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CHARACTERISTICS AND APPLICATION OF BIOPOLYMERS FOR MICROENCAPSULATION OF BIOLOGICALLY ACTIVE SUBSTANCES

Abstract

In the feeding of highly productive agricultural animals and poultry, an important role belongs to the quality and safety of compound feed products, for the production of which premixes or preparations of biologically active substances are used. Increasingly, probiotic supplements based on live microorganisms are used in the feeding of farm animals and poultry, which, among other things, provide stable immunity to infectious diseases without using antibiotics in the diet. As a result, the general condition improves and the productivity of animals and the quality of livestock products increase. Today, one of the urgent issues is the stabilization of probiotic microorganisms. Thus, the microencapsulation technology allows you to cover the particles of the probiotic supplement with an individual shell to obtain a product with a directed effect and an adjustable time of release of the constituent substance. The article deals with protein metabolism during feeding of highly productive breeds of cattle. Methods of cell stabilization and advantages of stabilized forms of probiotics are presented. Technologies and types of shell materials widely used for microencapsulation of probiotics are analyzed. The use of a specific microencapsulation technology or material depends on the properties of the specific probiotic strain. Organic and inorganic substances, synthetic materials and natural biodegradable polymers are used to stabilize the cells of microorganisms. The most popular biopolymers used for microencapsulation are considered in detail - alginates, carrageenans, chitosan, starch, gum arabic, pectin, gelatin, whey protein. The structural composition, production methods, functional characteristics, volumes of global production of the considered biopolymers are given. The use of microencapsulation of probiotic microorganisms with natural biopolymers for the purpose of stabilizing probiotics in animal feed is an innovative approach.

Key words: biopolymers, microencapsulation, probiotics, high-performance animals, biologically active substances, protein.

Introduction

Animal husbandry is important for ensuring the country's food security.

Recently, highly productive cows of Holstein, Ukrainian black-spotted and red-spotted Holstein breeds with a productivity of over 6,000 liters of milk per lactation have been bred in Ukraine. The uniqueness of high-yielding cows lies, first of all, in the high level of transformation of feed energy into milk, thanks to the diverse microbiota of the gastrointestinal tract.

Complex and interdependent processes take place in the rumen of highly productive cows, the violation of which can even lead to the death of the animal.

Part of the crude protein that is broken down in the rumen provides rumen microorganisms with ammonium and non-protein nitrogen. This contributes to the assimilation of carbohydrates and the synthesis of microbial protein (Fig. 1). The rest of the protein that is not broken down in the rumen (bypass) moves to the intestine, where it is hydrolyzed to amino acids. Detoxification of ammonia occurs in the liver, where urea is synthesized, which is excreted in the urine. Urea can also be reabsorbed in the renal tubules, return to the scar with saliva or through the mucosa, and be reused for digestion [1].

Recently, the production of feed probiotic supplements has increased, the feeding of which to large cattle leads to an improvement in the general condition of the animals, an increase in milk productivity and quality indicators of milk.

Probiotics are live microorganisms that, when administered naturally, have positive effects on the physiological, biochemical, and immune reactions of the animal's body through the optimization and stabilization of its microbiota [2].

An urgent issue is the stabilization of probiotic microorganisms, which is the fixation of cells of microorganisms in a certain phase with the possibility of interphase interaction. The physico-chemical features of this technology make it possible for polyenzyme systems to function for a long time, independent of exogenous factors.

Organic and inorganic substances, synthetic materials (polyethylene, nylon, polyurethanes) and natural biodegradable polymers (pectin, alginate, chitosan, carrageenan, etc.) can be used to stabilize the cells of microorganisms [3].

Cell stabilization methods are divided into three groups: binding on a solid carrier, inclusion in the spatial structure of the carrier, and stabilization using membrane technology [3].

Advantages of stabilized forms of probiotics:

• a high concentration of bacterial cells in the sorbent makes it possible to produce stable drugs;

- adsorption of metabolic products allows to increase the survival of bacterial cultures;
- stabilization gives cells resistance to freezing and freeze-drying;
- increases the survival rate of probiotic cultures in fermented milk products;
- makes bacterial cells more resistant to the influence of negative environmental factors, as well as the influence of gastric juice and bile acids during their survival in the gastrointestinal tract;
- increases the shelf life of probiotics [3].

