

INFLUENCE OF CHITOSAN ON MICROBIOLOGICAL DATA AND QUALITY CHARACTERISTICS OF SPREADABLE CURD CHEESE AND MAYONNAISE

Algirdas Liutkevičius¹, Vilma Speičienė¹, Gitana Alenčikienė¹, Dalia Sekmokienė², Renata Žvirdauskienė¹, Aldona Mieželiene¹

¹*Food Institute, Kaunas University of Technology*

Taikos 92, LT-51180 Kaunas; Phone +370 37 312 153; Fax. +370 37 312 393

E-mail: Algirdas.Liutkevicus@ktu.lt

²*Veterinary Academy of the Lithuanian University of Health Sciences*

Tilžės 18, LT-47181 Kaunas; Phone +370 37 362 695; E-mail: dalsek@lva.lt

Abstract. The studies of the usage of prebiotic dietary fibre (PDF) chitosan (further chitosan) in food industry, and especially its effect on safety and complex quality characteristics of food products are insufficient. Therefore, this paper represents the results obtained on the influence of chitosan on microbiological, physical and sensory data of food products, such as spreadable curd cheese and mayonnaise.

It has been established that the addition of chitosan showed the tendency to reduce the total plate count in the samples of spreadable curd cheese during storage. However the addition of chitosan did not have any effect on the growth of yeast and moulds in the above mentioned products.

Chitosan significantly increased the values of active acidity of spreadable curd cheese, and mayonnaise, but did not affect the spreadability of these products.

Chitosan did not demonstrate any effects on the texture and colour properties of fresh and 6-month stored samples of spreadable curd cheese and mayonnaise, however, it had negative effect on the sensory properties of the products: reduced general odour and flavour balance in fresh samples, decreased gloss and distribution in the mouth, and drying taste and sandy feeling appeared. Chitosan reduced thickness and creaminess of spreadable curd cheese during storage, however both control and test samples were evaluated as acceptable after 20 days of storage.

Enrichment of mayonnaise with chitosan reduced certain flavours and slightly changed texture properties of the products. The best test results of the sensory characteristics of mayonnaise enriched by chitosan were established after 4 months of storage.

Keywords: chitosan, spreadable cheese, mayonnaise, microbiological, sensory properties

Introduction. Food science and investigations made indicate that many of the EU population diets lack dietary fibre, especially PDF. It is a well known fact that the lack of PDF is a risk factor for many diseases. Following the provisions of Commission Regulation (EC) No. 432/2012 of 16 May 2012, which established a list of permitted health claims for foods, the consumption of PDF like chitosan up to 3 g per day helps to maintain normal cholesterol levels in blood.

Chitosan is a linear aminopolysaccharide consisting of (1,4)-linked 2-amino-deoxy- β -D-glucan derived from the deacetylation of chitin, which is a main component of the exoskeleton of crustaceans. Chitosan is biodegradable, biocompatible, biofunctional and non-toxic compound. Detailed studies of chitosan showed different properties of water soluble and water insoluble chitosan (Qin et al., 2006, Aranaz et al., 2009), and especially different antimicrobial properties. Antimicrobial activity of chitosan was demonstrated against various kinds of microorganisms in foods, such as mayonnaise (Oh et al., 2001), pork products (Sagoo et al., 2002), cooked food (Rodriguez et al., 2002). Investigations of Rhoades (Rhoades, Roller, 2000) showed that chitosan has effective antimicrobial activity in low pH products. Composition of food products had significant effect on chitosan antimicrobial activity, so for each product type additional observations should be made. Chitosan in mayonnaise may be used as a preservative that inhibits the growth of spoilage microorganisms (Oh et al., 2001).

Effect of chitosan addition on sensory properties of milk products investigated not too much. Gammariello with colleagues (Gammariello et al., 2011) found that if chitosan added in range from 0.010 % to 0.020 % had no significant effect on sensory properties (odour, taste, texture) of Straciatella cheese. Analogical data were established for Fior di latte cheese when amount of chitosan varied from 0.006 % to 0.024 % (Gammariello et al., 2010). But overall acceptability of Apulia spreadable cheese enriched by chitosan decreased during the storage (Gammariello et al., 2008). Analysis of literature prove statement made by other authors (Rhoades, Roller, 2000), that usage of chitosan in manufacture of food products of different composition must done with care, as negative changes in sensory quality can appear.

Aim of the research was to determine the effects of prebiotic dietary fibre – aminopolysaccharide chitosan – on microbiological, physico-chemical and sensory properties of food products, such as spreadable curd cheese and mayonnaise.

