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- RESEARCH ARTICLE -

Efficiency of a Herbal Liquid Extract Mixture for the Prevention of *Salmonella* Growth in Whipped Cream

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Abstract

In this study, a herbal liquid extract mixture (ASATİM® ST 1412; recommended for milk-based product and whipped cream by company, Kayseri, Turkey) was used to exhibit the inhibitory effect on *Salmonella* Enteritidis growth in whipped cream. For this aim, 8 different groups (K1, K2, K3, K4, K5, 0.1%, 1%, 10%) were designed and the samples were left 1 to 3 hours at room temperature. Then, the bacterial counts were obtained. The experiment was performed in duplicate. In the first experiment number of the bacteria of whipped cream (K1) showed roughly 1 logarithmic unit rising as from 4.6 to 5.5 in 3 hours. Calculated results were at the end of the 3rd hour for cream added *Salmonella* and the herbal liquid extract mixture of 0.1%, 1%, 10% to the samples; $3x10^7$cfu/g, $2.05x10^8$cfu/g and $1.3x10^5$cfu/g respectively for the first experiment. Then, in the results of second experiment these values were found as $<10^8$cfu/g, $4.5x10^8$cfu/g and $3.5x10^4$cfu/g respectively. Thus, 10% ratio herbal liquid extract mixture was much more effective on *Salmonella* growth than the other ratios. According to the results of this study, the commercial herbal liquid extract mixture is an alternative and natural method for precluding *Salmonella* growth in whipped cream.

Keywords:

Salmonella, whipped cream, herbal liquid, extract mixture

Article history:

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Introduction

Pastry products with cream are commonly used in the food and confectionery industries (Sharifzadeh, Hajsharifi-Shahreza, & Ghasemi-Dehkordi, 2016). Some foods like muscle meat

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have natural barriers which prevent or delay to the attack of microorganisms on the inner parts, while desserts and cakes containing milk or cream which are milk-based products are suitable media with high nutrient content, water activity (a_w) and pH values for microbial growth (Sharifzadeh et al., 2016; Alişarlı et al., 2002). Besides, pastry creams tend to contamination with several pathogenic microorganisms because of their ingredients and production methods like using raw cream (Sharifzadeh et al., 2016; Alişarlı et al., 2002; Ray, 2004; Pajohi-Alamoti et al., 2016; Kotzekidou, 2013). Therefore; pastry cream is the main cause of food poisoning among humans that is an important and common problem all around the world and *Salmonella* is one of the major pathogenic microorganisms in the pastry cream (Sharifzadeh et al., 2016; Ray, 2004; Pajohi-Alamoti et al., 2016).

Salmonella is a member of *Enterobacteriaceae* family, Gram-negative, nonsporulating, mesophile, facultative anaerobic microorganism and has more than 2000 serovars (Ray, 2004; Mahmoud, 2012). Although *Salmonella* is sensitive to low pH, some strains can survive in low pH due to fact that they have acid-resistance (Ray, 2004). *Salmonella* is a heat-sensitive pathogen and it can be eliminated with heat processes (Mahmoud, 2012; Kavaz Yüksel & Yüksel, 2015). This pathogen is found in the environment such as water, soil, sewage and gastrointestinal tract of animals and humans (Ray, 2004; Mahmoud, 2012). Meat, poultry, fish, egg, milk, dairy products, fruits and vegetables are transmission vector of *Salmonella* (Ray, 2004; Kotzekidou, 2013; Mahmoud, 2012; Kavaz Yüksel & Yüksel, 2015; Paião et al., 2013; Newell et al., 2010; Holliday et al., 2003). The most common recorded cause of diarrheal illnesses is *Salmonella* contagion in industrialized countries and all over the world (Ray, 2004; Kotzekidou, 2013; Mahmoud, 2012). In addition to that, the Ministry of Health in Italy was reported that *Salmonella* was the most important reason of the outbreaks (Argenio et al., 1999). *Salmonella* causes to Salmonellosis (*Salmonella* infection), which is infected by consuming *Salmonella*-contaminated foods and it can be fatal (Ray, 2004; Kotzekidou, 2013; Mahmoud, 2012; Kavaz Yüksel & Yüksel, 2015; Paião et al., 2013; Newell et al., 2010; Holliday et al., 2003). Therefore, it is essential to apply microbial control to foods such as pastry cream, which are suitable media for *Salmonella* growth based on both health and industrial prospects (Pajohi-Alamoti et al., 2016; Mahmoud, 2012).

