KAUNAS UNIVERSITY OF TECHNOLOGY

VESTA NAVIKAITĖ-ŠNIPAITIENĖ

PRESERVATION OF THE ACTIVE PROPERTIES OF ANTHOCYANINS AND ESSENTIAL OILS BY IMMOBILIZING IN ANIONIC POLYSACCHARIDES

Summary of Doctoral Dissertation
Technological Sciences, Chemical Engineering (05T)

2017, Kaunas

This doctoral dissertation was prepared at Kaunas University of Technology (KTU), Faculty of Chemical Technology, Department of Polymer Chemistry and Technology in the period of 2013–2017. The studies were supported by Research Council of Lithuania. A part of the research was performed at Zurich University of Applied Sciences (ZHAW) in Switzerland. This part of work was financially supported by the Scientific Exchange Programme (Sciex-NMS^{ch}). The research on anthocyanins was carried out by using high-performance liquid chromatography at Lithuanian University of Health Sciences (LSMU) during the course of KTU-LSMU researcher groups projects.

Scientific Supervisor: Assoc. Prof. Dr. Rima KLIMAVIČIUTĖ (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T).

Scientific Adviser: Assoc. Prof. Dr. Ramunė RUTKAITĖ (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T).

Editor: Armandas Rumšas (Publishing Office "Technologija")

Dissertation Defence Board of Chemical Engineering Science Field:
Prof. Dr. Petras Rimantas VENSKUTONIS (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T) – **chairman**;
Assoc. Prof. Dr. Veronique COMA (University of Bordeaux, Technological Sciences, Chemical Engineering, 05T);
Prof. Dr. Habil. Juozas Vidas GRAŽULEVIČIUS (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T);
Assoc. Prof. Dr. Jolita OSTRAUSKAITĖ (Kaunas University of Technology, Technological Sciences, Chemical Engineering, 05T);
Prof. Dr. Sonata TRUMBECKAITĖ (Lithuanian University of Health Sciences, Biomedical Sciences, Pharmacy, 08B).

The official defence of the dissertation will be held at 2:00 p.m. on 15th of December 2017 at the public meeting of Dissertation Defence Board of Chemical Engineering Science Field in Rectorate Hall at Kaunas University of Technology.

Address: K. Donelaičio St. 73-402, 44249 Kaunas, Lithuania.
Tel. no. (+370) 37 300 042; fax. (+370) 37 324 144; e-mail doktorantura@ktu.lt.

Summary of doctoral dissertation was sent on 15th of November, 2017.
The doctoral dissertation is available on the internet http://ktu.edu and at the library of Kaunas University of Technology (K. Donelaičio St. 20, 44239 Kaunas, Lithuania).

KAUNO TECHNOLOGIJOS UNIVERSITETAS

VESTA NAVIKAITĖ-ŠNIPAITIENĖ

ANTOCIANINŲ IR ETERINIŲ ALIEJŲ VEIKLIŲJŲ SAVYBIŲ IŠSAUGOJIMAS IMOBILIZUOJANT JUOS ANIJONINIUOSE POLISACHARIDUOSE

Daktaro disertacijos santrauka
Technologijos mokslai, chemijos inžinerija (05T)

2017, Kaunas

Disertacija rengta 2013–2017 metais Kauno technologijos universiteto (KTU) Cheminės technologijos fakulteto Polimerų chemijos ir technologijos katedroje. Mokslinius tyrimus rėmė Lietuvos mokslo taryba. Dalis tyrimų atlikta stažuojantis Ciuricho taikomųjų mokslų universitete (*Zurich University of Applied Sciences*, ZHAW) Šveicarijoje ir gavus Šveicarijos stipendijų fondo (*Sciex-NMS^{ch}*) paramą. Antocianinų tyrimai atlikti efektyviosios skysčių chromatografijos metodu Lietuvos sveikatos mokslų universitete (LSMU), vykdant bendrus KTU ir LSMU mokslininkų grupių projektus.

Mokslinė vadovė:

Doc. dr. Rima KLIMAVIČIŪTĖ (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T).

Mokslinė konsultantė:

Doc. dr. Ramunė RUTKAITĖ (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T).

Redagavo: Armandas Rumšas (leidykla „Technologija“)

Chemijos inžinerijos mokslo krypties disertacijos gynimo taryba:
Prof. dr. Petras Rimantas VENSKUTONIS (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T) – **pirmininkas**;
Doc. dr. Veronique COMA (Bordo universitetas, technologijos mokslai, chemijos inžinerija, 05T);
Prof. habil. dr. Juozas Vidas GRAŽULEVIČIUS (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T);
Doc. dr. Jolita OSTRAUSKAITĖ (Kauno technologijos universitetas, technologijos mokslai, chemijos inžinerija, 05T);
Prof. dr. Sonata TRUMBECKAITĖ (Lietuvos sveikatos mokslų universitetas, biomedicinos mokslai, farmacija, 08B).

Disertacija bus ginama viešame chemijos inžinerijos mokslo krypties disertacijos gynimo tarybos posėdyje 2017 m. gruodžio 15 d. 14:00 val. Kauno technologijos universiteto Rektorato salėje.

Adresas: K. Donelaičio g. 73-402, 44249 Kaunas, Lietuva.
Tel. + 370 37 300 042; faks. + 370 37 324 144; el. paštas doktorantura@ktu.lt

Disertacijos santrauka išsiųsta 2017 m. lapkričio 15 d.
Su disertacija galima susipažinti interneto svetainėje http://ktu.edu ir Kauno technologijos universiteto bibliotekoje (K. Donelaičio g. 20, 44239 Kaunas).

INTRODUCTION

Motivation of the research. The anthocyanins and essential oils are derived from plants and are denoted by anti-cancer, anti-inflammatory, antioxidant and antibacterial properties. What concerns their active properties, they are widely used in cosmetics, pharmaceuticals and food industry including active food packaging [1, 2, 3].

The anthocyanins are unstable compounds. In order to increase the stability of anthocyanins, they are immobilized into various carriers which not only protect molecules of anthocyanins from physical and chemical factors, but also maintain their activity [4]. However, these methods are expensive and related with loss of anthocyanins. Under acidic conditions ($1 < pH < 3$), anthocyanins exist in the form of red flavylium cation which could participate in ionic interaction with anionic groups of polymers. So far, only the formation of the stable suspension of anthocyanins and chondroitin sulfate complex nanoparticles has been described in scholarly literature [5]. However, it can be expected that other sulfated natural or modified polysaccharides, such as carrageenan or dextran sulfate, could also form complexes with anthocyanins. Both above mentioned polysaccharides are widely used in the formation of polymeric complexes with positively charged polymers or low molecular weight compounds [6, 7]. Ionic polymeric complexes are formed in aqueous solutions due to the electrostatic interaction between the oppositely charged groups of polymers and low molecular weight compounds. The formation of ionic complexes can be regarded as a green process because it takes place in water, at room temperature, without any organic solvent and with a limited energy input [8].

Production of active packaging materials belongs to the list of the innovative technologies of the contemporary society. Active compounds are released into headspace of packaging or harmful substances are absorbed from the headspace in order to prolong the shelf life of food [9]. One of the ways to obtain active packaging materials is to form coatings containing essential oils having antimicrobial or antioxidant activities on polymer films. Natural, modified natural or synthetic polymers can be used as coating binders.

The aim of the thesis. The aim of this research is the preservation of the active properties of anthocyanins and essential oils by immobilizing them in anionic polysaccharides.

In order to achieve the aim of the thesis, the following tasks were formulated:

1. To select the conditions for obtaining the complexes (soluble and in the form of nano- or microparticles) between dextran sulfate, κ- and ι-carrageenan and anthocyanins which are present in the aqueous extract of the wild bilberry.

2. To evaluate the stability and the antioxidant properties of anthocyanins introduced into complexes with dextran sulfate, κ- and ι-carrageenan, as well as to assess the release of anthocyanins from complexes into different media.

3. To investigate the interaction between flavylium cation of anthocyanins and sulfate groups of carrageenan by using the equilibrium adsorption method and two variables adsorption models in order to describe the adsorption data.

4. To propose a technological scheme for the production of cross-linked κ-carrageenan/anthocyanins complex microgranules.

5. To develop coatings containing essential oils and to determine their antibacterial and antioxidant activities through the vapor phase without direct contact with the investigative substrate.

6. To develop an active packaging prototype.

Novelty of the study. It was discovered that anthocyanins from the aqueous wild bilberry extract can form a complex with dextran sulfate, κ- and ι-carrageenan, first of all, due to the electrostatic interaction between the flavylium cation of anthocyanins and the sulfate groups of polysaccharides. The state of complexes (soluble, nano- or microparticles) depends on the weight ratio of the polysaccharide and anthocyanins and on their total concentration. At the optimal weight ratio of the polysaccharide to anthocyanins at 0.4 and the total concentration of dextran sulfate or carrageenans and anthocyanins equaling to or being less than 0.16 g/L and 0.07 g/L, respectively, stable nanosized complex particles dispersions were obtained. With an increase of the total concentration of the complex-forming substances, a darkly red precipitate of the complex was formed.

The purification of anthocyanins during immobilization on polymer carriers was demonstrated by using high-performance liquid chromatography.

The incorporation of anthocyanins into the complex with polysaccharides protected flavylium cation from hydration and increased the stability of the aqueous complex suspension. Anthocyanins are effectively released from the microgranules of the complex into the model intestinal and gastric media.

It was found out that the coatings containing thyme essential oil or eugenol demonstrated antibacterial activity against *E. coli* and *L. monocytogenes* and effective inhibition of free radicals without direct contact with the investigative substrate.

The practical value of the work. Lyophilized microgranules of the cross-linked κ-carrageenan/anthocyanins complex have been used to prepare a prototype of antioxidant and anti-inflammatory rectal suppositories. A technological scheme for the production of the cross-linked κ-carrageenan/anthocyanins complex microgranules was proposed.

Films with active coatings containing eugenol were developed and used for fresh beef packaging. It was found that fresh beef in such packaging was protected from lipid oxidation and meat discoloration.

Dissertation statements to be defended. During the adsorption of anthocyanins on sulfated polysaccharides, anthocyanins are isolated from the crude extract of the wild bilberry while preserving their antioxidant properties; later on, they can be released into various media.

Approval and publications of the results of the study. The results of the research have been published in 2 scientific articles in the journals indexed in *Clarivative Analytics Web of Science* database, 1 article has been published in other peer-reviewed scientific publications and 9 papers have been presented in the proceedings of scientific conferences.

Structure and content of the dissertation. The dissertation consists of an introduction, three chapters, conclusions, a list of references, and a list of publications relevant to the subject of the dissertation. The dissertation text covers 114 pages, it features 20 tables and 48 figures, 31 mathematical expressions and equations, and the list of references includes 240 bibliographic sources.

