MODIFYING PROPERTIES OF FEATHER KERATIN BIOPLASTIC FILMS USING KONJAC GLUCOMANNAN

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In order to diminish the brittleness of pure feather keratin films, in this research, blended films with improved physical properties were prepared using konjac glucomannan (GM). The influence of the GM ratio in the blended films on their final characteristics was studied. The chemical composition, thermal properties, as well as water swelling, moisture absorption and surface hydrophilic/hydrophobic character of the films were analyzed using Fourier-transform infrared spectroscopy, thermogravimetry, optical microscopy and goniometry. The results showed that konjac glucomannan improved the breaking strength and Young's modulus, as well as the water and moisture sorption and surface water contact properties of the blended films. The present study revealed the great potential of combining waste feather keratin and konjac glucomannan for improving the essential characteristics of films developed for different fields of application, such as in food packaging (dry or wet food packaging concepts) or medical applications.

Keywords: feather keratin, konjac glucomannan, blended films, physical properties, water contact angle

INTRODUCTION

Poultry feathers are one of the most aggravating by-products of the poultry industry. Each year, about 5 million tons of poultry feather wastes are produced, which are currently converted into low nutritional value pet food and, in some countries of the world, are sanitized and land-filled.[1,2] Continuous and intensive growth of the human population in the last 50 years in the world determines a linear increase of the animal products demand.[2] According to the Food and Agriculture Organization, in 2015, the global production of poultry meat amounted to 100.6 million tons, and the perspective for 2030 would reach 143.3 million tons.[3] In the EU, the Directive 1999/31/EC on the landfill of waste[4] restricts landfilling of wastes that contain large quantities of biodegradable materials with high burning values. The concept of zero emissions and zero waste was coined for developing production processes with minimized emissions and wastes. Therefore, finding an appropriate and wide application of poultry feathers will be one of the most important achievements in the area of industrial waste management and recycling related to the poultry industry in the future.

Feathers represent a large potential source of keratin, as this structural protein represents about 90% of their composition. Keratin is a biopolymer with a three-dimensional fibrous structure consisting of amino acids. High amounts (about 8%) of cysteine in feather keratin represent the most important property, which differentiates keratin from collagen or other structural proteins.[5,6] Cysteine residues are present as disulfide bonded dimeric amino acid cystine, which enables extensive cross-linking and a high amount of hydrophobic residues. The solubilization of keratin requires breaking the disulfide bonds. The numerous disulfide cysteine bonds present in keratin bind peptide chains permanently, making them resistant to enzymatic lysis.[6] Besides, keratin is insoluble in water, weak alkalis, acids and organic solvents. For keratin extraction, different physico-chemical and biological techniques have been tested, such as reduction, oxidation and processing with ionic liquids, enzymatic degradation *etc*.[7-9] The main drawback of keratin isolated from feathers that hampers its wider application is its rather low average molecular weight (around 10 kDa).[10]

Therefore, for developing functional fibers and films from keratin, for many applications such as in healthcare, water cleaning, food packaging *etc.*, it is extremely important to find ways to improve its mechanical properties and, at the same time, to preserve all its important bioactive features. Recently, many approaches have been focused on different combinations and blends of feather keratin with polymers, which could, synergistically, support the physical and chemical properties of new compounds/composites. Grkovic *et al.* prepared and investigated keratin/polyethylene oxide nanocomposites for potential biomedical applications.[11] Esparza and coworkers investigated keratin/poly(vinyl alcohol) blends in order to use them as scaffolds for tissue engineering.[12] Tanabe and coworkers[13] investigated chitosan/keratin films, and found out that chitosan addition significantly improved the strength of the prepared films. Furthermore, the films exhibited antibacterial properties and supported fibroblast attachment and proliferation. The same biopolymer combination was also investigated by other research groups.[14-16] Pardo-Ibanez *et al.*[17] developed renewable packaging by the combination of feather keratin with polyhydroxybutyrate (PHBV).