While *Salmonella* infection is prevented with lots of different methods, including adding antibiotic or antimicrobial compounds such as spice or essential oil (Mahmoud, 2012; Kavaz Yüksel & Yüksel, 2015; Paião et al., 2013), last studies have been focused on using spice liquid extract for preventing *Salmonella* growth, because of increasing antibiotic resistance of some food borne *Salmonella* strains (Mahmoud, 2012; Amrutha et al., 2017; Bernbom et al., 2009; Mahgoub et al., 2013; Perumalla & Hettiarachchy, 2011; Sanchez et al., 2006; Kelati et al., 2017). A chemical compound of the plant extract or essential oils has various important antimicrobial ingredients which are terpenoides, phenols, alkaloids and flavanoids (Mahmoud, 2012; Botsoglou et al., 2003; Exarchou et al., 2002; Burt et al., 2007). These components have a hydrophobicity which provides them to divide in the lipids of the cell membrane and mitochondria making them more permeable (Mahmoud, 2012; Burt et al., 2007). An example of these plants is a garlic (*Allium sativum*) that is used against to pathogenic bacteria, including *Salmonella, especially* in fish products (Bernbom et al., 2009; Kumar & Berwal, 1998). The other examples to benefit from antibacterial characteristics of medicinal and aromatic plants' extracts as *Rumex acetosella, Achillea millefolium, Plantago Lanceolata* (Burt et al., 2007; Kokoska et al., 2002; Macit & Köse, 2015; Shale et al., 1999; Stewart, 1996; Yaldız et al., 2010). *Rumex acetosella* is used as digestive, nutritive and cure of

wounds and bruises. But also its leave extract has antibacterial activity on both of Gram-negative and Gram-positive bacteria such as *Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae* (Hızlısoy et al., 2010; Shale et al., 1999). *Achillea millefolium* is consumed as painkiller, diuretic, antiviral, vermifural, antifebrile, neuroleptic, cure of prostate and as many similar treatment (Hızlısoy et al., 2010; Yaldız et al., 2010). In addition to these, extract of *Achillea millefolium* is effective on bacteria such as *Bacillus cereus* and *Staphylococcus aureus* (Kokoska et al., 2002). *Plantago Lanceolata* is used for cure of wound, eczema, callus, ambustion and for some other statements (Hızlısoy et al., 2010; Macit & Köse, 2015). Phenylpropanoid glycoside verbascoside which is found *Plantago Lanceolata* has an antimicrobial activity. Besides, antimicrobial compounds of *Plantago Lanceolata* affect to inhibit rumen fermentation and volatile acid composition of it. Furthermore, *Plantago Lanceolata* can change milk composition (Stewart, 1996).

In the light of this information, this study aimed using a commercial herbal liquid extract mixture which is composed of sorrel extract (*Rumex acetosella*), millfoil extract (*Achillea millefolium*), ribwort plantain extract (*Plantago Lanceolata*) (ASATİM® ST 1412;Kayseri, Turkey) for the preventing of *Salmonella* growth in whipped cream which is used commonly in the food industry.

Materials and Methods

The Herbal Liquid Extract Mixture and Bacteria

In the current study, a herbal liquid extract mixture containing **sorrel extract** (*Rumex acetosella*), **millfoil extract** (*Achillea millefolium*), **ribwort plantain extract** (*Plantago Lanceolata*) (ASATİM® ST 1412; recommended for milk-based product and whipped cream by company, Kayseri, Turkey) was used to exhibit the inhibitory effect on *Salmonella* Enteritidis ATCC 13076 growth in whipped cream (prepared under sterile conditions with milk as the company proposed; 75g powder whipped cream is mixed with 200 mL UHT milk).

Dropping Plate Technique

Eight different groups which were designed as total concentration of each one at 10 mL like that only pastry cream as **K1**, cream with *Salmonella* as **K2**, cream added only the herbal liquid extract mixture at 0.1%, 1% and 10% ratio as **K3**, **K4**, **K5** respectively, cream added *Salmonella* and the herbal liquid extract mixture of **0.1%**, **1%**, **10%** to the samples were called with their herbal liquid extract mixture. The samples were left 1, 2 and 3 hours at room temperature. Furthermore, there were three samples for each 8 groups by each hour and diluted them seven-fold serial dilution for enumeration. The test bacteria, *Salmonella*, was adjusted to 0.4 ($O.D._{600}$) which was calculated equal to 10^6cfu/mL in order to add to the samples. After that, dropping plate technique was performed by adding 0.01 mL proportion of each samples on to the Tryptic Soy Agar plates in duplicate. After inoculation, incubation temperature and time was chosen as 37℃ for 24 hours in accordance with the optimum growth condition of the bacterium.

