Ternary potato starch-furcellaran-gelatin film – a new generation of biodegradable foils

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Abstract: Foils were prepared from potato starch (S), furcellaran (F) and gelatin (G) (S/F/G foils) using glycerol as a plasticizer. Their mechanical properties, aqueous solubility, water content, water uptake, enzymatic hydrolysis, and thermal (DSC) properties were determined. The 0.16 mm thick foil has ~ 80 MPa mechanical resistance with an elongation at break of 27.8 %. The aqueous solubility and water uptake of the S/F/G foils reached 35 % and 145 %, respectively. The S/F/G foils were susceptible to hydrolysis with polysaccharide enzymes such as glucoamylase and Viscozyme L (a blend of arabanase, cellulase, β-glucanase, hemicellulase and xylanase), as well as trypsin, a proteinase.

Keywords: furcellaran, gelatin, starch, biodegradable films.

Trójskładnikowa folia: skrobia ziemniaczana-furcellaran-żelatyna jako nowa generacja biodegradowalnych folii

Streszczenie: Z mieszaniny skrobi ziemniaczanej (S), furcellaranu (F) i żelatyny (G) oraz glicerolu jako plastyfikatora otrzymywano trójskładnikową folię S/F/G. Zbadano właściwości mechaniczne, rozpuszczalność, zawartość wody, wodochłonność, podatność na hydrolizę enzymatyczną oraz właściwości termiczne (DSC) wytworzonej folii. Folia o grubości ok. 0,16 mm wykazywała wytrzymałość mechaniczną ~ 80 MPa, a jej wydłużenie przy zerwaniu wynosiło 27,8 %. Folia S/F/G charakteryzowała się małą rozpuszczalnością (ok. 35 %) i wodochłonnością (ok. 145 %). Zbadano kinetykę reakcji hydrolizy enzymatycznej folii S/F/G w obecności enzymów polisacharydowych: glukoamylazy i Viscozyme L (mieszanina enzymów arabinazy, celulazy, β-glukanazy, hemicelulazy, ksylanazy) oraz enzymu białkowego trypsyny.

Słowa kluczowe: furcellaran, żelatyna, skrobia, folie biodegradowalne.

Replacing plastics with biodegradable materials is one promoted way of reducing the volume of waste. Currently, a growing interest is noted in biodegradable packaging materials [1–3]. Attention was paid to foils designed from natural, versatile sources, such as polysaccharides and proteins. Foils made from such sources are proposed for enveloping small doses of food such as snacks and separating layers of components.

Materials made of proteins and polysaccharides are biodegradable, they have good barrier properties for odors, aromas and lipids and obstruct the migration of humidity. Moreover, they can be good carriers for fragrances, bactericides, antioxidants and dyes in food technology, as well for bactericides and fungicides in agriculture. Polysaccharide foils are highly stable and because of their hydrophilic character offer a weak barrier against water vapor.

Protein foils possess better mechanical properties and suppressed permeability for oxygen but not for other gases. These properties rationalize the attention paid to pure polysaccharide and polysaccharide – protein blends capable of forming stable and compact networks [4].

This paper presents the preparation and properties of foils made of furcellaran, potato starch and gelatin blended with one another in various proportions and combinations. Potato starch is one of the most common components of packaging materials. It is a versatile, cheap material offering foils of good mechanical sorption and optical properties. In combination with other polysaccharide and protein components it offers interesting barrier properties [5].

Like potato starch, furcellaran is also a natural, anionic polysaccharide. Its' structure and functional properties resemble these of carrageenans [6]. Its linear, non-branched chain is built of (1→3)-β-D-galactopyranose units bearing sulfate groups at the C-4 position, which are neutralized with calcium, magnesium and potassium ions [7], and a (1→4)-3,6-anhydro-α-D-galactopyranose unit. There are some reports of foils made from carrageenans [8, 9] but foils from furcellaran remain unknown.

Gelatin is a heterogenic substance of molecular weight from $10 \cdot 10^3$ to $400 \cdot 10^3$ [10]. It is composed of a unique sequence of amino acids, among them a considerable amount of glycine, proline and hydroxyproline units. The two latter amino acids provide the gelatinizing effect [11]. It is

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used as a functional additive to dairy products, fish, meat and bakery products and drinks. It is also useful as an encapsulating agent in the pharmaceutical industry [12].