Konjac glucomannan (GM) is a linear polysaccharide isolated from the tuber of *Amorphophallus konjac*. It is composed of β-D-manose and β-D-glucose with β-1.4 linkages in a molar ratio of 1.6:1. It has a low amount of acetyl groups, approx. 1 per 17 residues at C-6 position.[18] It is used mostly in the food industry as a thickening, gelling, stabilizing and emulsifying agent. In the last two decades, however, GM has become increasingly important in the development of edible films.[19] There have been reported investigations of many different GM combinations with various other biopolymers. Three-component glucomannan-chitosan-nisin edible films with antimicrobial properties have been developed by Li *et al.*[20] Cheng *et al.*[21] modified the physical properties of GM-carboxymethylcellulose edible films by alkali, while Jia and coworkers combined GM with chitosan and soy-protein-isolate.[22] Besides, GM was combined with whey-protein-isolate,[23] zein,[24] gelatin,[25] gellan gum[26] and agar.[27] In all the investigations, GM proved to support the mechanical and barrier properties of the developed films.

To the best of our knowledge, there have been reported no studies yet on the application of GM in feather-isolated keratin blend films. Thus, it is a great challenge to develop and characterize films from poultry feather keratin (K) and konjac glucomannan (GM) in order to find out whether synergism is developed between GM and keratin, and if the presence of GM has a positive influence on the physico-chemical properties of the blended films, in comparison with pure keratin films. Keratin was extracted from feather wastes and was applied together with GM, in different proportions, for forming the films. The chemical, thermal, mechanical and surface properties of the films were studied and discussed.

EXPERIMENTAL

Materials
Poultry feathers were obtained from a poultry meat production company (Perutnina Ptuj d.d.), as final waste in the production and processing of poultry meat. Feathers were cleaned industrially – washed on a grid with running water, which removed large impurities, such as secretions, blood, *etc.*

A food grade powder extract from *Amorphophallus konjac* was purchased from Farmalabor Srl. (Canosa Di Puglia, Italy).

Methods
Waste feather cleaning
Waste feathers were washed thoroughly at 60 °C by using non-ionic detergent (Sandoclean PC, Sandoz), followed by rinsing of the samples using warm and cold deionized water. The cleaned feathers were dried in a ventilated dryer at 40 °C for 72 hours. The feathers were then cut into small pieces (approx. 5 mm) and, in order to remove grease, they were extracted by petroleum ether in a Soxhlet apparatus. The dry and cleaned feathers were stored in closed containers.

Keratin extraction
The extraction procedure was applied according to Martelli *et al.*[28] in order to obtain keratin from the feathers. A quantity of cleaned feathers (17.5 g) was transferred into a three-necked flask, to which 200 mL of an aqueous solution containing 94.5 g of urea, 15 g of sodium dodecyl sulfate (SDS), 23 mL of 2-mercaptoethanol and 4.75 g of 2-amino-2 hydroximethil-propane-1,3-diol (TRIS) was added. The flask was then placed into a water bath at 50 °C. The solution was stirred constantly under nitrogen atmosphere for 1 h and then was filtered. The filtrate was dialyzed in 10 L of distilled water using a Spectra-pore cellulose dialysis membrane (MWCO 6000 Da, Carl Roth Zellutrans). The average protein concentration of the filtrates was determined using a protein assay kit (GoldAnalisa), and found to amount to around 3 g/100 mL.

Film preparation

Films were prepared by solution casting and evaporation from water solutions of pure keratin (K) and pure konjac glucomannan (GM), and by mixing the two in different ratios. The samples were denoted as: K/GM wt% (Table 1). Basic water solutions of the two polymers were prepared, each in a concentration of 1%, by constant stirring for 1 hour at room temperature. Glycerol was added to each solution as a cross-linker in a concentration of 0.06 g/g of each polymer. For the preparation of the blends, the basic solutions were mixed together in adequate proportions. 50 mL of each polymer solution was poured into polyethylene Petri dishes and evaporated in a vacuum dryer at 40 °C and 100 mbar.

Microscopy

A ZEISS Axiotech 25HD (+ pole) optical microscope, with an AxioCam MRC (D) high resolution digital camera, was used to analyze the morphological properties of the films and to determine the swelling of the films in water. Also, the cross-section area of the samples was observed to determine their mechanical properties.

FT-IR spectroscopy

FT-IR spectra were recorded on a Perkin Elmer spectrum GX FT-IR spectrometer with a Golden Gate ATR attachment and diamond crystal. The absorbance measurements were carried out within the range of 400-4000 cm^{-1}, with 16 scans and a resolution of 4 cm^{-1}.

