

ANTIMICROBIAL ACTIVITY OF SWEET BASIL AND THYME AGAINST *SALMONELLA ENTERICA* SEROTYPE ENTERITIDIS IN EGG-BASED PASTA

Jasmina Stojiljković¹, Miomir Nikšić², Nikola Stanišić³, Zoran Stojiljković⁴ and Goran Trajkovski⁵

¹College of Applied Studies, 17500 Vranje, Serbia

²Institute for Food Technology and Biochemistry, University of Belgrade, Faculty of Agriculture, 11080 Belgrade, Serbia

³Institute for Animal Husbandry, 11080 Belgrade-Zemun, Serbia

⁴ECOVIVENDI d.o.o., 11 000 Belgrade, Serbia

⁵University of Cyril and Methodius, Faculty of Agricultural Science and Food, Skopje, Macedonia

Abstract - *Salmonella enterica* serotype Enteritidis is known as one of the most common pathogenic bacteria causing salmonellosis in humans. Raw materials of animal origin (eggs, chicken meat) are frequent vectors that transmit this bacterium. Since eggs are used for the production of pasta, due to insufficient thermal treatment during pasta drying, they can be a potential risk to consumer health. Different essential oils of herbs can be used to reduce present pathogenic microorganisms. This paper compares a decrease in the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and *Salmonella enterica* serotype Enteritidis isolated from outbreaks of salmonellosis in egg-based pasta under the influence of thyme and sweet basil essential oils. The results indicate that the utilized oils were more effective against the epidemic strain than the ATCC strain. In addition, thyme oil caused a more significant inhibition of *Salmonella enterica* serotype Enteritidis during the production process.

Key words: egg-based pasta; *Salmonella* Enteritidis; sweet basil; thyme

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INTRODUCTION

Salmonellosis is an infectious disease of humans and animals. Although primarily intestinal bacteria, salmonellae are widespread in the environment and may commonly be found in farm effluents, human sewage and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries, but appears to be most prevalent in areas of intensive animal husbandry, especially that of poultry or pigs (OIE, 2008).

Salmonellosis can affect all species of domestic animals; young animals and pregnant animals are

the most susceptible. Enteric disease, often presenting as a bloody or profuse watery diarrhea with pyrexia, is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicemia, abortion, arthritis, necrosis of the extremities and respiratory disease, may be seen. The signs and lesions are not pathognomonic. Many animals, especially poultry and pigs, may also be infected but show no clinical illness (Wray and Wray, 2000).

Salmonella spp. are small, Gram-negative, rod-shaped, asporogenic bacteria that can grow on a wide range of media. Hence, they can be found in various unprocessed foods. Bacteria from *Salmonel-*

la species are intolerant to high concentrations of salt and nitrites, so they are usually not found in cured meat. On the other hand, thermally untreated eggs represent a very good substrate for the growth of *Salmonella* spp. Eggs can be infected by *Salmonella* via two ways of transmission, vertical and horizontal. Vertical transmission occurs when the egg contents are contaminated with *Salmonella* during the formation of the egg (Messens, 2005). The horizontal route includes trans-shell infection of the contents of eggs during their transit through the cloacae (EFSA, 2005; Martelli and Davies, 2012).

Inside the egg, the growth of *Salmonella* is facilitated by the temperature of storage. Eggs should be stored at a constant temperature that should not exceed 20°C (ACMSF, 1993; Martelli, 2012). *Salmonella* can grow at 20°C in the egg albumen, while it is unable to grow at temperatures less than 10°C. If *Salmonella* reaches the egg yolk, it can grow rapidly, even at room temperature (25°C) (Gantois et al., 2009; Martelli, 2012). Humans are most frequently intoxicated with salmonellae after they have consumed raw and undercooked eggs (Martelli, 2012).

During the production of egg-based pasta, drying is at 46°C. Since *Salmonella* Enteritidis can survive even higher temperatures (Blackburn et al., 1997) there is a potential risk of the contamination of the final product. Therefore, additional treatment can be performed in order to ensure the elimination of *Salmonella enterica* serotype Enteritidis in egg-based pasta.

