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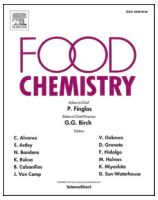

Utilisation of soybean post-production waste in single- and double-layered films based on furcellaran to obtain packaging materials for food products prone to oxidation

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ABSTRACT

Single-layered films, based on furcellaran and protein hydrolysates from soybean bran (1st layer), were obtained. Moreover, a procedure for the preparation of double-layered films was developed, in which an ethanol extract from soybean bran was deposited onto the 1st layer. It was checked how the addition of the 2nd layer affects the functional properties of the film. The addition of the 2nd layer increased the thermal properties, modulus of elasticity and antioxidant activity, while decreases were noted for tensile strength and elongation at break parameters. The films were used as packaging materials for storing butter and the active films did not extend the quality of butter during storage, however, they behaved in the same way as synthetic films. Therefore they have the potential to be used as packaging material instead of a synthetic film.

1. Introduction

These days, industries have problems with using synthetic preservatives to enhance the shelf-life of dairy products due to consumer awareness and the detrimental health effects of such additives. Butter is a dairy product that is consumed globally, directly and indirectly in a number of food products due to its therapeutic and nutritional significance. The main component of the butter should be fat, which plays a vital role in shelf stability, appearance, flavour, texture and nutritional value. Butter is more vulnerable to oxidative deterioration that further leads to the reduction of nutritional quality, also making the food unacceptable for consumers. The oxidation of fats not only deteriorates the quality of butter, but it also causes many human diseases, thus antioxidants are added to foods to prevent or delay oxidation. To resolve these problems, synthetic preservatives are being used (Arshad, Sameen, Huma, & Zia, 2020). However, data from literature indicate that the use of synthetic antioxidants in food may cause diseases such as liver damage or cancer among consumers (Chen, Yang, Sun, Niu, & Liu, 2012). This fact prompts the search for new sources of natural antioxidants. Protein hydrolysates coming from chemically or biologically broken-down food proteins are composed of peptides with a broad range of molecular weights. They are widely used as functional food ingredients and nutraceuticals. These hydrolysates may exhibit different functions, such as those antioxidative. Therefore, they could also be used as food preservatives. Protein hydrolysates with antioxidant activity from food protein sources as natural ingredients have been increasingly investigated due to their potential health benefits (Pan, Liu, Yang, Liu, Wang, & Wang, 2020).

Soybean (*Glycine* max L) is a source of protein, and from an economic point of view, its cultivation plays an important role world-wide. The latest report from the Foreign Agricultural Service/USDA Global Market Analysis estimated global soybean production in 2021/22 to be at 384.42 million metric tons. Soybean bran is a by-product of conventional soy milling. It is mainly used as a low-value ingredient in animal

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feed, a fibre source for dairy and beef cattle, in reduced fat diets for pets and as a bulk additive to feeds (Sessa, 2004). The chemical composition of the soybean husk/bran depends on the efficiency of the dehulling process. If high protein soy flour is produced, the dehulling process is more intensive to avoid contamination of the meal with husk pieces. As a consequence, soybean husks can contain varying amounts of protein, cellulose, hemicellulose, lignin and pectin, and they can be valuable by-products (Rojas, Siqueira, Miranda, Tardioli, & Giordano, 2014). A wide variety of polyphenols and peptides with antioxidant activity have been extracted from legume residues (Tassoni et al., 2020).These compounds have potential applications in the food industry as alternative natural antioxidant compounds and ingredients in new green-labelled products.

Furcellaran is a polysaccharide obtained from the red algae *Furcellaria lumbricalis*. Its film-forming properties may make it an interesting ingredient for the production of active packaging (Jamróz, Kulawik, et al., 2021; Tkaczewska et al., 2021). The objective of this study was to add the protein hydrolysate from soybean bran as antioxidant compounds into single- and double-layered furcellaran films. It was checked how the deposition of the prepared ethanol extract from soybean husk will affect the functional properties of tested films. A further aim of the study was to analyse the effect of antioxidant active coatings on the oxidation degree of butter stored at refrigerated and room temperature. To the best of our knowledge, there is no reported work on using protein hydrolysates from soybean and furcellaran to fabricate an antioxidant packaging film.

2. Materials and methods

2.1. Materials

Furcellaran (FUR) (type 7000) was purchased from Est-Agar AS (Karla village, Estonia). Glycerol was procured from P.P.H STANLAB sp. j. (Lublin, Poland).

Soybean bran - waste from the production of soy flour - was obtained from the Z.P.H.U Roma company (Poland) for preparation of the hydrolysate. The basic composition of soybean bran was determined according to AOAC methods and comprised the following parameters: dry weight 90.59%±0.02, protein 33.44%±4.13, fat 2.81%±0.05, ash 5.61%±0.02 insoluble dietary fibre 37.5%±1.30, soluble dietary fibre 7.3%±0.3 and carbohydrates 3.91%±0.02.

All chemical reagents were used as upon receipt, without being subjected to further purification. The distilled water applied in all experiments was obtained using the 3-stage Millipore Direct-Q 3UV purification system (Burlington, MA, USA).

2.2. Preparation of single- and double-layer films

Soybean bran was ground in a laboratory mill (MMK-06, MPM Poland) and the raw material prepared in this way was mixed with distilled water at a ratio of 1:20. The suspension was mixed with a magnetic stirrer (MR, Heidolph, Germany) at 350 rpm for 15 min. Hydrolysis of the soybean bran was conducted using food-grade enzymes Alcalase® (Novozymes, Bagsværd, Denmark). Hydrolysis conditions were determined on the basis of available literature: pH 8.0, 65 °C. The earlier-prepared suspension, heated to the set temperature and pH, was adjusted using 1 M NaOH. Adding enzyme preparations was 2% for protein content. Hydrolysis was performed for 180 min. During the first 15 min, the pH was continuously monitored, corrected with 1 M NaOH, and following, the pH was controlled every 15 min. The reaction was terminated by maintaining the hydrolysates at 95 °C for 15 min while the samples were centrifuged at 4,000 × g for 15 min at 15 °C. The soy bran hydrolysate was freeze-dried via LyoQuest (Telstar, Tokyo). The obtained hydrolysate was characterised by high antioxidant activity totalling 15.58 μM Trolox/mg (FRAP value), 29.05% (ability to reducing free radicals DPPH) and 95.15% (metal chelating activity), respectively. The content of polyphenols in the obtained hydrolysate was 488.73 gallic acid equivalent mg/mL.

