KAUNAS UNIVERSITY OF TECHNOLOGY

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WHEY PROTEIN EDIBLE FILMS WITH FUNCTIONAL ADDITIVES: COMPOSITION, PROPERTIES AND APPLICATION IN FOOD PRODUCTION

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

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IŠRŪGŲ BALTYMŲ VALGOMOSIOS PLĖVELĖS SU FUNKCINIAIS PRIEDAIS: SUDĖTIS, SAVYBĖS, PANAUDOJIMAS MAISTO PRODUKTŲ GAMYBOJE

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INTRODUCTION

Preserving and maintaining the quality of food products during their shelf life is one of the basic tasks for food producers. Different physicochemical treatments are designed and applied in food technologies, due to the preservation of the original quality of the raw materials and food products. However, there are many investigations going on for the improvement of food production technologies, finding environmental friendly and reliable multifunctional alternative treatments or technologies, which could help to control the moisture and gas transfer processes in food; as well as protect food from harmful microorganisms. One such alternative solution could be bio-polymeric edible films or coatings that would protect food products from undesirable moisture, oxygen, carbon dioxide, flavor and taste components migration, or even fat transfer processes in food. They may be the source of functional food ingredients as well, i.e. antimicrobial materials that improve food safety and stability, antioxidants that prevent oxidation of the fat, flavor/taste ingredients and pigment, which improve the sensual quality of the product, etc. Therefore, the bio-polymeric edible films and coatings could prolong the shelf time limits of food raw materials or products, keeping their quality more constant whilst at the same time decreasing food waste.

Whey protein is a by-product of cheese manufacture, having a high nutritional content value and exceptional functional properties to form gels, stabilize emulsions and foams. Due to such functional properties, this raw material could be used for the production of edible films and coatings. However, hydrophilic properties of whey protein films and coatings are the main issue for their practical applications. The aim must therefore be to identify the methods of whey protein modification, which would reduce hydrophilicity, water absorption and solubility of whey protein films and coatings and thus reduce their sensitivity to moisture. Another very important scientific challenge is to investigate the possibilities of the practical applications of edible films and coatings. There is very little scientific information about the impact of edible films and coatings on the quality of specific food products. It is necessary to carry out detailed investigations of their applications to the real food systems, i.e. to determine the effectiveness of their barrier and mechanical properties whilst maintaining the integrity of the food products, the slowdown of moisture and gas transfer processes and the changes of chemical composition during storage time, moreover to develop their preservative properties against rapid harmful microorganisms growth in the food.

The aim of this work was to develope the whey protein based edible films and coatings containing various functional additives and their application effectiveness on fresh fruits and poultry meat against physicochemical and microbial spoilage of these food products during storage time.

In order to achieve the aim of the work the reasearch had to complete these **goals**:

1. To investigate the impact of heat treatment and enzymatic cross-linking on physical and chemical properties of milk and whey protein solutions;
2. To investigate the impact of heat treatment and enzymatic cross-linking, as well as the impact of cationic polysaccharide and plasticizers on mechanical and barrier properties of whey protein edible films;
3. To investigate the impact of whey protein edible films containing antimicrobial additives on some of the food born and pathogenic microorganisms;
4. To investigate the impact of whey protein edible films and coatings containing functional additives on the quality of fresh apples during storage time;
5. To investigate the impact of whey protein edible coatings containing functional additives on the quality of fresh strawberries during storage time;
6. To investigate the preservative effect of whey protein edible films containing various functional additives against the chemical and microbiological spoilage of turkey meat during storage time.

Scientific novelty of the research. During the investigation of the enzymatic treatment impact on the whey protein dispersion, it was obtained that transglutaminase triggered the formation of higher molecular weight whey protein molecules, due to the longer incubation time and higher incubation temperature with higher amount of the enzyme in the system independent on the heat pre-treatment of whey protein dispersion. There was no impact of the simultaneous effect of heat treatment and enzymatic crosslinking on physical and chemical properties of whey protein edible films. To improve the formation of whey protein and chitosan edible coatings on the sliced apple surface, corona treatment was applied. Whey protein and chitosan blend edible films had an inhibitorial impact on the pathogenic, as well as on lactic acid bacterias; the positive impact of these films was determined on physical chemical and microbiological properties of real and model systems of sliced apples, strawberries and turkey meat. Whey protein and chitosan blend films containing quince or cranberry juice inhibited the growth of *P. expansum* and the development of *S. typhimurium*, *E. coli* and *C. jejuni* when applied on apples and turkey meat during storage time.

Practical significance of the research. Biopolymer edible films and coatings were designed using whey protein and polysaccharide chitosan, together with functional additives as plasticizers sorbitol or glycerol, and quince or cranberry juice, resulting in their mechanical and barrier properties being good. Therefore, such edible films and coatings can be successfully used to prolong the shelf life, as well as prevent the microbiological spoilage of some food products. A prototype technology for applying the coating on the fresh sliced apples was prepared; as a result the coated apples can be stored for 12 hours at 20 °C and for 24 hours at 4 °C without the deterioration of their physical properties. A prototype technology for applying the coating on fresh strawberries was prepared as well; as a result the ripening process of such coated strawberries can be retarded for 2 days at 20 °C and for 6 days at 4 °C and RH 80 %.

Publication of the research results. Results of the research are published in 2 journals included into the database of the Institute for Scientific Information (ISI), in 2 reviewed periodical scientific journals included into the list of other databases, and were presented at 5 international conferences.

Structure and contents of the dissertation. The dissertation consists of an introduction, literature survey, experimental part, results and discussion, conclusions, a list of references and a list of publications on the dissertation topic. The list of references includes 278 bibliographic sources. The main results are discussed on 48-105 pages, illustrated in 22 tables and 26 figures.

1. OBJECTS AND METHODS

Fig. 1 represents the research project and **Table 1** summarizes the analysis methods used to determine the impact of various factors on the properties of whey protein (WP) edible films and feasibility of their application.