Materials and methods

Products analysed: spreadable curd cheese and mayonnaise. They were purchased in the supermarkets of Lithuania. Chitosan applied for experiments was purchased from Pfannenschmidt GmbH (Germany). It was produced from shells of crustaceans, particle size – 100 % pass through 80 mesh, deacetylation degree – of min 90 %.

The packages of above mentioned food products were supplemented with chitosan powder. Spreadable curd cheese was enriched with chitosan by intense mixing for 1 minute, and mayonnaise was mixed for 3 min using a mixer SM 2188 (*Clatronic*, Germany). The amount of added chitosan in the test samples was 0.45 %. Both control samples and test samples (containing chitosan) were poured into 350 g polystyrene containers intended for food storage. Containers with samples were covered with aluminium folia, sealed, and stored at the temperature of 6 °C before testing.

Active acidity was measured by pH meter 538 using an electrode with integrated temperature sensor Sen Tix 97 T (WTW GmbH, Weilheim, Germany).

Texture of samples. Spreadability was defined as easiness of sample flow on the surface. Spreadability of samples was measured using universal texture analyser Universal Testing Machine Instron 3343 (Instron Engineering Group, High Wycombe, England) putting samples of 20 °C temperature into special conical containers. Samples were pressed at the speed of 5 mm/min using 1 kN power sensor and conical operating body where sample rises up the surface at the angle of 45°.

Colour characteristics were measured by colour meter CR-400 (Konica Minolta, Osaka, Japan). The parameters L*, a*, b* were measured in light reflection mode (lightness, redness and yellowness according to *CIELAB* scale). Standard light source C was used with radiation that is close to average sunlight.

Microbiological indicators. Total plate count was determined by applying the Petri dish method using microbiological growth medium (Plate Count Agar, Liofilchem) according to LST EN ISO 4833:2003 incubating the samples under aerobic conditions in a thermostat at 30±1 °C for 3 days.

The number of yeast and mould fungi was determined by seeding 0.1 ml of prepared test sample on to the surface of Petri dish on Dichloran Rose-Bengal Chloramphenicol (DRBC Agar, Liofilchem) Agar according to LST ISO 21527-1:2008 incubating the samples under aerobic conditions in a thermostat at 25±1 °C for 5 days.

Samples for microbiological analysis at 0 day were taken 4–5 h after chitosan addition.

Sensory analysis. For the assessment of sensory properties, a sensory profile test was applied. The essence of this test lies in a group of trained assessors analysing pre-selected products (samples) and selecting descriptors to describe sensory properties of product. Afterwards, scales were selected to assess the intensity of each property, and the intensity of each property of all the products was marked in a separate scale. Based on these data, a sensory profile was developed for each product showing the intensity of each sensory property. Based on these profiles, products can be compared according to their different properties and their intensity, and a link between sensory quality of products and their separate intensities may be established.

A group of 6–8 assessors participated in the test.

Assessors were selected and trained according to the standard ISO 8586. The assessment was performed in the individual booth of the sensory science laboratory of KTU Food Institute established according to the requirements of the standard ISO 8589. For data collection and analysis, software *Fizz Network* (Biosystems, France) was used.

For development of sensory profiles a fully balanced randomized sample presentation plan with two repetitions was applied. The intensity of each property of the studied products was assessed using 15-points scale: 1 – lowest intensity, 8 – moderate intensity, 15 – strong intensity.

Statistical analysis of data. In order to determine whether the impact of chitosan was significant, Student's test was applied. When assessing the effect of storage duration, analysis of variance was applied. If statistically significant different average values were estimated, Duncan's multiple range test was applied. It helped to determine the specific moment of the research when the average intensities of one or another property varied with the level of significance being 0.05. Data analysis was performed using statistical software „SPSS for Windows“, version 15.0 (SPSS Inc., IL, USA, 2006).

Results and discussion

Spreadable curd cheese. The microbiological test results of spreadable curd cheese, enriched by chitosan are presented in Table 1. Total plate count in all samples with chitosan was lower than in control, but the difference was not significant. During the storage, the total plate count in samples with chitosan showed the tendency to decrease also. Some reduction in total plate count may be related to antimicrobial effects of chitosan, especially against lactic acid bacteria, that are present in curd. No et al. (2002) has determined that chitosan significantly reduces the growth of gram-positive bacteria such as *Lactobacillus bulgaricus*, *Lactobacillus plantarium* and *Lactobacillus brevis*. The effect of chitosan reducing the growth of lactic acid bacteria was recorded when studying yogurt containing chitosan supplement (Seo et al., 2009). However, other authors claim that chitosan does not affect the growth of lactic acid bacteria in *Mozzarella* cheese (Altieri et al., 2005) or in a certain type of *Mozzarella* cheese called *Stracciatella* (Gammariello et al., 2009). It should be noted that in the mentioned studies chitosan content used was of 0.010–0.075 %, which is significantly lower in comparison to our work.

