

# NOVEL ANTIOXIDANT MATERIALS BASED ON POLYSACCHARIDES CONTAINING RESVERATROL AND SYRINGIC ACID

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## Abstract

Polysaccharides offer exceptional advantages for biomedical applications due to their natural biocompatibility, biodegradability, and lack of immune response. By incorporating active ingredients, researchers can tailor these materials' properties for specific uses. This study focused on developing enhanced biodegradable films using chitosan and konjac glucomannan as base materials, enriched with two natural antioxidants: resveratrol and syringic acid. Films containing these antioxidants at two different concentrations (10% and 20%) were obtained and thoroughly characterized using multiple analytical techniques. Infrared spectroscopy confirmed the successful incorporation of the active compounds, while scanning electron microscopy and atomic force microscopy revealed homogeneous surfaces with slightly increased roughness due to the additives. The antioxidant-enriched films demonstrated significant improvements in several properties. Most importantly, they showed strong antioxidant activity, with resveratrol and syringic acid working synergistically to enhance radical scavenging capabilities. The 20% concentration films exhibited markedly improved wettability, while mechanical properties were enhanced compared to the pure polymer blend. Although moisture vapor transmission decreased with the additives, this actually represents a beneficial barrier property for many applications. The films' swelling behavior proved particularly interesting, showing high swelling capacity at physiological pH (7.4) but significantly lower swelling at acidic pH (5.5). This pH-responsive behavior, combined with enhanced antioxidant properties, makes these materials especially promising for medical applications such as wound dressings. The obtained antioxidant-enhanced biopolymer films hold considerable potential in multiple industries, including medical devices, cosmetics, food products, and packaging applications. With further biological testing, these materials could advance wound care treatments by providing protective barrier functions and therapeutic antioxidant benefits.

**Keywords:** chitosan, konjac glucomannan, resveratrol, syringic acid, antioxidant films

[Engineering of Biomaterials 173 (2025) 06]

doi:10.34821/eng.biomat.173.2025.06

Submitted: 2025-05-05, Accepted: 2025-06-10, Published: 2025-06-13



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## Introduction

Polysaccharides are a broad group of natural macromolecules that are widely applied in food, packaging, the pharmaceutical industry, cosmetics, and biomedical engineering [1-3]. They possess beneficial properties such as biocompatibility, biodegradability, and non-toxicity [1,2]. Each carbohydrate polymer is distinguished by its unique characteristics. Many of them are capable of meeting a wide range of requirements, thereby facilitating their application across a diverse array of purposes [4,5]. An additional important aspect in this matter is the polysaccharides' functionalization by, for example, chemical modification, which expands their implementation [6]. Recent decades have seen a growing popularity of chitosan (CS) in a variety of applications, including shelf-life prolongation, its use in pharmaceuticals and cosmetics, environmental pollution protection, and even in the textile industry [6-8]. Chitosan is a chitin derivative; its source can constitute crustaceans' exoskeletons, cell walls of fungi, or insects [6,7,9]. It is a polycationic biopolymer, composed of D-glucosamine and N-acetyl-D-glucosamine units [10]. Chitosan demonstrates solubility within slightly acidic pH solutions, a condition under which amino groups undergo protonation. This characteristic classifies it as a polycation. Chitosan exhibits mucoadhesive properties, as well as antioxidant, and antibacterial activity [10-13]. Many physical forms of chitosan can be obtained, including films, foams, sponges, fibers, hydrogels, or beads [14]. They can be selected based on the specific requirements of the application.

Another valuable polysaccharide is konjac glucomannan (KGM). This biopolymer is derived from *Amorphophallus konjac* tubers [15,16]. The specimen is an example of the *Amorphophallus* genus, which comprises almost 170 species, principally distributed across Southeast Asia and Africa [17]. The main chain of glucomannan is built up from D-glucose and D-mannose units linked by  $\beta$ (1-4) glycosidic bonds. Additionally, randomly distributed acetyl groups are linked to mannose in the C-6 position, constituting 5 to 10% of the polymer [18,19]. KGM is a water-soluble macromolecule that shows high viscosity, good water-holding capacity, and excellent gel-forming properties in aqueous solutions [15]. This biopolymer is predominantly recognized as a dietary fibre [20,21]. Nevertheless, it is increasingly used in a variety of other domains, including the development of drug delivery systems, the manufacture of membranes, the production of cosmetics, and the creation of wound dressings [22-24]. The positive effects of konjac glucomannan on health are well documented. The most notable benefits include its ability to reduce blood sugar and lipids, regulate body weight, and reduce inflammation in the digestive system [17]. The protective and regenerative capabilities of konjac glucomannan on skin were confirmed by several studies on cells [24] and *in vivo* (topically applied [25], and orally administered [26]).

In addition to the advantages of natural polymers, such as the biodegradability and biocompatibility mentioned above, biopolymers have poor mechanical strength, and it is difficult to control their degradation process [27]. To overcome these obstacles, different types of modifications are carried out [28]. Chitosan and konjac glucomannan possess functional groups that can be used in the modification process [6,29,30]. One of the modification methods is blending, which allows the creation of a product with improved properties compared to the starting polymers that constitute the blend [31]. Chitosan and konjac glucomannan are well-miscible polymers that can be easily blended [32,33].