Determination of the antimicrobial activity of 17 essential oils against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juices indicated that the reduction in the number of bacteria can reach 50% (Friedman et al., 2004). In addition, significant inactivation of *Salmonella* Enteritidis in tomato juice was achieved by the previous addition of citric acid or cinnamon bark oil (Mosqueda-Melgar et al., 2008). The essential oil of cloves, cinnamon, bay and thyme were tested against *Listeria monocytogenes* and *Salmonella* Enteritidis in soft cheese; clove oil was found to be more effective against *Salmonella* Enteritidis in

full fat cheese than in cheese slurry. Cinnamaldehyde and thymol were effective against six *Salmonella* serotypes on alfalfa seeds (Burt, 2004). Also, *Salmonella* Enteritidis in various foods can be reduced by use of the essential oils of lemongrass, cinnamon leaf, geraniol, thyme, oregano, clove bud, allspice, bay leaf, palmarosa and marjoram oils (Duan and Zhao, 2009; Raybaudi-Massilia et al., 2006; Friedman et al., 2002; Burt, 2004).

The aim of this research was to determine the antimicrobial activity of the essential oils of sweet basil and thyme against *Salmonella enterica* serotype Enteritidis in egg-based pasta. In addition, the activity of the oils was investigated against both *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and the epidemic strain of *Salmonella enterica* serotype Enteritidis.

MATERIALS AND METHODS

Egg-based pasta technology and the sampling procedure

Egg-based pasta was made by the following recipe: 10 kg wheat grits and 2 kg wheat flour, 24 eggs, 3.2-3.4 L water and 0.010 kg β -carotene. Ingredients were mixed and 25 g of the dough was inoculated with 0.1 mL of the suspension of the investigated species of bacteria with the initial number of bacteria 10^9 CFU/g. After inoculation, different amounts of sweet basil and thyme essential oil (Fitofarm, Skopje, Republic of Macedonia) were added to the prepared dough, at final concentrations of 1%, 2.5% and 5%. Pasta was formed by extrusion and then dried in a chamber at a temperature of 46°C and relative humidity of 80% for 9 h. Afterwards the pasta was cooled at room temperature for 15 min and packed into PE bags. Samples of the pasta with and without the addition of oils were taken during the following production stages: dough making, dough extrusion, pasta drying, pasta cooling, pasta packaging.

Microorganisms

The antimicrobial activity of the oils was investigated with *Salmonella enterica* serotype Enteritidis (D)

ATCC 13076 from the MicroBioLogics, Ins. Joins ATCC Proficiency Standard Program, Minnesota, USA, and *Salmonella enterica* serotype Enteritidis 6084 isolated from outbreaks of salmonellosis in the National Institute of Public Health, Leskovac, Serbia.

Enumeration of bacteria

Determination of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and epidemic strain was performed according to the methods for microbiological analysis and super-analysis of food. A quantity of 25 g of pasta was mixed with 225 mL of selenite broth (Torlak, Belgrade, Serbia) and incubated for 24 h at 37°C. Inoculation was carried out by spreading 0.1 mL of the appropriate dilution on the surface of SS agar (Torlak, Belgrade, Serbia) plates. Enumeration of bacteria was performed after incubation at 37 °C during 24 h. All experiments were done in triplicate.

Statistical analysis

Data were subjected to analysis using MS Office Excel and the computer program SPSS 17. A two-factorial experiment was used to compare the significance of differences in means between the control and concentrations of 1%, 2.5% and 5% of sweet basil and thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and the epidemical strain *Salmonella enterica* serotype Enteritidis in egg-based pasta.

RESULTS

In the EU, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the serovars most frequently associated with human illness. Human *Salmonella* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *Salmonella* Typhimurium cases are mostly associated with the consumption of contaminated pig, bovine and poultry meat (EFSA, 2013).

In order to investigate the possible effect of the essential oil of sweet basil and thyme on the reduction of growth of *Salmonella enterica* serotype Ente-

ritidis in egg-based pasta the number of bacteria was determined during different stages of the production process. Pasta was inoculated with *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and the epidemic strain with an initial number of bacteria of 10⁹ CFU/g, and different concentrations of essential oils were added.

During the production process, the number of *Salmonella* Enteritidis (D) ATCC 13076 in egg-based pasta with sweet basil decreased by 1 log CFU/g (Fig. 1). The addition of 1% and 2% of essential oil of sweet basil had no influence on the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in the pasta. The addition of 5% of essential oil of sweet basil reduced the number of *S. Enteritidis* (D) ATCC 13076 during extrusion by 0.14 log CFU/g. The greatest reduction was observed during cooling, 0.76 log CFU/g, while in the final product it was 0.51 log CFU/g compared to the control. Comparison of the impact of different concentrations of sweet basil against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 is shown in Table 1.