Counting and Calculation of the Colonies

After 37℃ for 24 hour-incubation process, occured colonies were counted according to seen clearly and denumerability. Thus, colony forming units were calculated with regard to the following formula (Halkman & Ayhan, 2000):

$N=C / [V * (n_1 + 0,1* n_2) *d_1]$

In the formula;

N: Number of microorganisms in 1 g or 1 mL of food sample

C: Total number of colonies in all Petri dishes counted

V: Volume transferred to counted Petri dishes

n_1: The number of the counted Petri dishes for the first dilution

n_2: The number of the counted Petri dishes for the second dilution

d_1: Dilution ratio of the two consecutive diluted concentrates in which the count is done

*Beyond measure or 0 → These were rounded next upper dilution and shown as <next upper dilution

Results

The experiment was performed in two recurrences and the data obtained in this study are given in Table 1 and Table 2. The results of bacterial counts belong to only whipped cream as K1, cream with *Salmonella* as K2, cream added herbal liquid extract mixture at 0.1%, 1% and 10% ratio as K3, K4, K5 respectively, cream added *Salmonella* and herbal liquid extract mixture of 0.1%, 1%, 10% to the samples at the end of 1st, 2nd and 3rd hour for first experiment (Table 1).

Table 1. The results of bacterial counts belong to only whipped cream.

	1st Hour		**2nd Hour**		**3rd Hour**	
	N(cfu/g)	**Log**	**N(cfu/g)**	**Log**	**N(cfu/g)**	**Log**
K1	$4.0x10^4$	4.6	$1.9x10^5$	5.3	$3.4x10^5$	5.5
K2	$1.5x10^8$	8.2	$1.0x10^8$	8.0	$2.5x10^8$	8.4
K3	$1.6x10^5$	5.2	$2.7x10^5$	5.4	$3.0x10^5$	5.5
K4	$1.7x10^5$	5.2	$3.1x10^5$	5.5	$8.6x10^4$	4.9

K5	$4.5x10^4$	4.7	$7x10^4$	4.8	$1.4x10^5$	5.2
0.1%	$3.0x10^8$	8.5	$2.8x10^7$	7.4	$3.0x10^7$	7.5
1%	$1.5x10^8$	8.2	$8.6x10^6$	6.9	$2.1x10^8$	8.3
10%	$3.8x10^4$	4.6	$2.7x10^5$	5.4	$1.3x10^5$	5.1

As far as the Table 1, number of the bacteria of K1 which is non-added bacteria to whipped cream sample was calculated as $4x10^4$cfu/g at the end of the 1st hour and then almost 1 logarithmic unit increasing was observed and the colony number has reached to $3.4x10^5$cfu/g at the end of the 3rd hour. This means that, whipped cream has microbial risk when it is waited in room temperature. The other remarkable result was observed in the sample containing 10% herbal liquid extract mixture and 10^6cfu/g *Salmonella* as well. The amount of bacteria was calculated as $3.8x10^4$cfu/g, $2.7x10^5$cfu/g and $1.3x10^5$cfu/g at the end of the 1st, 2nd and 3rd hours respectively. The calculated number of the bacteria of K5 which is 10% herbal liquid extract and the bacteria-free whipped cream mixture was similar to the sample containing 10% herbal liquid extract mixture and 10^6cfu/g *Salmonella* except for 2 hours due to the fact that there was not a fluctuation. Furthermore, there was a slight increasing in the following hours as $4.5x10^4$cfu/g, $7x10^4$cfu/g and $1.4x10^5$cfu/g at the end of the 1st, 2nd and 3rd hours respectively. Likewise, the amount of bacteria of K3 sample increased proportionally from 1 to 3 hours, but it had higher amounts bacteria than K5 because of containing 0.1% ratio the herbal liquid extract mixture. When the number of the bacteria of K2 was investigated; it was calculated as $1.5x10^8$cfu/g at the end of the 1st hour. Then, it decreased at $1x10^8$cfu/g surprisingly at the end of the 2nd hour and it reached as $2.5x10^8$cfu/g at the end of the 3rd hour. The very similar result was observed in the sample containing 1% herbal liquid extract mixture and 10^6cfu/g *Salmonella*, it decreased and then rose over 3-hour period. The number of bacteria of K4 and added 0.1% herbal liquid extract mixture and 10^6cfu/g *Salmonella* in whipped cream were shown a decrease from the end of the 1st hour to end of the 3rd hour.

