

# Effect of the Addition of Chitosans with Different Molecular Structure on Fermentation Process and Viscosity Changes During Sour Cream Storage

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## Abstract

The effect of different health promoting chitosan-based additives (high molecular weight chitosan, carboxymethyl chitosan, chitosan oligosaccharide) with different molecular structure on pH changes during cream fermentation process and viscosity changes of sour cream during storage were studied. The inhibition of cream fermentation is much higher for chitooligosaccharide with low molecular weight and higher degree of deacetylation than that for high molecular weight chitosan with lower degree of deacetylation especially at a higher concentrations of additives. The increment of viscosity of sour cream with the increase of concentration of high molecular weight chitosan and decrease of viscosity with the increase of concentration of chitooligosaccharide have been observed. It was demonstrated that the effect of additives on viscosity can be explained by changing the interaction forces between negatively charged casein micells and by the start of gel formation during cream fermentation process at higher pH.

## Keywords

*Sour Cream; Chitosan; Viscosity; Storage; Fermentation*

## Introduction

Chitosan and its derivatives received increased attention due to their potential applications in the food industry as supplements with health beneficial properties (antitumoral, hypocholesterolaemic, antidiabetic, modulation of inflammation and immune response, antibacterial) [Friedman, 2010 - No, 2007 - Xia, 2011 - Kim, 2005 - Yin, 2009].

It is a challenge for food science on how to combine health promoting properties of chitosan with its beneficial properties for food production technology.

The term chitosan often leads to misunderstanding because it fails to identify a single substance with

specific molecular structure and is commonly used to refer to different copolymers with various content of acetylated and deacetylated units and various molecular weight [Roberts, 2007]. Various functional derivatives of chitosan can be prepared by chemical modification to create targeted molecular structure tailored to novel specific applications.

The electrostatic interactions between the protonated positively charged D-glucosamine units of chitosan and the negatively charged surfaces are responsible for the changes in the biochemical and biophysical processes in food at micro- and at nanolevel.

White bread, for example, is a complex multi-component and thermodynamically unstable food system. It has been demonstrated that adsorption of polycationic chitosan macromolecules on the negatively charged surfaces of bread components (starch, gluten) can be associated with the changes in the microstructure and physical properties of such a system [Kerch, 2008 – Kerch, 2010].

Milk in which fat globules are dispersed in water is a typical example of an oil-in-water emulsion. Food oil-in-water emulsions are also complex multi-component thermodynamically unstable systems and certainly polycationic chitosan macromolecules are expected to have an impact on the processes at the microscale and on the properties of these food products. The behaviour of chitosans in water and fermented milk solutions is expected to be distinct due to differences in composition.

There is an evident lack of information on the applications of chitosan and especially carboxymethyl chitosan and chitooligosaccharides in dairy products as well as on understanding of their mechanisms of

action related to the microstructure of dairy products. Chitosan macromolecules due to their antibacterial properties can interact with lactic acid bacteria and affect fermentation processes in dairy products.

Food products with low fat content are associated with a reduced risk of obesity and coronary heart diseases [Meunier-Goddik, 2004]. Dairy companies produce different products with reduced fat content, including sour cream. But the decrement of fat content decreases viscosity and often negatively affects sensory properties of food products. It is a challenge to maintain the quality and sensory characteristics of a food product at decreased fat content. So the use of food thickeners with health promoting properties can be beneficial for the improvement of dairy products quality and functionality. High molecular weight chitosan functions in acid environment as a thickener and stabilizer [Yin, 2009] and as a preservative are in use to extend the shelf life of various food products due to its antibacterial and antifungal properties [Wang, 1992 - Gomez-Rivas, 2004]. Casein micelles are particles with pH-dependent negative charge density. Lowering the pH to the isoelectric point of casein (pH 4.6) decreases the net electrostatic charge and repulsive steric interactions and enables the aggregation of casein micelles [Swaigood, 1996 - Zhong, 2007]. It can be expected that at the presence of cationic additives such as chitosan the casein charge will be decreased at higher pH. Chitosan can also bind most of metal ions through chelation.