Antibacterial activity of chitosan at pH below its pKa≈6.3 is mainly explained by the polycationic nature. Electrostatic interaction between the positively charged amino groups (NH₃⁺) of chitosan and the predominantly anionic components of bacteria surface can lead to disturbance of membrane integrity and functions (Kong et al., 2008). Also, it was proposed that low molecular weight chitosan can penetrate cell wall of bacteria and combine with DNA that inhibits the synthesis of mRNA and DNA transcription (Sudarshan et al., 1992).

As shown in Table 2 chitosan supplement in spreadable curd cheese increased the pH values of the product. In fresh samples after addition of chitosan pH

has increased from 5.12 to 5.45. During the storage both, demonstrated increase in pH values. the control samples and samples containing chitosan,

Table 1. Effect of chitosan on microbiological data of spreadable curd cheese

Day	Total plate count, CFU/1g		Yeast and moulds, CFU/1g	
	C*	CH**	C*	CH**
0	2.3×10^3 ^{aA}	1.1×10^3 ^{aA}	$<1.0 \times 10^1$ ^{aA}	$<1.0 \times 10^1$ ^{aA}
10	2.4×10^3 ^{aA}	9.0×10^2 ^{aA}	$<1.0 \times 10^1$ ^{aA}	$<1.0 \times 10^1$ ^{aA}
20	1.9×10^3 ^{aA}	8.1×10^2 ^{aA}	$<1.0 \times 10^1$ ^{aA}	$<1.0 \times 10^1$ ^{aA}

C* – control sample, CH** – sample with added chitosan
a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($p < 0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P < 0.05$), depending on chitosan addition.

Table 2. Effect of chitosan on active acidity, spreadability and colour characteristics of spreadable curd cheese

Day	pH		Force, N		Colour characteristics					
	C*	CH**	C*	CH**	L*		a*		b*	
					C*	CH**	C*	CH**	C*	CH**
0	5.12 ^{aA}	5.44 ^{aB}	0.88 ^{aA}	0.85 ^{aA}	86.38 ^{aA}	86.20 ^{aA}	-3.06 ^{aA}	-3.10 ^{aA}	11.24 ^{aA}	10.80 ^{aA}
10	5.14 ^{aA}	5.45 ^{aB}	0.90 ^{aA}	0.78 ^{aA}	87.25 ^{aA}	86.40 ^{aA}	-3.04 ^{aA}	-3.12 ^{aA}	11.40 ^{aA}	10.95 ^{aA}
20	5.22 ^{bA}	5.48 ^{aB}	1.07 ^{aA}	0.61 ^{aB}	88.42 ^{aA}	86.90 ^{aA}	-3.05 ^{aA}	-3.23 ^{aA}	11.60 ^{aA}	11.38 ^{aA}

C* – control sample, CH** – sample with added chitosan
a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($p < 0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P < 0.05$), depending on chitosan addition.

Table 3. Effect of chitosan on odour properties of spreadable curd cheese during the storage

Property	Moment of testing, days	Sample	
		Control	Containing chitosan
Odour			
overall	0	13.50 ^{aA}	12.58 ^{aB}
	10	13.25 ^{aA}	12.36 ^{aB}
	20	13.08 ^{aA}	11.08 ^{bB}
fresh	0	12.00 ^{aA}	11.17 ^{aA}
	10	11.25 ^{aA}	10.36 ^{aA}
	20	10.75 ^{aA}	9.25 ^{bA}
sour	0	10.00 ^{aA}	9.33 ^{aA}
	10	8.52 ^{bA}	7.24 ^{bA}
	20	6.42 ^{cA}	5.08 ^{cA}
balanced	0	12.33 ^{aA}	11.92 ^{aA}
	10	11.56 ^{aA}	10.58 ^{bA}
	20	11.00 ^{aA}	8.25 ^{cB}
herbal	0	12.83 ^{aA}	12.42 ^{aA}
	10	11.53 ^{bA}	10.52 ^{bA}
	20	10.83 ^{cA}	8.75 ^{cB}
nontypical	0	1.00 ^{bA}	1.00 ^{bA}
	10	1.55 ^{aA}	1.25 ^{bB}
	20	1.75 ^{aA}	1.50 ^{aB}

a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($P < 0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P < 0.05$), depending on chitosan addition.

