**Cell Biology**
2016; 4(6): 35-39
http://www.sciencepublishinggroup.com/j/cb
doi: 10.11648/j.cb.20160406.11
ISSN: 2330-0175 (Print); ISSN: 2330-0183 (Online)

# The Growth of *Salmonella* Enteritidis in Egg-Based Pasta with the Addition of Sweet Basil and Thymus

**J. Stojiljkovic[1, *], M. Trajcev[2], M. Petrovska[3], B. Petanovska Ilievska[2], G. Trajkovski[2]**

[1]College of Applied Studies, Filip Filipovic Vranje, Republic of Serbia

[2]Faculty of Agricultural Science and Food, University "St. Cyril and Methodius", Skopje, Republic of Macedonia

[3]Faculty of Medicine, University "St. Cyril and Methodius", Skopje, Republic of Macedonia

## Email address:

jasmina_vranje@yahoo.com (J. Stojiljkovic), bpetan88@yahoo.com (B. P. Ilievska)

[*]Corresponding author

## To cite this article:

J. Stojiljkovic, M. Trajcev, M. Petrovska, B. Petanovska Ilievska, G. Trajkovski. The Growth of *Salmonella* Enteritidis in Egg-Based Pasta with the Addition of Sweet Basil and Thymus. *Cell Biology*. Vol. 4, No. 6, 2016, pp. 35-39. doi: 10.11648/j.cb.20160406.11

**Received**: August 29, 2016; **Accepted**: November 2, 2016; **Published**: March 18, 2017

**Abstract:** *Salmonella enterica* serotype Enteritidis is known as one of the most common pathogenic bacteria causing salmonellosis with humans. Most frequently, raw materials of the animal origin (eggs, chicken meat) appear as a vector in the transmission of this bacterium. Since eggs are used for the production of egg-based pasta, and due to an insufficient thermal treatment during pasta drying they can be a potential risk for the consumer's health. Different pot herbs can be used in order to reduce potentially present pathogenic microorganisms. This paper compares a decrease of 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 thymus and sweet basil essential oil. The reduction of the number achieved during process ranges from 0.5 to 1.5 log CFU/g. The results indicated that utilized oils were more effective against epidemic strain then ATCC strain. Also, thyme oil caused more significant inhibition of *S.* Enteritidis during production process.

**Keywords:** Egg-Based Pasta, *Salmonella* Enteritidis, Sweet Basil, Thyme

## 1. Introduction

Salmonellosis is an important foodborne diseases and it manifests by acute gastroenteritis with short incubation period, and it caused by bacteria belonging to the genus *Salmonella* [1]. The symptoms of salmonellosis can be general, such as the abdominal pain, fever, nausea, vomiting and diarrhea, as well as special, manifested in the body dehydration, a headache and blood in stool. The symptoms are supposed to be caused by the endotoxin released from cells by the action of proteolytic enzymes and low pH values in the gaster.

*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 *Salmonella* species are intolerant to high concentrations of salt and nitrites, so they are usually not found in cured meat. On the other hand, thermal untreated eggs represent 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 almonella during the formation of the egg [2]. Horizontal rout includes transshell infection of the contents of egg during transit through the cloacae [3, 4].

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°C [4, 5]. *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) [4, 6]. Humans are most frequently intoxicated with *salmonellae* after they have consumed raw and undercooked eggs [4].

During the production of the egg-based pasta drying on the temperature of 46°C is performed. Since *Salmonella*

Enteridis can survive even higher temperatures [7] 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.

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 [8, 9]. 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) [10]. Essential oils of cinnamon, oregano and mustard are efficient in the reduction of the number *Salmonella* in beef [11], while a concentration of 2 μL/mL cinnamon, geranium, lemongrass and palmarosa oils decreased the number of *Salmonella* Enteritidis in fruit juice [12]. Mint oil at 5-20 μL/g is effective against *Salmonella* Enteritidis in low fat yoghurt and cucumber salat [13]. 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% [14]. Also, significant inactivation of *Salmonella* Enteritidis in tomato juice was achieved by previous addition of citric acid or cinnamon bark oil [15]. 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 [16]. Also, S. 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 [12, 16-18].

