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ANTIMICROBIAL PROPERTIES OF BERRY POWDERS IN CREAM CHEESE

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Abstract. This study aims to evaluate the efficiency in reducing pathogens using berry powders in a cream cheese recipe, as compared to the traditional method of manufacturing. This article presents a review of the microbiological hazards of dairy products and a case study of antimicrobial properties of berry powders on pathogenic microorganisms that can accidentally colonize cream cheese. The most relevant results in the reduction of pathogenic microorganisms were obtained in cream cheese samples with the addition of rosehip and aronia powders on *Salmonella Abony* ATCC 6017; hawthorn powder on *Staphylococcus aureus* ATCC 25923. All berry powder additions had major effects on *Escherichia coli* ATCC 25922. Research has shown that powders of berries can be used for the manufacture of dairy products with natural ingredients more resistant to accidental contamination and safe for consumption.

Keywords: *reducing pathogens, thermostation period, log CFU, growth of microorganisms.*

Introduction

Due to their unique composition and properties milk and other dairy products are excellent growth media for many spoilage and pathogenic microorganisms [1, 2].

Salmonella continues to be a major concern for the dairy industry because these bacteria have caused recent outbreaks of illness and have been isolated from various dairy products in the market place. *Salmonella* bacteria are generally not heat resistant and normally grow at 35 to 37 °C, but they can grow at much lower temperatures, provided that the incubation time is suitably extended. To minimize problems, foods should be kept at 2 to 5 °C or below at all times [3]. Lately several salmonellosis cases have been recorded due to consumption of raw milk (both regular and organic). It goes to prove that more of the animal origin strains have been gaining entrance and surviving in the raw milk. The

December 2016 recall of nonfat dry milk had a huge resonance regarding dairy contamination [4].

The questions to be asked are: does Salmonella gain entrance into raw milk from cows, and does pasteurization kill the bacteria? Salmonella does gain entrance into raw milk from cows, and in the majority of cases pasteurization is adequate to kill it. If some strains of *Salmonella* survive high heat treatments, how can we eliminate this problem in dairy products [4]?

Microbiological risks in foods such as milk and dairy products are addressed in several studies. Most research includes the influence of heat treatment or salt in monitoring this risk [5]. The objective proposed in State project 18.51.07.01A / PS was to investigate other methods of reducing and controlling microbiological risk in food. Previous studies [6, 7] have shown that berry powder has an inhibitory effect on pathogenic microorganisms (*S. aureus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *B. mesentericus*), including meat products [8].

The purpose of this study was to evaluate the effect of adding berry powders to the cream cheese recipe on pathogenic microorganisms that may accidentally infect the finished product.

Materials and methods

In the pilot laboratory of the Food Technology Department, Technical University of Moldova, cream cheese samples were prepared using various berry powders grown in the Republic of Moldova. In this study the following samples with berry powder additions were investigated microbiologically:

- ✓ Cream cheese - sample control – (S1);
- ✓ Cream cheese with 2% rose-hip powder – (S3);
- ✓ Cream cheese with 2% sea buckthorn powder – (S6);
- ✓ Cream cheese with 2% aronia powder – (S9);
- ✓ Cream cheese with 2% hawthorn powder – (S12).

Microbiological tests were carried out in the laboratory of the Microbiology and Immunology Department, "Nicolae Testemitanu" State University of Medicine and Pharmacy. For the contamination of the experimental samples of cream cheese the microbial strains (*Staphylococcus aureus* ATCC 25923, *Salmonella Abony* ATCC 6017, *Escherichia coli* ATCC 25922) were procured from the National Public Health Agency. Inoculation of microorganisms in cream cheese samples, thermostatization and monitoring of microbial growth was performed according to the following bibliographic sources [8-10].

Results and discussions

The predominant human bacterial pathogens that can potentially be transferred to milk include mainly *Listeria monocytogenes*, *Salmonella spp*., *Staphylococcus aureus* and *Escherichia coli*. Raw milk provides a potential growth medium for the development of these bacteria [11]. Although pasteurization destroys potential pathogenic microorganisms, post pasteurization processing can lead to the recontamination of dairy products. For hygienic reasons, most cheeses are produced from pasteurized milk; however, with a production of 700,000 tons per year, raw milk cheeses represent a significant proportion of ripened cheeses produced in Europe, particularly in Italy, France and Switzerland. Many artisan cheese makers, customers and researchers claim the benefits/advantages of raw milk cheeses, so it is important to know the consequences and possible implications of pasteurization on the ripening process, and ultimately on the sensory characteristics of cheese [12, 13].