To enhance various properties of materials, such as biological ones, low-molecular additives can be implemented. Resveratrol is a well-known substance with high antioxidant capacity, anti-cancer activity, anti-inflammatory effects, and anti-aging properties [34,35]. This stilbene derivative can be extracted from grapes, cranberries, peanuts, and Japanese knotweed [36-38]. It occurs in two isomer forms: *trans* and *cis*; only the *trans* isomer possesses positive biological activity. Several factors, for example, UV radiation, high temperature, or increased pH, can lead to the *trans* isomer transformation into the *cis* form [37,38]. Another valuable antioxidant is syringic acid. It belongs to the phenolic acid group. The compound and its derivatives occur naturally in a wide range of plant products, including olives, dates, pumpkin, as well as honey and red wine [39,40]. Syringic acid is a red wine marker, as an intermediate product of malvidin processing [41]. It is an effective free radical scavenger and exhibits antibacterial, anti-inflammatory, anti-diabetic, and anti-cancer properties [39,40].

In the present study, the properties of chitosan/konjac glucomannan films enriched with resveratrol and syringic acid were evaluated. Both antioxidants were implemented at two different concentrations. The materials were fully characterised by infrared spectroscopy, SEM and AFM imaging, mechanical testing, contact angle measurements, and swelling analysis. The permeation of water vapour through the films has also been studied, and radical scavenging assays have been carried out. The proposed combination of resveratrol and syringic acid in such a biopolymeric matrix has never been investigated. The addition of low-molecular-weight compounds influenced not only the antioxidant activity of the films but also modified their physicochemical and mechanical properties. The findings of this study demonstrate the potential of the proposed films for a variety of applications.

## Materials and Methods

### Films preparation

The initial solutions were prepared by dissolving chitosan (POL-AURA, Dywity, Poland) and konjac glucomannan (POL-AURA, Dywity, Poland) in 0.1M acetic acid (POCH, Gliwice, Poland), to obtain 2% (w/v) and 0.5% (w/v) solutions, respectively. The solutions were combined in a weight ratio of 80% (CS) to 20% (KGM) and stirred for 24 h, following the protocol outlined in our previous study [33]. Next, resveratrol (POL-AURA, Dywity, Poland) was dissolved in ethanol and incorporated into the polymeric mixture at mass fractions of 5% and 10% relative to the total polymer mass. Simultaneously, the same amounts of syringic acid (POL-AURA, Dywity, Poland) were added to the two solutions, obtaining a summary concentration of additives 10% (BRSA10) and 20% (BRSA20), respectively. The final solutions were stirred on a magnetic stirrer for 1 h and then poured onto polystyrene plates (10 x 10 cm). The prepared samples were allowed to dry at room temperature under constant conditions and without access to light. The process took 3 days.

### Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectra were recorded using Nicolette iS10 spectrometer with diamond ATR equipment (Thermo Fisher Scientific, Waltham, MA, USA). The parameters of the analysis were as follows: 64 scans, wavenumber range 400-4000 cm<sup>-1</sup>, 4 cm<sup>-1</sup> resolution.

### Scanning electron microscopy (SEM)

A scanning electron microscope (Quanta 3D FEG, D9399, FEI, Eindhoven, The Netherlands) was used to image the surface of the film samples. Prior to analysis, the samples were covered with gold to provide a conductive surface for electron beam interaction. The SEM images presented here were taken at a magnification of 150x.

### Atomic force microscopy (AFM)

An atomic force microscope with SPM sond (Digital Instruments Veeco Metrology Group, Santa Barbara, CA, USA) was employed for the film's topography imaging and roughness evaluation. The tapping mode of the microscope was implemented, and the measurements were carried out under ambient conditions. Roughness was determined from 5 μm x 5 μm AFM images using Gwyddion software (version 2.62).

### Mechanical tests

The mechanical properties of the materials were evaluated using the mechanical testing machine Z.05 (Zwick and Roell, Ulm, Germany). The samples were properly prepared, die-cut into a dumbbell shape, and tested according to the ASTM D882 standard. Tensile tests were carried out with 0.1 MPA initial force, 5 mm/min crosshead speed, and 50 mm/min starting position speed. Data were collected using TestXpert II 2017 software. All measurements were conducted under ambient conditions.

### Moisture Vapor Transmission Rate (MVTR)

The films were die-cut into fragments measuring Ø 33 mm and placed into containers filled with 10 mL of distilled water. Consequently, containers with positioned films were affixed with a ring of Ø 30 mm and sealed around with parafilm. Then each set was weighed (*m*<sub>1</sub>) and placed in an incubator at 37°C. After 72 h, the second measurement (*m*<sub>2</sub>) was taken. The methodology was adapted with some modifications from the study described by Minsart et al. [42]. The equation used to calculate water permeation through the films was as follows:

$$MVTR = \frac{m_1 - m_2}{t \cdot A} [g/24 h \cdot m^2] \quad (1)$$

*t* - times [24 h]; *A* - area of water evaporation [m<sup>2</sup>].

