

Possibilities for Application of Cellulose Derivatives under Cryoconservation of Probiotics

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Abstract

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Decoding mechanisms for hypo- and anabiosis at low temperature adaptations is of primary importance to the life activity of all organisms.

The collection of many scientific investigations in the area of cryobiology has determined lots of factors, destroying the biological systems under cryogenic treatment.

Up to the present there is no information available regarding the application of cellulose derivatives as cryoprotective media during freezing and lyophilization of foods and microorganisms- lactic acid bacteria, yeasts etc.

In the paper the authors present the possibilities for application of some types of modified synthetic hydrocolloids (Na-carboxymethylcellulose and methylcellulose) as cryoprotective media for probiotics cryoconservation.

Key words: freeze-drying, cellulose derivatives, lactic acid bacteria, cryoprotection

Introduction

Until now the achieved purposes in the area of cryobiology would not have been possible if there have not been found various protective compounds, the so-called cryoprotectors, that are able to eliminate either some or all factors destroying the biological systems by cryogenic treatment. Complex chemical and biological solutions, serums, polymers or other substances, that cause partial cell dehydration, are used as cryoprotectors. Cryoprotection is based on

examination of natural models, which shows that cells preliminarily subjected to partial dehydration are able to survive in the environment (Belouse et al., 1985).

The defensive properties of cryoprotectors depend mainly on their ability to form hydrogen bonds with the cell liquid phase in contrast with the large quantity of nonvolatile electrons with strong chemical reactional ability (Pushkar et al., 1984).

The mechanism for defensive action is due to the ability of nonelectrolyte cryoprotective solutions to decrease the effec-

tive concentration of salts during the phase of ice formation at each temperature, lower than the freezing point. As a result of adding cryoprotector to the frozen cells, the increase of salt concentration never reaches the critical level that might cause biological damage.

These data are of great importance because of their application in the sphere of low temperature cell conservation, cell suspensions, vegetable and animal products etc.

One of the contemporary directions in the area of food biotechnologies is the application of polymeric systems, including hydrocolloids.

The term "hydrophilic colloids" is used as a synonym of the term- rubber. These polymeric compounds can be dissolved and dispersed in warm or cold water and form strong viscose solutions and dispersions.

According to their origin, the hydrocolloids can be classified as follows:

- Natural - agar, alginates, pectins, carragenans, gelatins, arabian rubber etc.
- Modified or semisynthetic - chemical derivatives of natural substances, f.ex. modified starch or cellulose;
- Synthetic - synthetic chemical products, f.ex. polyvinylpyrolidin etc.

On the basis of scientifically grounded principles, these polymeric matrixes of natural and synthetic origin with specific properties have influence on:

- Biochemical processes in the respective biological system;
- Playing the role of protective substances with antimicrobial activity as a result of their barrier properties;
- Playing the role of polymeric matrixes for immobilization of biologically active compounds, enzymes, cell materials, medicinal substances etc.

A great number of the synthetic and semisynthetic hydrocolloids have been slightly used in the food industry. Natural and modified hydrocolloids (such as methylcellulose, carboxymethylcellulose, low-esterified pectin, propyleneglycol alginates etc.), are more often used as qualitative indicators of some food products. Under their influence the quantity of the free water can be changed, respectively various forms of water bonds that determine the physical properties of the products. Furthermore, they influence on the technological processes- slow the water evaporation, change the speed of water freezing in products, change the rheological properties etc., i.e. their properties are of essential importance during lyophilization of biomaterials of various origin.

In the Table 1 some of the technological properties of modified hydrocolloids are presented, especially those which are of great interest from the point of view of their cryogenic treatment.