Efficient pasteurization of milk eliminates the risk of pathogenic organisms, but does not destroy organisms that grow slowly or produce spores. While pasteurization destroys many microorganisms in milk, improper handling after pasteurization can recontaminate it [14, 15]. Both raw and pasteurized milk can be contaminated during bottling, shipment and storage. Pasteurization only destroys the pathogens in milk at the time of processing; if unsanitary conditions allow pathogens to re-enter the milk later, it will be contaminated again [16].

Heat is lethal to microorganisms, but each species has its own particular heat tolerance. During a thermal destruction process, such as pasteurization, the rate of destruction is logarithmic, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is proportional to the number of organisms present. The process is dependent both on the temperature of exposure and the time required at this temperature to accomplish the desired rate of destruction. Thermal calculations thus involve the necessity to know the concentration of microorganisms to be destroyed, the acceptable concentration of microorganisms that can remain after (e.g. spoilage organisms, but not pathogens), the thermal resistance of the target microorganisms (the most heat tolerant ones), and the temperature-time relationship required to destroy the target organisms [17].

Table 1 and Figures 1 and 2 include the obtained results in the evaluation of the influence of the berry powders (rose-hip, sea buckthorn, aronia and hawthorn) on *Salmonella Abony* ATCC 6017.

Table 1

Reducing the growth rate of pathogenic microorganisms in cream cheese samples with the berry powders additions.

Sample coding	**Estimated values**	**Incubation duration, h**		
		0	**24**	**48**
***Salmonella Abony* ATCC 6017**				
S1	CFU/1g product	$1.4*10^4$	$7.12*10^8$	$1.4*10^8$
	log CFU	4.146	8.85	8.15
S3	CFU/1g product	$1.4*10^4$	$1.1*10^7$	0
	log CFU	4.146	7.041	$-\infty$
S6	CFU/1g product	$1.4*10^4$	$4.2*10^7$	$5*10^6$
	log CFU	4.146	7.62	6.70
S9	CFU/1g product	$1.4*10^4$	0	0
	log CFU	4.146	$-\infty$	$-\infty$
S12	CFU/1g product	$1.4*10^4$	$6.6*10^7$	10^7
	log CFU	4.146	7.82	7
***Staphylococcus aureus* ATCC 25923**				
S1	CFU/1g product	$1.4*10^4$	$1.25*10^8$	$3.2*10^7$
	log CFU	4.146	8.097	7.50
S3	CFU/1g product	$1.4*10^4$	$3.8*10^7$	$1*10^6$
	log CFU	4.146	7.58	6

Continuation *Table 1*

S6	CFU/1g product	$1.4*10^4$	$4,0*10^7$	$2.8*10^7$
	log CFU	4.146	7.6	7.45
S9	CFU/1g product	$1.4*10^4$	$7.1*10^7$	$5.1*10^7$
	log CFU	4.146	7.85	7.71
S12	CFU/1g product	$1.4*10^4$	$2.8*10^7$	0
	log CFU	4.146	7.45	-∞
***Escherichia coli* ATCC 25922**				
S1	CFU/1g product	$1.4*10^4$	$1.9*10^7$	$8*10^6$
	log CFU	4.146	7.28	6.90
S3	CFU/1g product	$1.4*10^4$	$1.2*10^7$	0
	log CFU	4.146	7.08	-∞
S6	CFU/1g product	$1.4*10^4$	0	0
	log CFU	4.146	-∞	-∞
S9	CFU/1g product	$1.4*10^4$	$2*10^6$	0
	log CFU	4.146	6.30	-∞
S12	CFU/1g product	$1.4*10^4$	$6*10^6$	0
	log CFU	4.146	6.78	-∞

According to European legislation, raw milk must be processed within a maximum limit of 48 hours, to reduce the development of pathogens [18]. In raw-milk cheese production, checking the acidity (pH and titratable acidity) of the milk and the curd along the production chain can help minimize risk. If acidity develops rapidly (in the first 6, 8 or 12 hours since the start of processing), it means that a good part of the microorganisms dangerous to health and product quality have been limited [19].

