

KAUNAS UNIVERSITY OF TECHNOLOGY

DEIMANTĖ SIMANAVIČIŪTĖ

**IMMOBILIZATION OF PHENOLIC ACIDS AND NATURAL
EXTRACTS IN CATIONIC POLYSACCHARIDES**

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

2018, Kaunas

This doctoral dissertation was prepared at Kaunas University of Technology, Faculty of Chemical Technology, Department of Polymer Chemistry and Technology during the period of 2014–2018. The studies were supported by Research Council of Lithuania.

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

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**FENOLINIŲ RŪGŠČIŲ IR AUGALINIŲ EKSTRAKTŲ
IMOBILIZAVIMAS KATIJONINIUOSE POLISACHARIDUOSE**

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INTRODUCTION

Motivation of the research. Phenolic acids such as hydroxybenzenecarboxylic and hydroxycinnamic acids possess good antimicrobial and antioxidant properties, anti-inflammatory and anti-inflammatory effects, as well as effects on the cardiovascular system. However, these compounds are unstable, sensitive to heat, alkaline, oxidation and light, and therefore quickly lose their beneficial properties. The biological activity of phenolic acids could be preserved by the immobilization in polymeric carriers containing cationic groups, for example, in the microgranules of cross-linked cationic starch derivatives or chitosan powders. Both cationic starch derivatives and chitosan have positive charge groups in solution and could form ionic complexes with negatively charged low molecular weight compounds.

For the immobilization of phenolic acids, not only pure phenolic acids could be applied, but also natural extracts, because they contain a variety of phenolic compounds. For example, mushroom extracts have demonstrated antimicrobial, antioxidant and anti-inflammatory effects and could be used as ingredients of cosmetics [1]. The extracts from cherry and blackcurrant leaves have been applied as natural antimicrobial agents in meat products [2]. Murta fruit (*Ugni molinae* Turcz) extract has been encapsulated in cross-linked methyl cellulose and tested in active food packaging applications [3].

Nowadays, great attention is paid to natural additives and supplements with antioxidant properties. Also, bioactive compounds from natural extracts could be used in the production of edible films and active packaging. Such packaging would work by releasing bioactive compounds into various media and enriching them with natural antioxidant compounds.

The aim of the thesis. The aim of this research is the preservation of phenolic acids and natural extracts components antioxidant activity by their adsorption on cationic polysaccharides.

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

1. To select the suitable cationic polysaccharides for the immobilization of phenolic acids.
2. To investigate the interaction between cationic polysaccharides and phenolic acids or natural extracts by using the equilibrium adsorption method and applying various adsorption models.
3. To evaluate the antioxidant properties of phenolic acids and natural extracts introduced into complexes with cationic polysaccharides as well as to assess the release of phenolic acids and natural extracts from complexes into different media.

4. To evaluate the thermal stability of phenolic acids and natural extracts introduced into complexes with cationic polysaccharides.
5. To develop an active herbal packaging bag prototype.
6. To design a technological scheme for the production of herbal packaging material with a bioactive coating.

Novelty of the study. For the first time, it has been established that vanillic, caffeic, chlorogenic and rosmarinic acids as well as the chlorogenic acid isomers present in artichoke and green coffee bean extracts could form ionic complexes with cross-linked cationic starch or chitosan in water. The complexes are formed due to the electrostatic interaction between the carboxylic groups of phenolic acids and polysaccharide cationic groups.

The complexes of cationic starch derivatives or chitosan and vanillic, caffeic, chlorogenic and rosmarinic acids as well as the chlorogenic acid isomers included in natural extracts exhibit the prolonged antioxidant activity which is related with the gradual desorption of phenolic acids from microgranules or particles of obtained complexes.

Herbal tea packaging bags containing chitosan and green coffee bean extract complex exhibit an antioxidant effect and enriches the aqueous extracts of herbal teas with phenolic compounds as well as chlorogenic acid isomers.

The practical value of the work. The obtained chitosan and green coffee bean extract complex powder has been applied in the preparation of antioxidant herbal tea bag prototype. The tea bags with bioactive chitosan and green coffee bean extract coatings were used to package milk thistle fruits, Detoxset and motherwort herbs. The aqueous extracts of herbal teas packaged in the bags with bioactive coating have been enriched with an antioxidant chlorogenic acid isomers in comparison with the aqueous extracts of herbal teas packaged by using conventional bags. A technological scheme for the production of the herbal tea packaging material with a bioactive coating was designed.

Dissertation statements to be defended. During the adsorption of phenolic acids and components of natural extracts from aqueous solutions onto cationic polysaccharides, phenolic acids and natural extracts thermal stability is increased and antioxidant activity is preserved.

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 publication is published in other international databases, 1 article has been published in other peer-reviewed scientific publications, and 8 papers have been presented in the proceedings of scientific conferences. The two industrial tests were done in the company UAB “Švenčionių vaistažolės”.

Structure and content of the dissertation. The dissertation consists of an introduction, three chapters, conclusions, a list of references, a list of publications relevant to the subject of the dissertation and annex. The dissertation text covers 138 pages, it features 48 figures and 26 tables, 22 mathematical expressions, and the list of references includes 215 bibliographic sources.

Personal input of the author. The author modified natural starch, carried out adsorption of bioactive compounds onto biopolymers studies, analysed obtained results, examined antioxidant and thermal properties of the obtained complexes. The chromatographic analysis of natural extracts was carried out at the Lithuanian University of Health Sciences (LSMU) during the course of Researcher team project No. MIP-055/2015, financed by the Research Council of Lithuania. Scanning electron microscopy studies were carried out at the Institute of Materials Science of Kaunas University of Technology. The nuclear magnetic resonance spectroscopy investigations were performed at the Open Access Centre “Spectroversum” of Vilnius University.

1. RESEARCH METHODOLOGY

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

Preparation of cationic cross-linked starch derivatives. The molecular mass of anhydroglucopyranose units (AGP) was assumed as a mole of starch. Cationic cross-linked starch having tertiary amine groups (CTAS), was obtained by cross-linking reaction of potato starch with epichlorohydrin (EPCH) in aqueous reaction mixture containing NH_4OH at room temperature (20 ± 2 °C) for 24 h [4]. The molar ratio of AGP : EPCH : NH_4OH : NaOH : H_2O was 1 : 0.5 : 1 : 0.5 : 20.

Cationic cross-linked starch having quaternary ammonium groups (CCS) was prepared by two-step modification reaction [5]. In the first step starch was cross-linked with 0.1 mol/AGP of EPCH in the presence of NaOH at room temperature for 24 h, washed with cold water, dried and then cationized with 2,3-epoxypropyltrimethylammonium chloride (EPTMAC) in the presence of NaOH as catalyst at 45 °C for 24 h. The molar ratio of AGP : EPTMAC : NaOH : H_2O was 1 : 0.45 : 0.04 : 4.

After completion of the reaction, the obtained CTAS and CCS were washed with isopropanol-water (4:1 (v/v)) mixture until neutral reaction and dried at room temperature. The amount of introduced nitrogen was determined by the Kjeldahl method after purification by Soxhlet extraction with methanol for 16 h. The nitrogen content in CTAS and CCS was 1.5 % and 2.6 %, respectively.

Equilibrium adsorption studies. 0.1 g of CTAS, CCS or chitosan (ChS) were placed into an Erlenmeyer flask, and 100 mL of caffeic acid (CA), chlorogenic acid (CGA), rosmarinic acid (RA), artichoke extract (AE) or green coffee bean extract (GCBE) aqueous solution of a certain concentration were added. The flask was stoppered and shaken for 60 min at temperatures of 30 °C, 40 °C, 60 °C and fixed shaking intensity in a thermostatic bath with the temperature control of ± 1 °C (Mettmert GmbH, Germany). Then the mixture was filtered through a paper filter, and the residual concentration of phenolic acid or natural extract in the filtrate solution was estimated. The amount of the adsorbed phenolic acid or natural extract (q_e (g/g)) was calculated. The Langmuir [6], Freundlich [7], Dubinin-Radushkevich [8] adsorption models were used to describe the adsorption isotherms.

Investigation of phenolic acids or natural extracts release. 0.1 g of obtained complexes microgranules were placed into 50 mL of desorption solution and stirred at 300 rpm for 30 min at room temperature (20 ± 1 °C). Afterwards, the mixtures were filtered through a glass filter and the concentrations of released phenolic acids or natural extracts were determined. The distilled water, ethanol, 0.1 mol/L HCl solution and 0.1 mol/L acetate buffer solution of pH4.1, as well as 0.1mol/L phosphate buffer solution of pH6.8 were used as the desorption media.