The greatest decrease in the pasta after the addition of thyme essential oil at a concentration of 2.5% was observed during the extrusion phase, however, the final number *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 was slightly lower as compared to the control (Fig. 2). On the other hand, the addition of 5% of thyme oil led to a reduction of up to 1.29 log units, which was observed during drying. This concentration of thyme oil caused the decrease in the number of ATCC strain by 1 log unit during the extrusion and this difference remained mostly stable to the end of the analyzed process. Comparison of the impact of different concentrations of thyme against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 is shown in Table 2.

The results obtained during the investigation of the influence of sweet basil on the epidemic strain *Salmonella enterica* serotype Enteritidis in the pasta are presented in Fig. 3. Reduction in the number of epidemic strain was observed with the addition of 2.5 and 5% of sweet basil oil. Essential oil of sweet

Table 1. Comparison of impact of different concentrations of sweet basil on *Salmonella enterica* serotype Enteritidis (D) ATCC 13076.

Pairwise Comparisons						
Dependent Variable: <i>Salmonella</i> Enteritidis (D) ATCC 13076						
(I) Conc. of sweet basil	(J) Conc. of sweet basil	Mean Difference (I-J)	Std. Error	Sig. ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
Control	1%	44111111.1	35935036.9	0.226	-28141123.2	116363345.4
	2.5%	80777777.7*	35935036.9	0.029	8525543.4	153030012.1
	5%	144616666.6*	35935036.9	0.000	72364432.2	216868901.0
1% sweet basil	Control	-44111111.1	35935036.9	0.226	-116363345.4	28141123.2
	2.5%	36666666.6	35935036.9	0.313	-35585567.7	108918901.0
	5%	100505555.5*	35935036.9	0.007	28253321.1	172757789.9
2.5% sweet basil	Control	-80777777.7*	35935036.9	0.029	-153030012.1	-8525543.4
	1%	-36666666.6	35935036.9	0.313	-108918901.0	35585567.7
	5%	63838888.8	35935036.9	0.082	-8413345.4	136091123.2
5% sweet basil	Control	-144616666.6*	35935036.9	0.000	-216868901.0	-72364432.2
	1%	-100505555.5*	35935036.9	0.007	-172757789.9	-28253321.1
	2.5%	-63838888.8	35935036.9	0.082	-136091123.2	8413345.4

Based on estimated marginal means

*The mean difference is significant at the 0.05 level

b Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

Table 2. Comparison of impact of different concentrations of thyme on *Salmonella enterica* serotype Enteritidis (D) ATCC 13076

Pairwise Comparisons						
Dependent Variable: <i>Salmonella</i> Enteritidis (D) ATCC 13076						
(I) Conc. of thyme	(J) Conc. of thyme	Mean Diff. (I-J)	Std. Error	Sig. ^b	95% Confidence Interval for Difference ^b	
					Lower Bound	Upper Bound
Control	1%	-131244444.4	119289832.6	0.277	-371092728.1	108603839.2
	2.5%	54988888.8	119289832.6	0.647	-184859394.8	294837172.5
	5%	147572777.7	119289832.6	0.222	-92275505.9	387421061.4
1% thyme	Control	131244444.4	119289832.6	0.277	-108603839.2	371092728.1
	2.5%	186233333.3	119289832.6	0.125	-53614950.3	426081617.0
	5%	278817222.2*	119289832.6	0.024	38968938.5	518665505.9
2.5% thyme	Control	-54988888.8	119289832.6	0.647	-294837172.5	184859394.8
	1%	-186233333.3	119289832.6	0.125	-426081617.0	53614950.3
	5%	92583888.8	119289832.6	0.441	-147264394.8	332432172.5
5% thyme	Control	-147572777.7	119289832.6	0.222	-387421061.4	92275505.9
	1%	-278817222.2*	119289832.6	0.024	-518665505.9	-38968938.5
	2.5%	-92583888.8	119289832.6	0.441	-332432172.5	147264394.8

Based on estimated marginal means

* The mean difference is significant at the 0.05 level

b Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

basil in the concentration of 5% caused a decrease in the number of epidemic *Salmonella enterica* during storing of the egg-based pasta by 1 log unit. This is in agreement with the investigation of Barbosa et al. (2009) who achieved a reduction of 1-1.3 log units of *Salmonella* Enteritidis with the addition of essen-

tial oils. The concentration of 5% of sweet basil oil was more effective in the case of the epidemic strain than the ATCC strain. Comparison of the impact of different concentrations of sweet basil against *Salmonella enterica* serotype Enteritidis epidemic strain is shown in Table 3.