1. RESEARCH METHODOLOGY

The **materials and chemicals** used in this work were chemically or analytically pure reagents and were not additionally purified before use.

Extraction of anthocyanins. Defrosted bilberries and distilled water (weight ratio 1:1) were homogenized with an overhead stirrer (Eurostat, IKA, Germany). The obtained suspension was filtered through a nylon filter, then through a glass filter and finally centrifuged at 6000 rpm for 15 min, and kept in a freezer at –18 ºC. The total anthocyanins (ATC) concentration was determined by employing the pH differential method [10] and varied from 0.63 to 0.66 g/L.

Formation and characterization of polysaccharides and anthocyanins complexes. The desired amount of dextran sulfate (DESU) or carrageenan (CARG) aqueous solution of the same concentration as the ATC concentration in water extract, was added to the desired amount of the ATC water extract under stirring at 150 rpm for 30 min. The DESU/ATC, ι-CARG/ATC or κ-CARG/ATC complexes were characterized by different polysaccharide-to-ATC weight ratios which were equal to 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 or 2.0. The total concentration of the complex forming reagents for DESU/ATC and both CARG/ATC varied from 0.08 to 0.88 g/L and 0.07 to 0.70 g/L, respectively.

The water insoluble part of the complexes was eliminated by centrifugation at 9000 rpm for 60 min. The residual concentration of ATC in the solution was estimated, and the amount of ATC introduced into the complex was calculated.

The absorbance spectra of ATC or complex solutions were recorded by using a visible light spectrophotometer T60 (PG Instruments). The formation of complexes was confirmed by FT-IR spectroscopy by using a Perkin-Elmer Frontier spectrophotometer. The amounts of individual ATC immobilized into the complexes with polysaccharides were determined by HPLC.

A Delsa^{TM} Nano C particle size analyzer (Beckman Coulter) was used to measure the particle size of the complexes by evaluating the cumulative intensity distribution.

Preparation of cross-linked carrageenan. The microgranules of CARG were cross-linked with 0.3, 0.6, 0.8 and 1.0 mol of epichlorohydrin (EPCH) per mole of CARG in isopropanol–water mixture (2:1 v/v) in the presence of sodium hydroxide at room temperature for 6 h under stirring at 150 rpm, washed with the acidified isopropanol–water mixture until reaching the neutral pH value and dried at room temperature.

Equilibrium adsorption studies. 0.025 g of dry cross-linked CARG (ι-CARG-C or κ-CARG-C) was placed into an Erlenmeyer flask, and 100 mL of the ATC solution of the desired concentration was added. The flask was stoppered and shaken for 60 min at a temperature of 22 ºC, 30 ºC or 40 ºC and at a fixed shaking intensity in a thermostatic bath with the temperature control of ±1 °C (Memmert GmbH, Germany). Then the mixture was filtered through a glass filter, and the

residual concentration of ATC in the filtrate was estimated. The amount of the adsorbed ATC (qe (g/g)) was calculated. The Langmuir [11], Freundlich [12], Dubinin-Radushkevich [13] and Temkin [14] adsorption models were used to describe the adsorption isotherms.

Investigation of ATC desorption. The desired amount of freeze-dried microgranules of CARG-C/ATC was poured into 50 mL of desorption solution and stirred at 300 rpm for 30 min at room temperature (22±1 ºC). Afterwards, the mixture was filtered through a glass filter and the concentration of desorbed ATC was determined [10].

Determination of antioxidant activity. The antioxidant activity of ATC, DESU/ATC, CARG/ATC was evaluated by two methods, i.e. by using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS*) [15] or 2,2-diphenyl-1-picrylhydrazyl (DPPH) [16] reagents.

Preparation of active coatings. The aqueous emulsions (EM) consisting of water, 25 % (w/v) hydrophobically modified starch (S), and 12.5 % (w/v) or 25 % (w/v) of EO were prepared by using rotor-stator homogenizer (POLYTRON® PT 2500 E, Switzerland). After that, EM were mixed with acrylic binder *Premo(R)STAR OPV FDA-WP3P-00AH* (AC) at various weight ratios in order to obtain different amounts of EO in the coatings. Active coating mixtures with cellulose acetate (CA) as a binder were obtained by mixing 12 wt% CA solution in acetone and the desired amount of EO. The corona treated oriented-polypropylene (OPP) films were coated with the prepared coating mixtures by using a coating machine (ZEHNTNER ZAA 2300, Switzerland) and left to dry at room temperature for 1 hr. EO in the AC/S and CA coatings was ranging from 2.5 to 20 wt% and 7 % to 23 wt%, respectively.

Determination of antioxidant and antibacterial properties of coated films. The antioxidant and antibacterial properties of coated films were investigated trough the vapor phase in tightly closed Petri dishes by using vapor DPPH (VP-DPPH) and vapour diffusion (VD) methods [17], respectively, which have been developed at Zurich University of Applied Sciences. The films with active coatings were attached to the inside of the lid of a Petri dish and the substrate (DPPH solution or microorganisms spread on a solid medium) was placed into the bottom plate of a Petri dish thus avoiding direct contact with the active sample. The antioxidant activity of coated films was determined at different testing times and expressed as inhibition of DPPH (%). The antibacterial activity of the coated films was evaluated against *E. coli* and *L. monocytogenes*.

Preparation and analyses of antioxidant food packaging prototype. Steaks of fresh beef (*Longissimus Dorsi*) were placed into a high barrier tray (PS/EVOH/PE) packed under modified atmosphere (consisting of 80% O_2 and 20% CO_2) and sealed with PET/PE/EVOH/PE by using a tray sealer (Multivac T200, Switzerland). OPP film with active coating (187 cm^2) containing different amounts of eugenol was attached to the peel film. The samples were displayed in

a refrigerated cabinet under illumination at 2 ± 1 °C for 24 h, and thereafter stored in the dark for 14 days. During the storage time, the changes in meat color were being monitored by using spectrophotometer Konica Minolta CR-4000, and lipid oxidation in meat was quantified by using *Food TBARS Assay Kit* according to the protocol [18].

2. RESULTS AND DISCUSSION

2.1 Complexes of dextran sulfate and anthocyanins from bilberry extract

Complexes of DESU and ATC extracted from *Vaccinium myrtillus* were obtained during electrostatic interaction between the DESU sulfate groups and the cationic moieties of ATC (Fig. 2.1). With the formation of DESU/ATC complexes, a hypsochromic shift and the hyperchromic effect in the spectra of the complex solution was apparent when compared with the spectrum of ATC only (Fig. 2.2).

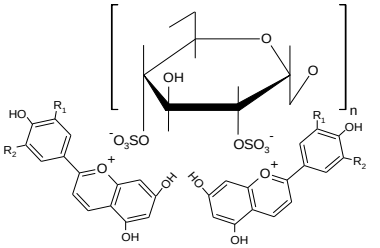

Fig. 2.1 Formation of an ionic complex between the sulfate groups of DESU and the flavylium cation of ATC

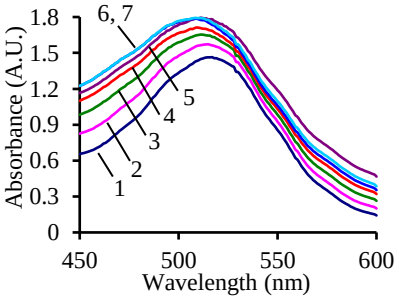

Fig. 2.2 Absorption spectra of ATC *(1)* and DESU/ATC complexes at different weight ratios: *2* – 0.1; *3* – 0.2; *4* – 0.3; *5* – 0.4; *6* – 0.8; *7* – 1. ATC concentration equaled 0.066 g/L, and the pH of the solutions was 3.2±0.2

The amount of ATC incorporated into the complex with DESU depended on the DESU and the ATC weight ratio and on their total concentration. As one

can see in Fig. 2.3, the amount of the incorporated ATC expressed as a percentage from the initial ATC concentration reached the maximum in the complex at the weight ratio DESU/ATC=0.8. At the same time, the amount of the incorporated ATC expressed as grams per one gram of DESU decreased with the increase of the DESU concentration in the complex. Moreover, the soluble complex remained in supernatant, and, therefore, the residual concentration of ATC was increased. If we take into account both parameters, the optimal weight ratio of DESU/ATC was 0.4. Consequently, about 1.7 g of ATC per gram of DESU (70% of the initial amount of ATC), or about $3.8 \cdot 10^{-3}$ moles of ATC (expressed as cyanidin-3-*O*-glucoside equivalents (cy-3-glc)) per gram of DESU, can be incorporated into a complex with such a composition.

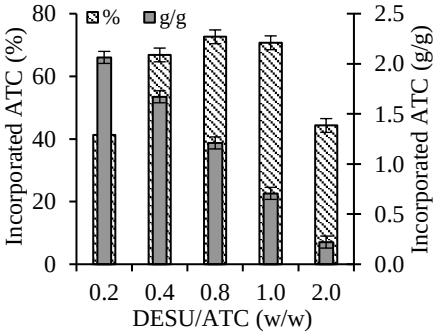

Fig. 2.3 Influence of the weight ratio DESU/ATC on the amount of ATC incorporated into complexes. The total concentration of DESU and ATC was 0.66 g/L

At the optimal weight ratio (DESU/ATC=0.4), the total concentration of complex forming reagents had a major influence on the form of the complex. The obtained results are presented in Fig. 2.4.

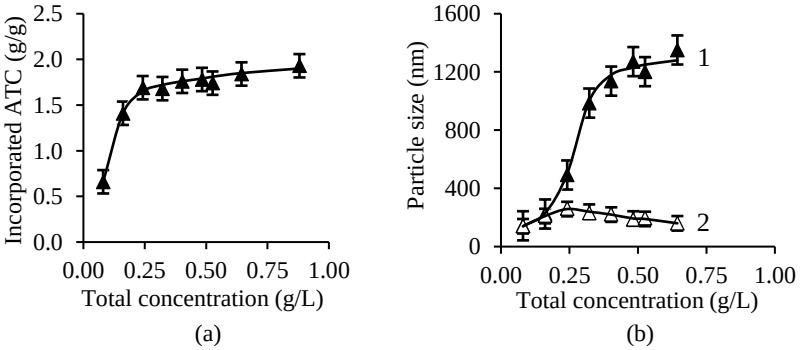

Fig. 2.4 Influence of total DESU and ATC concentration on the amount of ATC incorporated into a complex (a) and on the size of complex particles (b): *1* – before centrifugation; *2* – after centrifugation. DESU/ATC=0.4

At a higher total concentration of components, the formed complex precipitated, and the maximum amount of the incorporated ATC was about 1.7 g/g DESU (Fig. 2.4a). When the total concentration was equal to 0.16 g/L or less, a stable suspension of DESU/ATC complex nanoparticles was obtained (Fig. 2.4b).