The spreadability of the fresh curd was not affected by addition of chitosan (Table 2). Both, the force required to press the control samples and samples containing chitosan in cuvettes varied by approximately 0.85 N. The force required for control samples that have been stored for 20 days was higher ($P < 0.05$). However, in comparison to fresh samples the force values did not significantly change neither in control samples nor in the samples containing chitosan ($P > 0.05$). Based on the results obtained as regards to the force required to press we can make a conclusion that numerical values of control sample demonstrated a tendency to grow, while in the case of samples containing chitosan the numerical values have reduced.

It has been determined that addition of chitosan did not significantly affect colour characteristics of the fresh curd samples (Table 2): the values L^* , a^* and b^* were varying slightly ($P > 0.05$). During the storage period, none of colour characteristics has changed in both samples.

Sensory analysis of spreadable curd cheese was started from the assessment of odour, the intensity of which appeared to be affected by the addition of chitosan (Table 3). Overall intensity of odour in fresh samples containing chitosan was lower in comparison with the control

samples. It was established in fresh samples ($P < 0.05$) and the difference became more apparent in the samples stored for 20 days ($P < 0.01$). Despite this, the odour of the control sample had not significantly changed during the storage period. Addition of chitosan resulted in reduction of the overall odour intensity during the storage. Chitosan demonstrated no effects on the intensity of fresh and sour odour in both fresh samples. No foreign odours that were not typical for spreadable curd cheese were felt for fresh samples. During the storage, the intensity of herbal and sour odour had reduced and a weak non typical odour appeared in both samples. For the sample containing chitosan, the general odour intensity as well as fresh, sour, herbal odours and balance of odour have reduced during the storage. The non typical odour that is not characteristic for product had appeared in both samples.

As shown in Table 4. The texture properties of fresh samples were not varying. During the storage, the texture has not changed significantly in the control samples. However, decrease of thickness was observed in samples containing chitosan. The thickness of the sample containing chitosan stored for 20 days was determined to be lower in comparison to the control. Also this sample was ascertained to be less creamy.

Table 4. Effect of chitosan on texture properties of spreadable curd cheese during the storage

Property	Moment of testing, days	Sample	
		Control	Containing chitosan
Texture in mouth			
creaminess	0	12.25 ^{aA}	11.42 ^{aA}
	10	11.99 ^{aA}	11.01 ^{aB}
	20	11.83 ^{aA}	10.08 ^{aB}
thickness	0	11.42 ^{aA}	10.75 ^{aA}
	10	11.01 ^{aA}	9.23 ^{aA}
	20	10.33 ^{aA}	8.67 ^{bB}
sandiness	0	3.00 ^{aA}	4.75 ^{aA}
	10	3.25 ^{aA}	4.85 ^{aA}
	20	3.67 ^{aA}	5.92 ^{aA}
oral adhesion	0	7.42 ^{aA}	8.17 ^{aA}
	10	7.52 ^{aA}	7.68 ^{aA}
	20	7.67 ^{aA}	7.25 ^{aA}

a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($P < 0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P < 0.05$), depending on chitosan addition.

The data of spreadable curd cheese taste assessment presented in Table 5 revealed that added chitosan did not have any significant effect on the intensity of taste properties of fresh curd samples. Sweet and sour tastes were weaker for 10 days stored samples in comparison with fresh samples. However, some weak, non typical for curd cheese, taste appeared. Stored samples containing chitosan were ascertained to have less intense overall taste. Chitosan addition appeared to have no significant effect on aftertaste and oral adhesion properties.

Addition of chitosan appeared to have significant effect on taste and texture properties of product. However preliminary assessments of acceptability with a small

group of consumers revealed that fresh samples were equally acceptable (Table 6). In the stored samples containing chitosan the changes of textural properties of samples took place. It had a significant negative effect on the texture and overall acceptability of these samples; nevertheless, at the end of storage the samples remained still acceptable.

Mayonnaise. Microbiological analysis of mayonnaise revealed that chitosan did not influence the total plate count or the growth of yeast or moulds (Table 7). No variations were observed during the storage period in control or test samples. Microbiological stability of mayonnaise may be related to low pH of the samples

despite of the fact that chitosan increased pH in the fresh samples and the values of pH (5.12–5.48) further increased during the storage period. Spreadability of mayonnaise was not affected by chitosan addition (Table 7). Spreadability of fresh samples both control one and those containing chitosan, varied by approximately 0.55

N. During the storage no effect of chitosan on the product spreadability was noticed. However, in comparison to fresh samples spreadability has reduced ($P<0.05$) and it is demonstrated by the increased force required to spread the sample in the testing cuvette. Reduced spreadability may be related to maturity of mayonnaise.

