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SYNTHESIS OF QUERCETIN FUNCTIONALIZED CHITOSAN AND DETERMINATION OF ANTIOXIDANT PROPERTIES

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Abstract. This paper is dedicated to the synthesis of a copolymer with reducing properties obtained by functionalizing chitosan with quercetin and determining the antioxidant activity of the derivatives obtained depending on the molar mass of the polymer. For this purpose, low molecular weight chitosan was obtained by oxidizing commercial chitosan with hydrogen peroxide and further functionalization with quercetin by the covalent grafting method. The functionalization process was performed through the following steps: functionalization of chitosan with ethyl chloroformate to increase the reactivity of the amine group to the hydroxyl group of quercetin and grafting the quercetin molecule to the synthesized intermediate. The comparative antioxidant properties of the composite obtained by grafting technical chitosan with quercetin and by grafting low molecular weight chitosan were studied by the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) method. The obtained results indicate that a decrease in the molecular weight of chitosan contributed to its grafting with quercetin. As a result, the functionalized polymer composite acquired a higher antioxidant activity and can be successfully used to inhibit the oxidation of various organic substrates in the cosmetic, food and pharmaceutical industries.

Keywords: chitosan, functionalization, grafting, quercetin, antioxidant activity.

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Introduction

Chitosan is a linear polysaccharide composed of fragments of *D*-glucosamine and *N*-Acetyl-*D*-glucosamine linked by *β*-(1-4) bonds. It is obtained by deacetylation of chitin, the structural component of the fungal cell walls and the exoskeleton of crustaceans and insects. Chitosan possesses favourable physicochemical properties such as low toxicity, biodegradability, biocompatibility, antioxidant properties, antimicrobial activity, each of which favours its use in various fields of study [1,2]. However, as a bio functional material it has certain limitations, in particular due to its insolubility in certain conditions: chitosan is soluble in the acidic pH range, but insoluble in the neutral or basic range [3].

The most important improvement of the properties of the chitosan macromolecule is based on its functionalization with biologically active natural compounds, from the class of flavonoids. Quercetin (3,3,4,5,7-pentahydroxy flavone) is one of the most abundant flavonoids found in human diet. Quercetin boasts a plethora of biological properties, including antioxidant, anticancer, anti-inflammatory, antibacterial, antiviral, radical-scavenging, gastro protective and immune-modulatory activities [4-8]. Despite its multifaceted pharmacological benefits, quercetin's utility in the pharmaceutical industry is hampered by its limited solubility in water [9].

Over the past few decades, polyphenol-chitosan derivatives have raised the attention of scientists and became a research topic in the pharmaceutical, medicinal and food industries. In addition to the widely studied bioactivities, polyphenols are also well known for their antioxidant activity [9,10]. Different types of polyphenol-chitosan derivatives share common enhancements in their physicochemical and biologically active properties, including improved water solubility and stronger antioxidant activity. This improvement in antioxidant properties is attributed to the introduction of hydrophilic hydroxyl groups into the chitosan chain. As for polyphenols, it has been established that their bioactivities are determined by the molecular structure, in other words, the degree and position

of hydroxylation [11]. Therefore, it is assumed that the physicochemical properties and applications of polyphenol-chitosan compounds depend on the specific grafted polyphenol and its conjugation position. The study of new methods of functionalization of chitosan continues to attract special interest among the scientific community. Thus, three methods for obtaining chitosan-polyphenol derivatives were developed: a) ester-mediated modification; b) enzyme-mediated strategy; c) free radical induction grafting [11,12].

Ester-mediated modification has been adopted in the synthesis of polyphenol-chitosan derivatives in the last decade. Different crosslinking agents create a covalent bond between phenolic acid and chitosan. Among them, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, a water soluble coupling agent, is the most studied crosslinking agent widely used to initiate the covalent bond between phenolic acid carboxylic groups and phenolic groups, chitosan chain amines [13,14]. The enzyme-mediated route was considered an ecological procedure for the generation of chitosan derivatives. Some polyphenol oxidases, such as tyrosinase and laccase, are able to convert phenolic compounds to *o*-quinones [15]. These reactive species may undergo a non-enzymatic reaction and covalently bind to the amine nucleophilic group of chitosan by the formation of azomethines (Schiff-type reaction) or Michael mechanisms. Free radical induction is an advanced method of grafting flavonoids to the chitosan macromolecule [16].