Thermal analysis (TGA)

Mettler Toledo TGA/SDTA 851 apparatus was used to perform TGA analyses under the following conditions: temperature range from 30 to 500 °C, heating rate of 10 K/min, nitrogen flow of 200 mL/min.

Determination of mechanical properties

Vibrodyn 400 (Lenzing Instruments, GmbH & Co. KG) apparatus was used to determine the mechanical properties, breaking force and elongation of the films. The film samples were cut into strips with the approx. length of 6 cm and approx. width of 1 mm. Breaking strength was calculated based on the breaking force measurements and cross-section area determination of the specimens. The applied gauge length was 20 mm and the measurement speed was 10 mm/min.

Determination of moisture content

A Mettler Toledo HB43 laboratory analyzer was used to determine the moisture content of the film samples after 24 hours conditioning at a relative humidity of 65% and a temperature of 20 ± 2 °C. The apparatus performs simultaneous weighing and drying of samples in a closed container until the samples reach mass equilibrium.

Determination of swelling in water

The behavior of the films in water was analyzed by using two different procedures. In the first one, the film samples, cut into 1 x 1 cm pieces, were immersed into excess water on Petri dishes, and their behavior, *i.e.* swelling, twisting, dissolution, color change *etc.*, was observed in the time span of 2 h. In the second procedure, the behavior and the appearance of the film samples during the first 50 s of immersion into excess water were monitored by an optical microscope. The changes in thicknesses were measured in 10 s steps.

Table 1
Composition and denotation of film samples

Sample denotation	Keratin content (wt%)	Konjac glucomannan content (wt%)
K/GM 100/0	100	0
K/GM 70/30	70	30
K/GM 50/50	50	50
K/GM 30/70	30	70
K/GM 0/100	0	100

Determination of water contact angles

The OCA35 Contact Angle measurement system from Dataphysics (Germany) was applied for contact angle measurements. A drop of liquid was released onto the film surfaces and photographed. The tangent of the sessile drop profile at the three-phase contact point drawn onto the photo-print and the value of the contact angle were determined. All measurements were conducted at room temperature with a drop volume of 3 μL. To obtain the average value of the contact angles, 10 repetitions were performed for each of the film samples.

RESULTS AND DISCUSSION

Photographs of the films prepared from keratin, GM and their blends are presented in Figure 1. There were slight differences in transparency, color and texture between the film

samples of different compositions. The pure keratin film (K/GM = 100/0) (Fig. 1 a) was transparent, but much stiffer in comparison with other film samples. The sample containing 30 wt% of glucomannan (K/GM = 70/30), was yellowish, but still transparent. Increased amounts of GM (K/GM = 50/50, K/M = 30/70 and K/GM = 0/100) caused increased yellowness and turbidity, as well as better flexibility and softness.

The FTIR spectra of all the film samples are presented in Figure 2. The spectrum of the pure glucomannan film (K/GM 0/100) showed all the characteristic peaks of GM. The broad peak at 3100-3700 cm^{-1} was attributed to –O-H stretching, and the peak at 2882 cm^{-1} – to asymmetric stretching of C-H in the methylene groups.[29,30] A broad band at 1637 cm^{-1} was assigned to stretching of the C-O of the hydroxyl groups, and the band at 1375 cm^{-1} – to the angular deformation of C-H. The C-O ether bond stretching is revealed by the peak at 1150 cm^{-1}, and the C-O alcohol bond stretching – by the peaks at 1066 and 1011 cm^{-1}. The peaks at 874 and 808 cm^{-1} are characteristic of β-glucosidic and β-mannosidic linkages in konjac glucomannan.[29,31]