### Antioxidant tests

The antioxidant properties of the films were evaluated using two methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) (POL-AURA, Dywity, Poland) and ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (POL-AURA, Dywity, Poland) assays. Before analysis, the films were prepared in the same way: the samples were cut into fragments of comparable weight (~0.1000 g), then placed in 10 mL flasks and made up with ethanol to the mark, and then flasks were shaken for an hour to obtain the extracts. The results were presented as a Trolox equivalent antioxidant capacity (TEAC) per 100 g of sample.

### DPPH radical scavenging assay

DPPH at a concentration of 0.304 mM and 100 μM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich Søborg, Denmark) were prepared in ethanol (Stanlab Lublin, Poland). Subsequently, a calibration curve was prepared, obtaining the following Trolox concentrations: 0.00; 2.50; 7.51; 12.52; 17.53; 22.54; and 25.04 mg·mL<sup>-1</sup> in 10 mL flasks. Next, 1.5 mL of ethanol, 0.5 mL of DPPH solution, and 0.5 mL of each Trolox solution were added to the plastic cuvettes.

The mixtures were placed in a dark place for 15 min, and then the absorbance was measured with a Shimadzu UV-1601 UV-Vis spectrophotometer (Kyoto, Japan). Measurements were carried out at  $\lambda = 517$  nm using ethanol as a reference. The samples were prepared for the measurements in the same way, using 0.5 mL of extract instead of Trolox solution, and measured using the same parameters.

#### ABTS radical scavenging assay

ABTS aqueous solution at a concentration of 7 mM was prepared and mixed with 2.45 mM potassium persulfate ( $K_2S_2O_8$ ) solution in a 2:1 ratio, respectively. The mixture was incubated for 16 hours in a place without access to light. The initial mixture was then diluted, achieving a value of absorbance of about 1.0 at  $\lambda = 734$  nm. An ethanolic solution of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at a 5 mM concentration was prepared, and then a calibration curve was made in the following concentrations: 0.00, 12.40, 24.80, 62.00, 99.20, 148.80, 186.00, 223.20, and 248.00  $mg \cdot mL^{-1}$ . Subsequently, 0.1 mL of each concentration of Trolox and 3.9 mL of ABTS solution were mixed in plastic cuvettes and incubated for 10 min in a dark place. Afterwards, the absorbance of the obtained solutions was measured at  $\lambda = 734$  nm. Ethanol was used as a reference. The samples were measured in accordance with the methodology employed in the calibration curve.

#### Contact angle

The analysis was carried out using a goniometer with drop shape analysis equipment (DSA 10 produced by Kruss, Hamburg, Germany). Glycerin (G) and diiodomethane (D) contact angles were determined under room conditions. The Q-Dixon test was employed to identify and reject outliers. Based on the contact angle values for these two liquids, the surface free energy ( $\gamma_s$ ), as well as its components ( $\gamma_s^p$ ,  $\gamma_s^d$ ), was established following the Owens-Wendt method using the equation:

$$\gamma_L \cdot \frac{(1 + \cos\theta)}{2} = (\gamma_s^d \cdot \gamma_L^d)^{1/2} + (\gamma_s^p \cdot \gamma_L^p)^{1/2} \quad (2)$$

$\gamma_L^p$  - polar component of surface free energy;

$\gamma_L^d$  - dispersive component of surface free energy;

S - solid substrate;

L - liquid.

#### Swelling and degradation analysis

Before the analysis, the films were cut into squares of similar weight (0.005-0.0075g) and dried for 24 h at 45°C. Then, the squares were placed in containers with phosphate buffer saline (PBS) at two pH values: 5.5 ( $Na_2HPO_4$  from Chempur, Piekary Śląskie, Poland;  $NaH_2PO_4$  from Merck Darmstadt, Germany) and 7.4 (Life Technologies LTD, Renfrew, UK) at 37°C. The weight of the samples was measured after 0.25; 1; 2; 4, and 8 h. For each measurement, film pieces were removed from the buffer solution and gently dried of the excess fluid using a paper towel. To calculate the swelling degree, the equation was used:

$$\text{Swelling} = \frac{(m_t - m_0)}{m_0} \cdot 100\% [\%] \quad (3)$$

$m_t$  - the weight of the material after immersion in PBS [g];

$m_0$  - the initial weight of the material [g].