In order to increase the low solubility of chitosan in different solvents and thus to widen the fields of application, different chitosan derivatives with antioxidants were obtained and reported in the literature [17]. The aim of this work is to perform the functionalization of commercial chitosan and low molecular weight oxidized chitosan by grafting with quercetin and determination of its antioxidant properties by the DPPH method. Reducing the molecular weight of chitosan by oxidation favours its grafting with polyphenols. As a result, the functionalized polymeric composite can exhibit a higher antioxidant activity, so it can be successfully used to inhibit the oxidation of various organic substrates in the cosmetics, food and pharmaceutical industries.

Experimental

Materials

Commercial chitosan with a low-molecular weight of 704 kDa, quercetin (QR); 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical; dimethylformamide (DMA); acetic acid; ethyl chloroformate ($C_3H_5ClO_2$); chloroethanol amine (C_2H_6ClNO); triethylamine ($C_6H_{15}N$); hydrogen peroxide (H_2O_2); sodium hydroxide (NaOH), and sodium nitrite ($NaNO_2$) were purchased from Sigma Aldrich and were used without any further purification. Distilled water was used throughout the entire study.

Equipment

The *T80+ UV-Visible spectrophotometer* (Germany) with wavelength region (190 to 1100 nm) was used for the measurements of UV-Vis absorption of the solutions at room temperature (25 °C). *Fourier transform-infrared spectroscopy* (FTIR) spectra of chitosan, quercetin and functionalized compounds have been recorded on FTIR spectrometer (Bruker, Germany). A glass viscometer with a capillary diameter of 0.99 mm was used for the determination of molecular weight of commercial and synthesized chitosan. Additionaly, other lab equipment was used digital balance Kern-200 with accuracy of 0.0001 g; pH meter Consort C 3030 model; magnetic stirrer with hot plate Wise Stir; EBA-200 centrifuge; pipette controller IsoLab, Finnpipette Digital 200-2000 μL, measuring cylinders; pipettes, and volumetric flasks, beakers and 1 cm size of quartz cuvettes.

Methods

Calibration curve of quercetin

Aliquots of ethanoic stock solutions (0.5 g/L) were added to 0.025 L flasks to obtain different initial quercetin concentrations (from 0.001 up to 0.15 g/L) and their absorbance was measured (A_{415nm}= f(C)). The quercetin concentration was determined from the calibration curve, according to the regression equation y= 0.0188 [QR] - 0.0556, where the correlation coefficient is 0.9995. As for the sample of functionalized chitosan, quercetin copolymer has been similarly prepared. Using the linear equation, the concentration of quercetin grafted to chitosan was calculated at λ= 415 nm: [QR]= 14.94 mg/L.

Procedure for synthesis of low-molecular weight chitosan

Chitosan is a hygroscopic material which may contain over 10% water. Thus, the samples were dried in a vacuum drying oven at 40ºC until the difference in weight in subsequent measurements was lower than 0.5%. In first step of the procedure, the mixture was prepared: 2.0 g of dry chitosan in a 0.1 L volumetric flask. Further, 0.06 L of 2% acetic acid was poured into the flask, and 1% hydrogen peroxide was added

as the oxidant. To ensure the complete dissolution of the chitosan, the flask was closed and stirred during 24 h at room temperature. After 24 h, the viscosity decreased and the colour of the mixture turned to light purple. A solution of 1% NaOH was added dropwise to precipitate the oxidized chitosan.

To remove any undissolved residues, the chitosan solution was filtered through the paper filter placed in a glass funnel. The filter was rinsed once with solvent and twice with distilled water. Then, the wet filter was transferred to the weighing bottle and was dried in an air dryer. After two days, it was dried further under vacuum to a constant mass. The mass of undissolved chitosan was determined and applied for the calculation of the concentration correction. The filtrate was used for viscosity measurements [18].

Procedure for the functionalization of commercial chitosan with quercetin

In order to increase the reactivity of the amine group to the hydroxyl group of quercetin the 1 wt.% chitosan solution was transferred into a 0.1 L volumetric flask and the same amount of ethyl chloroformate was added. The mixture was stirred for 24 h to obtain a perfectly transparent solution. After 24 h the mixture was precipitated by centrifugation. The precipitate was vacuum dried in a weighing bottle until constant mass. The obtaining compound (0.35 g) was transferred into a 0.1 L volumetric flask and 0.0035 L solvent of chloroethanol amine and an equimolar amount of quercetin (0.0015 M) was poured into the flask. The flask was closed and the content was stirred for 24 h at room temperature to ensure the complete dissolution of the chitosan. During stirring, the mixture changed its colour from yellow to brown which demonstrates the grafting process of the quercetin molecule to the synthesized intermediate.

