

Chitosan Dilutable and Dilactin Forte: Assessment of Their Efficiency for Safety and Quality of Foodstuff

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Abstract. The modern natural food preservatives used to process and store foodstuff allow to ensure its safety and high quality. Chitosan and dilactin-forte are among such medicines. These preservatives are not only safe, but also are beneficial to a human body in virtue of their effects onto human digestive system. The article describes the results of the research conducted to identify the impact of these natural preservatives on safety of carrot (*Daucus carota* subsp. *sativus*)

1. Introduction

Traditionally, food products which are to soon appear on the shelves have to meet safety requirements and to avoid any risk of using products which could harm and (or) cause damage to life or health of consumers, any deterioration of environment. At the same time, food industry tends to produce and put into realization safe and qualitative foodstuff. Qualitative products are not only safe, but also correspond to all indicators the Russian Federation Technical regulations of the Customs Union (TR CU – 21–2011) states.

Foodstuff production uses natural preservatives such as salt, vinegar, vegetable oil, honey, pepper, garlic to preserve food from spoiling, poisonous smells and harmful effects. New technologies allow the industry to apply medicines received from natural raw materials, for example, chitosan and dilactin-forte. Their safe and highly effective usage is proved by numerous lab experiments [2–7]. Besides, studying new medicines indicates their clear advantages over traditional supplies due to special characteristics allowing to consider them both as preservatives and the additives improving human digestion.

The research of new natural preservatives' effects on various food products including conditions of their storage and processing is essential for both expanding their application and improving quality of food products. The presented research attempts to estimate efficiency of chitosan dilutable and dilactin-forte for ensuring safety of carrot (*Daucus carota* subsp. *Sativus*).

2. Material and Methods

Experimental research uses two natural preservatives: chitosan dilutable and dilactin-forte, and implies verification of their impact on safety of two types of carrot – whole and cut. Here is a brief description of the preservatives' characteristics and the experiment's features.

2.1. Preservatives description.

As mentioned above, two natural preservatives were chosen. However, the focus was set on studying the influence of chitosan.

2.1.1. Dilutable Chitosan. Chitosan is extracted from Crustacea's shells, insects' cuticles and mushroom cellular walls [8-10]. Chitosan dilutable is produced from chitosan by adding CH₂COOH (chloracetic acid) to NH₂ or by adding OH in the alkaline environment (this carboxymethyl product fully dissolves in water). Chitosan is widely used, including food industry, medicine, agriculture, cosmetology and bioengineering.



In food industry the preservative is used to thicken dietary foodstuff in production of sauces, pastes, juice, wines. It is referred to food fibers and creates solution of high viscosity in a stomach, shows properties of a sorbent and promotes to removal of radionuclides from an organism, possesses immunostimulating, anti-sclerous and anti-arthritis abilities, reduces cholesterol and sugar levels [11, 12]. Chitosan creates thin, flexible protective peels on surface of fruits, vegetables, fish, which are antimicrobial filters and have selective permeability for gases and water, which allows to prolong storage life of fresh and frozen products.

2.1.2. Dilactin-forte. This is a nutritional supplement comprised of lactic acid, acetic acid, propionic acid and their salts. Due to its antimicrobial, buffer, antioxidant, stabilizing functions as well as safe for human health.

Dilactin-forte is used to produce and store meat, fish, confectionery, bakery, in sauces, seasonings, mayonnaise.

2.2. Experiment features

The research was conducted to show the influence Chitosan and Dilactin-Forte have on carrot microbiological pollution.

2.2.1. Research preparation. Before the experiment started, substratum, 0.6 cm paper disks were prepared and sterilized, 10 kg of carrots, Chitosan dilutable (in a dry form) and Dilactin-Forte (in the liquid state) were prepared. The packing material (bopp-film) was chosen and 15x20 cm packages were welded at a speed of 2 m/s at the temperature of 140 °C by means of the CBS-900 constant heating welder.

2.2.2. Experiment course. The first part of the experiment was made to compare the natural preservatives (Chitosan dilutable and Dilactin-Forte) influence on E.coli.

E.coli was seeded into agar-agar substratum in the sterilized Petri dishes. Each dish got 1ml3 E.coli and 15-20 ml layer of the substratum was added above. A disco-diffusive method was applied to assess the influence: paper disks containing preservatives were located on a surface of the stiffened agar containing E.coli. Diffusion of the preservative into agar led to formation of a suppression zone around the disks, showing the preservatives efficiency.