Determination of antioxidant activity. The antioxidant activity of obtained complexes was evaluated by two methods, i.e. by using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid [9] or 2,2-diphenyl-1-picrylhydrazyl [10] reagents and evaluating free radical (ABTS⁺ or DPPH[•]) scavenging activity.

Chromatographic analysis. Analysis of the samples was carried out with Acquity H-class UPLC system (Waters, USA) equipped with Acquity BEH column (2.1×50mm, 1.7 μm). Injection volume was 1 μL. Xevo TQD MS/MS detector (Waters, USA) was used to obtain MS and MS/MS data. The verification of phenolic markers was carried using a developed UPLC-MRM method within parallel checks of retention (using 3 gradients), MS and UV spectra of analytical standards. Quantified compounds were detected using Acquity eLambda PDA detector. The quantification method was external standard calibration. The determination of chlorogenic, neochlorogenic and total phenolic compound concentration was made by using the equation of linear calibration of the chlorogenic standard solutions at 325 nm.

Preparation and analyses of bioactive coating prototype. 5% ChS/GCBE solution containing 0.1860 g/g of adsorbed GCBE was obtained by dissolving the appropriate amount of ChS/GCBE complex powders in 1% acetic acid solution. The tea bag filter paper "Dynapore" 121/2 band (Glatfelter, Germany) was coated with the prepared ChS/GCBE complex solution by using flat mesh template and left to dry at room temperature. Three different types of herbal teas at 2g each were placed into the filter paper with active coating by forming tea packages. Then 150 mL of boiling water was poured over each tea bag. The antioxidant activity of the aqueous extracts was evaluated by using modified 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS⁺) method. The released amount of total phenolic compounds and individual caffeoylquinic acid derivatives was estimated by chromatographic analysis.

2. RESULTS AND DISCUSSION

2.1 Synthesis of cross-linked cationic starches

During the synthesis of CCS quaternary ammonium groups are attached to the main backbone as the side chains (Fig. 2.1, a), and degree of substitution (DS) could be rightly calculated from the amount of introduced nitrogen. CCS used in this investigation was found to have 2.6% of introduced nitrogen and calculated DS was equal to 0.42. If during starch cross-linking the ammonium hydroxide is added to the reaction mixture the cross-links containing nitrogen could be formed both between three and two AGPs of starch (Fig. 2.1, b) [11, 12]. Furthermore, tertiary amino groups could be attached to the AGPs of starch without formation of cross-links. For this reason, in the case of CTAS, it was difficult to precisely calculate DS from the estimated amount of introduced nitrogen. In our calculation, we admitted that all types of tertiary amino groups could be formed in equal

portions, i.e. an average DS was found to be 0.44 when the amount of nitrogen introduced in CTAS was 1.5%.

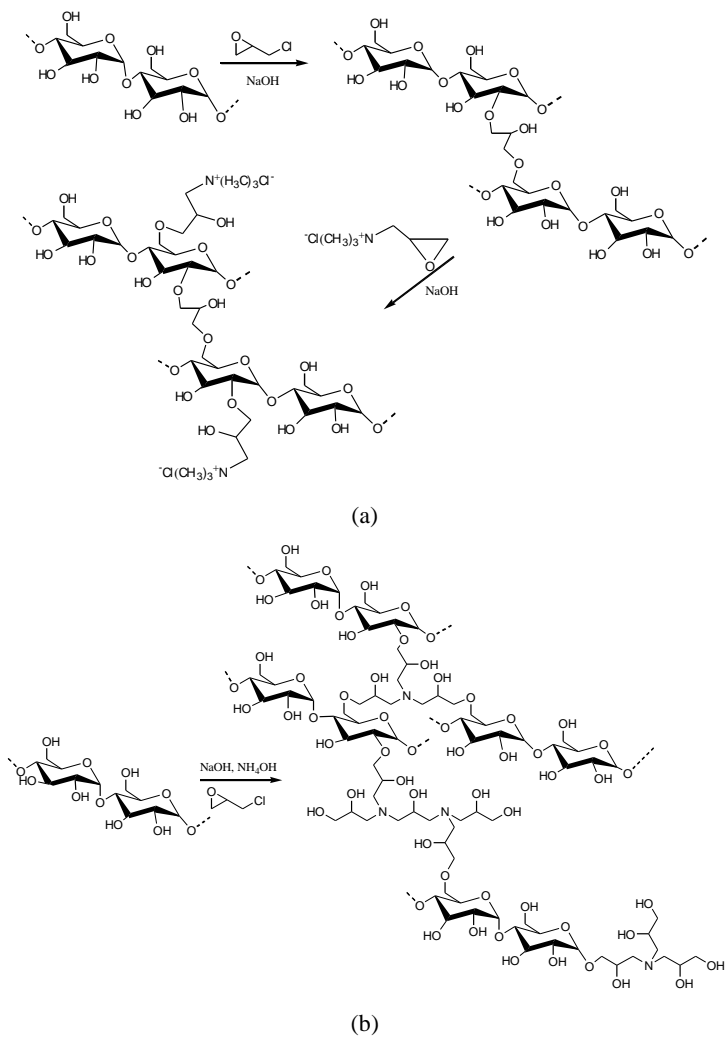


Fig. 2.1 Synthesis of CCS (a) and CTAS (b)

2.2 Equilibrium adsorption of chlorogenic acid onto cationic cross-linked starch derivatives having quaternary ammonium and tertiary amine groups

The differences in basicity of quaternary ammonium and tertiary amino groups would have an influence on the effectiveness of their interactions with anionic groups of CGA. In order to compare the sorption properties of CCS and CTAS, CGA adsorption onto CCS and CTAS microgranules was investigated by employing the equilibrium adsorption method, and the Langmuir, Freundlich, Dubinin–Radushkevich models were additionally used to describe the adsorption isotherms (see Table 2.1). The obtained adsorption isotherms of CGA onto CCS and CTAS at different temperatures are presented in Fig. 2.2.

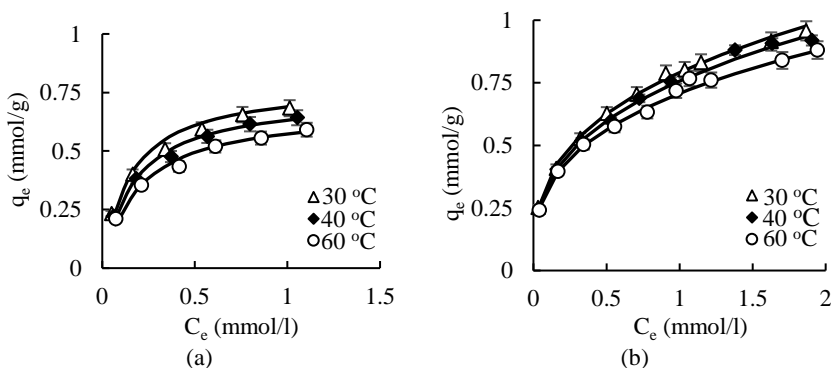


Fig. 2.2 Adsorption isotherms of CGA onto CTAS (a) and CCS (b) at different temperatures. Symbols represent experimental data and dashed lines represent fitted curves of the Langmuir adsorption model

Table 2.1 Parameters of three models for CGA adsorption onto CCS and CTAS at different temperatures

T (°C)	Langmuir model			Freundlich model		Dubinin– Radushkevich model	
	Q_L (mmol/g)	K_L (1/mol)	R^2	n_F	R^2	E_{DR} (kJ/mol)	R^2
Adsorption onto CTAS (DS = 0.44)							
30	0.79	6630	0.9962	3.3	0.9908	12.8	0.9950
40	0.74	5860	0.9969	3.2	0.9889	13.0	0.9928
60	0.69	5130	0.9954	3.1	0.9866	13.2	0.9888
Adsorption onto CCS (DS = 0.42)							
30	1.11	2770	0.9934	3.0	0.9971	11.6	0.9980
40	1.10	2650	0.9921	3.0	0.9963	11.7	0.9940
60	1.02	2640	0.9882	3.1	0.9966	12.0	0.9926

According to the Langmuir adsorption model CGA was adsorbed on the active centres of both modified starches, i.e. on quaternary ammonium groups of CCS or tertiary amine groups of CTAS. The driving forces of adsorption were electrostatic interactions between cationic groups of modified starches and carboxylic groups of CGA. The calculated values of Q_L for CGA adsorption on CCS were about 1.5 times higher than those for CGA adsorption on CTAS at all adsorption temperatures. The values of Freundlich constant n_F and Dubinin–Radushkevich adsorption energy E_{DR} indicated that CGA adsorption on both cationic starches was favourable and followed the ion-exchange mechanism. Furthermore, the values of n_F and E_{DR} were slightly higher in the case of CGA adsorption on CTAS and indicated that weakly basic tertiary amino groups were more preferable in CGA adsorption.

