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EFFECT OF COMPOSITION AND PROPERTIES OF CHITOSAN-BASED EDIBLE COATINGS ON MICROFLORA OF MEAT AND MEAT PRODUCTS

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ABSTRACT

Background. Analysis of the properties of various chitosan grades has resulted in a working hypothesis that chitosan can be used as part of protective film-forming coatings for meat and meat products. The aim of this study was the research of composition, properties and antibacterial activity of chitosan-based coatings used for cold storage of meat and meat products.
Material and methods. Protective coatings, developed by the authors, based on organic acids and chitosan with food gelatin, or distarch glycerol, or wheat fiber, or sodium alginate, or guar gum have been used as research material. The coatings were applied on the surfaces of retail cuts of veal and rabbit meat, boiled sausages, smoked sausages and smoked-boiled pork brisket. Antimicrobial activity of the solutions was evaluated in vitro. Microbial indicators of the mixtures were also determined by the zone of inhibition assay. Dynamic viscosity, the activation energy of viscous flow and pH of mixtures of fluids were measured. During the storage of meat and meat products total viable count of microorganisms was determined.
Results. Polymer solutions of chitosan:starch and chitosan:gelatin are technologicaly compatible, solutions of chitosan:fiber are two-phase colloidal systems. Coatings did not alter the samples inherent flavour characteristics. All coatings reduced total viable count of microorganisms compared to control samples without coating. Composition based on 2% solution of chitosan and organic acids and 2% gelatin solution in a ratio of 1:1 has the strongest bacteriostatic effect for meat and meat products. Including potassium sorbate and sodium benzoate in gelatin and chitosan solutions mixture for protective coating was not found reasonable, because of their lower bacteriostatic effect. Combined application of vacuum and protective coatings provided the strongest suppressing effect on microflora in all samples.
Conclusions. The chitosan-based edible coatings developed can be used to increase the shelf life and improve the microbial safety parameters of meat and meat products. The results of this study indicate that coatings have bigger stabilising effect on samples with the least amount of basic hurdle factors, in other words on most shelf unstable products.

Key words: chitosan, microbiological stability, protective coating, meat

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INTRODUCTION

Meat and meat products are favorable nutritive media for bacteria (Pseudomonas, Enterobacteriaceae, Micrococcaceae, Bacillus, Clostridium, Lactobacillus, etc.), yeast (Cryptococcus, Candida, Rhodotorula, etc.) and fungi (Mucor, Thamnidium, Penicillium, etc.) [Borch et al. 1996, Doulgeraki et al. 2012, Dillon 1998, Samelis 2006]. Microflora growth on these products reduces their quality, nutritional value and duration of cold storage particularly at low positive temperatures approaching cryoscopic. In this context, it looks prospective to reveal and research application of substances with antibacterial and antifungal properties, to which pathogens and spoilage organisms do not develop resistance and that would be harmless to human beings [Bilska et al. 2012].

The best researched new technologies of preserving the quality and safety of meat are non-thermal inactivation methods, such as high hydrostatic pressure, new packaging systems (modified atmosphere and active packaging), natural antimicrobial components and biopreservation [Ozimek et al. 2010, Tyburcy et al. 2010, Bilska 2011]. All these innovative technologies are developed for their having a mild effect on food products and being at the same time energy-saving, environmentally friendly and ensuring a natural product appearance while eliminating microflora activity [Zhou et al. 2010].

In a number of studies chitosan of various molecular weights is regarded as a promising natural antimicrobial component [Kong et al. 2010, No et al. 2007, Raafat and Sahl 2009]. With respect to human organism chitosan and its cleavage products (N-acethylglucosamine and glucosamine) are natural and harmless [Arai et al. 1968, Kean and Thanou 2010]. At the same time chitosan has mycostatic, bacteriostatic and antioxidant properties [Feng et al. 2008]. Such characteristics of chitosan make it usable for controlling microbial spoilage of meat and meat products [Samelis 2006].

