**Ewa Kopania,
Maria Wiśniewska-Wrona,
Justyna Wietecha**

Galactoglucomannans (GGMs) Extracted from Spruce Sawdust for Medical Applications

Institut of Biopolymers and Chemical Fibres
ul. M. Skłodowskiej-Curie 19/27, 90-570 Łódź, Poland
E-mail: celuloza@ibwch.lodz.pl

Abstract
The article presents a method of extracting galactoglucomannas (GGMs) from softwood (spruce). GGMs were extracted using thermal and enzymatic treatment in an aqueous environment. The GGMs extracted, depending on the extraction method, were characterized by different compositions of simple carbohydrates i. e. glucose, galactose and mannose, as well as by the average molecular weight. Evaluation of the composition of GGMs obtained was performed using GC/MS and SEC. The biopolymer composites obtained by combining GGMs with microcrystalline chitosan (MCCh), showed suitability for constructing dressing materials in the form of sponges. The studies were performed in order to evaluate antibacterial properities of composite realtive to Escherichia coli standard Gram (-) and their susceptibility to enzymatic and hydrolytic degradation. The research confirmed the usefulness of MCCh/GGMs composities for constructing dressing materials.

Key words: *softwood, galactoglucomannans microcrystalline chitosan, thermal treatment, enzymatic treatment, biopolymer composities.*

List of abbreviations

GGM – galactoglucomannans
MKCh – microcrystalline chitosan
G/MKCh – sponge with the participation of microcrystalline chitosan
GGM-S – galactoglucomannans from spruce sawdust after thermal treatment
MKCh/GGM – biocomposite of microcrystalline chitosan with the inclusion of galactoglucomannans

Inroduction

At present a tendency exists to explore new active biomaterials based on natural polymers, such as chitosan or totally new connections with other natural polymers, such as wood hemicellulose. In pulp production a particular part of hemicelluloses is dissolved and removed from the cooking liquor. The composition of the cooking liquor depends on the method of cooking. The content of hemicelluloses in softwood is 26% on average and is mostly hexosanes, whereas in hardwood it is 20 - 33%, with most of them being pentosanes and uronic acids [1, 2].

Galactoglucomannas, belonging to hemicelluloses, are important to numerous branches of industry (pulp and paper, textile, pharmaceutical, food and agriculture). Their usefulness is mainly the result of the ratio of galactose, glucose and mannose residue amounts, the average degree of polymerisation and their ability to deposit on the surface of cellulose materials [3 - 5]. GGMs can be fully used as bioactive substances to prepare antibacterial dressing materials with susceptibility to enzymatic and hydrolytic degradation [6].

Galactoglucomannans extracted from plant materials can create new natural composites with chitosan.

Chitosan is a polysaccharide which now plays a particular role in medicine. It is a derivative of chitin which is produced in the process of chemical deacetylation in the presence of concentrated NaOH or KOH at a temperature of 100 °C or higher [7]. Chitosan (β-1,4-2-amino-2-deoxy-D-glucopyranose) is a non-toxic, biodegradable and antibacterial polymer.

A specific property of chitosan is its bioactivity, which determines its medical application. The bioactivity is associated with a number of phenomena and processes such as biodegradation, the effect on the living cell membrane, and stimulating natural immunity. The biological activity of chitosan is dependent on the susceptibility of the polymer to enzymatic degradation in the presence of lysozyme and N-acetyl-D-β-glucosaminidase. These enzymes are found in human body fluids and produce bioactive oligomers of N-acetyl-D-glucosamine. Earlier studies have shown that hexamine which includes N-acetyl-D-glucosamine accelerates wound granulation, thereby accelerating the healing process [8].

It was found that galactoglucomannans isolated from raw materials could form new natural chitosan biocomposites [8].

As with chitosan, galactoglucomannans show biodegradable and biocompatible properties and can be connected to chitosan by hydrogen bonds. Chitosan readily reacts to GGMs in an acid medium to form intermolecular hydrogen bonds with the amino group of chitosan and carboxyl group of galactoglucomannans. The reaction between these groups leads to a water-insoluble complex. The activity of this complex depends primarily on the deacetylation degree. A higher deacetylation degree increases the "biological activity" of the MCCh/GGMs complex [9, 10].