## Results and Discussions

#### Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of the prepared films are presented in FIG. 1. The subsequent analysis of the infrared spectra indicated the presence of characteristic bands corresponding to the groups present in the initial polymers that constitute the blend (CB). Broad band at about 3184  $cm^{-1}$  indicates N-H and O-H vibrations [43]. Another band at 2874  $cm^{-1}$  is characteristic of C-H bonds present in polysaccharides [44]. Moreover, bands at 1630  $cm^{-1}$ , 1537  $cm^{-1}$ , and 1374  $cm^{-1}$  come from chitosan's C=O stretching, N-H bending, and C-N stretching vibrations, respectively [45,46]. Another spectral region includes stretching vibrations of the C-O-C and C-C from polymer skeletons in 1200-900  $cm^{-1}$  [47]. Furthermore, the bands at 1152  $cm^{-1}$  and 897  $cm^{-1}$  are associated with glycosidic bonds [48].

Some changes can be observed in the spectra of the films with additives. An additional band at 964  $cm^{-1}$  identified as resveratrol's trans olefin bond [49] can be distinguished in both modified samples. Additional narrow bands in the region 1456-1317  $cm^{-1}$  are associated with the methyl groups present in the syringic acid structure [50]. New bands and shifts in the infrared spectra, when compared to the CB sample, are marked in FIG. 1.

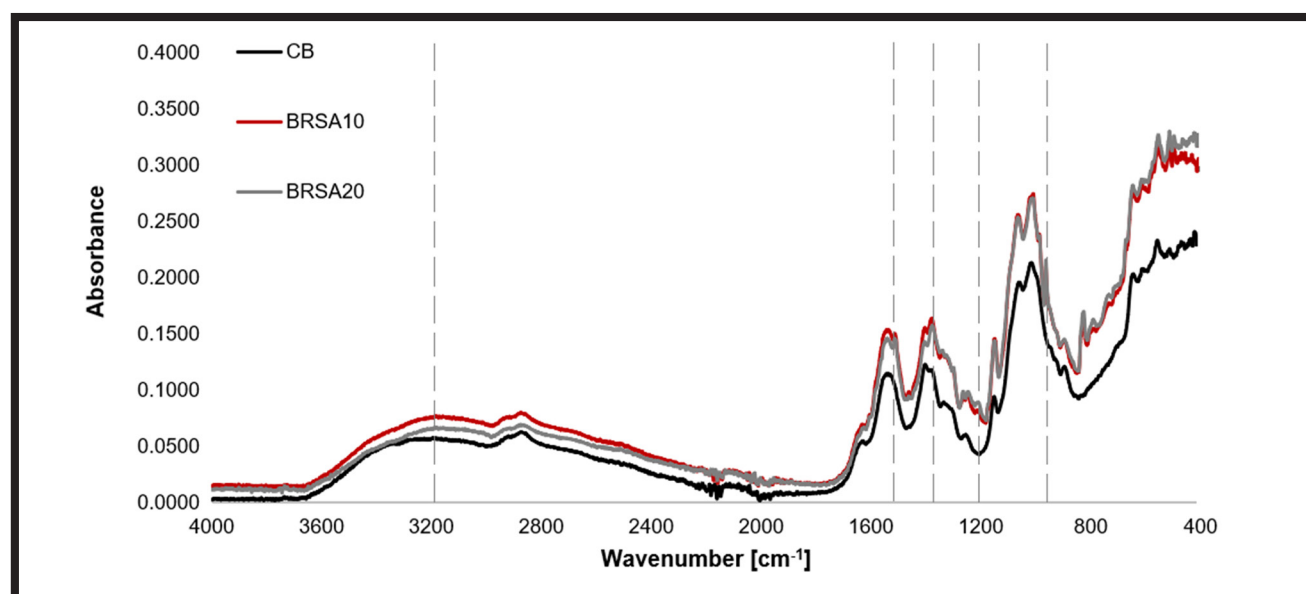


FIG. 1. Infrared spectra of the obtained films (CB – control blend without additives; BRSA10 – blend with 5% of resveratrol and 5% of syringic acid; BRSA20 – blend with 10% of resveratrol and 10% of syringic acid).