In meat industry one of the most important scientific areas for research and application of chitosan is the study of its antibacterial and antifungal properties and the development of protective coatings on the basis of this polysaccharide with myco- and bacteriostatic or myco-and bactericidal properties. Analysis of the properties of various chitosan grades has resulted in a working hypothesis that chitosan can be used as part of protective film-forming coatings for meat and meat products.

The aim of this study was the research of composition, properties and antibacterial activity of chitosan-based coatings used for cold storage of meat and meat products.

MATERIAL AND METHODS

Protective coatings, developed by the authors, based on organic acids and chitosan with various polymers added have been used as material under research.

The composition and properties of the coatings

Development of the composition of protective coatings used chitosan derived from crab chitin by alkaline deacetylation. The degree of deacetylation (C_d) of chitosan was 0.83, molecular weight 120 kDa, ash 0.56, solubility 99.99.

Besides chitosan the composition of coatings included food gelatin or polysaccharides of plant origin: modified starch – distarch glycerol, wheat fiber Vitacel WF400, sodium alginate or guar gum. Chitosan was dissolved in 1% acetic acid solution. Coatings included 2% chitosan solution in an aqueous solution of organic acid, two percent aqueous solutions of gelatin, cellulose and starch. Chitosan solution was mixed with a solution of each of the other polymers in a ratio of 3:1, 2:1, 1:1, 1:2 and 3:1. Coatings are multicomponent because attempt is made to affect various components and systems of a microbial cell. The most important hurdle factors used for food preservation are temperature, water activity (a_w), acidity (pH), preservatives and competitive microflora [Leistner 2000]. Within the resulting coatings organic acids reduce pH, biopolymers reduce a_w due to their own water-holding capacity, chitosan is an antimicrobial component of natural origin.

The coatings were applied on the surfaces of retail cuts of veal and rabbit meat, boiled sausages, smoked sausages and smoked-boiled pork brisket. The samples were packed in polystyrene trays and stretch-wrap or vacuum bags with pressure $-0.85 \cdot 10^5$ Pa (X-Vac, Komet Maschinenfabrik GmbH, FRG). Refrigerated storage was performed at 4 ±1°C.

Methods

Contribution of each of the hurdle factors in overall antimicrobial activity of the solutions was evaluated in vitro: 2 ml of the mixture were added to 20 ml of the molten medium (meat extract peptone agar (MPA), SRCAMB, Obolensk, Russia). After solidification of the medium test strains of *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (museum cultures of Microbiology Department of North-Western State Medical University named after I.I. Mechnikov, Saint-Petersburg, Russia) were inoculated on the surface. The bacterial inoculum concentration was ~10^9 CFU·ml^{-1}. Samples were kept at the temperature of 37°C and the growth of cultures of microorganisms was observed.

Microbial indicators of the mixtures were also determined by the zone of inhibition assay. An infectious background of *P. fluorescens* (strains pt and 228 RRj, inoculum concentration ~10^5 CFU·ml^{-1}) was created on potato-sucrose medium (potato-dextrose agar, ARRIAM, Saint-Petersburg, Russia) by dash method, and mixtures of various dilution ratios: 1:1, 1:10, 1:100, 1:1000 were placed in the wells (d holes ≈ 10 mm).

Dynamic viscosity η, Pa·s, the activation energy of viscous flow E_a, kJ·mol^{-1} and pH of mixtures of fluids were also measured. Evaluation of rheological properties of mixtures was made with a rotary viscometer Rheotest 2.1 (Rheotest Messgeräte Medingen GmbH, FRG). pH was determined on a laboratory pH-meter with a scale of 0.01. Water activity was measured by wet and dry hygrometer method [Rahman 2009]. Activation energy of viscous flow was determined according to rheological measurements of viscosity at constant shear stress lg τ = 2.2, using the formula [Eyring et al. 1941]:

$$E_a = R \frac{\ln \eta_{20} - \ln \eta_{20}}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where:
$T_1 = 20 + 273 = 293$ K,
$T_2 = 30 + 273 = 303$ K.