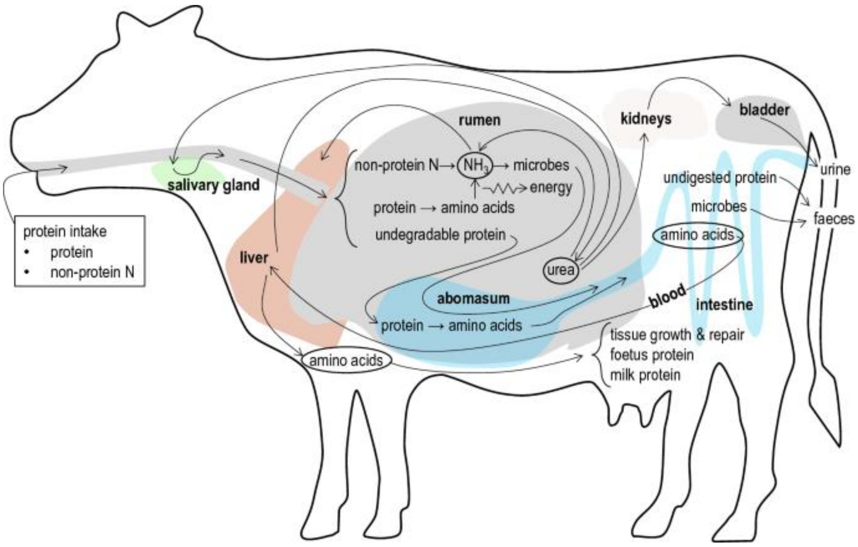

Fig. 1. Protein metabolism during cattle feeding

The purpose of the study

The purpose of the work is to analyze and generalize information about the sources, properties and prospects of using biopolymers for microencapsulation of biologically active substances.

Results and its discussion

Microencapsulation is a technological process of packaging solid particles of a substance in an individual shell, which allows you to obtain a product with a directed effect and an adjustable time of the start of the release of the constituent substance, which is achieved by applying shells with different physicochemical properties.

Technologies and types of shell materials that are widely used for microencapsulation of probiotics are listed in table. 1. The use of a specific microencapsulation technology or material depends on the properties of a specific probiotic strain [4].

Table 1 – Microencapsulation technologies of probiotics [4]

Technology				
Spray drying	Spray cooling	Coating with air suspension	Extrusion	Simultaneous extrusion
Shell material				
Water-soluble polymers	Lipids, wax	Insoluble and water-soluble polymers, lipids, wax	Water-soluble polymers	Water-soluble polymers
Stages of microencapsulation				
introduction of cells of microorganisms into the solution	the introduction of cells of microorganisms into the melt	preparation of the coating melt (solution)	dissolution cells in the polymer solution	serving cells in the internal inlet nozzle (nozzle)
↓ spraying into an aerosol	↓ spraying into an aerosol	↓ fluidization of cells of microorganisms	↓ submission of the solution into the bath-collector	↓ feeding the polymer to the external inlet of the nozzle
↓ evaporation of the solvent	↓ cooling $t < t_{melting}$	↓ spraying small drops of material	↓ cross-linking of the polymer with divalent ions	↓ dripping cells and polymer in the bath collector
↓ department powder	↓ coating hardening	↓ drying	↓ compression polymer by thermal activation	↓ cross stitching polymer with divalent ions
		↓ freezing	↓ complex form ation with polyelectrolytes	↓ polymer thickening by thermal activation
		↓ crystallization of the coating with the base inside		

The most popular biopolymers used for microencapsulation are alginates, carrageenans, chitosan, starch, gum arabic, pectin, gelatin and whey protein.

Alginates are most often used for encapsulation of microorganisms. These are biopolymers that are extracted from the walls of brown algae (Phaeophyceae), Ascophyllum nodosum, Laminaria hyperborea, Laminaria japonica, Macrocystis pyrifera and Laminaria digitate, and some bacteria (Azotobacter vinelandii, Pseudomonas spp.) [3, 5].

Alginates are produced with a wide range of molecular weight, which depends on solubility, swelling time, and viscosity. The viscosity of alginate solutions decreases with increasing temperature, and depolymerization may occur at high temperatures. Gelation of alginate is explained by the association of blocks built from α-L-guluronic acid with the participation of a cation [6].