The furcellaran (2.5 g/400 mL H_2O) dispersion was prepared. Consequently, the film-forming dispersion was mixed with the protein hydrolysate from soybean (5 g) and glycerol (4 mL). The dispersion was stirred at 50 °C for 30 min, and 100 mL of distilled water was added. The furcellaran/protein hydrolysate from soybean film was labelled as FUR + HSOY (single-layered film).

The double-layer films were prepared analogously to the method developed by Tkaczewska et al. (2021), with modifications. The FUR (2.5 g/400 mL H_2O) dispersion was prepared. Afterwards, the film forming dispersion was mixed together with HSOY (5 g) and glycerol (4 mL). The dispersion was stirred at 50 °C for 30 min. The obtained film-forming dispersion was poured into specially prepared rectangular moulds (30 cm × 20 cm) and left for 3 h until gel formation. Then, a 10% soy bran solution (20 g/200 mL) was prepared in 70% ethanol (marked as ethanol extract of soy bran). The achieved extract was filtered and then 200 mL of this extract placed on the previously poured FUR/HSOY gel, which was allowed to dry for 24 h. The ethanol extract of soybean husk accounted for 28.57% of the whole mass of the solution.

The concentration of the ethanol extract from the soybean husks added to the double-layered films was selected based on literature data (Chung, Ji, Canning, Sun, & Zhou, 2010) and preliminary studies assessing the antioxidant properties of coatings with different concentrations of the extract.

After the drying process, the active films were removed from the dishes and the bioactive side of the film was marked with tape and labelled as the FUR + HSOY/SOY extract (double-layered film).

2.3. Polyphenols and antioxidant properties of the soybean bran ethanol extract and obtained coatings

2.3.1. Analysis of polyphenols in extract of soy bran

For the determination of polyphenols samples (~0.5 g) homogenized with solvents as ethanol/water/ascorbic acid (70:29:1, *v/v/v/m*) were used for extraction as previously described by Wojdyło, Nowicka, Turkiewicz, and Tkacz (2021). Finally, before analysis the extract was filtered by hydrophilic PTFE membrane (0.20 μm, Millex Simplicity Filter; Merck, Germany). Polyphenols were analyzed using an Acquity UPLC system (Waters Corp., Milford, MA, USA) equipped with a photodiode (PDA) with binary solvent manager (Waters Corp., Milford, MA, USA) series according to the method (Wojdyło, Oszmiański, & Czemerys, 2007). The mixture was separated in a BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters Corp.; Ireland) and used for analysis. Quantification was achieved by standard compounds (0.05 to 0.5 mg/mL; $r^2 \leq 0.998$)) made from genistein-7-glucuronide. The data were collected by Empower 2.0 Manager (Waters Corp., Milford, MA, USA) software. All samples were done in triplicate.

2.3.2. Ferric reducing antioxidant power (FRAP)

The film extracts (15 mg/mL) were prepared by adding 150 mg of the films to 10 mL of distilled water, and then heated to a temperature of 45 °C. The tubes with film extracts were placed in a 50 °C water bath and shook for 10 min to ensure that the films were totally dissolved.

A measure of the reducing power regarding the soy bran ethanol extract or film extract was performed according to the method described by (Khantaphant & Benjakul, 2008), with some modifications. Following, 150 μL of the soy bran ethanol extract or film extract was incubated at 37 °C with 2.85 mL of the FRAP reagent containing 10 mM of TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM of $FeCl_3$. Absorbance values were read at 595 nm after 30 min, using the Helios Gamma UV-1601 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The results were calculated as the μmol of Trolox equivalent per 1 mg of film.

2.3.3. DPPH radical scavenging

For the $DPPH^{\bullet}$ assay, the soy bran ethanol extract or film extract (7,5

mg/1mL) was mixed with 0.1 mM DPPH$^{\bullet}$ in an ethanol solution using a 0.2:2.8 ratio (v/v) and incubated in the dark for a 30-min period. Consequently, absorbance of the solution (A_{sample}) was measured at 517 nm (Helios Gamma, Thermo Fischer Scientific, USA) and compared with blanks, in which the film extract was replaced with distilled water (A_{blank}). The results are expressed as % of inhibition:

$$DPPH\ radical\ scavenging\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

2.3.4. Metal ion chelating ability

The film ability to chelate ferrous ions was evaluated using the method proposed by Xie, Huang, Xu, and Jin (2008). One mL of ethanol extract of soy bran or films extract was first mixed with a 50-μl 2 mM $FeCl_2$ solution and 1.85 mL of 5 mM ferrozine. After 10 min, the absorbance of the reaction mixture (A_{sample}) was measured at 562 nm and compared with blanks, in which the film extract was replaced with distilled water (A_{blank}). The ferrous ion chelating ability was calculated using the following formula:

$$Chelating\ ability(\%) = 1 - (\frac{A_{sample}}{A_{blank}}) \times 100\%$$

2.4. Measurement of film thickness

Film thickness was measured via a manual instrument: Mitotuyo, No. 7327 (Kawasaki, Japan). The measurement was carried out to the nearest 1 μm for 5 points, equally distributed around a circle, 10 mm from its edge.

2.5. Water content and solubility

The methodology for measuring solubility and water content of single- and double-layered films was presented earlier (Jamróz, Kulawik, et al., 2021).