It will be demonstrated in this paper that mechanisms of action can vary essentially with the changes in molecular weight and degree of deacetylation of chitosan resulting in alternative potential applications. The lack of knowledge in this field is evident and reliable successful applications will be possible only after the peculiarities of mechanism of action and related effect on properties for the different chitosans with specific molecular structure are identified. Relatively little is known also about the mechanism of action and related applications of chitooligosaccharides and a number of chitosan derivatives with various grafted side chains [Mourya, 2010] as additives in different food products [Kim, 2005 - Yin, 2009].

In order to improve the current state of knowledge about applications of chitosan and its derivatives in dairy products, it is necessary to investigate the interaction mechanisms between chitosans with varying molecular structure and various sour cream

ingredients, and to determine the influence of these interactions on the fermentation process and viscosity changes during sour cream storage.

The objective of this research is to demonstrate the effect of chitosan and its derivatives with different molecular structure on the lactic acid bacteria growth during fermentation process and to estimate, through the measurements of viscosity changes during storage, the interaction of casein micelles and fat globules with chitosan macromolecules under conditions of varied chitosan concentration in sour cream.

## Materials and Methods

### *Sour Cream Preparation*

Milk with fat content 2.5% was mixed with sour cream with fat content 35% to standardize the fat content to the 12% level. Pasteurized (85 - 95°C, 20 s), homogenized (8,0–12,5 Mpa, 60–65°C) cream formulations for sour cream are cooled to 20–24°C, a culture CHN-19 (Chr. Hansen A/S, Hørsholm, Denmark) containing mesophilic lactic acid bacteria *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc* spp., was added and mixed properly with various concentrations (0.1%, 0.5% and 1.0%) of high molecular weight chitosan, carboxymethyl chitosan or chitooligosaccharide and incubated at this temperature for 12 hours. Lactic acid bacteria converted lactose to lactic acid. Lactic acid reduced the pH from the natural pH of milk (pH 6.5–6.6) to the pH 4.6 and lower.

### *Additives*

Different additives—carboxymethyl chitosan, chitooligosaccharide, chitosan have been investigated: Chitosan—degree of deacetylation 70%, viscosity 2435 cP (Primex ehf, Siglufjördur, Iceland), Carboxymethyl chitosan – degree of deacetylation 77%, molecular weight 82000 (Kitozyme, Herstal, Belgium), Chitooligosaccharide—degree of deacetylation 85%, molecular weight < 3000 (Biotech, Nizhny Novgorod, Russia).

### *Experimental Analysis*

The pH of the samples was measured during fermentation using 3520 pH Meter (Barloworld Scientific Ltd. T/As Jenway, Dunmow, Essex, UK).

Viscosity measurements at temperature +20 ±0.5 °C

have been carried out after fermentation and after 7 days sour cream storage at temperature 5°C using a Brookfield DV-III Ultra Programmable Rheometer (Brookfield E.L.V. GmbH, Lorch, Germany). The relative viscosity has been defined as the ratio of the viscosity value after 7 days storage to the viscosity value immediately after fermentation.

Each experiment has been repeated three times, and the average value and the standard deviation have been calculated.

## Results and Discussion

### Effect of Chitosan Derivatives on the Changes in pH and on the Inhibition of Lactic Acid Bacteria Growth during Fermentation

Fig.1 shows pH changes during fermentation. Addition of chitooligosaccharide lowered the initial pH of milk from 6,6 to 6,1, but addition of high molecular weight chitosan increased the initial pH of milk from 6,6 to 6,8.