HPLC analysis of dextran sulfate/anthocyanins complexes

The formation of DESU/ATC complexes was confirmed by HPLC. The summarized results are presented in Table 2.1. According to HPLC analysis, the amount of individual ATC incorporated into a complex varied from 73.7% in the case of malvidin-3-*O*-glucoside to 90.8% in the case of delphinidin-3-*O*-arabinoside. The total amount of ATC determined by HPLC was higher than that obtained by employing the pH method.

Table 2.1 The amount of individual ATC incorporated into the complex with DESU (DESU/ATC=0.4, the total concentration 0.63 g/L) was calculated from the chromatography data

Individual ATC	Concentration (g/L)		Amount of incorporated ATC	
	Initial	After complex formation	g/g of DESU	% from the initial level
Cyanidin-3-*O*-arabinoside	0.059	0.009	0.20	84.7
Cyanidin-3-*O*-galactoside	0.080	0.017	0.25	78.0
Cyanidin-3-*O*-glucoside	0.093	0.023	0.28	74.8
Delphinidin-3-*O*-arabinoside	0.057	0.005	0.21	90.8
Delphinidin-3-*O*-galactoside	0.076	0.009	0.26	87.7
Delphinidin-3-*O*-glucoside	0.084	0.012	0.28	85.3
Malvidin-3-*O*-glucoside	0.104	0.027	0.31	73.7
Petunidin-3-*O*-glucoside	0.069	0.009	0.24	86.5
Peonidin-3-*O*-glucoside	0.045	0.012	0.13	74.2
Total	0.667	0.123	2.160	81.6
pH method	0.630	0.140	1.960	77.8

Analyses of dextran sulfate/anthocyanins complex stability

The stability of the DESU/ATC=0.4 complex suspension was investigated and compared with the stability of the bilberry water extract having the same ATC concentration of 0.3 g/L. The influence of the storage time (12 days) on the residual concentration of ATC (in %) and the relative radical scavenging capacity (RRSC in %) calculated from the data of the DPPH radical scavenging activity was determined. It is shown in Fig. 2.5. After 12 days, the ATC concentration in the DESU/ATC complex suspension decreased by about 12%, while the ATC concentration in the water extract decreased by 35% (Fig. 2.5a). At the same time, the RRSC of the ATC introduced into the complex was effectively preserved (Fig. 2.5b).

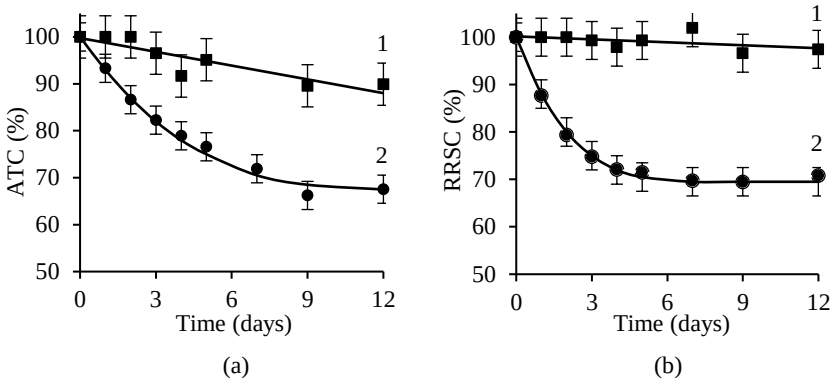

Fig. 2.5 Decrease of the ATC concentration (a) and the relative radical scavenging capacity (RRSC) (b) depending on the storage time: 1 – DESU/ATC=0.4; 2 – ATC. Storage in the dark at 4 ºC temperature. ATC concentration was 0.3 g/L

2.2 Complexes of carrageenan and anthocyanins from bilberry extract

κ-CARG and ι-CARG having one and two sulfate groups, respectively, are natural polysaccharides chosen to form complexes with flavylium cation of ATC present in the wild bilberry water extract (Fig. 2.6). The composition of complexes was evaluated as the weight ratio of CARG to ATC (expressed as Cy-3-glc).

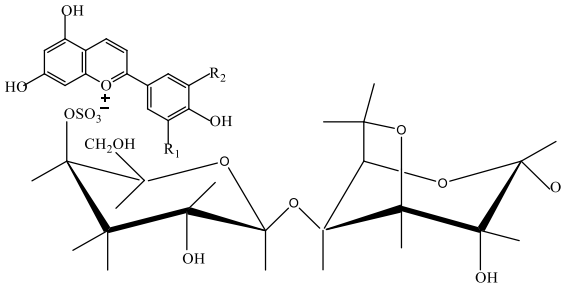

Fig. 2.6 Ionic complex formation between sulfate groups of κ-CARG and the flavylium cation of ATC

The form of complexes (nanoparticle suspension or solid precipitate) depended on both the weight ratio and the total concentration of complex forming reagents. Complexes with different κ-CARG or ι-CARG to ATC weight ratios at a constant total concentration of 0.7 g/L were formed. It was determined that the optimal weight ratio both in the case of κ-CARG/ATC and ι-CARG/ATC was the same; it equaled to 0.4 (Fig. 2.7).

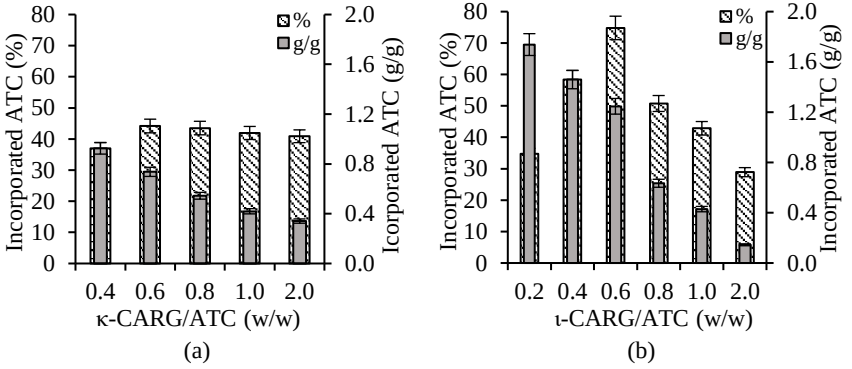

Fig. 2.7 Influence of the CARG/ATC weight ratio on the amount of ATC incorporated into a complex. The total reagent concentration was 0.7 g/L

The influence of the total concentration of CARG and ATC on the amount of ATC incorporated into the complex was evaluated at the optimal CARG/ATC weight ratio of 0.4 (Fig. 2.8). At the higher total concentration, all the formed complexes precipitated, and the maximum amount of the incorporated ATC was about 0.95 g/g and 1.6 g/g in the case of κ-CARG/ATC and ι-CARG/ATC, respectively. At the optimal weight ratio of CARG/ATC and the total ATC and CARG concentration equal to or less than 0.07 g/L, stable κ-CARG/ATC or ι-CARG/ATC complex nanoparticle dispersions were obtained.

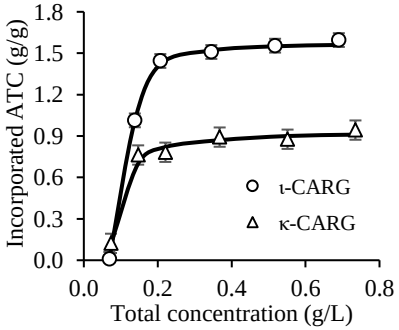

Fig. 2.8 Influence of the total reagent concentration on the amount of ATC incorporated into the CARG/ATC=0.4 complex

Analyse of the stability of carrageenan/anthocyanins complexes

The stability of the CARG/ATC=0.4 complex suspension was investigated and compared with the stability of the bilberry water extract featuring the same ATC concentration of 0.0445 g/L. The influence of the storage time on the residual

concentration of ATC was determined (Fig. 2.9). After 10 days, the ATC concentration in ι-CARG/ATC and κ-CARG/ATC complexes suspensions decreased by about 10% and 20%, respectively, while the ATC concentration in the water extract decreased by 33%. At the same time, the AOEF (determined by employing the ABTS* method) of the ATC introduced into the complex was effectively preserved. After 240 h, AOEF of ι-CARG/ATC and κ-CARG/ATC suspensions and ATC solution decreased by 20%, 25% and 41%, respectively.

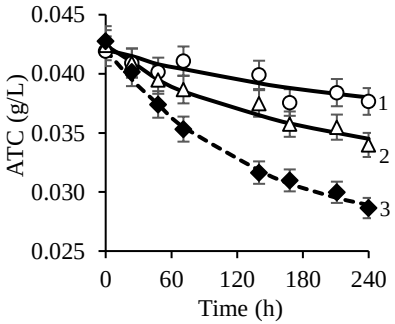

Fig. 2.9 Decrease of ATC concentration depending on the storage time. *1* – ι-CARG/ATC=0.4; *2* – κ-CARG/ATC=0.4; *3* – ATC. Storage in the dark at a temperature of 4 ºC

Cross-linked CARG derivatives for anthocyanins adsorption

In order to decrease the solubility of CARG in water, CARG was modified by the chemical cross-linking reaction with epichlorohydrin (EPCH). It was found that the optimal amount of EPCH was 0.3 mol/mol CARG. Such cross-linked CARG (CARG-C) was used for the equilibrium adsorption of ATC from the bilberry water extract.

Equilibrium adsorption of ATC on cross-linked CARG

The ATC adsorption on the CARG-C microgranules was investigated by employing the equilibrium adsorption method, and the Langmuir, Freundlich, Dubinin–Radushkevich and Temkin adoption models were additionally used to describe the adsorption isotherms. The obtained isotherms of ATC adsorption on ι-CARG-C or κ-CARG-C at different temperatures are presented in Fig. 2.10. According to the Langmuir adsorption model, the flavylium cation of ATC was adsorbed on the active centers of ι-CARG-C or κ-CARG-C, i.e. on their sulfate groups. The driving forces of the adsorption were electrostatic interactions between the latter groups and the cation species of ATC. The amount of the adsorbed ATC increased with an increase of the adsorption temperature and was 1.3–1.7 times higher on the ι-CARG-C containing two sulfate groups (Table 2.2).