During the storage of meat and meat products total viable count (TVC) was determined.

All the experiments were performed with at least three replicates, the data was processed by methods of mathematical statistics with theoretical frequency of 0.95.

Food products samples coating methods

The possibility of applying the protective film-forming mixture on meat products immediately after heat treatment and after cooling down to 20°C was examined. For this purpose emulsified meat products without casing were produced by conventional recipe with a series of parallel samples making at least 10 copies. Cooling was carried out at 20 ±2°C, drying was carried out at a temperature of 4 ±1°C, both of them with natural convection. Two coating methods were examined – coating by immersion and by aerosol spraying. Experiments were made with the composition of a film-forming coating solution of chitosan and gelatin in the ratio of 1:1. Changes in the mass of a product before and after application of the mixture and its drying and mixture of composition were determined. Prepared samples were placed in cold storage at 4 ±1°C and analyzed for microbiological parameters during storage.

RESULTS AND DISCUSSION

In order to determine the interaction and stability of solution mixture systems their rheological characteristics were investigated. Figure 1 and Figure 2 show flow curves of mixtures of fluids.

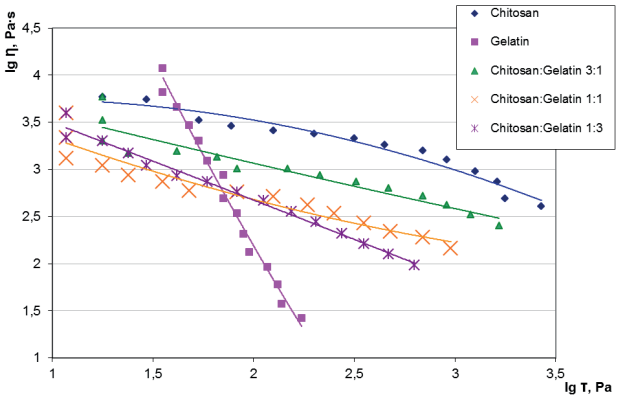

Fig. 1. Flow curves of solutions of chitosan, gelatin and their mixtures at 20°C

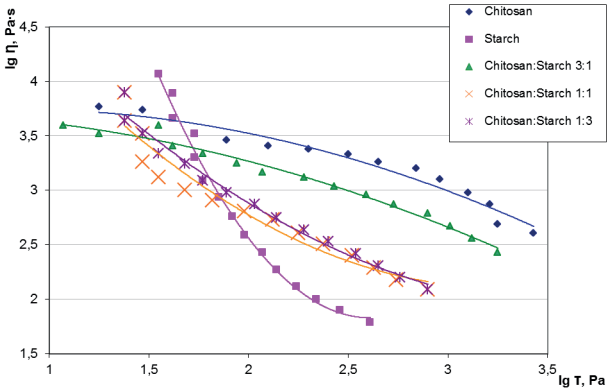

Fig. 2. Flow curves of solutions of chitosan, starch and their mixtures at 20°C

With the increase of shear stress the viscosity of all systems under investigation decreases due to gradual destruction of the spatial network of hydrogen bonds as a result of breach of the structure of macromolecules water environment, reduce of hydrophobic hydration and intermolecular interactions, unfolding of a polymer chain by hydrodynamic flow and relative orientation of macromolecules along the flow axis.

As can be seen from Figure 1 and Figure 2 chitosan solutions have the highest viscosity compared with gelatin and starch solutions. The influence of shear stress on viscosity of gelatin solutions is less pronounced than for the other two polymers.

Activation energy of viscous flow of various mixture solutions is shown on Figure 3 and Figure 4.

Activation energy of viscous flow can serve as an indirect characteristic of the strength of a structure in a solution. With introduction of one part (25%) of gelatin solution the activation energy of the mixture does not change. With further increase of the amount of gelatin the solution in mixture there is a sharp increase in activation energy, therefore, a gel is formed or, in other words, the structure of the solution becomes more stable. Gelatin liquefies when heated due to a conformational change of its macromolecules. In a process of cooling gelatin solutions form a physical grid, which, when heated, disintegrates because of the thermal motion of molecules.