The FTIR spectrum of the pure keratin film (K/GM 100/0) showed the characteristic bands attributed predominantly to the peptide bonds of amines and amides, labeled as amide A (2800-4000 cm^{-1}), amide I (1600-1700 cm^{-1}), amide II (1480-1580 cm^{-1}) and amide III (1220-1330 cm^{-1}).[32,33] The broad transmission band at 3274 cm^{-1} is assigned to the stretching vibrations of the O-H and N-H bonds (amide A). The bands at 2918 cm^{-1} and 2850 cm^{-1} are assigned to symmetrical CH$_3$ stretching vibrations.[34,35] The strong transmission peak at 1630 cm^{-1} is attributed to C=O vibration of the amide I with α-helix conformation, and the peak at 1529 cm^{-1} comes from N-H bending and C-H stretching vibration of amide II in β-sheet conformation.[35] The peak at 1219 cm^{-1} is characteristic of the stretching vibrations of C-N (amide III).[36,37]

By comparison of the spectra of pure keratin and konjac glucomannan films with those of the blended films, it can be seen that, with an increasing amount of glucomannan, the absorption bands at app. 3280 cm^{-1} and at 2924 cm^{-1} broadened and shifted to higher wave numbers, which confirmed the introduction of the -OH groups of glucomannan. There is also an increasingly expressed peak at 1023 cm^{-1}, which is characteristic of C6-OH bonds. With the addition of higher amounts of glucomannan, the characteristic peaks of mannose at 874 cm^{-1} and 808 cm^{-1} also appeared. At the same time, the reduced intensities of the keratin peaks at 1630 cm^{-1} and at 1530 cm^{-1}, which are characteristic of the vibration of the amide N-H groups, and the intensity of the peak at 1220 cm^{-1}, which is characteristic of the valence vibrations of the C-N bond, are noted. With a higher GM ratio, the peak at 1630 cm^{-1} shifted towards higher wave numbers. This was expressed most clearly in the case of the samples K/GM 30/70 and 70/30, indicating the formation of new hydrogen bonds between the keratin and glucomannan macromolecules.[24,26,38,39]
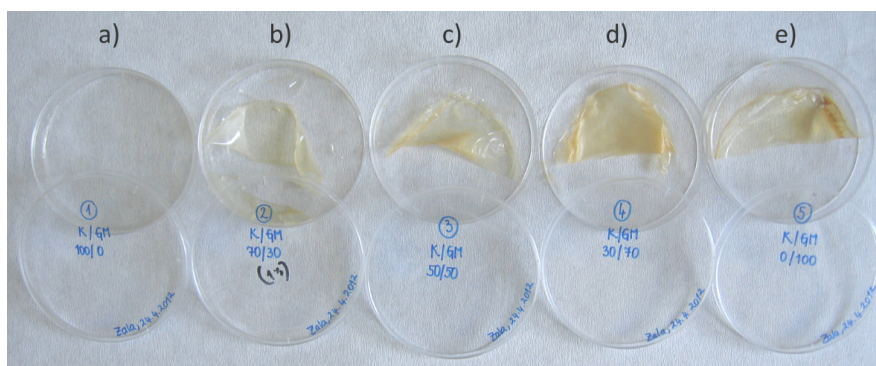