EXPERIMENTAL PART

Materials

– Furcellaran ($M_w = 255 \cdot 10^3$), a product of Est-Agar AS (Karla village, Estonia);
– potato starch, Superior Standard (WPPZ S.A., Luboń, Poland);
– gelatin from Canadian fish skin (Sigma-Aldrich, Poznań, Poland, catalogue no. G7765);
– glycerol from Sigma-Aldrich (Poznań, Poland);
– trypsin from porcine pancreas, lyophilized powder, BioReagent, suitable for cell culture, 1000–2000 BAEE units/mg solid (Sigma-Aldrich, Poznań, Poland);
[*ca.* 270 BAEE units correspond to 1 international unit (U) at 25 °C]
– glucoamylase OPTIDEX® L-400 401-04122-001 (Genencor International, USA) isolated from fungus with activity of 365 U/g at pH 4.0–5.5 and 35–40 °C;
– Viscozyme L was the product of Sigma-Aldrich (Poznań, Poland).

Foil preparation

A blend of furcellaran (0.2 % w/v), gelatin (0.2 % w/v) and potato starch (0.6 % w/v) in 50 cm^3 H_2O was heated at 90 °C for 20 min followed by an admixture of glycerol (0.05 % w/v). The ingredients were mixed near to the isoelectric point of the furcellaran/gelatin complex, pH 5.0 (in a ratio 1 : 1) [13]. The mixture was poured into polyester Petri dishes (ϕ = 80 mm) then dried for two days in the oven at 50 °C. The dry foils were collected from the dishes. Prior to each experiment, the foils were conditioned in a desiccator over saturated aq. $Mg(NO_3)_2$ solution [14] at 20 °C and relative humidity *RH* of 50 %.

Enzymatic hydrolysis

Hydrolysis of polysaccharides

Furcellaran (0.1 g/100 cm^3 H_2O), potato starch (0.3 g/100 cm^3 H_2O), furcellaran/starch mixture in the 1 : 3 ratio (0.4 g/100 cm^3 H_2O) and the foil (0.5 g/100 cm^3 H_2O) were enzymatically digested either with glucoamylase (0.25 cm^3) (activity 365 U/g), or Viscozyme L (0.25 cm^3) (activity of 100 FBU/g).
[One fungal beta-glucanase unit (FBU) is the amount of enzyme that, according to the above outlined standard conditions, releases glucose or reducing carbohydrate with a reduction capacity equivalent to 1 μmol glucose/min.]

The reaction mixtures were incubated at 37.0 °C and the reaction course was monitored spectrophotometrically by controlling the level of reducing sugars by the Miller method utilizing 3,5-dinitrosalicylic acid. Absorbance at 520–540 nm was recorded [15].

Hydrolysis of proteins

Gelatin (0.1 g/100 cm^3 H_2O) and foils (0.5 g/100 cm^3 H_2O) were digested with trypsin (0.025 g/10 cm^3 H_2O) (activity of 1000–2000 BAEE units/mg). The reaction course was monitored by titration according to Sorensen (the formol method) [16]. Suspensions of the gelatin and foils with trypsin were incubated in the water bath at 37.0 °C. Samples (2 cm^3) of the reaction mixtures, as well as control (enzyme-free), were transferred into a 50 cm^3 flask containing formaldehyde (4 cm^3) and phenolphthalein (5 drops). These solutions were titrated with 0.02 mol/dm^3 aq. NaOH solutions up to the development of a pale pink color stable for 1 min. The amount of consumed titrant was recalculated for the amount [μmoles] of protons of carboxylic groups (1 cm^3 of 0.02 mol/dm^3 aq. NaOH solution is equivalent to 20 μmoles of COOH groups).

Additionally, blends of glucoamylase (0.25 cm^3) and trypsin (0.025 g) in 200 cm^3 H_2O and Viscozyme L (0.25 cm^3) with trypsin (0.025 g) in 200 cm^3 H_2O were prepared. The foil samples (0.5 g) were added to these solutions in order to elucidate the simultaneous digestion of the polysaccharide and protein components of the foils.

Methods of testing

Foil thickness

The thickness of the foils was measured using a manual instrument, Mitotuyo, No. 7327 (Kawasaki, Japan). The measurements were performed to 1 μm precision in 5 points equally distributed around a circle, 10 mm from its edge. The average value of these estimations was accepted as the foil thickness.

Foil solubility

Foil samples (20 x 20 mm) were dried for 2 h at 105 °C then, after weighing, they were transferred into distilled water (200 cm^3) and maintained at room temperature for 24 h. Then the foils were taken from the suspension, dried for 2 h at 105 °C and weighed. The solubility was calculated from Eq. (1)

$$\text{Solubility} = (m_0 - m_r)/m_0 \cdot 100\ (\%) \qquad (1)$$

where: m_0, m_r – weights (g) of samples prior to and after the solubility test, respectively.
The estimations were triplicated.