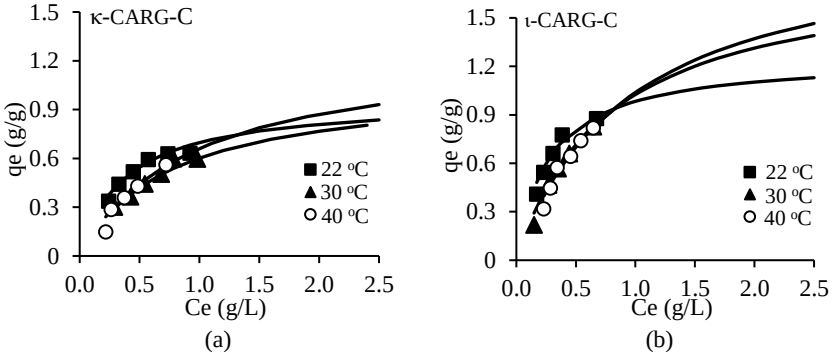

Fig. 2.10 Adsorption isotherms of ATC on CARG-C at different temperatures. The symbols stand for experimental data whereas the lines represent the fitted curves of the Langmuir adsorption model

Table 2.2 Parameters of the Langmuir model for ATC adsorption on κ-CARG-C and ι-CARG-C at different temperatures

T (°C)	Q_L (g/g)	K_L (1/g)	*EF* (mol/equiv)	R^2
κ-CARG-C				
22	0.96	2.63	0.87	0.9934
30	1.06	1.31	0.96	0.9934
40	1.28	1.07	1.16	0.9823
ι-CARG-C				
22	1.25	3.64	0.74	0.9807
30	1.82	1.29	1.07	0.9998
40	2.02	1.06	1.18	0.9797

The values of Freundlich constant n_F and Dubinin–Radushkevich adsorption energy E_{DR} indicated that ATC adsorption was moderately difficult both on ι-CARG-C and κ-CARG-C, especially at higher temperatures; physical forces also begin to play a role in the ATC adsorption alongside with the electrostatic interaction between the ATC flavylium cation and the sulfate groups of CARG. The values of Temkin adsorption energy ΔE_T indicate that the differences in the behavior of sulfate groups of κ-CARG-C and ι-CARG-C as active centers are negligible (Table 2.3).

Table 2.3 Parameters of three models for ATC adsorption on κ-CARG-C and ι-CARG-C at different temperatures

T (°C)	Freundlich model		Dubinin–Radushkevich model		Temkin model	
	n_F	R^2	E_{DR} (kJ/mol)	R^2	ΔE_T (kJ/mol)	R^2
κ-CARG-C						
22	2.24	0.9781	9.57	0.9824	13.6	0.9852
30	1.73	0.9924	8.59	0.9941	14.4	0.9913
40	1.50	0.9995	8.39	0.9982	10.5	0.9963
ι-CARG-C						
22	2.30	0.9776	8.92	0.9726	12.4	0.9786
30	1.54	0.9977	8.27	0.9992	10.5	0.9994
40	1.33	0.9976	7.93	0.9986	9.0	0.9968

The thermodynamic characteristics of ATC adsorption on ι-CARG-C and κ-CARG-C have been evaluated (Table 2.4). The negative values of ΔG^o indicate that ATC (to be precise, the flavylium cation) adsorption on both ι-CARG-C and κ-CARG-C is spontaneous. However, the absolute values of ΔG^o are not high, and they show that the affinity of the flavylium cation to the CARG-C is not high. The negative values of the changes in enthalpy ΔH^o and entropy ΔS^o show that ATC adsorption on both CARG-C is exothermic, and that the order of the system increases during the adsorption process.

Table 2.4 Thermodynamic parameters of ATC adsorption on κ-CARG-C and ι-CARG-C

T (°C)	ln K_C	K_C	R^2	ΔG^o (kJ/mol)	ΔH^o, (kJ/mol)	ΔS^o, (J/mol K)	R^2
κ-CARG-C							
22	0.767	2.15	0.8990	-1.88			
30	0.512	1.67	0.9694	-1.29	-15.2	-45	0.9217
40	0.407	1.50	0.9760	-1.06			
ι-CARG-C							
22	1.097	3.00	0.9025	-2.67			
30	0.965	2.62	0.9976	-2.43	-15.1	-42	0.9570
40	0.700	2.01	0.9787	-1.82			

Desorption of ATC from carrageenan/anthocyanins complexes

ATC was desorbed from ι-CARG-C/ATC having 0.091 g of adsorbed ATC per g of ι-CARG-C into different media: distilled water, 0.1 mol/L HCl solution (the modeling medium of the stomach), 0.1 mol/L phosphate buffer pH=6.8 (the modeling medium of the intestines), ethanol (EtOH) and a mixture of EtOH and 0.1 mol/L HCl. The obtained results show that the amount of desorbed ATC depends on the desorption medium. After 30 min, a large part of ATC was desorbed into the EtOH:HCl= 2:3 mixture (Table 2.5). It should be emphasized,

that 64% and 86% of ATC from the initial amount was desorbed into 0.1 mol/L HCl solution and phosphate buffer with pH=6.8, respectively (Table 2.6). The similar results were obtained when ATC was desorbed from κ-CARG-C/ATC having 0.091 g of adsorbed ATC per g of κ-CARG-C (Table 2.7).

Table 2.5 Influence of the desorption solution on the amount of ATC desorbed from ι-CARG-C/ATC

Composition of desorption solution (by volume)	ATC concentration in desorption solution (mg/L)	ATC amount after desorption (%)*
EtOH	no desorption	0
HCl	44±0.1	64
EtOH:HCl=4:1	27±0.1	39
EtOH:HCl=3:2	62±0.1	91
EtOH:HCl=2:3	66±0.2	96
EtOH:HCl=1:4	53±0.3	77

*ATC concentration (calculated from the added amount of ι-CARG-C/ATC) was 69 mg/L

Table 2.6 Influence of the desorption buffer solution on the amount of ATC desorbed from ι-CARG-C/ATC

Buffer solution	pH of buffer solution	ATC concentration in desorption solution (mg/L)	ATC amount after desorption (%)*
Acetate	3.7	27±0.1	40
	4.1	37±0.2	54
	5.6	33±0.1	48
Phosphate	5.8	66±0.2	96
	6.8	59±0.2	86
Distilled water		41±0.3	59

*ATC concentration (calculated from the added amount of ι-CARG-C/ATC) was 69 mg/L

Table 2.7 Influence of the desorption buffer solution on the amount of ATC desorbed from κ-CARG-C/ATC

Desorption solution	ATC amount after desorption	
	(mg/L)	(%)*
Distilled water (pH=5.0)	28±0.1	40
0.1 M HCl (pH=1.0)	43±0.3	61
EtOH:HCl=2:3	69±0.1	99
Phosphate buffer (pH=6.8)	63±0.1	90

*ATC concentration (calculated from the added amount of κ-CARG-C/ATC) was 70 mg/L

HPLC analysis of solutions after ATC adsorption on CARG-C and desorption from CARG-C/ATC microgranules

The HPLC-UV method was chosen to investigate the solution after ATC adsorption on ι-CARG-C microgranules as well as the solution after ATC desorption from ι-CARG-C/ATC microgranules into EtOH:HCl(0.1 M)=2:3 mixture. The summarized results are presented in Table 2.8. The data of the HPLC analysis implies that ATC is isolated from the crude water extract of wild bilberries during the adsorption on ι-CARG-C, whereas the main phenolic–chlorogenic acid remains in the adsorption solution. After the desorption from ι-CARG-C/ATC, sufficiently pure EtOH:HCl solution of ATC was obtained. These results confirmed the dissertation statement under defense that during the adsorption on sulfated polysaccharides, anthocyanins are isolated from the crude extract of the wild bilberry, and then anthocyanins can be released into various media.

Table 2.8 Amounts of individual ATC or chlorogenic acid adsorbed on ι-CARG-C microgranules and then desorbed into EtOH:HCl=2:3 solution as calculated from chromatography data

ATC	Initial (mg/L)	After adsorption (mg/L)	Adsorbed		ATC in desorption solution (C_{max}=366 mg/L)	
			(mg/L)	(%)*	(mg/L)	(%)**
Cyanidin-3-*O*-arabinoside	71	5	66	93	20	86
Cyanidin-3-*O*-galactoside	92	9	83	90	29	84
Cyanidin-3-*O*-glucoside	114	13	101	89	36	84
Delphinidin-3-*O*-arabinoside	76	4	72	95	21	86
Delphinidin-3-*O*-galactoside	91	5	86	94	30	84
Delphinidin-3-*O*-glucoside	108.5	7	101.5	93	36	83
Malvidin-3-*O*-arabinoside	22	2	20	91	12	73
Malvidin-3-*O*-galactoside	34.5	4	30.5	89	12	83
Malvidin-3-*O*-glucoside	101.5	13	88.5	87	38	80
Peonidin-3-*O*-arabinoside	7	1	6	89	2	87
Peonidin-3-*O*-galactoside	10	1	9	90	7	65
Peonidin-3-*O*-glucoside	48.5	6	42.5	87	15	83
Petunidin-3-*O*-arabinoside	21	1	20	95	5	89
Petunidin-3-*O*-galactoside	34.5	2	32.5	94	9	87
Petunidin-3-*O*-glucoside	78.5	6	72.5	93	25	84
Total	910	79	830	91	297	81
Chlorogenic acid	633	613	20	3	19	-

* ATC concentration from the initial ATC amount

** ATC concentration from the adsorbed ATC amount

Application of cross-linked κ-carrageenan/anthocyanins complex microgranules

Lyophilized microgranules of the cross-linked κ-carrageenan/anthocyanins complex containing 0.17 g/g of ATC were used to prepare the prototype of rectal suppositories with cocoa butter as a base at Lithuanian University of Health Sciences. The control release of ATC from suppositories into a model of the intestinal medium was emphasized as a positive feature which could be employed in the production of intestinal preparations with high bioavailability.

A technological scheme for the production of cross-linked κ-carrageenan/anthocyanins microgranules was proposed and consists of three stages (Fig. 2.11). During the first and second stages, cross-linked microgranules of κ-CARG-C are obtained, and the aqueous bilberry extract is prepared, respectively. In the third stage, ATC from the aqueous bilberry extract are adsorbed onto κ-CARG-C.

κ-CARG is cross-linked with EPCH in alkaline isopropanol:water mixture (2:1 volume ratio) in reactor (*1*) for 6 h at room temperature (~ 20 ° C) with constant agitation. After that, the reaction mixture is washed with acidified isopropanol:water mixture until pH 7-8 of the filtrate in the centrifuge (*3*), dried (*5*) and grained (*6*). The obtained κ-CARC-C microgranules can be stored (*7*) or used for ATC adsorption. ATC from the aqueous bilberry extract are adsorbed onto κ-CARG-C in the reactor (*12*) for 30 min at room temperature (~ 20 ° C) with constant agitation. After the adsorption the obtained κ-CARG-C/ATC microgranules are washed and filtrated in the centrifuge (*14*), lyophilized (*16*), and stored (*17*).