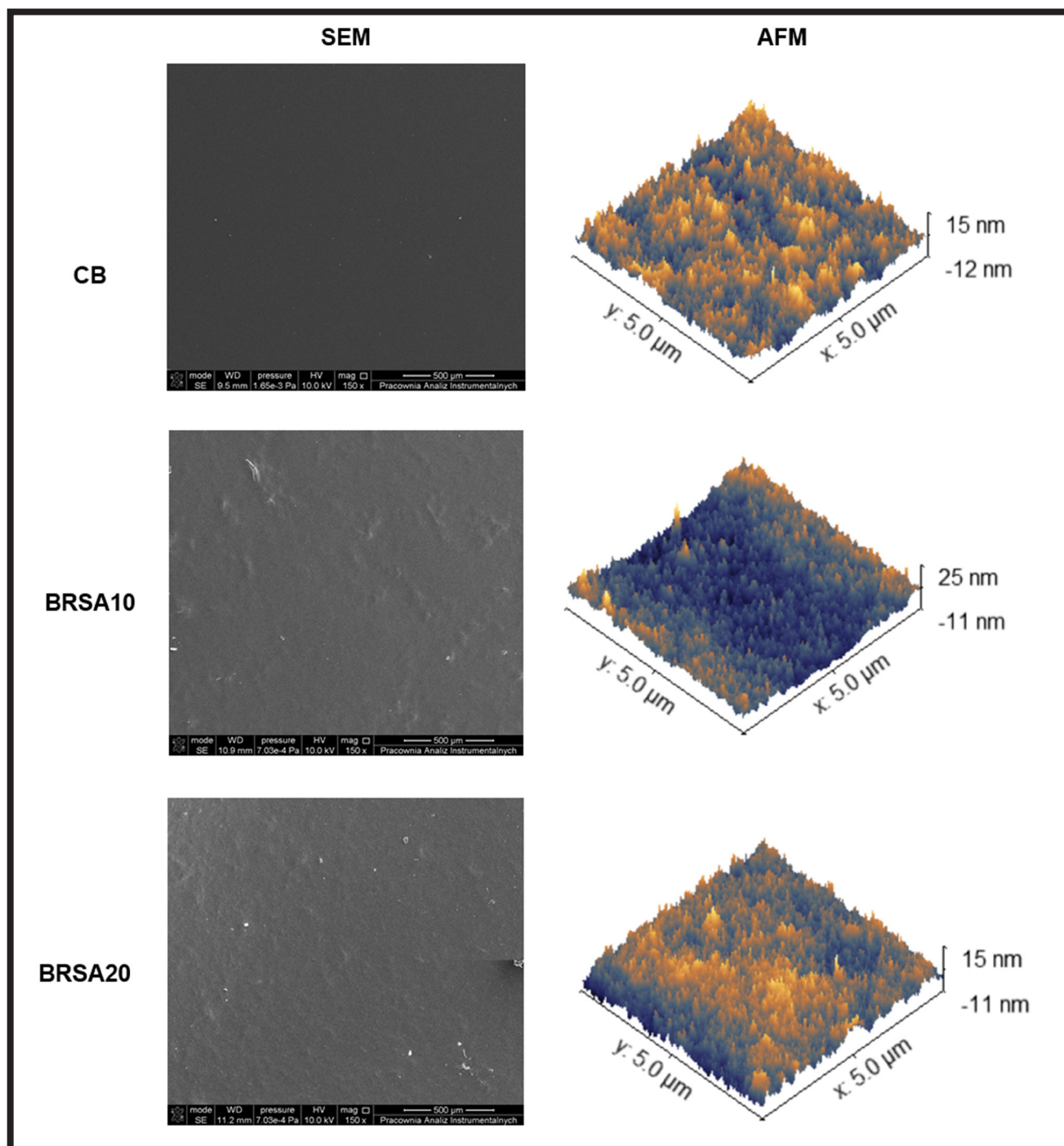
### Microscopy imaging (SEM and AFM)

The results of microscopic imaging are summarized in FIG. 2. The surface of the CB sample is characterised by a smooth and homogenous texture. It was demonstrated that the addition of antioxidant substances may result in a slight reduction in surface smoothness. Changes in surface structure are similar for samples with both concentrations of additives (BRSA10 and BRSA20). The observed smoothness changes may be mainly associated with the presence of resveratrol. Pastor et al. [51] found that resveratrol incorporation into the chitosan/methylcellulose films resulted in surface irregularities. This phenomenon is attributed to the rearrangement of the polymer chains and the disruption of the polymer network by resveratrol, as the authors suggest [51].

AFM images allowed the evaluation of films' topographical characteristics, as well as, the roughness determination (TABLE 1). The topographic images of all samples show a high degree of densely packed hills. The addition of resveratrol and syringic acid to the blend resulted in a slight enhancement in the films' roughness. The BRSA10 sample was characterized by the highest roughness value.

**TABLE 1. Roughness parameters for the tested samples.**

Sample	$R_q$ [nm]	$R_a$ [nm]
CB	$3.13 \pm 0.03$	$2.49 \pm 0.01$
BRSA10	$4.68 \pm 0.85$	$3.64 \pm 0.45$
BRSA20	$3.35 \pm 0.94$	$2.65 \pm 0.76$