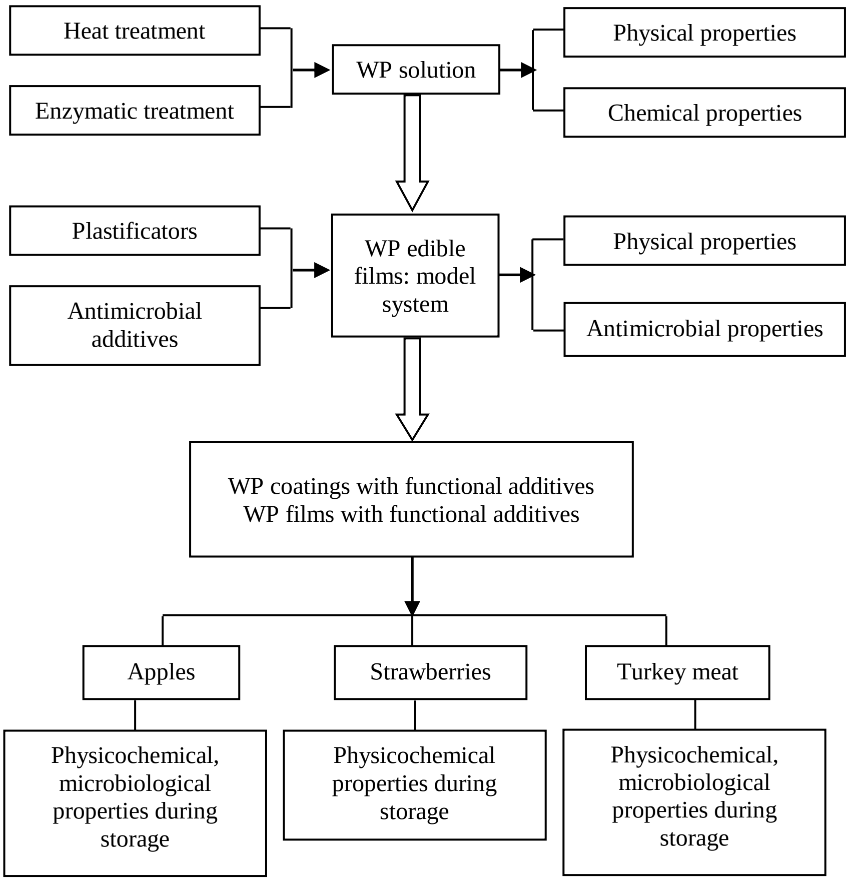

Fig. 1. Research scheme

Table 1. Research objects, analyzed properties and analysis methods

Objects	Properties	Methods
Skim milk	Protein polymerization	Gel chromatography
WP solution	Opacity	Spectrophotometry
	WP concentration	Spectrophotometry
	Morphology	Atomic force microscopy
	Particles mass (kDa)	Gel electrophoresis (SDS-PAGE)
	Rheological properties	Flow determination Dynamic viscosity
WP films	Mechanical burst strength and elasticity	Texture analysis: puncture test
	Water vapor permeability	Gravimetric method
	Oxygen permeability	ASTM F2622-08 standard
	Solubility	Gel electrophoresis (SDS-PAGE)
	Antimicrobial properties	Diffusion in agar medium
Apples	Color	Spectrophotometry
	Morphology	Photography
	Mechanical hardness	Texture analysis: puncture test
	Moisture content	Gravimetric method
	Amount of phenol compounds	Folin-Ciocalteu method
	Antimicrobial properties against *P. expansum*	Inoculation on model and real systems
Strawberries	Color	Spectrophotometry
	Morphology	Photography
	Mechanical hardness	Texture analysis: puncture test
	Weigh	Gravimetric method
	Amount of phenol compounds	Folin-Ciocalteu method
	Amount of vitamin C	Titration
Turkey meat	Moisture content	Gravimetric method
	pH	Potentiometric method
	Volatile fatty acids	Distillation with water steam
	Total viable count	Inoculation on agar medium
	Antimicrobial properties against *S. typhimurium, E. coli, C. jejuni*	Inoculation on selective medium

2. RESULTS AND DISCUSSION

2.1. The influence of composition and manufacture conditions on physicochemical and microbiological properties of whey protein edible films containing functional additives

Barrier and mechanical properties of whey protein films are defined by their composition and microstructure, which is formed during the chemical or physical pretreatments of whey protein. Therefore, it was considered to examine the impact of heat treatment and enzymatic cross-linking by transglutaminase on the properties of the whey protein system. Transglutaminase (TG) catalyzes protein polymerization reactions during the formation of covalent bonds between protein glutamine and lysine residues, or between protein glutamine residues and primary amines.

Scientific publications show contradictory information about the polymerization of whey proteins by TG. Some of the authors declare that $-NH_2$ groups of the whey proteins are easier accessible for TG when thiol groups in the proteins are blocked by thermal denaturation. Other authors have demonstrated whey protein effective polymerization with TG without prior denaturation of the proteins SH groups. The interpretation and comparison of different scientific experimental data is difficult, due to the different concentrations of enzyme and whey proteins in the analyzed systems. Therefore, the research was interested to find out:

1) the optimal content of TG and temperature of enzymatic treatment for milk proteins polymerization;
2) the influence of heat treatment and enzymatic cross-linking conditions on physicochemical changes of whey proteins solution and films made therefrom.

<u>The impact of heat treatment and enzymatic cross-linking on the physicochemical properties of milk protein and whey protein solutions</u>

Finding the answer to the first question, pasteurized (80 °C, 2 min) skim milk (3.4 % protein) has been treated with 1, 2, 4 U/g protein TG enzyme at 40 °C and 50 °C for 20, 30 and 60 min. The analysis of polymerization degree shows a higher content of di-, tri-, and polymers in the system when a higher amount of enzyme and longer incubation time at a higher temperature was applied. On the contrary, the content of protein monomers decreased respectively. It is obvious that the longer incubation time of the proteins system with a higher amount of enzyme results creates a greater quantity of protein covalent ε-(γ-glutamyl) lysine bonds. For instance, during milk incubation at 40 °C for 60 min with 1 U/g protein TG protein, the polymerization degree was 8.34 % and with 4 U/g protein TG – 26.62 % higher than in the control sample without TG. As a result of the skim milk proteins polymerization degree analysis, a further investigation of whey protein solution was treated with 4 U/g proteins TG at 40 °C for 60 min.

Three different conditions of whey protein heat and enzymatic treatments were applied to get the answer for the second question:

1) whey protein solution was heat treated in a different heat mode (samples No. 1, 2, 3);
2) whey protein solution was enzymatic treated with TG for a different incubation time mode (samples No. 4, 5);
3) whey protein solution was heat pre-treated and treated with TG (sample No. 6).

The conditions for the preparation of the whey protein solutions are presented in **Fig. 2**. The physicochemical changes in the whey protein solutions were evaluated by analyzing the optical density, rheological properties, particle size, and atomic force microscopy images. Evaluation of the protein polymerization degree was carried out by polyacrylamide gel electrophoresis.

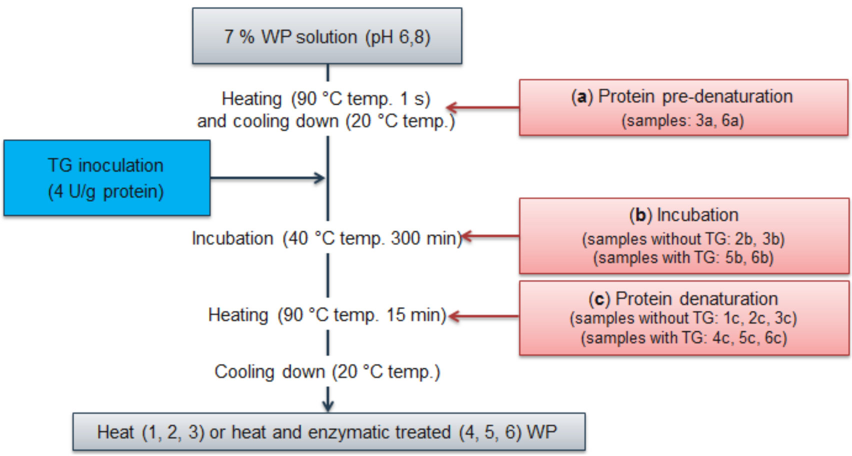

Fig. 2. Heat and enzymatic treatments of whey protein solution

The conformational changes initiated by the thermal energy tertiary structure of whey protein has been changed and large aggregates were formed. As a result, opacity of the whey protein solutions increased. Due to the transglutaminase initiated protein cross-linking, opacity of the TG treated whey protein solution increased even more. The highest opacity was observed in the samples where whey proteins were incubated with TG for a long time (300 min); in this case, the proteins unfolded greatly and exposed larger amounts of free thiol groups, which caused better accessibility for TG and more intensive cross-linking reactions. The formation of whey protein polymers was investigated carrying out gel electrophoresis (**Fig. 3**).