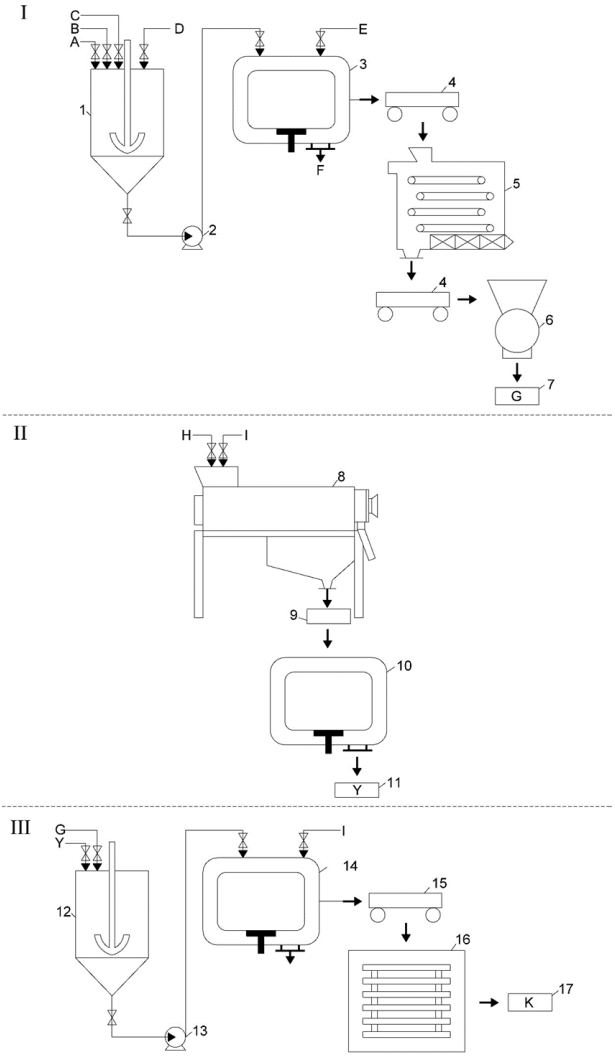

Fig. 2.11 A technological scheme for the production of κ-CARG-C/ATC microgranules: *1, 12* – a reactor; *2, 13* – a pump; *3, 10, 14* – a centrifuge with a filter; *4, 15* – a trolley; 5 – a dryer; *6* – a mill; *7, 11, 17* – product packaging and storage; *8* – an extractor; *9* – a container; *16* – a freeze dryer; *A* – mixture of isopropanol and water; *B* – solution of sodium hydroxide; *C* – EPCH; *D* – κ-CARG; *E* – acidified mixture of isopropanol and water; *F* – filtrate; *G* – κ-CARG-C; *H* – wild bilberry; *I* – water; *Y* – aqueous wild bilberry extract; *K* – κ-CARG-C/ATC microgranules

2.3 Packaging materials containing essential oils

Antibacterial coatings

The antibacterial activity of thyme EO, clove EO, eugenol, and linalool, against *E. coli* and *L. monocytogenes* was assessed by agar diffusion method. The obtained results showed that thyme essential oil (TH) is the most active EO against the selected microorganisms, and it was used for immobilization in coatings.

Corona-treated oriented polypropylene films were coated with compositions consisting of TH and acrylic binder/hydrophobically modified starch (AC/S). The antibacterial activity of coated films was investigated by performing VD assay against *E. coli* and *L. monocytogenes* (Fig. 2.12).

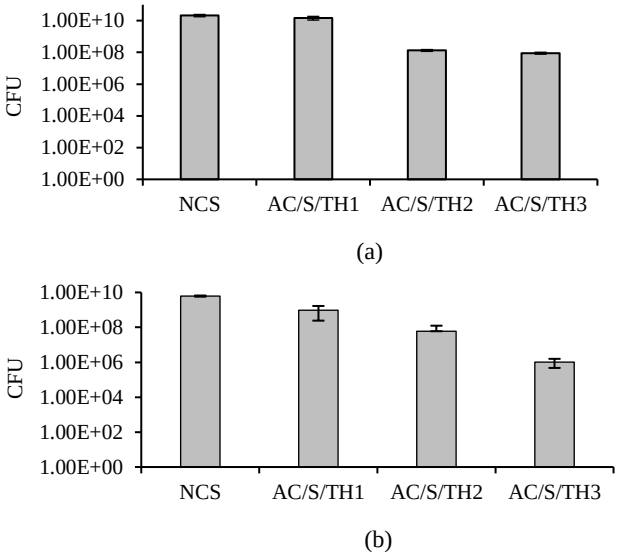

Fig. 2.12 The antibacterial activity of AC/S/TH coatings against *E. coli* (*a*) and *L. monocytogenes* (*b*) by VD assay. Amount of TH: AC/S/TH1 – 5.85±0.3 g/m^2; AC/S/TH2 – 10.56±0.17 g/m^2; AC/S/TH3 – 21.59±1.12 g/m^2

The results showed that the antibacterial activity of coated films against *E. coli* and *L. monocytogenes* depended on the amount of the incorporated TH. The AC/S coatings with highest amount of TH showed the highest antibacterial activity. As it could be seen from the obtained results, AC/S/TH3 coatings containing 21.59±1.12 g/m^2 of EO inhibited cells of *E. coli* and *L. monocytogenes* around the 1000-fold from normal growth (negative control sample (NCS)).

Antioxidant coatings

The antioxidant activity of EO (clove, basil, ginger, rosemary, caraway) and its main components (eugenol, linalool, methyl chavicol, eucalyptol and α-pinene) was investigated by employing the DPPH method. The obtained results showed that clove essential oil (CL) and eugenol (EU) were the most active materials which thus were used for immobilization in the coatings.

Corona-treated oriented polypropylene films were coated with compositions consisting of CL or EU and cellulose acetate (CA) or AC/S as binders. The antioxidant activity of coated films obtained by using the VP-DPPH method are presented in Figs. 2.13 and 2.14.

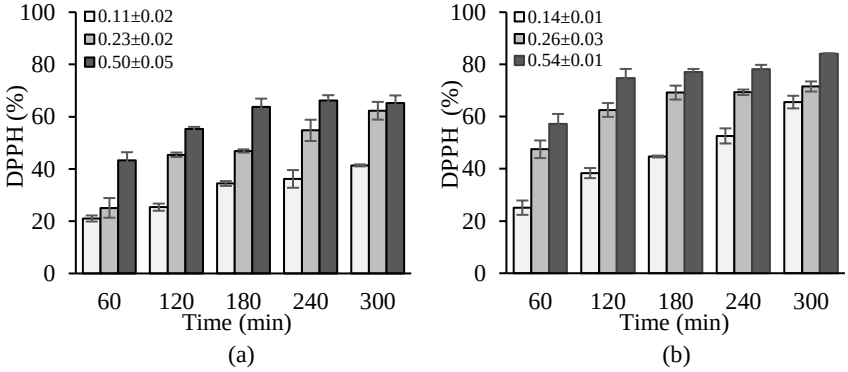

Fig. 2.13 Time-dependent antioxidant activity of AC/S/CL (*a*) and AC/S/EU (*b*) coated films containing different concentration of CL or EU (expressed as g/m^2 and indicated in the Figures)

The antioxidant activity depended on the concentration of the active component in the coating and testing time, as well as regarding the type of binder. Coatings having EU had a higher antioxidant activity in comparison with those having CL. When AC/S was used as a binder, after 300 min, inhibition of DPPH free radicals depending on the amount of immobilized EU varied from 65% to 84% (Fig. 2.13). CA based coatings showed especially high antioxidant activity in this test (Fig. 2.14). With increase of CL concentration in CA/CL coatings from 0.18 to 0.77 g/m^2 the inhibition of DPPH after 180 min increased from 87% to 92% (Fig. 2.14 a). Whereas, the increase of EU concentration from 0.19 to 0.82 g/m^2 yielded the inhibition of DPPH from 90 to 94% (Fig. 2.14 b).

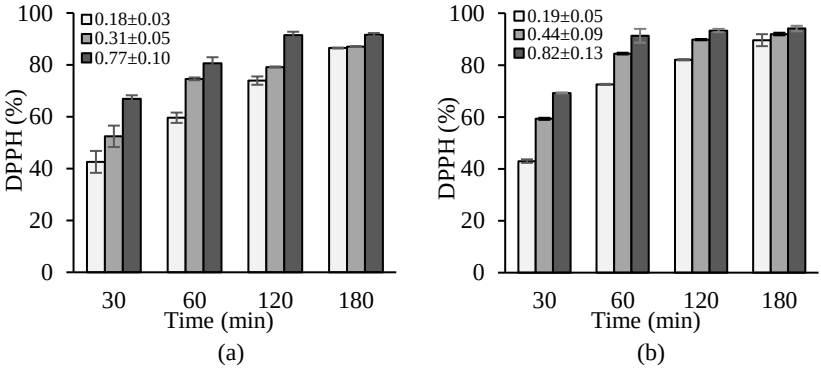

Fig. 2.14 Time-dependent antioxidant activity of CA/CL (*a*) and CA/EU (*b*) coated films containing different concentrations of CL or EU (expressed as g/m^2 and indicated in the Figures)

Antioxidant food packaging prototype

Based on the antioxidant activity of coated OPP films, EU was chosen as the most effective antioxidant for food packaging application. OPP films with two AC/S/EU coatings containing different amounts of EU (0.32±0.03 g/m^2 and 6.40±0.14 g/m^2), and CA/EU coating with 0.65±0.08 g/m^2 of EU were used to prepare an active food packaging prototype for fresh beef steaks. Control samples were packaged in the packaging without the active coating. The color changes and the lipid oxidation of beef were evaluated during the storage period of 14 days under modified atmosphere (80% O_2 and 20% CO_2) at 2±1 °C.

The color investigation revealed that the red color of beef was successfully preserved in the active packaging (see Table 2.9).