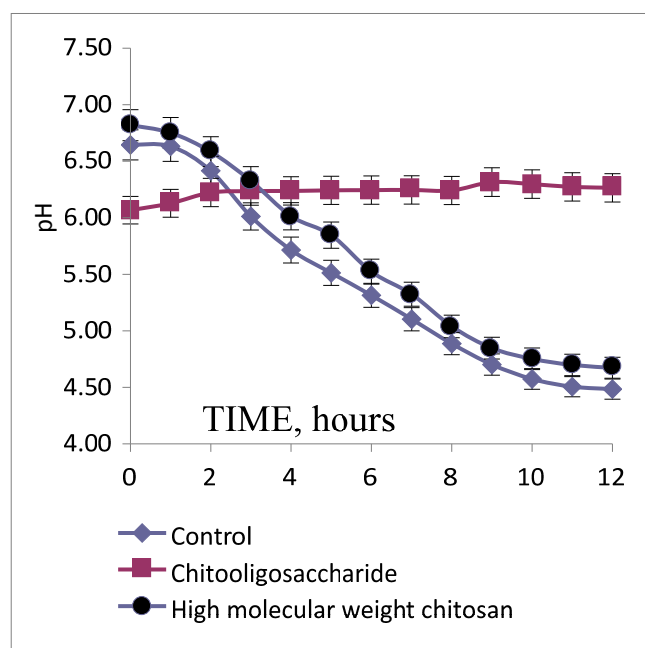


FIG.1 pH CHANGES WITH FERMENTATION TIME FOR CONTROL SAMPLE AND SAMPLES CONTAINING 1% HIGH MOLECULAR WEIGHT CHITOSAN AND CHITOLIGOSACCHARIDE

Chitooligosaccharide with low molecular weight at the concentration 1% completely prevents fermentation and pH don't decrease essentially. These data support the hypothesis that the mechanism of chitooligosaccharide antibacterial action involves interaction between the polycations of chitooligosaccharide and the anions on the bacterial

surface that changes the membrane permeability [Tsai, 1999]. But high molecular weight chitosan only slightly inhibits fermentation at the same concentration, resulting in a bit higher pH values. It was earlier observed that chitosan and chitooligosaccharide lactate inhibit fermentation during dough processing [Rakcejeva, 2011]. The process of fermentation will be allowed to proceed faster if concentration of chitooligosaccharide is decreased from 1.0% to 0.1%, Fig.2.

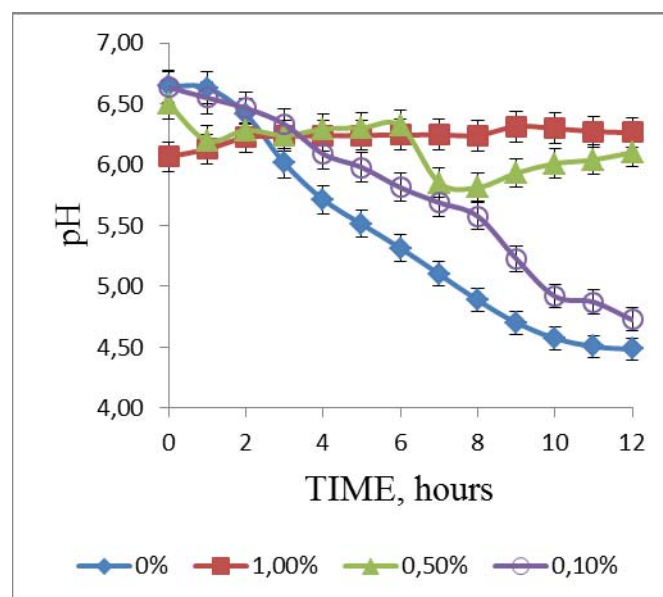


FIG.2 pH CHANGES WITH FERMENTATION TIME FOR THE SAMPLES CONTAINING DIFFERENT CONCENTRATIONS OF CHITOLIGOSACCHARIDE

It was demonstrated [Tsai, 2004] that chitooligosaccharides have much weaker antimicrobial activity against *Escherichia coli* than chitosan. Our data, presented in Fig.1 and Fig.2, show that these data by Tsai [Tsai, 2004] and the previously reported results indicating that high molecular weight chitosan markedly inhibits the growth of lactic acid bacteria and shows higher antibacterial activities compared to chitosan oligomers cannot be considered to be universally applicable.