DPPH assay for the determination of the antioxidant activity

The antioxidant activity of the functionalized chitosan: quercetin copolymer was further investigated by the DPPH method at λ =517 nm. DPPH concentrations (%) in all analysed systems were calculated according to the Eq. (1).

$$W(DPPH) = \left(\frac{A_t}{A_o}\right) x\ 100\% \quad (1)$$

where: A_t - absorbance at time t,
A_o - absorbance at initial time.

Determination of the intrinsic viscosity of chitosan

The limiting or intrinsic viscosity [η], is related to the molecular weight of the polymer by the semi-empirical Mark Houwink (Eq.(2)) [18].

$$[\eta] = KM\alpha\ v \quad (2)$$

where, K and α are constants for a given polymer, solvent and temperature. Generally, $0.5< \alpha< 0.8$ for flexible random coils in a good solvent, $0.8 < \alpha < 1.0$ for inherently stiff molecules (*e.g.* cellulose derivatives, DNA, *etc.*) and $1.0< \alpha< 1.7$ for highly extended chains (*e.g.* polyelectrolytes in solutions of very low ionic strength) [19].

All measurements were performed in triplicate and the standard deviation was calculated. P-values below 0.05 were considered statistically significant.

Results and discussion

Grafting of quercetin to commercial and low molecular weight chitosan was obtained by oxidation with hydrogen peroxide. Initially, the chitosan was coupled with ethyl chloroformate to increase the reactivity of the amine group. The interaction took place by substituting a hydrogen atom from the primary amine group and the hydrochloric acid formed was further removed by adding triethanolamine. Subsequently, the obtained compound was grafted with quercetin by forming a covalent bond. This complex interacted at the 4′hydroxyl groups on the ß ring of quercetin and the ethylic alcohol was removed [20].

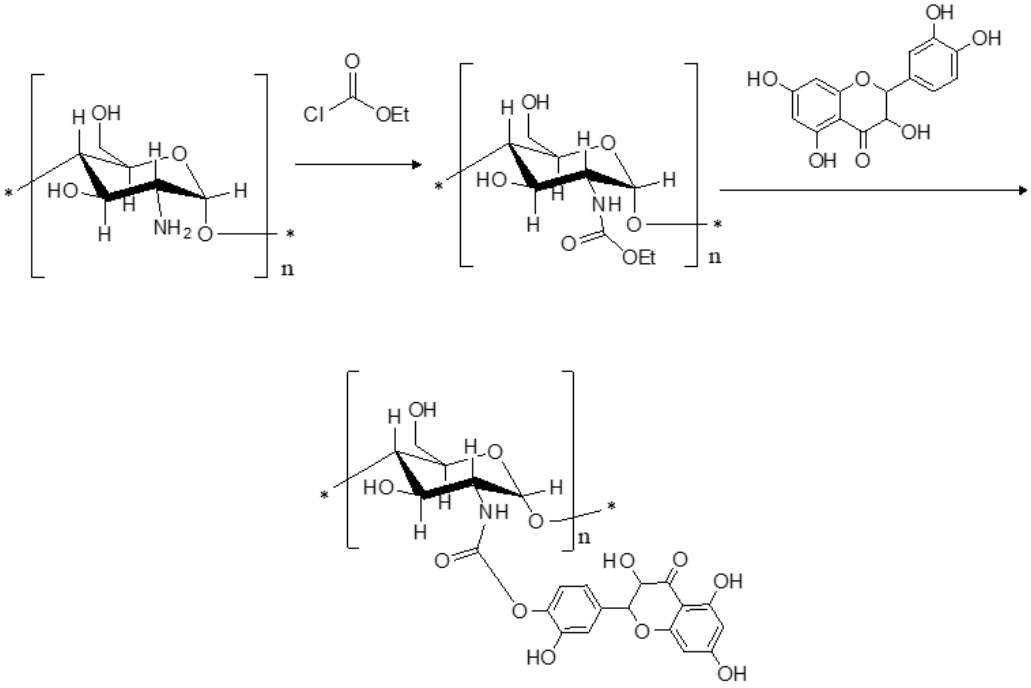
Morphological characterization of chitosan sample

The grafting process was performed on both commercial chitosan and low molecular weight chitosan, synthesized by oxidation of the commercial one. Comparing the data obtained by determining the molecular weight in the case of two polymers (commercial chitosan and oxidized chitosan), it was noticed that the molecular weight of chitosan obtained by oxidation is lower. This can be explained by a higher degree of deacetylation compared to technical chitosan.