Water absorptivity

Samples of foils (20 x 20 mm) were dried for 2 h at 105 °C, weighed, then immersed in distilled water (200 cm^3) for

24 h. Then, they were removed from the bath, dried with a filter paper and weighed.

The absorptivity was calculated from Eq. (2)

$$\text{Absorptivity} = (m_1 - m_0)/m_0 \cdot 100\ (\%) \qquad (2)$$

where: m_0, m_1 – weights (g) of samples prior to and after bathing, respectively.

Estimations were run in five replications.

Differential scanning calorimetry (DSC)

DSC experiments were performed with a Mettler-Toledo 821e (USA) calorimeter equipped with a Haake intracooler under a constant flow of argon (80 cm^3/min) within the temperature range of 25–400 °C at the heating rate of 10 °C/min. Samples (4–5 mg) were placed in 0.04 cm^3, hermetically closed aluminum pans.

Water content

The estimation was made by a DSC technique. The water contents in foils and mixtures of substrates were calculated from the estimated heat of water evaporation (Q_{vap}) from the measured samples. The measurements were carried out in the range of 25–200 °C in 0.04 cm^3 aluminum pans with 50 μm holes. The rate of the temperature increase was 5 deg/min. The water content resulted from the estimated mass enthalpy of evaporation of deionized water (ΔH_{vap} = –2216 J/g).

Mechanical properties

Mechanical properties were determined with a TA.XT2i Stable Micro System (Surrey, UK) texturometer. Thus, foil samples were conditioned for 7 days at 20 °C and relative humidity RH = 50 %. In order to determine the tensile strength (σ, MPa) and elongation at break (ε, %), conditioned 25 x 100 mm foils were fixed in the instrument clamps mounted at a distance of 30 mm. The rate of deformation was 0.2 mm/s. The results were statistically evaluated by applying analysis of variance at the level of α = 0.05. The measurements were run in 5 replications.

Scanning electron microscopy (SEM)

The SEM technique provided an examination of the surface of the biopolymer foils. A JEOL JSM-7500F instrument (Massachusetts, USA) was used.

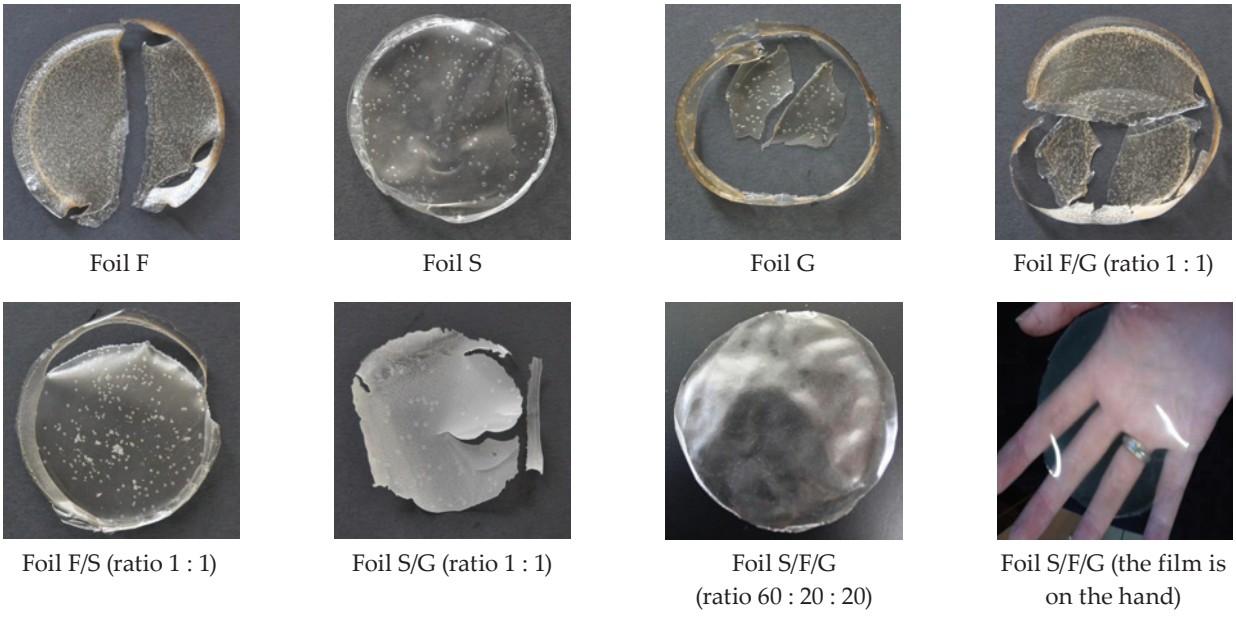
Statistics

All data were expressed as mean ± standard deviation ($n \geq 3$). Differences among data mean values were tested for statistical significance at the $p < 0.05$ level using analysis of variance and the Fisher's test.