Advantages of chitosan oligomers as antibacterial agents over the high molecular weight chitosan have been demonstrated recently [Benhabiles, 2012].

The exact mechanism of antifungal and antibacterial properties of chitosan and its derivatives is not still completely understood [Dutta, 2011] and various interpretations of experimental evidence may be considered. Badawy and Rabea [Badawy, 2011] in the recent review article summarized a number of possible mechanisms of chitosan antibacterial action. Evidently

that combination of different mechanisms is very probable and changes in molecular weight and deacetylation degree in chitosans, and degree of substitution and molecular structure and position of a substituent in glucosamine units of chitosan derivatives, as well as a pH of solution can lead to different intensity of different mechanisms for chitosan and lactic acid bacteria interaction.

Our data show that inhibition of lactic acid production by lactic acid bacteria can be explained by the mechanisms that involve changes in the cell membrane permeability due to interactions between the positively charged polycationic chitosan molecules and negatively charged microbial cell outer membranes and the mechanisms that involve penetration into the bacteria which play a major role when chitooligosaccharides are used as additives. The antibacterial activity of high molecular weight chitosan is much lower compared with low molecular weight chitosans. Goy [Goy, 2009] supposed that in the case of chitosan the mechanisms changing cell membrane permeability, mechanisms that involve penetration into bacteria and the ability of chitosan to bind and remove trace metals, which are essential trace elements for bacterial growth, act simultaneously but at different intensities. The experimental evidence, in Fig.1, provides support for the hypothesis that intensities of mechanisms that involve changes in membrane permeability and penetration into cell are low in the cases when molecular weight of chitosan is high enough. Mechanism that involves adsorption of high molecular weight chitosan molecules to lactic acid bacteria surface with the formation of a polymeric layer which prevents nutrients from entering the cell without penetration into cell better explains experimental data presented in Fig.1.

*Lactococcus lactis* and *Leuconostoc* spp., are Gram-positive bacteria and it is suggested that Gram-positive bacteria are inhibited by the binding of lower molecular weight chitosan derivatives to DNA or RNA, but high molecular weight chitosan cannot pass through cell membranes and forms a film that prevents cells against nutrient transport through the microbial cell membrane [Jarmila, 2011].

Changes in membrane permeability because of cross-linkage between the polycations of chitosan and the anions on the bacterial surface observed by Tsai and Su [Tsai, 1999] take place at lower pH compared with our data when the presence of chitooligosaccharide prevented decrease of pH during fermentation, as

shown in Fig.1. Thus it is important to emphasize that in our studies low molecular weight chitooligosaccharide prevented fermentation due to bactericidal effect also at higher pH. The similar results have been reported for chitosan-lactate derivatives regarding antimicrobial action of hydrolyzed chitosan against lactic acid bacteria [Savard, 2002].

In the studies of dongchimi fermentation, the growth of *Leuconostoc* spp. was reported to decrease after the addition of chitosan due to its antimicrobial effect [Lee, 2010]. It is noticeable that the most remarkable inhibition of lactic bacteria growth was observed when higher concentrations of chitosans with higher degrees of deacetylation have been added. In the current study, the degrees of deacetylation of high molecular weight chitosan, carboxymethyl chitosan and chitooligosaccharide were distinct. So our experimental data together with the data presented by Lee [Lee, 2010] allow for the conclusion that only high concentrations and high degrees of deacetylation (commonly associated with low molecular weight) of chitosan can essentially inhibit fermentation processes. Our data show that during fermentation high molecular weight chitosan interacts mainly with casein micelles and milk fat, but chitooligosaccharides interact mainly with lactic acid bacteria. Our experimental data suggest that in fermentation of dairy products the most appropriate is the use of low concentrations of the high molecular weight chitosan with low degree of deacetylation.

