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INHIBITING OF ACCIDENTAL PATHOGENIC MICROBIOTA IN MEAT PRODUCTS WITH BERRY POWDERS

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Abstract. This article presents a case study of antimicrobial properties of berry powders on pathogenic microorganisms that can accidentally colonize meat and meat products. We tested the inhibiting properties of rose-hip and hawthorn on the growth of pathogenic and opportunistic pathogenic microorganisms (*S. aureus* ATCC 25923, *Salmonella* Abony ATCC 6017, *Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922). We found out that introducing rose-hip and hawthorn powder in the sausage recipe decreased the microorganism growth rate on purposely contaminated samples. By studying Lag and Logaritmic phases of strain growth rate we found that hawthorn has a greater bacteriostatic effect on *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 strains, and rose-hip has a greater bacteriostatic effect on *Salmonella* Abony ATCC 6017, *Klebsiella pneumoniae* ATCC 13883 strains.

Keywords: *pathogenic microorganisms, antimicrobial effect, meat products, rose-hip, hawthorn.*

Introduction

Worldwide, poisoning and food poisoning pose one of the most severe public health and industrial development problems [1]. The delayed phase of bacterial growth is important from a medical and food safety perspective, but it is difficult to study due to low cell density and metabolic rate [2]. The theoretical and experimental models for delay phase dynamics, such as the Baranyi model [3], are widely used in the field of food safety to estimate the duration of the delay phases in a particular food product. Globally, it is estimated that *Salmonella* is responsible for 80.3 million cases of food-borne illness [4]. A major problem threatening the food industry is the contamination with food microbes of human origin resulting from inappropriate handling and processing. Microbial contamination reduces the shelf-life and quality of foods that lead to food infections and outbreaks of food poisoning, some of which may be fatal [5]. Continuous monitoring of food processing is essential to avoid possible health problems [6, 7]. *Staphylococcus aureus* is one

of the major pathogens in food products, which frequently causes diseases as a result of consuming food contaminated with the staphylococcal toxin [8]. *E. coli* can enter the meat during processing. If contaminated meat is not treated at a temperature of 71 °C, bacteria can survive and infect the consumer. This is the most common way people in Canada become infected with *E. coli*. Any food product that has come into contact with the raw meat could also be infected [9]. A definition of quality that is applicable to the food industry is to ensure that the product is safe for consumption and that the composition of the food does not present a favorable environment for recontamination. Many bacterial species produce responses to environmental stress. The main factors affecting microbial growth and bacterial survival are pH, water activity (a_w) and temperature [10].

Based on risk assessment, manufacturers should decide what measures or combination of measures should be implemented to achieve the microbiological risk reduction objective. Some of these measures can be easily applied, while others require significant investment. We propose the use of berry additives in order to reduce the microbiological risk and to control the quality and safety of the foodstuffs. The Lag phase is a period without growth that occurs when stationary phase bacteria are transferred to a fresh environment. Bacteria in the delayed phase seem inert: their biomass does not increase. The low number of cells and low metabolic activity make it difficult to study this phase. As a consequence, it was not studied as well as other bacterial growth phases [11].

In this context, a case study aiming to study the hawthorn and rose-hip influence on the growth rate of pathogenic and opportunistic pathogenic microorganisms (*S. aureus, Salmonella, Klebsiella pneumoniae, E. coli*) in meat products was conducted.

Previous studies indicate that hawthorn, rose-hip and sea-buckthorn have antimicrobial properties [12, 13, 14]. The addition of powders or extracts from these fruits in the manufacturing of pastry and confectionery products controls the microbiological risk, diminishing the growth of *B. mesentericus* and *B. subtilis* sporulated bacteria.

Materials and methods

The research was carried out within State project 18.51.07.01A/PS "*Decreasing contamination of raw materials and food products with pathogenic microorganisms*".

For the contamination of the products the microbial strains (*S. aureus* ATCC 25923*, Salmonella Abony* ATCC 6017*, Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922) were procured from The National Public Health Agency.

As a substrate for contamination, the sausage samples obtained in laboratory conditions within the Department of Food Technology, Technical University of Moldova. Microbiological tests were carried out in the laboratory of the Department of Microbiology and Immunology, State University of Medicine and Pharmacy "Nicolae Testemițanu".