Activation energies of chitosan:starch solutions are in line with activation energy of the chitosan solution. However, the system chitosan:starch 1:1 shows a sharp increase of activation energy that characterizes stabilization of the structure. It can be assumed that in this system a kind of interaction takes place. The same can explain the lower viscosity of this solution that abnormally falls out of solutions viscosity series for chitosan > 3:1 > 1:1 > 1:3 > polymer, which can be seen in Figure 2.

Mixtures of chitosan:starch and chitosan:gelatin are clear solutions that do not separate during storage. Films dried from these mixtures are transparent, which indicates the technological compatibility of polymer solutions.

Solutions chitosan:fiber are two-phase colloidal systems of "polymer-filler" type. Fiber is inert filler, which breaks the intermolecular interaction, helping

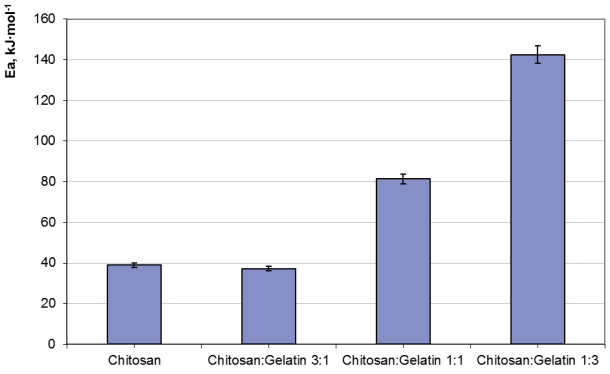

Fig. 3. Dependence of activation energy of viscous flow on the composition of chitosan:gelatin solutions mixtures

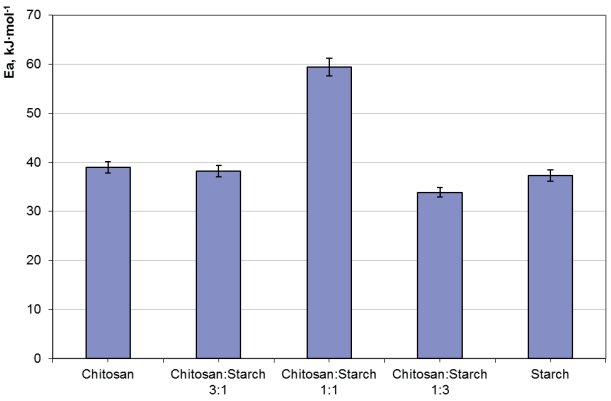

Fig. 4. Dependence of activation energy of viscous flow on the composition of chitosan:starch solutions mixtures

to reduce strength and elasticity of the film. A film from this mixture is smooth on one side and rough on the other one, indicating phase separation.

The amount of the film-forming mixture used when applied by immersion exceeded the amount of the same mixture used when applied by spraying by an average of 33%. In addition, losses due to periodic mixture changes in dipping container when applying by immersion were not taken into account. From the changes of the mass of the samples after cooling it was noted that almost all mixture remains on the product when the coating is applied by spraying, while some part of the mixture is lost when applied by dipping. The mixture excess appears to get on the product after immersion and it flows down later. When the temperature is lowered the mixture becomes more viscous, which promotes the formation of more uniform coating layer, the samples covered after cooling of the product showed the best consumption of the mixture and microbiological results. Total viable count in samples treated by spraying reached 10^3 CFU·g^{-1} after 12 days of cold storage, while for the samples treated by immersion the same was reached after 9 days. Thus, spraying after cooling of meat products was selected as the most effective method of applying the mixtures to maximize the shelf life of the product.

Biopolymers in the protective coatings can bind water and reduce its activity in the surface layer of the product. After applying the polypeptide-polysaccharide coatings on emulsified meat products without a casing a_w decreased from 0.85 to 0.69 and from 0.98 to 0.73 for two different recipes, respectively. Reducing the water activity of the surface layer is an important factor in maintaining the microbiological stability of meat products during storage, since the contamination and growth of microflora mainly starts from the surface of a product [Sperber 1983].