Flexibility of chitosan molecular chain is increased with the molecular weight increase, which in fact decreases the number of available antibacterial groups [Mourya, 2010], and as a consequence, decreases inhibitory ability, and at the same time improves binding ability with milk fat and improves interaction with negatively charged surfaces of casein micelles.

Fig.3. shows that viscosity of sour cream is increased with the rise of concentration of high molecular weight chitosan and viscosity decreases with the increase of concentration of chitooligosaccharide due to inhibition of fermentation. Intermediate viscosity has been observed for sour cream with carboxymethyl chitosan used as an additive. The effect of polyampholytic macromolecules of carboxymethyl chitosan that are negatively charged and smaller in size on viscosity changes is lower compared with a positively charged polycationic chitosan that interacts electrostatically with the negatively charged surfaces

and through charge neutralization and bridging modifies interaction among ingredients of a food system.

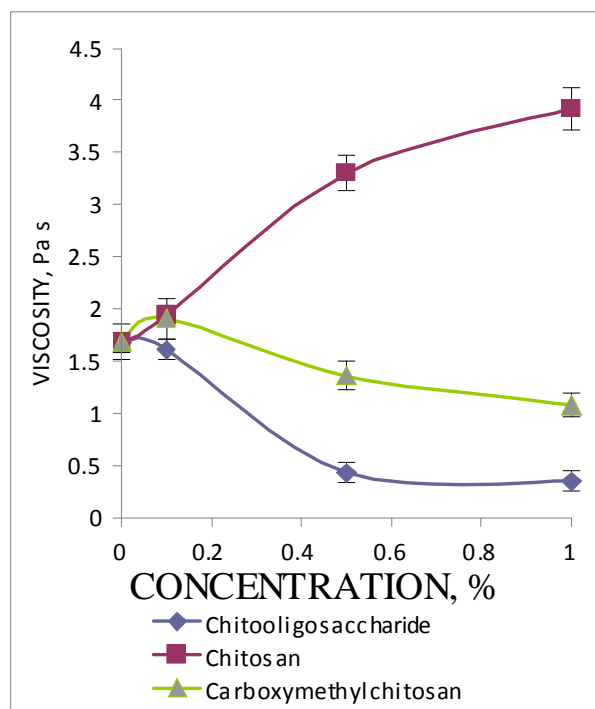


FIG.3 SOUR CREAM VISCOSITY DEPENDENCE ON THE CONCENTRATION OF CHITOSAN, CHITOLIGOSACCHARIDE AND CARBOXYMETHYL CHITOSAN

Similar to the addition of starch-lipid composite to yogurt [Singh, 2009], high molecular weight chitosan, but not chitooligosaccharide or carboxymethyl chitosan, can cause faster aggregation and high molecular weight chitosan can be used as a good thickener, fat replacer and stabilizer for the production of sour cream. Chitosan adsorption to negatively charged casein micelle surface is also possible. It is reported [Beysseriat, 2006 - Aranaz, 2009] that adsorption to fat droplets in *in vitro* human digestion model increases with growing chitosan molecular weight due to cationic loops and tails and difference in conformation of the high molecular weight chitosan molecule. Fat droplets are also present in milk, as an oil-in-water emulsion, so the similar physical behavior at microscale and difference between high molecular weight chitosan and low molecular weight chitosan are expected and demonstrated in Fig.3.

Aggregation of fat globules is prevented by the negative charge on the surface and the presence of steric hindrance layer [Everett, 2007]. The addition of high molecular polycationic chitosan can increase viscosity due to the increment of aggregation of fat globules because of the changes in electrostatic

repulsion between negatively charged surfaces.