The concentration of grafted quercetin was found to be higher for low molecular weight chitosan compared to commercial chitosan. The formation of new functional groups was established by the FTIR analysis method.

Figure 1 displays the FTIR spectrometric analysis of commercial chitosan, quercetin and the synthesized chitosan-quercetin copolymer.

According to the IR spectrum data, the attachment of quercetin is confirmed by the presence of a signal in the spectrum, characteristic of the C=C aromatic ring stretch bands of quercetin at 1610 cm^{-1}, as well as slight changes in the 1100-1400 cm^{-1} region. In the chitosan spectrum, a medium intensity thin peak is observed at 2920 cm^{-1}, characteristic for the C-H stretch band of methyl group. In the product spectrum, this signal merges into a broader one at 2860 cm^{-1}, indicating the presence of additional frequency absorption of C-H, which may be related to the vibrations of aromatic C-H bonds. At the same time, there are characteristic peaks in the chitosan spectrum at 894 cm^{-1} for wagging of the amine groups and broad bend of out-of-plane OH at 1023 and 1070 cm^{-1}; two amide bands at 1647 cm^{-1} (I amide band, C=O) and 1568 cm^{-1} (II amide band).



Scheme 1. The mechanism of interaction of chitosan with quercetin at room temperature.

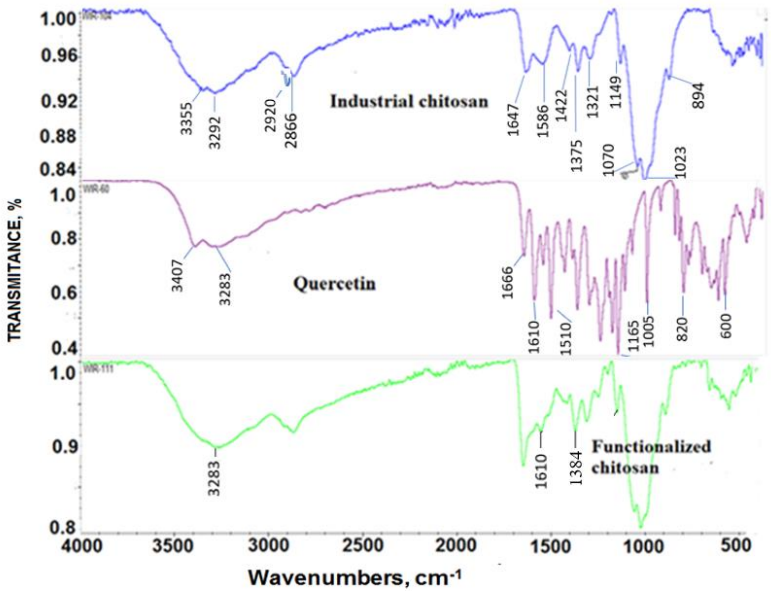


Figure 1. FTIR spectra of commercial chitosan, quercetin and functionalized chitosan.

However, the peak ratio characteristic of chitosan and quercetin indicates that the concentration of the latter is small compared to the concentration of the polymer. The absence of the OH groups stretching band of quercetin at 3407 cm^{-1} also indicates a small concentration of the latter in the polymer.

Figure 2 shows FTIR spectra of low molecular weight chitosan, quercetin and functionalized polymer. For instance, the spectra of the low molecular weight chitosan polymer grafted with quercetin show a significant difference from the FTIR spectra of free chitosan and quercetin.