RESULTS AND DISCUSSION

Preliminary studies were performed with simple, monocomponent foils made of furcellaran (F), gelatin (G) or potato starch (S), binary F/G, S/F and S/G foils, as well as with ternary S/F/G foils. The selection of the composition of the foil under study proceeded with extensive studies of the ternary materials composed of potato starch, gelatin and furcellaran combined in a wide range of proportions [13].

The ternary S/F/G foil was composed of starch (0.6 % w/v), furcellaran (0.2 % w/v) and gelatin (0.2 % w/v). All simple and binary foils exhibited poor functional properties. When dried, they stuck to the glass support. They were dull-yellow, not uniform, fragile and their texture was irregular. Among the prepared foils, the ternary



Foil F | Foil S | Foil G | Foil F/G (ratio 1 : 1)

Foil F/S (ratio 1 : 1) | Foil S/G (ratio 1 : 1) | Foil S/F/G (ratio 60 : 20 : 20) | Foil S/F/G (the film is on the hand)

Fig. 1. Appearance of foils

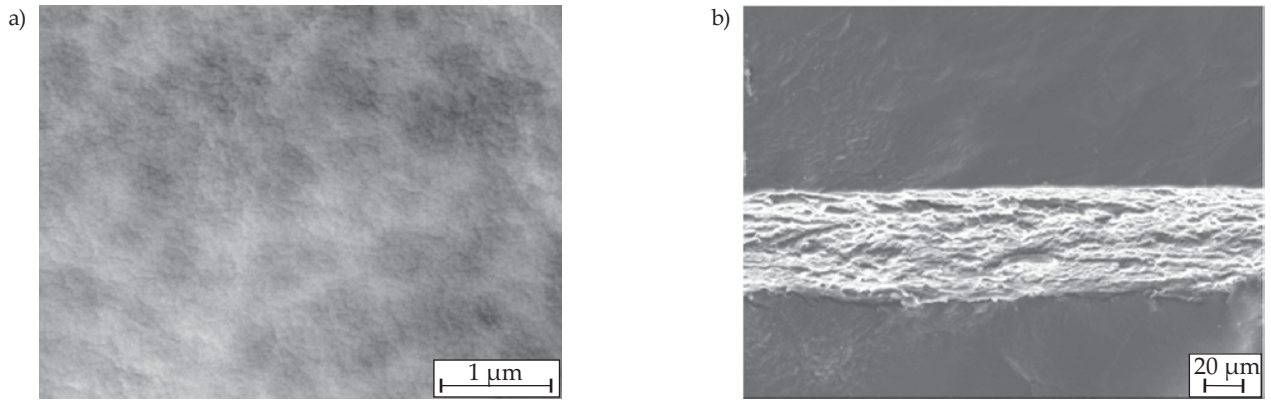


Fig. 2. Micrographs (SEM) of the internal macrostructure of S/F/G films: a) surface, b) cross section

S/F/G foil composed of 60 % S, 20 % F and 20 % G was superior (Fig. 1). Therefore, the study of the properties of ternary foils focused on the 60/20/20 S/F/G foil.

The S/F/G foils had a good appearance and uniformity. Following [17], 0.05 % w/v glycerol was added as the plasticizer. Preliminary studies showed that such admixture of glycerol provided a superior plasticizing effect for the foil.

Up to date, there are no reports on S/F/G foils. The ternary 60/20/20 S/F/G foils prepared within this project were 0.15–0.17 mm thick. Figure 2 presents micrographs of the surface (a) and cross-section (b) of this foil. Generally, thin films of the ternary S/F/G blend had uniform structures.

The mechanical properties, σ and ε, of the foils reached about 80 MPa and 27.8 %, respectively. It is commonly accepted [18] that packaging foils from biopolymers should be characterized by σ and ε values between 10–50 MPa and 10–100 %, respectively. Thus, the S/F/G foil satisfies these conditions.

Comparison of these properties with other foils prepared from polysaccharides and/or proteins might be of interest. Prepared foils of gelatin and pectin had σ = 106.7 MPa [19], and the reported binary gelatin/carrageenan foil had σ = 104 MPa and ε = 7 % [8]. Jridi *et al*. [20] investigated gelatin/chitosan foils of varying proportions of components and found that their σ and ε varied within the ranges 44–46 MPa and 1.47–3.96 %, respectively. A higher proportion of chitosan in such foils increased the σ parameter and, simultaneously, decreased the ε parameter.