The thermodynamic characteristics of CGA adsorption onto CCS and CTAS were evaluated (Table 2.2). The negative values of ΔG indicated that adsorption of CGA both on CCS and CTAS was spontaneous. However, the values of ΔG in the case of CGA adsorption on CTAS were more negative and confirmed the higher affinity of CTAS to CGA. Moreover, the higher negative value of the change of enthalpy ΔH showed that adsorption of CGA onto CTAS was more exothermic process than CGA adsorption on CCS. The value of the change of entropy ΔS is the measure of changes in the system order during the adsorption process. In the case of CGA adsorption onto CTAS the more negative of ΔS showed that the order of systems increased in the great extent.

Table 2.2 Thermodynamic parameters of CGA adsorption onto CCS and CTAS

T ($^{\circ}C$)	K_c	ΔG (kJ/mol)	R^2	ΔH (kJ/mol)	ΔS (J/mol·K)	R^2
Adsorption onto CTAS (DS = 0.44)						
30	12.40	-6.4	0.9952			
40	9.46	-5.9	0.9915	-21.2	-49.0	0.9960
60	7.57	-5.4	0.9891			
Adsorption onto CCS (DS = 0.42)						
30	7.84	-5.2	0.9936			
40	7.06	-5.1	0.9768	-8.1	-9.6	0.9999
60	5.87	-4.9	0.9750			

2.3 Immobilization of hydroxycinnamic acids onto cationic cross-linked starch having quaternary ammonium groups

Equilibrium adsorption of CA, CGA and RA onto CCS

The differences in chemical structure determine the properties of phenolic acids, first of all, their antioxidant activity [13, 14], as well as their pK_a . According to literature [15, 16], the pK_a values of CA, CGA and RA are equal to 4.47, 3.58 and 2.92, respectively. The higher is the value of pK_a , the smaller is the extent of dissociation at a given pH value. The influence of phenolic acids acidity on their interactions with strong basic groups of CCS could be investigated by employing

the equilibrium adsorption method, and the Langmuir, Freundlich, Dubinin–Radushkevich models can be used to describe the adsorption isotherms. The obtained adsorption isotherms of CA, CGA and RA onto CCS at different temperatures are presented in Fig. 2.2 and Fig. 2.3.

According to the Langmuir adsorption model, phenolic acids were adsorbed on the active centres of CCS, i.e. on the quaternary ammonium groups. The driving forces of adsorption were interactions between these groups and carboxylic groups of acids. As can be seen from data in Table 2.1 and Table 2.3, the values of the Langmuir sorption capacity Q_L decreased with an increase of adsorption temperature, and at a constant temperature they were closely related to the dissociation constant of investigated acid. The higher was the value of pK_a , the lower was the value of Q_L , i.e. the amount of adsorbed acid decreased in the order which was contrary to the order of increasing value of pK_a . The calculated values of the Dubinin-Radushkevich mean free energy E_{DR} confirmed the ion-exchange mechanism. The values of the Freundlich constant n_F indicated the favourability of adsorption.

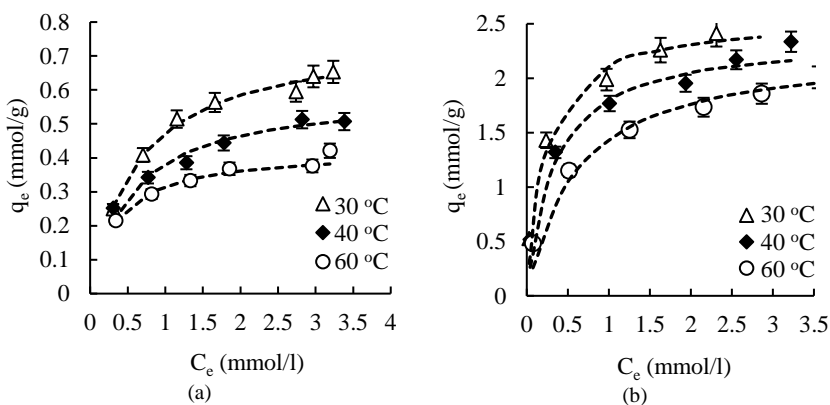


Fig. 2.3 Adsorption isotherms of CA (a) and RA (b) onto CCS at different temperatures. Symbols represent experimental data and dashed lines represent fitted curves of the Langmuir adsorption model

Table 2.3 Parameters of three models for phenolic acids adsorption onto CCS with DS=0.42 at different temperatures

T (°C)	q_e (mmol/g)	Langmuir model			R^2	Freundlich model		Dubinin–Radushkevich model	
		Q_L (mmol/g)	K_L (l/mol)	EF (mol/ekv)		n_F	R^2	E_{DR} (kJ/mol)	R^2
Adsorption of CA ($pK_a = 4.47$)									
30	0.65	0.76	1660	0.42	0.9957	2.55	0.9501	10.1	0.9698
40	0.51	0.59	1870	0.32	0.9927	3.15	0.9964	12.2	0.9925
60	0.42	0.42	2990	0.23	0.9987	3.32	0.9932	13.2	0.9986
Adsorption of RA ($pK_a = 2.92$)									
30	2.38	2.68	3240	1.46	0.9837	2.82	0.9989	12.2	0.9962
40	2.34	2.38	3080	1.30	0.9937	2.89	0.9926	12.6	0.9930
60	2.01	2.28	1700	1.25	0.9947	3.54	0.9957	13.4	0.9972

The thermodynamic characteristics of CA, CGA adsorption onto CCS have been evaluated (Table 2.2 and Table 2.4). The negative values of ΔG indicated that adsorption of CA, CGA and RA onto CCS was spontaneous. The absolute values of ΔG were closely related to the pK_a values of acids, i.e. to the ionization of acids in adsorption solution. The negative values of the change of enthalpy ΔH showed that adsorption of acids onto CCS was exothermic process. In the case of CA adsorption onto CCS the positive value of ΔS showed that the order of systems decreased. In contrary, during the CGA and RA adsorption onto CCS, the order of systems increased, particularly, in the case of RA adsorption.

Table 2.4 Thermodynamic parameters of phenolic acids adsorption onto CCS

T (°C)	ΔG^o (kJ/mol)	R^2	ΔH (kJ/mol)	ΔS (J/mol·K)	R^2
Adsorption of CA ($pK_a = 4.47$)					
30	-3.06	0.9624			
40	-3.19	0.9938	-1.57	5.09	0.9714
60	-3.26	0.9987			
Adsorption of RA ($pK_a = 2.92$)					
30	-9.44	0.9581			
40	-8.88	0.9904	-30.9	-70.6	0.9970
60	-7.34	0.9978			

Equilibrium adsorption of CA, CGA and RA from their solutions containing NaOH

The equilibrium adsorption isotherms of CA, CGA and RA from the solutions containing equimolar amounts of NaOH at temperature of 40 °C and the calculated parameters of the Langmuir, Freundlich and Dubinin-Radushkevich adsorption models as well as ΔG^o values along with the values of R^2 are given in Fig. 2.4 and Table 2.5. The addition of NaOH to acids solutions increased the ionization of their carboxylic groups, and, consequently, the intensity of electrostatic interaction between CCS quaternary ammonium groups and ionized carboxylic groups of phenolic acids, as well as, the favourability of adsorption.

The obtained data confirmed that the sorption properties of investigated phenolic acids depended on the degree of carboxylic groups' ionization. In the case of the adsorption of ionized acids onto CCS, the parameters of adsorption models and the affinity are mostly related to the structure of the acids as opposed to adsorption from initial acids solutions where pK_a of acids was a keynote parameter.