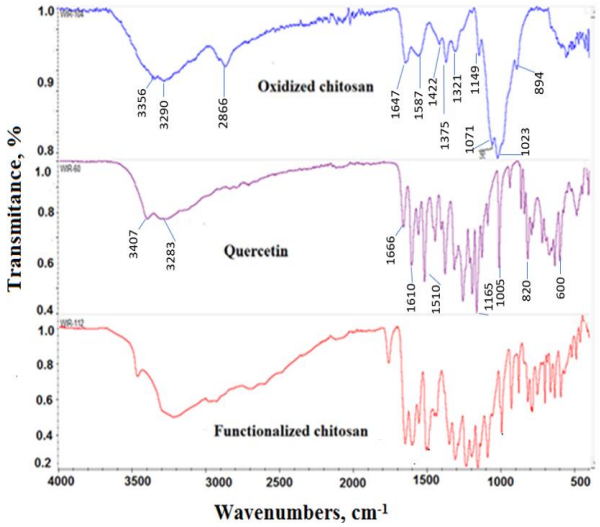
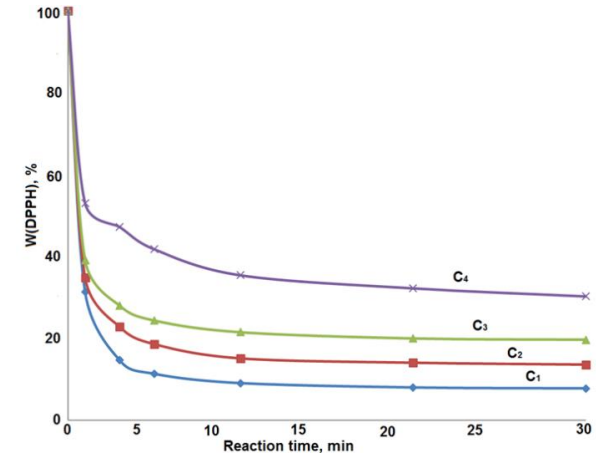


Figure 2. FTIR spectra of low molecular weight chitosan, quercetin and functionalized polymer.
1 - Phenol group (3300 cm^{-1}),
2 – hydroxyl group (1375 cm^{-1}),
3 - aromatic ring (1600 cm^{-1}),
4 - Etheric bond (1150 cm^{-1}).

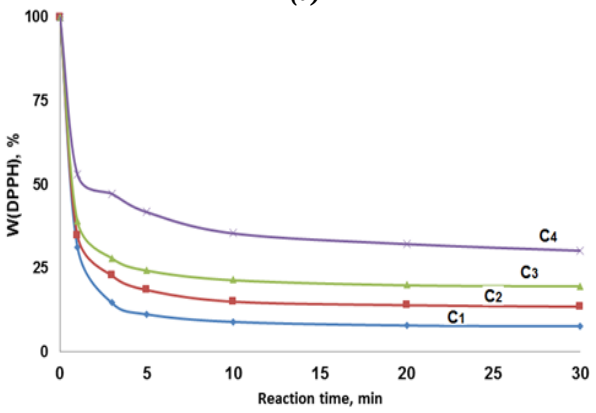
The product spectrum shows a band at 3432 cm^{-1}, characteristic of the OH groups stretching bend of quercetin. The disappearance of a broad and intense absorption in the region of 1071-1023 cm^{-1} indicates the formation of amide bonds, and the intensity of absorption bands at frequencies characteristic of amide I and amide II bands has also increased. Signals characteristic of quercetin appear in the 600-894 cm^{-1} region, and the peak of wagging of the NH group has become less intense, confirming the reduction of free amino groups. Thus, based on the analysis of the FTIR spectra, it can be concluded that the functionalization reaction of chitosan with quercetin occurred primarily at the NH_2 group [21], and from the product's spectrum, it can be inferred that the attachment of quercetin to low molecular weight chitosan occurred to a greater extent than to the commercial chitosan.

Antioxidant activity evaluation

Figure 3 shows the dependence W(%) (DPPH) = *f(t)* on the variation of the concentrations of the synthesized composite. It was calculated W(%) of DPPH based on the initial concentration of functionalized commercial chitosan (Figure 3*(a)*) and compared to W(%) of DPPH based on the concentration of the composite obtained from oxidized chitosan (Figure 3*(b)*). Using the graphical method EC_{50} was calculated for the functionalized commercial chitosan (EC_{50} = 0.5) and for the composite obtained from oxidized chitosan (EC_{50}= 0.2), Figure 4. To compare the antioxidant activity of the compound obtained by grafting commercial chitosan with quercetin, the amount of quercetin grafted to the commercial chitosan macromolecule was calculated.



(a)



(b)

Figure 3. Variation of [DPPH] in interaction with quercetin-grafted commercial *(a)* and oxidized chitosan *(b)*.
C_1=0.052 g/L, C_2=0.030 g/L, C_3=0.021 g/L, C_4=0.0105 g/L.