Salmonella has long been recognized as an important human health problem of economic significance in animal and humans. A total of 108,614 confirmed cases of salmonellosis were reported in the European Union in 2009, although cases attributed to *S. enteritidis* have decreased during the last years [20]. However, *Salmonella* infections have not declined over the past 15 years in USA [21]. Dairy products along with meat and eggs are the most common causes of food-borne infection by *Salmonella*. Salmonellosis from contaminated milk and dairy products has been associated with inadequate pasteurization and post-process contamination. Most cheeses, including raw or pasteurized milk cheeses, properly manufactured and aged, appear to pose no significant health risk of *Salmonella* infection [22].

There is extensive literature describing strains of lactic acid bacteria (LAB) from traditional dairy products able to inhibit the most important cheese pathogens (*L. monocytogenes, S. aureus, Klebsiella pneumoniae, Salmonella typhimurium, Bacillus subtilis*, and *Pseudomonas aeruginosa*) in media laboratory [19, 23]. Single strains of *L. garvieae* and *Lactococcus lactis* inhibited *S. aureus* early in a cheese matrix [24, 25].

Bertrand et al. [26] investigated the effect of raw milk cheese consumption on the enteric microbiota. The study by Fstanz [27] and Podolak et al. [14] is also of interest.

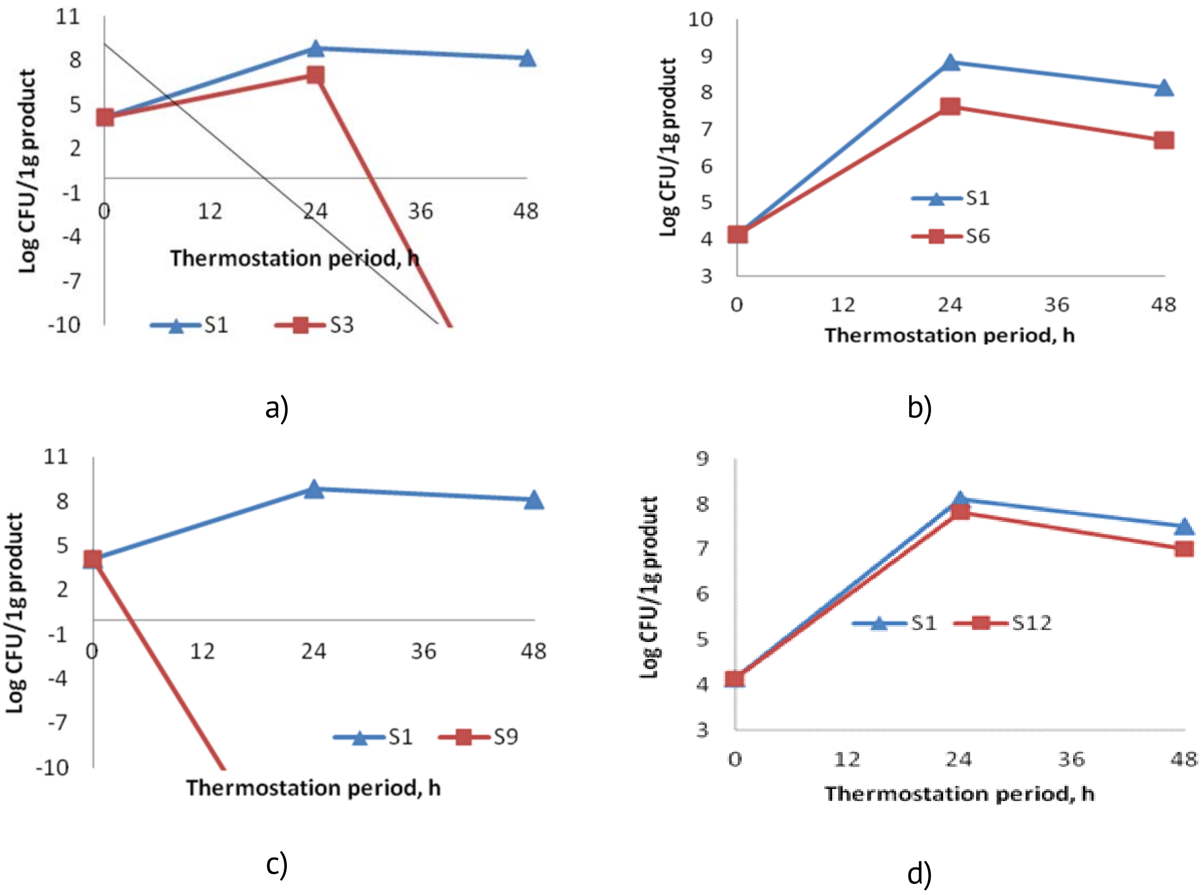

Figure 1. Comparison of the growth rate reduction of *Salmonella Abony* ATCC 6017 in the control sample and the samples with berry powders additions during 48 hours:
a) Cream cheese: sample control – (S1) and with rose-hip powder – (S3);
b) Cream cheese: sample control – (S1) and with sea buckthorn powder – (S6);
c) Cream cheese: sample control – (S1) and with aronia powder – (S9);
d) Cream cheese: sample control – (S1) and with hawthorn powder – (S12).

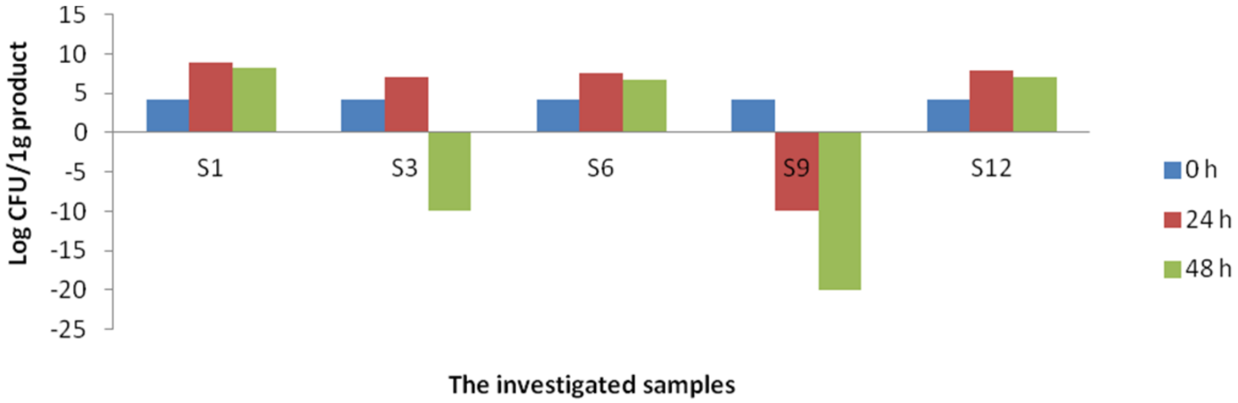

Figure 2. Modification of the content of ***Salmonella Abony*** ATCC 6017 inoculated in cream cheese samples at thermostat for 48 hours and temperature 37 ºC.

The factors that determine the growth of *Salmonella spp*. are included in Table 2 [14, 28]. It is known that groups of grown microorganisms in food have the optimum, minimum and maximum pH. Table 2 lists the approximate pH ranges for growth in laboratory media for selected organisms relevant to food. Generally, pH interacts with other parameters in food to inhibit growth. The pH can interact with factors such as a_w, salt, temperature, redox potential and preservatives to inhibit growth of pathogens and other organisms. The pH of food significantly impacts the lethality of heat treatment of the food. Less heat is needed to inactivate microbes as the pH is reduced [29, 30].