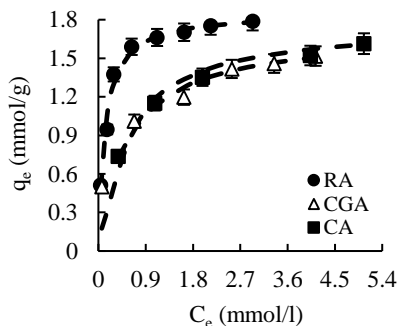


Fig. 2.4 Adsorption isotherms of CA, CGA and RA onto CCS from the solutions with the equimolar amounts of NaOH at temperature of 40 °C. Symbols represent experimental data and dashed lines represent fitted curves of the Langmuir adsorption model

Table 2.5 Parameters of adsorption models and ΔG value for the adsorption of ionized phenolic acids onto CCS at temperature of 40 °C

Parameter	Type of phenolic acid		
	CA	CGA	RA
pH of adsorption solution	6.7	6.8	6.5
q_e (mmol/g)	1.61	1.52	1.79
Langmuir model			
Q_L (mmol/g)	1.78	1.71	1.86
K_L (1/mol)	1850	1780	7960
EF (mol/ekv)	0.97	0.94	1.02
R^2	0.9944	0.9927	0.9996
Freundlich model			
n_F	4.76	3.72	9.14
R^2	0.9897	0.9942	0.9474
Dubinin-Radushkevich model			
E_{DR} (kJ/mol)	14.0	13.8	20.7
R^2	0.9933	0.9970	0.9548
ΔG (kJ/mol)	-8.14	-8.66	-21.4
R^2	0.9892	0.9869	0.9508

Release of CA, CGA and RA from CCS/CA, CCS/CGA and CCS/RA complexes

CA, CGA and RA were released from CCS/CA, CCS/CGA and CCS/RA having 0.028 g, 0.085 g and 0.114 g of adsorbed CA, CGA and RA per 1 g of CCS, respectively, into the different media i.e. distilled water, ethanol, 0.1 mol/L HCl solution (a model of gastric medium), 0.1 mol/L acetate buffer pH=4.1 and 0.1 mol/L phosphate buffer pH=6.8 (a model of the intestinal medium). The obtained results show that the amount of released phenolic acids depends on the desorption medium. After 30 min, a large part of phenolic acids was released into the 0.1 mol/L HCl solution. Moreover, 41–64% and 63–82% of phenolic acids from the initial amount in the complexes were released into 0.1 mol/L acetate buffer pH=4.1 and 0.1 mol/L phosphate buffer pH=6.8, respectively.

Table 2.6 Influence of the desorption solution on the amount of CA, CGA and RA released from CCS/CA, CCS/CGA and CCS/RA

Desorption solution	Amount of released phenolic acid, (mg/L) / (%*)		
	CA	CGA	RA
0.1 mol/L HCl solution	44 / 80.8	134 / 84.9	164 / 79.7
0.1 mol/L acetate buffer (pH=4.1)	33 / 60.6	102 / 64.5	85 / 41.4
0.1 mol/L phosphate buffer (pH=6.8)	35 / 63.4	123 / 82.3	143 / 69.8
Distilled water	2 / 2.8	2 / 1.1	1 / 0.4
Ethanol	0 / 0	0 / 0	0 / 0

* $C_{\max \text{ CA}} = 55 \text{ mg/l}$, $C_{\max \text{ CGA}} = 157 \text{ mg/l}$, $C_{\max \text{ RA}} = 205 \text{ mg/l}$

Antioxidant activity of CA, CGA, RA adsorbed onto CCS

The antioxidant activity of CCS/CA, CCS/CGA and CCS/RA was evaluated by the DPPH' method, and expressed as time dependent DPPH' radical scavenging activity (%). The antioxidant activity of CA, CGA and RA solutions also was determined. The obtained data are presented in Fig. 2.5. Immobilization of phenolic acids onto CCS prevented the rapid loss of antioxidant activity and secured the prolonged radical scavenging activity which could be related to gradual release of CA, CGA and RA from CCS microgranules.

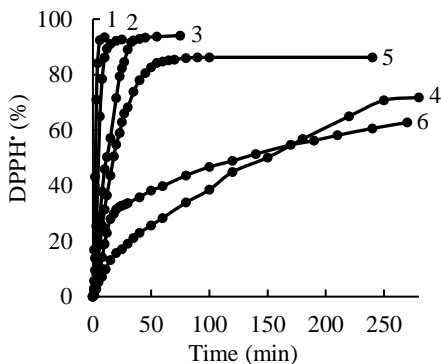


Fig. 2.5 DPPH[•] radical scavenging activity depending on time: 1 - RA; 2 - CCS/RA; 3 - CA; 4 - CCS/CA; 5 - CGA; 6 - CCS/CGA. Concentration of phenolic acids alone or added with CCS/CA, CCS/CGA or CCS/RA in DPPH solution was 0.013 g/L, 0.038 g/L, 0.051 g/L, respectively

2.4 Immobilization of artichoke and green coffee bean extracts onto cationic cross-linked starch having quaternary ammonium groups

Equilibrium adsorption of AE and GCBE onto CCS

AE and GCBE contained 8.1% and 74.6% of phenolic compounds, respectively. CGA was predominant compound in the extracts and comprised 2.3% and 49.5% from the total amount of phenolics, respectively. CCS microgranules were used as the matrix for the immobilization of CGA as well as other phenolic substances from the aqueous solutions of AE and GCBE. The amount of adsorbed AE and GCBE was determined by evaluating the differences of AE or GCBE concentrations in the adsorption solutions before and after the adsorption by using UV spectroscopy. The obtained adsorption isotherms of AE and GCBE onto CCS at different temperatures are presented in Fig. 2.6. The Langmuir adsorption model was used to describe the adsorption results (Table 2.7).

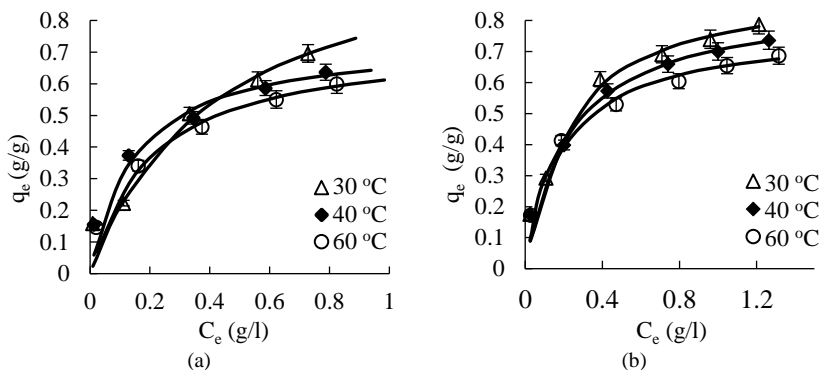


Fig. 2.6 Adsorption isotherms of AE (a) and GCBE (b) onto CCS at different temperatures. Symbols represent experimental data and lines represent fitted curves of the Langmuir adsorption model

Table 2.7 Parameters of Langmuir model for AE and GCBE adsorption onto CCS at different temperatures

T ($^{\circ}\text{C}$)	Q_L (g/g)	K_L (l/mol)	R^2
Adsorption of AE			
30	1.10	2.35	0.9916
40	0.75	6.69	0.9938
60	0.74	4.93	0.9969
Adsorption of GCBE			
30	0.93	4.45	0.9983
40	0.87	4.31	0.9996
60	0.78	5.06	0.9963

According to the Langmuir adsorption model anionic substances present in the extracts are adsorbed onto the quaternary ammonium groups of CCS. To determine the amount of adsorbed chlorogenic acid isomers, the solutions after adsorption experiments performed at 30 °C temperature were analyzed by using UPLC-UV-MS/MS method. The chromatographic analysis results revealed that only 8% and 17% of the total amount of adsorbed AE and GCBE, respectively, is characteristic to chlorogenic acid isomers. This clearly indicates that other phenolic compounds are also adsorbed on CCS and competing with CGA during the adsorption.

UPLC analysis of adsorption solutions

The UPLC-UV method was chosen to get more information about the composition of AE and GCBE solutions before and after adsorption on CCS. It was found that the initial artichoke extract contains 1.5% of chlorogenic acid, 0.6% luteolin 7-O-glucoside, 0.5% of cryptochlorogenic acid, 0.3% of

neochlorogenic acid, and 0.2% of cynarine. During the interaction of the aqueous solution of AE with CCS microgranules 80.4% of chlorogenic acid 76.7% of neochlorogenic acid, 82.0% of cryptochlorogenic acid and 82.3% of cynarine and 38.4% of luteolin 7-O-glucoside were adsorbed. When testing GCBE adsorption solutions, only the predominant chlorogenic, neochlorogenic and cryptochlorogenic acids were assessed because it was found that these acids embrace of 32.5%, 6.6% and 10.4% of GCBE content, respectively. It was found that 84.9% of chlorogenic acid, 83.1% of cryptochlorogenic acid and 81.4% of neochlorogenic acid were adsorbed onto the microgranules of CCS in the aqueous medium.

Release of AE and GCBE form CCS/AE and CCS/GCBE complexes

Release of phenolic compounds from CCS was followed by measuring UV absorbance intensity of desorption solutions at 325 nm. Different desorption media were used: distilled water, ethanol, 0.1 mol/L HCl solution (a model of gastric medium), 0.1 mol/L acetate buffer pH=4.1 and 0.1 mol/L phosphate buffer pH=6.8 (a model of the intestinal medium). After 30 minutes, the largest part of AE and GCBE phenolics from the complexes was released into the 0.1 mol/L HCl solution as well as into the phosphate buffer. The release of phenolics from the complexes also proceeded into the acetate buffer, and was insignificant or didn't proceed in distilled water and ethanol.