Table 2.9 Effect of active packaging on color changes of fresh beef packed under modified atmosphere during the storage at 2 ± 1 ºC

Color characteristic	Packaging	Time (day)				
		0	3	7	10	14
Brightness *L* (NBS)	Control	41.08±0.91	41.24±2.82	42.52±2.20	43.81±1.40	44.33±3.38
	AC/S/EU1	41.08±0.91	40.64±1.68	40.87±1.93	43.39±2.34	44.06±2.12
	AC/S/EU2	41.08±0.91	41.27±1.50	41.08±2.00	43.83±2.28	44.06±1.88
	CA/EU	41.08±0.91	41.52±1.74	41.28±1.20	43.27±1.91	43.86±1.91
Chromaticity coordinate *a* (NBS)	Control	35.36±0.54	30.59±1.68	25.15±1.06	23.28±1.92	18.44±3.36
	AC/S/EU1	35.36±0.54	32.17±1.55	29.99±1.30	26.80±1.29	26.53±1.08
	AC/S/EU2	35.36±0.54	29.20±1.86	26.06±1.11	27.40±1.72	26.10±1.51
	CA/EU	35.36±0.54	31.25±1.41	25.94±1.02	26.51±1.95	26.03±1.07
Chromaticity coordinate *b* (NBS)	Control	14.87±0.58	18.36±1.35	15.59±1.32	15.51±2.06	14.21±1.19
	AC/S/EU1	14.87±0.58	18.60±1.07	16.89±0.92	17.00±0.86	16.60±0.92
	AC/S/EU2	14.87±0.58	17.63±1.42	15.75±0.92	17.11±1.48	16.26±0.94
	CA/EU	14.87±0.58	18.35±0.97	15.28±0.80	16.59±1.40	16.38±1.02
Hua angle H^o_{ab} (degree)	Control	22.80±0.75	30.96±1.32	31.76±1.79	33.60±2.74	38.11±4.96
	AC/S/EU1	22.80±0.75	30.02±0.86	29.38±1.02	32.38±0.56	32.03±1.29
	AC/S/EU2	22.80±0.75	31.10±1.10	31.15±1.37	31.96±1.62	31.93±1.03
	CA/EU	22.80±0.75	30.42±0.58	30.49±0.63	32.04±1.82	31.70±1.19
Vividness of color C_{ab} (NBS)	Control	38.36±0.62	35.69±1.99	29.60±1.42	28.01±2.41	23.36±2.99
	AC/S/EU1	38.36±0.62	37.17±1.80	34.43±1.47	31.74±1.52	31.30±1.23
	AC/S/EU2	38.36±0.62	34.12±2.25	30.46±1.24	32.31±2.08	30.75±1.69
	CA/EU	38.36±0.62	36.25±1.68	30.10±1.25	31.29±2.20	30.55±1.32
Total color difference ΔE_{ab} (NBS)	Control	-	6.79±1.23	10.65±0.93	12.66±1.85	17.56±3.62
	AC/S/EU1	-	5.50±0.65	6.17±1.05	9.49±0.95	9.75±1.21
	AC/S/EU2	-	7.23±1.04	9.62±0.96	9.17±1.53	10.06±1.48
	CA/EU	-	5.90±0.64	9.56±0.93	9.66±1.46	10.10±1.00

EU amount in coating: EU1 – 0.32±0.03 g/m^2; EU2 – 6.40±0.14 g/m^2; EU – 0.65±0.08 g/m^2

The lipid oxidation was evaluated by the 2-thiobarbituric acid reactive substances (TBARS) assay and expressed as milligrams of malondialdehyde (MDA) per kilogram of beef. The influence of the active packaging type on the lipid oxidation in beef steaks during the storage is shown in Fig. 2.15.

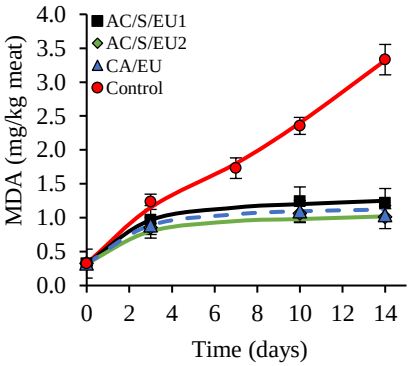

Fig. 2.15 MDA amount in beef stored in different packaging under modified atmosphere during the storage at 2±1 ºC (TBARS assay)

The initial amount of oxidized lipids in the fresh beef was 0.32±0.03 mg/kg of MDA. During the storage, MDA in beef kept in control packaging increased significantly to 1.71 mg/kg and 3.33 mg/kg after 7 and 14 days, respectively. Meanwhile, the lipid oxidation of beef kept in antioxidant packaging was much lower with amounts of MDA ranging from 1.01 mg/kg to 1.22 mg/kg. It was reported that the amount of MDA equal to 1.5 mg/kg is closely related to perceptible and unacceptable off-odor of meat [19]. Therefore, the tested antioxidant packaging prototype with EU is a promising technology offering an increase of the display life of beef.

CONCLUSIONS

1. Ionic complexes consisting of such polysaccharides as dextran sulfate, κ- or ι-carrageenan and anthocyanins present in the wild bilberry aqueous extract were obtained due to electrostatic interaction between the flavylium cations of anthocyanins and the anionic sulfate groups of polysaccharides. The amount of anthocyanins incorporated into the complex depended on the weight ratio of the polysaccharide and anthocyanins and on their total concentration. When the highest amount of anthocyanins was incorporated into the complex, the weight ratio of polysaccharide to anthocyanins was equal to 0.4. At the optimal weight ratio, polysaccharide and anthocyanins complex particle size depended on the total concentration of the polysaccharide and anthocyanins. High-performance liquid chromatography experiments confirmed that all individual anthocyanins present in the aqueous extract of the wild bilberry are taking part in the formation of complexes with polysaccharides.

2. Incorporation of anthocyanins into the complex with polysaccharides not only protected flavylium cation from hydration but also increased the stability of the aqueous complex suspension. Anthocyanins can be released from the complex into different media, including 0.1 M hydrochloric acid as a model of gastric medium and a phosphate buffer with pH=6.8 as a model of the intestinal medium.

3. Microgranules of the carrageenan/anthocyanins complex can be obtained by adsorption of anthocyanins from the wild bilberry extract onto cross-linked carrageenan derivatives. 0.3 mol of epichlorohydrin to one mole of carrageenan in the reaction mixture is sufficient to obtain cross-linked carrageenan derivatives with optimal properties. Two-variable mathematical adsorption models have been applied to describe the isotherms of equilibrium adsorption of anthocyanins on cross-linked carrageenans derivatives: the Langmuir sorption model confirmed electrostatic interaction between the flavylium cations of anthocyanins and the sulfate groups of cross-linked carrageenans. The values of the Freundlich constant n_F and the Dubinin–Radushkevich adsorption energy E_{DR} indicated that anthocyanins adsorption was moderately difficult on cross-linked carrageenans. At higher temperatures the physical forces also begin to play a role in the anthocyanins adsorption alongside with the electrostatic interaction. The low values of changes in the Gibbs free energy ΔG^o showed that the affinity of the flavylium cation to the cross-linked carrageenan was not high.

4. Lyophilized microgranules of the cross-linked κ-carrageenan/anthocyanins complex have been used to prepare a prototype of rectal suppositories, and a technological scheme for the production of microgranules has been proposed.

5. The active coatings comprised of thyme essential oil or eugenol and cellulose acetate or acrylic binder/hydrophobically modified starch on polymer films have been developed, and their antibacterial activity against *E. coli* and *L.*

monocytogenes has been demonstrated. They effectively inhibited free radicals without direct contact with the investigative substrate.

6. A prototype of antioxidant packaging has been developed. Fresh beef packed by using cellulose acetate/eugenol or acrylic binder/hydrophobically modified starch/eugenol coated polymer films has been preserved from discoloration and lipid oxidation for 14 days.

REFERENCES

1. TAYLOR, C., M. WALLACE, M., GIUSTI, M. Anthocyanins in Health and Disease. CRS Press, Taylor and Francis Group, Boca Raton London, New York, 2014, p. 2. ISBN 9781439894712

2. SHARIFI-RAD, J., et al. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. *Molecules.* 2017, 22(1), 70. Access via doi:10.3390/molecules22010070

3. JOSÉ-LUIS, R. Essential Oils: What They Are and How the Terms Are Used and Defined. In: *Essential Oils in Food Preservation, Flavor and Safety*. 2016, Elsevier, 3-10. Access via: 10.1016/B978-0-12-416641-7.00001-8

4. CAVALCANTI, R. N., D. T. SANTOS, M. A. A. MEIRELES. Non-Thermal Stabilization Mechanism of Anthocyanins in Model and Food System. *Food Research International*. 2011, 44(2), 499-509. Access via doi: 10.1016/j.foodres.2010.12.007

5. JEONG, D., and K. NA. Chondroitin sulphate based nanocomplex for enhancing the stability and activity of anthocyanins. *Carbohydrate Polymers*. 2012, 90, 507-515. Access via doi: 10.1016/j.carbpol.2012.05.072

6. LIU, J., X. ZHAN, J. WAN, Y. WANG, C. WANG. Review for Carrageenan-Based Pharmaceutical Biomaterials: Favourable Physics Feature versus Adverse Biological Effects. *Carbohydrate Polymers*. 2015, 121, 27-36. Access via doi: 10.1016/j.carbpol.2014.11.063

7. COSTALAT, M., L. DAVID, T. DELAIR. Reversible Controlled Assembly of Chitosan and Dextran Sulfate: a New Method for Nanoparticles Elaboration. *Carbohydrate Polymers*. 2014, 102, 717-728. Access via doi: 10.1016/j.carbpol.2013.10.098

8. CHEOW, W. S., and K. HADINOTO. Green Preparation of Antibiotic Nanoparticle Complex as Potential Anti-Biofilm Therapeutics via Self-Assembly Amphiphile-Polyelectrolyte Complexation with Dextran Sulfate. *Colloids and Surface B; Biointerfaces*. 2012, 92, 55-63. Access via doi: 10.1016/j.colsurfb.2011.11.024

9. *REGULATION (CE) No. 450/2009* (29/05/2009). Access via http://eurlex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32009R0450

10. FULEKI, T., and F. J. FRANCIS. Quantitative Methods for Anthocyanins. Determination of Total Anthocyanins and Degradation Index for Cranberries Juice. *Journal of Food Science*. 1968, 33, 78-83. Access via doi: 10.1111/j.1365-2621. 1968.tb00888.x

11. LANGMUIR, I. Adsorption of Gases on Plane Surfaces of Glass: Mica and Platinum. *Journal of the American Chemical Society*. 1918, 40, 212-223. Access via doi: 10.1021/ja02242a004

12. FREUNDLICH, H. M. F. Über die Adsorption in Lösungen. Zeitschrift fürPhysikalische *Chemie*. 1906, 57, 385-470.

13. DUBININ, M. M. Modern Theory of Voluminal Filling of Micropores of Carbonadsorbent. Izvestija Akademii Nauk SSSR. *Chimija*. 1991, 1, 9–30, in Russian.

14. TEMKIN, M. I. Adsorption Equilibrium and the Kinetics of Processes on Non-Homogeneous Surfaces and in the Interaction between Adsorbed Molecules. *Zhurnal Fizicheskoi Khimii*. 1941, 15, 296-332.

15. MILLER, N. J. et al. A Novel Method for Measuring Antioxidant Capacity and its Application to Monitoring the Antioxidant Status in Premature Neonates. *Clinic Science*. 1993, 84, 407-412.

16. BRAND-WILLIAMS, W., M. E. CUVELIER, C. BERSET. Use of a Free Radical Method to Evaluate Antioxidant Activity. *Lebensmittel-Wissenschaft Technologie*. 1995, 28, 25-30.

17. ANDREA, P. Entwicklung und Etablierung von Methoden zum Testen der Wirksamkeit antimikrobieller Verpackungen. In (Bachelorarbeit), 2014, unveröffentlicht.