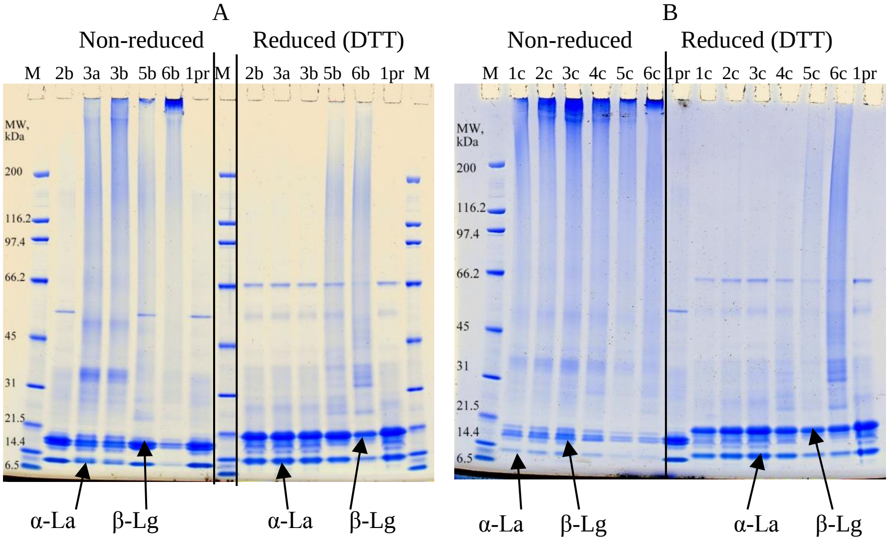

Fig. 3. The impact of heat (samples No. 1, 2, 3) and heat-enzymatic (samples No. 4, 5, 6) treatments on whey protein molecular weight. A – whey protein molecular weight: a – after pre-denaturation, b – after incubation; B – whey protein molecular weight after the final denaturation (c); 1pr – native whey protein molecular weight; M – proteins standard (6.5 – 200 kDa); MW – molecular weight

Whey protein gel electrophoresis illustrates the molecular changes of proteins after their heat and enzymatic treatments. Comparing the native whey protein fractions in well No. 7, in sample No. 3 various sizes of whey protein polymers were created during thermal denaturation, moreover, even a larger amount of higher molecular weight macromolecules were in samples No. 4, 5 and 6, which were formed due to the addition of transglutaminase (**Fig. 3 A**). After the final denaturation of the whey proteins (**Fig. 3 B**), high molecular weight macromolecules were formed in all samples, however, after the reduction of the disulphide bonds with dithiothreitol (DTT) it seems that TG made a strong impact on the formation of whey protein polymers in sample No. 6 and in sample No. 5 there was formed only a small amount of high molecular weight polymers.

Gel electrophoresis results are verified by the viscosity. **Fig. 4** represents viscosity changes of whey protein solutions influenced by heat treatment and enzymatic cross-linking. After the final denaturation of whey proteins, the viscosity of the samples No. 5 and 6 was much higher than the other samples, confirming that transglutaminase initiated the formation of whey protein macromolecules. The higher viscosity of sample No. 5 compared with sample

No. 6 could be obtained due to the formation of a uniform size and larger molecules, which were detected by electrophoresis. In this case, the dispersive properties of whey protein solutions caused their viscosity characteristics; mono dispersive or low dispersive systems are more viscose than poly dispersive ones.

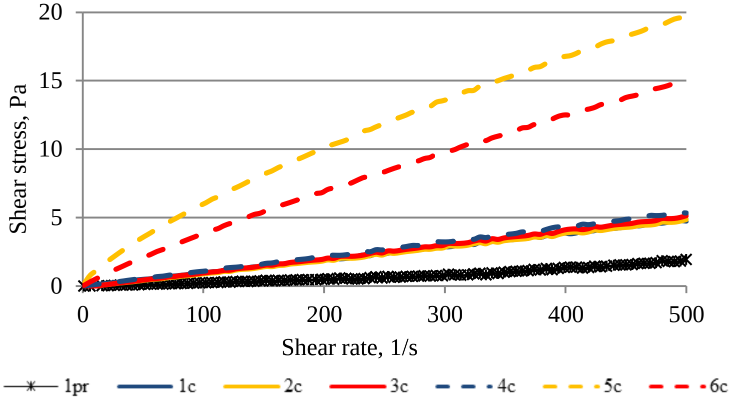

Fig 4. Flow curves of heat and heat-enzymatic treated whey protein solutions

<u>The impact of heat treatment and enzymatic cross-linking on the physicochemical properties of whey protein films</u>

After investigation of the whey protein microstructure, the impact of heat treatment and enzymatic cross-linking of whey protein on the barrier and mechanical properties (**Table 2**) of the films made therefrom were analyzed. The manufacture process of the films is presented in **Fig. 5**.

Table 2. Water vapor permeability and mechanical properties of the films made of heat treated and enzymatic cross-linked whey protein solutions

Film No.	Water vapor permeability (WVP)*	Tensile strength (TS)**	Elongation (E)**
	$g/cm^2 \cdot 24h$	MPa	%
1c	0.074 ± 0.002^{A}	2.685 ± 0.178^{B}	80.95 ± 8.09^{A}
2c	0.094 ± 0.001^{B}	2.245 ± 0.647^{AB}	86.97 ± 7.48^{A}
3c	0.112 ± 0.003^{C}	2.592 ± 0.196^{AB}	82.94 ± 4.31^{A}
4c	0.074 ± 0.002^{A}	2.512 ± 0.235^{AB}	82.76 ± 2.44^{A}
5c	0.094 ± 0.000^{B}	1.901 ± 0.731^{A}	89.10 ± 9.67^{A}
6c	0.109 ± 0.003^{C}	2.810 ± 0.344^{B}	84.84 ± 7.50^{A}

$^{A, B, C}$ – values in one column marked with different capital letters have significant difference ($p \leq 0.05$; *Dunnett T3, **Dunnett C).

Physical properties of the films mostly depend on the microstructure of their components. Transglutaminase did not have any positive significant impact on the water vapor permeability of whey protein films, though heat treatment increased it.

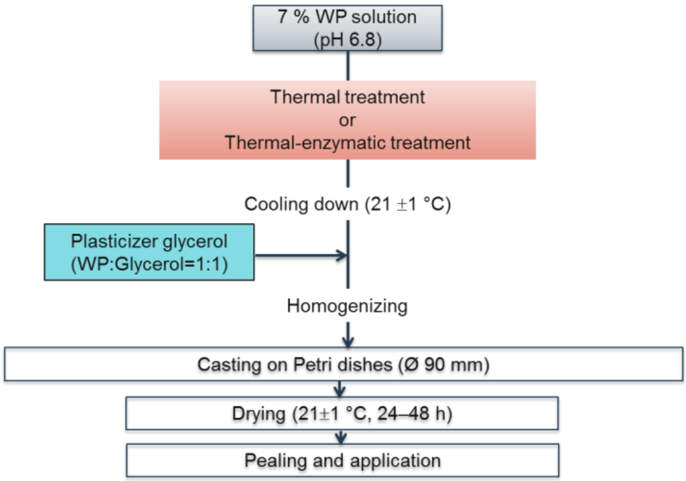

Fig 5. Heat and enzymatic treated whey protein films manufacture procedure

Mechanical properties of the films are very important characteristics, due to their influence on the effectiveness of the films behavior as a barrier. Mechanical properties of heat treated and enzymatic cross-linked whey protein films remained stable, except for the film made of the enzymatic treated whey proteins which were not pre-denatured (sample No. 5). Film No. 5 had a lower tensile strength and a slightly higher elasticity in comparison with the control films. All films had very low oxygen permeability, which was 20 times lower than the low density polyethylene oxygen permeability; however there was no significant difference between the control film and the films made of heat treated and enzymatic cross-linked whey protein.