From the reference strains (*S. aureus, S. Abony, K. pneumoniae* and *E. coli*), microorganism suspensions were prepared according to the McFarland 0.5 turbidity standard. This turbidity corresponds to approximately 1×10^8 CFU·mL, then decimal 10^{-3} and 10^{-6} dilutions were performed for inoculations in the sausage samples [15].

Nutritional environments used Muller Hinton agar, Endo and Mannitol salt agar. One milliliter of microorganism suspension corresponding to the McFarland 0.5 turbidity standard prepared from the reference strains (*S. aureus, S. abony, K.pneumoniae* and *E. coli*) was added to one gram of ground sausage sample. From each type of sausage, four samples were prepared. The intentionally contaminated samples were incubated in the thermostat

at 37 °C for 24 hours, 48 hours, 72 hours and 96 hours. Upon expiration of the incubation time set, the increased colonies were counted in the tested Petri dishes and the microbial growth rate was calculated [16]. In order to determine the antibacterial effect of biologically active compounds of rose-hip and hawthorn in sausages, we have to analyze the multiplication kinetics of *S. aureus, S. abony, K. pneumoniae* and *E. coli in situ*. Two phases of the microorganism growth curve were studied: *Lag* and *Exponentia*l phase.

Results and discussions

In order to study whether berries have a bactericidal or bacteriostatic effect in meat products, different samples of sausage with rose-hip and hawthorn additives have been prepared under laboratory conditions at the Department of Food Technology. The sausage samples were intentionally contaminated with *S. aureus* ATCC 25923*, Salmonella Abony* ATCC 6017*, Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922 strains. The microbial growth in situ (sausage) was identified, in control samples (without added berry powder) as well as in those with rose-hip and hawthorn additives. Incubation was carried out at 37 °C. The growth rate of pathogenic microorganisms was identified after 24, 48, 72 and 96 hours. Table 1 presents the monitoring results of the pathogenic strains *in situ* growth.

Table 1.

Growth of *in situ* microorganisms (sausages with an addition of biologically active substances from rose-hip and hawthorn)

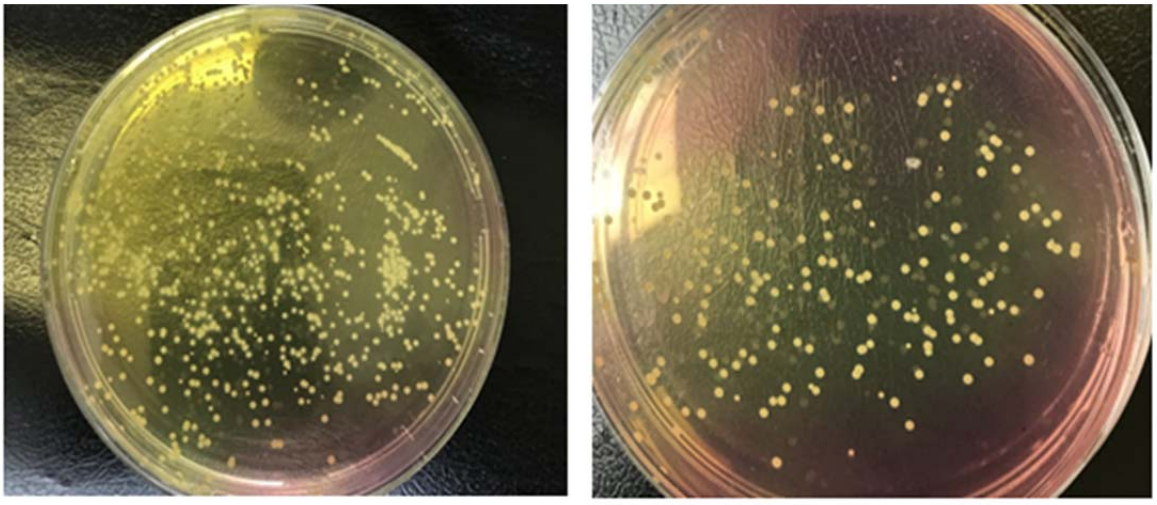
	Strains / Sample	***S.aureus* ATCC 25923**		***Salmonella Abony* ATCC 6017**		***Klebsiella pneumoniae* ATCC 13883**		***E. coli* ATCC 25922**	
		Nr. of colonies		**Nr. of colonies**		**Nr. of colonies**		**Nr. of colonies**	
		10^{-3}	**10^{-6}**	**10^{-3}**	**10^{-6}**	**10^{-3}**	**10^{-6}**	**10^{-3}**	**10^{-6}**
After 24h	**control**	552	78	diffuse	>700	diffuse	168	diffuse	488
	with rose-hip additive	228	16	diffuse	120	diffuse	88	diffuse	64
	with hawthorn additive	96	1	diffuse	248	diffuse	103	diffuse	88
After 48h	**control**	>1000	268	diffuse	>800	diffuse	346	diffuse	596
	with rose-hip additive	440	23	diffuse	228	diffuse	114	diffuse	264
	with hawthorn additive	176	3	diffuse	480	diffuse	144	diffuse	152
After 72h	**control**	>1000	396	diffuse	difuz	diffuse	412	diffuse	>700
	with rose-hip additive	560	49	diffuse	392	diffuse	300	diffuse	960