The film-forming properties of alginates are used to apply a protective coating to tablets and dragees. In recent years, alginates have been used for medicinal preparations in the form of capsules and microcapsules, for which previously gelatin was mainly used. The introduction of alginates into the gelatin mass allows creating a qualitatively new type of capsules with selective solubility in certain parts of the gastrointestinal tract.

Alginates are practically safe, are not absorbed into the blood, and are well tolerated by the body. Water-soluble alginates in solutions from 4 to 6% form practically non-toxic gels, in higher concentrations - gelatinous or pasty mixtures.

More than 25,000 tons of alginates are produced and consumed annually in the world. Sodium alginate is in the greatest demand, the main producers of which are the USA, France, China and Japan [7].

Characteristics of sodium alginate are given in Table 2.

Table 2 – Characteristics of sodium alginate [8]

Indicator	Norm
Appearance	fibrous or granular powder
Color	white or yellowish
Scent	almost odorless
Viscosity, mPa	800 - 900
pH	6,0 - 8,0
Mass loss during drying, %	not more 15
Water-insoluble substances, %	not more 2
Mass fraction of the main substance, %	90,8 - 106,0
Sulfate ash content, %	18 - 27
Formaldehyde, mg/kg	not more 50
Arsenic, mg/kg	not more 3
Lead, mg/kg	not more 5
Mercury, mg/kg	not more 1
Cadmium, mg/kg	not more 1
The total content of bacteria, CFU/g	≤ 5000
Fungi and yeast, CFU/g	≤ 500

Carrageenans are natural sulfated polysaccharides found in red seaweed Chondrus Crispis, Eucheuma Species, Gigartina Species, etc. By chemical nature, carrageenan is close to agaroids and is an unbranched sulfated heteroglycan, the molecules of which are built from the residues of D-galactopyranose derivatives with a strict alternation of α-(1,3)- and β-(1,4)-bonds between them [9].

Depending on the degree of polymerization and esterification, carrageenans are classified into three groups:

- κ-carrageenan (kappa-carrageenan) forms strong, hard gels;
- λ-carrageenan (lambda-carrageenan) does not have a gel-forming variety, it forms gels in a mixture with proteins, not water; used to promote viscosity in sweet dough, dairy products;
- ι-carrageenan (iota-carrageenan) is an elastic gel that is not capable of syneresis and has thixotropic properties.

Carrageenans with different amounts of sulfate groups, which combine with metal ions in different ways, are obtained from plant sources [10].

Due to their physical and chemical properties, carrageenans have a wide range of applications. About 70% of carrageenans produced in the world are used in the food industry: in the production of dairy (chocolate milk, sherbets, homemade cheese, cheese pastes, baby food), meat and fish products (canned food, sausage casings, jelly coatings), seasonings, non-alcoholic beverages, bakery products (bread dough, fruit cakes, sugar glazes) and confectionery products [11-12].

Carrageenans are used as gelling agents and emulsion stabilizers in milk and water-based systems, as well as to improve the properties of other gels due to the ability of carrageenans to form complexes with other hydrocolloids [13-14].

About 60 thousand tons of carrageenans are produced annually in the world, the main industrial production is concentrated in Indonesia, the Philippines, the USA, France, Canada and Chile.

Chitosan is a polysaccharide, dietary fiber of animal origin, which is obtained by alkaline treatment of chitin. By its nature, chitin is one of the three most common polysaccharides, after cellulose and starch, which have found wide use in the food industry. Researchers and entrepreneurs see the same potential for chitin [15].

Significant interest in the natural polymer chitosan is due, first of all, to its presence of a number of unique properties, such as biocompatibility, biodegradability, non-toxicity, high sorption capacity for metal ions [16].

Today, the volume of chitosan production in the world reaches 3500 tons per year. Unlike other polysaccharides, chitosan has a primary amino group in its composition, which makes it possible to create a wide range of derivatives on its basis under acceptable synthesis conditions, and also gives it the properties of a chelating polymer. The use of chitosan in the food industry is due to its biological activity, emulsifying, oil-retaining and fat-retaining ability, as well as the ability to improve the rheological characteristics of the food mass [17-19].

An important property of this polysaccharide is its ability to interact with proteins, form emulsions, gels, act as a stabilizer and antioxidant [20].