Table 2.8 Influence of desorption medium on the amount of AE and GCBE phenolics released from CCS/AE and CCS/GCBE

Desorption medium	Amount of released phenolic acid, (mg/L) / %	
	AE	GCBE
0.1 mol/L HCl solution	249 / 84.8	214 / 73.8
0.1 mol/L acetate buffer (pH=4.1)	121 / 41.2	130 / 45.0
0.1 mol/L phosphate buffer (pH=6.8)	191 / 65.0	171 / 59.1
Distilled water	3 / 1.0	0

*from $C_{max} = 290$ mg/L

Antioxidant activity of AE and GCBE adsorbed onto CCS

The antioxidant activity of CCS/AE and CCS/GCBE was evaluated by ABTS⁺ method, and expressed as time dependent ABTS⁺ radical scavenging activity (%). The antioxidant activity of AE and GCBE solutions also was determined. The amount of natural extracts in the individual solutions and in those containing CCS/AE and CCS/GCBE complexes microgranules was the same. The obtained data are presented in Fig. 2.7. The prevalence of phenolic acids in microgranules of CCS/AE and CCS/GCBE complexes predetermined their high antioxidant activity and prevented rapid loss of antioxidant properties.

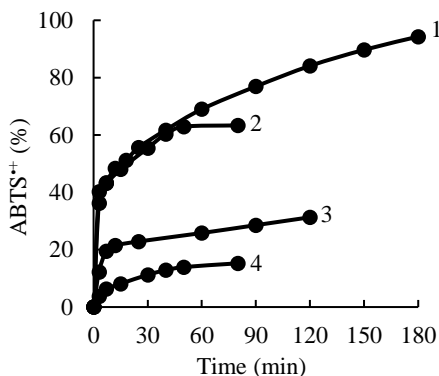


Fig. 2.7 ABTS^{•+} radical scavenging activity depending on time: 1 – CCS/GCBE; 2 – GCBE; 3 – CCS/AE; 4 – AE. Concentration of natural extracts alone or added with CCS/AE or CCS/GCBE in ABTS^{•+} solution was 0.0154 g/L

2.5 Thermal stability of complexes of caffeic, chlorogenic, rosmarinic acids or artichoke and green coffee bean extracts and cationic cross-linked starch having quaternary ammonium groups

The thermograms of complexes were recorded under a nitrogen atmosphere. The characteristic degradation temperatures and mass losses are given in Table 2.9. As can be seen, the tested complexes have a higher initial degradation temperature in comparison with the thermal degradation of phenolic acids or natural extracts itself. The immobilization of CA, CGA, RA, AE, GCBE onto CCS increased the thermal stability of these active compounds by 31–99 °C.

Table 2.9 Characteristic data for the thermal degradation of CA, CGA, RA, AE, GCBE, CCS and their complexes

Sample name	Initial thermal degradation temperature (°C)	Mass loss (%)
CA	219	83
CGA	206	71
RA	245	55
AE	186	56
GCBE	177	68
CCS/CA	279	79
CCS/CGA	277	79
CCS/RA	276	78
CCS/AE	277	77
CCS/GCBE	276	79
CCS	273	84

2.6 Immobilization of chlorogenic acid, artichoke and green coffee bean extracts onto chitosan

Equilibrium adsorption of CGA, AE and GCBE onto ChS

The CGA, AE and adsorption onto ChS was investigated by employing the equilibrium adsorption method, and the Langmuir adsorption model was used to describe the adsorption isotherms. The obtained adsorption isotherms of CGA, AE and GCBE onto ChS at different temperatures are presented in Fig. 2.8.

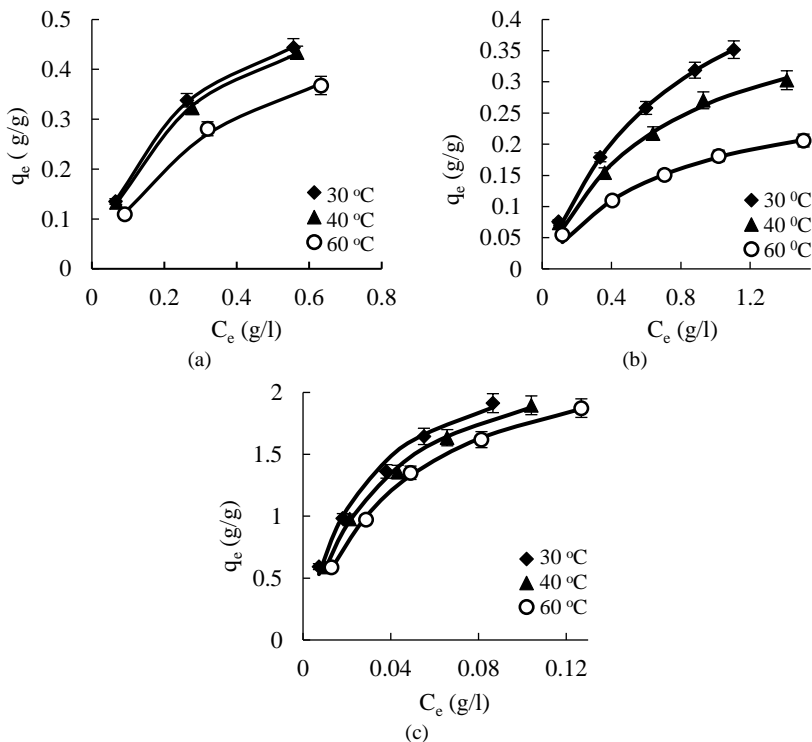


Fig. 2.8 Adsorption isotherms of CGA (a), AE (b) and GCBE (c) onto ChS at different temperatures. Symbols represent experimental data and lines represent fitted curves of the Langmuir adsorption model

Table 2.10 Parameters of Langmuir model for CGA, AE and GCBE adsorption onto ChS at different temperatures

<i>T</i> (°C)	<i>Q_L</i> (g/g)	<i>K_L</i> (l/mol)	<i>R</i> ²
Adsorption of CGA			
30	0.63	4.24	0.9995
40	0.62	3.98	0.9994
60	0.60	2.54	0.9924
Adsorption of AE			
30	0.61	1.24	0.9998
40	0.46	1.45	0.9939
60	0.31	1.40	0.9991
Adsorption of GCBE			
30	2.45	38.20	0.9914
40	2.49	29.52	0.9984
60	2.51	22.89	0.9990

According to the Langmuir adsorption model CGA and phenolic components of the extracts were adsorbed onto the primary amine groups of ChS (see Table 2.10). To determine the amount of adsorbed chlorogenic acid isomers (chlorogenic, neochlorogenic and cryptochlorogenic acids) from AE and GCBE, the solutions obtained after adsorption experiments at 30 °C temperature were analyzed by using UPLC-UV-MS/MS method. The chromatographic analysis results showed that only 1.97% and 6.24% of the total amount of adsorbed AE and GCBE, respectively, can be related to chlorogenic acid isomers adsorption, and indicated that other compounds were competing with those phenolic acids during the adsorption onto CCS.

UPLC analysis of adsorption solutions

The UPLC-UV method was chosen to obtain more information about the composition of AE and GCBE solutions after adsorption onto ChS. It was determined during interaction of the AE with ChS powder 53.1% of chlorogenic acid, 52.1% of neochlorogenic acid, 67.4% of cryptochlorogenic acid were adsorbed. In the case of GCBE, it was found that 68.0% of chlorogenic acid, 74.3% of cryptochlorogenic acid and 76.4% of neochlorogenic acid were adsorbed onto the powder of ChS from the aqueous medium.

Release of CGA, AE and GCBE from ChS/CGA, ChS/AE and ChS/GCBE complexes

The release of CGA and phenolic compounds from ChS/CGA, ChS/AE and ChS/GCBE complexes having 0.186 g, 0.116 g and 0.180 g of adsorbed CGA, AE and GCBE per 1 g of ChS, respectively, into the different media was assessed. The following desorption media were used: distilled water, ethanol, 0.1 mol/L HCl solution (a model of gastric medium), 0.1 mol/L acetate buffer pH=4.1 and 0.1

mol/L phosphate buffer pH=6.8 (a model of the intestinal medium). The released amount was identified by assessing UV absorbance intensity at 325 nm.

After 30 minutes, ChS/CGA, ChS/AE and ChS/GCBE complex powders were dissolved in 0.1 mol/L HCl solution. ChS/AE complex powder also was dissolved in 0.1 mol/L acetate buffer pH=4.1. Meanwhile, 82.7% and 92.6% of CGA and GCBE phenolics, respectively, were released from the complexes into 0.1 mol/L acetate buffer. Moreover, 88%, 50.2% and 42.5% of CGA, AE and GCBE, respectively, were released from the complexes into 0.1 mol/L phosphate buffer pH=6.8. The release of phenolics from the complexes was insignificant or didn't proceed into distilled water and ethanol.