Table 2

Limits for growth of pathogenic microorganisms when other condition are near optimum*

Indicators	Minimum	Optimum	Maximum
		Salmonella ssp.	
Temperature, °C (F)	5 (41)	35 - 37 (95 - 99)	45 - 47 (113 - 117)
***p*H**	3.8	7-7.5	9.5
Water activity	0.93	0.99	>0.99
		Staphylococcus aureus growth	
Temperature, °C (F)	7 (45)	35 - 40 (95 - 104)	48 (118)
***p*H**	4.0	6.0-7.0	10.0
Water activity	0.83	0.98	0.99
		Staphylococcus aureus toxin	
Temperature, °C (F)	10 (50)	40 - 45 (104 - 113)	46 (115)
***p*H**	4.5	7.0- 8.0	9.6
Water activity	0.88	0.98	0.99
		Enteromorarrhagic E. coli	
Temperature, °C (F)	10 (50)	40 - 45 (104 - 113)	46 (115)
***p*H**	4.4	6.9-7.0	9.0
Water activity	0.95	0.99	

*Sources: ICMSF 1980, p 101. [https://www.canr.msu.edu/uploads/234]

A literature review showed that pathogenic microbes may be present in raw milk and in various types of dairy products, and these pathogenic microbes may also be present in the finished product, as they can enter industrial products during processing, packaging, distribution and storage [31, 32]. Some individuals are chronic carriers of *Salmonella* and if such an individual comes in contact with the anticaking agents (at the time of their manufacturing), especially dry blended products, the *Salmonella* can gain entrance into the anticaking agents and thus into the finished cheese. It is recommended to use low pH, high-heat spray dried anticaking agents, which are handled by workers to eliminate subdued pathogens [4]. The key factors to reduce the risk of *S. typhimurium* contamination are the implementation of appropriate hygiene measures to minimize the contamination of raw milk and proper storage of cheeses (e.g. water buffalo mozzarella cheese) at refrigeration temperature [33]. There are also alternative methods of controlling the microbiological risk in dairy products [34, 35].

Table 1 and Figures 3 and 4 include the results in evaluating the influence of berry powders (rose-hip, sea buckthorn, aronia and hawthorn) on *Staphylococcus aureus* ATCC 25923.

Staphylococcus aureus is a ubiquitous pathogen, thus, the sources of this bacteria for dairy products contamination are diverse [36]. This bacterium is commonly found in a wide variety of mammals and birds and can be transferred to food mainly by dairy animals that have mastitis and by human carriers during food processing [37].