Table 5. Effect of chitosan on the taste properties of spreadable curd cheese during the storage

Properties	Moment of testing, days	Sample	
		Control	Containing chitosan
Taste			
overall	0	13.00 ^{aA}	12.67 ^{aA}
	10	13.02 ^{aA}	12.23 ^{aA}
	20	13.08 ^{aA}	12.00 ^{aB}
sweet	0	6.67 ^{aA}	7.08 ^{aA}
	10	4.25 ^{bA}	4.56 ^{bA}
	20	3.50 ^{cA}	3.67 ^{cA}
sour	0	9.42 ^{aA}	8.50 ^{aA}
	10	7.56 ^{bA}	7.11 ^{bA}
	20	6.50 ^{cA}	4.92 ^{cA}
salty	0	6.50 ^{aA}	7.08 ^{aA}
	10	5.99 ^{aA}	6.25 ^{bA}
	20	5.25 ^{aA}	5.33 ^{bA}
herbal	0	11.58 ^{aA}	11.50 ^{aA}
	10	10.45 ^{aA}	10.25 ^{aA}
	20	10.08 ^{aA}	8.42 ^{bA}
pasteurisation	0	5.58 ^{aA}	5.83 ^{aA}
	10	5.01 ^{aA}	4.42 ^{aA}
	20	4.08 ^{aA}	3.42 ^{bA}
non typical	0	1.00 ^{aA}	1.33 ^{aA}
	10	1.25 ^{abA}	1.58 ^{abA}
	20	1.50 ^{bA}	2.00 ^{bA}
Aftertaste			
overall	0	9.50 ^{aA}	9.42 ^{aA}
	10	8.99 ^{aA}	9.03 ^{aA}
	20	8.42 ^{aA}	8.75 ^{aA}
oral adhesion	0	6.58 ^{aA}	7.50 ^{aA}
	10	6.75 ^{aA}	8.36 ^{aA}
	20	6.92 ^{aA}	9.00 ^{aA}

a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($P<0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P<0.05$), depending on chitosan addition.

Table 8 presents the results of mayonnaise odour assessment. Overall odour intensity of a fresh sample containing chitosan was the same as the control. However starting from 2 months of storage overall odour intensity of the samples became different. The odour of the sample containing chitosan was weaker in comparison with control sample. Overall odour intensity in control samples did not significantly changed during the storage ($P>0.05$), whereas the odour of stored mayonnaise samples containing chitosan was weaker compared to fresh samples. The similar tendency was observed when assessing the intensity of sour and heavy odours. The intensity of the mentioned odours in fresh samples containing chitosan was the same as in the control

samples. However, after 2 months of storage the intensity of odour in these samples was lower in comparison with control samples. The effect of chitosan on the fatty odour was only observed after storing the samples for up to 4 months ($P<0.01$). Non typical to mayonnaise odour was not identified. However, after 4 months of storage some non typical odour emerged. As a result, mayonnaise stored for 6 months was not subjected to sensory analysis.

Thickness of samples felt in mouth was not affected by chitosan addition (Table 9). This property remained stable even after storing the samples for 4 months. Nevertheless chitosan supplement had significant effect on other appearance and texture properties of mayonnaise. Samples containing chitosan became less glossy after 2

months of storage and this tendency remained after 4 months storage. Chitosan additive reduced visual homogeneity ($P<0.05$) and graininess felt in the mouth ($P<0.001$) in the fresh samples. This tendency remained in the samples that were stored for 2 and 4 months. The

effect of chitosan on the distribution in mouth and oral adherence was observed only after 4 months of storage. Samples containing chitosan were more poorly distributed in mouth; they adhered to the walls of oral cavity.

Table 6. Effect of chitosan on acceptability of spreadable curd cheese during the storage

Acceptability	Moment of testing, days	Sample	
		Control	Containing chitosan
Taste	0	14.08 ^{aA}	13.50 ^{aA}
	10	13.86 ^{aA}	13.01 ^{aA}
	20	13.33 ^{aA}	12.50 ^{aA}
Texture	0	14.08 ^{aA}	13.33 ^{bA}
	10	13.69 ^{aA}	12.12 ^{abA}
	20	13.33 ^{aA}	11.67 ^{abB}
Overall	0	14.00 ^{aA}	13.25 ^{bA}
	10	13.69 ^{aA}	12.01 ^{abA}
	20	13.33 ^{aA}	11.83 ^{abB}

a, b – average values marked by different letters in the columns for different indicators, statistically significantly different ($P<0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators, statistically significantly different ($P<0.05$), depending on chitosan addition.