18. FOOD TBARS ASSAY KIT (protocol). Oxford Biomedical Research, Inc. Access via http://www.funakoshi.co.jp/data/datasheet/OBR/FS50.pdf

19. MARTÍNEZ, L., D. DJENANE, I. CILLA, J. A. BELTRÁN, P. RONCALÉS. Antioxidant Effect of Rosemary, Borage, Green Tea, Puerh Tea and Ascorbic Acid on Fresh Pork Sausages Packaged in Modified Atmosphere. Influence of the Presence of Sodium Chloride. *Journal of the Science of Food and Agriculture*. 2006, 86(9), 1298-1307. Access via doi: 10.1002/jsfa.2492

LIST OF PUBLICATIONS ON THE DISSERTATION TOPIC

Publications on the dissertation topic in the journals of the main list of *Clarivate Analytics Web of Science* database

1. Klimavičiūtė, Rima; **Navikaitė, Vesta**; Jakštas, Valdas; Ivanauskas, Liudas. Complexes of dextran sulfate and anthocyanins from *Vaccinium myrtillus*: formation and stability // Carbohydrate polymers. Oxford: Elsevier. ISSN 0144-8617. 2015, vol. 129, p. 70-78.
2. **Navikaitė, Vesta**; Simanavičiūtė, Deimantė; Klimavičiūtė, Rima; Jakštas, Valdas; Ivanauskas, Liudas. Interaction between κ- and ι-carrageenan and anthocyanins from *Vaccinium myrtillus* // Carbohydrate polymers. Oxford: Elsevier. ISSN 0144-8617. 2016, vol. 148, p. 36-44.

Articles in other peer reviewed research publications

1. **Navikaitė, Vesta**; Klimavičiūtė, Rima. Nanocomplexes of dextran sulfate and anthocyanins from *Vaccinium myrtillus* // Chemistry and Chemical Technology: proceedings of the international conference, 25 April, 2014 / Kaunas University of Technology. Kaunas: Technologija, 2014. ISSN 2351-5643. 2014, p. 257-259.

Conference proceedings

1. **Navikaitė, Vesta**; Klimavičiūtė, Rima. Nanocomplexes of anionic polymers and anthocyanins from Vaccinium myrtillus // Baltic Polymer Symposium 2014 : Laulasmaa, Estonia, September 24-26, 2014 : program and abstracts / Tallinn University of Technology. Tallinn: Tallinn University of Technology, 2014. p. 103.
2. **Navikaitė, Vesta**; Yildirim, Selçuk; Wolfram-Schilling, Evelyn; Rüegg, Nadine; Rutkaitė, Ramunė. Development antioxidant coatings using essential oils for food packaging applications // MATBIM 2015 [e-resource] : 3rd international meeting on material/bioproduct interaction, 17-19, June 17–19, Zaragoza, Spain: book of abstracts. [S.l.: s.n.], 2015. p. [1-2].
3. Klimavičiūtė, Rima; **Navikaitė, Vesta**; Jakštas, Valdas.; Ivanauskas, Liudas. Formation and properties of carrageenan-anthocyanins complexes // Polysaccharides and polysaccharide-based advanced materials: from science to industry : 4th EPNOE international polysaccharide conference, October 19–22, 2015, Warsaw, Poland: book of abstracts. Lodz: Institute of Biopolymers and Chemical Fibres, 2015. p. 231.
4. **Navikaitė, Vesta**; Rutkaitė, Ramunė; Yildirim, Selçuk; Rüegg, Nadine; Wolfram, Evelyn. Biopolymers as carriers of eugenol for antioxidant food packaging // BiPoCo 2016 : 3rd international conference Bio-based polymers

and composites, August 28 – September 1, 2016, Szeged, Hungary : abstract book. [Budapest: BiPoCo, 2016]. p. 393-394.

5. **Navikaitė, Vesta**; Klimavičiūtė, Rima; Jakštas, Valdas; Ivanauskas, Liudas. Immobilization of anthocyanins on cross-linked carrageenan // BiPoCo 2016 : 3rd international conference Bio-based polymers and composites, August 28 – September 1, 2016, Szeged, Hungary : abstract book. [Budapest: BiPoCo, 2016]. p. 234-235.
6. Jakštas, Valdas; Klimavičiūtė, Rima; **Navikaitė, Vesta**; Ivanauskas, Liudas; Bernatonienė, Jurga. Pharmaceutical improvement of pharmaceutical characteristics of anthocyanins using the incorporation into the Iota or Kappa carrageenans // Vital nature sign [e-resource]: 10th international scientific conference, May 19–20, 2016, Vilnius, Lithuania: abstract book / editors: Tiso, Nicola and Kaškonienė, Vilma. [S.l.: s.n.]. ISSN 2335-8653. 2016, p. 106.
7. **Navikaitė, Vesta**; Rutkaitė, Ramunė; Yildirim, Selçuk; Wolfram, Evelyn; Rüegg, Nadine. (2016). Polysaccharide and essential oils coatings for active food packaging materials // Baltic polymer symposium 2016 : Klaipeda, September 21-24, 2016 : programme and abstracts / Kaunas University of Technology, Vilnius University, Klaipeda University. Kaunas: Kaunas University of Technology, 2016, ISBN 9786090212356. p. 88.
8. **Navikaitė-Šnipaitienė, Vesta**; Rutkaitė, Ramunė; Yildirim, Selçuk; Wolfram, Evelyn; Rüegg, Nadine. Antioxidant and antimicrobial food packaging coatings // Chemistry and chemical technology 2017: proceedings of the international conference, April 28, 2017, Kaunas. Kaunas: Kauno technologijos universitetas. ISSN 2538-7359. 2017, p. 53.
9. Rutkaitė, Ramunė; Ivanauskas, Liudas; Jakštas, Valdas; Klimavičiūtė, Rima; **Navikaitė-Šnipaitienė, Vesta**; Simanavičiūtė, Deimantė. Modified polysaccharide derivatives for immobilization of bioactive phenolic compounds // 25th Polychar 2017 World Forum on Advanced Materials, Kuala Lumpur, Malaysia, October 9–13, 2017 [e-resource]. [S.l.: s.n.]. 2017, p. 1.

OTHER PUBLICATIONS

1. Danilovas, Paulius Pavelas; **Navikaitė, Vesta**; Rutkaitė, Ramunė. Preparation and characterization of potentially antimicrobial polymer films containing starch nano- and microparticles // Materials science = Medžiagotyra / Kaunas University of Technology, Academy of Sciences of Lithuania. Kaunas: KTU. ISSN 1392-1320. 2014, Vol. 20, No. 3, p. 283–288.
2. Danilovas, Paulius Pavelas; **Navikaitė, Vesta**; Rutkaitė, Ramunė. Gamtinio ir modifikuoto krakmolo jodoforai // Medžiagų inžinerija 2013 : respublikinė konferencija: konferencijos pranešimų santraukos. November 15, 2013,

Kaunas / Kauno technologijos universitetas, Lietuvos medžiagų tyrinėtojų asociacija. Kaunas: Technologija. ISSN 2345-0886. 2013, p. 52–53.

3. Rutkaitė, Ramunė; Babelytė, Miglė; Danilovas, Paulius Pavelas; **Navikaitė, Vesta**. Diphilic properties of propylene oxide and octenyl succinic anhydride modified starches and their coatings // Chemistry and Chemical Technology 2015: programme and proceedings of the international conference, Vilnius, Lithuania, January 23, 2015 / Vilnius University, Lithuanian Academy of Sciences, Kaunas University of Technology, Center for Physical Sciences and Technology. [S.l.: s.n, 2015], ISBN 9786094594618. p. 317-320.
4. **Navikaitė, Vesta**; Jonuškienė, Ilona; Rukuižienė, Žaneta; Rutkaitė, Ramunė. Electrospun cellulose acetate fibers containing essential oils for active food packaging // Chemistry and chemical technology: international conference of Lithuanian Society of Chemistry: Lithuanian Academy of Science, Vilnius, Lithuania, April 28–29, 2016: book of abstracts / Fizinių ir technologijos mokslų centras, Vilniaus universitetas, Lietuvos mokslų akademija, Kauno technologijos universitetas. [S.l.: s.n, 2016], ISBN 9786099551135. p. 230.
5. Danilovas, Paulius Pavelas; Jonuškienė, Ilona; **Navikaitė, Vesta**; Rukuižienė, Žaneta; Rutkaitė, Ramunė. PO-002-(103) - antioxidant and antimicrobial electrospun cellulose acetate fibers for active food packaging // MATBIM 2017: 4th international meeting on Material/Bioproduct Interaction, April 26–28, 2017, Porto, Portugal: book of abstracts. Porto: [s.n.]. 2017, p. 141-143.
6. Danilovas, Paulius Pavelas; Budnikas, Tomas; **Navikaitė, Vesta**; Rutkaitė, Ramunė; Jonuškienė, Ilona. PO-022-(102) - plasticized cellulose acetate films for active food packaging applications // MATBIM 2017 [e-resource]: 4th international meeting on material/bioproduct interaction, 26th-28th April, 2017, Porto, Portugal: book of abstracts. [S.l.: s.n.]. 2017, p. 200-202.

REZIUMĖ

Temos aktualumas

Iš augalų išgaunami antocianinai ir eteriniai aliejai pasižymi priešvėžinėmis, uždegimo slopinamosiomis, antioksidacinėmis, antibakterinėmis savybėmis. Dėl šių savybių jie plačiai naudojami ne tik kosmetikoje, farmacijoje, bet ir maisto pramonėje, pavyzdžiui, veikliosioms maisto pakuotėms gaminti [1, 2, 3].

Antocianinai yra nepatvarūs junginiai, todėl stabilumui padidinti jie gali būti imobilizuojami įvairiuose nešikliuose, kurie apsaugo antocianinų molekules nuo fizinių ir cheminių veiksnių poveikio ir padeda išlaikyti antocianinų aktyvumą [4]. Tačiau šie būdai susiję su didelėmis sąnaudomis ir antocianinų nuostoliais. Terpėje, kurios pH vertė yra tarp 1 ir 3, antocianinai egzistuoja raudonos spalvos flavilio katijono pavidalu ir gali sudaryti joninius kompleksus su anijoninių grupių turinčiais polimerais. Kol kas literatūroje yra aprašyti tik stabilūs nanodydžio antocianinų ir chondroitino sulfato kompleksai [5]. Tačiau tokius kompleksus galėtų sudaryti ir kiti sulfatogrupių turintys gamtiniai ar modifikuoti polisacharidai, pavyzdžiui, karageninai ar dekstrano sulfatas. Tiek vieni, tiek kiti yra plačiai naudojami polimeriniams kompleksams su teigiamojo krūvio polimerais ar joniniams kompleksams su mažamolekuliais teigiamojo krūvio grupių turinčiais junginiais gauti [6, 7]. Joniniai polimerų kompleksai susidaro vandeniniuose tirpaluose dėl elektrostatinės sąveikos tarp priešingų ženklų krūvio grupių turinčių polimerų ir mažamolekulių junginių kambario temperatūroje, t. y. nenaudojant energijos išteklių, neteršiant gamtos organiniais tirpikliais, todėl toks kompleksų susidarymas priskiriamas prie aplinkai nekenksmingų procesų [8].