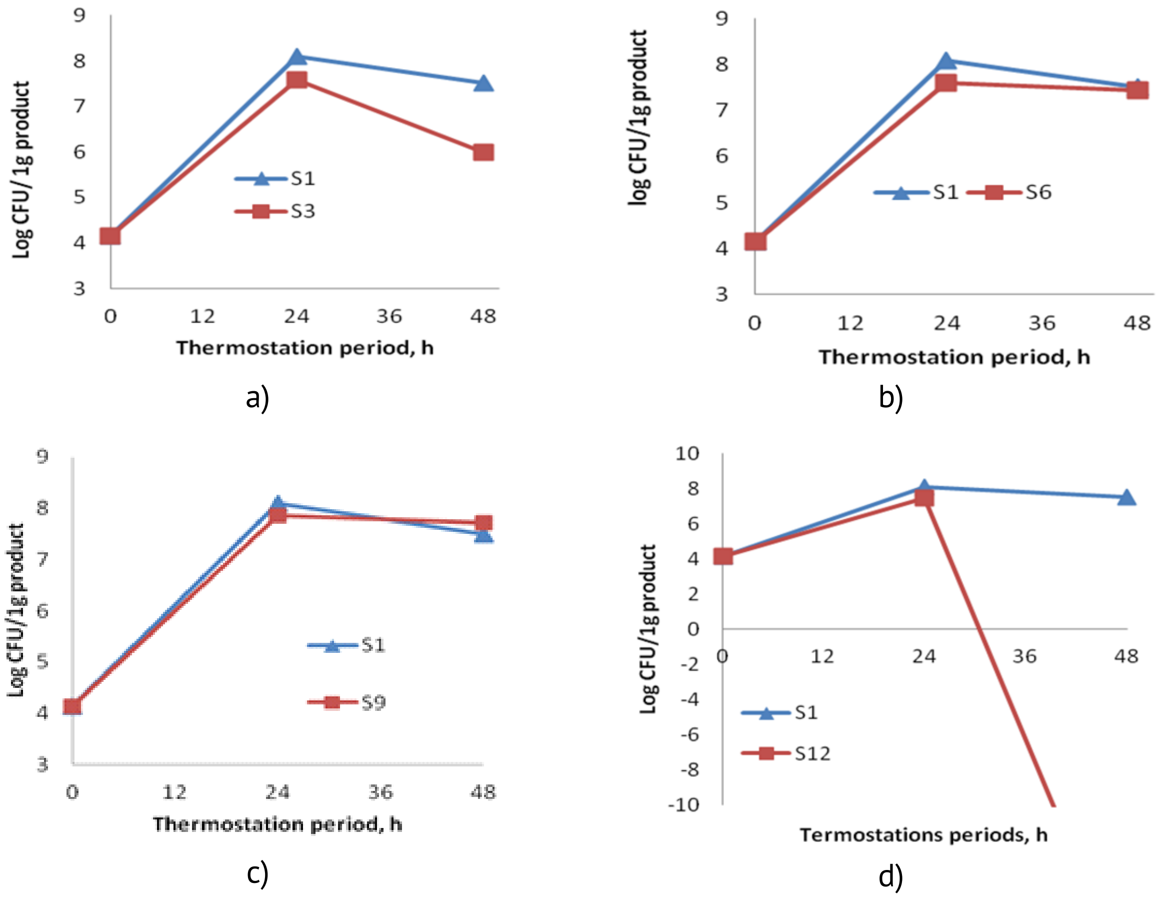

Figure 3. Comparison of the growth rate reduction of *Staphylococcus aureus* ATCC 25923 in the control sample and the samples with berry powders additions during 48 hours:
a) Cream cheese: sample control – (S1) and with rose-hip powder – (S3);
b) Cream cheese: sample control – (S1) and with sea buckthorn powder – (S6);
c) Cream cheese: sample control – (S1) and with aronia powder – (S9);
d) Cream cheese: sample control – (S1) and with hawthorn powder – (S12).

Contamination of *Staphylococcus aureus* can have a broad occurrence in raw dairy products, with frequencies between 5 and 100% in cheeses [38].

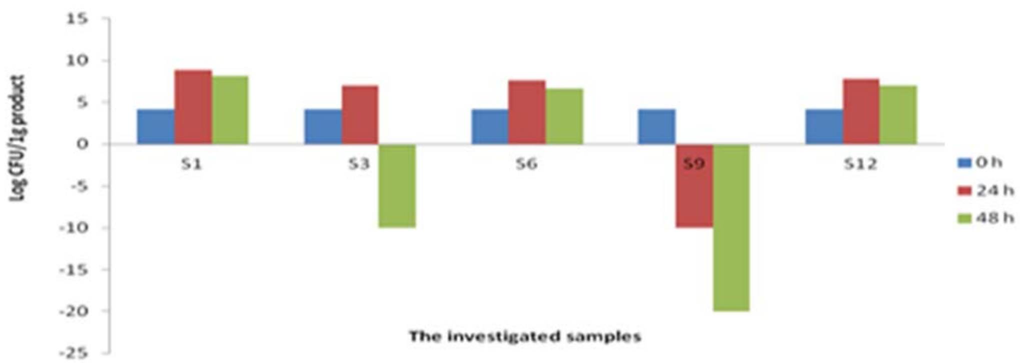

Figure 4. Modification of the content of *Staphylococcus aureus* ATCC 25923 inoculated in cream cheese samples at thermostat for 48 hours and temperature 37 °C.

The number of *Staphylococcus aureus* in raw milk or other dairy products needs to be less than 10^4 CFU g^{-1}, according to the United States Food and Drug Administration regulations [39].Investigations indicated that *Escherichia coli* O157:H7 is an emerging cause of food borne illness and that young dairy cattle are a reservoir for it. A particularly dangerous type is referred to as enterohemorrhagic *E. coli* (EHEC).

Infection with EHEC strains is often associated with food borne outbreaks traced from milk, dairy products and other foods leading to hemorrhagic colitis (bloody diarrhea) and hemolytic uremic syndrome in humans [40]. Table 1 and Figures 5 and 6 include the results after evaluating the influence of berry powders (rose-hip, sea buckthorn, aronia and hawthorn) on *Escherichia coli* ATCC 25922.