Figure 1: Pure keratin, GM and blend films: a) K/M 100/0, b) K/GM 70/30, c) K/GM 50/50, d) K/GM 30/70, and e) K/GM 0/100
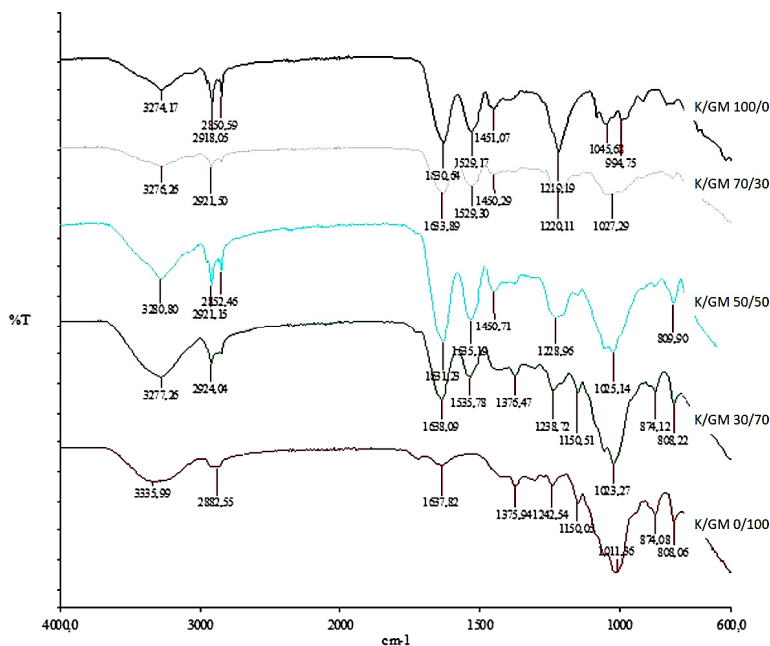
Figure 2: FTIR spectra of pure keratin (K/M = 100/0), pure glucomannan (K/GM = 0/100), and their blend films: K/GM = 70/30, K/GM = 50/50 and K/GM = 30/70
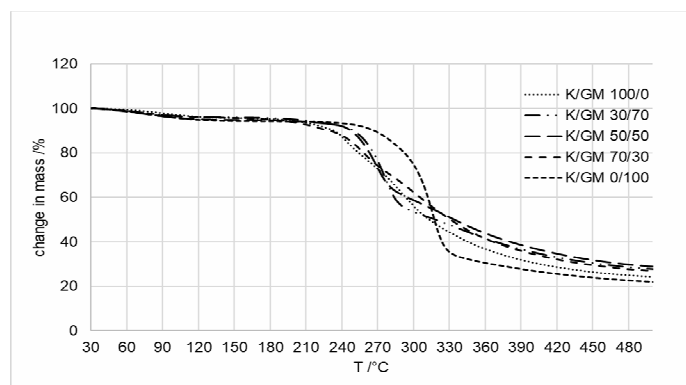

Figure 3: Thermogravimetric curves for samples: K/GM 100/0, K/GM 70/30, K/GM 50/50, K/GM 30/70 and K/GM 0/100

Thermal properties

The thermal properties of the films were investigated by thermogravimetric analysis. The results are shown in Figure 3.

The first change in mass of the keratin film (K/GM 100/0) started at about 75 °C, whilst for the K/GM 0/100 film, it occurred at app. 50 °C. As regards the other three blended films (K/GM 70/30, K/GM 50/50 and K/GM 30/70), the first decrease in mass happened at around 55 °C. In the temperature range from 50 to app. 200 °C, the masses of all the samples were reduced by about 6%. This confirmed the evaporation of weakly bonded water within the samples.

For the pure keratin film, the main thermal decomposition range was between 210 °C and 310 °C. Within this range, the sample lost about 53% of its weight. In the temperature range from 210 °C and 250 °C, the process of melting, or denaturation, of the α-helical fraction of keratin usually takes place.[40] In the same temperature range, the destruction of the disulfide bonds and elimination of H_2S, as well as the thermal pyrolysis of chain linkages, peptide bridges and skeletal degradation of keratin occurs.[35,41] At the temperature of 500 °C, the residual mass of keratin was about 24%.

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The pure glucomannan film (K/GM 0/100) showed the main weight loss in the temperature range from 270 °C to 330 °C, which was about 60 °C higher than for the pure keratin film sample. The weight loss of the film sample K/GM 0/100 within this temperature range was about 59%, and the change was faster than in the case of pure keratin. At 500 °C, the residual mass of the the pure glucomannan film was around 22%.

The main decomposition of all the three blended films K/GM 70/30, K/GM 50/50 and K/GM 30/70 started at app. 240 °C. However, the mass loss in this range was more gradual, in comparison to that of pure GM, and at 500 °C, the residual mass of these samples was around 27%. The temperature at which the main decomposition process of the blended films started was app. 30 °C higher in comparison with that for the pure keratin film, and further decreases of the mass with increasing temperature were much more gradual than in the case of the pure GM film. The results showed that the combination of keratin and GM in the blended films created a synergism regarding the thermal degradation resistance.

The mechanical properties of the films were determined based on the measurements of breaking force and elongation using a Vibrodyn (Lenzing Technik) dynamometer. The results are presented in Figures 4-6.