Table 2.11 Influence of desorption medium on the amount of CGA, AE and GCBE phenolics released from ChS/CGA, ChS/AE and ChS/GCBE

Desorption medium	Released amount, (mg/L) / %		
	CGA	AE	GCBE
0.1 mol/L HCl solution	Dissolve	Dissolve	Dissolve
0.1 mol/L acetate buffer (pH=4.1)	259 / 82.7	Dissolve	283 / 92.6
0.1 mol/L phosphate buffer (pH=6.8)	275 / 88.0	104 / 50.2	129 / 42.5
Distilled water	14 / 4.5	82 / 6.4	13 / 4.2

*from $C_{max\ CGA} = 313\ mg/L$, $C_{max\ AE} = 208\ mg/L$, $C_{max\ GCBE} = 305\ mg/L$

Antioxidant activity of CGA, AE and GCBE adsorbed onto ChS

The antioxidant activity of ChS/CGA, ChS/AE and ChS/GCBE was evaluated by the ABTS⁺ method, and expressed as time dependent ABTS⁺ radical scavenging activity (%). The antioxidant activity of CGA, AE and GCBE solutions also was determined. The amount of CGA and natural extracts in the individual solution and those containing ChS/CGA, ChS/AE and ChS/GCBE complexes microgranules was the same. The obtained data are presented in Fig. 2.9. CGA, AE and GCBE immobilization onto ChS prevented the rapid loss of antioxidant properties and determined higher antioxidant activity of obtained complexes.

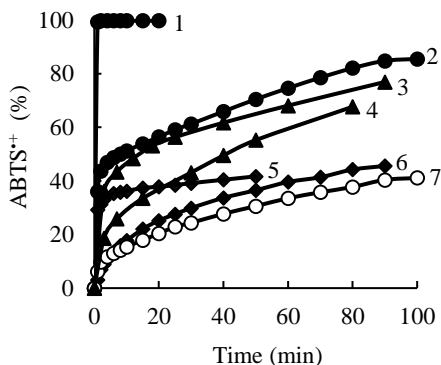


Fig. 2.9 ABTS^{•+} radical scavenging activity depending on time: 1 – CGA; 2 – ChS/CGA; 3 – GCBE; 4 – ChS/GCBE; 5 – AE; 6 – ChS/AE; 7 – ChS. Concentration of CGA and natural extracts alone or added with ChS/CGA, ChS/AE or ChS/GCBE in ABTS^{•+} solution was 0.0038 g/L

Thermal stability of CGA, AE, GCBE and ChS complexes

The thermograms of complexes were recorded under nitrogen atmosphere. The characteristic degradation temperatures and mass losses are given in Table 2.12. As could be seen, the immobilization of CGA and natural extracts onto ChS increased the thermal stability of these active compounds by 67–100 °C.

Table 2.12 Characteristic parameters for the thermal degradation of CA, CGA, RA, AE, GCBE, CCS and their complexes

Sample name	Initial thermal degradation temperature (°C)	Mass loss (%)
CGA	209	71
AE	184	56
GCBE	177	68
ChS/CGA	276	60
ChS/AE	284	60
ChS/GCBE	276	60
ChS	282	63

2.7 Bioactive coating prototype

ChS/GCBE complex powder containing 0.1860 g of adsorbed GCBE per 1 g ChS was used to prepare bioactive coating on herbal tea packaging bag (see Fig. 2.10). The chromatographic analysis showed that the amount of total content of phenolic compounds and individual caffeoylquinic acid derivatives (chlorogenic, neochlorogenic and cryptochlorogenic acid) was significantly increased i.e. by 0.4 – 47.14 times in water when using tea packaging with a bioactive coating. Aqueous

extracts of herbal tea packages with a bioactive coating after 5 minutes also showed 0.5–33% higher antioxidant activity.

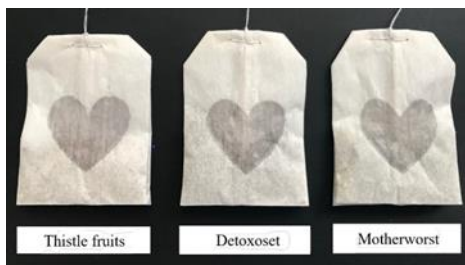


Fig 2.10 The herbal tea packages with biologically active coating

A technological scheme for the production of ChS/GCBE complex powder and filter paper band with biologically active coating was designed (Fig 2.11). During the first stage, the ChS/GCBE complex powder is obtained. In the second stage, the tea filter paper band is coated with the prepared ChS/GCBE complex solution.

ChS and GCBE powders are stored in the tanks 1 and then dosed with weight dispensers 2. A certain amount of ChS (*B*) is supplied to the reactor 9. Meanwhile, the certain amount of GCBE (*A*) is placed into the dissolver 5, in which the required amount of water is added (*C*). Then, the obtained GCBE solution is dosed by using a volume dispenser 6 and transported by a membrane pump 7 to a periodic reactor 9, wherein the GCBE solution is adsorbed onto ChS powder at a temperature of 20–25 °C. The duration of the adsorption process is 60 minutes. After adsorption, the aqueous suspension of ChS/GCBE is transported to a drum vacuum filter 10 by a membrane pump 7, in which the ChS/GCBE complex powder is separated from the GCBE solution. The removed filtrate (*D*) is transported to the drain by the centrifugal pump 13, while the ChS/GCBE powder by a screw conveyor 11 is supplied to the rotary dryer 12, where it is dried at 80–90 °C. The obtained ChS/GCBE complex powder (*E*) can be stored or further used for the formation of bioactive coating.

In the second stage, the ChS/GCBE coating is formed on the tea filter paper band. The ChS/GCBE complex powder in the dissolver 14 is dissolved in a 1% aqueous acetic acid solution. The obtained solution by using the membrane pump 7 is delivered to a flexography printing machine 17. In the device 15 printing forms are produced. The filter paper rolls are stored in the storage 16. The ChS/GCBE solution by using printing forms is applied onto the filter paper band, which then is transported to a convection dryer 19 by a transport shaft 18. Thereafter, the filter paper band with bioactive coating by a transport shaft 18 is supplied to the cutting device 20. The rolls of bioactive packing material are transported to the production warehouse.

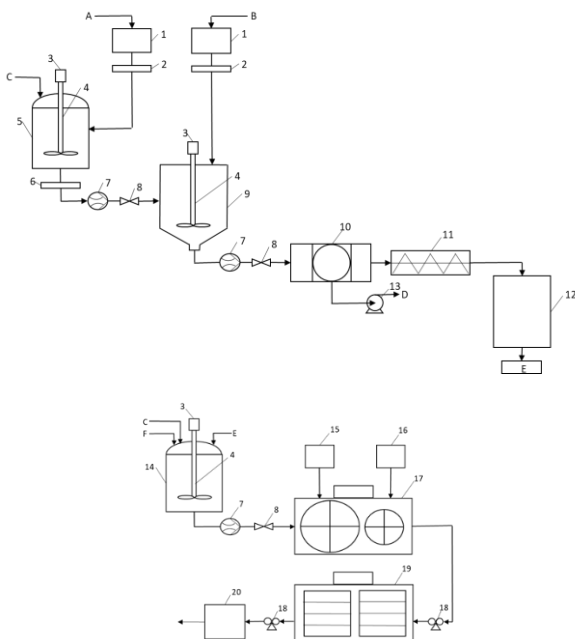


Fig 2.11 A technological scheme for the production of ChS/GCBE complex powder and filter paper band with biologically active coating: 1 – tank; 2 – weight dispenser; 3 – motor; 4 – mixer; 5 - dissolver; 6 – volume dispenser; 7 – membrane pump; 8 – valve; 9 – reactor; 10 – drum vacuum filter; 11 – screw conveyor; 12 – rotary dryer; 13 – centrifugal pump; 14 – dissolver; 15 – production of printing forms; 16 – storage of filter paper; 17 – flexography printing machine; 18 – transport shaft; 19 – convection dryer; 20 – cutting device. *A* – GCBE, *B* – ChS, *C* – water, *D* – filtrate; *E* – ChS/GCBE complex powder, *F* – acetic acid

CONCLUSIONS

1. Ionic complexes were formed in water between chlorogenic acid and cross-linked cationic starches having quaternary ammonium or tertiary amine groups due to electrostatic interactions. The amount of chlorogenic acid incorporated into the complex depended on the nature of the cationic groups. The amount of phenolic acid adsorbed onto microgranules was higher in the case of the use cross-linked cationic starch having quaternary ammonium groups.