**FIG. 2. The visualization of films' surface and topography.**

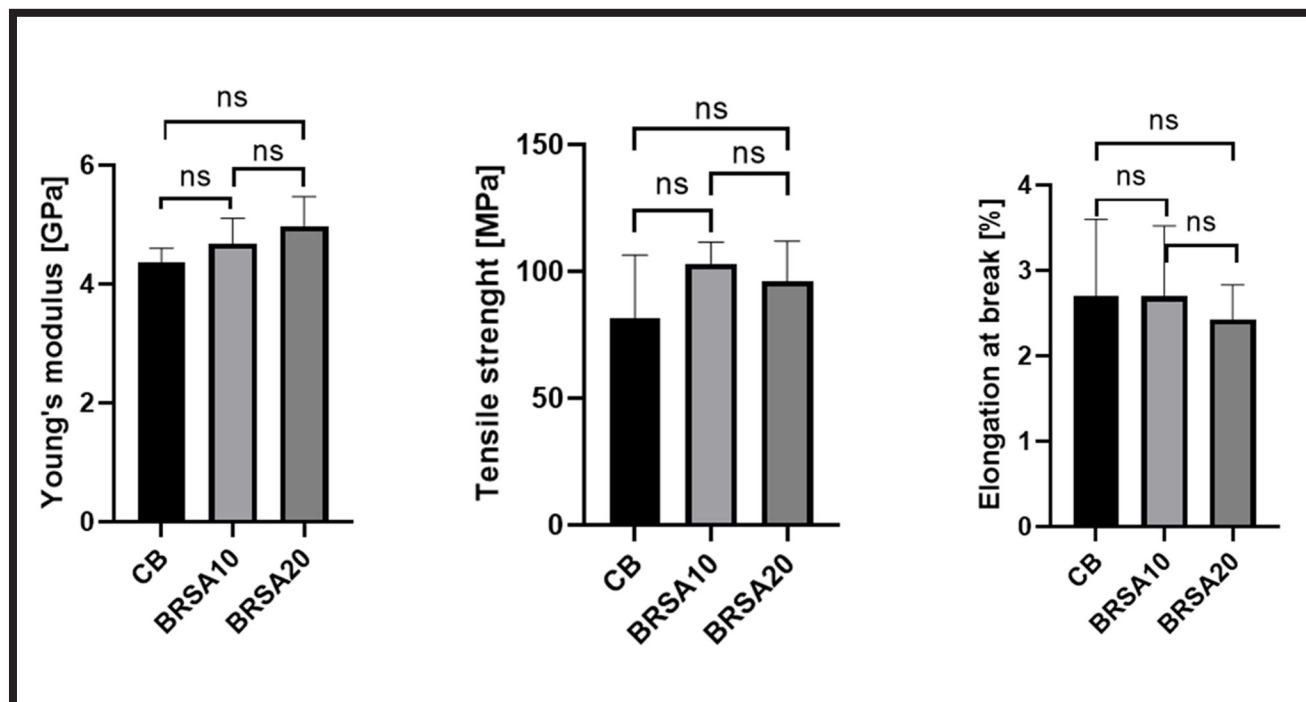


FIG. 3. The results of the mechanical parameters evaluation in the tensile test of CB, BRSA10, and BRSA20 films. Statistically significant differences are indicated as follows: ns - not significant.

#### Mechanical tests

The results of the mechanical properties evaluation are presented in FIG. 3. The tests revealed that the addition of resveratrol and syringic acid slightly improved the mechanical properties of the films. Primarily, the higher the content of the additives, the higher Young's modulus. In addition, the tensile strength is increased for the samples with antioxidant compounds, with the highest value for the BRSA10 sample. However, a small decrease in the elasticity of the films can be observed as the concentration of antioxidant compounds is higher. In a study by Pastor et al. [51], where resveratrol was added to the polymeric matrix, the mechanical properties of films were decreased. Furthermore, similar observations were made by Samprasit et al. [52], where polyvinyl alcohol/chitosan films containing resveratrol and mangostin were examined. On the other hand, in a study by Zhang et al. [53], the emulsions containing syringic acid grafted pectin and soy protein isolate were used to improve polymeric films' properties. The authors suggested that decreased elongation at break after emulsion implementation can be attributed to the creation of intermolecular hydrogen bonds that cause lower mobility [53].

The films analyzed in this study, composed of two polysaccharides and two active agents, exhibit complex intermolecular interactions. In this case, an increased number of hydrogen bonds can be the reason for mechanical characteristic changes.

#### Moisture Vapor Transmission Rate (MVTR)

Subsequently, the MVTR parameter was evaluated, and the results indicated that the addition of antioxidant compounds reduced the moisture permeation through the samples (FIG. 4). Depending on the application, this parameter may have different values. For example, the MVTR value for cutaneous applications, such as the application of wound dressings, remains undefined. Dry wounds and wounds with high exudation levels will have different characteristics. The factors influencing water transmission through the material may be the density of the structure and the nature of the additive [54].

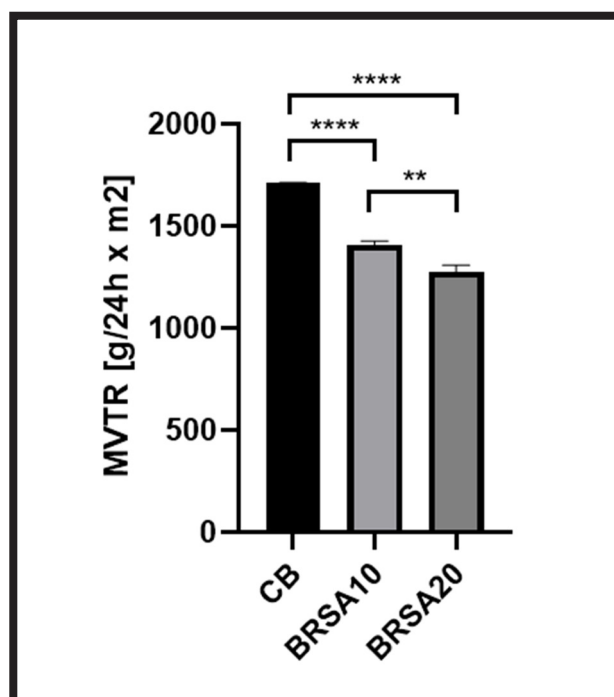


FIG. 4. The results of the moisture vapor transmission rate for the tested films. Statistically significant differences were as follows: \*\*  $p = 0.001$ ; \*\*\*\*  $p < 0.0001$ .