Table 7. Effect of chitosan on the microbiological indicators, active acidity and spreadability of mayonnaise

Parameter	Sample	Months			
		0	2	4	6
Total plate count, CFU/1g	Control	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA
	Containing chitosan	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA
Yeast and moulds, CFU/1g	Control	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA
	Containing chitosan	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA	$<1.0 \times 10^1$ aA
pH	Control	2.82 ^{aA}	2.88 ^{bA}	2.92 ^{bA}	2.79 ^{aA}
	Containing chitosan	4.10 ^{abB}	4.16 ^{bbB}	4.21 ^{bbB}	4.10 ^{abB}
Force, N	Control	0.57 ^{aA}	0.71 ^{bA}	0.79 ^{bA}	0.84 ^{bA}
	Containing chitosan	0.55 ^{aA}	0.71 ^{bA}	0.88 ^{bA}	0.87 ^{bA}
Colour characteristic					
L*	Control	88.08 ^{aA}	87.05 ^{aA}	86.24 ^{aA}	86.94 ^{aA}
	Containing chitosan	87.80 ^{aA}	86.72 ^{aA}	86.11 ^{aA}	86.56 ^{aA}
a*	Control	-3.57 ^{aA}	-3.60 ^{aA}	-2.97 ^{aA}	-3.01 ^{aA}
	Containing chitosan	-3.65 ^{aA}	-3.58 ^{aA}	-2.93 ^{aA}	-2.99 ^{aA}
b*	Control	22.79 ^{aA}	22.86 ^{aA}	22.53 ^{aA}	22.41 ^{aA}
	Containing chitosan	22.74 ^{aA}	23.10 ^{aA}	23.02 ^{aA}	22.69 ^{aA}

a, b, c – average values marked by different letters in the columns for different indicators, statistically significantly different ($P<0.05$), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different ($P<0.05$), depending on chitosan addition.

When assessing taste properties of mayonnaise it has been established that chitosan addition reduced overall intensity of taste. Samples of this type demonstrated weaker sour taste (Table 10). Sweet and egg-like odour were not significantly affected by chitosan, whereas salty and spicy odours in the 4-month stored samples felt weaker. After storing the samples for up to 4 months a non typical taste that is not characteristic to fresh mayonnaise emerged and it was more intense in the samples containing chitosan. Therefore samples stored for

6 months were not tested applying sensory analysis not only due to altered odour that was mentioned before but also due to altered taste properties.

Intensity of overall aftertaste differed only in fresh samples: it was more intense in the samples containing chitosan. However this difference was not statistically reliable in the stored samples. Chitosan additive caused more intense astringent and drying bitter aftertaste that was most intensely felt in the samples stored for 4 months.

Table 8. Effect of chitosan on odour properties of mayonnaise during storage

Property	Moment of testing, months	Sample	
		Control	Containing chitosan
Taste			
overall	0	13.08 ^{aA}	12.33 ^{bA}
	2	13.13 ^{aB}	11.63 ^{abA}
	4	13.08 ^{aB}	10.50 ^{aA}
sour	0	12.33 ^{aA}	11.67 ^{bA}
	2	12.38 ^{aB}	9.63 ^{aA}
	4	12.31 ^{aB}	9.00 ^{aA}
heavy	0	9.75 ^{aA}	8.33 ^{aA}
	2	9.75 ^{aB}	7.13 ^{aA}
	4	9.00 ^{ab}	7.50 ^{aA}
fatty	0	8.75 ^{aA}	8.42 ^{bA}
	2	7.13 ^{aA}	6.00 ^{aA}
	4	8.46 ^{ab}	7.42 ^{abA}
non typical	0	1.00 ^{aA}	1.00 ^{aA}
	2	1.38 ^{aA}	1.38 ^{aA}
	4	5.38 ^{bA}	4.75 ^{bA}

a, b – average values marked by different letters in the columns for different indicators, statistically significantly different (P<0.05), depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different (P<0.05), depending on chitosan addition.

Table 9. Effect of chitosan on the appearance and texture properties of mayonnaise during storage

Properties	Moment of testing, months	Sample	
		Control	Containing chitosan
Appearance			
glossiness	0	12.42 ^{aA}	11.58 ^{bA}
	2	11.38 ^{aA}	9.25 ^{aB}
	4	11.85 ^{aA}	10.92 ^{bB}
integrity	0	13.17 ^{aA}	12.17 ^{bB}
	2	12.13 ^{aA}	10.75 ^{bB}
	4	12.15 ^{aA}	8.67 ^{aC}
sponginess	0	11.33 ^{aA}	12.08 ^{bA}
	2	11.63 ^{aA}	11.13 ^{abA}
	4	11.85 ^{aA}	10.25 ^{aC}
Texture in mouth			
thickness	0	11.00 ^{aA}	11.42 ^{aA}
	2	11.88 ^{aA}	11.13 ^{aA}
	4	12.00 ^{aA}	12.00 ^{aA}
graininess	0	2.08 ^{aA}	3.25 ^{aC}
	2	1.50 ^{aA}	2.88 ^{aB}
	4	2.62 ^{aA}	7.25 ^{bC}
distribution in mouth	0	11.83 ^{aA}	11.17 ^{aA}
	2	11.13 ^{aA}	10.00 ^{aA}
	4	11.54 ^{aA}	10.67 ^{ab}
oral adherence	0	7.67 ^{aA}	8.17 ^{aA}
	2	7.25 ^{aA}	7.25 ^{aA}
	4	8.69 ^{aA}	10.00 ^{bB}