Investigation of the films solubility revealed that all films had very low solubility in water; it was necessary to use the reduction agent DTT in order to disrupt their structure. The lowest solubility was determined for film No. 6 composed of pre-denatured and TG treated whey proteins, however the dissolution of film No. 5 was also very low and the high molecular weight macromolecules remained undisrupted due to the cross-links. Nevertheless, based on gel electrophoresis results, during the drying stage of the films, the oxidation process of whey protein occurred, resulting in the formation of 20–50 kDa molecular weight oligomers.

The impact of functional additives on the physical and antimicrobial properties of whey protein films

Since there was no significant positive impact of TG on the mechanical properties of whey protein films observed, plasticizers glycerol (G) and sorbitol (S) were subsequently investigated (**Table 3**). Plasticizers increase the mobility of the polymer chains and dependent on the composition and molecular structure of plasticizers, they can improve the mechanical properties of the biopolymeric films and even increase or decrease their hydrophobicity. **Fig 6**. represents the manufacture process of whey protein films containing functional additives.

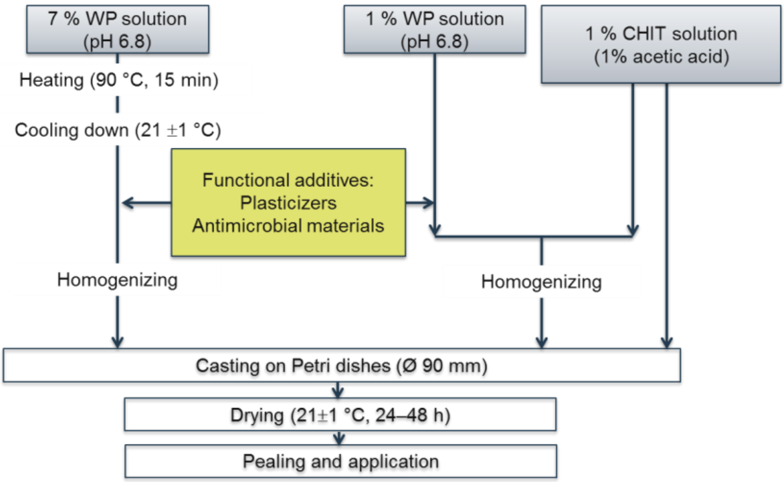

Fig 6. Whey protein films containing functional additives manufacture process

During the investigation of sorbitol (S) and glycerol (G) plasticizers on the whey protein films physical properties, it was found that TS of films containing S was 59 % higher and E was 9 % higher in comparison with films containing G. The increase of S with 30 % caused a TS increase to 87 % and slight decrease of E to 8 %. The mixture of plasticizers in the ratio G:S=1:1 or 1:4 caused even higher TS values, however E values did not differ significantly.

In comparison with whey protein films containing S or G, the whey protein and chitosan (CHIT) mixture (WP/CHIT) films were significantly stronger and the high elasticity remained.

Water vapor permeability (WVP) analysis results show that the least WVP were films containing sorbitol and that there was no difference between the different amounts of S in the film composition.

Table 3. Water vapor permeability and mechanical properties of the films made of whey proteins and various plasticizers

Film	Water vapor permeability (WVP)*	Tensile strength (TS)**	Elongation (E)**
	$g/cm^2 \cdot 24h$	MPa	%
WP/G (1:1)	0.074 ± 0.002^{A}	3.872 ± 0.320^{A}	$33.13 \pm 3,20^{CD}$
WP/S (1:1)	0.040 ± 0.002^{B}	6.153 ± 0.615^{B}	$36.05 \pm 2,77^{D}$
WP/S (1:1,3)	0.039 ± 0.001^{B}	11.534 ± 0.313^{C}	33.29 ± 0.91^{CD}
WP/G/S (G:S=1:1)	0.059 ± 0.002^{C}	16.348 ± 0.838^{D}	29.765 ± 1.60^{C}
WP/G/S (G:S=1:4)	0.046 ± 0.002^{B}	14.961 ± 0.526^{D}	33.13 ± 1.30^{CD}
WP/CHIT	0.067 ± 0.002^{D}	36.478 ± 1.970^{E}	8.23 ± 0.42^{B}
WP/CHIT/G (WP:G=1:1)	0.090 ± 0.003^{E}	$32.458 \pm 5.460d^{EF}$	38.97 ± 11.21^{CD}
WP/CHIT/S (WP:S=1:1)	0.060 ± 0.001^{C}	47.261 ± 3.264^{F}	35.26 ± 7.56^{CD}
CHIT	0.043 ± 0.002^{B}	31.277 ± 2.952^{E}	4.56 ± 1.01^{A}

$^{A, B, C, D, E, F}$ – values in one column marked with different capital letters have significant difference ($p \leq 0.05$; *Dunnett T3, **Dunnett C).

Functional additives providing the antimicrobial properties to the films can enable them to protect food products from microbiological spoilage and thereby improve food safety. Therefore, the antimicrobial properties of the films were investigated by adding in to the whey protein solution various synthetic or natural antimicrobial materials: propolis, nisin, calcium lactate, potassium sorbate, benzoic or ascorbic acids, quince or cranberry juice. Quince or cranberry juices were sources for natural antimicrobial substances that are rich in organic acids and phenols.

The analysis of antimicrobial properties of the films was carried out using pathogenic microorganisms *Salmonella typhimurium, Salmonella agona* and *Campylobacter jejuni*, lactic acid bacterias *Lactobacillus sakei* and *Lactobacillus plantarum*, and fungus *Penicillium expansum* (**Table 4**).

WP films without antimicrobial additives only had an impact against *S. agona* and *C. jejuni* growth. Meanwhile, WP/CHIT and CHIT films inhibited the growth of all analyzed microorganisms, except fungus.

Concerning the growth of the analyzed microorganisms around (inhibition zone formation) and under WP/CHIT/S films containing antimicrobial additives on agar medium, propolis, nisin, calcium lactate, potassium sorbate, benzoic and ascorbic acids, in some cases provided and in other cases improved the WP films antimicrobial properties, and the WP/CHIT inhibitory effect against microorganisms was increased.

Table 4. Antimicrobial properties of whey protein films containing different antimicrobial substances

Film	*S. typhimurium*	*S. agona*	*P. expansum*	*L. sakei*	*L. plantarum*	*C. jejuni*
	Inhibition zone, including the diameter of the sample, mm					
CHIT	N	N	0	N	13.33±1.03 A	N
WP/CHIT/S	N	N	0	14.50±0.84 A	17.17±1.17 BC	N
WP/CHIT/S/ propolis (1 %)	N	N	N	15.50±0.55 AB	16.33±1.86 B	N
WP/CHIT/S/ nisin (2500 IU/g)	N	N	N	38.50±1.76 C	48.33±1.97 D	N
WP/CHIT/S/ calcium lactate (1 %)	N	N	N	14.33±0.52 A	15.00±1.41 AB	N
WP/CHIT/S/ benzoic acid (0,03 %)	N	N	N	14.50±0.55 A	16.83±0.75 BC	N
WP/CHIT/S/ ascorbic acid (0,03 %)	N	N	N	15.67±1.21 AB	19.17±1.72 C	N
WP/CHIT/S/ potassium sorbate (1 %)	15.00±0.63	15.83±1.17	20.17±0.41	16.33±1.03 B	17.67±1.51 BC	28.17±2.23

N – microorganisms did not grow under the film, but there was no inhibition zone observed.
A, B, C, D – values in one column marked with different capital letters have significant difference ($p \leq 0.05$, Tukey).