2. The complexes of vanillic, caffeic, chlorogenic, rosmarinic acids and cross-linked cationic starch have been obtained by adsorption of phenolic acids from their aqueous solutions onto cross-linked cationic starch microgranules at equilibrium adsorption conditions:

2.1 The Langmuir adsorption model confirmed electrostatic interaction between the carboxylic groups of phenolic acids and the quaternary ammonium groups of cross-linked cationic starch. The values of the Freundlich constant n_F and the Dubinin–Radushkevich adsorption energy E_{DR} indicated that phenolic acids adsorption onto cross-linked cationic starch having quaternary ammonium groups was favourable and phenolic acids were adsorbed due to ion-exchange mechanism.

2.2 The sorption properties of phenolic acids depended on the degree of ionization of carboxylic groups. The amount of adsorbed phenolic acids increased with the decrease of pK_a value, i.e. in the following order: caffeic acid < chlorogenic acid < rosmarinic acid.

3. The complexes of phenolic compounds present in artichoke and green coffee bean extracts and cross-linked cationic starch or chitosan have been obtained by adsorption of natural extracts components from aqueous solutions onto cross-linked cationic starch microgranules or chitosan particles at equilibrium adsorption conditions:

3.1 The Langmuir adsorption model confirmed that chlorogenic acid isomers present in natural extracts were adsorbed onto quaternary ammonium groups of cross-linked cationic starch or primary amine groups of chitosan.

3.2 It was proved by ultra-performance liquid chromatography analysis, not only chlorogenic acid isomers were adsorbed from natural extracts aqueous solutions, but also other phenolic compounds, because artichoke and green coffee bean extracts contain 8.1 % and 74.6 % of phenolic compounds respectively, among which chlorogenic acid isomers comprise only 2 % and 49.5 % from the total amount of phenolics respectively.

4. Vanillic, caffeic, chlorogenic, rosmarinic acids as well as chlorogenic acid isomers and other anionic phenolic compounds present in artichoke and green coffee bean extracts can be gradually released from the obtained complexes into different media, including 0.1 M hydrochloric acid (a model of gastric medium)

and pH 6.8 phosphate buffer (a model of the intestinal medium), therefore complexes exhibited the prolonged antioxidant activity.

5. The immobilization of phenolic acids and natural extracts components onto cationic starch microgranules and chitosan powders increased their thermal stability by 31 – 99 °C and 67 – 100 °C, respectively.

6. The developed herbal tea packaging bag prototype with chitosan and green coffee bean extract coating has exhibited antioxidant properties:

6.1 Ultra-performance liquid chromatography analysis results indicated that aqueous extracts of milk thistle fruits, Detoxoset and motherwort herbs kept in packaging bags with bioactive coating after 5 min were enriched with total phenolic compounds by 17.3–81.5% and with individual chlorogenic acid isomers i.e. neochlorogenic acid by 1.5–8.1 times, chlorogenic acid by 1.9–47.1 times and cryptochlorogenic acid by 0.8–28.5 times.

6.2 The antioxidant activity of aqueous extracts of herbal teas packaged in the bags with bioactive coating after 5 min was higher by 0.5–33.2% compared to those obtained by using packaging bags without the coating.

6.3 A technological scheme for the production of chitosan and green coffee bean extract complex powder and herbal tea packaging material with a bioactive coating was designed.

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

1. **Simanavičiūtė, Deimantė**; Klimavičiūtė, Rima; Rutkaitė, Ramunė. Equilibrium adsorption of caffeic, chlorogenic and rosmarinic acids on cationic cross-linked starch with quaternary ammonium groups // *International Journal of Biological Macromolecules*. Amsterdam : Elsevier. ISSN 0141-8130. 2017, vol. 95, p. 788-795. [IF(E): 3,671 (2016)]
2. **Simanavičiūtė, Deimantė**; Liudvinavičiūtė, Dovilė; Klimavičiūtė, Rima; Rutkaitė, Ramunė. Cross-linked cationic starch derivatives for immobilization of chlorogenic acid // *European Polymer Journal*. Oxford : Elsevier. ISSN 0014- 3057. 2017, vol. 93, p. 833-842. [IF(E): 3,531 (2016)]

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2. **Simanavičiūtė, Deimantė**; Liudvinavičiūtė, Dovilė; Klimavičiūtė, Rima; Rutkaitė, Ramunė. Cationic starch derivatives for immobilization of bioactive phenolic acids // *BiPoCo 2016 : 3rd international conference "Bio-Based Polymers and Composites, August 28 - September 1, 2016, Szeged, Hungary : abstract book*. [Budapest] : [BiPoCo]. 2016, p. 222-223.

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REZIUMĖ

ĮVADAS

Temos aktualumas

Fenolinės hidroksibenzenkarboksirūgštys ir hidroksicinamono rūgštys pasižymi geromis antimikrobinėmis ir antioksidacinėmis savybėmis, antivėžiniu ir uždegimą slopinančiu poveikiu bei teigiamai veikia širdies ir kraujagyslių sistemą. Tačiau šie junginiai yra nestabilūs, jautrūs karščiui, šarmams, oksidacijai ir šviesai, todėl greitai praranda naudingąsias savybes. Biologiškai aktyvioms savybėms išsaugoti fenolinės rūgštys galėtų būti imobilizuotos katijoninių grupių turinčiuose polimeriniuose nešikliuose, pavyzdžiui, tinklinio katijoninio krakmolo darinių mikrogranulėse ar chitozono milteliuose. Tiek katijoninio krakmolo dariniai, tiek chitozanas tirpale turi teigiamą krūvį ir gali sudaryti joninius kompleksus su mažamolekuliais, neigiamą krūvį turinčiais junginiais.

Fenolinių rūgščių imobilizavimui galima naudoti ne tik grynąsias rūgštis, bet ir augalinius ekstraktus, nes juose gausu įvairių fenolinių junginių. Grybų ekstraktai, kaip fenolinių junginių šaltiniai, pasižymi antimikrobiniais, antioksidaciniais ir priešuždegiminiais poveikiais ir gali būti naudojami kaip kosmetikos priedai [1]. Vyšnių ir juodųjų serbentų lapų ekstraktai naudojami kaip natūralūs antimikrobiniai priedai mėsos produktuose [2]. Murta vaisiaus (lot. *Ugni molinae Turcz*) ekstraktas, įkapsuliuotas tinklintoje metilceliuliozėje, naudojamas aktyvioms maisto pakuotėms gaminti [3].

Šiuo metu didelis dėmesys skiriamas natūraliems maisto priedams ir papildams, kurie pasižymi antioksidacinėmis savybėmis. Augaliniuose ekstraktuose esančios bioaktyviosios medžiagos galėtų būti panaudojamos valgomų plėvelių ir veikliųjų pakuočių gamyboje. Tokios pakuotės veiktų atpalaiduodamos bioaktyviuosius junginius į įvairias terpes, taip jas praturtindamos antioksidacinėmis savybėmis, pasižyminčiomis gamtinėmis medžiagomis.

Darbo tikslas

Šio darbo tikslas – išsaugoti fenolinių rūgščių ir augalinių ekstraktų komponentų antioksidacinį veiklumą adsorbavus juos katijoniniais polisacharidais.

Darbo tikslui pasiekti suformuluoti šie **uždaviniai**:

1. parinkti tinkamus katijoninius polisacharidus fenolinių rūgščių imobilizavimui;
2. ištirti sąveiką tarp katijoninių polisacharidų ir fenolinių rūgščių bei augalinių ekstraktų pusiausvyrosios adsorbcijos sąlygomis, jai aprašyti taikyti adsorbcijos modelius;

3. įvertinti katijoninių polisacharidų ir fenolinių rūgščių bei augalinių ekstraktų kompleksų antioksidacines savybes ir atpalaidavimą įvairiose terpėse;
4. įvertinti katijoninių polisacharidų ir fenolinių rūgščių bei augalinių ekstraktų kompleksų terminį stabilumą;
5. sukurti veikliosios vaistažolių talpyklos prototipą;
6. pasiūlyti vaistažolių pakavimo medžiagos su bioaktyviaja danga gamybos technologinę schemą.

Mokslinis darbo naujumas

Pirmą kartą nustatyta, kad vanilino, kavos, chlorogeno ir rozmarino rūgštys bei vandeniniuose artiškų ir žaliųjų kavos pupelių ekstraktuose esantys chlorogeno rūgšties izomerai gali sudaryti joninius kompleksus su tinkliniu katijoniniu krakmolu ar chitozanu. Kompleksai susidaro dėl elektrostatinės sąveikos tarp fenolinių rūgščių karboksigrupių ir polisacharidų katijoninių grupių.