2.6. Water vapour transmission rate (WVTR)

The methodology for testing the WVTR of single- and double-layered films was presented in our previous work (Jamróz, Tkaczewska, et al., 2021).

2.7. Determination of contact angle

The determination of contact angle was conducted as previously described by Jamróz, Tkaczewska, et al. (2021).

2.8. Surface colour measurement

The methodology for surface colour measurement of single- and double-layered films was presented earlier (Jamróz, Kulawik, et al., 2021).

2.9. Mechanical properties

The methodology for measuring mechanical properties of tested films was presented earlier (Jamróz, Tkaczewska, et al., 2021). Determination of tensile properties was carried out based on the standards for plastics, the general principles of which are presented in ISO 527–1. Test conditions were in agreement with ISO standard 527–3:1995.

2.10. Scanning electron microscopy (SEM)

FUR/CMC + HGEL film morphology was analysed using the JEOL JSM − 7500F Field Emission Scanning Electron Microscope, equipped with a Retractable Backscattered-Electron detector (RBEI) and EDS (energy dispersive spectra) detection system of characteristic X-ray radiation – the INCA PentaFetx3 EDS system. To conduct the observation of double-layered film conformation sample cross-sections were made.

2.11. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis of the films was conducted using the DSC 204F1 (Phoenix Netsch GmbH, Germany) differential scanning calorimeter. The film samples were hermetically sealed in aluminium pans and heated in a calorimeter from 25 to 300 °C at a rate of 10 °C/min. The empty aluminium pan was used as a standard. Thermal transition temperatures and enthalpy were calculated using the Proteus Analysis software (Netzsch GmbH, Germany). Enthalpy values were expressed in J/g. Each sample was calculated in triplicate.

2.12. UV–Vis spectroscopy analysis

UV–Vis analysis was carried out using a UV-5500 spectrophotometer (UV 5500 Metash, Shanghai Xiwen Biotech, China), and the absorbance spectrum was registered between 200 nm and 800 nm.

2.13. Evaluation of butter storage quality

2.13.1. Butter treatment

Evaluation of active packaging effectiveness was performed on the example of butter. Butter having a fat content of 82% from one production series (Mlekovita, Wysokie Mazowieckie, Poland) was purchased in a large-area store. The 50 g of butter was wrapped in each type of the active films and synthetic ones (LDPE coating). The surface of the butter packed in the double-layer film was in contact with the active coating layer (with ethanol extract of soybean bran). In order to facilitate the observation of oxidative changes, selected samples were stored at room temperature (~25 °C) and analysed every 3 days (for a 12-day storage period). The second group of tested samples was stored in refrigerated conditions (+4°C) and analysed every 6 days (for 12 days). Prior to each analysis, the film was taken off from the butter sample. The butter samples were homogenised in the R2 Robot Coupe blender (Vincennes, France).

2.13.1.1. Fatty acid composition. The fat was extracted using the method developed by Folch, Lees, and Sloane-Stanley (1957), and then esterified by the method proposed by Ledoux, Chardigny, Darbois, Soustre, Sébédio, and Laloux (2005). The fatty acid methyl esters were analysed using the TRACE 1300 GC (Thermo Scientific, USA), equipped with a FID detector on a BPX-70 60-m × 0.25-mm × 0.20-mm column (Phenomenex, Torrance, USA). Helium, with a 5 mL/min flow, was used as a carrier gas. The split flow was set to 10 mL/min. The applied temperatures were 240 °C for the detector and 220 °C for the feeder. Baseline temperature was 60 °C, which was maintained for 3 min, then there was a temperature increase 7 °C/min until reaching 200 °C, and following, this was maintained for 20 min: dispenser temp.: 220 °C, detector temperature: 250 °C. The identification of fatty acid methyl esters was done by comparing retention times with Supelco 37 FAME Mix standards (Sigma-Aldrich, USA). Analysis on each sample was performed in duplicate. Two injections into GC were made per duplicate (n = 2x2x3).

2.13.1.2. Oxidation rate of butter lipids. TBARS analyses were conducted as previously described by Jamróz, Kulawik, Guzik, and Duda (2019). The determination of peroxidate value was done using the Acetic Acid-Chloroform method (AOAC Official Method 965.33 "Peroxide Value of Oils and Fats" AOCA-AOAC Method). Acid value was determined via the titration method (AOAC Official Method 969.17 Acid Value of Butterfat" IDF-ISOAOAC Method).

2.14. Statistical analysis

Statistical analysis was carried out via Statistica 13 software (Stat Soft, Inc., USA). *t*-Student tests were conducted to determine the differences between single and double-layered films. Water contact angle was analysed using nonparametric test U Mann-Whitney after conducting the Shapiro-Wilk test, in which non normal distribution of results was detected.

The results of butter analyses were conducted performing the Shapiro-Wilk test and after confirming the normality of the results two-way ANOVA test with sample variant and storage time as two factors. To determine the differences between means Tukey's post-hoc test was used at $P < 0.05$. In cases where the distribution was not normal, Box-Cox transformation was applied and the ANOVA analysis was repeated.

3. Results and discussion

The preparation of double-layered films, by deposition of active compounds on the 1st layer, was developed by our team earlier (Jamróz, Kulawik, et al., 2021; Tkaczewska et al., 2021). In this study, the 1st layer was furcellaran and soybean hydrolysate, while the active 2nd layer in the film was a soybean husk ethanol extract with high antioxidant potential.

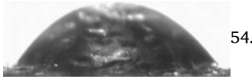
According to Ashaolu (2020), soy by-products should be used as ingredients in functional foods and nutraceuticals. Accordingly, it seemed advisable to use waste from soybean flour production as an active ingredient in the coatings.

The obtained films were homogeneous with a delicate yellow–brown colour. The thickness of the FUR + HSOY film was 0.09 ± 0.01 mm, while the application of the second layer in the form of soybean husk extract increased the film thickness (0.14 ± 0.01 mm). The reason for this is the enrichment of the film with additional solid elements.