a, b, – average values marked by different letters in the columns for different indicators. statistically significantly different (P<0.05). depending on the duration of storage period.
A, B, C– average values marked by different letters in the rows for different indicators. statistically significantly different (P<0.05).

Table 10. Effect of chitosan on taste properties of mayonnaise during the storage

Properties	Moment of testing, months	Sample	
		Control	Containing chitosan
Taste			
overall	0	13.25 ^{aA}	12.75 ^{aB}
	2	13.25 ^{aA}	12.38 ^{aB}
	4	13.08 ^{aA}	11.92 ^{aC}
sweet	0	7.83 ^{aA}	8.17 ^{aA}
	2	8.25 ^{aA}	8.13 ^{aA}
	4	6.92 ^{aA}	6.67 ^{aA}
sour	0	11.25 ^{abA}	9.67 ^{baB}
	2	9.63 ^{aA}	7.75 ^{aB}
	4	12.08 ^{baA}	8.00 ^{aC}
salty	0	7.25 ^{aA}	7.58 ^{aA}
	2	6.88 ^{aA}	6.87 ^{aA}
	4	7.54 ^{aA}	6.92 ^{aB}
heavy	0	6.25 ^{aA}	5.92 ^{aA}
	2	7.00 ^{aA}	6.75 ^{aA}
	4	6.00 ^{aA}	5.33 ^{aB}
egg-like	0	8.92 ^{baA}	9.08 ^{baA}
	2	8.63 ^{baA}	7.25 ^{abA}
	4	6.54 ^{aA}	6.42 ^{aA}
non typical	0	2.00 ^{aA}	3.08 ^{aA}
	2	2.13 ^{aA}	3.25 ^{aA}
	4	5.85 ^{baA}	7.92 ^{baB}
Aftertaste			
overall	0	9.67 ^{abA}	11.00 ^{abC}
	2	8.25 ^{aA}	9.25 ^{aA}
	4	11.23 ^{baA}	12.17 ^{baA}
drying	0	5.58 ^{baA}	8.67 ^{abB}
	2	3.00 ^{aA}	5.88 ^{aB}
	4	4.15 ^{abA}	10.17 ^{baC}
bitter	0	6.67 ^{baA}	8.50 ^{abB}
	2	6.00 ^{aA}	8.00 ^{aB}
	4	7.31 ^{baA}	9.83 ^{baC}

a, b – average values marked by different letters in the columns for different indicators, statistically significantly different (P<0.05), depending on the duration of storage period.
A, B, C – average values marked by different letters in the rows for different indicators, statistically significantly different (P<0.05), depending on chitosan addition.

Table 11. Effect of chitosan on the acceptability of mayonnaise during the storage

Acceptability	Moment of testing, months	Sample	
		Control	Containing chitosan
Taste	0	12.58 ^{baA}	11.17 ^{baA}
	2	11.38 ^{baA}	10.25 ^{baA}
	4	8.69 ^{aA}	5.83 ^{aB}
Texture	0	13.42 ^{aA}	12.92 ^{baA}
	2	12.88 ^{aA}	12.25 ^{abA}
	4	12.69 ^{aA}	10.58 ^{aB}
Overall	0	12.75 ^{baA}	11.33 ^{baA}
	2	11.75 ^{baA}	10.25 ^{baA}
	4	8.54 ^{aA}	5.92 ^{aB}

a, b – average values marked by different letters in the columns for different indicators statistically significantly different (P<0.05) depending on the duration of storage period.
A, B – average values marked by different letters in the rows for different indicators statistically significantly different (P<0.05), depending on chitosan addition.

Weak astringent/drying taste that was identified during our studies confirms the findings of other authors. When studying the effects of different amounts of chitosan on the sensory properties of yogurt, it has been established that 0.7 % chitosan additive content caused a clearly identifiable astringent taste even in fresh yogurt samples. Lower concentrations of chitosan did not demonstrate any negative effects on the taste (Seo et al., 2009). Seo et al., (Seo et al., 2011) noted that astringent flavour that is characteristic to pure chitosan is more or less felt in the products enriched with it.

