g) was mixed with 25 mL of chloroform/acetic acid (2:3 v/v) followed by 1 mL of saturated potassium iodide solution. The reaction mixture was allowed to stand for 5 min in the dark. Distilled water (75 mL) was added to the mixture, which was then titrated with 0.01 mol/L sodium thiosulfate using 1 mL of 10 g/L starch solution as an indicator. POV was calculated using the following Equation:

$$\text{POV (meq } O_2\,kg^{-1}) = (a - b) \times N \times 100/w \quad (1)$$

where: *a* and *b* are the volumes (mL) of sodium thiosulfate used for the sample and blank (distilled water) titrations respectively, *N* is the concentration of sodium thiosulfate (mol/L) and *w* is the sample weight (g).

2. 3. Iodine values (IV) measurements

The IV values of lard were measured according to PN-EN ISO 3961:2013-10 [33]. The sample (1-2 g) was mixed with 20 mL of cyclohexane: acetic acid (1:1 v/v) followed by 25 mL of Wijs solution. The reaction mixture was allowed to stand for 1 hour in the dark. After an incubation period, 20 mL of 10% KI and 150 mL of distilled water were added to the mixture, which was then titrated with 0.1 mol/L sodium thiosulfate to a light brown-yellow colour. Then 1 mL of 1 mL of 10 g/L starch solution was added and the mixture was continuously titrated until it became colourless. The iodine value was calculated using the following Equation:

$$IV = 12.69 \times N \times (a - b)/w \quad (2)$$

Where *a* and *b* are the volumes of sodium thiosulfate used for the sample and blank (distilled water) titrations, respectively, *N* is the concentration of sodium thiosulfate (mol/L), w is the sample weight (g) and 12.69 is a calculation coefficient.

2. 4. Acid values (AV) measurements

The acid value (AV) values of lard were measured according to PN-EN ISO 660:2010 [34]. The sample (1-2) g was mixed with 50 mL of ethanol: diethyl eter (1:1 v/v). The mixture was titrated with 0.1 mol/L KOH solution in the presence of phenolphthalein, as an indicator. The acid value was calculated using the following Equation:

$$AV = 56.1 \times (a - b) \times N/w \quad (3)$$

where: *a* and *b* are the volumes of potassium hydroxide used for the sample and blank (distilled water) titrations, respectively, *N* is the concentration of potassium hydroxide (mol/L), w is the sample weight (g) and 56.1 is a calculation coefficient.

2. 5 Statistical analysis

All determinations were carried out in triplicate as a minimum. Statistical significance was determined using an analysis of variance (ANOVA) followed by a Duncans's test. The values were considered as significantly different when $p < 0.05$. All analyses were performed with Statistica version 10 (StatSoft Polska, Kraków, Poland).

3. RESULTS AND DISCUSSION

Food products are very susceptible to rancidity caused by the oxidation of lipids that contain unsaturated fatty acids that can be attacked by oxygen free radicals. Antioxidants are added to foods to intercept and react with these free radicals at a faster rate than the lipid substrate. Nevertheless, the current incorporation of antioxidants throughout the entire food matrix in one large initial dose is not an efficient process due to the oxidation occurring at the surface and high initial doses of antioxidant having a pro-oxidant effect [35]. Therefore, one emerging technology is the use of antioxidant active packaging, where the antioxidant is incorporated into a packaging material with the purpose of being delivered to the food surface during commercialization, at an appropriate rate. Most of the active packaging developments base their work on the mass transportation properties of plastic materials (sorption, migration, and permeation), and the release of the active agents depends on several factors, such as the type of polymer and type of food. However, the presence of synthetic antioxidants in food is questionable, owing to the potential risks. This has been encouraged by strong consumer demand, as synthetic compounds are frequently perceived as undesirable or harmful. Natural antioxidants are preferred to artificial substances, especially by consumers [4,7]. Moreover, the use of active antioxidant packaging that incorporates natural antioxidants presents important advantages. The addition of a natural compound to the packaging may reduce the need to use synthetic antioxidants in the plastic, reducing the risk of potential toxicity by migration [1].

Animal fats have long been recognized as a raw material for food and industrial applications [36]. Lard is a form of pig fat which in a saturated or unsaturated form derived from its adipose tissue and may be used in its raw form as frying medium or after modification of its physical properties, as shortening for baking applications and is often used in food production as an emulsion, shortening, or as a substitute to butter, margarine or cooking oils. Usually, it is extracted from the back skin, muscle, surrounding digestive organs, surrounding the kidneys of pig. Scientifically, lard is known as triglyceride, it is mainly consists of fats or fatty acid. It contains considerable proportions of palmitic, stearic, oleic and linoleic acids. There are also small amounts of palmitoleic, traces of linoleic, arachidonic and myristic acids [37]. Lard is a common choice due to its cheap market price and easy availability [38-40]. The application of antioxidants and antioxidant-gelatin composite films for lard are known from literature. Mihaylova and Schalow used quercetin-containing flavonoid extract obtained from *Sophora japonica* flower for the antoxidant stabilization of lard [41]. Antonietta Paleari *et al*. covered lard with antioxidant spices and