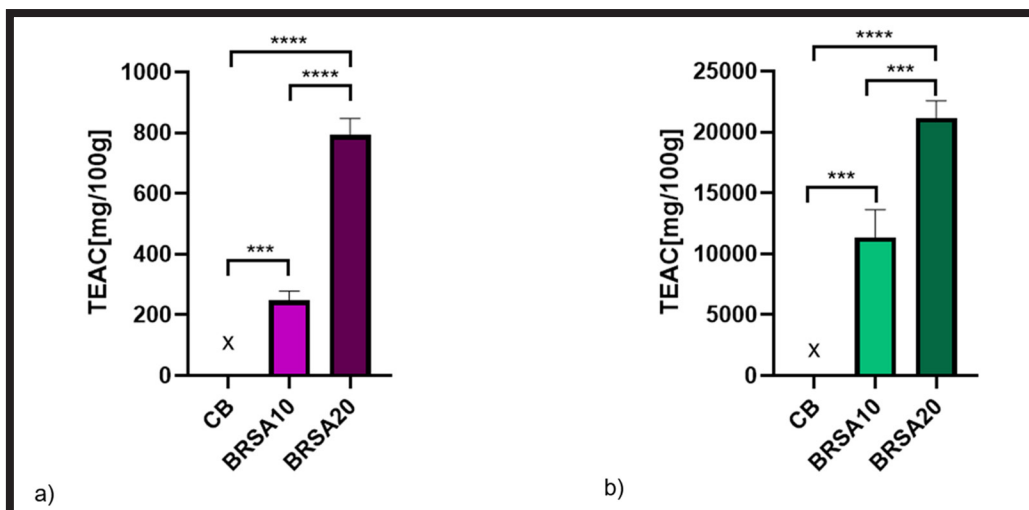


FIG. 5. The results of: a) DPPH radical scavenging assay, b) ABTS radical scavenging assay. The results were statistically significant (\*\*\*  $p < 0.0006$ ; \*\*\*\*  $p < 0.0001$ ).

TABLE 2. The values of the contact angle and surface free energy for the tested films.

Sample	$\Theta^G$	$\Theta^D$	$\gamma_s$ [mJ/m <sup>2</sup> ]	$\gamma_s^d$ [mJ/m <sup>2</sup> ]	$\gamma_s^p$ [mJ/m <sup>2</sup> ]
CB	101.88 ± 2.90	67.9 ± 3.43	24.29 ± 1.80	24.15 ± 1.71	0.13 ± 0.08
BRSA10	98.34 ± 3.95	62.97 ± 5.03	26.98 ± 2.70	26.74 ± 2.56	0.23 ± 0.15
BRSA20	74.46 ± 8.69	63.05 ± 2.62	29.78 ± 3.95	21.16 ± 0.15	8.59 ± 4.14

### Antioxidant tests

The initial film (CB) exhibits no antioxidant activity, while the films containing antioxidant compounds possess a high free radical scavenging potential. Both methods gave comparable results; the higher the concentration of the active ingredients, the higher the antioxidant activity (FIG. 5). The BRSA20 film shows the highest antioxidative capacity. It can be assumed that a synergistic effect is exhibited by resveratrol and syringic acid against DPPH radical, since a film containing a double concentration of these substances demonstrates antioxidant activity that exceeds the level that could be predicted by the simple addition of TEAC values. The antioxidant potential of resveratrol and syringic acid is associated with the high content of hydroxyl groups [55,56].

### Contact angle

The wettability of the blend film and the films with additives was investigated by the measurement of the materials' contact angle using the sitting drop method. It was determined that CB exhibits a hydrophobic character. The addition of antioxidants resulted in a reduction of the contact angle for the materials. The higher the additives content in the film, the lower the contact angle. Furthermore, BRSA20 is the only sample that demonstrates hydrophilic properties. It also has the highest value of the polar component of the surface free energy. The presence of hydrophilic -OH groups in resveratrol and syringic acid suggests the possibility of these groups orienting themselves on the surface of the film. This is particularly relevant when the infrared spectra do not reveal a high amount of interactions between the active substances and the matrix. The results are summarized in TABLE 2.

### Swelling and degradation analysis

Swelling analysis was carried out in two solutions of different pH (5.5 and 7.4). Swelling was observed in all samples, irrespective of the pH of the solution. BRSA20 exhibited the highest swelling degree at pH 7.4, reaching almost 1000% in the first few minutes of analysis (FIG. 6).