To sum up, none of the samples which were treated with the herbal liquid extract mixture changed as much as K1 (containing only whipped cream) that was shown 1 logarithmic unit increasing. The results of the first experiment was proved that the herbal liquid extract mixture with different ratio is effective in *Salmonella* growth in whipped cream.

The results of bacterial counts belong to only whipped cream as K1, cream with *Salmonella* as K2, cream added herbal liquid extract mixture at 0.1%, 1% and 10% ratio as K3, K4, K5 respectively, cream added *Salmonella* and herbal liquid extract mixture of 0.1%, 1%, 10% to the samples at the end of 1st, 2nd and 3rd hour for second experiment (Table 2).

Table 2. The results of bacterial counts belong to only whipped cream.

	1st Hour		**2nd Hour**		**3rd Hour**	
	N(cfu/g)	**Log**	**N(cfu/g)**	**Log**	**N(cfu/g)**	**Log**
K1	$<10^3$	<3.0	$1.0x10^4$	4.0	$<10^3$	<3.0
K2	$4.5x10^9$	9.7	$2.0x10^8$	8.3	$2.5x10^9$	9.4
K3	$2.5x10^5$	5.4	$3.0x10^5$	5.5	$3.4x10^5$	5.5
K4	$<10^3$	<3.0	$1.0x10^5$	5.0	$1.8x10^6$	6.3
K5	$<10^3$	<3.0	$8.6x10^5$	5.9	$<10^3$	<3.0
0.1%	$4.5x10^8$	8.7	$2.0x10^9$	9.3	$<10^8$	<8.0
1%	$1.5x10^8$	8.2	$<10^7$	<7.0	$4.5x10^8$	8.7
10%	$<10^3$	<3.0	$3.5x10^5$	5.5	$3.5x10^4$	4.5

With reference to the Table 2, number of the bacteria of the samples which were entreated with the herbal liquid extract mixture (0.1%, 1% and 10 %) have not never reached too high amount like K2 which was bacteria and whipped cream mixed. It was observed that, somehow number of the bacteria did not change too much such as nearly 1 logarithmic unit, the others were roughly 2 logarithmic unit, increasing between 1st and 2nd hours like K4, K5 and the sample containing 10% herbal liquid extract mixture and the bacteria. When considered K1, the number of the bacteria was almost stable in 3-hour period, which were calculated as $<10^3$, $1x10^4$cfu/g, $<10^3$ respectively. The result was shown a little alteration by comparing with results of first experiments. It can be said that, the lowest number of the bacteria is in the sample containing 10% herbal liquid extract mixture and 10^6cfu/g *Salmonella* according to the table and also it is seen in Figure 1. In other words, the most effective amount of the herbal liquid extract mixture was 10% portion for holding down the number of bacteria. Which is a very similar result with the first trial. When compared the data obtained in the study, it can be said clearly the bacterial numbers were not calculated same for both two experiments. The reason of it should be that *Salmonella* has a quick and different growth rate at the suitable media. However, it is also obvious that the sample containing herbal extract at 10% has a significant inhibitory effect on *Salmonella*.