Starch is a polysaccharide consisting of a large number of monosaccharide glucose residues connected by glycosidic bonds. It has two types of molecules - amylose (usually 20-30%) and amylopectin (usually 70-80%). Both forms consist of polymers of α-D-glucose residues. Unlike amylopectin, amylose performs a useful function as a hydrocolloid. Chemical modifications of starches such as cross-linking, oxidation, acetylation and hydroxypropylation can provide certain beneficial changes in functionality. Resistant starch, i.e. starch that is not digested, is an ideal surface for the adhesion of probiotic microorganisms, their storage and passage through the gastrointestinal tract. Mixing starch with κ-carrageenan, alginate, xanthan, and low molecular weight sugars are often used in microencapsulation because they reduce the reactivity of starch. Starch derivatives, mainly hydrolyzed forms such as dextrins and maltodextrins, are also often used as carriers for spray and freeze drying [3].

Ukraine is one of the world's leading producers of corn starch. In recent years, there has been a tendency to increase the production of corn starch in the country. This is due to the growing demand for corn starch as a stable raw material for the food and non-food industries [21].

Gum arabic is a hydrocolloid from acacia exudate. Due to the high content of natural dietary fibers (up to 90% in terms of dry matter) and low energy value (2kcal/g), gum arabic is recommended for use in health and dietary products, performs a prebiotic and hypoglycemic function, contributes to the maintenance of human immunity by stimulating growth and development of its bifido- and lactobacilli, can be associated with other important components of nutraceuticals, in particular with polyphenols and mineral substances.

The functional and technological properties of gum arabic are due to the peculiarities of its structure. According to its chemical structure, gum arabic belongs to glycoproteins, the molecules of which contain fragments of polysaccharide and protein nature. The total protein content is about 2% (sometimes 1%), but in individual fractions the protein can be up to 25%. The polysaccharide fraction of gum arabic consists of residues of galactose (45-46%), arabinose (23-24%), rhamnose (13-14%) and glucuronic acid (14-16%) with the presence of small amounts of arabofuranose and methylglucuronic acid units. The protein component plays an important role in the functionality of gum arabic. The simultaneous presence of hydrophilic carbohydrates and hydrophobic protein gives the gum arabic molecule emulsifying and stabilizing properties [22-23].

The total production of gum arabic is estimated at 70 tons per year. Europe is the largest market for chewing gum, with France and Great Britain being the main importers. Outside of Europe, the largest market for gum arabic exists in the United States [24].

Pectin is a component of plant cell walls that protects against the invasion of microorganisms. Pectins consist of α-D-galacturonic acid, which is interrupted by rhamnose residues. A significant difference between pectins is the content of methyl esters in them. Pectins with a high degree of esterification form gels due to hydrophobic interaction and the formation of hydrogen bonds between pectin molecules. Pectins with a low methoxyl content form gels in the presence of di- and polyvalent cations, which form crosslinks and neutralize the negative charge of the pectin molecule [3, 25].

The world production of pectin is concentrated in Europe (Germany, Switzerland, etc.), South America (Argentina, Brazil), South Africa, China, Iran, etc. The volume of production is about 30 thousand tons per year. CP Kelco (USA) is the world's largest producer of pectin. The largest pectin factory in the world, Kobenhagen Pektinfabric (Denmark), plays a significant role in global production. The German company "Herbstreith & Fox KG" (Germany) occupies the second place in terms of production volume [26-27].

Natural pectins of fruits and berries are an important part of the human diet, the basis of many medicines and biologically active supplements. The use of pectin in drug technology is based on its functional characteristics and technological purpose. The use of pectin for the encapsulation of medicines ensures a gentle (gentle) mode of their absorption in the gastrointestinal tract. Due to the presence of carboxyl groups of galacturonic acid, pectins can bind heavy metal ions in the alimentary tract, followed by the removal of insoluble complexes from the body [27].

Gelatin is a high-molecular-weight polypeptide obtained from the partial hydrolysis of collagen, which forms the main component of connective tissues of animals, such as bones and skin [28-30].

Collagen hydrolytic processes can be divided into three groups: physical, chemical and enzymatic [31-34].

Functional properties of gelatin are related to chemical characteristics. Gel strength, viscosity and melting point of gelatin depend on its molecular mass distribution and amino acid composition. The amino acids proline and hydroxyproline play an important role in the renaturation of gelatin subunits during gelation. As a result, gelatin with a high amino acid content has a higher gel strength and melting point.

Table 3 shows a comparative analysis of the amino acid composition of gelatin of different origin.