The lowest swelling ability was exhibited by the CB sample. In a slightly acidic environment, the films had significantly lower swelling potential (FIG. 7). All samples reached a maximum degree of swelling in one hour. Thereafter, a decrease in the values was observed, which may indicate the initiation of a degradation process. The swelling properties are determined by the polymer chain arrangement and the density of the structure. The presence of amine groups in chitosan is subject to pH-dependent protonation or deprotonation, resulting in alterations to the arrangement and density of polymer chains. This, in turn, leads to changes in the swelling ability of the resulting films [57].

### Conclusions

This study successfully developed biodegradable films by combining chitosan and konjac glucomannan with two powerful antioxidants: resveratrol and syringic acid. Through comprehensive testing, we found that incorporating these antioxidant compounds significantly enhanced the films' properties while maintaining their structural integrity.

The addition of resveratrol and syringic acid transformed the original blend material in several important ways. Most notably, the films gained substantial antioxidant capabilities, with the two compounds appearing to work together synergistically against DPPH radicals. This enhanced antioxidant activity represents a major improvement over the base material.

The physical properties of the films also changed in a positive way. While the surface became slightly rougher with the antioxidant additions, the mechanical strength improved. The films became more wettable and showed an increased swelling capacity, particularly at physiological pH (7.4). Additionally, the materials demonstrated reduced moisture vapor transmission, indicating better barrier properties.

These combined characteristics make the films particularly promising for medical applications, especially as wound dressings. The high swelling capacity at body pH, improved wettability, and reduced water vapor permeability are all desirable features for skin contact applications. However, the enhanced properties also suggest potential uses in multiple industries, including biomedical devices, cosmetics, and food packaging.

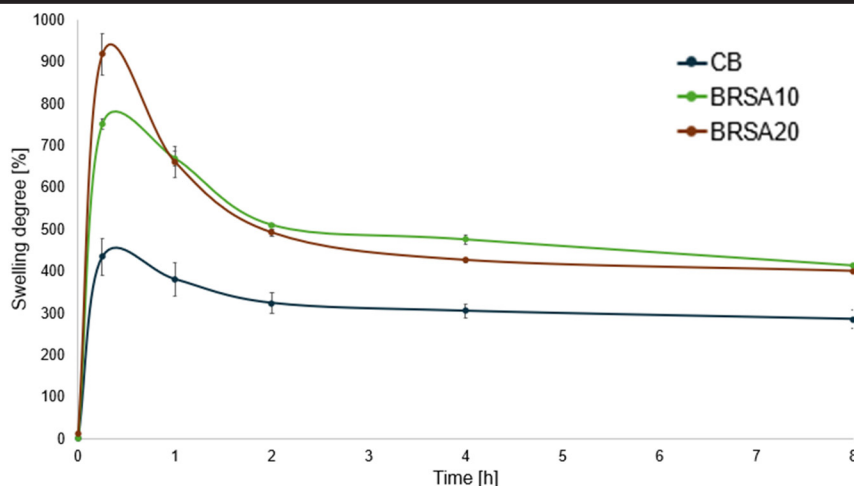


FIG. 6. The results of the swelling analysis for the film materials carried out at pH = 7.4.

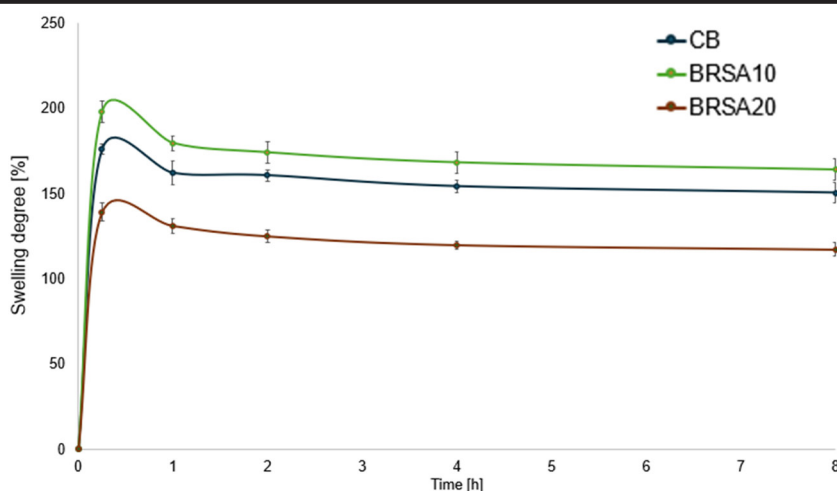


FIG. 7. The results of the swelling analysis for the film materials carried out at pH = 5.5.

While these initial results are encouraging, further research is needed to fully explore the materials' potential and optimize their performance for specific applications. The successful incorporation of natural antioxidants into biopolymer films opens new possibilities for developing functional materials that combine sustainability with enhanced performance.

## Acknowledgements

*This research was supported by IDUB in the 6th Edition of Grants4NCUStudents of Nicolaus Copernicus University in Toruń (project number 4101.00000070).*

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