The aqueous solubility of the S/F/G foil is essential for its practical application. The foil exhibited a limited solubility that, as shown in Fig. 3, is time dependent within the first hour. In this period it reached 35 % and then remained unchanged.

For comparison, foils prepared from fish gelatin [21] and starch foils [4] were completely water soluble. Jridi *et al*. [20] reported that the aqueous solubility of gelatin/chitosan foils of varying proportions decreased with higher chitosan proportions and, at optimum composition, reached 35 %. The limited (35 %) solubility of the foil confirmed the stability of the polysaccharide-protein polymer network, that is, the S/F/G composition was suitable for the manufacturing of biodegradable foils.

The thermal properties of the physical mixtures of the components and of the relevant foil are demonstrated in Fig. 4. The comparison of the diagrams provided evidence for the binding between all three components in the final product. The thermal stability of the foil was

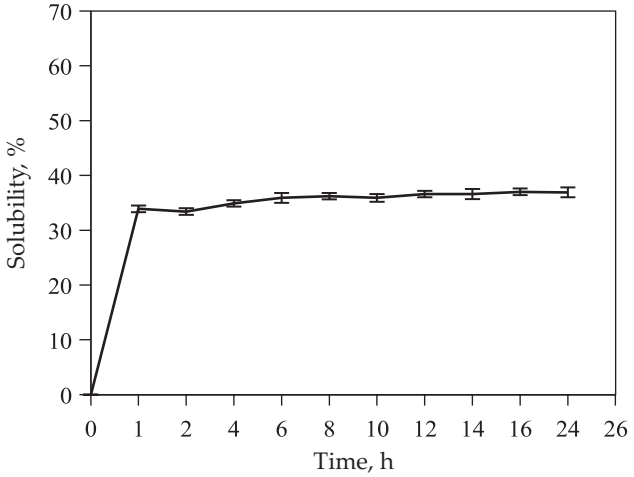


Fig. 3. Aqueous solubility of the S/F/G foil within 24 h; mean values ± standard deviations, *n* = 3

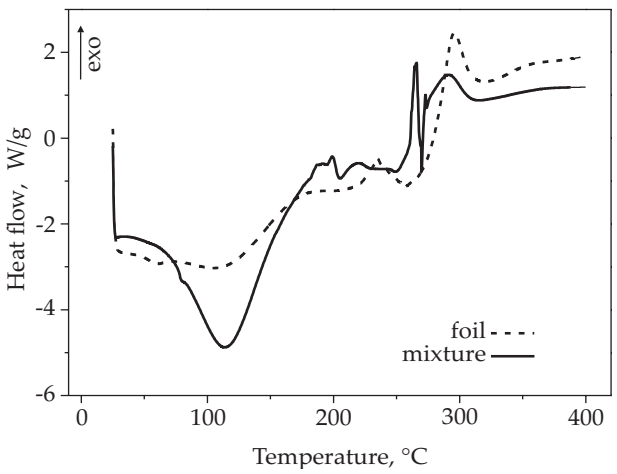


Fig. 4. DSC curves of S/F/G foil (broken line) and ternary furcellaran/starch/gelatin blend (solid line)

T a b l e 1. The water content of the S/F/G foil, and the physical mixtures of starch/furcellaran/gelatin

	Sample weight, mg	Q_{vap}, mJ	Water content, %
Mixture of the components starch/furcellaran/gelatin	4.96	-1197	10.9
S/F/G foil	4.45	-459	4.6

T a b l e 2. Reaction rate constants for the hydrolysis of furcellaran, starch, furcellaran/starch blend and the S/F/G foil with glucoamylase

Hydrolysis	Reaction rate constants, mg/(min · cm^3)			
	$k_1 \cdot 10^{-3}$	$k_2 \cdot 10^{-3}$	$k_3 \cdot 10^{-3}$	$k_4 \cdot 10^{-3}$
Furcellaran	12.5	1.4	–	–
Starch	3.3	2.6	11.6	–
Starch/furcellaran	273.0	21.6	8.2	43.0
S/F/G foil	1.0	18.0	4.0	30.4

higher than that of every separate component (ΔG for the physical blend of the components reached 111.5 kJ/g, whereas for the foil that value was 155.0 kJ/g).

The water content of the S/F/G foil, and the physical mixtures of the components of starch/furcellaran/gelatin, were calculated from the measured mass enthalpy of vaporization of deionized water, which is: ΔH_{vap} = -2216 J/g.