The biological activity of GGMs depends on their chemical structure, especially on the galactosyls chain length and deacetylation degree. These factors have an important influence on the increase in activity of GGMs and their use as a potential antibacterial agent in dressing materials [11]. In addition, GGMs are most often

used as a carrier of medicaments which increase their activity and reduce adverse side effects [12, 13].

The aim of this work was to study the application possibility of isolated GGMs from spruce sawdust. The GGMs obtained were used for the preparation of useful composite materials. The hemicelluloses obtained were converted into functional GGMs and biocomposites with selected GGMs. The GGMs and biocomposities/GGMs had to maintain controlled structure parameters with simultaneous indication of their suitability for the construction and modification of dressing materials in the form of sponges. The sponges obtained were characterised by improved strength, antibacterial properties, sorption parameters and susceptiblity to enzymatic and hydrolytic degradation.

Experimental part

Raw material

Softwood – spruce (*Picea abies*) was utilised for the purposes of this research

Galactoglucomannans (GGMs)

We used (GGMs) extracted from sawdust of spruce wood. Two main processing methods were used to extract GGMs: the thermal and thermal/enzymatic methods, with selected enzymes from groups of cellulases and hemicellulases according to the methodology described in literature [14]. The characteristics of the isolated GGMs are shown in ***Table 1***.

Chitosan

We used chitosan from Primex ehf. The characteristics of chitosan are shown in ***Tables 2*** and ***3***.

For the preparation of biopolymer composites, microcrystalline chitosan (MCCh) was used with the following physico-chemical parameters (***Table 4***).

For the study of the enzymatic and hydrolytic degradation of the biopolymer composites, the following were used:
- phosphate buffer pH 7.4,
- Lysozyme, also known as muramidase from egg white, EC 3.2.1.17, by Merck, activity 50,000 U/mg. The enzyme was used in the biodegradation process of the composite materials (sponges).

Research methodology

Preparation of microcrystalline chitosan (MCCh)

Microcrystalline chitosan (MCCh) was prepared by agglomeration from solution using a method continuously developed at the Institute of Biopolymer and Chemical Fibres (IBWCh) [15], with the use of a continuous reactor - Dispax Reactor Labor-Pilot 2000/4 by IKA (Germany).

Preparation of biopolymer composites to produce dressing materials

To prepare biopolymer composites, GGMs were added to a suspension of microcrystalline chitosan in the form of aqueous solutions in a proportion of 10, 15, 20 wt%. with respect to chitosan. Then glycerin was added to the polymer mixture in an amount of 0.4 part weight to 1 part weight of biocomposite (based on the dry weight of polymer). The mixture was homogenised for 10 to 15 min using a high speed stirrer type T 50 IKA at 2.000 - 3.000 r.p.m.

Method of preparation of dressing materials in the form of sponges

Dressing material was prepared in the form of a sponge (by drying techniques) in a laboratory lyophiliser - ALFA Christ 1-4 (Germany). Lyophilisation was carried out in the temperature range of -20 to 10 °C and pressure of 10 - 70 Pa. The time of drying was 20 - 24 hours.

Sterilisation of biopolymer composites as sponges

The sterilisation of biopolymer composites was performed using two methods:
- by fast electrons at the Institute of Applied Radiation Chemistry, Lodz University of Technology, using a dose of 25 kGy radiation
- using a steam autoclave at 121 °C for 15 minutes.

Study of the antibacterial activity of sponges with microcrystalline chitosan and sponges with the inclusion of galactoglucomanns

Testing of the antibacterial activity of MCCh sponges and biocomposite sponges was carried out at the Microbiological Laboratory of IBWCh in accordance with Procedure PB-1, Issue. VI – Examination of antibacterial activity of fibers and textiles by quantitative and qualitative methods (JIS L 1902:2002) (IBWCh) [16].