The preservatives' efficiency was assessed by mass fractions of 1-5%. After a 24-hour incubation of dishes in the thermostat at a temperature of 35-37 °C the data was taken. The disco - diffusive method showed that most effective on E.coli was chitosan in mass fractions in 1%, 2% and 3%. The results determined these concentrations for the second part of the experiment. The second part of the experiment was aimed to state the effect of Chitosan in controlling microbiological pollution of food products.

According to the results of the first part of the experiment Dilactin-Forte was excluded as a less effective preservative, which allowed to choose carrot *Daucus carota* subsp. *savitus* as a sample of food product.

Carrots were used as whole samples or cut in straws of 2-3 cm wide and 5-6 cm length. Samples of 100 g were processed by pre-prepared preservative, then were packed and kept in the refrigerator at 4 °C. Besides preparation of samples microbiological analysis of product samples was held on the start of the experiment to state the amount of yeast and mold, coliforms, QMAFAnM, Salmonella and L.monocytogenes. Also, the same analyses were carried out for Chitosan by a mass fraction of 1%.

Comparative analyses of samples to identify Salmonella bacteria and Listeria monocytogenes were made by the standard methods of the analysis and the polymerase chain reaction (PCR). PCR research was conducted by extracting the DNA from the microorganisms by selection of specific primers of the microorganisms, temporary and temperature parameters of the analysis. The discriminatory analyses on detection of pathogenic microorganisms of the Salmonella and Listeria monocytogenes group showed their absence in the samples studied.

QMAFAnM, coliforms and yeast/mold analysis were carried out by using method of limit cultivations and deep seeds into substratum.

Coliforms' analysis incubated for 24 hours in the thermostat at epy temperature of 37 °C.

QMAFAnM analysis were incubated in the thermostat at the temperature of 37 °C for 3 days.

Yeast/mold analysis were incubated in the thermostat at the temperature of 25 °C for 5 days.

The analysis of microbiological pollution of foodstuff was carried out by selecting 1 gr of a sample and performing 5 tenfold cultivations. First and second cultivations were used for yeast/mold analysis; 2-5 were used for yeast/mold analysis. (The results are specified in Table 1).

All analyses were made three times.

Table 1

0 day analysis results (average numbers), colony-forming units (CFU)

Coliforms	QMAFAnM		Yeast/mold		<i>Salmonella</i>	<i>Listeria monocytogenes</i>	Chitosan dilutable 1%
Negative	4c	5c	1c	2c	None	None	1
	9	1	80	8			

During the experiment Coliforms, QMAFAnM and yeast/mold analysis were carried out on first, third, fifth, seventh and tenth days of storage. (The results are specified in Table 2).

Table 2

Analysis results, CFU

		1 day											
		Coliforms			QMAFAnM						Yeast/mold		
		1s	2s	3s	1s		2s		3s		1s	2s	3s
					2c	4c	2c	4c	2c	4c			
1 %	Whole	Negative			75	0	180	2	110	1	10	2	7
	Cut				227	3	100	1	150	2	32	5	15
2 %	Whole				15	0	4	0	10	0	1	0	0
	Cut				93	3	550	2	240	3	30	10	15
3 %	Whole				16	0	10	0	11	0	3	0	1
	Cut				27	0	8	0	15	0	2	3	2
		3 day											
		Coliforms			QMAFAnM						Yeast/mold		
		1s	2s	3s	1s		2s		3s		1s	2s	3s
					3c	5c	3c	5c	3c	5c			
1 %	Whole	Positive			100	0	0	0	110	1	5	1	6
	Cut				90	0	119	1	150	1	6	5	8
2 %	Whole				70	1	0	0	85	0	30	25	30
	Cut				100	0	100	0	120	1	400	500	450
3 %	Whole				1	0	13	0	5	0	0	0	0
	Cut				3	0	2	0	2	0	2	4	3
		5 day											
		Coliforms			QMAFAnM						Yeast/mold		
		1s	2s	3s	1s		2s		3s		1s	2s	3s
					3c	5c	3c	5c	3c	5c			
1 %	Whole	Positive			210	2	2	0	115	1	3	4	3
	Cut				200	1	85	1	150	2	0	12	8
2 %	Whole				110	1	250	1	165	1	35	35	40
	Cut				10	1	400	15	150	2	25	500	30
3 %	Whole				20	0	15	0	11	0	0	7	5
	Cut				23	0	35	0	25	0	5	7	6