In the food industry widely used is Na-carboxymethylcellulose (CMC) that is obtained by consecutively processing pure cellulose with NaOH and chloracetate. The process of producing this cellulose derivative can be controlled as to be obtained products with different degree of substitution of hydroxyl groups, polymerization and evenness of the substitution along the whole length of the cellulose molecule. These indicators determine the properties of the product, i.e. the dissolution of derivatives and properties of their solutions. Theoretically, OH-groups can be substituted by each glucose ring. The level of substitution varies from 0.4 to 1.2 while in the food industry - in the range from 0.65 to 0.85 averagely for the whole cellulose molecule (Figure 1).

Up to present moment no data can be

Table 1
Technological properties of some modified cellulose derivatives

Hydrocolloids	Water bonding and emulsion stabilization	Improvement of texture	Stabilization density	Stabilization of milk emulsions and suspensions	Gelling properties	Improvement of dry foods rehydration
Na-carboxy methyl-cellulose	+	+	+	+	+	+
Methyl-cellulose	+	+	+	+	+	+

found regarding the application of cellulose derivatives as cryoprotective media during freezing and lyophilization of foods and microorganisms lactic acid bacteria, yeasts etc. (O'Brien et al., 1999). The variety and specificity of polymeric compounds on one hand, together with the differences among separate groups of microorganisms, differences among strains from one and the same type, requires the most suitable cryoprotective medium be found through set of experiments.

Main objective: Investigations of possibilities for the application of some types modified synthetic hydrocolloids (Na -

carboxymethylcellulose and methylcellulose), as cryoprotective media during cryopreservation of probiotics.

Materials and Methods

For the objective of the experiment, the following hydrocolloids have been used:

1. Na - carboxymethylcellulose ("Tylopur C 1000 p") - E 4466, imported from "Clariant", Germany
2. Methylcellulose "Avicel" - FMC (USA)

The above-mentioned hydrocolloids were selected because of their wide ap-

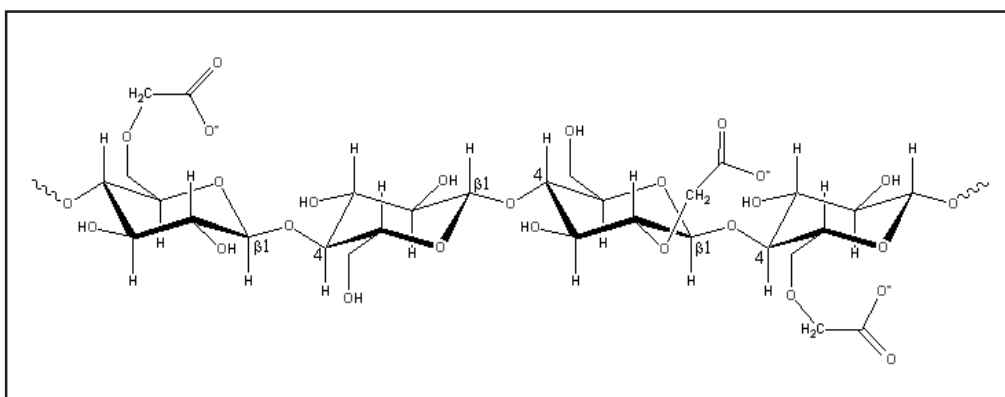


Fig. 1. Chemical structure of carboxymethylcellulose

plication in the food industry: safety, neutral taste and aroma, high ability to enter into water -bonding activity and formation of gel -like products (Obretenov, 2002).

The quantitative content of cellulose derivatives was considered with their chemical composition, colloid properties, as well as the chemical composition of the media and specifics of the biochemical activity of lactic acid bacteria.

In the investigation conducted we have used the following lactic acid microorganisms, provided by the available collection of Institute of Cryobiology and Food Technology (ICFT) and National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC).