Testing cytotoxic of dressing sponge

Research of cytotoxic effects in direct contact was carried out at the Nofer Institute of Occupational Medicine, Lodz, the Department of Toxicology and Carcinogenesis of the Laboratory of Molecular Toxicology. Research was performed on mouse fibroblast cells, line BALB/3T3, clone A31 (Mouse embryo, ATCC CCL-163), in neutral red uptake assay (NRU),

Table 1. *Characteristics of GGMs isolated from spruce wood shavings after thermal and enzymatic treatment (enzyme Hemicellulase).*

Symbol	After treatment	Average Mw, Da	Carbohydrate content – mass ratio		
			Glucose	Galactose	Mannose
GGM-S	thermal	39 253	1	13	2
GGM-2	enzymatic	113 316	206	1	1

Table 2. *Physicochemical properties of starting chitosan.*

Symbol of chitosan	Mv, kDa	SD, %	Ash, %	WRV, %
ChitoClear hqg 95	373.0	81.0	0.31	156.0

Table 3. *Molecular characteristics of starting chitosan (ChitoClear hqg 95) and microcrystalline chitosan (MCCh/HQG); $\bar{M}_n$ – number average molar mass, $\bar{M}_w$ – mass average molar mass, Pd – polidyspersity.*

Symbol of chitosan	$\bar{M}n$, kDa	$\bar{M}w$, kDa	Pd	Percentage fractions Mw × 10-3, %						
				< 5	5-50	50-100	100-200	200-400	400-800	>800
ChitoClear hqg 95	26.3	130.7	5.78	4	35	17	20	14	8	2
MKCh/HQG	24.8	119.1	5.54	4	35	18	20	15	7	1

Table 4. *Physicochemicals parameters of mcrocrystalline chitosan (MCCh).*

Symbol	Starting of chitosan	$\bar{M}_v$, kDa	SD, %	Cocncentration of polymer, %	WRV, %	pH
MKCh/HQG	ChitoClear hqg 95 (TM 3445)	310.0	81.0	2.68 -3.89	650.0	7.0-7.3

under 24 hour incubation, according to PN-EN ISO 10993-5:2009 "Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity".

Degradability evaluation of selected dressing materials in the form of sponges

Hydrolytic degradation was carried out according to Polish Standard PN-EN ISO 10993-13:2002.

Degradability evaluation was performed on dressing materials in the form of sponges made from MCCh with the addition of GGMs.

Enzymatic degradation. Lysozyme of 200 μg/cm^3 was used for enzymatic degradation. Preparations were removed from the bath after 1, 3, 7, 14 and 21 days, filtered in a Büchner funnel, and then washed in distilled water at 50 °C with the addition of 70% ethyl alcohol. The preparations were dried to constant weight in a lyophiliser. The degradation process of composite sponges was evaluated based on changes in the pH-value, weight loss and concentration of aminosccharides produced by enzymatic and hydrolytic degradation of chitosan contained in the composite. Photographic documentation was made for all preparations.

Determination of average molecular weight of chitosan

- Determination of the average molecular weight of chitosan was made according to the viscosimetric method: SPR/BPB/5 IBWCh procedure [17].
- Determination of the average molecular weight of chitosan was made by gel chromatography SEC/GPC according to the SPR/BLF/ IBWCh procedure [18].

Determination of chitosan deacetylation degree

Determination of the chitosan deacetylation degree (SD) was made in the UV spectrum by the first derivative method according to the SPR/BLF/21 IWBCh procedure [19].

Determination of ash content

The ash content of chitosan was determined by weight based on the residue after ashing the sample at 800 °C according to SPR/BLF/6 IBWCh [20].

Determination of chitosan in the preparation

The polymer content of microcrystalline chitosan was determined according to the SPR/BPB/11 IBWCh procedure [21].

Determination of the water retention value (WRV)

The water retention value was determined gravimetrically according to the SPR/BPB/14 IBWCh procedure [22].

Determination of physico-mechanical parameters of composite materials in the form of sponge

The mechanical properties were determined at the accredited Laboratory of Metrology IBWCh, (certified accreditation AB 338). The basic mechanical parameters of the composite materials were evaluated according to the following standards [23, 24]:

- PN-EN ISO 4593:1999 – thickness in mm;
- PN-EN ISO 527-3:1998 – tensile strength in MPa and elongation at max. tension in %.

Determination of the amount of aminosccharides released

Aminosccharides of the dressing materials obtained after hydrolytic and enzymatic degradation were determined by the colorimetric method using 3.5-dinitrosalicylic acid (DNS). The method is based on measurnig the absorbance (E) of the test samples in relation to the zero sample at a wavelength of $\lambda = 540$ nm by a Helios γ (USA) spectrometer [25].