The lowest breaking strength (16.8 ± 1.4 MPa) was recorded for the film sample made from pure keratin, and the highest – for the film made from pure konjac glucomannan (66.8 ± 2 MPa). From the diagram in Figure 4, the clear trend of the breaking strength increase with the increase in the GM ratio can be observed, most probably owing to the increased amounts of intermolecular interactions between the keratin and GM
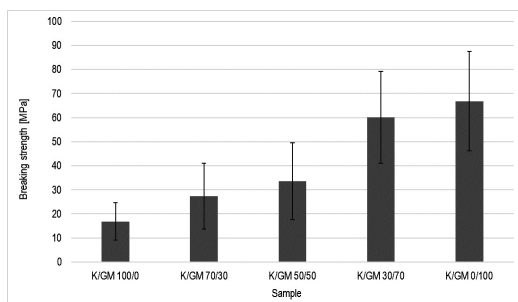
macromolecules.[20] The significantly higher average molecular weight of GM (Mw ~ 200 kDa), compared to that of feather keratin (Mw ~ 10 kDa), provided higher amounts of weak intermolecular interactions, *i.e.* hydrogen bonds and physical chain-to-chain interaction between GM macromolecules, as well as between GM and keratin macromolecules, which led to higher breaking strengths of the blended films.[27,42] Owing to the rather high standard deviations of the measurements, the differences in breaking strengths between the film samples K/GM 70/30 and K/GM 50/50, as well as between K/GM 30/70 and K/GM 0/100, could not be considered as significant.

A significant increase of Young's modulus after the addition of GM could also be observed (Fig. 5). The Young's modulus of the pure keratin film was rather low (212 ± 99.1 MPa), compared to those of the GM and blended films (higher than 700 MPa). Films prepared from proteins, such as keratin, gluten and casein, are usually brittle, owing to the presence of extended hydrogen bonding, ionic and hydrophobic interactions.[43] Additionally, the low average molecular weight of feather keratin could cause higher amounts of irregularities and voids in the supramolecular structure. This could be lessened by the addition of cross-linking agents, such as glycerin, sorbitol or polyethylene glycol.[44]

In this research, the amounts of added glycerol were rather low in comparison to those used in other similar investigations, in order to trace better the influence of GM on the film structure. A small amount (30%) of GM in the film composition already determined a significant (app. 4 times) increase in the Young's modulus of the blended films to around 800 MPa.

Figure 4: Breaking strength (MPa) of samples K/GM 100/0, K/GM 70/30, K/GM 50/50, K/GM 30/70 and K/GM 0/100
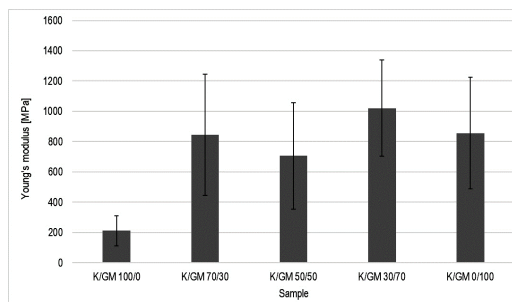
Figure 5: Young's modulus (MPa) of samples K/GM 100/0, K/GM 70/30, K/GM 50/50, K/GM 30/70 and K/GM 0/100
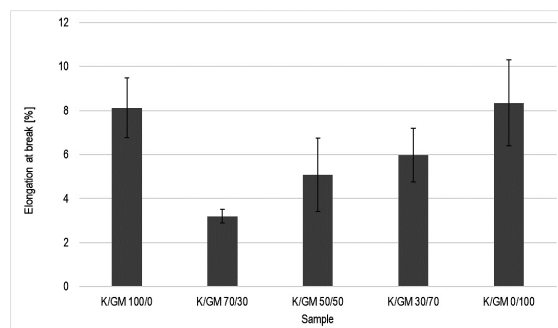
Figure 6: Elongation at break (%) for samples K/GM 100/0, K/GM 70/30, K/GM 50/50, K/GM 30/70 and K/GM 0/100

Table 2
Moisture adsorption equilibrium at RH 65 ± 2% and T = 20 ± 2 °C for pure keratin and GM (K/GM 100/0 and K/GM 0/100) and blend film samples (K/GM 70/30, K/GM 50/50, K/GM 30/70)

Sample	Moisture content, %
K/GM 100/0	7.8 ± 0.2
K/GM 70/30	9.2 ± 0.5
K/GM 50/50	10.1 ± 0.2
K/GM 30/70	11.2 ± 0.9
K/GM 0/100	14.4 ± 0.2