The acidities of biopolymers aqueous solutions that are part of coatings make 6.0-7.3. The mixtures pH was decreased to 4.0-4.2 by adding organic acids. Development of microflora on the product surface should be significantly suppressed at these acidity values [Baranyi and Roberts 1994]. Chitosan is also a part of the composition as natural antibacterial ingredient. The contribution of each of these hurdle factors in total bacteriostatic effect determined *in vitro* is shown in Table 1.

Table 1. Effect of composition on antimicrobial properties of the mixtures

Composition	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Gelatin and organic acid solutions	–	+	–
Gelatin, organic acid and chitosan solutions	–	–	–
Control (molten medium)	+	+	+

+ – growth, – – no growth.

Bacteria of the genera *Bacillus*, *Staphylococcus*, *Escherichia* found in meat are transferred from the hands of workers and equipment, skins and intestines of animals, as well as from surrounding air [Gill 1998, Rahkio and Korkeala 1997]. Many representatives of these genera are pathogenic to human beings. A hurdle factor sufficient to suppress the development of *B. subtillis* and *E. coli* was the reduced acidity, due to the presence of organic acids in protective coatings. Growth stop of *S. aureus* was achieved by the combined action of acid and chitosan.

Pseudomonas aeruginosa, *P. putida* and *P. fluorescens* can extracellularly cleave glucose of meat through gluconate and 2-keto-gluconate followed by Entner-Doudoroff pathway metabolism, due to the presence of glucose dehydrogenase and gluconate dehydrogenase in their membranes [Conway 1992]. This gives pseudomonads competitive advantages over other microorganisms in the initial stage of development of meat spoilage during storage in refrigerated air. All chitosan-based mixtures slowed down the growth of *P. fluorescens* in the zone of inhibition assay. However, mixtures with gelatin and chitosan showed the smallest growth areas of microflora in comparison with samples without protective mixtures and in comparison with the other protective mixtures.

Since coatings polymer composition affects their antimicrobial activity, mixtures with solutions of sodium alginate and guar gum were studied. These coatings did not alter the samples inherent flavour characteristics either. Retail cuts of veal before coating had pH 5.9, after coating pH of the average sample (cuts

80 ±3 g) dropped by 0.2-0.4. The acidity of the samples did not change during storage period.

Total viable count in retail cuts of veal with various coatings (solutions of organic acids and gelatin and chitosan, sodium alginate and chitosan, guar gum and chitosan in various ratios) in polystyrene trays and stretch-wrap during refrigerated storage is shown on Figure 5. All coatings reduced microflora growth compared to control samples in polystyrene trays and stretch-wrap without coating. The polypeptide-polysaccharide coating had the strongest bacteriostatic effect of all coatings studied. Microbiological indicators of polysaccharide coatings with sodium alginate and guar gum became better with increasing of chitosan proportion in the mixture. Samples with guar gum in coatings compositions had more intensive microflora growth compared to samples with alginate-based coatings, which may reflect various interaction mechanisms of these polysaccharides with chitosan. If polysaccharide and chitosan interact in such a way that active groups of chitosan are inhibited, then bacteriostatic properties of the composition are reduced. Another reason may be the greater availability of guar gum for the enzyme system of microorganisms compared to sodium alginate.

Only one mixture of polysaccharide coating was used for storing retail rabbit meat cuts – mixture of solutions of sodium alginate and chitosan in ratio of 1:1. Similar to veal samples the biggest total viable count (Fig. 6) was recorded in samples without protective coating. Total viable count in uncoated veal samples was 1.5 times higher than in samples with sodium alginate-based coatings and 2.0 times higher than in the samples with gelatin-based coatings after 9 days of refrigerated storage.