Research results and discussion

Preparation of GGMs using forms and biocomposites with GGM content with controlled structure parameters

Polymer biocomposites of microcrystalline chitosan (MCCh) were prepared as a gel suspension. The MCCh (characteristics see ***Table 2***) were prepared by agglomeration with a solution of chitosan using the continuous method. Physicochemical parameters and GPC analysis of microcrystalline chitosan are shown in ***Tables 3*** and ***4***.

Biopolymer composites with bioactive galactoglucomannans (GGMs), useful for functionalisation of dressing materials, were obtained from spruce sawdust as a result of thermal (sample GGM-S ***Table 1***) and enzymatic treatment using a commercial formulation of Hemicellulase (sample GGM -2, see ***Table 1***).

Microcrystalline chitosan (MCCh) with characteristics contained in ***Table 3*** was used as a basic component of the biocomposite. Biopolymer composites MKCh/GGM were prepared according to the methodology described. GGMs were added to a suspension of microcrystalline chitosan in a dissolved form in quantities of 10, 15, 20 wt%. in relation to the content of chitosan in the formulation.

In the case of preparing biocomposites used for the construction of dressing materials in the form of sponges in order to ensure appropriate flexibility and drape of the dressing, a plasticiser (glycerin) was added to the mixture of polymers in a quantity of 0.4 part. weight to 1 part weights of the biocomposite (based on the dry weight of polymers).

The project aim was to develop a composite of MCCh/GGMs to produce dressing materials in the form of sponges with high tensile, antibacterial and sorption properties, as well as vulnerable to enzymatic and hydrolysis degradation.

Application of bioactive GGMs to obtain the functionalisation of selected dressing materials

The biopolymer composites MCC/GGMs were tested for use in dressing materials in the form of sponges. GGMs with the characteristics contained in ***Table 1*** were used to make the biocomposite. The sponge dressing was prepared according to the methodology described. The biocomposite sponges produced were evaluated for their physico-mechanical and sorption properties. They were also examined for their susceptibility to hydrolytic enzymatic degradation in the presence of lysozyme. Biological studies were also performed on selected biocomposites, and included the evaluation of cytotoxic activity.

Assessment of physico-mechanical properties of dressing materials in the form of sponges with the participation of galactoglucomannans

The strength and sorption properties of the GGMs biocomposities were examined. We evaluated dressings materials containing GGMs and MCCh at 10, 15, 20% wt. In order to ensure sufficient flexibility and drape of the biocomposities prepared, glycerin was added to the

Tabela 5. *Physical and mechanical parameters of dressing materials in the form of sponges with the inclusion of galactoglucomannans.*

Symbol of sample	Thickness, mm	Tensile strength, MPa	Elongation at max. tension, %	WRV, %
G/MCCh	**2.77 ± 0.07**	**0.07 ± 0.01**	**4.54 ± 1.2**	**141**
Coefficient of variation	2.06	2.65	21.6	nd
G/MCCh + 10% GGM-2	**3.13±0.16**	**0.05±0.01**	**4.70±0.15**	**142**
Coefficient of variation	3.99	4.93	7.99	nd
G/MCCh + 15% GGM-2	**2.88±0.16**	**0.05±0.01**	**5.91±1.3**	**146**
Coefficient of variation	4.62	6.18	17.3	nd
G/MCCh + 20% GGM-2	**3.04±0.10**	**0.06±0.01**	**9.56±1.57**	**158**
Coefficient of variation	2.57	13.2	13.2	nd
G/MCCh + 10% GGM-S	**2.88±0.05**	**0.08±0.01**	**6.60±1.6**	**114**
Coefficient of variation	1.53	6.92	19.9	nd
G/MCCh + 15% GGM-S	**3.28±0.21**	**0.04±0.01**	**5.19±1.4**	**131**
Coefficient of variation	5.18	7.62	22.2	nd
G/MCCh + 20% GGM-S	**3.61±0.10**	**0.06±0.01**	**6.92±1.7**	**140**
Coefficient of variation	2.13	15.20	19.4	nd

Tabela 6. *Antimicrobial activity of dressing materials in the form of sponges (quantitative method).*

