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THE EFFECT OF THE TECHNOLOGICAL PROCESS AND ESSENTIAL OIL OF BASIL AGAINST *SALMONELLA ENTERICA* SEROTYPE ENTERITIDIS (D) ATCC 13076 IN EGG BASED PASTA

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Abstract: *Salmonella* cause illness in humans and animals. Most *Salmonella* serotype are naturally occurring in food. Egg and egg products are an important part of the human diet. Since eggs are used for the production of egg pasta, and due to an insufficient thermal treatment during pasta drying they can be a potential risk for the consumer's health. Different essential oils of herbs can be used in order to reduce potentially present pathogenic microorganisms. The aim of this paper is to describe the impact of the technological process of production of pasta with eggs under the influence of sweet basil on a decrease of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076. There is a significant effect of concentration of basil against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 ($p<0.05$). Also there is a significant impact of the process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 ($p<0.05$). There is not significant differences in the effects of the concentration of basil and technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in the production of pasta ($p = 0.737$).
Keywords: technological process, basil, pasta, *Salmonella enterica* serotype Enteritidis (D) ATCC 13076

1. INTRODUCTION

Salmonellosis is one of the most common and widely distributed food-borne diseases. It constitutes a mayor public health burden and represents a significant cost in many countries. Millions of human cases are reported word-wide every year and the disease results in thousands of deaths (Herikstad et al. 2002). Infectious with *Salmonella* is estimated to cause to 1.4 million illnesses, 31 000 hospitalisation and 1100 deats each year in the U.S. (Mead et al. 1999), the costs of wich may be upwards of $ 2 billion (Frenzen et al. 1999).

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). Horizontal rout includes transshell infection of the contents of egg during transit through the cloacae (EFSA 2005; Martelli and Davies 2012).

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

During the production of the egg-based pasta drying on the temperature of 46 ºC is performed. Since *Salmonella* Enteridis can survive even higher temperatures (Blackburn et al. 1997) there is a potential risk of the contamination of the final product. So, additional treatment can be performed in order to ensure 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 of the number of bacteria can reach 50% (Friedman et al. 2004). Also, significant inactivation of *Salmonella* Enteritidis in tomato juice was achieved by previous addition of citric acid or cinnamon bark oil (Mosqueda-Melgar et al. 2008). Essential oil of clove, cinnamon, bay and thyme were tested against *Listeria* monocytogenes and *Salmonella* Enteritidis in soft cheese; clove oil was found more effective against *Salmonella* Enteritidis in full fat cheese than in cheese slurry. Cinnamaldehyd and thymol were effective against six Salmonella serotypes on alfaalfa seeds (Burt 2004). Also, *Salmonella* Enteridis in various foods can be reduced by the use of 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 is the determination of individual stages of the tecnological process of production pasta with eggs with basil (mixing, extrusion, drying of pasta, cooling, packaging, storage and distribution) against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076.

2. MATERIAL 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 the inoculation, different amounts of sweet basil essential oil (Fitofarm, Skopje, Republic of Macedinia) were added to the prepared dough, at the final concentrations of 1%, 2.5% and 5%. Pasta was formed by extrusion and then dried in the chamber at the temperature of 46 ºC and relative humidity of 80% for 9h. Afterwards pasta was cooled at the room temperature for 15 min and packed into PE bags. Samples of the pasta with and without the addition of oils were collected during following production stages: dough making, dough extrusion, drying of pasta, pasta cooling, pasta packaging.

Microorganisms: The antimicrobial activity of the oils was investigated with following bacteria *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 from the MicroBioLogics, Ins. Joins ATCC Proficiency Standard Program, Minesota, USA.

Enumeration of bacteria: The determination of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 was performed according to the Methods of carrying out microbiological analysis and super-analysis of food. The quantity of 25g of pasta was mixed with 225 mL of selenite broth (Torlak, Belgrade, Serbia) and incubated for 24 h at 37ºC. Inoculation was carried out by spreading of 0.1 mL of the appropriate dilution on the surface of SS agar (Torlak, Belgrade, Serbia) plates. The enumeration of bacteria was performed after the incubation on 37 ºC during 24 hours.