Table 3. Comparison of impact of different concentrations of sweet basil on *Salmonella enterica* serotype Enteritidis epidemic strain

Pairwise Comparisons						
Dependent Variable: Epidemic strain <i>Salmonella</i> Enteritidis						
(I) Conc. of sweet basil	(J) Conc. of sweet basil	Mean Difference (I-J)	Std. Error	Sig. ^a	95% Confidence Interval for Difference ^a	
					Lower Bound	Upper Bound
Control	1%	24950000.0	106189918.3	0.815	-188559140.8	238459140.8
	2.5%	-16994444.4	106189918.3	0.874	-230503585.2	196514696.3
	5%	115083333.3	106189918.3	0.284	-98425807.4	328592474.1
1% sweet basil	Control	-24950000.0	106189918.3	0.815	-238459140.8	188559140.8
	2.5%	-41944444.4	106189918.3	0.695	-255453585.2	171564696.3
	5%	90133333.3	106189918.3	0.400	-123375807.4	303642474.1
2.5% sweet basil	Control	16994444.4	106189918.3	0.874	-196514696.3	230503585.2
	1%	41944444.4	106189918.3	0.695	-171564696.3	255453585.2
	5%	132077777.7	106189918.3	0.220	-81431363.0	345586918.5
5% sweet basil	Control	-115083333.3	106189918.3	0.284	-328592474.1	98425807.4
	1%	-90133333.3	106189918.3	0.400	-303642474.1	123375807.4
	2.5%	-132077777.7	106189918.3	0.220	-345586918.5	81431363.0

Based on estimated marginal means

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

Table 4. Comparison of impact of different concentrations of thyme on *Salmonella enterica* serotype Enteritidis epidemic strain

Pairwise Comparisons						
Dependent Variable: Epidemic strain <i>Salmonella</i> Enteritidis						
(I) Conc. of thyme	(J) Conc. of thyme	Mean Difference (I-J)	Std. Error	Sig. ^a	95% Confidence Interval for Difference ^a	
					Lower Bound	Upper Bound
Control	1%	2533333.3	54968805.8	0.963	-107988858.2	113055524.9
	2.5%	8922222.2	54968805.8	0.872	-101599969.3	119444413.8
	5%	103695555.5	54968805.8	0.065	-6826636.0	214217747.1
1% thyme	Control	-2533333.3	54968805.8	0.963	-113055524.9	107988858.2
	2.5%	6388888.8	54968805.8	0.908	-104133302.7	116911080.4
	5%	101162222.2	54968805.8	0.072	-9359969.3	211684413.8
2.5% thyme	Control	-8922222.2	54968805.8	0.872	-119444413.8	101599969.3
	1%	-6388888.8	54968805.8	0.908	-116911080.4	104133302.7
	5%	94773333.3	54968805.8	0.091	-15748858.2	205295524.9
5% thyme	Control	-103695555.5	54968805.8	0.065	-214217747.1	6826636.0
	1%	-101162222.2	54968805.8	0.072	-211684413.8	9359969.3
	2.5%	-94773333.3	54968805.8	0.091	-205295524.9	15748858.2

Based on estimated marginal means

a Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments)

As was observed for the sweet basil oil, the epidemic strain is less resistant to the addition of thyme oil than the ATCC strain (Fig. 4). The addition of 2% of thyme oil had little influence on the growth of the epidemic strain. The increase in the concentration of oil to 5% caused stronger inhibition and a reduction in the number of epidemic strain from 8.42 to

7.23 log CFU/g during extrusion. The number of *Salmonella* Enteritidis in the final product was lower by 1.52 log units in the pasta with 5% of thyme oil compared to the control. Comparison of the impact of different concentrations of thyme against *Salmonella enterica* serotype Enteritidis epidemic strain is shown in Table 4.