3.1. Water properties

The water properties of the film were determined by analysing their

Table 1
Properties of single- and double-layered films.

	FUR + HSOY	FUR + HSOY/SOY EXTRACT
Water properties		
Water content [%]	13.85[a] ± 0.21	12.30[b] ± 0.37
Solubility [%]	100[a]	100[a]
WVTR [g/m^2 × d]	784.58[a] ± 21.78	795.65[a] ± 3.68
Water contact angle [°]	 33.84[a] ± 1.00	Soy extract  54.44[b] ± 1.82 FUR + HSOY  38.56[c] ± 1.01
Thermal properties		
T_m [°C]	166.9[a] ± 5.7	154.1[a] ± 3.1
ΔH [J/g]	141.58[a] ± 17.50	169.97[a] ± 19.43
Color properties		
L*	82.36[a] ± 0.42	80.59[b] ± 0.81
a*	0.18[a] ± 0.03	0.94[b] ± 0.13
b*	29.28[a] ± 0.49	31.66[b] ± 0.83
ΔE	–	3.06
Appearance		
Mechanical properties		
Max. breaking load [N]	5.56[a] ± 0.82	4.12[b] ± 0.62
Tensile strength [kN/m]	0.37[a] ± 0.05	0.27[b] ± 0.04
Elongation at break [%]	36.56[a] ± 4.42	26.98[b] ± 7.38
Modulus of elasticity [MPa]	8.65[a] ± 0.57	30.82[b] ± 5.83
Antioxidant properties		
FRAP [μM Trolox/mg]	6.60[a] ± 0.04	8.40[b] ± 0.02
DPPH [%]	10.26[a] ± 3.03	18.55[b] ± 0.02
Metal chelating activity [%]	99.97[a] ± 0.01	99.97[b] ± 0.01

[a,b,c] – different letters in particular rows indicate significant differences between means ($P < 0.05$).

water content and solubility (Table 1). FUR + HSOY films had a water absorption of 13.85%, and the addition of soybean husk extract significantly affected this parameter, causing reduction in its value. After 24 h in water, the films were 100% soluble. Unfortunately, although high solubility is crucial for the fast packaging degradation, it is not advantageous to be used in packaging of materials. The water vapour permeability of the film is also a quite significant parameter when storing food products. Both films have a high WVTR factor. The 2nd layer addition did not affect the WVTR parameter (Table 1).

To evaluate the method of applying the 2nd active layer to the 1st one, the contact angle of the 1st and 2nd film layers was determined (Table 1). The FUR + HSOY film had a contact angle value of 33.84°.

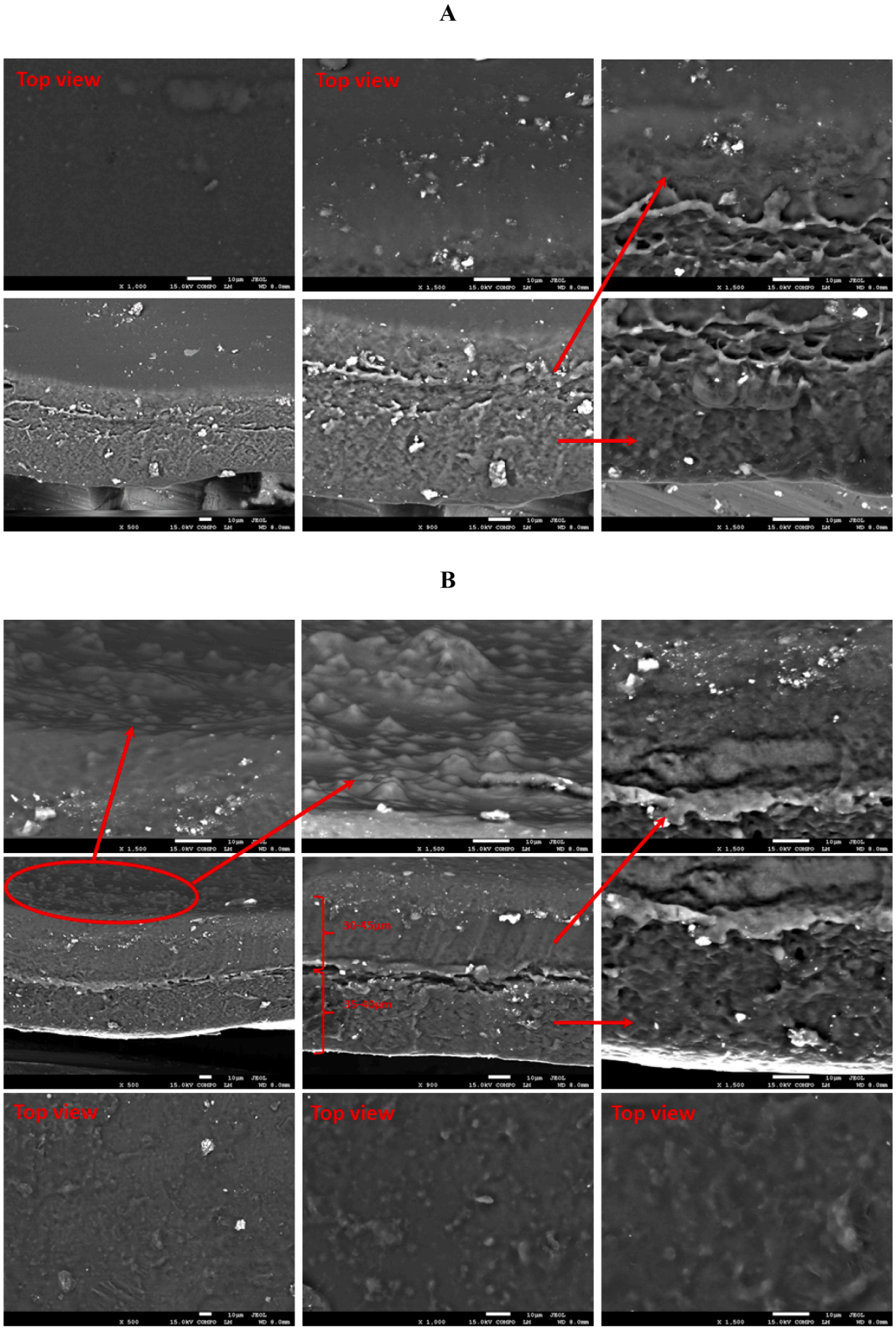


Fig. 1. SEM photograph of (A) FUR + HSOY and (B) FUR + HSOY/SOY EXTRACT.