Statistical Analysis: Data were subjected to analysis using MS Office Excel and the computer programme SPSS 17. Two-factorial experiment showed the significance of differences in means between control and concentration of 1%, 2,5% i 5% of sweet basil and technological proces against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta.

3. RESULTS AND DISCUSSION

In the EU, *Salmonella* Enteritidis and *Salmonella* Typhimurium are the serovars most frequently associated with human illness (Guard-Petter 2001). Human *Salmonella* Enteritidis cases are most commonly associated with the consumption of contaminated eggs and poultry meat (EFSA 2013). In order to investigate possible effect of essential oil of sweet basil to the reduction of the growth of *Salmonella enterica* serotype Enteritidis 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 with the initial number of bacteria of 10^9 CFU/g and different concentrations of essential oil were added.

During the production process the number of *Salmonella* Enteritidis (D) ATCC 13076 in egg-based pasta with sweet basil decreased for 1 log CFU/g (Fig 1). The addition of 1% and 2% of essential oil of sweet basil had no influence to the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in pasta. Addition of 5% of essential oil of sweet basil reduced the number of S. Enteridis (D) ATCC 13076 during extrusion for 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 (Fig 1).

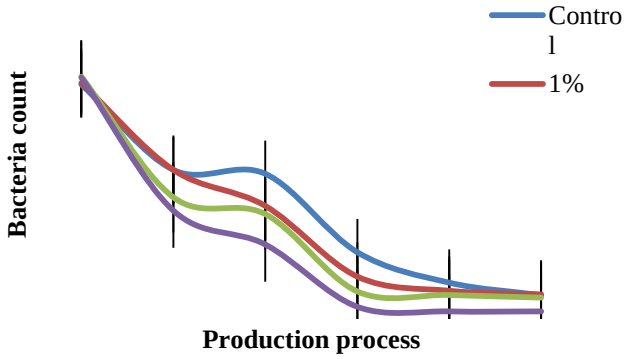

Fig 1. Survival of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in pasta with sweet basil during proces

The impact of technological process of production of pasta with eggs against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 is shown in Table 1.

Table 1. Impact of the technological process against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta

Pairwise Comparisons						
Dependent Variable: *Salmonella enterica* serotype Enteritidis (D) ATCC13076						
(I) Proces	(J) Proces	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Mixing	Ekstrusion	453333333,333*	44011252,244	,000	364842779,845	541823886,821
	Drying	549833333,333*	44011252,244	,000	461342779,845	638323886,821
	Cooling	856916666,667*	44011252,244	,000	768426113,179	945407220,155
	Packaging	913250000,000*	44011252,244	,000	824759446,512	1001740553,488
	Storage	933258333,333*	44011252,244	,000	844767779,845	1021748886,821
Ekstrusion	Mixing	-453333333,333*	44011252,244	,000	-541823886,821	-364842779,845
	Drying	96500000,000*	44011252,244	,033	8009446,512	184990553,488
	Cooling	403583333,333*	44011252,244	,000	315092779,845	492073886,821
	Packaging	459916666,667*	44011252,244	,000	371426113,179	548407220,155
	Storage	479925000,000*	44011252,244	,000	391434446,512	568415553,488
Drying	Mixing	-549833333,333*	44011252,244	,000	-638323886,821	-461342779,845
	Ekstrusion	-96500000,000*	44011252,244	,033	-184990553,488	-8009446,512
	Cooling	307083333,333*	44011252,244	,000	218592779,845	395573886,821
	Packaging	363416666,667*	44011252,244	,000	274926113,179	451907220,155
	Storage	383425000,000*	44011252,244	,000	294934446,512	471915553,488
Cooling	Mixing	-856916666,667*	44011252,244	,000	-945407220,155	-768426113,179
	Ekstrusion	-403583333,333*	44011252,244	,000	-492073886,821	-315092779,845
	Drying	-307083333,333*	44011252,244	,000	-395573886,821	-218592779,845
	Packaging	56333333,333	44011252,244	,207	-32157220,155	144823886,821
	Storage	76341666,667	44011252,244	,089	-12148886,821	164832220,155
Packaging	Mixing	-913250000,000*	44011252,244	,000	-1001740553,488	-824759446,512
	Ekstrusion	-459916666,667*	44011252,244	,000	-548407220,155	-371426113,179
	Drying	-363416666,667*	44011252,244	,000	-451907220,155	-274926113,179
	Cooling	-56333333,333	44011252,244	,207	-144823886,821	32157220,155
	Storage	20008333,333	44011252,244	,651	-68482220,155	108498886,821
Storage	Mixing	-933258333,333*	44011252,244	,000	-1021748886,821	-844767779,845
	Ekstrusion	-479925000,000*	44011252,244	,000	-568415553,488	-391434446,512
	Drying	-383425000,000*	44011252,244	,000	-471915553,488	-294934446,512
	Cooling	-76341666,667	44011252,244	,089	-164832220,155	12148886,821
	Packaging	-20008333,333	44011252,244	,651	-108498886,821	68482220,155
Based on estimated marginal means						
*. The mean difference is significant at the ,05 level.						
b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