Regarding safe and healthy food, there is a great interest in natural sources of functional preservatives. Therefore, one of the research focuses was on the natural antibacterial substances available in quince and cranberry juice.

Due to the high acidity of the juices, chitosan was dissolved with juice and mixed with a whey protein solution following the manufacture of the films. WP/CHIT and CHIT films containing quince and cranberry juice had good antifungal properties against *P. expansum* and WP/CHIT films containing quince and cranberry juice had an inhibitory effect against pathogenic bacterias *S. typhimurium*, *S. agona* and *C. jejuni*, and lactic acid bacterias *L. sakei* and *L. plantarum* (**Table 5**).

Regarding the mechanical properties of WP/CHIT and CHIT films containing quince and cranberry juice (**Table 6**), the addition of juice significantly decreased the TS values of CHIT films regardless of the amount and type of juice. WP/CHIT films showed no significant difference when comparing TS values with those of CHIT films. However, the addition of cranberry and quince juice to the composition of WP/CHIT films had a significant effect on this parameter. The negative influence of juice content on the TS of the WP/CHIT films was more evident. In all the cases, the TS of WP/CHIT films was higher than that of CHIT, however, the difference was negligible. The highest tensile strength was recorded for WP/CHIT films with cranberry juice (1:19) and WP/CHIT films with quince juice (1:16).

Significant differences were recorded between the elasticity of CHIT and WP/CHIT films (**Table 6**). The addition of cranberry and quince juice caused a significant increase in the elasticity of films. Taking into account that the films were produced without addition of plasticizers, it can be predicted that sugars and the pectin present in juice can act as plasticizers and cause higher elasticity of the films. However, the effect of the added juice amount on this parameter of the films was not so evident.

Table 5. Antimicrobial properties of whey protein-chitosan films containing quince and cranberry juice

Film	*S. typhimurium*	*S. agona*	*L. sakei*	*L. plantarum*	*C. jejuni*
	Inhibition zone, including the diameter of the sample, mm				
WP/CHIT/Quince	18.17±2.14^{A}	25.17±0.75^{A}	18.50±0.84^{A}	16.67±1.37^{A}	27.50±1.36^{A}
WP/CHIT/Cranberry	14.33±0.52^{B}	19.00±0.63^{B}	17.17±1.17^{B}	19.50±1.22^{B}	25.00±1.10^{B}

$^{A, B}$ – values in one column with different capital letters have significant difference (p≤0.05, Tukey).

Table 6. Mechanical properties of whey protein-chitosan films containing quince and cranberry juice

Film	Tensile strength (TS), MPa	Elongation (E), %
CHIT (Quince 1:16)	8.802±0.044^{CD}	37.13±0.14^{EFG}
CHIT (Quince 2:15)	5.091±0.023^{ABCD}	52.21±1.49^{DEFG}
CHIT (Quince 3:14)	3.670±0.024^{ABC}	59.59±1.57^{CDE}
WP/CHIT (Quince 1:16)	9.914±0.062^{D}	84.05±1.56^{B}
WP/CHIT (Quince 2:15)	5.548±0.050^{ABC}	93.49±2.65^{AB}
WP/CHIT (Quince 3:14)	4.111±0.064^{ABC}	86.98±7.92^{AB}
CHIT (Cranberry 1:19)	7.661±0.057^{BCD}	55.28±0.47^{DEF}
CHIT (Cranberry 2:18)	3.412±0.028^{AB}	67.43±0.48^{CD}
CHIT (Cranberry 3:17)	1.365±0.010^{A}	58.24±2.68^{CDE}
WP/CHIT (Cranberry 1:19)	10.273±0.057^{D}	86.66±2,18^{AB}
WP/CHIT (Cranberry 2:18)	4.275±0.022^{ABC}	89.55±1.05^{AB}
WP/CHIT (Cranberry 3:17)	3.602±0.023^{ABC}	110.93±0.36^{A}

$^{A, B, C, D, E, F, G}$ – values in one column marked with different capital letters have significant difference (p≤0.05, Dunnett C).

2.2. The impact of whey protein edible films and coatings containing functional additives on the quality of some food products

The aim of this part of the study was to investigate the effect of edible coatings and films application on some food products during storage time.

<u>The impact of whey protein edible coatings on fresh apples</u>

The investigation was done on fresh sliced apple slices coated by a double layer coating made of whey protein and chitosan respectively. The moisture lost, changes of phenolic compounds, mechanical properties and surface browning of apple slices were analyzed. Analysis results showed that the double layer coatings can prevent apple slices from browning (1) for 12 hours during storage time at 21±1 °C under RH 30–50 %, (2) for 14 hours at 21±1 °C under RH 75 %, and (3) for 24 hours at 4±1 °C under RH 80–90 %. The moisture loss of the coated apple slices decreased (1) 12 % during 24 hours storage at 21±1 °C under RH 30–50 %, (2) 14 % during 48 hours storage at 21±1 °C under RH 75 %, and (3) 6 % during 72 hours storage at 4±1 °C under RH 80–90 %. The amount of phenolic compounds were higher in the coated apple slices in comparison with the control samples without coating during storage for 48 hours at 21±1 °C under RH 75 %. Mechanical strength of coated apple slices when compared with the control samples was lower at 21±1 °C and higher at 4±1 °C due to the different occasion of water molecules migration in apple tissues under different storage conditions. In conclusion, a double layer coating had a positive effect on the physical properties of apple slices during storage under different conditions.

The impact of the drying effect of air flow caused by an electric field (8 kV) on the physicochemical properties of coated and uncoated apple slices was analyzed. The electric field had a positive effect on all samples regarding slower browning of the apple surface and slower moisture loss, however there was no significant difference between the physicochemical properties of coated in comparison with uncoated (control) apple slices.

The second part of the investigation was done analyzing the antifungal effect of CHIT and WP/CHIT edible films containing a different amount of cranberry and quince juice on a simulation model (apple puree and agar mixture) and native apple systems. The tests were carried out using *P. expansum* inoculated into the simulation model media and the native apple, respectively. Examined films were placed on the inoculated samples and the colony diameter on each sample was measured during incubation for 264 hours. The growth curves of *P. expansum* based on the colony diameters were characterized by a lag phase followed by linear growth for all the examined films. The shortest lag phase and the highest growth rate were recorded for the control samples (a simulated model and native apple systems not covered by a film). A significant ($P \leq 0.05$) inhibition effect of both CHIT and WP/CHIT films containing quince and cranberry juice on the inhibition of *P. expansum* was recorded regarding both analyzed systems. The presence of cranberry juice in the composition of CHIT and WP/CHIT films resulted in a longer lag phase and a lower *P. expansum* growth rate on the simulation model media in comparison with films made with the addition of quince juice. It can be presumed that the differences in organic acids and the phenolic compounds content in the quince and cranberry juice were the reason of the observed results. Cranberry juice is characterized by higher amounts of anthocyanins and benzoic acid, while quince juice contains more ascorbic and sorbic acids. The absence of anthocyanins and small amounts of benzoic acid in quince juice can possibly cause a lower effect of the films made with that juice. However, no clear differences in antifungal properties of the films containing cranberry or quince juice were determined when the experiment was conducted on the apples. In conclusion, addition of quince and cranberry juice to the chitosan and whey proteins with chitosan films as natural antifungal agents has a potential to prolong the shelf life of apples.