The water content of the foil S/F/G is less than half that of the physical mixtures of the components (Table 1).

Using glucoamylase and Viscozyme L, enzymatic hydrolysis was performed on the S/F/G foil, S/F blend, as well as for all three separate compounds. Additionally, the protein component was digested with trypsin.

One could see in Fig. 5 that furcellaran, which was relatively stable to the enzymatic digestion, increased the susceptibility of potato starch to the enzymatic digestion with glucoamylase. This could be a result of interactions between both these anionic polysaccharides. The S/F/G foil was considerably more resistant to the enzyme. Moreover, all three separate compounds were fully digested after 200 min whereas the S/F/G foil was digested to 45 % over 280 min and, after that time, further hydrolysis was fully suppressed. Figure 5 also shows that the hydrolysis proceeded stepwise. Furcellaran was digested in two steps, starch hydrolyzed in three steps and the S/F/G foil decomposed in four steps. In every step there was a linearity of the reducing sugar content against time, indicating the zero-order of the reactions induced by the maximum saturation of the substrate with the enzyme.

T a b l e 3. Reaction rate constants for the hydrolysis of furcellaran, starch, furcellaran/starch blend and S/F/G foil with Viscozyme L

Hydrolysis	Hydrolysis rate constants, mg/(min · cm^3)			
	$k_1 \cdot 10^{-3}$	$k_2 \cdot 10^{-3}$	$k_3 \cdot 10^{-3}$	$k_4 \cdot 10^{-3}$
Furcellaran	59.0	1.4	–	–
Starch	119	12.3	5.6	–
Starch/furcellaran	99.9	7.8	–	–
S/F/G foil	11.0	2.9	21.2	1.7

Reaction rate constants for each polysaccharide component reaction step are reported in Table 2.

Low values of the hydrolysis rate constants for the S/F/G foils compared to relevant values for its components and the starch/furcellaran blends implied a high resistance of the foil to these enzymes.

Figure 6 presents the course of the hydrolysis of the S/F/G foil, starch/furcellaran blend and furcellaran and starch separately with Viscozyme L.

Figure 6 reveals that starch, furcellaran and their blend completely hydrolyzed within 200 min and 45 % of the S/F/G foils hydrolyzed within 300 min. Viscozyme L hydrolyzed furcellaran and starch/furcellaran blend in two steps, starch was hydrolyzed with that enzyme in three steps and the S/F/G foil in four steps (Table 3).

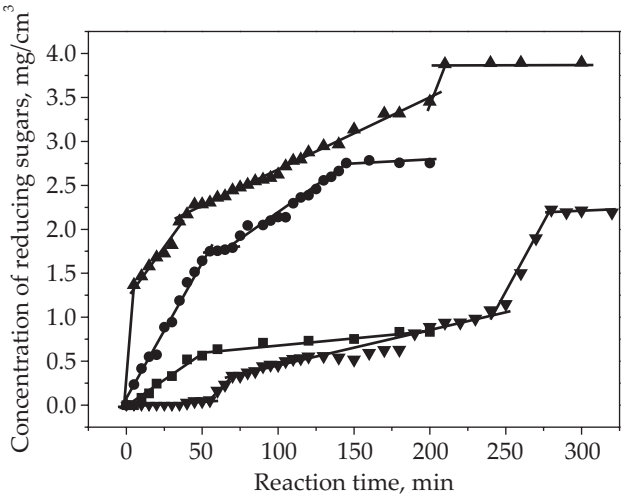


Fig. 5. Time-dependent level of reducing sugars in materials digested with glucoamylase: S/F/G foil (▼), starch/furcellaran blend (▲), furcellaran (■), starch (●)

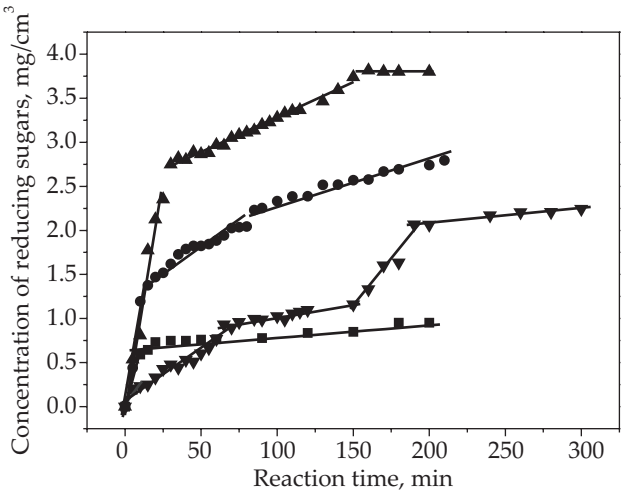


Fig. 6. Time-dependent level of reducing sugars in materials digested with Viscozyme L: S/F/G foil (▼), starch/furcellaran blend (▲), furcellaran (■), starch (●)