The impact of different concentration of sweet basil in pasta with eggs on a decrease of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 is shown in Table 2.

Table 2. Impact of different concentration of basil against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta

Pairwise Comparisons						
Dependent Variable: *Salmonella enterica* serotype Enteritidis (D) (D) ATCC 13076						
(I) Concentration of sweet basil	(J) Concentration of sweet basil	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound

Control	1%	44111111,111	35935036,979	,226	-28141123,256	116363345,479
	2,5%	80777777,778*	35935036,979	,029	8525543,410	153030012,145
	5%	144616666,667*	35935036,979	,000	72364432,299	216868901,034
1% sweet basil	Control	-44111111,111	35935036,979	,226	-116363345,479	28141123,256
	2,5%	36666666,667	35935036,979	,313	-35585567,701	108918901,034
	5%	100505555,556*	35935036,979	,007	28253321,188	172757789,923
2,5% sweet basil	Control	-80777777,778*	35935036,979	,029	-153030012,145	-8525543,410
	1%	-36666666,667	35935036,979	,313	-108918901,034	35585567,701
	5%	63838888,889	35935036,979	,082	-8413345,479	136091123,256
5% sweet basil	Control	-144616666,667*	35935036,979	,000	-216868901,034	-72364432,299
	1%	-100505555,556*	35935036,979	,007	-172757789,923	-28253321,188
	2,5%	-63838888,889	35935036,979	,082	-136091123,256	8413345,479
Based on estimated marginal means						
*. The mean difference is significant at the ,05 level.						
b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Table 3. Impact of the technological process and sweet basil against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 in egg-based pasta

Tests of Between-Subjects Effects					
Dependent Variable: *Salmonella* Enteritidis (D) ATCC13076					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8218303736527777800,000[a]	23	357317553762077310,000	30,745	,000
Intercept	11313652400138936000,000	1	113136524001389360000,000	973,473	,000
Concentration of sweet basil	202077155972222432,000	3	67359051990740808,000	5,796	,002
Proces	7887924334027776000,000	5	157758486680555520,000	135,742	,000
Concentration * Proces	1283022465277777760,000	15	8553483101851851,000	,736	,737
Error	557853213333333180,000	48	11621941944444442,000		
Total	20089809349999997000,000	72			
Corrected Total	8776156949861110800,000	71			
a. R Squared = ,936 (Adjusted R Squared = ,906)					