aromatic herbs [42]. Yeo *et al*. applied free radical scavengers such as α-tocopherol, BHT, sesamol, tert-butylhydroquinone in thermally-oxidized lard [43]. Dziedzic *et al*. used polyhydroxydihydrochalcones as antioxidants for lard [44]. Jongjareonrak et al. applied modified fish skin gelatin films incorporated with BHT and α-tocopherol on plastic cups containing lard [7].

Recent studies have focused on techniques to develop active gelatin-based packaging films and coatings, including antimicrobial, antioxidant and other agents which can enhance the biological features of food [1]. Liu *et al*. developed gelatin-based films packaged with sunflower oils with different free/encapsulated tea polyphenols in chitosan nanoparticles [45]. Their results showed a reduction in the oxidation of sunflower oil obtaining lower peroxide (POV) and thiobarbituric acid reactive substance (TBARS) values for oils packed in the films. In addition, an improvement in antioxidant activity when using and optimum ratio of free and encapsulated additives was demonstrated over a long period of storage (6 weeks) as well as the preservation of the functional properties of the developed films. Gómez-Estaca *et al*. [46] observed that pigskin gelatin film with oregano and rosemary extracts delayed the lipid oxidation of cold smoked sardine coated with the films in storage at 5 °C.

Kakei *et al*. noted that chitosan-gelatin incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil improved the lipid stability of minced trout fillet [47]. Chottanom *et al*. [48] noted that gelatin-starch pouches modified with plant extract decreased the hydrolytic rancidity rate of meat products. Lee *et al*. [5] applied a fish skin gelatin film containing *Moringa oleifera* leaf extract to the packaging of Gouda cheese and noted lower POV and TBARS values in covered samples after a period of storage. Alparslan *et al*. [13] observed that a gelatin film enriched with laurel essential oil was suitable for the preservation of rainbow trout fillet, retarded fish lipid oxidation and the ability of laurel essential oil to preserve the film depended on its ratio. Also Alparslan *et al*. [12] used gelatin coatings enriched with orange leaf essential oil for the preservation of shrimps, and noted that coatings effectively preserved lipid stability. On the other hand, Antoniewski *et al.* [49] found that a gelatin coating had no effect on lipid oxidation in salmon fillet stored at 4 °C for 14 days. Ahmad *et al*. [3] used modified gelatin films with lemongrass essential oil to wrap sea bass slices. During storage, a significant increase in TBARS was observed in control samples than compared to the samples wrapped with non-modified and modified films. This result suggested that the lipid oxidation in sea bass slices could be delayed when a lemongrass essential oil film was applied, probably due to the antioxidant activity of the lemongrass essential oil, as well as the low oxygen permeability characteristics of the modified film. Those results are in line with our results in our previous study, which showed that modified melanin/gelatin films have a significantly lower oxygen transmission rate in comparison to the non-modified films [31].

According to Ahmad *et al*. [3] the antioxidant activities of the essential oils have been attributed to assorted mechanisms, including the prevention of radical chain initiation, the binding of transition metal ion catalysts, the decomposition of peroxides and the interaction with the free radicals. Melanins are also known from their antioxidant activity as well as metal ions chelating ability [50-54]. Also there are several reports that packaging materials and coatings containing melanins have antioxidant activity [35,55,56].

The mechanism of action of these natural antioxidants in contact with food are related to lipid oxidation reactions. In addition, they are focused on phenolic groups present in the melanin structure. Hydrogen atoms from phenol hydroxyl groups could react with peroxyl

radicals produced at the early stages of the oxidation mechanism to yield stable phenoxyl radicals and, consequently result in the termination of the lipid peroxidation chain reactions. However, understanding the antioxidant activity mechanism of melanins in films is a hard task as this activity depends on the electronic and steric effects on their ring substituents, the strength of hydrogen-bonding interactions between the phenolic groups and the solvent, and the interactions with the film matrix and the packaged food [1].

POV was used to monitor the oxidation of lard packaged with gelatin coatings, both with and without the addition of incorporated melanin. Peroxide value, an oxidative rancidity parameter is measured from hyroperoxide content. The hyperoxide species generally occurs in the initial phase of oxidative rancidity and then reacts with additional lipid molecules to form other reactive chemical species [48]. As can be seen in Table 1, the POV of lard without film coating (uncoated) increased continuously and rapidly within a short time under controlled incubation. However, the POV of lard covered with films coatings increased slowly, even in the case of non-modified coatings. The differences between all the samples were statistically significant ($p<0.05$). This phenomenon can be attributed to the effect of the coatings in retarding oxygen of the lard. During the entire incubation period the POV of lard coated with coatings containing melanin was lower than that of lard coated with coatings devoid of melanin. It is tempting to suggest that it was due the antioxidant activity of melanin.