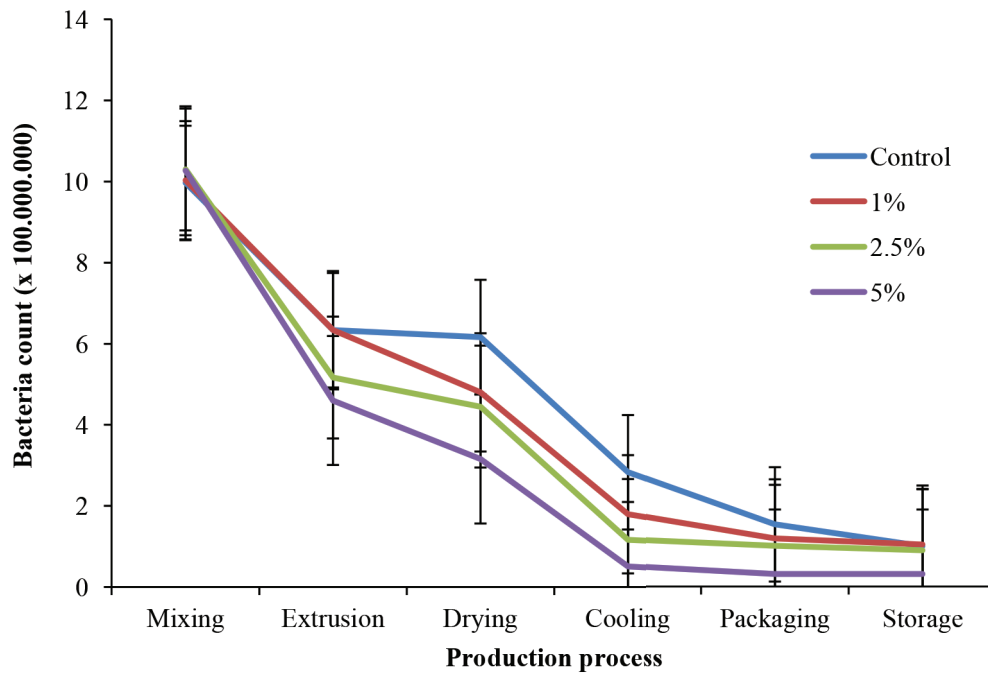


Fig. 1. Survival of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in pasta with essential oil of sweet basil

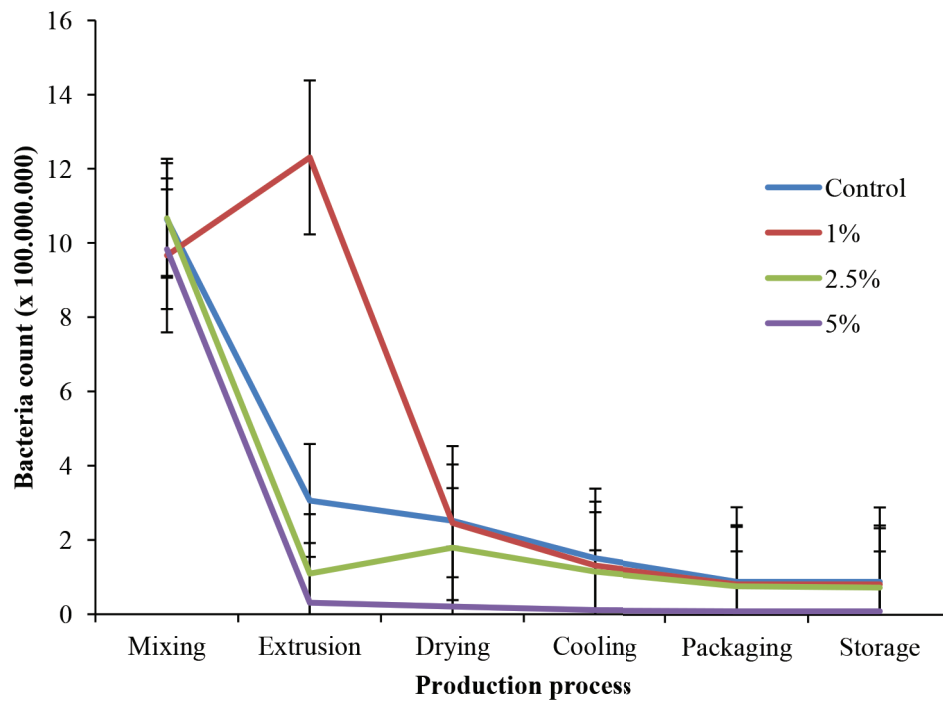


Fig. 2. Survival of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in pasta with different concentrations of thyme