Table 1. continuation

	with hawthorn additive	222	4	diffuse	>1000	diffuse	760	diffuse	344
After 96h	**control**	diffuse	416	diffuse confluent	diffuse	diffuse confluent	560	confluent	diffuse
	with rose-hip additive	diffuse	280	diffuse confluent	>1000	diffuse confluent	372	confluent	diffuse
	with hawthorn additive	144	3	diffuse confluent	>1000	diffuse confluent	896	confluent	364

After 24 hours in the samples inoculated with suspensions of strain *S. aureus* ATCC 25923, the following results were obtained: for 10^{-3} dilution in the control sample, 552 colonies grew, whereas in the sample with hawthorn additive, only 78 colonies, and in the one with rose-hip additive – 228 colonies. For the suspension of microorganisms with 10^{-6} dilution: in the control sample, 78 colonies grew, and in the rose-hip additive sample – 16 colonies and in the hawthorn additive one – 1 colony respectively. In the following incubation days, the growth rate of pathogenic micro-organisms was also different. In some Petri plates there was an abundant increase that spread across the entire surface.

Figures 1-5 show some Petri plates of the total number of plaques inoculated with different strains of microorganisms and incubated at 37 °C. Microorganisms grown in each Petri plate after 24 hours, 48 hours, 72 hours and 96 hours were counted, the result was expressed In CFU (colony forming units).



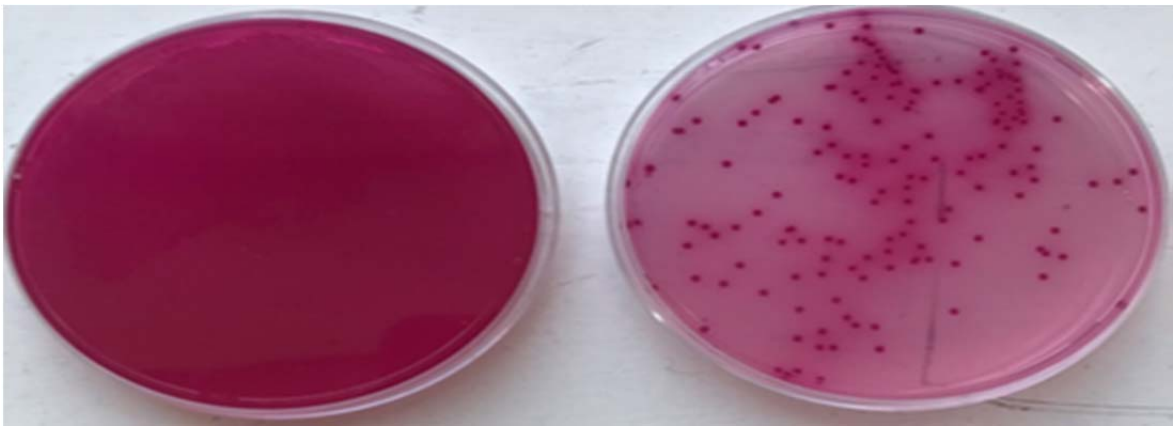
a) b)

Figure 1. Growth of *S. aureus* ATCC 25923 strains in sausage samples tested after 24h: *a)* control sample; b) hawthorn sample.