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

# 2. Materials and Methods

### *2.1. 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 and thyme 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.

### *2.2. Enumeration of Bacteria*

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 and *Salmonella enterica* serotype Enteritidis 6084 isolated from outbreaks of salmonellosis in the National Institute of Public Health, Leskovac, Serbia.

The determination of the number of *Salmonella enterica* serotype Enteritidis (D) ATCC 13076 and epidemic strain 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 37°C. 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°C during 24 hours. All experiments were done in triplicate.

# 3. Results and Discussion

In order to investigate possible effect of essential oil of sweet basil and thyme 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 and epidemic strain with the initial number of bacteria of $10^9$ CFU/g and different concentrations of essential oils were added.

During the production process the number of Salmonella decreased for 1 log CFU/g (Table 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 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.

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

| | Different concentration of sweet basil | | | |
|---|---|---|---|---|
| | **Control** | **1%** | **2.5%** | **5%** |
| | **Log CFU/g** | | | |
| **The stage of technological process** | | | | |
| Mixture | 9.00±0,05 | 9.00±0,1 | 9.00±0,18 | 9.00±0,06 |
| Exstrusion | 8,8±0,06 | 8,79±0,1 | 8,71±0,03 | 8,66±0,03 |
| Drying | 8,79±0,01 | 8,68±0,05 | 8,65±0,03 | 8,50±0,03 |
| Cooling | 8,45±0,001 | 8,25±0,02 | 8,06±0,03 | 7,69±0,12 |
| Packaging | 8.00±0,6 | 8,05±0,2 | 8.00±0,07 | 7,47±0,13 |
| Storing and distribution | 8.00±0,05 | 8.00±0,11 | 7,95±0,07 | 7,49±0,01 |

The results obtained during the investigation of the influence of sweet basil to epidemic strain *Salmonella enterica* serotype Enteritidis in the pasta are presented in Table 2.

***Table 2.*** *Survival of epidemic strain Salmonella enterica serotype Enteritidis in the pasta made with different concentration of sweet basil.*

| | Different concentration of sweet basil | | | |
|---|---|---|---|---|
| | **Control** | **1%** | **2.5%** | **5%** |
| **The stage of the technological process** | **Log (cell number per gram)** | | | |
| Mixture | 8.97±0.03 | 9.00±0.09 | 9.02±0.10 | 8.79±0.55 |
| Exstrusion | 8.63±0.26 | 8.74±0.03 | 8.53±0.30 | 7.55±0.09 |
| Drying | 8.67±0.04 | 8.55±0.17 | 8.54±0.17 | 7.41±0.04 |
| Cooling | 8.32±0.01 | 8.04±0.44 | 8.94±0.09 | 7.59±0.21 |
| Packaging | 8.00±0.05 | 7.99±0.01 | 7.76±0.13 | 7.27±0.18 |
| Storing and distribution | 7.99±0.01 | 7.98±0.01 | 7.74±0.02 | 7.04±0.12 |

Reduction of the number of epidemic strain was observed with the addition of 2.5 and 5% of sweet basil oil. Essential oil of sweet basil in the concentration of 5% causes the decrease in the number of epidemic *Salmonella enterica* during storing of egg-based pasta for 1 log unit. This is in agreement to the investigation of Barbosa et al., [19], who achieved the reduction of 1-1.3 log units of *Salmonella* Enteritidis with the addition of essential oils. Concentration of 5% of sweet basil oil was more effective in the case of epidemic strain then ATCC strain.

The greatest decrease in the pasta with the addition of essential oil of thyme in the concentration of 2.5% was observed during the extrusion phase, but the final number was slightly lower compared to control (Table 3). On the other hand, addition of 5% of thyme oil lead to the reduction of up to 1.29 log units which was observed during drying. This concentration of thyme oil caused the decrease of the number of ATCC strain for 1 log unit during the extrusion and this difference remained mostly stable till the end of the analyzed process.