Addition of chitosan did not have any significant effect on the acceptability of taste and texture (Table 11) in fresh and 2-month stored samples. However, after 4 months of storage samples containing chitosan were assessed as being less acceptable ($P < 0.001$). Overall acceptability of these samples reduced only after 4 months of storage.

Conclusions

1. Addition of a biologically active ingredient – chitosan – did not have notable effect on the total plate count and growth of yeast and moulds in mayonnaise. However addition of chitosan to spreadable curd cheese was related to tendency in the total plate count reduction and demonstrated no impact on the growth of yeast and moulds.

2. Chitosan supplement prominently increased the values of active acidity in spreadable curd cheese and mayonnaise. However it demonstrated no impact on spreadability of these products.

3. Chitosan supplement did not have any effect on the texture and colour characteristics in both fresh and stored samples of spreadable curd cheese and mayonnaise.

4. Chitosan supplement demonstrated some negative effects on the following sensory properties of the studied products. In the samples of spreadable curd cheese containing chitosan, thickness as well as creaminess was reduced. Despite of it, both control and test samples of spreadable curd cheese were assessed as being acceptable during the entire testing period (20 days).

5. Chitosan supplement in mayonnaise caused reduction of certain odours and tastes, slightly changed texture properties. With respect to the results of sensory analysis obtained, the storage duration for mayonnaise containing chitosan should be reduced to 4 months.

References

1. Altieri C., Scrocco C., Sinigaglia M., Nobile M. A. Use of chitosan to prolong mozzarella cheese shelf life. *Journal of Dairy Science*. 2005. 88. P. 2683–2688.
2. Aranaz I.; Mengibar M.; Harris R.; Panos I., Miralles B.; Acosta N., Galed G., Heras A. Functional characterization of chitin and chitosan. *Current Chemical Biology*. 2009. 3. P. 203–209
3. Commission Regulation (EC) No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction

of disease risk and to children's development and health. *Official Journal of the European Union*. 2013 05 12. P. L 136/1–L 136/40.

4. Gammariello D., Chillo S., Mastromatteo M., Di Giulio S., Attanasio M., Del Nobile M. A. Effect of Chitosan on the Rheological and Sensorial Characteristics of Apulia Spreadable Cheese. *Journal of Dairy Science*. 2008. 91. P. 4155–4163.

5. Gammariello D., Conte A., Attanasio M., Del Nobile M. A. A study on the synergy of modified atmosphere packaging and chitosan on stracciatella shelf life. *Journal of Food Process Engineering* 2011. 34. P. 1394–1407.

6. Gammariello D., Conte A., Del Nobile M. A. Assessment of chitosan and extracts of lemon and sage as natural antimicrobial agents during Fior di latte cheesemaking. *International Journal of Dairy Technology*. 2010. 63. P. 530–537.

7. ISO 4121 Sensory analysis – Guidelines for the use of quantitative response scales.

8. Oh H. I., Kim Y. J., Chang E. J., Kim J. Y. Antimicrobial characteristics of chitosans against food spoilage microorganisms in liquid media and mayonnaise. *Bioscience Biotechnology and Biochemistry*. 2001. 65. P. 2378–2383.

9. LST EN ISO 4833:2003 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 °C.

10. LST ISO 21527-1:2008 Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95.

11. No H. K., Park N. Y., Lee S. H., Meyers S. P. Antibacterial activity of chitosan and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology*. 2002. 74. P. 65–72.

12. Qin C., Li H., Xiao Q., Liu Y., Zhu J., Du Y. Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate Polymers*. 2006. 63. P. 367–374.

13. Rhoades J., Roller S. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. *Applied and Environmental Microbiology*. 2000. 66. P. 80–86.

14. Sagoo S., Board R., Roller S. Chitosan inhibits growth of spoilage micro-organisms in chilled pork products. *Food Microbiology*. 2002. 19. P. 175–182.

15. Seo M. H., Chang Y. H., Lee S., Kwak. H. S. The physicochemical and sensory properties of milk supplemented with ascorbic acid-soluble nano-chitosan during storage. *International Journal of Dairy Technology*. 2011. 64. P. 57–63.

16. Seo M. H., Lee S. Y., Chang Y. H., Kwak H. S. Physicochemical, microbial, and sensory properties of yogurt supplemented with nanopowdered chitosan during storage. *Journal of Dairy Science*. 2009. 92. P. 5907–5916.

17. Sudarshan N. R., Hoover D. G., Knorr D. Antibacterial action of chitosan. *Food Biotechnology*. 1992. 6. P. 257–272.

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