Based on the results obtained by counting the colonies from the Petri plates, the number of microorganisms was calculated depending on the dilutions of the inoculated strain suspensions and the growth curve of each strain was constructed for a period of 4 days (96 hours). The bacterial growth curve represents the number of live cells in a bacterial population over a period of time.

a) b)

Figure 2. Growth of *E. coli* ATCC 25922 strains in sausage samples tested after 48h: a) control sample; b) hawthorn sample.

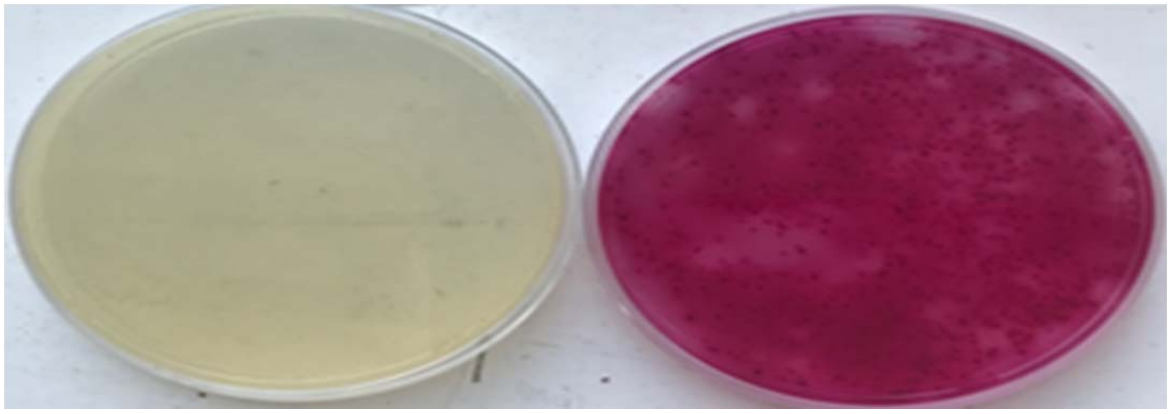


a) b)

Figure 3. Growth of *Klebsiella pneumonia* ATCC 13883 strains in sausage samples tested after 48h: a) control sample; b) hawthorn sample.

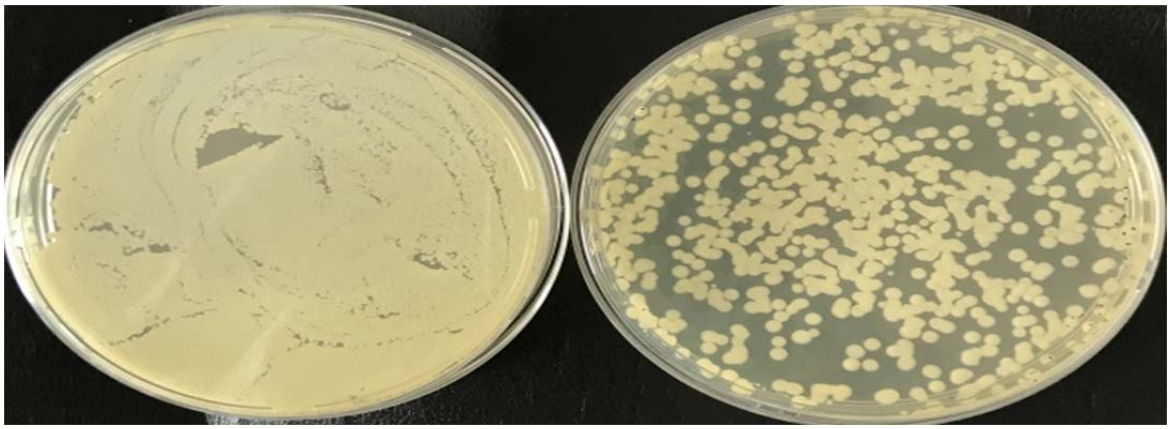
The *Lag* phase (adaptation phase) is characterized by cellular activity but not growth. A small group of cells are placed in a nutrient-rich environment that allows them to synthesize proteins and other molecules necessary for replication. These cells grow in size, but there is no cell division in this phase [17, 18].