Gelatin is an important hydrocolloid and is used as a stabilizer, thickener, emulsifier, foaming and wetting agent for food, pharmaceutical, medical and technical applications due to its surface-active properties, as well as for encapsulation of food additives and active substances [38-39].

Gelatin capsules protect active substances from moisture, high temperature or other extreme conditions, thus increasing their stability [40].

Whey protein is a protein obtained from whey and buttermilk [15, 41].

Whey protein is albumin, so it dissolves in water at a pH of 6.6. The hydration capacity of whey protein concentrates is 0.45–0.52 g of water/g of protein [41, 42].

The annual world production of whey proteins is about 600,000 tons [41].

Table 3 – Amino acid composition of gelatin of different origins (number of residues/1000 total amino acid residues) [35-37]

Amino acids	Pig skin	Cattle skin
Asx (asparagine residues and aspartic acid)	46	44
Threonine (Thr)	18	17
Serin (Ser)	36	29
Glx (glutamine residues and glutamic acid)	83	74
Glycine (Gly)	355	341
Alanine (Ala)	116	115
Valin (Val)	24	21
Methionine (Met)	5	5
Isoleucine (Ile)	12	11
Leucine (Leu)	25	25
Tyrosine (Tyr)	3	1
Phenylalanine (Phe)	14	12
Histidine (His)	5	5
Lysine (Lys)	29	26
Arginine (Arg)	53	48
Proline (Pro)	90	123
Hydroxylysine (Hyl)	-	7
Hydroxyproline (Hyp)	86	96
In total	1000	1000

Whey protein is extremely popular due to its ability to form films and is used as a protective material during spray drying, which is the final step in the preparation of water-soluble microcapsule systems [3].

Conclusions

Microencapsulation is a technological process of covering the outer surface of particles with film-forming substances, which will make it possible to obtain a product with a directed effect and an adjustable duration of action.

The use of natural biopolymers in the microencapsulation technology for the stabilization of probiotic microorganisms is an innovative approach to increasing the efficiency of the use of probiotic additives in the feeding of farm animals.

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ХАРАКТЕРИСТИКА ТА ЗАСТОСУВАННЯ БІОПОЛІМЕРІВ ДЛЯ МІКРОКАПСУЛЮВАННЯ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Анотація

В годівлі сільськогосподарських високопродуктивних тварин і птиці важлива роль належить якості та безпечності комбікормової продукції, для виробництва якої застосовують премікси або препарати біологічно активних речовин. Все частіше в годівлі сільськогосподарських тварин і птиці використовують пробіотичні добавки на основі живих мікроорганізмів, які в тому числі, забезпечують стійкий імунітет до інфекційних хвороб, не використовуючи в раціоні антибіотиків. В результаті покращується загальний стан та підвищується продуктивність тварин та якість тваринницької продукції. Сьогодні одним з актуальних питань є стабілізація пробіотичних мікроорганізмів. Так, технологія мікрокапсулювання дозволяє покривати частинки пробіотичної добавки індивідуальною оболонкою для одержання продукту зі спрямованою дією та регульованим часом вивільнення складової речовини. В статті розглянуто метаболізм протеїну при годівлі високопродуктивних порід великої рогатої худоби. Наведено методи стабілізації клітин та переваги стабілізованих форм пробіотиків. Проаналізовано технології та види матеріалів оболонки, які широко використовуються для мікрокапсулювання пробіотиків. Використання конкретної технології мікрокапсулювання або матеріалу залежить від властивостей конкретного пробіотичного штаму. Для стабілізації клітин мікроорганізмів використовують речовини органічної та неорганічної природи, синтетичні матеріали та природні біодеградовані полімери. Детально розглянуто найбільш популярні біополімери, які використовують для мікрокапсулювання – альгінати, карагінани, хітозан, крохмаль, гуміарабік, пектин, желатин, сироватковий протеїн. Наведено структурний склад, способи отримання, функціональні характеристики, об'єми світового виробництва розглянутих біополімерів. Використання мікрокапсулювання пробіотичних мікроорганізмів природними біополімерами, з метою стабілізації пробіотиків в годівлі сільськогосподарських тварин, є інноваційним підходом.

Ключові слова: біополімери, мікрокапсулювання, пробіотики, високопродуктивні тварини, біологічно активні речовини, протеїн.

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