- *Lactobacillus bulgaricus*, strain 3556;
- *Streptococcus thermophilus*, strain 1374
- *Lactobacterium acidophilus*; strain 1379
- *Bifidobacterium bifidum*, strain 1370;

Analyses:

1. Physicochemical analysis:
 - Solubility of hydrocolloids- in compliance with a certificate;
 - Determination of the dry content substance after lyophilization- modified method - electronic scales "Sartorius" equipped with infrared heating of the samples is used;
 - Viscosity -viscosimeter "RHEOMAT 108"- "Contraves"
2. Microbiological investigations:
 - **Determination the total number of viable lactic acid bacteria-** according to the method of the limited dilutions by Mc Crady;

• **Calculating the survivability of lactic acid microflora (%)** -according to the following formula:

$\% \text{ survived cells} = (\text{number of survived cells} \times \text{number of cells } 0) \cdot 100,$

where,

x- moment of calculating survivability (at the 48-th hour after samples lyophilization)

0- moment before lyophilization (number of viable cells before lyophilization).

• **Microscopic picture** of the preparations used, samples coloured in methylen blue by Löffler.

3. Technological approaches:

Technological investigations, respectively freeze-drying of lactic acid bacteria, have been conducted in a laboratory freeze-drier with conductive heating. The layer thickness of cultures is 6-8 mm, and the residual pressure in the freeze-drier is in the range from 10^{-1} - 10^{-2} mm Hg (Tsvetkov et al., 1985). The bacteria cultures were preliminary frozen in aluminum and glass containers. As a medium for strain cultivation was used skimmed cow milk. Cultivation of the strains *Lb. bulgaricus* and *Str. thermophilus* has been implemented at temperature 43° C within 5-9 hours whereas strains *Lactobacterium acidophilus* and *Bifidobacterium bifidum* at temperature 37° C within 5-8 hours.

During the technological experiments we have tested and developed four variants, both are control samples.

1. Control 1- strains of lactic acid bacteria without cryoprotector.

2. Control 2- strains of lactic acid bacteria with 10% solution of sucrose, as a proved cryoprotective medium in practice.

3. Variant 1- strains of lactic acid bacteria with 0,5% solution of Na - carboxymethyl cellulose

3. Variant 2- strains of lactic acid bacteria with 0,5% solution of Methylcellulose.

The survivability of the lactic acid bacteria in the process of freezing in fridge cameras (-35° C - 40° C), has been traced and in the subsequent lyophilization and conservation as well.

Results and Discussion

In the process of investigations and experiments conducted we have been determined high survivability of the lactic acid microflora in all types of samples, containing cellulose derivatives, by optimal concentrations at freezing and after freeze-drying. The results are presented in Figure 2 and shows that the lowest survivability of microorganisms is both in the

control sample 1 - without cryoprotector and the control sample 2 - with 10% solution of sucrose as a cryoprotective medium.

Consequently, the modified synthetic hydrocolloids, used by us in the course of experiments, show colligative properties, i.e. more effectively bond water in the product in comparison with the conventional cryoprotector - sucrose (Lee et al., 1981). This might be due to the hydrogen bonds stabilization, existing between hydrocolloids and proteins which can not be destroyed during freezing and lyophilization.

The results prove also that under the influence of cryoprotectors of hydrocolloid origin, the resistance of microorganisms has been increase during lyophilization.

	<i>Lb. bulgaricus</i>	<i>Str. thermophilus</i>	<i>Lb. Acidophilus</i>	<i>Bifidobacterium bifidum</i>
Control 1	23.2	70.4	40.5	48.9
Control 2	40.8	81.5	56.8	50.6
Variant 1	50.3	90.4	68	67.8
Variant 2	45.6	87.9	67.4	65.3