On the other hand, the outbreaks of *Listeria monocytogenes* and *Staphylococcus aureus* (*S. aureus*) are enterotoxin producers. *Salmonella spp*. and *Escherichia coli O157: H7* result from direct or indirect contamination of cheese. *S. aureus*, one of the pathogenic microorganisms that can be found in cheese, is a significant part of the poisonings from cheese [41].

The growth of *E. coli* can occur at temperatures between 7 – 46 °C, pH of 4.4 – 10.0 and a minimum water activity of 0.95 when other conditions are near optimum.

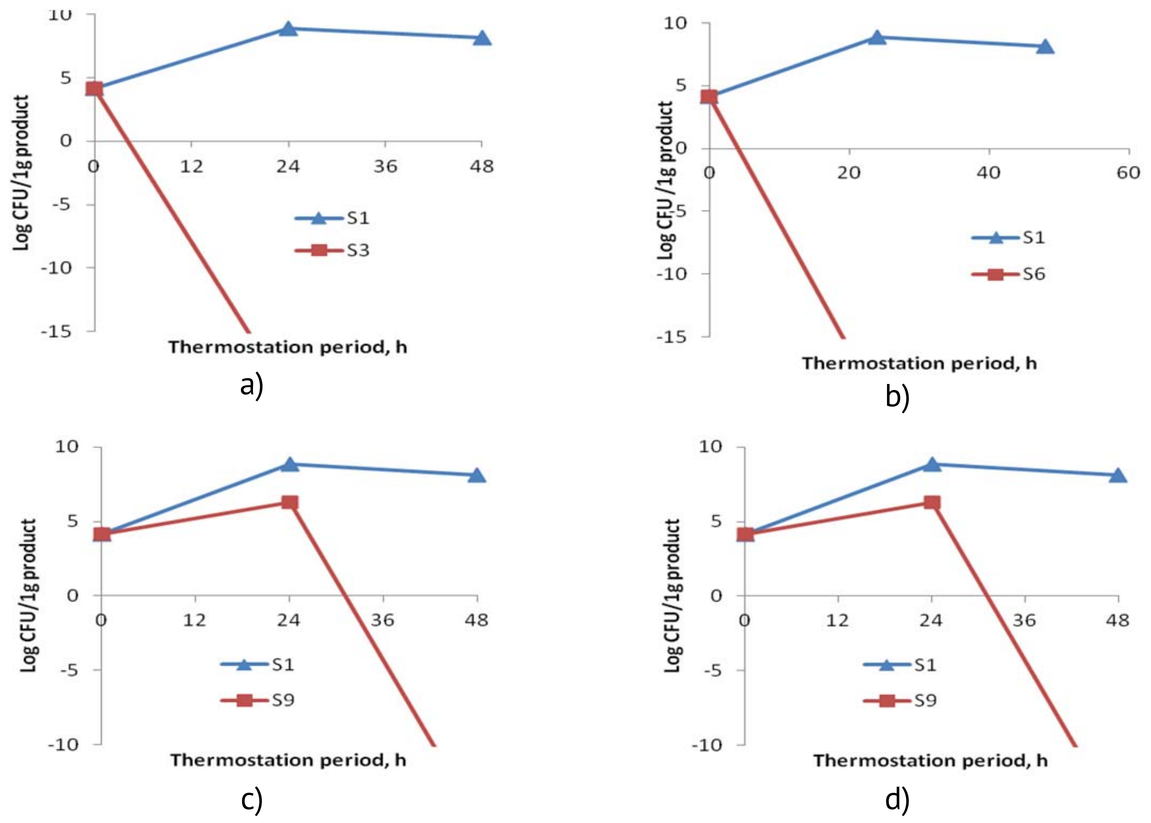

Figure 5. Comparison of the growth rate reduction of *Escherichia coli* ATCC 25922 in the control sample and the samples with berry powders additions during 48 hours:
a) Cream cheese: sample control – (S1) and with rose-hip powder – (S3);
b) Cream cheese: sample control – (S1) and with sea buckthorn powder – (S6);
c) Cream cheese: sample control – (S1) and with aronia powder – (S9);
d) Cream cheese: sample control – (S1) and with hawthorn powder – (S12).