With the addition of high molecular weight GM, the new intermolecular interactions improved the Young's modulus of the samples significantly. Such interactions were proved by the FTIR spectra, especially, in the case of the samples K/GM 30/70 and 70/30, indicating the formation of new hydrogen bonds between the keratin and GM macromolecules. The high average values of the Young's modulus of these two blended film samples were most probably the consequence of these intermolecular bonds.[45]

Elongation at break of all the film samples was rather low (Fig. 6). This was, on the one hand, determined by the low amounts of added glycerol, which plays a significant role in the elasticity of films.[27,44] The pure keratin and GM films showed the highest elongation at break among all the samples (around 8%). The lowest elongation at break was observed for the film that contained only 30% GM, and, with the increasing amounts of GM in the blended films, the elongation at break increased from around 3% for K/GM 70/30 to app. 6% for K/GM 30/70. It is known that the mechanical properties of blended polymer films are influenced by the compatibility between the component polymers,[46] therefore, generally lower elasticity of blended films is expected.

The results of moisture content determination are presented in Table 2. The presented results are the average values determined from measurements of three parallel samples. From Table 2, it follows that the least hygroscopic film sample is the pure keratin film, since its moisture content at equilibrium was around 7.8%. The low moisture sorption of the keratin film is again the proof of the strong intermolecular interactions *via* disulfide and H– bonds and, therefore, low amounts of free hydrophilic (hydroxide, carboxyl, amide) functional groups. As expected, the most hygroscopic was the pure konjac glucomannan film, which, under the defined conditions (65 ± 2% RH and 20 ± 2 °C), adsorbed around 14.4% of moisture. The presence of GM in the blended films influenced the increase in their moisture adsorption abilities. The reason for that is, in the first place, the introduction of large amounts of GM hydroxyl groups, which were accessible to water molecules. Owing to the high average molecular weight of GM, these amounts are high even in the blended film samples with the lowest GM ratio. On the other hand, the GM macromolecules in the blended films modify the supramolecular structure of the blended films by way of increasing the amounts of amorphous regions in comparison with the pure keratin film sample.[24]

The film composition influenced the behavior of the samples in excess water significantly. The keratin film (K/GM 100/0) showed no other changes than slight bending, even after 2 h in water, while it remained transparent and of the same dimensions. Partly, this is also a

consequence of the rather low amounts of glycerol applied in this research, because it is known that the concentration of glycerol in feather keratin films impacts significantly the amount of adsorbed water.[43] On the other hand, the konjac glucomannan film (K/GM 0/100) started to swell immediately after immersion into water, and after 2 h, the majority of the sample was dissolved. As for the blended film samples, the change in transparency appeared from totally transparent at the beginning to opaque white after 2 h, and only a slight bending could be traced.

Because of the rather quick dissolution of some film samples, long lasting exposure to excess water was not an appropriate approach. Therefore, the behavior of samples during the short exposure to water was monitored, as an indicator of water accessibility. The changes of film thickness during the first 50 s of immersion into excess water were measured in time steps of 10 s, using an optical microscope. The results are presented in Figure 7. It can be clearly seen from Figure 7 that the samples with the highest amounts of GM (70 and 100 wt%) swell in water more quickly, and, after 50 s, reach the highest thickness, in comparison with the samples with lower amounts of GM or with the pure keratin film. This proved a more accessible (*i.e.* porous/amorphous) structure of the K/GM film to water. Most probably, due to the low average

molecular weight of feather keratin and the high amount of potential intermolecular binding sites, a supramolecular structure was formed, which was less accessible to water molecules.[24,35]

The surface wettability of the films was monitored by measuring the contact angles (CA) with water. The results were collected in Table 3.