FUR films with the addition of gelatin hydrolysates had a higher contact angle value (50.9°) (Jamróz, Kulawik, et al., 2021), which can be attributed to the presence of greater interactions between the gelatin hydrolysate and the polysaccharide. The obtained result confirms the highly hydrophilic nature of the single-layered film. The application of a layer of soybean husk ethanol extract caused the contact angle value to increase by almost 60%. Moreover, there was a slight increase in the value of the contact angle for the FUR + HSOY layer. However, there was no change in the nature of tested films, as they remained hydrophilic. Our research team achieved completely different results in this study (Jamróz, Kulawik, et al., 2021), where the application of the Ala-Tyr active layer resulted in a reduction of the contact angle. This behaviour can be attributed to the type of applied layer. Furthermore, the results suggest that the film with the soybean husk ethanol extract layer causes less water absorption via the film surface.

3.2. Microscopic photographs

SEM images of tested films were presented in Fig. 1. The FUR + HSOY film (Fig. 1**A**) shows a homogeneous structure with visible individual solid elements, which may be an unbonded soybean hydrolysate. Kchaou, Jridi, Benbettaieb, Debeaufort, and Nasri (2020), who added cuttlefish (*Sepia officinalis*) skin protein hydrolysates to gelatin films, came to similar conclusions. The application of the extract to the FUR + HSOY layer indicates that a double-layered film was formed (Fig. 1**B**). In the earlier work (Jamróz, Kulawik, et al., 2021), the deposition of the Ala-Tyr aqueous solution on the furcellaran-gelatin hydrolysates layer did not indicate the cross-section of the two separate layers, while in this work, the deposition of the ethanol extract indicated the formation of two layers. In the SEM pictures, it irregular ethanol layer with many solid elements can be observed. During the preparation of the ethanol solution, insoluble parts of the soybean bran may have been left. The clear separation of the layers in the double-layered films in the SEM photos has already been shown (Jamróz, Cabaj, et al., 2021; Jamróz, Tkaczewska, et al., 2021).

3.3. Thermal properties

DSC analysis was used to measure the peak melting temperature (T_m) and the enthalpy change (ΔH) of tested films (Table 1). The addition of the ethanol layer did not affected the thermal stability of the FUR/HSOY film. The ΔH values increased with the addition of the layer enriched with soybean husk extract, indicating an increasing tendency of thermostability, however, these changes were not statistically significant. A completely different trend was observed by Hajji et al. (2021) by introducing shrimp and crab protein hydrolysates to chitosan–gelatin films. The slight decrease noted by these authors in the temperature value of the double-layered films may be related to the disturbance of the interaction of the FUR/HSOY layer with the ethanol soy extract layer, which may further result in an increase in the free volume between the layers and the mobility of molecules.

3.4. Mechanical properties

The mechanical properties of the tested films were determined (Table 1). The addition of the active layer caused a significant decrease, both in terms of mechanical strength and flexibility. This is probably connected with weakening of the homogeneous structure and the deterioration of interfacial adhesion. The type of active layer applied has great influence on the mechanical properties of the double-layered films. TS values of the obtained single- and double-layer films are much lower than TS values of LDPE films (not<10 MPa) (Szlachetka, Witkowska-Dobrev, Baryła, & Dohojda, 2021). The addition of the Ala-Tyr aqueous solution layer improved the mechanical properties of the FUR film with the gelatin hydrolysate (Jamróz, Kulawik, et al., 2021). Application of the ethanol layer deteriorated the intermolecular effects of the film, and this contributed to the deterioration of the dense structure of the film and its brittleness. Similar conclusions were reached by de Morais Lima, Bianchini, Guerra Dias, da Rosa Zavareze, Prentice, and da Silveira Moreira (2017) who added fish protein hydrolysates to chitosan-xanthan gum films. The authors concluded that the slight decrease in tensile strength can be attributed to the formation of disulphide bridges, hydrophobic interactions and hydrogen bridges in the proteins, which may make the film brittle.

3.5. Colour properties

The colour of the tested films was assessed (Table 1). Slight changes in the colour of the film were observed after the application of the soybean husk extract. The double-layered film had lower L* values (it was darker) and higher a* (reddish/greenish) and b* (yellowish/bluish) values. ΔE-values greater than 3 may indicate colour differences that are quite distinct and can be perceived by an inexperienced observer. The ΔE value of double-layered films, measured in relation to the FUR + HSOY film, is 3.06. Moreover, the differences in colour can be seen with the naked eye (Table 1). The dark brown colour of the hydrolysate and the yellow colour of the soybean husk ethanol extract significantly influenced the colour of the furcellaran-based film. Y.-K. Lee, Ko, Davaatseren, and Hong (2016) reached similar conclusions when using soy protein hydrolysates from two different soybean species for pork chops.

3.6. UV–Vis spectrum

UV light can lead to undesirable reactions in food products, such as lipid oxidation, discolouration and loss of nutrients, therefore, protection against UV radiation is an important functional property of packaging materials (Nouri, Yaraki, Ghorbanpour, Agarwal, & Gupta, 2018). The UV–Vis absorption spectra of films are demonstrated in Fig. 2. Pure furcellaran films show no absorption in the entire spectrum (Jamróz, Kopel, et al., 2019). The addition of HSOY to the film and the application of a second layer of soybean husk ethanol extract increased the absorption within the range of 200–350 nm, which may make this type of film a suitable material for sunlight blocking applications (You, Zhang, Liu, & Lei, 2016).