The impact of whey protein edible coatings on fresh strawberries

The impact of double layer coatings WP/S|WP/S, WP/S|CHIT and WP/CHIT/S|WP/CHIT/S on the quality of fresh strawberries was investigated. Coatings on the surface of strawberries were formed by dipping the berries in the coating solutions and drying each layer constantly. Samples were stored at 20 °C under RH 80 % for 6 days and at 4 °C under RH 80 % for 13 days. Edible coatings caused a slower increase of the phenolic compounds, a slower decrease of total weight and a slightly higher mechanical strength of coated strawberries in comparison with uncoated strawberries. As a result, the ripening process and senescence of strawberries were delayed. The greatest impact of the preservation of physicochemical properties of strawberries had a double layer WP/S|CHIT coating on samples at 20 °C under RH 80 %. Nevertheless, storage conditions had more impact on the amount of ascorbic acid in strawberries then the coating during storage for 6 days, because the effect of the coatings on the concentration of ascorbic acid in strawberries had been seen more than after 7 days storage at 4 °C and differences between the different compositions of the coatings were not observed.

In conclusion, more efficient protection of the coatings was determined during storage at 20 °C than at 4 °C due to the slower biochemical processes during storage at the low temperature. Edible coatings and especially WP/S|CHIT can slow down spoilage of strawberries.

The impact of whey protein edible films on raw turkey meat

The impact of WP, CHIT edible films and WP/CHIT edible films containing various antimicrobial additives as propolis, nisin, calcium lactate, potassium sorbate, benzoic or ascorbic acids, quince or cranberry juice on bacterial contamination of turkey meat was investigated. The total bacterial count of turkey meat during storage for 6 days at 5 °C was analyzed. All edible films demonstrated an antimicrobial effect on the total bacterial count, except WP and WP/CHIT/nisin films, which had a weaker impact in comparison with WP/CHIT films containing other antimicrobial additives. For further investigation, turkey meat samples were additionally contaminated with *Salmonella typhimurium*, *Escherichia coli* and *Campylobacter jejuni* and covered with examined edible films. The growth of pathogenic microorganisms in the samples wereanalyzed during storage for 6 days at 5 °C (*S. typhimurium* and *E. coli*) and 16 °C (*S. typhimurium* and *C. jejuni*). The largest antimicrobial impact of the examined films was obtained during samples stored at 16 °C; all examined edible films had diverse antimicrobial impact against *S. typhimurium* and *C. jejuni*. Regarding samples stored at 5 °C, there were no significant antimicrobial impact of the examined edible films on *S. typhimurium* growth during the experiment and only CHIT and WP/CHIT films without antimicrobial additives demonstrated a significant decrease of *E. coli* growth on turkey meat after the 3^{rd} day of the experiment. WP/CHIT films containing quince and cranberry juice had the most significant antimicrobial impact on the total bacterial count of turkey meat during storage at 5 °C and contaminated pathogenic microorganisms in turkey meat during storage at 5 °C and 16 °C. Quinces and cranberry juice can be a very useful natural source of antimicrobials and may help to protect raw food products from microbiological spoilage, thereby extending their shelf life.

Finally, the impact of WP/CHIT/S edible films containing calcium lactate, quince or cranberry juice on the physicochemical properties of turkey meat during storage for 3 days at 16 °C was studied. The obtained results showed a positive impact of the examined edible films on the physicochemical properties of turkey meat. Edible films caused pH stability of turkey meat, decreased the exclusion of volatile fatty acids and slowed down the protein hydrolysis process in turkey meat. As a result, the chemical spoilage of turkey meat was delayed.

3. CONCLUSIONS

1. Heat pre-treatment did not have a significant impact on the dispersity and viscosity of transglutaminase treated skim milk (3.4 %) and whey protein solutions; incubation time and temperature with this enzyme as well as the amount of enzyme had a significant impact on these properties. As a result, higher molecular weight macromolecules were composed due to the crosslinking of milk protein influenced by the longer incubation time (20–60 min) and higher incubation temperature (40 °C and 50 °C) with the higher amount of transglutaminase (1–4 U/g protein).
2. Mechanical and barrier properties of edible films made of heat and enzymatic treated whey protein were not significantly different in comparison with the edible films made of whey protein without enzyme. Functional additives as sorbitol and glycerol (ratio 1:1) or as chitosan made a significant impact on the mechanical strength of the films, by increasing it respectively to 16.348±0.838 MPa and 47.261±3.264 MPa, while water vapor permeability was decreased to 0.039±0.001 g/cm^2·24h by adding sorbitol into the system.
3. The investigation of the antimicrobial properties of whey protein films against *S. typhimurium*, *S. agona*, *P. expansum*, *L. sakei*, *L. plantarum* and *C. jejuni* revealed that whey protein films have no antimicrobial properties, and films containing chitosan inhibited the growth of all the analyzed microorganisms except *P. expansum*. Whey protein edible films containing propolis, nisin, calcium lactate, potassium sorbate, benzoic or ascorbic acids had an antimicrobial impact on the analyzed microorganisms. Whey protein and chitosan blend films containing natural quince or cranberry juice slowed down the growth of *P. expansum* and inhibited all the analyzed pathogenic and lactic acid bacterias.
4. Double layer edible coating made of whey protein and chitosan blend prevented fresh sliced apples from browning 12–24 hours, reduced surface hardening of apple slices, decreased their moisture lost with 6–14 % and influenced the retention of a higher amount of phenolic compounds during storage. The coating formation method of drying in an air flow or in an air flow together with a corona wind did not have any significant impact on the physical properties of the coated apple slices. However, the formation of the coating in an electrical field was faster, therefore the browning of the apple surface was delayed. The investigation of inhibitory properties of whey protein and chitosan blend, as well as chitosan edible films containing quince or cranberry juice against contaminated *P. expansum* growth in the model system of apple puree showed a better inhibitory effect in the films containing cranberry juice (from 25.0±4.2 % to 100.0 %) than containing quince juice (from 21.0±7.1 % to 44.5±7.5 %). Regarding the analysis of whey protein and chitosan blend, as well as chitosan films containing quinces

and cranberry juice inhibitory effect against contaminated fungus grow on the natural apple system, it was found that all investigated films slowed down the growth rate of fungus, though none of the films stopped fungus growth completely.

5. The total weight of strawberries coated by various compositions of edible films decreased slower and the ripening process also descreased, in addition the senescence was delayed due to the slower increase of phenolic compounds in comparison with the uncoated strawberries. The greatest impact on the quality of strawberries was observed with a double layer whey protein and chitosan blend coating. However, a more efficient protection of the coatings was determined during the storage at 20 °C than at 4 °C, due to the slower biochemical processes during the storage at the low temperature. In this case, the storage conditions had a greater impact on the amount of ascorbic acid in strawberries than the coating. The effect of the coatings on the concentration of ascorbic acid in strawberries had been observed for at least 7 days at 4 °C, though the differences between the different compositions of the coatings were not observed.
6. Wey protein and chitosan blend, as well as chitosan films containing synthetic antimicrobial substances as calcium lactate, potassium sorbate, benzoic or ascorbic acids, in addition to natural antimicrobial materials as propolis, quince or cranberry juice had a significant impact on the decrease of the total count of bacteria’s on turkey meat for 6 days; the total count of bacteria’s detected on turkey meat did not exceed 10^6 CFU/1g during storage for 6 days. Furthermore, films with antimicrobial materials had a significant positive influence on the inhibition of contaminated pathogenic microorganisms such as *S. typhimurium*, *E. coli* and *C. jejuni* on turkey meat and slowed down their growth for 6 days. The impact of whey protein and chitosan blend edible films containing calcium lactate, quince or cranberry juice on the physicochemical properties of turkey meat was positive, due to the delayed chemical spoilage. Edible films caused pH stability, decreased the exclusion of volatile fatty acids and slowed down the protein hydrolysis in turkey meat.