Vacuum packaging of rabbit meat cuts has significantly reduced microflora development during refrigerated storage (Fig. 7). Vacuum packaging is a very strong hurdle factor. Total viable count in rabbit meat cuts without protective coating and with regular packaging was about 4 times higher than in vacuum packaged uncovered cuts after 9 days of refrigerated storage. Total viable count was 2.7 times different in samples with protective coatings with and without vacuum packaging after 9 days of storage at 4 ±1°C. Protective coatings together with vacuum bags reduced TVC in rabbit meat cuts 1.7-1.9 times.

Gelatin and chitosan-based composition was enriched with potassium sorbate (0.5%) and sodium benzoate (0.5%). The idea was to enhance coatings

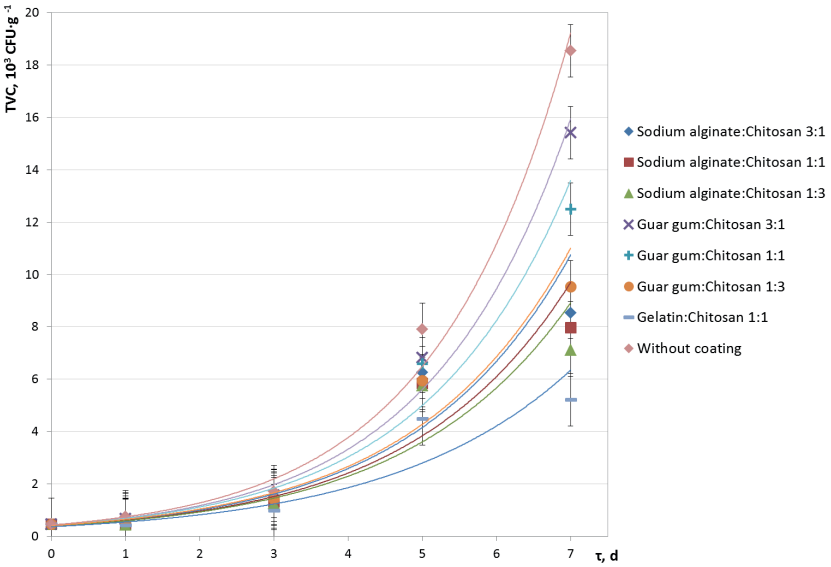

Fig. 5. Total viable count in veal cuts with chitosan-based edible coatings during refrigerated storage at 4 ±1°C

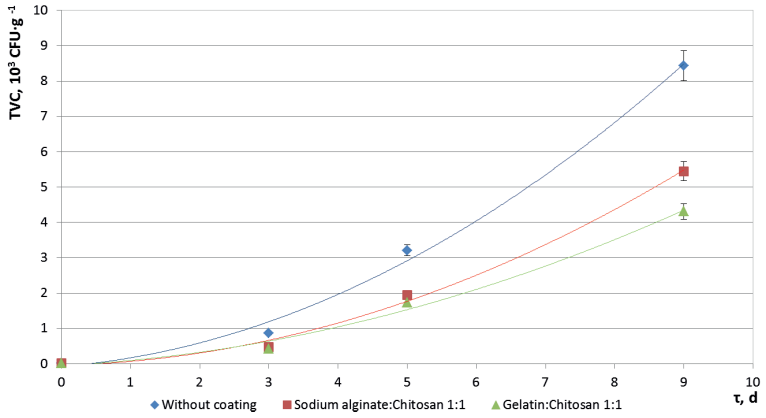

Fig. 6. Total viable count in rabbit meat cuts with chitosan-based edible coatings during refrigerated storage at 4 ±1°C