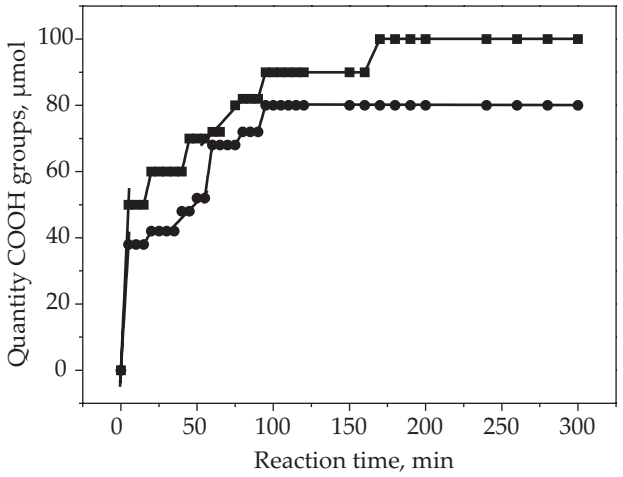


Fig. 7. Time-dependent content of the carboxylic groups in gelatin (■), S/F/G foil (●) hydrolyzed with trypsin

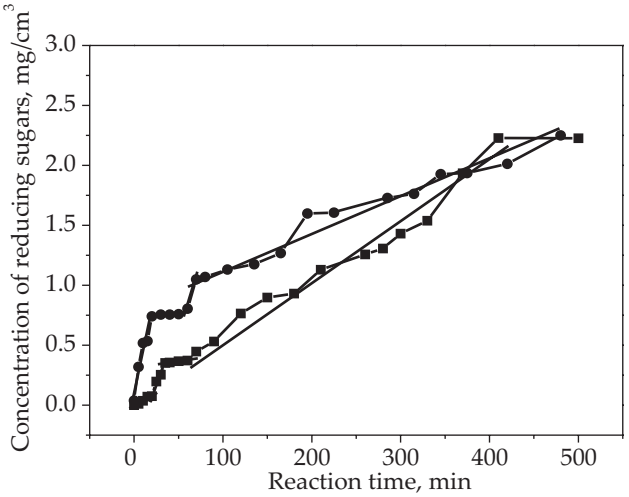


Fig. 8. Time-dependent reducing sugar content on the hydrolysis of the S/F/G foil with Viscozyme L-trypsin (●), glucoamylase--trypsin (■) blends

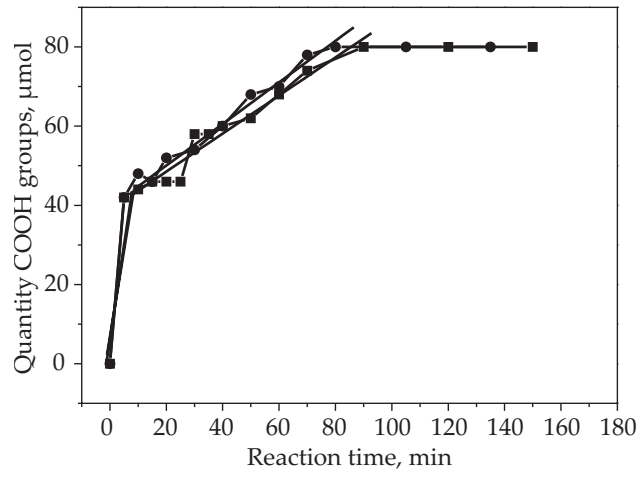


Fig. 9. Time-dependent content of the carboxylic groups in the S/F/G foil hydrolyzed with Viscozyme L-trypsin (●), glucoamylase-trypsin (■) blends

T a b l e 4. Reaction rate constants for hydrolysis with trypsin of gelatin and S/F/G foil

Hydrolysis	Rate constant, μmol/min				
	k_1	k_2	k_3	k_4	k_5
Gelatin	2.0	2.0	0.5	1.6	1.0
S/F/G foil	0.8	0.48	3.2	0.8	1.6

T a b l e 5. Rate constants for particular steps of the hydrolysis of the polysaccharide components of the S/F/G foil digested with glucoamylase-trypsin and Viscozyme L-trypsin blends