		7 day											
		Coliforms			QMAFAnM						Yeast/mold		
		1s	2s	3s	1s		2s		3s		1s	2s	3s
				4c	5c	4c	5c	4c	5c	2c	2c	2c	
1 %	Whole	Positive		150	0	90	1	110	10	4	5	7	
	Cut			25	1	10	0	15	1	6	7	9	
2 %	Whole			35	2	10	1	10	1	50	80	65	
	Cut			0	0	28	3	35	3	300	440	400	
3 %	Whole			10	0	5	0	11	0	5	3	4	
	Cut			10	0	7	0	8	0	7	10	5	
		10 day											
		Coliforms			QMAFAnM						Yeast/mold		
		1s	2s	3s	1s		2s		3s		1s	2s	3s
				4c	5c	4c	5c	4c	5c	2c	2c	2c	
1 %	Whole	Positive		270	3	110	1	110	1	5	7	8	
	Cut			30	2	35	3	32	2	10	12	15	
2 %	Whole			35	4	10	3	10	1	125	150	115	
	Cut			15	1	35	3	27	3	500	450	500	
3 %	Whole			15	1	10	1	11	1	4	5	5	
	Cut			10	1	7	1	15	1	9	10	8	

For results analysis average numbers were counted. (The results are shown in Table 3).

Table 3

Average numbers, CFU						
QMAFAnM	Whole					
	1%	12167	70000	100000	116667	166667
	2%	967	51667	100000	183333	266667
	3%	1233	6333	15333	86667	100000
		1 day	3 day	5 day	7 day	10 day
	Cut					
	1%	15900	66667	133333	166667	233333
	2%	29433	106667	600000	210000	233333
	3%	1667	2333	27667	83333	100000
		1 day	3 day	5 day	7 day	10 day
Yeast/mold	Whole					
	1%	633	400	400	467	667
	2%	33	2833	3667	6500	13000
	3%	133	0	400	400	467
		1 day	3 day	5 day	7 day	10 day

	Cut					
	1%	1733	633	667	733	1233
	2%	1833	45000	18500	38000	48333
	3%	233	300	600	733	900
		1 day	3 day	5 day	7 day	10 day

During the experiment cultivations used for analysis could change due to the results of the previous ones.

3. Results and Discussion

The results of the conducted research are presented in Figures 1 – 4. They demonstrate that the effect of chitosan dilutable on microbiological pollution of carrot has the following characteristics:

Comparison of chitosan dilutable and dilactin-forte influence on E.coli revealed higher efficiency of the first in suppression of microorganisms' activity.

The greatest efficiency during the second part of an experiment was shown by 3% solution of chitosan dilutable. Its use allowed to increase expiration date of samples by four days for QMAFAnM indicator comparing to 1% and 2% solutions. 3% solution of Chitosan also had the greatest efficiency on yeast and mold bacteria and allowed to increase the product's expiration date by 10 days.

It also should be noted that Chitosan is more efficient to samples of the whole carrots in comparison with cut carrots.