Salmonellae are readily destroyed by heat pasteurization of foods at high a_w. As the a_w is reduction with addition of solutes or by removal of water, heat resistance increases markedly. In foods such chocolate, several seconds on 105 ºC may be required to reduce *Salmonella* counts by 1 log CFU/g. There is a high probability of infections at doses of $>10^5$ cells but in foods containing high levels of fat and/or protein such as chocolate, salami and cheddar cheese infection can result from ingesting as few as <10-100 cells (Teunis et al. 2010). In the EU in 2004-2009 was 8 outbreaks with pasta (25% with *Salmonella* spp.), (EFSA 2012). To minimize the potential risk of

salmonellosis due to the consumption of egg and egg products, good manufacturing and handling practices in production of pasta with eggs should always be observed.

Initial populations of 430-930 and 1.5-24 cells of Salmonella /100 g pasta (MPN Most Probable Number) of pasta (12% moisture) decreased to 0.4-23 and <0.3-1.5 cells/100 g respectively, during storage at room temperature for 360 days (Rayman et al. 1979).

After drying the pasta at 46 ° C, humidity of 80% for a period of 9 hours and the inhibition of the various concentrations of the essential oil of basil on SS agar was colony, which indicates that the conditions for the drying of pasta against *Salmonella* Enteritidis (D) ATCC 13076 were not rigorous and that this strain of pathogenic bacteria is not destroyed. After cooling and packaging of pasta in plastic bags presence of Salmonella was also recorded (Fig 1).

Comparison within the group (different process: mixing, extrusion, drying, cooling, packaging, and storage and distribution) in the two-factorial experiment showed that there was statistically significant at $p < 0.05$ between the mixing and all other processes ($p = 0.000$), between extrusion and other processes ($p = 0.000$), and all other drying processes ($p = 0.000$), cooling with mixing, extrusion and drying ($p = 0.000$), packaging with mixing, extrusion and drying ($p = 0.000$) and storage and distribution with mixing, extrusion and drying ($p = 0.000$) (Table 1).

Essential oils of spices and herbs have been used as food additives, as flavoring agent and as 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 the 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 the concentration of basil at 2.5%, the mean difference was significant ($p<0.05$), for the control and basil concentration of 5% ($p<0.05$). For a concentration between 1% and 5% significance level of the difference was $p<0.05$ (Table 2). Essential oils of cinnamon, oregano and mustard are efficient in the reduction of the number *Salmonella* in beef (Turgis, et al. 2008), while a concentration of 2 μL/mL cinnamon, geranium, lemongrass and palmarosa oils decreased the number of *Salmonella* Enteritidis in fruit juice (Raybaudi-Massilia et al. 2006). Essential oils of *Thymus vulgaris*, *Mentha piperita*, *Rosmarinus officinalis*, showed strong antimicrobial activity (both bacteriostatic and bacteriocidal effect) against *Salmonella* Enteritidis and *Escherichia coli* in concentrations ranged from 0,125 to 2% (v/v) (*Niculae et al. 2009*).

There is not significant differences in the effects of the concentration of basil and technological process on *Salmonella* Enteritidis ATCC 13076 (Table 3) in the production of pasta ($p = 0.737$). There is a significant effect of concentration of basil on *Salmonella* Enteritidis ATCC 13076 ($p = 0.002$). Also there is a significant impact of the process on *Salmonella* Enteritidis ATCC 13076 ($p = 0.000$).

4. CONCLUSION

This study has been described the individual stages of the tecnological process of production pasta with eggs with basil (mixing, extrusion, drying of pasta, cooling, packaging, storage and distribution) against *Salmonella enterica* serotype Enteritidis (D) ATCC 13076. There is not significant differences in the effects of the concentration of basil and technological process on *Salmonella* Enteritidis ATCC 13076 (Table 3) in the production of pasta ($p = 0.737$). There is a significant effect of concentration of basil on *Salmonella* Enteritidis ATCC 13076 ($p = 0.002$). Also there is a significant impact of the process on *Salmonella* Enteritidis ATCC 13076 ($p = 0.000$).

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