***Table 3.*** *Survival of Salmonella enterica serotype Enteritidis (D) ATCC 13076 in the pasta made with different concentration of thyme.*

| | Different concentration of thyme | | | |
|---|---|---|---|---|
| | **Control** | **1%** | **2.5%** | **5%** |
| **The stage of technological process** | **Log CFU/g** | | | |
| Mixture | 9.03±0.01 | 8.96±0.19 | 9.01±0.16 | 8.98±0.14 |
| Exstrusion | 8.49±0.01 | 8.48±0.03 | 8.03±0.13 | 7.48±0.02 |
| Drying | 8.31±0.35 | 8.38±0.10 | 8.25±0.07 | 7.02±0.77 |
| Cooling | 8.18±0,03 | 8.09±0.20 | 8.06±0.03 | 7.02±0.11 |
| Packaging | 7.95±0.05 | 7.91±0.05 | 7.88±0.01 | 6.97±0.02 |
| Storing and distribution | 7.95±0.003 | 7.91±0.05 | 7.86±0.004 | 6.96±0.04 |

As it was observed for sweet basil oil, epidemic strain is less resistant to the addition of thyme oil compared to ATCC strain (Table 4). The addition of 2% of thyme oil had little influence to the growth of epidemic strain. The increase of the concentration of oil to 5% caused stronger inhibition and the reduction of 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 for 1.52 log units in the pasta with 5% of thyme oil compared to the control.

***Table 4.*** *Survival of epidemic strain Salmonella enterica serotype Enteritidis in the pasta made with different concentration of thyme.*

| | Different concentration of thyme | | | |
|---|---|---|---|---|
| | **Control** | **1%** | **2.5%** | **5%** |
| **The stage of the technological process** | **Log CFU/g** | | | |
| Mixture | 8.99±0.16 | 9.05±0.01 | 9.01±0.06 | 8.97±0.23 |
| Exstrusion | 8.42±0.03 | 8.15±0.50 | 7.66±0.85 | 7.23±0.13 |
| Drying | 7.87±0.65 | 8.04±0.04 | 7.96±0.23 | 7.00±0.06 |
| Cooling | 7.93±0.06 | 7.90±0.08 | 7.87±0.01 | 6.83±0.07 |
| Packaging | 7.88±0.01 | 7.85±0.06 | 7.85±0.06 | 6.78±0.03 |
| Storing and distribution | 7.86±0.01 | 7.83±0.06 | 7.79±0.04 | 6.34±0.81 |

Although there is literature data of strong antimicrobial activity of basil oil indicating that the number of *Salmonella* Enteritidis strain can be reduced for 3 log units [20], obtained results showed the reduction of 0.5 units and 1 log unit for ATCC and epidemic strain, respectively. Addition of thyme oil had stronger antimicrobial effect since it caused the reduction of the number of epidemic strain of approximately 1.5 log units from the beginning of the process.

# 4. Conclusion

In this paper the survival of *S.* Enteritidis in egg-based pasta was investigated. As the eggs were not thermally treated, nor were they dried, they represent the critical control point. As far as the egg-based pasta production process is concerned, the production steps can influence a decrease in the number of *Salmonella*. The addition of essential oils of sweet basil and thyme can induce the reduction of the number of *S.* Enteritidis in egg-based pasta. The results indicated that utilized oils were more effective against epidemic strain then ATCC strain. Also, thyme oil caused more significant inhibition of *S.* Enteritidis during production process. Further investigation concerning applied concentrations of oil should be performed in order to achieve greater reduction of *S.* Enteritidis.

# References

[1] OIE terrestrial manual, 2004.

[2] W. Messens, "Eggshell penetration by *Salmonella*: A review". World's Poultry Science J., 2005, pp. 71-84.

[3] EFSA, "Opinion of the scientific panel on biological hazards on the request from the commission related to the microbiological risks on washing of table eggs", 2005 pp. 1-39.

[4] F. Martelli and R. H. Davies, "*Salmonella* serovars isolated from table egges: A overview", Food Research International., 2012, pp. 745-754.

[5] ACMSF, "Repot of *Salmonella* in eggs". Advisory committee on the microbiological safety of foodmicrobiology reports, 1993.