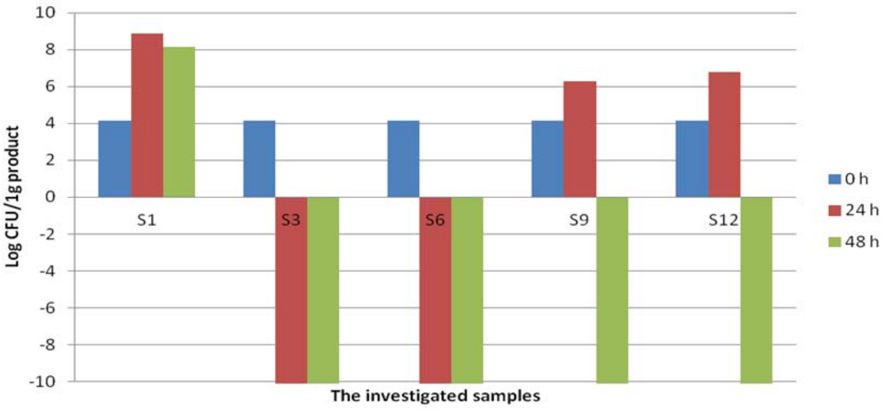

Figure 6. Modification of the content of *Escherichia coli* ATCC 25922 inoculated in cream cheese samples at thermostat for 48 hours and temperature 37 °C.

Some Shiga toxin-producing *Escherichia coli* (STEC) strains can survive at pH 2.5 – 3.0 for over 4 hours. STEC is capable to survive frozen storage at -20°C, however, it is readily inactivated by cooking [42, 43].

STEC can be a contaminant of milk sourced from infected herds. As raw milk cheese production does not include a process that reliably inactivates pathogens, the microbiological quality of raw milk is critical. Other risk factors include temperature control of the raw milk, acidification process, curd cooking, maturation/ripening, salt concentration, water activity, pH and nitrate [44, 45]. *Staphylococcus aureus, Salmonella spp., Listeria monocytogenes* and *Escherichia coli O157:H7* are the most frequent potential pathogens associated with milk or dairy products in industrialized countries [46-48].

The food industry is looking for alternatives to satisfy consumer demand for safe foods with a long shelf-life capable of maintaining the nutritional and organoleptic quality. The application of antimicrobial compounds-producing protective cultures may provide an additional parameter of processing in order to improve the safety and ensure food quality, keeping or enhancing its sensorial characteristics. In addition, strong evidence suggests that certain probiotic strains can confer resistance against infection with enteric pathogens [49]. Thus, it is proposed to use berry powders to control the microbiological risk that can occur in cream cheese at an unconditional deviation.

Conclusions

Various published studies have shown that several food borne pathogens are present in raw milk of different dairy species, and these pathogenic microbes can also be present in manufactured products, because they can enter the manufactured products opportunistically during processing, packaging, distribution and storage processes. The factors that determine the growth of pathogenic microorganisms in dairy products are: temperature, pH, water activity and presence of antimicrobial substances. The efficiency of a certain antimicrobial solution will also depend on the type, genus, species, and strain of the target microorganism. Likewise, it will also depend on environmental factors such as pH, water activity, temperature, atmosphere composition, initial microbial load, and acidity of the food substrate. The test results evaluated the antimicrobial effect of berry powders on pathogenic microorganisms (*Salmonella Abony* ATCC 6017; *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922). It was determined that the addition of berry powders (rose-hip, aronia, sea buckthorn and hawthorn) can keep the growth rate of the microorganisms, including the pathogens, under control. Most microorganisms inoculated in tested cream cheese samples with the addition of berry powders were destroyed after 48 hours of thermostation at 37 **°C**. The calculated CFU log was minus infinity. The most relevant antimicrobial effect was seen for berry powders on *E. coli* strains inoculated in tested cream cheese samples. The additions of rose-hip and aronia powders manifested major antimicrobial effect on *Salmonella* strains. The addition of hawthorn powder manifested major antimicrobial effect on *Staphylococcus aureus*. Therefore, the use of added berry powders in the recipe for the production of dairy products can have two meanings: improving the nutritional value of the food and increasing the product shelf-life by keeping the microbiological risk under control. The results of this study highlighted the need to improve and implement hygienic practices. Moreover, further research is needed to fully study the antimicrobial properties of berry powders in dairy production.

Acknowledgments

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