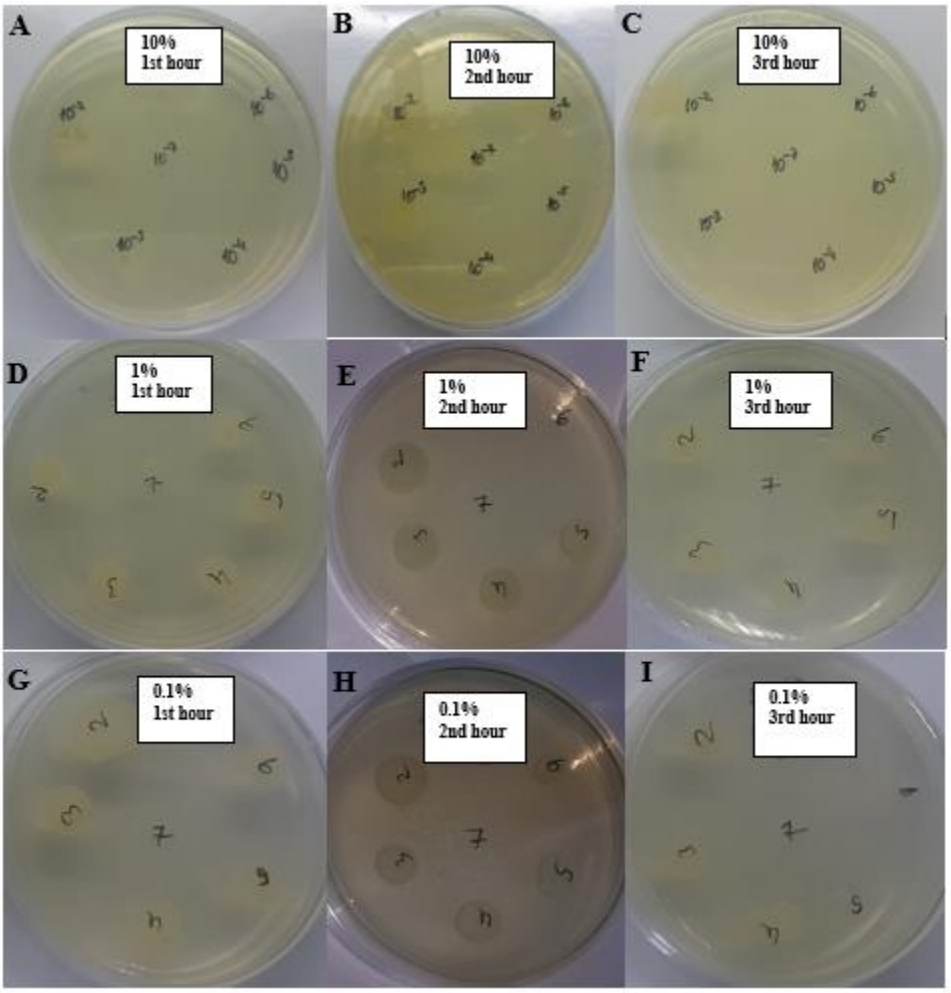

Figure 1. Counted colonies of the second experiment, after 37℃ for 24 hours incubation of the sample containing 10%, 1% and 0.1% herbal liquid extract mixture and 10^6cfu/g *Salmonella* at top-down which were seen clearly and denumerability. A, D and G: The Petri dishes incubated for 1 hour. B, E and H: The Petri dishes incubated for 2 hours. C,F and I: The Petri dishes incubated for 3 hours.

Discussion

The results of that study are shown similarity with the other studies which are used the same herbal extract in the studies. For example; in 2010, Hızlısoy H. et al. demonstrated that 5% diluted of the herbal extract is an effective cleaning of chicken carcasses from *Campylobacter jejuni* (Hızlısoy et al., 2010). According to another study which was done by Tornuk F. et al. (2011) was indicated that washing treatment of raw meat surface with the herbal extract reduced the number of *Listeria monocytogenes* and *Escherichia coli* O157:H7 almost 3 log cfu/cm^2 (Tornuk et al., 2011).

Also, in the literature some experiments are seen remarkable when compared to our study. One of them was performed by A. Sharifzadeh et al. (2016) and they showed evaluation of microbial contamination (for coliforms, *E. coli*, *Staphylococcus aureus*, and yeast) of cream-filled pastries in confectioneries of Chaharmahal Va Bakhtiari (Iran) with their study that the number of

bacteria (total) was 8.21 logarithmic units (Sharifzadeh et al., 2016). Also, in the study of P. Kotzekidou (2013); samples of desserts with dairy cream were analysed and according to the results; aerobic colony counts ranged from 10^3 to $<10^9$ cfu/g and the majority of samples (76.7%) contained *Enterobacteriaceae* with population dissipation in the range of 10^3 to $<10^7$ cfu/g; some samples had *enterococci* and *Staphylococcus spp.* which was counted as $>10^5$ cfu/g (Kotzekidou, 2013). Similarly, M. Alişarlı et al. (2002) searched about the determination of the microbiological quality of some dairy desserts. They calculated the number of microorganisms in cream, pastries higher than other milk-based desserts (Alişarlı et al., 2002). Thus, it can be deduced that pastry creams are suitable media for microorganism growth and they can cause food poisoning.

