FORMS OF PROTECTIVE CHITOSAN-BASED COATINGS FOR FISH PRODUCTS AND OTHER FOOD STUFF

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Abstract

It was investigated that coatings based on chitosan and other polysaccharides having antimicrobial properties and being biodegradable can be successfully used for preserving quality and extending shelf-life of fish produce. Composite coatings with preset properties based on chitosan as a basic poly-electrolyte complex and containing sodium alginate, pectin, zosterin, copolymer of polyvinylpyrrolidone and crotonic acid have been created. The concentration of solutions, method and conditions of applying them on fish products have been defined. The physical and chemical properties, microbiological, organoleptic changes in fish products packed into chitosan-based films and coatings have been studied. Biological testing of the chitosan based coatings for their resistance to microorganisms' caused biodegradation has been performed. Such qualities of chitosan-based films and coatings as natural origin, non-toxicity, anti-microbial properties, good rheological characteristics and biodegradability predetermine good prospects for their use in preserving fish produce.

Introduction

In conditions when enhanced requirements are applied to environmental ecology a great significance is attached to the problem of utilizing garbage consisting of packaging materials (synthetic polymers, cardboard) that are resistant to biodegradability and decomposition. Great significance is given to the creation of easily utilized packing materials, edible films providing protection of food produce from microbial contamination, oxidizing effect, drying etc.

Until now the majority of packaging materials is being based on polymers and this fact does not help to solve the problem of protecting the environment as the assimilation period of synthetic materials in soil lasts for several decades.

It seems vital to develop such multi-purpose packaging materials for food products that would protect them from microbial contamination, influence of atmospheric oxygen, loss on drying, etc. and being at the same time safe and easily utilizable non-toxic biodegradable and sanitary and environmentally safe.

Protective coatings used to package food products initiate other enhanced requirements. First of all: mechanical strength, chemical resistance and high technological properties.

Packing materials used to preserve food products shall be gas-, steam,- and aroma tight, light-, thermo-, frost- and fat resistant and bactericidal.

In this connection chitosan-based packing materials and films deserve special attention as chitosan is biodegradable possess high sorption properties and appropriate rheological characteristics necessary to prepare packing materials.

There is abundant evidence of the survival rate of gram-positive and gram-negative bacteria after being treated by chitosan. It is shown that destruction rate of E-coli makes 38-88%, of B. subtilis – up to 100%, of Ps.aureofaciens – 80 – 90% and depends upon its molecular mass [2, 3].
The mechanism of antibacterial attack against microorganisms is explained by chitosan influence on the integrity of microbe cells exterior membrane when their permeability reaches levels incompatible with life. [4].
It seems very promising to obtain composite solutions and films on the basis of chitosan and various poly-acids that would permit to combine properties of different polymers, enhance strength, change adhesive characteristics, impart biological activity and biodegradability of packing materials.

Materials and methods

Traditionally obtained edible chitosan, sodium alginate, apple pectin, vinylpyrrolidon and crotonic acid were used to get composition solutions for making the films and applying them on products.
The biopolymers used are not only biodegradable which significantly helps to solve the problem of utilization but also are edible which makes it unnecessary to remove packaging materials from products. More over, eating them helps to purify an organism by removing heavy metals and radionuclide.
Chitosan has been obtained out of sea water (crab, shrimp) and freshwater (Gammarus Pulex) crustaceans by the “cold” method developed by the VNIRO [1]. The samples of chitosan have been analyzed for moisture content, mineral (ash) content, deacetylation rate, solubility in acetic acid, molecular mass, dynamic and characteristic viscosity.
Algin acids – polysaccharides obtained from brown algae the molecules of which are built out of residues of D-mannuronic and L-guluronic acids the ration of which and distribution of monomer links along the chain depends upon the source of obtaining. Algin acids and their salts (sodium alginate, potassium alginate) are widely used in food industry medicine, biotechnology. They demonstrate immune-modulating and bactericidal activity, help to remove heavy metals and radionuclide without disturbing metabolism of calcium, normalize functioning of thyroid gland as they contain iodine, improve carbohydrate metabolism, reduce lipids content in blood.
Pectin – linear 1 $\rightarrow$ 4 polygrammnogalacturated, consisting of α-D – galacturinic and α-L – rhamnopyranosic residues; it is contained in many fruit and vegetables. Pectin is used in food industry as a chelating agent to prevent loss on drying. It demonstrated bactericidal properties in respect of acute intestine diseases pathogens without changing normal micro flora.
Copolymer vinilpyrolidon with crotonic acid is a component of Catapol antiseptic remedy being in general a catamine AB polymer chain. It is a powerful remedy with high disinfecting, moistening, and anticorrosive properties and absolutely safe. Characteristics of the enlisted components are given in the Table 1.