Symbol sample	*Escherichia coli (ATCC 11229)*	
	Activity	
	Bacteriostatic	Bactericidal
G/MCCh (reference)	2.1	- 1.7
G/MCCh + 10% GGM-2	4.2	0.6
G/MCCh + 15% GGM-2	3.7	0.0
G/MCCh + 20% GGM-2	4.5	0.4
G/MCCh + 10% GGM-S	2.4	-1.2
G/MCCh + 15% GGM-S	2.9	-0.8
G/MCCh + 20% GGM-S	2.7	-1.4

mixture in an amount of 0.4 part weight to 1 part weight of the composite (based on the dry weight of polymer). We used different types of GGMs:

- GGM-2 obtained from spruce sawdust after enzymatic treatment (for characteristics see ***Table 1***),
- GGM-S obtained from spruce sawdust after thermal treatment (for characteristics see ***Table 1***).

The control sample contained sponge made from MCCh without GGMs. The test results are shown in ***Table 5***.

We can conclude that the addition of GGM of 10, 15 and 20 wt% relative to the chitosan in the composite significantly affects the flexibility of the sponge dressing - elongation at maximum stress (***Table 5***).

It is visible that the best dressing sponge flexibility was exhibited by the sample with a symbol of G/MCCh + 20% GGM-2, for which the elongation at maximum stress was 9.56%, more than twice higher than the control. The study also showed that the composite containing GGMs also affected the sorption capacity of sponge dressings. In the case of formulations containing GGM-2 in the quantity of 20 wt%, the WRV was about 10% higher than that of the dressing material formed from chitosan. The resultant low strength value of sponges can be compensated using sponge as a dressing hybrid with a nonwoven substrate.

Antimicrobial activity of selected dressing material containg galactoglucomannans under *in vitro* conditions

One of the main problems in the proper treatment of wounds is the possibility of various kinds of infections. An alternative for eliminating this problem is the application of dressings exhibiting antibacterial activity that constitutes effective protecion against microorganism development.

At this stage of the research we evaluated antibacterial activity with respect to bacterium *Escherichia coli* Gram (-), of selected dressing material in the form of a sponge with different inclusions of GGMs (for GGM-2 & GGM-S characteristics see ***Table 1***). The study also evaluated the activity of sponges obtained from microcrystalline chitosan without GGMs. *E. coli* ranked among to enterobacteriaceae, which inhabit human and animals gastrointestinal tract and often cause development of various infections, which clinical symptoms and course are dependent on the place of their occurrence [26].

The assessment of antibacterial activity was carried out for preparations of biocomposites after sterilisation by steam at 121 °C for 15 minutes. The test results are summarised in ***Table 6***.

It was found that the sponge composite and control sample tested (sponge MCCh without GGMs) showed only bacteriostatic properties towards *Escherichia coli* bacteria, as evidenced by activity values (***Table 6***). The increase in GGM composite content did not significantly affect the change in activity of the sponge dressings.

Determining the cytotoxicity of dressing material in the form of sponges under in vitro conditions

Dressing materials used to treat various types of wounds should adequately ensure the maintenance of a moist environment around the wound, a constant temperature similar to that of the human body and lightly acidic pH. Additionally they must protect the damaged spot against external sources of infection. The factors mentioned above have an impact on the stimulating effect of the processes taking place in the next stages of wound healing and reduce pain associated with the wound not only by moisture limiting the stimulation of nerve fibers but also through acidic pH, which reduces the production of prostaglandin PGE2, sensitising nerve endings [11]. Due to the direct contact of the dressing material with the skin and damaged soft tissue, it must be made of biocompatible polymeric materials. Therefore selected biocomposites of MCCh/GGMs in the form of sponges were evaluated by carrying out cytotoxic effects under *in vitro* conditions.

Dressing was evaluated in the form of sponges (G/ + 20% MCCh GGM-2), which showed the best funcional and bacteriostatic properties.

Research of cytotoxicity showed that the extract of test material G/ + 20% MCCh GGM-2 after 24 hours of incubation exhibits cytotoxic properties on BALB/3T3 fibroblast cells clone A31. However, it should be stressed that in spite of the extract showing a cytotoxic effect under *in vitro* conditions, it does not mean that this extract will exhibit cytotoxicity *in vivo*.