Vanilino, kavos, chlorogeno ir rozmarino rūgščių bei augaliniuose ekstraktuose esančių chlorogeno rūgšties izomerų kompleksai su tinkliniu katijoniniu krakmolu ar chitozanu pasižymi pailginto veikimo antioksidaciniu poveikiu, kuris siejamas su fenolinių rūgščių laipsnišku atpalaidavimu iš sudarytų kompleksų mikrogranulių ir dalelių.

Vaistažolių talpykla su chitozано ir žaliųjų kavos pupelių ekstrakto komplekso danga pasižymi antioksidaciniu poveikiu ir praturtina vaistažolių vandeninę ištrauką fenoliniais junginiais, taip pat chlorogeno rūgšties izomeriais.

Praktinė darbo vertė

Pagaminti chitozано ir žaliųjų kavos pupelių ekstrakto komplekso milteliai panaudoti antioksidacinėmis savybėmis pasižyminčios vaistažolių talpyklos prototipui sukurti. Sukurtos vaistažolių talpyklos su bioaktyviaja chitozано ir žaliųjų kavos pupelių ekstrakto danga buvo panaudotos margainių vaisiams bei „Detoxoset“ ir sukatzolės vaistažolėms supakuoti. Vaistažolių, įdėtų į talpyklas su bioaktyviaja danga, vandeninės ištraukos buvo praturtintos antioksidaciniu poveikiu pasižyminčiais chlorogeno rūgšties izomeriais, lyginant su vandeninėmis ištraukomis, gautomis vaistažolės įdėjus į įprastą talpyklą. Pasiūlyta vaistažolių pakavimo medžiagos su bioaktyviaja danga gamybos technologinė schema.

Ginamasis disertacijos teiginys

Fenolines rūgštis ir augalinių ekstraktų komponentus adsorbuojant iš vandeninių tirpalų katijoniniais polisacharidais yra padidinamas fenolinių rūgščių ir augalinių ekstraktų terminis stabilumas ir išsaugomas antioksidacinis veiklumas.

Darbo rezultatų apibavimas

Doktorantūros studijų metu 2 publikacijos disertacijos tema paskelbtos *Clarivate Analytics Web of Science* duomenų bazės žurnaluose, turinčiuose citavimo indeksą, 1 publikacija kitų tarptautinių duomenų bazių leidiniuose, 1 publikacija paskelbta kituose recenzuojamuose leidiniuose ir 8 straipsniai tarptautinių konferencijų pranešimų medžiagoje. Atlikti du gamybiniai bandymai įmonėje UAB „Švenčionių vaistažolės“.

Darbo apimtis

Daktaro disertaciją sudaro įvadas, 3 skyriai, išvados, literatūros sąrašas, publikacijų disertacijos tema sąrašas, padėka ir priedai. Bendra apimtis – 138 puslapiai. Darbe pateikti 48 paveikslai, 26 lentelės, 22 matematinės išraiškos. Literatūros sąrašą sudaro 215 šaltinių.

Autorės indėlis

Autorė modifikavo gamtinį krakmolą, vykdė bioaktyviųjų medžiagų adsorbcijos biopolimerais tyrimus ir juos analizavo, ištyrė gautų kompleksų antioksidacines ir termines savybes. Augalinių ekstraktų chromatografiniai tyrimai atlikti Lietuvos sveikatos mokslų universitete vykdant bendrą LMT mokslininkų grupių projektą Nr. MIP-055/2015. Skenuojamosios elektroninės mikroskopijos tyrimai atlikti Kauno technologijos universiteto Medžiagų mokslo institute. Branduolių magnetinio rezonanso tyrimai atlikti Vilniaus universiteto APC „Spectroversum“.

IŠVADOS

1. Vandenyje vykstant elektrostatinei sąveikai tarp tinklinių katijoninių krakmolų tretinių amonio grupių ar ketvirtinių amino grupių ir chlorogeno rūgšties susidaro joniniai kompleksai. Prijungtos chlorogeno rūgšties kiekis priklauso nuo katijoninių grupių prigimties. Ketvirtinių amonio grupių turinčio tinklinio katijoninio krakmolo granulėmis fenolinės rūgšties yra adsorbuojama daugiau.
2. Vanilino, kavos, chlorogeno, rozmarino rūgščių ir ketvirtinių amonio grupių turinčio tinklinio katijoninio kompleksai gauti adsorbuojant fenolines rūgštis iš vandeninių tirpalų tinklinio katijoninio krakmolo mikrogranulėmis pusiausvyros sąlygomis:
 - 2.1 Lengmiūro adsorbcijos modelis patvirtino elektrostatinę sąveiką tarp fenolinių rūgščių karboksigrupių ir tinklinio katijoninio krakmolo ketvirtinių amonio grupių. Freundlichio konstantos n_F bei Dubinino-Radushkevicho adsorbcijos energijos E_{DR} vertės parodė, kad sąlygos fenolinių rūgščių adsorbcijai ketvirtinių amonio grupių turinčiu tinkliniu katijoninio krakmolu yra palankios ir fenolinės rūgštys yra adsorbuojamos dėl jonų mainų;

- 2.2 fenolinių rūgščių sorbcinės savybės priklauso nuo karboksigrupių jonizacijos laipsnio. Adsorbuotų fenolinių rūgščių kiekis didėja, mažėjant pKa vertei ir kinta tokia tvarka: kavos rūgštis < chlorogeno rūgštis < rozmarino rūgštis.
3. Artišokų ir žaliųjų kavos pupelių ekstraktuose esančių fenolinių junginių ir ketvirtinių amonio grupių turinčio tinklinio katijoninio krakmolo ar chitozano kompleksai gauti adsorbuojant augalinių ekstraktų komponentus iš vandeninių tirpalų tinklinio katijoninio krakmolo mikrogranulėmis ar chitozano dalelėmis pusiausvyros sąlygomis:
- 3.3 Lengmiūro adsorbcijos modelis patvirtino augaliniuose ekstraktuose esančių chlorogeno rūgšties izomerų adsorbciją tinklinio katijoninio krakmolo ketvirtinėmis amonio grupėmis ar chitozano pirminėmis amino grupėmis;
- 3.4 efektyviosios skysčių chromatografijos metodu įrodyta, kad iš augalinių ekstraktų vandeninių tirpalų adsorbuojami ne tik chlorogeno rūgšties izomerai, bet ir kiti ekstraktuose esantys fenoliniai junginiai, nes artišokų ir žaliųjų kavos pupelių ekstraktų sudėtyje atitinkamai yra 8,1 % ir 74,6 % fenolinių junginių, tarp kurių chlorogeno rūgšties izomerai atitinkamai sudaro 2,3 % ir 49,5 %.
4. Iš sudarytų kompleksų mikrogranulių vanilino, kavos, chlorogeno, rozmarino rūgštys ar artišokų ir žaliųjų kavos pupelių ekstraktuose esantys chlorogeno rūgšties izomerai ir kiti anijoniniai fenoliniai junginiai gali būti laipsniškai atpalaiduojami į skirtingas terpes: į 0,1 M druskos rūgšties (modelinė skrandžio terpė) ir fosfatinį buferinį tirpalą, kurio pH vertė yra 6,8 (modelinė žarnyno terpė), todėl kompleksai pasižymi pailginto veikimo antioksidaciniu poveikiu.
5. Fenolines rūgštis ir augalinių ekstraktų komponentus imobilizavus tinklinio katijoninio krakmolo mikrogranulėse ir chitozano milteliais, jų terminis stabilumas atitinkamai padidėjo 31–99 °C ir 67–100 °C.
6. Sukurtas vaistažolių talpyklos su chitozano ir žaliųjų kavos pupelių ekstrakto danga prototipas pasižymėjo antioksidacinėmis savybėmis:
- 6.1 efektyviosios skysčių chromatografijos metodu įrodyta, kad margainių vaisius, „Detoxoset“ ir sukatzolės vaistažolės supakavus į talpyklas su bioaktyviąja danga, po 5 min. vaistažolių vandeninėje ištraukoje 17,3–81,5 % padidėja bendras fenolinių junginių ir pavienių chlorogeno rūgšties izomerų kiekiai: neochlorogeno rūgšties 1,5–8,1, chlorogeno rūgšties 1,9–47,1, o kriptochlorogeno rūgšties 0,8–28,5 karto;

- 6.2 vaistažolių talpyklų su bioaktyviaja danga vandeninių ištraukų po 5 min. antioksidacinis efektyvumas buvo 0,5–33,2 % didesnis nei talpyklų be dangos;
- 6.3 pasiūlyta chitozano ir žaliųjų kavos pupelių ekstrakto komplekso miltelių ir vaistažolių pakavimo medžiagos su bioaktyviaja danga gamybos technologinė schema.

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