3.7. Antioxidant activity of films

Packaging with antioxidant properties is one of the main types of active packaging that inhibits the oxidation process of food ingredients, and thus, can extend their shelf-life (Jamróz, Tkaczewska, et al., 2021). The antioxidant properties of all films are shown in Table 1.

Both of the tested films were characterised by good antioxidant properties measured by the FRAP method, and the values for double-layer films with the addition of soy bran extract were significantly higher than for single-layer films. A similar relationship was observed when examining antioxidant properties with the DPPH$^{\bullet}$ test. The films without the addition of the extract showed a significantly lower ability to scavenge free radicals than the double-layer film. No significant differences were noted in the metal ion chelating ability between the tested films. Both types of films showed very high antioxidant activity measured by this test, amounting to 99.97%. The antioxidant properties of the tested single-layer films result from the content of soybean bran hydrolysate, which is characterised by very high antioxidant properties. The increase in this activity in the double-layer films is due to the presence of the second layer, which is an extract of the same soybean bran. The 10% soybean bran extract had an iron ion reduction of 0.311 ± 0.01 mM/L Fe_2SO_4, a free radical scavenging ability of 78.4%±0.08, and a metal ion chelating capacity at the level of 55.85%±5.08. The high antioxidant properties of the obtained extract are mainly due to the high content of polyphenolic compounds. Polyphenols are one of the most important groups of compounds widely present in soy husk.

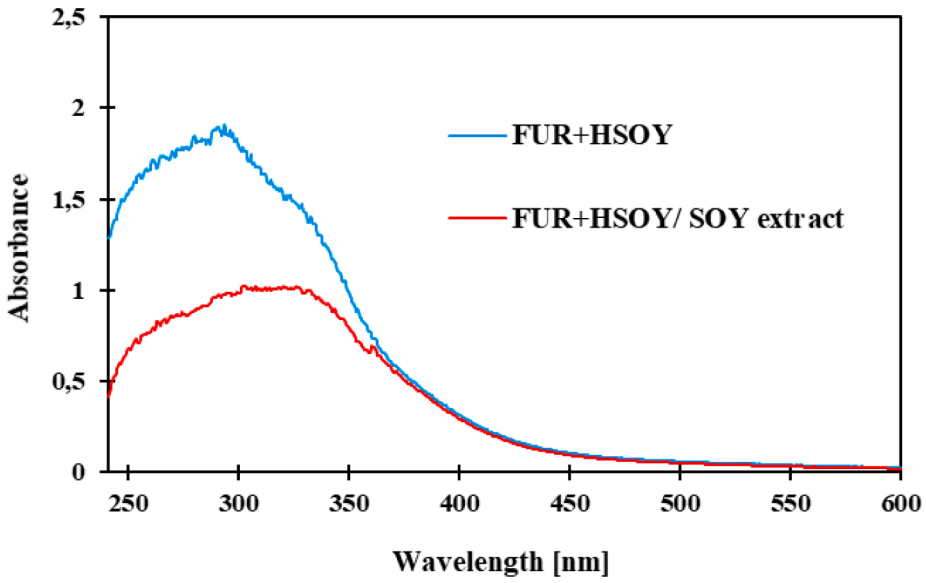


Fig. 2. UV–Vis spectrum of FUR + HSOY and FUR + HSOY/HSOY extract films.

Polyphenolics in soy husk were detected and identified using LC-ESI-MS QTof with PDA-visible data. The 23 compounds identified in soya hulls belonged primarily to isoflavones and phenolic acids (Suppl. Table 1). Among isoflavonoids mainly genistein (*m/z* 269.05) were the most abundant derivatives, then daidzein (*m/z* 253.06), glycitein (*m/z* 283.07), and hesperetin (*m/z* 301.09). Additionally some phenolic acids were detected in the analyzed sample. Most of the identified components found in this study have been previously reported in the composition of soybeans by Dueñas, Hernández, Robredo, Lamparski, Estrella, and Muñoz (2012), Kang, Hick, and Price (2007), S.-J. Lee et al. (2008) and March, Miao, Metcalfe, Stobiecki, and Marczak (2004). The total content of phenolic compounds was 6775.37 mg/kg, where isoflavonoids equal to 5874.48 mg/kg which accounts for 86.7% of total compounds. In the previous literature data (Tyug, Prasad, & Ismail, 2010), it has been shown that soy husk extract has been proven to be a good source of bioactive compounds, especially chlorogenic, ferulic, gallic, syringic and vanilic acids.

Comparing the antioxidant activity of both tested films with the antioxidant activity of the pure soybean hydrolysate, a decrease in the activity of the hydrolysate after its addition to the film can be observed. A similar phenomenon was observed in our previous research, where gelatin hydrolysate from carp skin was added to the films (Jamróz, Kulawik, et al., 2021). Lower film antioxidant activity can be explained by interactions between the furcellaran matrix and active molecules in the film, limiting their release (Kchaou et al., 2020). Moreover, similar results were achieved by Giménez, Gómez-Estaca, Alemán, Gómez-Guillén, and Montero (2009), who noted that squid gelatin hydrolysates demonstrated a lower level of antioxidant capacity within films than in free form (added to a filmogenic solution in an analogous amount). It is probable that this occurred due to interactions taking place between the peptides and film matrix formed through the process of hydrogen bonding.

3.8. Evaluation of butter oxidative stability

Rancidification is a significant problem in the storage of butter. This process, because of lipolysis (free fatty acid release) and fatty acid oxidation, causes a reduction in the taste and nutritional values of butter, therefore, causing economy-related losses to the dairy industry as well as problems with regard to food distribution. Lipolytic changes take place in milk fat as a consequence of triacylglycerol hydrolysis through lipases.