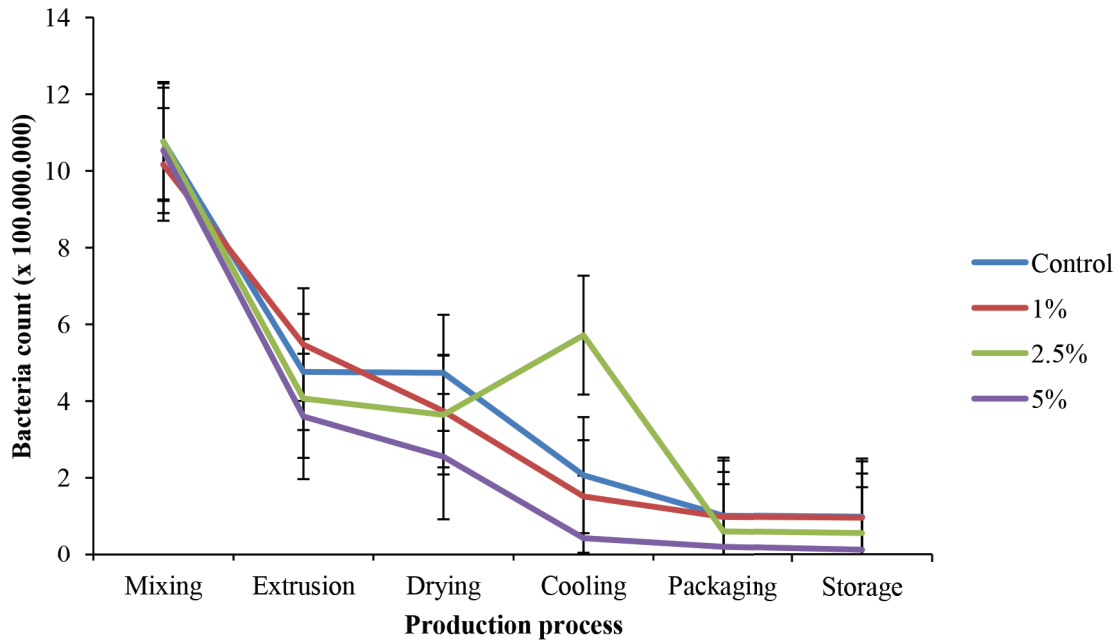


Fig. 3. Survival of epidemic strain *Salmonella enterica* serotype Enteritidis in pasta with different concentrations of sweet basil

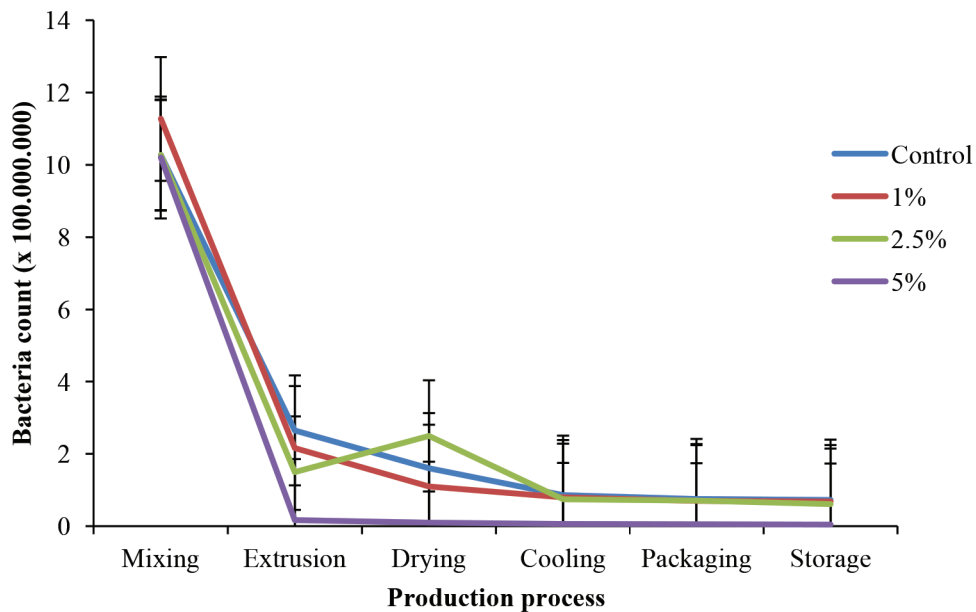


Fig. 4. Survival of epidemic strain *Salmonella enterica* serotype Enteritidis in pasta with different concentrations of thyme