In order to compare the antioxidant activity of the quercetin – functionalized compound with the antioxidant activity of quercetin, the amount of functionalized polyphenol with low molecular weight chitosan was determined. It has been established that as the molecular mass of the chitosan macromolecule decreases, its solubility in the neutral environment increases, as well as its ability to be functionalized with various compounds, and its antioxidant activity. The amount of quercetin in the composition of functionalized chitosan was calculated (y = 0.702 x or y = 44.88 mg/L). Thus, it was found that the amount of quercetin in the composition of chitosan (low molecular weight) is much higher compared to functionalized commercial chitosan where [QR] =14.94 mg/L.

Similar to the case of commercial chitosan, the antioxidant activity of oxidized chitosan, grafted with quercetin was studied. According to the experimental results display in Figure 4, EC_{50} was calculated for quercetin-functionalized chitosan. The EC_{50} value is equal to 0.2 which is 2.5 times lower than for the grafted commercial copolymer (EC_{50} = 0.5). The lower the EC_{50}, the more active the composite, the less antioxidant is consumed to reduce the concentration of DPPH by 50%, respectively.

Intrinsic viscosity of chitosan

The molecular weight of commercial and synthesized chitosan was determined by the viscometric method. Subsequently, the molecular weight of the polymer synthesized by oxidation was calculated using the Mark-Houwink equation (Eq.(2)). From the Figure 5 it was established that intrinsic viscosity [η] is 1.8985.

Since $Log\ [\eta] = log\ k + \alpha\ log\ M$ (log 1.8985 = log 0.0018 + 0.93 log M), the molecular mass of the synthesized chitosan was established as 131472 g/mol. Thus, it was established that the oxidized chitosan was of a lower molecular weight compared to commercial chitosan.

Figure 6 shows the determination of the prolongation effect of quercetin functionalized to chitosan. Practical studies were performed using the dialysis method using the semipermeable membrane recommended in pharmaceutical practice. The quercetin solution was found to pass through semipermeable membranes more rapidly and the quercetin-functionalized chitosan solution passed over a longer period of time. It was found that quercetin passes through the semipermeable membrane within 50-60 min, and still its concentration remained almost constant, while quercetin grafted to the polymeric material crossed the membrane within 4-5 h [20].

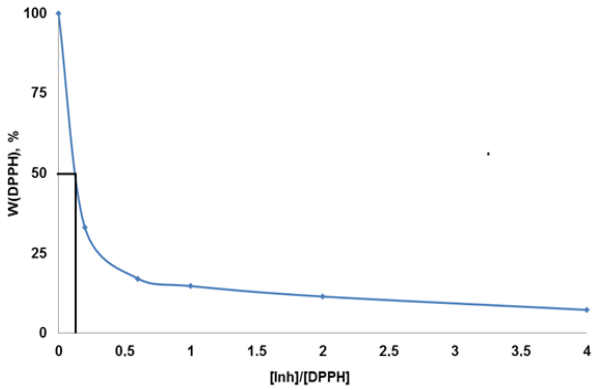


Figure 4. The dependence of DPPH concentration of the [Inh]/[DPPH] according to the concentration.

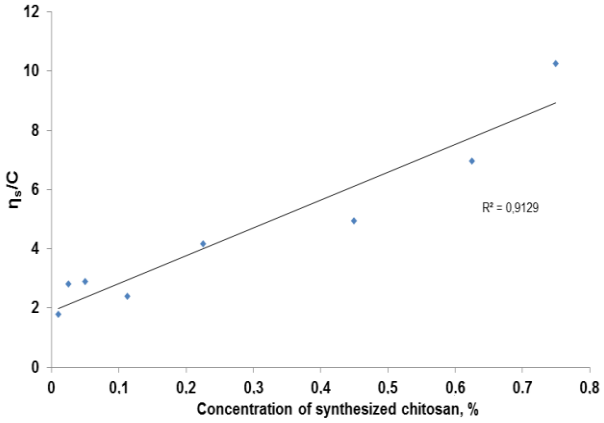


Figure 5. Dependence *ηs/C* on the concentration of synthesized chitosan at room temperature.

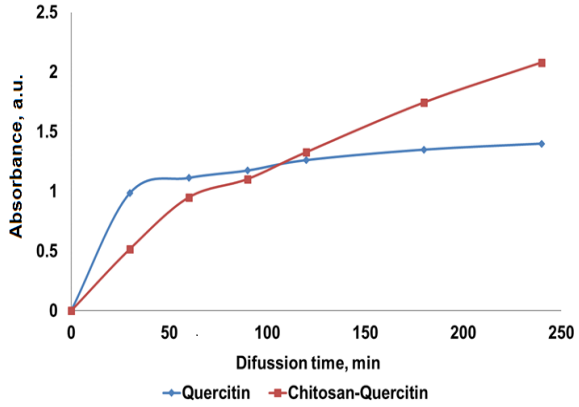


Figure 6. Time variation of absorbance of quercetin and functionalized chitosan in the process of passing through the semipermeable membrane.