Enzyme blend	Rate constant, mg/(min · cm^3)			
	$k_1 \cdot 10^{-3}$	$k_2 \cdot 10^{-3}$	$k_3 \cdot 10^{-3}$	$k_4 \cdot 10^{-3}$
Glucoamylase--trypsin	32.4	1.3	11.8	3.9
Viscozyme L-trypsin	4.3	14.3	3.0	4.8

T a b l e 6. Hydrolysis rate constants of the protein component of the S/F/G foil hydrolyzed with the glucoamylase-trypsin and Viscozyme L-trypsin blends

Enzyme blend	Rate constant, μmol/min	
	k_1	k_2
Glucoamylase--trypsin	4.4	0.30
Viscozyme L--trypsin	4.8	0.48

Slow hydrolysis of the S/F/G foil could result from obstructed access of the enzyme to the foil polysaccharides. Figure 7 demonstrates the course of the hydrolysis of gelatin in the S/F/G foil involving trypsin.

Relevant hydrolysis rate constants are given in Table 4.

Initially, hydrolysis of the S/F/G foil was slow but it gradually accelerated most likely because of time dependent facilitated access of the enzyme to the reaction sites.

Additionally, the S/F/G foil was hydrolyzed with two enzymes, that is with glucoamylase-trypsin and Viscozyme L-trypsin blends. Figure 8 presents the course of the hydrolysis of the polysaccharide component of that foil.

Independently of the applied blend, the course of the hydrolysis of furcellaran and starch in the foil spread over 4 steps. The corresponding rate constants are collected in Table 5.

Figure 9 presents the course of the enzymatic hydrolysis of the protein component of the S/F/G foil with the glucoamylase-trypsin and Viscozyme L-trypsin blends.

The hydrolysis of the protein component, that is, gelatin in the S/F/G foil proceeded in two steps. Figure 9 documents that the reaction in the initial step lasting 10 min was the fastest. Table 6 reports the relevant rate constants for each step.

SEM micrographs of the original foil and the same foil after 30 min hydrolysis are presented in Fig. 10. The film consists of three components, however enzyme Viscozyme L is active against two, and only those areas are

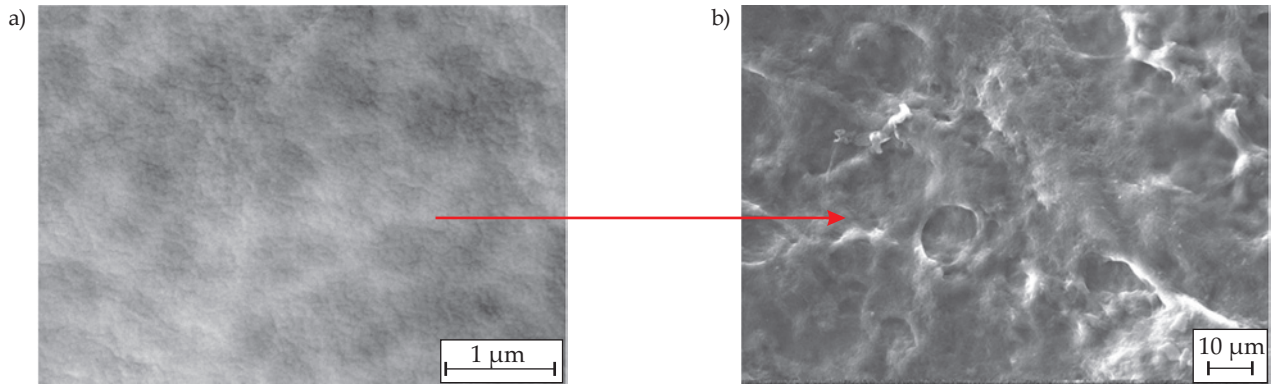


Fig. 10. Micrographs (SEM) of: a) foil prior to enzymatic hydrolysis, b) foil in the course of enzymatic hydrolysis with Viscozyme L

hydrolyzed. After 30 min hydrolysis with enzyme Viscozyme L, which produces a local effect, the structure of S/F/G foil starts to be heterogeneous. Also, steric conditions are important, that is, the enzyme ability to access substrate and the possibility of diffusion products.

CONCLUSIONS

In the present paper we have demonstrated that mixing potato starch, furcellaran, and gelatin rendered films with a homogeneous structure due to their high compatibility, as revealed by thermal and microstructure analysis of the composite films. For their mechanical endurance, elongation, water uptake, aqueous solubility and susceptibility to enzymes, ternary potato starch/furcellaran/gelatin (S/F/G) foils are suitable as packaging materials. This is a preliminary investigation, further studies need to be carried out (for example: durability of films, water vapor permeability, antioxidant and antimicrobial properties), so that the films can be used as biodegradable packaging in future.

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