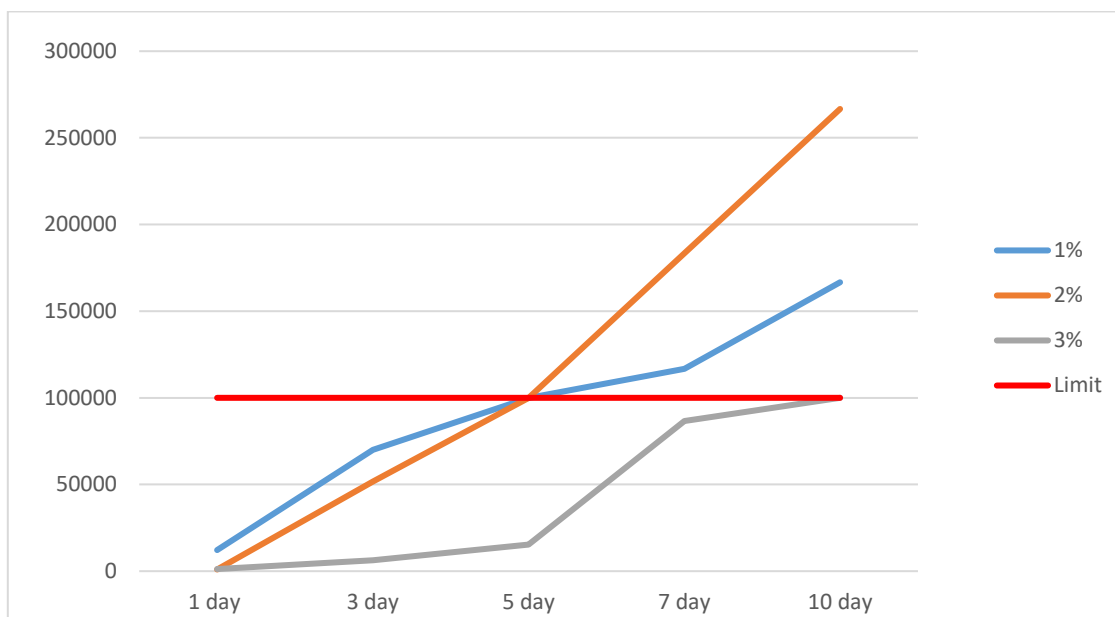


Figure 1. QMAFAnM (average numbers) whole carrot, CFU

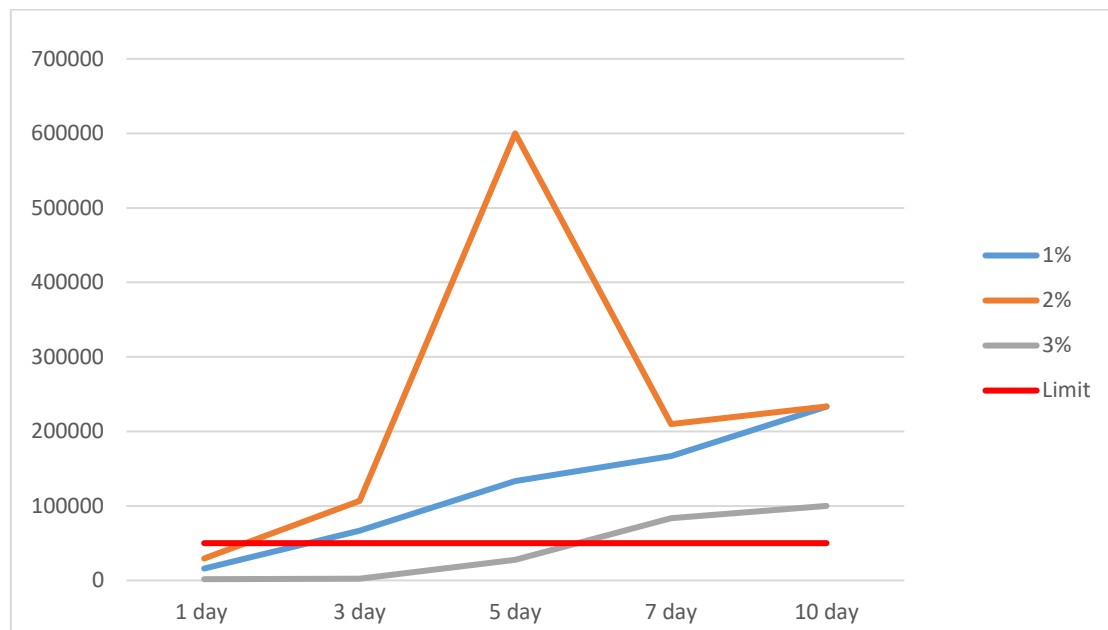


Figure 2. QMAFAnM (average numbers) cut carrot, CFU

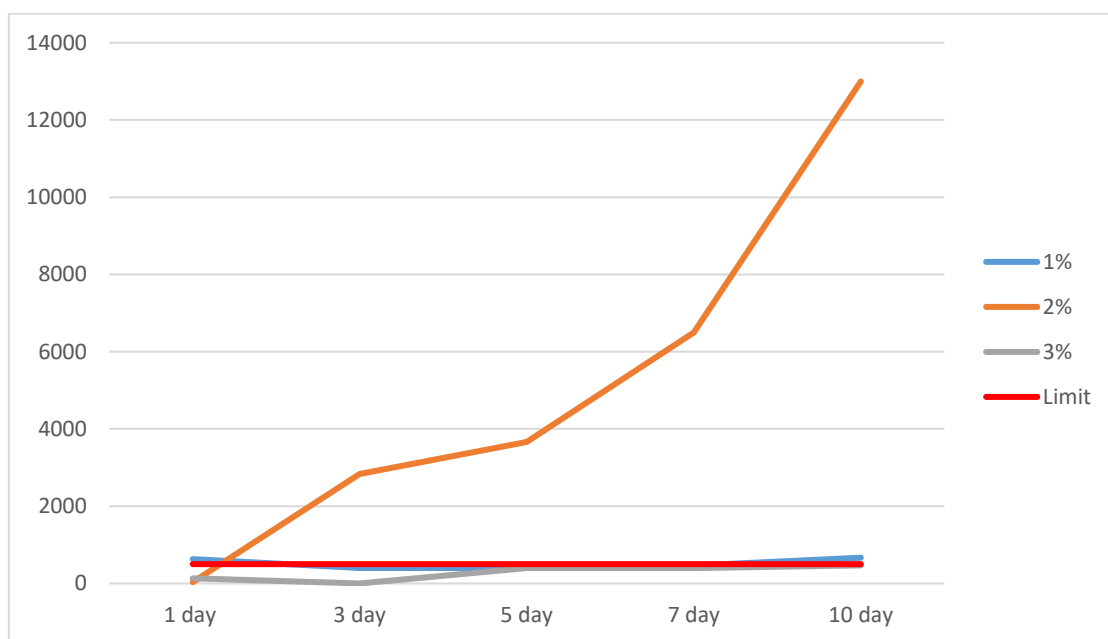


Figure 3. Yeast/mold (average numbers) whole carrot, CFU

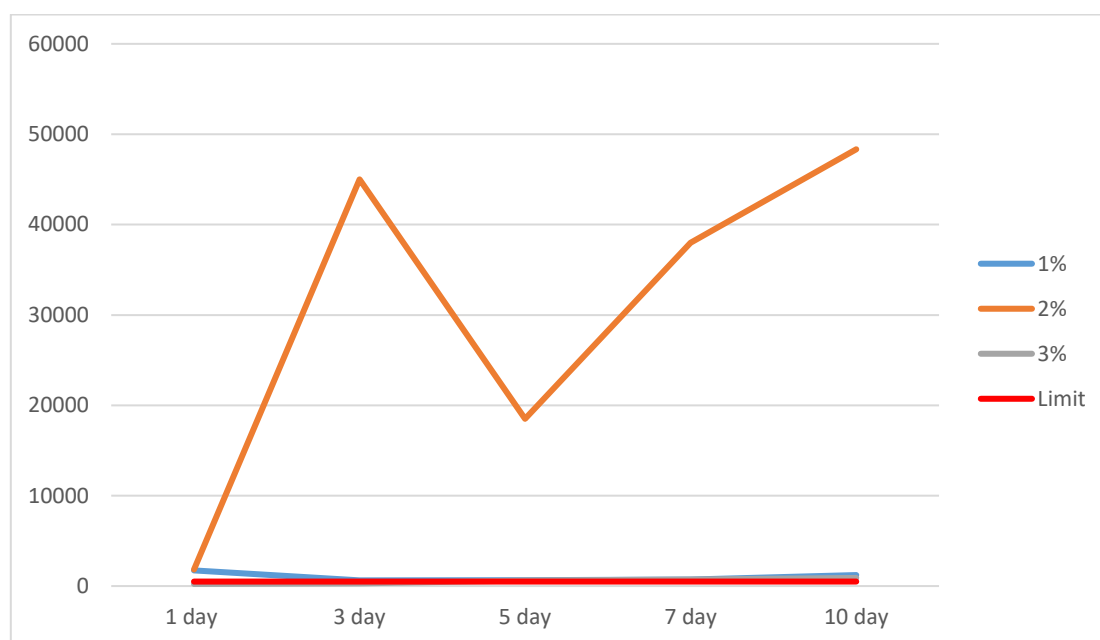


Figure 4. Yeast/mold (average numbers) cut carrot, CFU

4. Conclusion

The analysis of the experiment results allows to draw the following general conclusions:

1) Results of world-wide research and practical use of chitosan show that in comparison with other preservatives (including natural ones) this medicine has considerable advantages: it is safe for human body and improves food quality due to its unique properties.

2) During the carrot experiment it was revealed that the chitosan's influence on *E.coli* is much higher than that of dilactin-forte.

Chitosan solution in 3% mass fraction turned out to be the most efficient one, its use allowed to extend the QMAFAnM indicated samples expiration date by 4 days in comparison to solutions in 1% and 2% mass fraction.

The use of this medicine compared to contents indicator in yeast and mold samples showed that chitosan of 3% mass fraction is also most effective, its use increased the product's expiration date by 10 days).

The experiment proved natural preservatives are most efficient for whole samples of carrot (in comparison with cut ones).

3) It should be noted that the effect of chitosan in different mass solutions on various foodstuffs needs further research and studying. It's important to outline the most effective concentration of solution for each product type. Besides, research focused on developing new technologies to extract chitosan from different types of raw materials should also be conducted as it could allow to decrease its prime cost.

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