Table 1 – Characteristics of polymers used to prepare films and solutions for applying on food products

Polymer	Moisture, %	Ash content, %	Solubility, %	N_{amin}, %	RD, %	C00H, %	MM	η_{dyn}, сПз 2% sol.	[η], pp/g
Chitosan	10.20	0.46	99.7	8.48	98	0.00	206500	3173.61	9.00
Sodium Alginate	13.50	17.38	100	-	-	17.60	62500	13.98	1.25
Pectin	11.20	-	100	-	-	12.14	-	290.82	2.50
Copolymer	9.54	-	100	-	-	21.00	15000	1.33	0.12

The film coatings have been applied on fresh-frozen fillets (pike-perch, cod, polar cod), hot-smoked (Baltic herring, pink salmon) and cold-smoked (herring, mackerel) fish.
Protective properties of the film coatings have been estimated in the course of refrigerated storage being compared with the reference sample being kept in a standard manufacture package.

The following qualitative characteristics have been analyzed: moisture content, water-retaining ability, volatile matters nitrogen (VMN), acid and peroxide numbers, microbiological and organoleptic properties.

Results and discussions

Protective coatings based on chitosan, algin acid, pectin and copolymer have been manufactures in the form of films and solutions being applied on products by dipping or spraying.
The manufactured composite films had the following characteristics:
- chitosan-copolymer (ХАН-СП) based on combination of 3% chitosan and 2% acetic acid solutions in 1:0.1 proportion, 25 mcm film thickness, E-modulus of elasticity equal to 6.16 GPa, ultimate strength σ_n=126 MPa, breaking strength σ_p=128 MPa, elongation at rupture ε_p=19%;
- chitosan-pectin (ХАН-П) formed out of 4%-ого pectin solution and 2%-ого chitosan solution by the method of layer-after-layer application on a plate, proportion of chitosan and pectin being 1:2; 30 mcm film thickness; E=7.11 GPa, σ_p=95 MPa, ε_p=1.6%;
- chitosan-alginate (ХАН-А) was formed by layer-after-layer application of a 4% sodium alginate on a plate and 2%-ого chitosan solution in proportion 1:2; 30 mcm film thickness; E=5.42 GPa, σ_n=93 МПа, σ_p=99 MPa, elongation at rupture ε_p=17%.

With the purpose to improve strength the films immediately after formation had been warmed up within half an hour at 120 ^{o}C. It was found that film properties significantly depend on their composition and forming pH [5,6].
Application by dipping was made with the use of the following solutions: 1-3% chitosan solution in 2% acetic acid, 1% copolymer solution, 1-2% solution of apple pectin, 3% sodium alginate solution.
The ХАН and ХАН-СП coatings were applied on fish products (frozen cod fillets) by triple dipping in the following order: one dip into copolymer, pectin and sodium alginate and two dips into 1-% ХАН solution. After each dip fish was dried up in cold air flow.
While using the air-drop spraying method solutions were applied on fish by means of an atomizing device. For this purpose 1%-ХАН solution (thriple dipping) was used or 1-% pectin solution , or 3% sodium alginate solution was applied layer-after-layer and after that 1% ХАН solution was applied two times. After each spraying samples of fish were dried up in cold air flow. The results of the study are given in the Table 2.
The given data show that all the solutions and film materials help to preserve moisture in products as its content practically has not changed. Peroxide and acid numbers, volatile matters nitrogen has changed insignificantly during the four month storage period.
The microbiological characteristics of the stored products are within the set norms, their organoleptic indices comply with good quality of these types of products.
The biological resistance of chitosan films with respect to E.coli, Aspergillus niger bacteria and soil microorganisms has been studied. It was found that to make chitosan films biodegradable you need not only bacteria but oxygen as well – in anaerobic conditions the films do not act as nutritious substrate.