The *Exponential (logarithmic)* phase: after the delay phase, the bacterial cells enter the exponential or logarithmic phase. This is the moment when the cells divide by binary division and duplicate after each generation of time. Metabolic activity is high, as DNA, RNA, cell wall components and other substances necessary in growth are generated for division. In this growth phase, antibiotics and disinfectants are most effective, as these substances usually target bacterial cell walls or the synthesis process for proteins necessary in DNA transcription and RNA translation [2, 19]. In the study conducted, we tested the effect of biologically active substances on the Lag phase of certain pathogenic microbial strains.



a) b)

Figure 4. Growth of *Salmonella Abony* ATCC 6017strains in sausage samples tested after 72h: a) control sample; b) hawthorn sample.



a) b)

Figure 5. Growth of *Klebsiella pneumoniae* strains in sausage samples tested after 96h: a) control sample; b) hawthorn sample.

Figure 6 shows the *Lag* and *Exponential* growth phases of the tested microorganisms. From these phases of the microorganism growth curve, we can see the influence of various berry additives on decreasing the growth of pathogenic microorganism strains that may accidentally get on/in the meat products.

The bacterial *Lag* phase phenomenon was first described at the end of the nineteenth century, when the "latent period" was described in studies of the effects of temperature on *Salmonella enterica serovar Typhi Surprisingly* [20]. The *Lag phase* is the least well-understood growth phase, primarily due to the lack of data describing the physiological and molecular processes underlying it [17]. The assumption was that the delay phase allows for the adaptation necessary for bacterial cells to start activating in the new environmental conditions [21, 22].

Based on the experimental data analysis (Figures 1-5), the kinetic growth curves of the pathogenic microorganisms were plotted (Figure 6). From Figure 6, it is clear that hawthorn and rose-hip additives in sausage samples increased the *Lag phase* for the inoculated microorganism strains and diminished the growth rate of the pathogenic microorganisms.

In *S. Aureus* (Figure 6a), the addition of hawthorn powder completely inhibits the growth of microorganisms during the evaluated period. For *E. Coli* (Figure 6d) the hawthorn is also most effective for stagnating the development of the pathogen microbiota on meat products. In the case of *Klebsiella pneumoniae* (Figure 6b), the effect of the rose-hip powder is the most striking. For S*almonella Abony pneumoniae*, the *Lag phase* is observed over the course of 80 hours (rose-hip powder sausage) and about 40 hours for products with hawthorn (Figure 6c). The experimental results of this study confirm the results of another study on the antimicrobial properties of rose-hip and hawthorn powder on the pathogenic microbiota with *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia*. The plant powders researches have shown promising antimicrobial potential against pathogenic microorganisms and can be used in the food industry to reduce microbial contamination of raw materials and foods [23].

When bacteria are inoculated in fresh media, they often show a period without known growth as the delay phase [18]. The *Lag phase* is interesting as a fundamental biological process in which bacterial physiology adapts to a new environment. The *Lag* phase is also of interest in areas such as food safety - where the delay phase is a factor in determining the shelf life of foods [24, 25].