Main conclusion

Whey protein edible films with good mechanical and barrier properties were developed adding various functional additives as chitosan, sorbitol and glycerol into the whey protein system. It was observed that quince and cranberry juice as functional additives can provide the antimicrobial properties to the whey protein or whey protein and chitosan blend films and coatings and therefore prevent the microbial spoilage of food, prolong its shelf life and increase its safety.

LIST OF SCIENTIFIC PUBLICATIONS ON THE THEME OF THE DISSERTATION

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IŠRŪGŲ BALTYMŲ VALGOMOSIOS PLĖVELĖS SU FUNKCINIAIS PRIEDAIS: SUDĖTIS, SAVYBĖS, PANAUDOJIMAS MAISTO PRODUKTŲ GAMYBOJE

REZIUMĖ

Maisto produktų kokybės išsaugojimas jų realizacijos metu yra vienas pagrindinių uždavinių maisto produktų gamintojams. Maisto technologijose sukurta ir pritaikyta įvairių fizikinių ir cheminių apdorojimo priemonių, naudojamų siekiant kuo ilgiau išsaugoti pirminę maisto žaliavų ir produktų kokybę. Vis dėlto ir toliau nuolat ieškoma tobulesnių, draugiškų aplinkai, saugių vartotojų sveikatai, patikimų daugiafunkcinių alternatyvų, galinčių ne tik kontroliuoti drėgmės ar dujų pernašos procesus maiste, bet kartu apsaugoti jį nuo žalingų mikroorganizmų poveikio. Viena iš tokių alternatyvių priemonių gali būti biopolimerinės valgomosios plėvelės ar dangos, apsaugančios nuo drėgmės, deguonies, anglies dioksido, riebalų ir nepageidaujamų aromato bei skonio komponentų pernašos procesų maiste. Jos gali būti ir įvairių maisto funkcinių ingredientų šaltinis, pavyzdžiui, antimikrobinėmis savybėmis pasižyminčių medžiagų, kurios padidina maisto produktų saugą ir stabilumą, antioksidantų, stabdančių riebalų oksidaciją, aromato/skonio medžiagų ir pigmentų, kurie pagerina produkto kokybę. Todėl biopolimerinės valgomosios plėvelės ir dangos galėtų būti naudojamos pailginti maisto žaliavų ar produktų laikymo terminus, išlaikyti jų kokybę realizacijos metu, taip pat leistų išvengti problemų, susijusių su į aplinką išmetamomis maisto pramonės atliekomis.

Išrūgų baltymai yra sūrių gamybos šalutinis produktas, turintis didelę maistinę vertę ir pasižymintis išskirtinėmis funkcinėmis savybėmis formuojant gelius, stabilizuojant emulsijas, putas. Dėl tokių funkcinių savybių ši antrinė žaliava galėtų būti panaudota valgomosioms plėvelėms ir dangoms gaminti. Tačiau išrūgų baltymų plėvelių ir dangų hidrofilinės savybės yra pagrindinis trukdis norint šias plėveles pritaikyti praktiniam panaudojimui. Siekiama surasti tokių išrūgų baltymų modifikavimo priemonių, kurios sumažintų iš jų pagamintų plėvelių ir dangų hidrofiliškumą, drėgmės absorbciją bei tirpumą vandenyje ir tokiu būdu susilpnintų jų jautrumą drėgmei.

Kitas labai svarbus mokslinis uždavinys yra valgomųjų plėvelių ir dangų praktinio panaudojimo galimybių ištyrimas. Mokslinėje spaudoje yra nedaug duomenų apie valgomųjų plėvelių ir dangų poveikį konkrečių maisto produktų kokybei. Būtina atlikti išsamius jų pritaikymo realiose maisto sistemose tyrimus: įvertinti jų barjerinių ir mechaninių savybių efektyvumą išlaikant maisto produkto vientisumą, stabdant drėgmės bei dujų pernašos procesus, cheminės sudėties pokyčius laikymo metu, apsaugant nuo spartaus žalingų mikroorganizmų vystymosi.

Šis darbas skirtas ištirti įvairių funkcinių priedų įtaką baltymų plėvelėms ir dangoms bei įvertinti plėvelių praktinio panaudojimo galimybes.

Darbo tikslas – sukurti ir ištirti valgomąsias išrūgų baltymų plėveles ir dangas, skirtas apsaugoti įvairiems maisto produktams nuo nepageidautinų fizikinių cheminių ir mikrobiologinių pokyčių laikymo metu.

Darbo tikslui pasiekti buvo suformuluoti tokie **uždaviniai**:

1. Ištirti šilumos ir fermento transglutaminazės įtaką fizikinėms cheminėms pieno ir išrūgų baltymų tirpalų savybėms.
2. Ištirti išrūgų baltymų apdorojimo šiluma ir fermentu transglutaminaze, taip pat katijoninio polisacharido chitozano bei įvairių plastiklių įtaką išrūgų baltymų valgomųjų plėvelių mechaninėms ir barjerinėms savybėms.
3. Ištirti išrūgų baltymų valgomųjų plėvelių su įvairiais priedais, turinčiais antimikrobinėmis savybėmis pasižyminčių medžiagų, antimikrobinį poveikį prieš kai kuriuos mikroorganizmus.
4. Ištirti išrūgų baltymų valgomųjų plėvelių ir dangų su įvairiais funkciniais priedais poveikį šviežių obuolių kokybei laikymo metu.
5. Ištirti išrūgų baltymų valgomųjų dangų su įvairiais funkciniais priedais poveikį šviežių braškių kokybei laikymo metu.
6. Ištirti išrūgų baltymų valgomųjų plėvelių su įvairiais funkciniais priedais poveikį kalakutienos cheminiam ir mikrobiologiniam gedimui bei mikrobiologinei saugai.

Darbo mokslinis naujumas. Ištyrus fermento transglutaminazės poveikį išrūgų baltymų tirpalų dispersiškumui, nustatyta, kad didesnės molekulinės masės baltymų dariniai susiformavo didinant transglutaminazės kiekį, inkubavimo trukmę ir temperatūrą nepriklausomai nuo išankstinio baltymų denatūravimo šiluma. Nenustatyta patikimų skirtumų tarp plėvelių, pagamintų iš transglutaminaze apdorotų ir šiuo fermentu neapdorotų išrūgų baltymų tirpalų, fizikinių savybių. Pirmą kartą formuojant dangas ant pjaustytų obuolių, buvo pritaikytas išrūgų baltymų ir chitozano dangos džiovinimas elektriniame lauke. Nustatyta, kad išrūgų baltymų ir chitozano mišinio plėvelės inhibitoriškai veikė kai kurias patogenines bakterijas ir pieno rūgšties bakterijas. Jų teigiama įtaka buvo nustatyta realių maisto sistemų – pjaustytų obuolių, braškių ir kalakutienos – fizikinėms cheminėms savybėms bei mikrobiologinei saugai. Šių plėvelių sudėtį praturtinus svarainių ir spanguolių sultimis, jos slopino mikroskopinio grybo *P. expansum* augimą bei sulėtino patogeninių mikroorganizmų *S. typhimurium*, *E. coli* bei *C. jejuni* augimą tiek modelinėse maisto sistemose, tiek obuoliuose bei kalakutienoje.