[6] I. Gantois, R. Ducatelle, F. Pasmans, F. Haesbrouck, R. Gast., T. J. Humphrey et al. "Mechanisms of egg contamination by *Salmonella* Enteritidis", FEMS Microbiology Reviews., 2009, pp. 718/738.

[7] C. de W, Blackburn, L. M. Curtis, L. Humpheson., C. Billon, and P. J. McClure, "Development of thermal inactivation models for *Salmonella* Enteritidis and *Escherichia coli* O157:H7 with temperature, pH and NaCl as controlling factors", International J. of Food Microbiol., 1997, pp. 31–44.

[8] M. Škrinjar and T. N. Nemet, "Antimicrobial effects of spices and herbs essential oils", Acta Period. Tehnol., 2009, pp. 195-209.

[9] M. M. Tajkarami, S. A. Ibrahim, and D. O. Cliver, "Antimicrobial harb and spice compounds in food", Food Contr., 2010, pp. 1199-1218.

[10] M. Niculae, M. Spînu, C. D. Şandru, F. Brudaşă, D. Cadar, B. Szakacs, I. Scirtu, P. Bolfă, and C. I. Mateş, "Antimicrobial potential of some *Lamiaceae* essential oils against animal multiresistnal bacteria", Lucrări Scintifice Medicină Veterinară Timoşoara., 2009, pp. 170-175.

[11] M. Turgis, J. Borsa, M. Millette, S. Salmieri, and M. Lacroix, "Effect of Selected Plant Essential Oils or Their Constituents and Modified Atmosphere Packaging on the Radiosensitivity of *Escherichia coli* O157:H7 and *Salmonella* Typhi in Ground Beef", J. Food Prot., 2008, pp. 516-521.

[12] R. M. Raybaudi-Massilia, J. Mosqueda-Melgar, and O. Martin-Belloso, "Antimicrobial Activity of Essential Oils on *Salmonella* Enteritidis, *Escherichia coli*, and *Listeria innocua* in Fruit Juices", J. Food Prot., 2006, pp. 1579-1586.

[13] C. Tassou, E. H. Drosinos, and G. J. E. Nychas, "Effects of essential oil from mint (Mentha piperita) on *Salmonella* Enterititidis and *Listeria monocytogenes* in model food systems at 4°C and 10°C", J. of Applied Bacteriol., 1995, pp. 593-600.

[14] M. Friedman, P. R. Henika, C. E. Levin, and R. E. Mandrell, "Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice", J Agric Food Chem., 2004, pp. 6042-6048.

[15] J. Mosqueda-Melgar, R. M. Raybaudi-Massilia, and O. Martín-Belloso, "Inactivation of *Salmonella enterica* ser. Enteritidis in tomato juice by combining of high-intensity pulsed electric fields with natural antimicrobials", J Food Sci., 2008, pp. 47-53.

[16] S. Burt, "Essential oils: Their antibacterial properties and potential applications in food" – A review, International J. of Food Microbiology., 2004, pp. 223-253.

[17] J. Duan and Y. Zhao, "Antimicrobial efficiency of essential oil and freeze-thaw treatments against *Escherichia coli* O157:H7 and *Salmonella enterica* ser. Enteritidis in strawberry juice", J. Food Sci., 2009, pp. 131-137.

[18] M. Friedman, P. R. Henika, and R. E. Mandrell, "Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*", J Food Prot., 2002, pp. 1545-60.

[19] L. N. Barbosa, V. L. M. Rall, A. A. H. Fernandes, P. I. Ushimaru, I. S. Probst, and A. Fernandes, "Essential Oils Against Foodborne Pathogens and Spoilage Bacteria in Minced Meat", Foodborne Pathog. Dis., 2009, pp. 725–728.

[20] P. Rattanachaikunsopon and P. Phumkhachorn, "Antimicrobial activity of basil (*Ocimum basilicum*) oil against *Salmonella* Enteritidis in vitro and in food", Bioscience Biotechnol. and Biochem., 2010, pp. 1200-1204.