To slow down the oxidative processes in butter, active packaging with antioxidant properties can be used. Therefore, the tested films were applied to samples of butter from cows (Fig. 3).

3.8.1. Fatty acid composition

The changes in fatty acid composition of lipids provide an indirect measure of susceptibility to lipid oxidation (Maskan & Karataş, 1998). The profile of fatty acids in samples of butter stored in different conditions is presented in **Suppl. Tables 2 and 3**.

In fresh butter, the most abundant fatty acid was palmitic acid (C16:0) (33.52%), followed by oleic (C18:1n–9) (22.16%), myristic (C14:0) (11.15%), stearic (C18:0) (10.50%), lauric (C12:0) (3.48%), capric (C10:0) (3.15%) and butyric acid (C4:0) (3.22%). Despite the ranging variation factors which influence the fatty acid profile of cow butter, the results are consistent with those reported for cow's milk butter and cow's yoghurt butter in previous studies (Smykov, Topnikova, & Danilova, 2021; Staniewski, Ogrodowska, Staniewska, & Kowalik, 2021).

The type of packaging was not found to affect fatty acid profile or the quantity of each fatty acid during the 3rd, 6th or12th day, when the butter was stored at room temperature. Only on the 9th day of storage, there was an increase noted for the content of short-chain fatty acids (C4: 0, C6:0, C8:0, C10:0) in the butter samples packed in double-layer coatings with regard to the butter samples packed in the synthetic film. According to (Méndez-Cid, Centeno, Martínez, & Carballo, 2017), the release of short-chain fatty acids during storage could be relevant for sensory characteristics because they could provoke rancid flavouring of the butter.

Fig. 3. Appearance of butter in active and synthetic films.

However, due to the fact that these observed differences were not statistically significant in relation to the other studied groups, it may be assumed that they do not affect the butter quality. The fatty acid profile of butter stored at chilled temperature for 12 days was also not statistically different with respect to the type of applied packaging. The only difference that was observed was a statistically significant decrease in the total of polyunsaturated fatty acids in the butter packed using double-layer films on the 12th day of storage, which may indicate more advanced oxidation processes in this group. Nonetheless, to confirm such conclusions, further research using the accelerated shelf-life testing method is needed (Méndez-Cid et al., 2017).

3.8.2. *Evaluation of butter oxidative stability*

The formation of secondary oxidation products (aldehydes and carbonyl compounds) which trigger the off-flavour attribute of a food product was measured using the TBARS test (Adilah, Noranizan, Jamilah, & Hanani, 2020). The changes of TBARS values for butter packed in different films during storage at different temperatures are presented in Table 2.

It has been found that the TBA index increased statistically on day 12 for samples packed in synthetic and single-layer films stored at chilled temperature, compared to fresh butter (day 0). Such an increase was not observed for the samples stored in double-layer films. In a previous study (Labuza & Bergquist, 1983), it has been reported that lipid oxidation is exponentially related to temperature, which is why it was expected that the samples stored at room temperature would show a greater increase in TBARS compared to samples subjected to chilled storage. Surprisingly, no statistically significant increase in the TBARS index was found for the butter samples in various coatings during storage at room temperature. The only statistically significant difference was the increase of this index on the 12th day of storing the butter samples packed in the double-layer films (1.22 mg malonaldehyde/kg). The double-layer film under study was enriched using a soy bran extract with high polyphenol content. It was able to migrate to the butter. The degradation pathway concern some phenolic compounds may generate phenolic aldehydes, which may further provoke a similar response to that in the case of malonaldehyde during the lipid oxidation of butter evaluated via TBARS determination (Oussalah, Caillet, Salmiéri, Saucier, & Lacroix, 2004). This would probably result in an increase of TBARS values, which would mask the antioxidant effect of the double-layer coatings. In order to verify this hypothesis, further research should be carried out using a different type of food matrix.

The primary oxidation products of butter can be measured as peroxide value (PV). Oxidation of butter during storage for 12 days in cold and at room temperature is represented in Table 2. The results showed that all samples increased in PV and reached a maximum value after 12 days of storage. There was no statistically significant effect of the type of packaging on the peroxide value of butter stored at room temperature. This may be because the storage duration was too short, during which it was difficult to observed the occurring differences.

At the end of the storage period, butter packed in double-layer films and stored at 4°C showed the highest PV compared to other samples. This is a surprising result as in the research hypothesis, it was assumed that the addition of soy bran extract would increase the polyphenol content in the coating, which would further result in an antioxidant effect among food products. Due to the fact that food is a very complicated matrix, it is difficult to predict what interactions will occur between its ingredients and antioxidants and what will be the result of this be (Aruoma, 1996). Moreover, it may also be assumed that the antioxidant properties of the soybean bran extract decreased after complexation with the coating ingredients, and therefore, no *in vivo* effect was shown.

According to Kalinowska, Gryko, Wróblewska, Jabłońska-Trypuć, and Karpowicz (2020), the oxidant activity of polyphenol compounds changed from anti- to pro-oxidant when the lifetime of phenoxyl radicals is prolonged due to spin-stabilising effects induced by metal cations. Furthermore, the pro-oxidant activity of polyphenol extracts increased with their concentration. Thus, it may be assumed that a too high concentration of polyphenols in the double-layer coating may cause more advanced oxidative changes in the butter packed with this film.

According to various data in literature on the subject (Adilah et al., 2020; Arshad et al., 2020; Mahdi & Bassiri, 2015), the addition to the coatings of plant extracts as the antioxidant source could slow down PV during the storage of butter and margarine. However, on the basis of the obtained results, it can be concluded that the addition of soybean bran extracts applied as the second coating layer, despite causing an increase

Table 2
Fat quality parameters of the room-temperature and cold-stored butter (mean values ± standard errors).