Conclusion

The carried out studies showed that to obtain films and coatings of good quality the ash content of chitosan shall not exceed 1.5%, deacetilation rate shall be within 0.80 – 0.90, optimal dynamic viscosity of 3-% solution shall be within 1000 – 15000 MPa which corresponds to molecular mass of 150000 – 200000.
It was found that chitosan and poly-acid solutions can be used for manufacturing films and application of coatings on fish products in the form of solutions both by means of dipping and by spraying (atomizing) as well. Such coatings help to preserve good quality of fish produce for a long shelf-life.

Biological testing of chitosan-based coatings with respect to a natural mixture of microorganisms made it possible to conclude that biological degradation of the used material will take rather short time in natural conditions.

Table 2 – Changing of physical-chemical and microbiological characteristics of fish products during storage

Characteristic	Shelf-life, months	Frozen fillets of cod, (dipping)				Cold-smoked herring, (packing into films)			Hot-smoked Pink salmon, (spraying)				
		reference	ХАН	ХАН-П	ХАН-СП	reference	ХАН	ХАН-П	reference	ХАН	ХАН-П	ХАН-А	ХАН-СП
Moisture content, %	1	81.9	81.5	83.4	81.4	68.5	67.1	68.2	66.7	66.1	64.3	67.5	67.3
	2	82.5	82.1	84.0	82.2	67.7	66.9	68.3	66.6	65.9	67.4	68.2	67.0
	4	82.9	81.8	83.9	82.3	67.1	67.8	67.2	68.0	66.0	-	-	-
Water-retaining, Ability, %	1	53.8	54.5	49.4	43.8	62.1	63,4	63.2	52.7	54.3	61.3	65.1	73.0
	2	54.1	43.0	45.4	44.2	63.2	66.1	63.4	54.8	55.0	70.5	73.3	70.1
	4	53.8	50.0	44.7	43.8	63.5	67.0	59.1	59.9	60.8	-	-	-
Volatile matters nitrogen, mg/100 г	1	8.2	7.8	7.3	8.0	-	-	-	-	-	-	-	-
	2	7.9	7.8	7.2	8.2	-	-	-	-	-	-	-	-
	4	7.9	8.4	7.5	8.9	-	-	-	-	-	-	-	-
Acid number, mg КОН/g	1					31.2	27.0	24.7	24.8	22.7	23.8	23.4	22.3
	2					32.4	27.2	25.1	26.3	23.0	26.2	26.0	25.1
	4					34.8	30.1	25.3	28.4	25.2	-	-	-
Peroxide number	1					27.0	23.6	29.3	29.1	23.0	2.7	1.0	1.6
mol ½ O/kg	2					35.8	33.8	36.5	33.7	29.4	5.9	8.0	5.5
	4					40.1	37.9	38.0	43.5	37.2	-	-	-
КМАФАнМ, КОЕ/г	1	$2.5\cdot10^3$	$<10^1$	$1.8\cdot10^3$	-	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$5\cdot10^2$	$<10^1$	$<10^1$
	2	$4.8\cdot10^3$	$<10^1$	$1,3\cdot10^2$	-	10^1	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$1,3\cdot10^2$	$<10^1$	$<10^1$
	4	$5.4\cdot10^3$	$<10^1$	$<10^1$	-	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	-	-	-

References

[1] Antarctic krill: Reference book/ Edited by V.M. Bykova. – M.: VNIRO , 2001. – 207 p.

[2] Gerasimenko D.V., Avdienko I.D., Bannikova G.E. et al. Antibacterial activity of low molecular chitosan//Modern trends in chitin and chitosan studies: Materials of the Seventh International Conference. – M.: VNIRO, 2003, p.233 – 238.

[3] Sudarshan N.R., Hoover D.G., Knorr D. Antibacterial action of chitosan // Food Biotechnol. – 1992. – V.6. - № 3. – P.257 – 272.

[4] Vaara V. Agent that increase the permeability of the outer membrane // Microbiol. Revs. – 1992. – V.56. - № 3. – P.395 – 411.

[5] G.A. Vikhoreva, L.S.Galbreikh , Films and fibres// "Chitin and chitosan: obtaining, properties and application" Edited by K.G.Skrybin, G.A. Vikhoreva, V.P. Varlamov, "Nauka", M., 2002, 365 p.

[6] V. Krasavtsev, G. Maslova, E. Degtyareva, V. Bykova, L. Noudga // 5th Asia Pacific chitin-chitosan Symposium. Bangkok, Thailand, 2002, p.121.