Evaluation of the susceptibility of selected dressing material in the form of sponges to hydrolytic and enzymatic degradation

One of the most important features of biological dressings is the biodegradation ability and resorption of decomposition products in the human body, which further enables their application in the treatment of tissue damage.

Chitosan, which is one of the main components of the polymer biocomposites developed, are prone to the action of specific hydrolytic enzymes. A result of the enzyme action, is formed in the human body by bio-active resorbable low molecular products of degradation (mono- and active oligoamino glucoses) – showing, among others, stimulating properties of the wound healing process, to facilitate the reconstruction of the damaged tissue and vascularisation. An enzyme which has the ability to degrade chitosan is lysozyme, present in human body fluids [8, 27].

Hydrolytic and enzymatic degradation was tested in the form of a sponge with symbol G/ + 20% MCCh GGM-2, which includes galactoglucomannans. GGMs were obtained from spruce wood sawdust after enzymatic treatment with a commercial preparation (Hemicellulase).

The enzymatic and hydrolytic degradation was evaluated based on pH changes, the percentage loss of weight and concentration of aminosccharides. Evaluation of the susceptibility to enzymatic and hydrolytic degradation was carried out for 21 days. The time of hydrolytic and enzymatic degradation was determined on the basis of studies previously carried out by the Team of Biomaterials IBWCh [28 - 30] and literature review [8]. The test results are shown in ***Tables 7*** and ***8***.

Table 7. *Results of hydrolytic degradation of biocomposite preparations G/MCCh + 20% GGM-2/H.*

Degradation time, days	pH	Loss of weight, %	Concentration of aminosccharidess, µg/cm3
0	7.40	0.00	0.00
1	7.44	23.24	4.07
7	7.41	24.60	5.94
21	7.42	25.69	7.87

Table 8. *Results of enzymatic degradation of biocomposite preparation G/MCCh + 20% GGM-2/E.*

Degradation time, days	pH	Loss of weight, %	Concentration of aminosccharides, µg/cm3
0	7.40	0.00	0.00
1	7.40	33.55	7.81
3	7.39	34.54	12.65
7	7.38	36.78	13.26
14	7.43	37.73	23.10
21	7.41	38.84	26.21

The results presented in ***Table 7*** indicate that the sponge with GGMs test (symbol G/MCCh + 20% GGM-2) in the amount of 20% weight are slightly susceptible to hydrolytic degradation. The biggest loss in mass of 23% was observed after keeping the sponges in phosphate buffer for 24 h. In the following days of the test duration, the loss of mass changed slightly, with an increase to 24 - 26%. The progression of degradation was accompanied by the release of minor amounts of aminosccharides. The amount of aminosccharides after 21 days of degradation was 7.87 µg/cm3. After 21 days of keeping the dressing sponge in phosphate buffer, no important structural changes in the sponges were observed, as confirmed by the macroscopic pictures (***Figure 1***).

The results also confirm the susceptibility of the biocomposite G/MCCh + 20% GGM-2 to enzymatic degradation (***Table 8***). The biggest attack on the enzyme preparation followed after one day of the test. The loss of weight of the preparation was more than 33.55%. After a further 7, 14 and 21 days the loss increased by approximately 9%.

At the same time, which is very important, during the degradation a fairly intense release of aminosccharides was observed, whose concentration after 21 days was about 26.21 µg/cm3. During the degradation of the dressing sponges no changes in pH were observed. After 21 days of degradation in the phosphate buffer no significal structurasl changes of the dressing sponges were observed, as confirmed by the macroscopic photos of the sponges (***Figure 2***).