The casein micelles are covered by a hairy layer of  $\kappa$ -caseins providing steric stabilization and it was observed [Müller-Buschbaum, 2007] that at high added positively charged calcium concentration, this negatively charged  $\kappa$ -casein layer has collapsed, attraction between micelles has increased and neighboring micelles have aggregated. It was also suggested that collapse of the outer hairy-layer of kappa-casein can be due to a different pH and concentrations of sodium cations ( $\text{Na}^+$ ) from NaCl. The voluminosity of the casein micelles increased with addition of NaCl and decreased when pH was decreased [Karlsson, 2005]. The similar mechanism can be proposed when polycationic chitosan is added to dairy products. The voluminosity of the casein micelles, and as a consequence viscosity of sour cream can be increased with addition of positively charged chitosan. Chitosan may displace caseins from the surface of fat globules and promote aggregation allowing the fat globules to bond together and to form a network. Fat-binding capacity of chitosan also increases with the rise of molecular weight [Xia, 2011].

Combination of different mechanisms is possible—polycationic chitosan molecules can suppress electrostatic repulsion resulting in aggregation of casein micelles and high molecular weight chitosan macromolecules can bridge fat globules. Macromolecules of high molecular weight chitosan at higher concentrations overlap and form entanglements resulting in a substantial increase of sour cream viscosity due to a network formation. Fat binding ability of chitosan and cross-links formed at higher pH also contribute to the formation of a stronger gel and a more dense aggregate structure.

The relationship between effects of various applications of chitosan and its degree of deacetylation has not been completely clarified so far [Aranaz, 2009]. Fig.4 shows that sour cream viscosity after fermentation decreases with the increase of degree of deacetylation of chitosan additives at high concentrations and don't depend heavily on degree of deacetylation at low concentrations. So the viscosity dependence on chitosan concentration in sour cream is different from viscosity dependence of chitosan in aqueous acetic acid solutions where viscosity rises with the increasing degree of deacetylation of chitosan [Wang, 1994] and is consistent with the data on viscosity dependence on the degree of deacetylation reported for medium molecular weight chitosan

[Sjöholm, 2009].

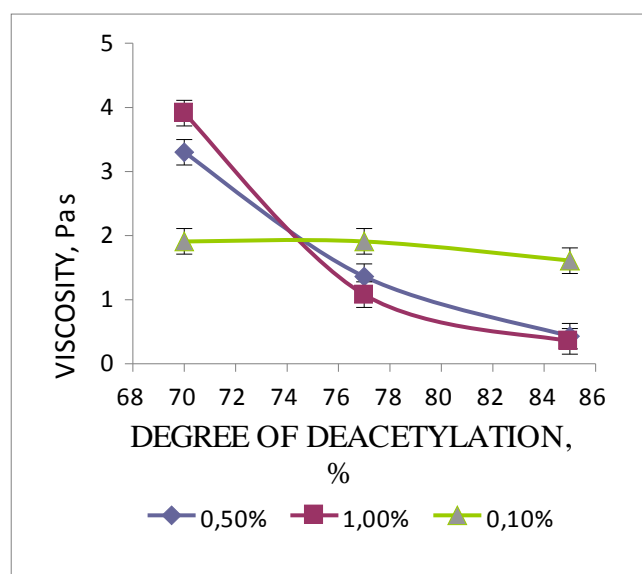
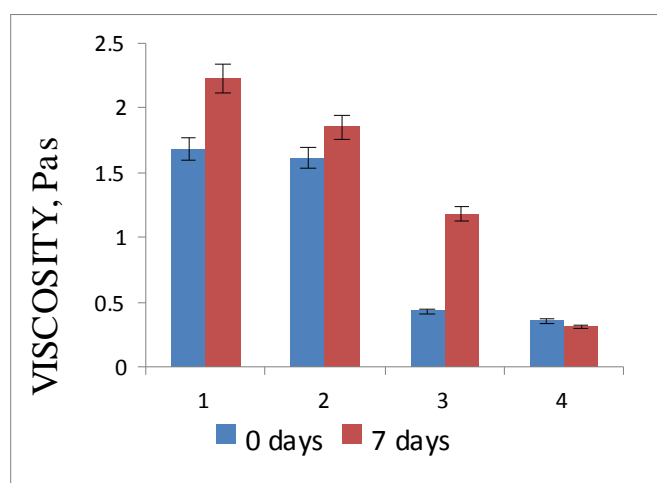
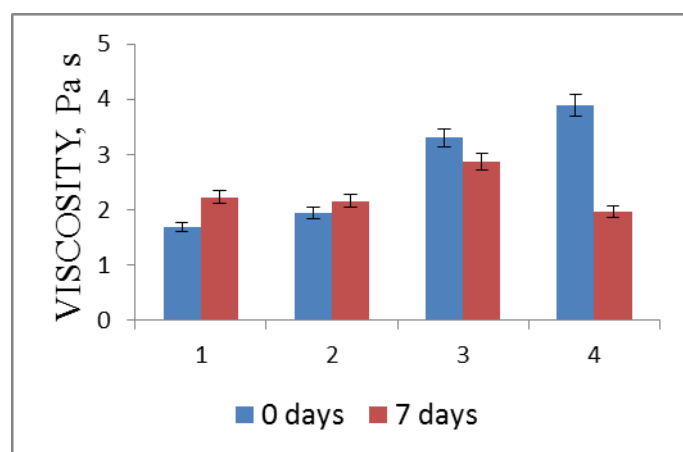