Veikliųjų pakuočių gamyba yra viena iš inovatyvių technologijų. Tokiose pakuotėse imobilizuotos aktyviosios medžiagos veikia atsipalaiduodamos į pakuotės vidų arba sugerdamos pakuotėje esančias kitas medžiagas, taip apsaugodamos maisto produktus nuo aplinkos poveikio ir pailgindamos jų tinkamumo vartoti trukmę [9]. Antimikrobinių ir antioksidacinių savybių turinčios maisto pakuotės gali būti gaunamos ant jų plėvelės formuojant eterinių aliejų turinčias dangas. Tokioms dangoms gauti kaip plėvėdariai gali būti naudojami gamtiniai, modifikuoti gamtiniai ar sintetiniai polimerai.

Darbo tikslas

Šio darbo tikslas – išsaugoti antocianinų ir eterinių aliejų veikliąsias savybes imobilizuojant juos anijoniniuose polisachariduose.

Darbo tikslui pasiekti suformuluoti šie **uždaviniai:**

1. Parinkti sąlygas tirpiems, nano- ar mikrodalelių dekstrano sulfato, κ-karagenino ir ι-karagenino kompleksams su vandeniniame mėlynių ekstrakte esančiais antocianinais gauti.

2. Įvertinti į kompleksus su dekstrano sulfatu ar κ-karageninu ir ι-karageninu sujungtų antocianinų stabilumą, antioksidacines savybes ir atpalaidavimą į įvairias terpes.
3. Ištirti sąveiką tarp antocianinų flavilio katijono ir karageninų sulfatogrupių pusiausvirosios adsorbcijos sąlygomis, jai aprašyti taikyti dviejų kintamųjų adsorbcijos modelius.
4. Pasiūlyti optimalių savybių antocianinų ir karageninų kompleksų gamybos technologinę schemą.
5. Sukurti eterinių aliejų turinčias dangas, nustatyti jų antibakterinį ir antioksidacinį efektyvumą nesant tiesioginio kontakto su tiriamuoju substratu (per garų fazę).
6. Sukurti veikliosios pakuotės prototipą.

Mokslinis darbo naujumas

Nustatyta, kad vandeniniame mėlynių ekstrakte esantys antocianinai gali sudaryti joninius kompleksus su sulfatogrupių turinčiais dekstrano sulfatu ar κ- bei ι-karageninais, visų pirma, dėl elektrostatinės sąveikos tarp antocianinų flavilio katijono ir polisacharido sulfatogrupių. Gauto komplekso pobūdis priklauso nuo jį sudarančių junginių masių santykio ir bendrosios jų koncentracijos: kai polisacharido ir antocianinų masių santykis yra optimalus – 0,4 g/g – ir bendroji dekstrano sulfato ir antocianinų komplekso koncentracija yra ≤0,16 g/l, o karageninų ir antocianinų – ≤0,07 g/l, gaunama stabili komplekso nanodalelių suspensija; padidinus bendrąją kompleksą sudarančių medžiagų koncentraciją, dalis komplekso iškrinta tamsiai raudonos spalvos nuosėdomis.

Efektyviosios skysčių chromatografijos metodu įrodyta, kad antocianinai yra išgryninami juos imobilizuojant polimeriniuose nešikliuose.

Antocianinų imobilizavimas kompleksuose su dekstrano sulfatu ar karageninais apsaugo antocianinų flavilio katijoną nuo hidratacijos ir padidina vandeninių komplekso suspensijų stabilumą saugant. Iš antocianinų ir polisacharidų kompleksų mikrogranulių antocianinai yra efektyviai atpalaiduojami į modelines skrandžio ir žarnyno terpes.

Nustatyta, kad čiobrelių eterinio aliejaus ar eugenolio turinčios dangos pasižymi antibakteriniu poveikiu *E. coli* bei *L. monocytogenes* bakterijoms ir stipriu laisvųjų radikalų sujungimu, nesant tiesioginio kontakto su tiriamuoju substratu, veikiant aktyviosios medžiagos garams.

Praktinė darbo vertė

Pagamintos liofilizuotos κ-karagenino ir antocianinų mikrogranulės panaudotos antioksidacinėmis ir uždegimo slopinamosiomis savybėmis pasižyminčių rektalinių žvakučių prototipui sukurti.

Pasiūlyta antocianinų imobilizavimo κ-karagenino mikrogranulėse technologinė schema.

Sukurtas antioksidacinės pakuotės prototipas: šviežia jautiena supakuota polimerine plėvele, padengta eugenolio turinčia danga, kuri apsaugo jautieną 14 dienų nuo lipidų oksidacijos ir mėsos spalvos pokyčių.

Ginamasis disertacijos teiginys

Antocianinai, adsorbuojami iš vandeninio mėlynių ekstrakto sulfatogrupių turinčiais polisacharidais, yra išgryninami išsaugant jų antioksidacines savybes ir vėliau gali būti atpalaiduojami į įvairias terpes.

Darbo rezultatų aprobavimas

Doktorantūros studijų metu 2 publikacijos disertacijos tema paskelbtos *Clarivate Analytics Web of Science* duomenų bazės žurnaluose, turinčiuose citavimo indeksą, 1 straipsnis publikuotas kituose recenzuojamuose mokslo leidiniuose, 9 straipsniai paskelbti mokslinių konferencijų pranešimų medžiagoje.

Darbo apimtis

Daktaro disertaciją sudaro įvadas, 3 skyriai, išvados, literatūros sąrašas, publikacijų disertacijos tema sąrašas ir padėka. Bendra apimtis – 114 puslapių. Darbe pateikti 48 paveikslai, 20 lentelių, 31 matematinė išraiška ir lygtis. Literatūros sąrašą sudaro 240 šaltinių.

IŠVADOS

1. Vykstant elektrostatinei sąveikai tarp polisacharidų – dekstrano sulfato, κ- ar ι-karageninų – sulfatogrupių ir vandeniniame mėlynių ekstrakte esančių antocianinų flavilio katijono, susidaro joniniai kompleksai. Į kompleksą sujungtų antocianinų kiekis priklauso nuo polisacharido prigimties, kompleksą sudarančių medžiagų masių santykio ir bendrosios jų koncentracijos. Daugiausia antocianinų į kompleksą yra sujungiama, kai polisacharido ir antocianinų masių santykis yra 0,4. Polisacharidų ir antocianinų komplekso dalelių dydis, esant optimaliam medžiagų masių santykiui, priklauso nuo bendrosios polisacharido ir antocianinų koncentracijos tirpale. Efektyviosios skysčių chromatografijos metodu įrodyta, kad kompleksų su polisacharidais susidarymo procese dalyvauja visi vandeniniame mėlynių ekstrakte esantys skirtingi antocianinai.

2. Antocianinų imobilizavimas kompleksuose su dekstrano sulfatu ar karageninais apsaugo antocianinų flavilio katijoną nuo hidratacijos ir padidina vandeninių komplekso suspensijų stabilumą saugant. Iš gautų kompleksų mikrogranulių antocianinai gali būti atpalaiduojami į įvairias terpes, tarp jų į 0,1 M druskos rūgšties (modelinė skrandžio terpė) ir fosfatinį buferinį tirpalą, kurio pH vertė yra 6,8 (modelinė žarnyno terpė).

3. Karageninų ir antocianinų kompleksų mikrogranulės gali būti gaunamos mėlynių ekstrakte esančius antocianinus adsorbuojant tinkliniais karageninų

dariniais. Optimalių savybių tinkliniams karageninų dariniams gauti reakcijos mišinyje užtenka 0,3 molio epichlorhidrino moliui karagenino. Pusiausvirosios adsorbcijos sąlygomis gautoms antocianinų adsorbcijos tinkliniais karageninų dariniais izotermoms aprašyti pritaikyti dviejų kintamųjų matematiniai adsorbcijos modeliai. Lengmiūro adsorbcijos modelis leido patvirtinti elektrostatinę sąveiką tarp flavilio katijono ir tinklinių karageninų sulfatogrupių. Freundlicho konstantos n_F bei Dubinino ir Radushkevicho adsorbcijos energijos E_{DR} vertės parodė, kad sąlygos antocianinų adsorbcijai tinkliniais karageninais nepakankami palankios ir aukštesnėje temperatūroje antocianinai adsorbuojami ne tik dėl jonų mainų, bet ir fizikinėmis jėgomis. Nedidelės neigiamos Gibso laisvosios energijos pokyčio $\Delta G°$ vertės parodė, kad antocianinų flavilio katijono giminiškumas tinkliniams karageninams nėra didelis.

4. Pagamintos liofilizuotos tinklinio κ-karagenino ir antocianinų mikrogranulės panaudotos rektalinio preparato prototipui sukurti ir pasiūlyta šių mikrogranulių gamybos technologinė schema.

5. Sukurtos čiobrelių eterinio aliejaus ar eugenolio turinčios celiuliozės acetato ar akrilinio plėvėdario ir hidrofobiškai modifikuoto krakmolo dangos ant polimerinės plėvelės, pasižyminčios antibakteriniu poveikiu *E. coli* bei *L. monocytogenes* bakterijoms ir stipriu laisvųjų radikalų sujungimu nesant tiesioginio kontakto su tiriamuoju substratu.

6. Sukurtas antioksidacinės pakuotės prototipas: šviežia jautiena supakuota polimerine plėvele, padengta celiuliozės acetato ir eugenolio ar akrilinio plėvėdario, hidrofobiškai modifikuoto krakmolo ir eugenolio danga. Mėsa šioje pakuotėje 14 dienų buvo apsaugota nuo lipidų oksidacijos ir nepageidaujamų spalvos pokyčių.

Trumpa informacija apie autorę

Vesta Navikaitė-Šnipaitienė gimė 1988 m. rugpjūčio 22 d. Kaune.
El. paštas: vesta.navikaite@ktu.lt; vesta.navikaite@gmail.com

Išsilavinimas:

1995–2007	Vidurinis išsilavinimas Kauno „Nemuno“ vidurinėje mokykloje
2007–2011	Taikomosios chemijos bakalauro studijos Kauno technologijos universiteto Cheminės technologijos fakultete
2011–2013	Chemijos inžinerijos magistrantūros studijos Kauno technologijos universiteto Cheminės technologijos fakultete
2013–2017	Chemijos inžinerijos doktorantūros studijos Kauno technologijos universiteto Cheminės technologijos fakulteto Polimerų chemijos ir technologijos katedroje

UDK 547.458 + 547.973 + 547.913] (043.3)

SL344. 2017-10-27, 2,5 leidyb. apsk. l. Tiražas 50 egz.
Išleido Kauno technologijos universitetas, K. Donelaičio g. 73, 44249 Kaunas
Spausdino leidyklos „Technologija“ spaustuvė, Studentų g. 54, 51424 Kaunas