Conclusion

The quercetin-grafted chitosan was synthesized. Grafting was performed with both commercial chitosan and low molecular weight chitosan, which was synthesized by oxidation of the commercial sample. The concentration of quercetin-grafted chitosan was found to be higher for low molecular weight chitosan compared to

commercial chitosan. Oxidized quercetin-grafted chitosan had higher solubility compared to quercetin-grafted commercial chitosan. The formation of new copolymers of commercial and oxidized chitosan was established by the FTIR method.

The DPPH method was used to determine the antioxidant activity of free quercetin, commercial chitosan and oxidized quercetin-grafted chitosan. The antioxidant activity of the quercetin-functionalized compound was compared with the antioxidant activity of the grafted commercial chitosan and it was established that the oxidized quercetin-grafted chitosan has higher antioxidant properties than the quercetin-grafted commercial chitosan This difference in antioxidant activity is due to the higher amount of quercetin grafted to the oxidized chitosan macromolecule compared to the grafted commercial chitosan.

The antioxidant activity of the low molecular weight chitosan graft polymer was 2.5-fold higher than that of the quercetin-grafted commercial chitosan. It has been determined that oxidized quercetin-grafted chitosan had a higher solubility compared to quercetin-grafted commercial chitosan.

During the study of the prolongation effect of quercetin-grafted chitosan, it was found that free quercetin passed through the semipermeable membrane within 50-60 min, and its concentration remained almost constant while quercetin grafted to the polymeric material crossed the membrane within 4-5 h.

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References

1. Pokhrel, S.; Yadav, P.N. Functionalization of chitosan polymer and their applications. Journal of Macromolecular Science, Part A, 2019, 56(5), pp. 450-475. DOI: https://doi.org/10.1080/10601325.2019.1581576
2. Hao, W.; Li, K.; Ma, Y.; Li, R.; Xing, R.; Yu, H.; Li, P. Preparation and antioxidant activity of chitosan dimers with different sequences. Marine Drugs, 2021, 19(7), pp. 366–380. DOI: https://doi.org/10.3390/md19070366
3. Tondwal, R.; Singh, M. Chitosan functionalization with a series of sulphur-containing α-amino acids for the development of drug-binding abilities. Journal of Applied Polymer Science, 2018, 135(12), pp. 1–14. DOI: https://doi.org/10.1002/app.46000
4. Kim, J.K.; Park, S.U. Quercetin and its role in biological functions: An updated review. EXCLI Journal, 2018, 17, pp. 856–863. DOI: https://doi.org/10.17179/excli2018-1538
5. Alizadeh, S.R.; Ebrahimzadeh, M.A. O-Glycoside quercetin derivatives: Biological activities, mechanisms of action, and structure–activity relationship for drug design, a review. Phytotherapy Research, 2022, 36(2), pp. 778–807. DOI: https://doi.org/10.1002/ptr.7352
6. Lyu, Y.-L.; Zhou, H.-F.; Yang, J.; Wang, F.-X.; Sun, F.; Li, J.-Y. Biological activities underlying the therapeutic effect of quercetin on inflammatory bowel disease. Mediators of Inflammation, 2022, 2022, 5665778, pp. 1–8. DOI: https://doi.org/10.1155/2022/5665778
7. Tian, R.; Jin, L.; Zhou, L.; Zeng, X.-P.; Lu, N. Quercetin attenuated myeloperoxidase-dependent HOCl generation and endothelial dysfunction in diabetic vasculature. Journal of Agricultural and Food Chemistry, 2021, 69(1), pp. 404–413. DOI: https://doi.org/10.1021/acs.jafc.0c06335
8. Batiha, G.E.-S.; Beshbishy, A.M.; Ikram, M.; Mulla, Z.S.; El-Hack, M.E.A.; Taha, A.E.; Algammal, A.M.; Elewa, Y.H.A. The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: quercetin. Foods, 2020, 9(3), pp. 374–389. DOI: https://doi.org/10.3390/foods9030374
9. Zhang, H.; Yang, J.; Zhao, Y. High intensity ultrasound assisted heating to improve solubility, antioxidant and antibacterial properties of chitosan-fructose Mallard reaction products. LWT - Food Science and Technology, 2015, 60(1), pp. 253–262. DOI: https://doi.org/10.1016/j.lwt.2014.07.050
10. Schreiber, S.B.; Bozell, J.J.; Hayes, D.G.; Zivanovic, S. Introduction of primary antioxidant activity to chitosan for application as a multifunctional food packaging material. Food Hydrocolloids, 2013, 33(2), pp. 207–214. DOI: https://doi.org/10.1016/j.foodhyd.2013.03.006
11. Kim, E.S.; Kim, D.Y.; Lee, J.-S.; Lee, H.G. Quercetin delivery characteristics of chitosan nanoparticles prepared with different molecular weight polyanion cross-linkers. Carbohydrate Polymers, 2021, 267, 118157, pp. 1–9. DOI: https://doi.org/10.1016/j.carbpol.2021.118157
Liu, Y.; Zhang, B.; Javvaji, V.; Kim, E.; Lee, M.E.; Raghavan, S.R.; Wang, Q.; Payne, G.F. Tyrosine-