FIG.4 SOUR CREAM VISCOSITY DEPENDENCE ON THE DEGREE OF DEACETYLATION OF ADDED CHITOSAN DERIVATIVES

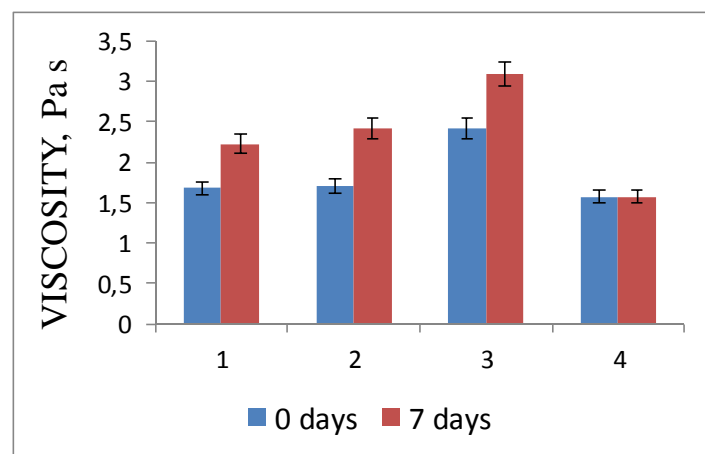
#### Viscosity Changes during Sour Cream Storage



a)



b)



c)

FIG.5 VISCOSITY CHANGES AFTER 0 AND 7 DAYS STORAGE OF SOUR CREAM CONTAINING DIFFERENT CONCENTRATIONS OF CHITOOLIGOSACCHARIDE (a), HIGH MOLECULAR WEIGHT CHITOSAN (b) AND CARBOXYMETHYLCHITOSAN (c): 1 – 0%; 2 – 0.1%; 3 – 0.5%; 4 – 1.0%