Darbo praktinė vertė. Sukurtos ir pagamintos geromis mechaninėmis ir barjerinėmis savybėmis pasižyminčios valgomosios plėvelės ir dangos iš biopolimerų – išrūgų baltymų bei chitozano su sorbitolio, glicerolio, svarainių bei spanguolių sulčių priedais. Šios sudėties plėvelės ir dangos gali būti sėkmingai naudojamos kai kurių maisto produktų vartojimo terminams pailginti ir mikrobiologinei saugai užtikrinti. Parengta prototipinė pjaustytų obuolių dengimo valgomąja danga technologija, leidžianti išlaikyti šviežiai pjaustytus obuolius 12 val. 20 °C temperatūroje ir 24 val. 4 °C temperatūroje be išvaizdos ir skonio pakitimų. Taip pat sukurta prototipinė braškių dengimo valgomąja danga technologija, leidžianti sustabdyti uogų nokimo ir senėjimo procesus 2 paras 20 °C temperatūroje ir 6 paras 4 °C temperatūroje esant 80 % RH.

IŠVADOS

1. Transglutaminaze (TG) paveikto lieso pieno (3,4 % baltymų) ir išrūgų baltymų tirpalo (7 % baltymų) dispersiškumo ir klampos pokyčiai nepriklausė nuo išankstinio baltymų denatūravimo šiluma, bet priklausė nuo TG kiekio, inkubacijos trukmės ir temperatūros. Nustatyta, kad išrūgų baltymų tirpale didelės molekulinės masės baltymų junginiai susiformavo išlaikius tirpalą su TG 40 °C temperatūroje 300 min, o liesame piene baltymų polimerizacijos laipsnis didėjo, didėjant fermento kiekiui (1–4 TV/g baltymų), ilgėjant jo veikimo trukmei (20–60 min) bei didėjant išlaikymo su fermentu temperatūrai (40–50 °C).
2. Palyginus mechanines bei barjerines savybes išrūgų baltymų plėvelių, pagamintų iš TG paveiktų ir nepaveiktų baltymų tirpalų, statistiškai patikimų skirtumų nenustatyta. Išrūgų baltymų plėvelių stiprumas padidėjo įdėjus plastiklių mišinio (sorbitolio ir glicerolio 1:1) priedo (16,348±0,838 MPa) ir chitozano priedo (47,261±3,264 MPa), o vandens garų pralaidumas sumažėjo iki 0,039±0,001 $g/cm^2 \cdot 24h$ pridėjus plastiklio sorbitolio.
3. Ištyrus plėvelių inhibitorinį poveikį prieš *S. typhimurium, S. agona, P. expansum, L. sakei, L. plantarum, C. jejuni* mikroorganizmus, nustatyta, kad išrūgų baltymų plėvelės be priedų antimikrobinėmis savybėmis nepasižymėjo, o chitozano ir išrūgų baltymų bei chitozano mišinio (1:1) plėvelės slopino visų tirtų mikroorganizmų vystymąsi, išskyrus *P. expansum*. Išrūgų baltymų plėvelėms inhibitorinį poveikį prieš analizuotus mikroorganizmus suteikė konservantai: propolis, nizinas, kalcio laktatas, kalio sorbatas, benzenkarboksirūgštis bei askorbo rūgštis. Išrūgų baltymų ir chitozano mišinio plėvelių sudėtį praturtinus svarainių ir spanguolių sultimis, jos inhibitoriškai veikė ir patogenines bakterijas *S. typhimurium, S. agona, C. jejuni*, ir pieno rūgšties bakterijas *L. sakei, L. plantarum* bei slopino *P. expansum* augimą.
4. Pjaustytus obuolius padengus dvisluoksne išrūgų baltymų ir chitozano danga, sulėtėjo obuolių rudavimo reakcijos (12–24 val.), sumažėjo paviršiaus sluoksnio kietumas ir drėgmės nuostoliai (6–14 %), obuoliuose išliko daugiau fenolinių junginių. Nustatyta, kad džiovinant dangas elektrinio lauko aplinkoje obuolių fizikinės savybės statistiškai reikšmingai nepakito, bet danga susiformavo greičiau. Svarainių ir spanguolių sulčių priedas išrūgų baltymų bei išrūgų baltymų ir chitozano plėvelėse sulėtino *P. expansum*

augimą modelinėje obuolio sistemoje atatinkamai nuo 21,0±7,1 % iki 44,5±7,5 % ir nuo 25,0±4,2 % iki 100,0 %. Skirtumus lėmė skirtingas antocianinų ir benzenkarboksi rūgšties kiekis sultyse. Tiriant plėvelių slopinantį poveikį *P. expansum* obuolių natūralioje sistemoje, nustatyta, kad visos tirtos plėvelės slopino mikroskopinio grybo augimą (nuo 44,5±3,4 % iki 70,2±3,9 %), tačiau visiškai jo sustabdyti nei vienu atveju nepavyko.

5. Ištyrus įvairios sudėties dvisluoksnių valgomųjų dangų poveikį braškėms, nustatyta, jog nokimo ir senėjimo procesai uogose su dangomis vyko lėčiau nei braškėse be dangos – užfiksuota lėtesnė fenolinių junginių, tarp jų ir antocianinų, sintezė ir lėtesnis masės mažėjimas. Didžiausią apsauginį poveikį braškėms padarė dvisluoksnė išrūgų baltymų bei chitozano danga, tačiau efektyviau jos apsauginis poveikis pasireiškė 20 °C nei 4 °C temperatūroje dėl žemoje temperatūroje sulėtėjusių biocheminių procesų. Dangų poveikis askorbo rūgšties koncentracijai braškėse buvo pastebėtas uogas laikant ilgiau kaip 7 paras, tačiau skirtumo tarp skirtingos sudėties dangų nustatyta nebuvo.
6. Išrūgų baltymų ir chitozano bei chitozano plėvelės, pagamintos tiek su cheminiais konservantais (kalcio laktatu, kalio sorbatu, benzenkarboksirūgštimi bei askorbo rūgštimi), tiek su propoliu, svarainių arba spanguolių sultimis ir uždėtos ant šviežios kalakutienos, stabdė mikrobiologinį mėsos gedimą 6 paras – bendras bakterijų skaičius visuose mėginiuose buvo $<10^6$ KSV/1 g – ir mažiausiai 6 paras sulėtino patogeninių mikroorganizmų *S. typhimurium*, *E. coli* bei *C. jejuni*, kuriais buvo užkrėsta kalakutiena, vystymąsi. Nustatytas išrūgų baltymų ir chitozano plėvelių su kalcio laktatu ir svarainių arba spanguolių sultimis slopinantis poveikis kalakutienos cheminiam gedimui. Plėvelės slopino kalakutienoje lakiųjų riebalų rūgščių susidarymą laikymo metu, todėl plėvelėmis apdengtos kalakutienos baltymų hidrolizė vyko lėčiau nei kalakutienoje be plėvelės.

Apibendrinanti išvada

Pagamintos geromis mechaninėmis ir barjerinėmis savybėmis pasižyminčios išrūgų baltymų valgomosios plėvelės ir dangos su chitozano, sorbitolio bei glicerolio priedais. Nustatyta, kad spanguolių bei svarainių sulčių priedai išrūgų baltymų ir chitozano valgomųjų plėvelių ir dangų sudėtyje sulėtina kai kurių maisto produktų gedimą, pailgina jų tinkamumo vartoti laiką ir užtikrina jų mikrobiologinę saugą.

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