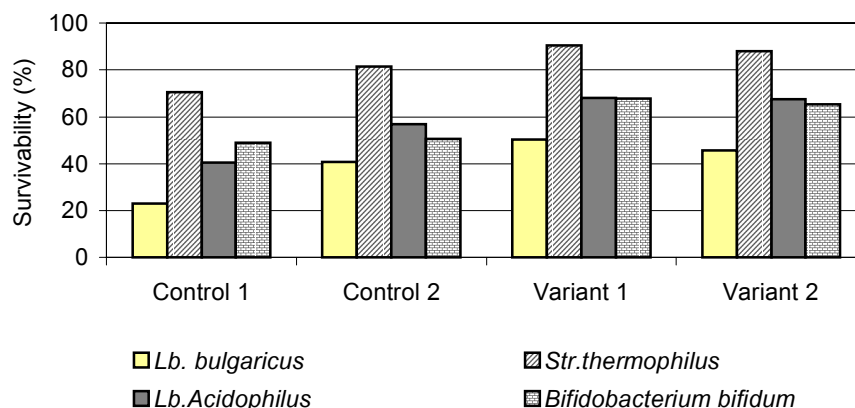


Fig. 2. Influence of modified cellulose derivatives on survivability of lactic acid bacteria after freeze-drying

	<i>Lb.</i> <i>bulgaricus</i>	<i>Lb.</i> <i>acidophilus</i>	<i>Bifidobact.</i> <i>bifidum</i>	<i>Str.</i> <i>hermophilus</i>
Control 1	20.3	37.0	40.0	68.2
Control 2	38.4	48.2	44.2	80.0
Variant 1	49.0	67.0	62.5	87.0
Variant 2	44.2	65.4	64.0	84.0

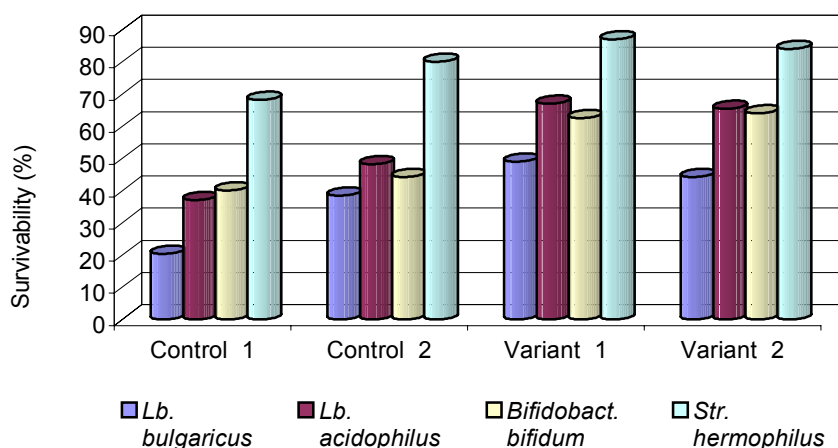


Fig.3. Influence of modified cellulose derivatives on survivability of lactic acid bacteria during 24-month shelf-life

A subject of interest is the changes that appear in the activity of lactic acid microorganisms during extended shelf life (Carpenter et al., 1996).

The investigations conducted into lyophilized lactic acid bacteria, preserved in vacuum packages of 3-layered aluminum foil, have not indicated any changes in their survivability and activity during 12-monthly shelf life under normal conditions and after 24 months - while the survivability of *Lb. bulgaricus*, *Lactobacterium acidophilus* and *Bifidobacterium bifidum*, respectively decrease to 70 % in the control sample 1 without cryoprotector, and 40 % in the Control sample 1, Variant 1, and Variant 2, containing cryoprotectors, as presented in fig.3.

Regarding the survivability of *Str. thermophilus* during extended shelf life (78%), our data coincide with those obtained by other authors (Hechly, 1991; Hughes et al., 1991). The data confirm the high stability of cocci under thermal treatment in comparison with the rod-like lactic acid bacteria which are thermolabile.

Conclusions

Modified cellulose derivatives- Na-CMC and methylcellulose are effective cryoprotective media for lactic acid bacteria treatment at low temperatures.

Playing the role of cryoprotectors, they supply higher degree of survivability of lactic acid bacteria during extended shelf

life in comparison with the conventional cryoprotector- sucrose.

The application of Na- CMC and methylcellulose during lyophilization of lactic acid bacteria is of essential importance not only for the increasing of their resistance but for producing milk acid products, enriched with plant fibers that make them even more healthy.

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