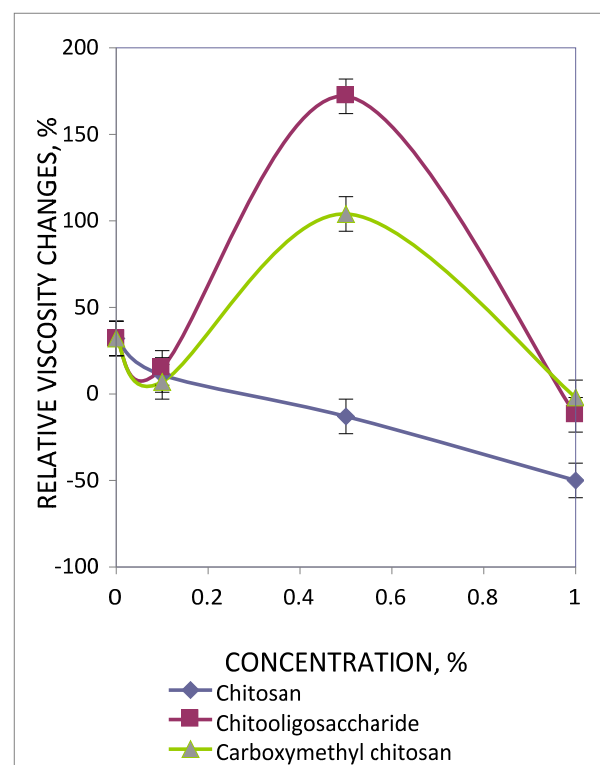


FIG.6 CHANGES OF RELATIVE VISCOSITY OF SOUR CREAM DURING STORAGE AT DIFFERENT CONCENTRATIONS OF CHITOSAN, CHITOOLIGOSACCHARIDE AND CARBOXYMETHYLCHITOSAN

Chitosan and its derivatives modify interaction between fat globules and casein micelles during storage. The highest increase of viscosity during storage has been observed for sour cream with low initial viscosity at chitooligosaccharide concentration 0.5% shown in Fig.5a and at carboxymethyl chitosan concentration 0.5% in Fig.5c. Decrease of viscosity

during storage has been observed for sour cream with higher initial viscosity at high molecular weight chitosan concentration 0.5% and especially at concentration 1.0% in Fig.5b. It was shown that the decrement of viscosity during storage can be related with the decrease of levels of minerals in milk [Bienvenue, 2003]. Therefore, the mechanism of viscosity reduction during storage can be related also to the ability of high molecular weight chitosan to bind minerals. These results are consistent with results of Chattopadhyay and Inamdar [Chattopadhyay D, 2010] that the higher molecular weight chitosan shows higher chelation power compared with lower molecular weight chitosans.

The effect of the decrease of viscosity during storage is more pronounced with the increase of chitosan concentration shown in Fig.5b. The changes of relative viscosity of sour cream during storage at different concentrations of chitosan and its derivatives are shown in Fig.6. The relative viscosity of sour cream decreases with the increase of chitosan concentration. Non monotonic sour cream relative viscosity dependence on concentration with a maximum observed at 0.5% for chitooligosaccharide and carboxymethyl chitosan, can be explained by the fact that the isoelectric point of chitosan oligomers depends on their concentration [Aider, 2006].

## Conclusions

Viscosity of sour cream rises with the increase of concentration of high molecular weight chitosan and the decrement in anomalous viscosity has been observed with the increase of concentration of chitooligosaccharide. Chitooligosaccharides at concentrations higher than 0.1% inhibit fermentation due to the interaction with lactic acid bacteria. Our data show that during fermentation process high molecular weight chitosan interacts mainly with casein micelles and milk fat, but chitooligosaccharides interact mainly with lactic acid bacteria. Combination and variation in intensity of different antibacterial mechanisms is evident for chitosans with different molecular structures. Only high concentrations and degrees of deacetylation (commonly associated with low molecular weight) of chitosan can essentially inhibit fermentation processes. The increase of sour cream viscosity with the increment of positively charged high molecular weight chitosan concentration can be explained by partial compensation of electrostatic repulsion between negatively charged

casein micelles and increase of aggregation of fat globules and casein micelles. Fat binding ability of chitosan and cross-links formed for the system at a higher pH, also contribute to the formation of a stronger gel and a more dense aggregate structure. The lowest viscosity after fermentation and 7 days storage has been observed for sour cream containing chitooligosaccharide. The sour cream viscosity after fermentation decreases with the increase of degree of deacetylation of chitosan additives at high concentrations and don't depend essentially on degree of deacetylation at low concentrations. During storage viscosity of sour cream containing more than 0.5% of high molecular weight chitosan decreases, but viscosity of sour cream containing 0.5% of chitooligosaccharide and 0.5% of carboxymethyl chitosan increases.

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