mediated grafting and crosslinking of natural phenols confers functional properties to chitosan. Biochemical Engineering Journal, 2014, 89, pp. 21–27.
DOI: http://doi.org/10.1016/j.bej.2013.11.016

12. Aljawish, A.; Chevalot, I.; Jasniewski, J.; Revol-Junelles, A.M.; Scher, J.; Muniglia, L. Laccase-catalysed functionalisation of chitosan by ferulic acid and ethyl ferulate: evaluation of physicochemical and biofunctional properties. Food Chemistry, 2014, 161, pp. 279–287. DOI: https://doi.org/10.1016/j.foodchem.2014.03.076
13. Lei, F.; Wang, X.; Liang, C.; Yuan, F.; Gao, Y. Preparation and functional evaluation of chitosan-EGCG conjugates. Journal of Applied Polymer Science, 2014, 131(3), pp. 1–8.
DOI: https://doi.org/10.1002/app.39732
14. Xie, M.; Hu, B.; Wang, Y.; Zeng, X. Grafting of Gallic acid onto chitosan enhances antioxidant activities and alters rheological properties of the copolymer. Journal of Agricultural and Food Chemistry, 2014, 62(37), pp, 9128–9136.
DOI: https://doi.org/10.1021/jf503207s
15. Torres, E.; Marín, V.; Aburto, J.; Beltrán, H.I.; Shirai, K.; Villanueva, S.; Sandoval, G. Enzymatic modification of chitosan with quercetin and its application as antioxidant edible films. Applied Biochemistry and Microbiology, 2012, 48(2), pp. 151–158.
DOI: https://doi.org/10.1134/S0003683812020123
16. Argüelles-Monal, W.M.; Lizardi-Mendoza, J.; Fernández-Quiroz, D.; Recillas-Mota, M.T.; Montiel-Herrera, M. Chitosan derivatives: introducing new functionalities with a controlled molecular architecture for innovative materials. Polymers, 2018, 10(3), pp. 342–375.
DOI: https://doi.org/10.3390/polym10030342
17. Czechowska-Biskup, R.; Wach, R.A.; Rosiak, J.M.; Ulański, P. Procedure for determination of the molecular weight of chitosan by viscometry. Progress on chemistry and application of chitin and its derivatives, 2018, XXIII, pp. 45–54.
DOI: https://doi.org/10.15259.PCACD.23.04
18. Kwaambwa, H.M.; Goodwin, J.W.; Hughes, R.W.; Reynolds, P.A. Viscosity, molecular weight and concentration relationships at 298 K of low molecular weight cis-polyisoprene in a good solvent. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2007, 294(1-3), pp. 14–19.
DOI: https://doi.org/10.1016/j.colsurfa.2006.07.041
19. Gonta, M.; Sirbu, E.; Robu, S.; Gonta, A.; Mocanu, L. Functionalization of flavonoids (Quercetin) to chitosan matrix and determination of antioxidant activity of obtained bio-composites. 4th International Conference on Nanotechnologies and Biomedical Engineering, Proceedings of ICNBME-2019, 2019, 77, pp. 355–359. DOI: https://doi.org/10.1007/978-3-030-31866-6_66
20. Meyers, R.A. Ed. Encyclopedia of Analytical Chemistry. John Wiley & Sons: Berlin, 2006, pp. 1–29. DOI: https://doi.org/10.1002/9780470027318.a0117