All the films showed hydrophilic surfaces, since all the contact angles with water were lower than 90°. The pure keratin film (K/GM 100/0) showed the least hydrophilic surface among all the samples. The amino acid composition of the central part of feather keratin macromolecules was proved to be mostly hydrophobic.[47] According to previous work,[48] the hydrophobic and hydrophilic amino acids in keratin totaled about 61% and 39%, respectively. Furthermore, films prepared from other similar proteins, such as fibroin[49] or zein,[50] also showed rather high water contact angles. The pure konjac glucomannan film (K/GM 0/100), on the other hand, showed high surface hydrophilicity with low water contact angles of around 30°. There were no significant differences between the water contact angles of the three blended films. It could be concluded that the addition of glucomannan into the blend influences the increase of hydrophilicity of the film samples significantly (Fig. 8).
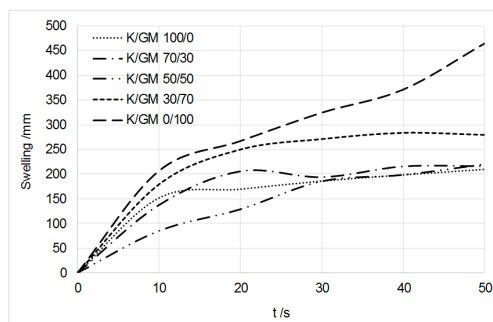

Figure 7: Swelling of film samples (K/GM 100/0, K/GM 70/30, K/GM 50/50, K/GM 30/70 and K/GM 0/100) during short immersion into excess water

Table 3
Contact angles of pure keratin and konjac glucomannan (K/GM 100/0; K/GM 0/100) and blended film samples (K/GM 70/30, K/GM 50/50, K/GM 30/70) with water

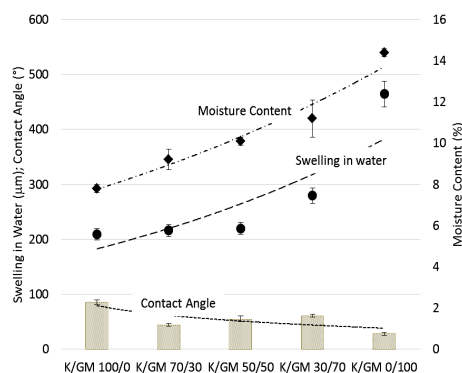| Sample	CA, °
K/GM 100/0	85.2 ± 4.3
K/GM 70/30	44.4 ± 2.9
K/GM 50/50	55.0 ± 4.9
K/GM 30/70	60.9 ± 2.3
K/GM 0/100	28.0 ± 2.5

Figure 8: Dependence of hydrophilic/hydrophobic character (moisture content, swelling in water, contact angles) on the amounts of added konjac glucomannan (GM) in blended films

CONCLUSION

The main drawbacks of the films prepared from pure feather keratin are their low breaking strength and brittleness. Therefore, in this study, feather keratin and konjac glucomannan were combined, in order to develop biopolymer films with satisfying physico-chemical properties. The solution casting and evaporation method was applied for film preparation, increasing the konjac glucomannan (GM) ratio from 30 to 70 wt%. All the physico-chemical properties of the films were altered significantly when compared to those of the pure keratin films.

The FTIR spectra showed typical bands for pure keratin and glucomannan, and especially for the blended film samples K/GM 70/30 and 30/70, which confirmed the formation of hydrogen bonds between the keratin and glucomannan macromolecules. This was also proved by the thermal analyses, as the temperature at which the main decomposition process of the blended films started was app. 30 °C higher in comparison with that for the pure keratin film.

The breaking strength of the blended film samples increased gradually with the increasing amounts of added GM, and, in the case of the sample K/GM 30/70, it was about 400% higher compared to that of the pure keratin film. On the other hand, the Youngs' modulus increased significantly (by about 4 times) when the lowest GM ratio was used (K/GM 70/30).

The about 20 times higher average molecular weight of konjac glucomannan, when compared to that of keratin, most probably had a prevailing influence on the supramolecular structure of the blended films in a way that changed the amounts of regions accessible to water molecules significantly. Thus, the blended films showed 15 to 40% higher moisture absorption, and about 18% higher swelling in water, in comparison with the pure keratin film. Furthermore, the surface hydrophilicity of the film samples increased with the amount of GM added, thus, the contact angles with water decreased by about 35%.

The results of this research revealed the high potential of combining the two biopolymers, konjac glucomannan and feather keratin. The addition of glucomannan endowed the blended films with properties that are essential in different fields of applications, such as in food packaging or for medical applications.

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