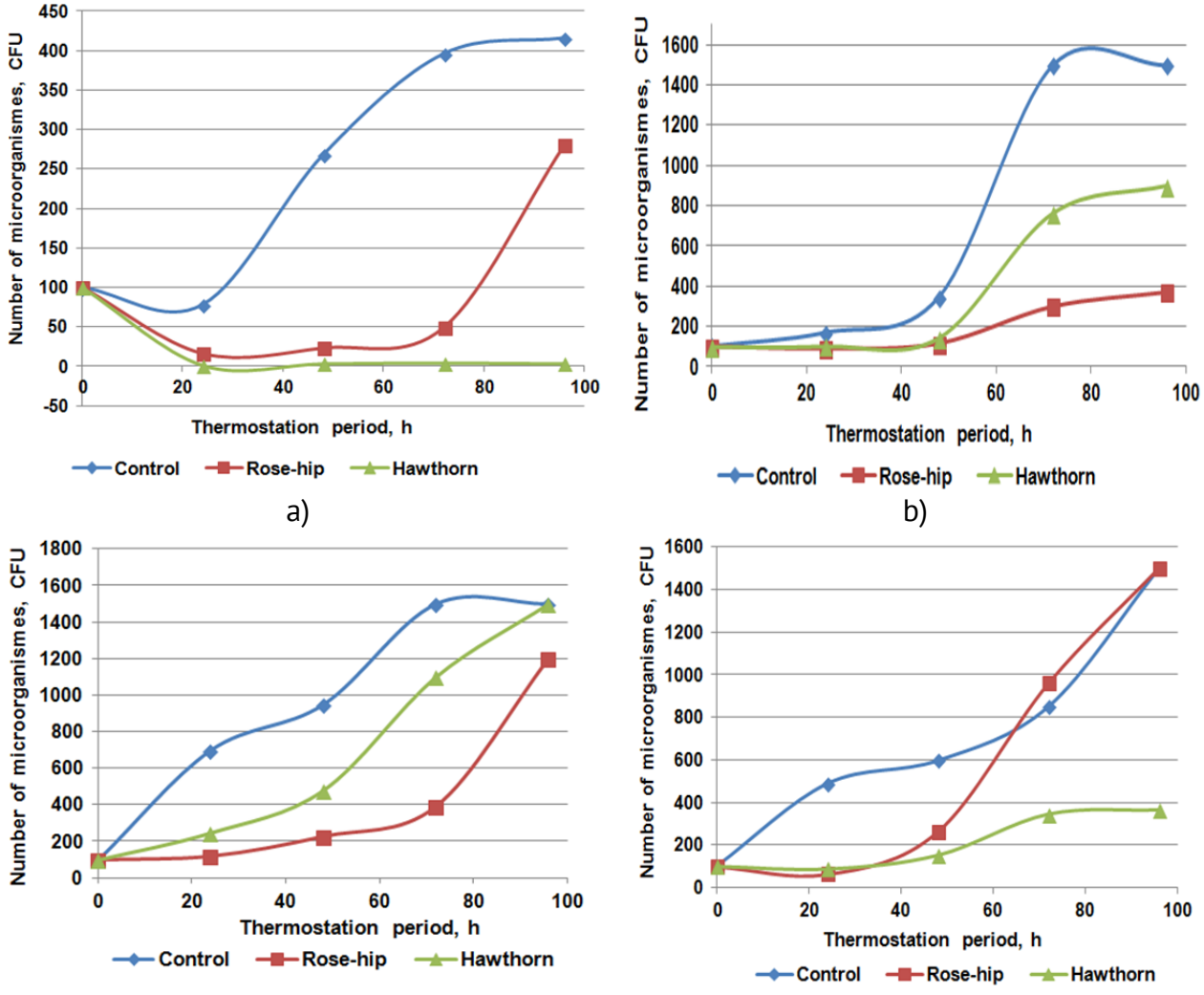


Figure 6. The Lag and Exponential phase of pathogenic strains in the tested sausage samples (testing period of 96 h): a) *S. aureus* ATCC 25923; b) *Klebsiella pneumoniae* ATCC 13883; c) *Salmonella Abony* ATCC 6017; d) *E. coli* ATCC 25922.

Conclusions

As a result of the conducted tests, we determined that rose-hip and hawthorn additives in the sausage recipe can control the growth rate of microorganisms, including pathogenic ones. This has been determined by evaluating the multiplication of microorganism strains such as *S. aureus* ATCC 25923, *Salmonella Abony* ATCC 6017, *Klebsiella pneumoniae* ATCC 13883 and *E. coli* ATCC 25922.

By studying the *Lag* and *Logarithmic* growth phases of pathogenic microbial strains we determined that the hawthorn has a greater bacteriostatic effect on strains of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 and the rose-hip has a greater bacteriostatic effect on *Salmonella strains Abony* ATCC 6017, *Klebsiella pneumoniae* ATCC 13883.

So the use of berry additives in the meat products recipe can mean two things: an improved nutritional value of the product and an increased product shelf-life by keeping the microbiological risk under control.

The extension of the *Lag* phase in the sausage samples with the berry powder additives demonstrates that these products can have a longer shelf-life, a factor that is extremely important from a food safety perspective.

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