Conclusions

1. The participation of galactoglucomannas in a composite of chitosan/GGMs in amounts of 10, 15 and 20 wt% relative to the chitosan significantly affected the flexibility of the sponge dressing obtained (elongation at maximum stress). The best flexibility was

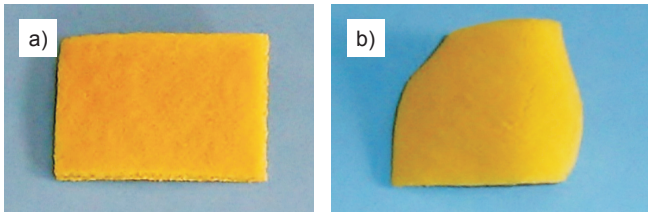

Figure 1. *Macroscopic photos of dressing sponges after hydrolytic degradation (for a period of 0 - 21 of days); G/MKCh + 20% GGM-2/H a) starting sample, b) sample after 21 days of degradation.*

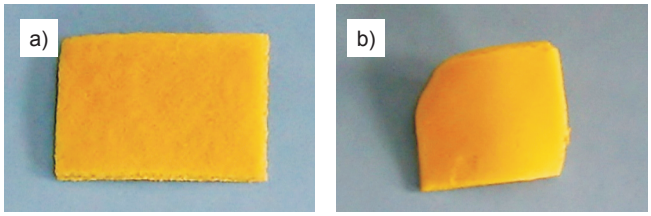

Figure 2. *Macroscopic photos of dressing sponges after enzymatic degradation (for a period of 0 - 21 of days); G/MKCh + 20% GGM-2/E a) starting sample, b) sample after 21 days of degradation.*

shown by a sponge dressing containing 20% GGMs. GGMs were isolated from the spruce wood after enzymatic treatment with Hemicellulase enzyme (sample GGM-2). The elongation at maximum stress was two times higher than that of the control sample. WRV reached a value about 10% higher than that of dressing material produced from the initial chitosan.

2. The composite sponges and control sample (MCCh sponge without GGM) showed only bacteriostatic properties against bacteria *Escherichia coli*. The increase in GGMs in the composite did not significantly affect the change in activity of the sponge dressings.
3. Composite sponges with the addition of GGMs showed a cytotoxic effect at a concentration of 100%. It should be emphasised that the cytotoxic effect of sponges *in vitro* does not mean that the action occurs *in vivo*.
4. Sponge biocomposites containing GGMs are susceptible to enzymatic degradation in the presence of lysozyme. After one day's action the enzyme caused a loss of mass of the preparation of more than 33.55%, and after 21 days - more than 38,84%. A the same time, during the degradation a release of aminosccharides was observed. The concentration of aminosccharides after 21 days was about 26.21 μg/cm^3.

Summary

As a result of the thermal and enzymatic treatment of spruce sawdust, galactoglucomannans of varied compositions of monosaccharides and molecular mass were obtained.

The research confirmed the usefulness of GGMs for the production of biocomposites with MCCh intended for the formation of dressing materials.

Acknowledgements

We would like to thank NCN for funding the work, conducted as part of N N508 619838: 'Biologically active galactoglucomannans acquired from the Polish conifer tree research project.