	Storage time [days]	TBARS [mg malonaldehyde/kg]		Peroxide value [mg]		Acid value [mg KOH/g]	
Room-temperature stored (25 °C)							
Fresh butter		0.34^{bc}	±0.02	0.52^{d}	±0.10	0.33^{e}	±0.06
CONTROL							
	3	0.21^{c}	±0.04	0.63^{d}	±0.22	0.97^{bcd}	±0.04
	6	0.68^{abc}	±0.03	2.80^{bc}	±0.52	0.99^{bcd}	±0.05
	9	0.59^{bc}	±0.05	3.48^{b}	±0.43	1.48^{bc}	±0.17
	12	0.59^{bc}	±0.04	7.18^{a}	±0.59	2.87^{a}	±0.20
FUR + HSOY	3	0.20^{c}	±0.04	1.20^{cd}	±0.20	0.79^{de}	±0.02
	6	0.57^{bc}	±0.27	3.09^{bc}	±0.27	0.91^{cde}	±0.04
	9	0.83^{ab}	±0.15	3.34^{b}	±0.26	1.56^{b}	±0.05
	12	0.60^{bc}	±0.06	8.49^{a}	±0.09	2.35^{a}	±0.02
FUR + HSOY/SOY EXTRACT	3	0.24^{c}	±0.02	0.48^{d}	±0.07	0.93^{cde}	±0.08
	6	0.45^{bc}	±0.04	2.70^{bc}	±0.25	1.05^{bcd}	±0.04
	9	0.84^{ab}	±0.17	2.87^{bc}	±0.32	1.57^{b}	±0.03
	12	1.22^{a}	±0.14	4.56^{ab}	±0.77	2.87^{a}	±0.31
Cold-temperature storage (+4°C)							
Fresh butter	0	0.34^{c}	±0.02	0.52^{c}	±0.10	0.33^{d}	±0.06
	6	0.48^{c}	±0.02	0.83^{c}	±0.32	0.97^{bc}	±0.09
CONTROL	12	1.02^{ab}	±0.11	1.37^{c}	±0.28	1.25^{ab}	±0.08
FUR + HSOY	6	0.63^{bc}	±0.10	0.41^{c}	±0.13	0.75^{c}	±0.04
	12	1.31^{a}	±0.22	3.96^{b}	±0.28	1.34^{a}	±0.06
	6	0.45^{c}	±0.11	0.83^{c}	±0.12	0.86^{c}	±0.04
FUR + HSOY/SOY EXTRACT	12	0.79^{bc}	±0.02	6.57^{a}	±0.23	1.28^{a}	±0.02

Control – control wrapped in cling film; FUR + SOY – butter wrapped in single-layer film; FUR + HSOY/SOY EXTRACT – butter wrapped in double-layer film.
^{a,b,c} – different letters indicate significant differences between means ($P < 0.05$).

in antioxidant film properties, does not allow to achieve the beneficial oxidation effects in the case of packed butter.

Acid value (AV) is a routine parameter in the description of fats and oils. An increase in the amount of free fatty acids in the fat sample shows hydrolysis of triglycerides. Determination of the butter's acid value after 12 days of storage in refrigerator and room-temperature conditions allowed to demonstrate that covering butter with the synthetic and soy protein hydrolysate coating did not have impact on the quality index of butter (Table 2).

On the starting day of the experiment, acid value (AV) was 0.33 mg KOH/g. After 12 days of storage, AV increased to 1.25–1.34 mg KOH/g and 2.35–2.87 mg KOH/g for the cold storage and room temperature storage groups, respectively. As earlier stated, active films are characterised by high antioxidant properties. Thus, it was assumed that they hinder the lipid oxidation process, and therefore, reduce the speed of acid number growth. The application of films causes the formation a protective layer, making the butter samples free of direct contact with the external surroundings, especially that of oxygen, which is the most linked to the process of lipid oxidation.

Hydrophilic edible coatings or films can act as a barrier to oxygen and carbon dioxide (Sun et al., 2019). The lack of statistically significant differences between the studied groups may be explained in the following: the control sample was covered using a synthetic film which, similarly as in the case of innovative coatings, formed a barrier against oxygen and other gases.

The application of an additional layer using the extract in furcellaran films increased the film's antioxidant activity while, at the same time, not affecting its ability to undergo lipid oxidation inhibition in the butter sample during storage. These results could be explained by the delayed release of the active molecules from the film matrix or by the interactions established between the film matrix and the active molecules in the film, which limited their *in vivo* effect (Kchaou, Jridi, Benbettaieb, Debeaufort, & Nasri, 2020).

4. Conclusion

In this study, single-layered films based on furcellaran and protein hydrolysates from soybean bran were successfully produced, as well as double-layered films in which a second layer based on soybean bran ethanol extract was deposited onto the first layer. The addition of the second layer improved UV-barrier properties and hydrophobicity. The antioxidant activity has been enhanced, which suggests the potential use of the film as active packaging against oxidation of packaged food. Therefore, the produced films were used as active packaging materials for storing butter. Despite the high antioxidant activity of the designed single-layer films, they are not effective in inhibiting the oxidation of butter lipids. However, it may be concluded that the samples packed in these films do not differ in quality from the samples packed in synthetic films. Completely edible films made of fully biodegradable waste materials are a promising alternative to synthetic packaging. Both the decrease in the amount of polyunsaturated fatty acids and the higher TBARS and PV indices of butter packed in double-layer films noted on the 12th day of storage may indicate that despite high *in vitro* antioxidant properties, these coatings may show pro-oxidative *in vivo* activity. Nonetheless, further research is required to verify this hypothesis.

CRediT authorship contribution statement

Ewelina Jamróz: Conceptualization, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Joanna Tkaczewska:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. **Marzena Zając:** Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Paulina Guzik:** Investigation, Methodology. **Lesław Juszczak:** Investigation, Methodology. **Agnieszka Kawecka:** Investigation, Methodology. **Katarzyna Turek:** Investigation, Methodology. **Małgorzata Zimowska:** Investigation, Methodology. **Aneta Wojdyło:** Investigation, Methodology.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132883.

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