Although there are literature data on the strong antimicrobial activity of basil oil indicating that the number of *Salmonella* Enteritidis strain can be reduced by 3 log units (Rattanachaiakunsopon and

Phumkhachorn, 2010), the obtained results showed a reduction of 0.5 units and 1 log unit for the ATCC and epidemic strains, respectively. The addition of thyme oil had a stronger antimicrobial effect since it

caused a reduction in the number of epidemic strain of approximately 1.5 log units from the beginning of the process.

DISCUSSION

Salmonellosis is a leading foodborne disease worldwide. A wide range of foods has been implicated in such diseases. However, foods of animal origin, especially poultry and poultry products, including eggs, have been consistently implicated in sporadic cases and outbreaks of human salmonellosis (FAO/WHO, 2002). The infectious dose is usually greater than 10^2 to 10^3 organisms and may vary with the age and health status of the host. In some cases, it can be as few as 15 to 20 cells (D' Aoust, 2000). Investigations in a number of countries have revealed that, when fresh, positive eggs contain about <50 *Salmonella* Enteritidis per egg. Growth in egg contents can occur because of storage related changes and become rapid once *Salmonella* can gain access to the egg yolk (Advisory Committee on the Microbiological Safety of Food, 2001). To minimize the potential risk of salmonellosis by the consumption of egg and egg products, good manufacturing and handling practices in the production of pasta with eggs should always be observed.

Essential oils of spices and herbs have been used as food additives, flavoring agents and natural food preservatives since ancient times. A number of spices have antimicrobial activity against different types of microorganisms (Škrinjar and Nemet, 2009; Tajkarami et al., 2010). Comparison of the effect of different concentrations of sweet basil against *Salmonella* enterica serotype Enteritidis (D) ATCC 13076 in egg-based pasta showed that the mean difference was significant at the 0.05 level between the control, basil concentration of 1%, 2.5% and 5%. Between the control and a concentration of basil of 2.5%, the mean difference was significant for the control and basil concentration of 5% ($p < 0.05$). For concentrations between 1% and 5% the significance level of the difference was $p < 0.05$.

Essential oils of cinnamon, oregano and mustard are efficient in the reduction of the number of *Salmonella* in beef (Turgis, et al., 2008), while a concentration of 2 $\mu\text{L}/\text{mL}$ of cinnamon, geranium, lemongrass and palmarosa oils decreased the number of *Salmonella* Enteritidis in fruit juice (Raybaudi-Massilia et al., 2006). The effect of different concentrations of thyme against *Salmonella* enterica serotype Enteritidis (D) ATCC 13076 in egg-based pasta showed that between concentrations of 1% and 5% of thyme there was no significant difference ($p < 0.05$). Mint oil at 5-20 $\mu\text{L}/\text{g}$ is effective against *Salmonella* Enteritidis in low fat yoghurt and cucumber salad (Tassou et al., 1995).

Comparison within a group (different concentrations of basil) in a two-factorial experiment showed that among controls, basil concentrations of 1%, 2.5% and 5%, and the dynamics of the epidemic strain of *Salmonella* Enteritidis in egg-based pasta there was no significant difference. Essential oils of *Thymus vulgaris*, *Mentha piperita* and *Rosmarinus officinalis* showed strong antimicrobial activity (both bacteriostatic and bactericidal effect) against *Salmonella* Enteritidis and *Escherichia coli* in concentrations ranging from 0.125 to 2% (v/v) (Niculae et al., 2009). Comparison within the group (of varying concentrations of thyme) in a two-factorial experiment showed that among the control, concentrations of 1%, 2.5% and 5% of thyme and the dynamics of the epidemic strain of *Salmonella* Enteritidis there was no significant difference.

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