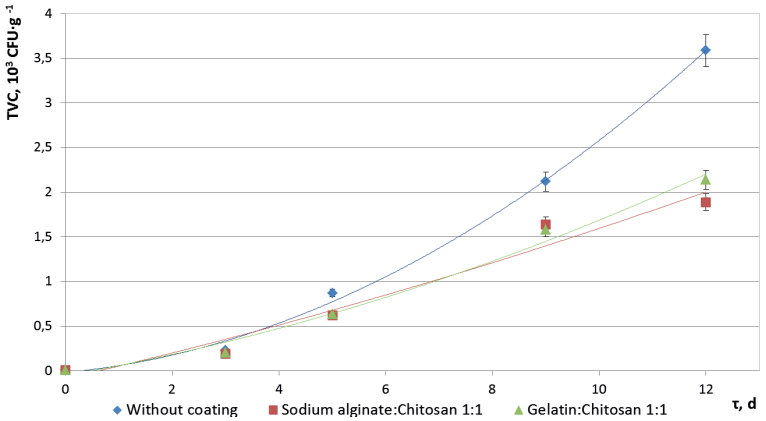

Fig. 7. Total viable count in rabbit cuts with chitosan-based edible coatings during refrigerated storage at 4 ±1°C in vacuum bags

bacteriostatic effect. Coatings with chemical preservatives had bacteriostatic effect too, but TVC in samples of sliced boiled sausages covered with this type of mixture was bigger than in samples covered with chitosan and gelatin only mixtures after 14 days of refrigerated storage. Organoleptic signs of spoilage were found in samples with chemical preservatives two days earlier too.

Films dried from mixtures with potassium sorbate and sodium benzoate had heterogeneous structure. Films made of gelatin and chitosan solutions are even and transparent in contrast to films with potassium sorbate and sodium benzoate, that had multiple small cracks and turbidity. Chemical and structural interactions in mixture are probable reasons for changing the appearance of these films, which are also responsible for reducing the total antimicrobial effect of the system. Thus including in potassium sorbate and sodium benzoate in gelatin and chitosan solutions mixture for protective coating is not reasonable.

Packaging efficiency rate, R_{ef} (Table 2) was calculated for assessment of various packaging options impact on meat products microflora during refrigerated storage:

Table 2. Packaging efficiency rates of various meat products packaging options

	Boiled sausage 'Russkaya' $\tau_{storage}$ = 15 d	Smoked sausage 'Braunshweigskaya' $\tau_{storage}$ = 19 d	Smoked--boiled pork brisket $\tau_{storage}$ = 19 d
Coating with gelatin:chitosan 1:1	1.5	1.1	1.3
Vacuum bags	2.2	1.8	1.5
Coating with gelatin:chitosan 1:1 and vacuum bags	2.5	2.0	2.1

$$R_{ef} = \frac{TVC_{contr}}{TVC_i}$$

where:
TVC_{contr} – total viable count in samples packed in polystyrene trays and stretch-wrap only,
TVC_i – total viable count in samples packed in additional i-th packaging option (samples with vacuum bags were packed without trays and stretch-wrap).

Coating had the greatest impact on microflora of emulsified sausage 'Russkaya' (Table 2) with a significant amount of water in the composition of 56%. On the other hand, smoked sausage 'Braunshweigskaya' and smoked-boiled pork brisket have bigger fat amount – 42 and 47%, respectively. In addition, these meat products, especially sausage, have another hurdle factor – smoking, so they are basically more resistant during storage. Vacuum packaging significantly lowered the intensity of microflora development in all meat products. Combined application of vacuum packaging and protective coatings provided the strongest suppressing effect on microflora in all samples.

CONCLUSIONS

Therefore, chitosan-based edible coatings developed can be used to increase the shelf life and to improve the microbial safety parameters of meat and meat products. It is shown that one of the polysaccharide and polysaccharide-polypeptide mixtures for protective coatings studied has the strongest bacteriostatic effect and it has a composition based on 2% solution of chitosan and organic acids and 2% gelatin solution in a ratio of 1:1. The results of this study indicate that coatings have stronger stabilising effect on samples with the least amount of basic hurdle factors, in other words on most shelf unstable products. Some additional studies on the assessment of new meat product shelf-life should be done when applying coatings for a specific technology.

There is some theoretical and practical interest in the further study of protective coatings with antioxidant and antimicrobial properties against other species, genera and strains of bacteria and fungi during storage at temperatures approaching cryoscopic.

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