Table 1. The influence of pure gelatin and gelatin/melanin coatings on lard peroxide values (POV) [meq O_2 kg^{-1}]

	Uncovered	Control	0.1% melanin	0.5% melanin	1% melanin
0 day	1.45±0.11	1.45±0.11	1.45±0.11	1.45±0.11	1.45±0.11
7 day	28.44±0.13	7.93±0.20	5.67±0.09	1.83±0.04	1.88±0.06
14 day	73.92±0.25	25.22±0.33	7.22±0.18	2.44±0.07	2.33±0.15
21 day	111.12±1.45	30.11±0.55	9.91±0.22	2.77±0.09	2.49±0.05

The acid value (AV) is a number that expresses the quantity of potassium hydroxide, in milligrams, required to neutralize the free acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids or acid phosphates. The acid value is often a good measure in the breakdown of the triacylglycrols into free fatty acids, which has an adverse effect on the quality of many lipids. Acid value is the measure of hydrolytic rancidity. In general, it gives an indication about edibility of the fat. As can be seen in Table 2, the AV of lard without film coating (uncoated) increased continuously and rapidly within a short time under controlled incubation. However, the AV of lard covered with films coatings increased slowly, even in the case of non-modified coatings, indicating that gelating coatings and melanin/gelatin coatings influenced the amount of free fatty acids in lard samples. The differences between all the samples were statistically significant ($p<0.05$).

Table 2. The influence of pure gelatin and gelatin/melanin coatings on lard acid values (AV) [mg KOH/g]

	Uncovered	Control	0.1% melanin	0.5% melanin	1% melanin
0 day	1.31±0.11	1.31±0.11	1.31±0.11	1.31±0.11	1.31±0.11
7 day	1.77±0.14	1.68±0.09	1.45±0.33	1.37±0.06	1.35±0.04
14 day	2.84±0.25	1.99±0.15	1.78±0.13	1.45±0.03	1.44±0.09
21 day	3.15±0.07	2.52±0.08	2.22±0.05	1.55±0.04	1.52±0.12

Iodine number indicates the degree of unsaturation i.e. the number of double bonds present at the length of the chain. Iodine value is low in animal fats and high in vegetable oils. The higher the iodine value, the lower the melting point. As can be seen in Table 3, the IV of lard without film coating (uncoated) decreased continuously under controlled incubation. However, the IV of lard covered with films coatings decreased slowly, even in the case of non-modified coatings, indicating that gelating coatings and melanin/gelatin coatings influenced the amount of unsaturated bonds in lard samples. The differences between all the samples were statistically significant ($p<0.05$).

Table 3. The influence of pure gelatin and gelatin/melanin coatings on lard iodine values (IV) [g/100 g]

	Uncovered	Control	0.1% melanin	0.5% melanin	1% melanin
0 day	58.11±1.11	58.11±1.11	58.11±1.11	58.11±1.11	58.11±1.11
7 day	52.34±1.98	57.13±3.15	56.98±1.67	56.33±2.34	57.45±2.17
14 day	45.22±1.45	52.84±3.19	55.12±3.12	55.23±3.78	56.11±1.56
21 day	43.23±2.45	50.13±2.63	51.34±2.88	52.18±2.52	53.66±1.34

The preventive effect on lard stabilization was possibly also caused by the increased hydrophobicity of the coatings matrix in the presence of melanin. In a previous study, the contact angle of films increased from 53.3° (unmodified film) 72.9° (1% of melanin in film) [31]. Similar mechanism has been proposed by Jongjareonrak *et al*. [7], who used hydrophobic BHT and α-tocopherol for the modification of fish skin gelatin films used for lard packaging. Gelatin films might function as a barrier to oxygen permeability on the lard surface. Therefore, only a small amount of oxygen could penetrate into the lard, leading to a lower oxidation rate. On the other hand, Jonjareonrak *et al*. [7] concluded that the addition of (BHT) and *α*-tocopherol had a negligible antioxidant effect on lard. It is tempting to suggest

that when they covered the cup containing the lard with films incorporated with BHT or α-tocopherol only changed the permeability of oxygen, and the antioxidants could not directly affect the oxidation of lard because of the separation between them and the lard, which was speculated also by Bao *et al*. [32] Their research found a direct connection between product and antioxidant coating, thus possibly maximising the effect of melanin against lard oxidation in our study.

4. CONCLUSION

Oxidation of lard was effectively retarded when covering with gelatin coatings containing fungal melanin. Hence, modified gelatin coatings can be applied to preserve of lipid stability.

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