References

1. Surewicz W. Podstawy technologii mas włóknistych, Ed. WNT, Warszawa (1971).
2. Wandelt P. Technologia celulozy i papieru. Technologia mas włóknistych, część 1, Ed WSiP, Warszawa (1983).
3. Willför S, Sundberg K, Tenkanen M, Holmbom B. Spruce-derived mannans – A potential raw material for hydrocolloids and novel advanced natural materials, *Carbohydrate Polymers* 2008; 72: 197–210.
4. Palm M, Zacchi G. Separation of hemicellulosic oligomers from steam-treated spruce wood using gel filtration, *Separation and Purification Technology*, 36, 191–201(2004)
5. Xu Ch, Eckerman Ch, Smeds A, Reunanen M, Eklund PC, Sjöholm R, Willför S. Carboxymethylated spruce galactoglucomannans: preparation, characterisation, dispersion stability, water-in-oil emulsion stability, and sorption on cellulose surface, *Nordic Pulp and Paper Research J* 2011; 26, 2: 167-178.
6. Mestechkina LNM, Egorov AV, Anulov OV, Shcherbukhin VD. Study of Galactoglucomannans from the Seed of *Cercis canadensis*", *Mikrobiologiya* 2005; 41, 3: 324–329. Original Russian Text Copyright © 2005 by Mestechkina, Egorov, Anulov, Shcherbukhin.
7. Piątkowski M. Chemical modification of chotosan under microwave irradiation, *Czasopismo Techniczne*, Wyd. Politechniki Krakowskiej, 1Ch, in Polish (2008)
8. Nordtveit RJ, Vårum KM, Smidsrød O. Degradation of partially N-acetylated chitosans with hen egg white and human lysozyme, *Carbohydrate Polymers* 1996; 29: 163-167.
9. Ye X, Kennedy JF, Li B, Xie BJ. Condensed state structure and biocompatibility of the konjac glucomannan/chitosan blend films, *Carbohydrate Polymers* 2006; 64: 532-538.
10. Zhang Y, Xie B, Gan X. Advance in the applications of konjac glucomannan and its derivatives, *Carbohydrate Polymers* 2005; 60: 27-31.
11. Alonso-Sande M, Teijeiro-Osorio D, Remuñán-López C, Alonso MJ. Glucomannan, a promising polysaccharide for biopharmaceutical purposes, *European Journal of Pharmaceutics and Biopharmaceutics* 2009; 72: 453-462.
12. Carien E, Beneke M, Alvaro M, Viljoen JH, Hamman H. Polymeric Plant-derived Excipients in Drug Delivery, *Molecules* 2009; 14: 2602-2620.
13. Alonso-Sande M, Cuña M, Remuñán-López C, Teijeiro-Osorio D, Alonso- Lebrero JL, Alonso M-J. Formation of new glucomannan–chitosan nanoparticles and study of their ability to associate and deliver proteins, Macromolecules 2006; 39: 4152-4158.
14. Kopania E, Milczarek S, Bloda A, Wietecha J, Wawro D. Extracting Galacto-glucomannans (GGMs) from Polish Softwood Varieties, *Fibres & Textiles in Eastern Europe* 2012; 20, 6B(96): 160-166.
15. Polish patent P-281975 (1989).
16. Procedure PB-1, wyd. IV – Examination of antibacterial activity of fibers and textiles quantitative and qualitative methods (JIS L 1902:2002) (IBWCh).
17. Procedure SPR/BPB/5 – Determination average molecular weight of chitosan, GLP Nr. G-016 (IBWCh).
18. Procedura SPR/BLF/10 - Estimation of molecular weigh distribution, average molecular weigth and polydispersity of cellulose and chitosan by gel chromatography method (GPC/SEC), GLP Nr. G-016 (IBWCh).
19. Procedure SPR/BLF/21- Estimation of deacetylation degree by the method of first differential coefficient of the UV spectrum, GLP Nr. G-016 (IBWCh).
20. Procedura SPR/BLF/6 – Determination of ash content, GLP Nr. G-016 (IBWCh).
21. Procedure SPR/BPB/11 – Determination of the chitosan content in microcrystalline chitosan (IBWCh).
22. Procedura SPR/BPB/14– Determination WRV of starting chitosan and microcrystallie chitosan, GLP Nr. G-016 (IBWCh).
23. PN-EN ISO 4593:1999 – Plastic-Film and scheeting – Determination of thickness by mechanical scanning.
24. PN – EN ISO 527-3:1998 – Plastics - Determination of Tensile Properties - Part 3: Test Conditions For Films And Sheets.
25. Procedura SPR/BBP/5 – Estimation of aminosccharides by colorimetry with 3,5 dinitrosalicylic acid, GLP Nr. G-016 (IBWCh).
26. Kołodyński J. Fundamentals of bacteriology, Issue. Uniwersytetu Wrocławskiego, 169-174, (1998).
27. Michalak J, Andziak P. The application of modern dressings in the treatment of difficult to heal wounds, Medipress, (2000).
28. Wiśniewska-Wrona M**,** Niekraszewicz A, Struszczyk H, Guzińska K. Estimation of Polymer Compositions Containing Chitosan for Veterinary Applications, *Fibres & Textiles in Eastern Europe* 2002; 10, 3(38): 82-85.
29. Kucharska M, Ciechańska D, Niekraszewicz A, Wiśniewska–Wrona M, Kardas I. Potential use of chitosan–based materiale in medicine, *Progress on chemistry and application of chitin and its derivatives* 2010; XV: 169-176.
30. Wiśniewska-Wrona M, Pałys B, Bodek KH. Study on the usability of polymer complexes in the form of films applied in bedsore treatments, *FIBRES & TEXTILES in Eastern Europe* 2012; 20, 6B,(96): 149-153.

Received 07.10.2013 Reviewed 29.01.2014