According to the study of S.L. Holiday et al. (2003) in which sweet cream whipped salted butter, sweet cream whipped unsalted butter, salted light butter, yellow fat spread and light margarine were used as designing products to determine the survival of pathogens, five serotypes of *Salmonella* which were Anatum, Enteritidis, Montevideo, Muenchen, and Typhimurium were inoculated two different types. These were high humidity and temperature abuse studies consisted of 20 mL of mixed-serotype or mixed-strain suspensions deposited in each indentation on the surface of test products followed by exposure to 85% relative humidity at 37℃ for 1 h and temperature. And physical abuse studies consisted of 2 mL of mixed-serotype or mixed strain suspensions added to 200 g of warm (37℃) test products and pummeled for 3 min. The bacterial numbers were 7.22, 7.82, 7.26, 7.32, 7.11 7.27 logarithmic units for 1 hour incubation and 3.91, 4.26, 4.47, 4.41, 4.37, 4.22 logarithmic units for 3 minutes incubation respectively for *S.*Anatum, *S.*Enteritidis, *S.*Montevideo, *S.*Muenchen, *S.*Typhimurium and mixture type (Holliday et al., 2003). When these results compared to ours the results of K2 samples were more higher, due to the higher first inoculation amount of the bacteria. On the other hand, the data of these studies are close and they show that dairy or milk-based products, such as butter and whipped cream are convenient to *Salmonella* growth.

M. Kumar and J.S. Berwal (1998) calculated minimum inhibitory concentration (MIC) of garlic (*Allium sativum*) against *Staphylococcus aureus*, *Salmonella* Typhi, *Escherichia coli* and *Listeria monocytogenes*. Then the results were found like that *E. coli* and *Staph. aureus*, inhibition occurred quickly and up to the 5% level of garlic, there was an almost 80% inhibition and then, a significant inhibition was seen, for *L. monocytogenes*, an important fall in growth was observed with rising garlic level and finally for *Salmonella* Typhi, a rapid reduction in growth was seen, with 50% inhibition at 1% garlic and 90% inhibition at 10% (Kumar & Berwal, 1998). Besides, Z. Kalaycıoğlu et al. (2017) demonstrated that, turmeric extract incorporation increased the physical and antibacterial characteristics of chitosan films due to the fact that, turmeric incorporated chitosan film decreased by 2 logarithmic units to number of *Salmonella* over 3 hour periods, although only chitosan film decreased that roughly 1 logarithmic unit (Kalaycıoğlu et al., 2017). E. Kakouri et al. (2017) indicated that minimum 1.5 mg of *Crocus sativus L. tepals* hydrolysed extract has antimicrobial effect on *Enterococcus faecium*, *Listeria monocytogenes*, *Staphylococcus xylosus*, *St. simulans*, *Salmonella enterica* and *Escherichia coli* by using Well Diffusion Assay (Kakouri et al., 2016). Y. Shahbazi (2016) found that *Ziziphora clinopodioides* essential oil (ZEO) in 2% portion and nisin at 500 IU mL^{-1} had an important antibacterial effect on *Salmonella* Typhimurium and *Staphylococcus aureus* growth in milk (Shahbazi, 2016). These studies and the current study indicated that certain amounts of herbal extracts are significant effects on pathogen

microorganisms which contaminate food. Therefore, using herbal extracts should be one of the important solutions for preventing microorganism growth in foods.

As far as the results of the present study and other studies in the literature, milk products such as whipped creams are pretty suitable growth media for pathogen microorganisms especially *Salmonella*. Thus, some treatments are necessary for like as these food products. Then, using of herbal mix extracts on the degenerative foods is a novel and effective method.

According to our study, it can be reported that 10% ratio of herbal liquid extract mixture in whipped cream has the most inhibitory effect on the bacteria when the result compared with the control sample. The producing company of the herbal liquid extract mixture proposes that the herbal liquid extract mixture should be added from 0.2% to 1% ratio to milk-based product and whipped cream. This approach is acceptable, so that in this study, high level of *Salmonella* was inoculated to the samples at the beginning which is an unusual situation.

In conclusion, in the daily use of whipped cream sample we can offer that in case of any risk, adding this herbal extract will be helpful to avoid food poisonings.

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