## IMPACT OF HEAT ADAPTATION ON SOME FOODBORNE PATHOGENS

 $\mathbf{BY}$ 

#### WAFAA MONGY MOHAMED ABD EL-HALIM

B. Sc. Agri. Sc. (Food Science and Technology), Ain Shams University, 2013

A Thesis Submitted in Partial Fulfillment
Of
The Requirements for Degree of

MASTER OF SCIENCE in Agricultural Sciences (Food Science and Technology)

> Department of Food Science Faculty of Agriculture Ain Shams University

#### **Approval sheet**

# IMPACT OF HEAT ADAPTATION ON SOME FOODBORNE PATHOGENS

### By

### WAFAA MONGY MOHAMED ABD EL-HALIM

B. Sc. Agri. Sc. (Food Science and Technology), Ain Shams University, 2013

#### This thesis for M.Sc. Degree has been approved by:

Dr.	Mahmoud Abd Allah Mohamed Saleh
	Head Researches of Food science Technology, Food Technology
	Research Institute
Dr.	Alaa Abd El-Rashid Mohamed
	Prof. of Food Science and Technology, Faculty of Agriculture, Ain
	Shams University
_	
Dr.	Amal Ahmed Mohamed Hassan
	Prof. of Food Science and Technology, Faculty of Agriculture, Ain
	Shams University
Dr	Mohamed Farag Khallaf
DI.	
	Prof. Emeritus of Food Science and Technology, Faculty of
	Agriculture, Ain Shams University

**Date of examination:** 31/10/2019

# IMPACT OF HEAT ADAPTATION ON SOME FOODBORNE PATHOGENS

## WAFAA MONGY MOHAMED ABD EL-HALIM

B. Sc. Agri. Sc. (Food Science and Technology), Ain Shams University, 2013

#### **Under the supervision of:**

#### **Dr. Mohamed Farag Khallaf**

Prof. of Food Science and Technology, Department of Food Science and Technology, Faculty of Agriculture, Ain Shams University (Principal supervisor)

#### Dr. Amal Ahmed Mohamed Hassan

Prof. of Food Science and Technology, Department of Food Science and Technology, Faculty of Agriculture, Ain Shams University

#### **ABSTRACT**

Wafaa Mongy Mohamed Abd EL-Halim: Impact of Heat Adaptation on Some Foodborne Pathogens. M.Sc. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2015.

The fundamental causes of foodborne disease are inappropriate food processing. When bacteria are subjected to thermal and pH stresses during growth, sublethal stresses can occur that may lead to differences in their subsequent tolerance to thermal treatments. In this work, the effect of prior heat and acid shock on the thermotolerance of three pathogenic bacteria (Staphylococcus aureus, Salmonella typhimurium and Listeria monocytogenes) in both of a model system and a food system were studied. The bacterial strains under study were grown at 37°C, i.e., the optimum temperature (control), sublethally heated at 47°, 52°, 57° and 60°C for various times, or sub-fatal acid treatment (at pH 4.5 or 5.5) for various times, then heat treated at 68°C for 15 min. The D<sub>68</sub>-values were calculated for the three strains, the estimated average of the  $D_{68}$ -values for the control was 4.83 min for Staphylococcus aureus, 5.41 min for Salmonella typhimurium and 5.20 min for Listeria monocytogenes, while the D<sub>68</sub>-values for heat shocked treatments ranged from 5.43 to 10.23 min for S. aureus, 6.18 to 9.2 min for S. typhimurium and 5.41 to 9.72 min for L. monocytogenes. The  $D_{68}$ -values for acid shock cells also calculated for the three strains, the estimated average of the D<sub>68</sub>-values of acid shocked cells for S. aureus ranged from 12.20 to 30.30 min, and for S. typhimurium 13.58 to 26.5 min and 14.64 to 26.5 for L. monocytogenes. The detection of cellular proteins of the three strains after heat or acid shock was examined to identify new proteins that were induced in response to stress, which in turn gave cells resistance to subsequent lethal stress. The current results also, indicated new bands that in S. aureus were 8-16, in S. typhimurium 7-15 and in L. monocytogenes 9 - 14 new bands were induced, by applying heat shock treatments with molecular weights ranged from 9 to 225 KDa. After acid shock treatment the results, also,

indicated new bands that in *S. aureus* 3-6, in *S. typhimurium* 4-11 and in *L. monocytogenes* 1-11 bands were induced with molecular weights ranged from 17 to 145 KDa. The induced heat and acid shock proteins further confirmed increased of thermotolerance. The cell walls of the strains were examined by using Scanning Electron Microscope (SEM), to detect the changes caused by thermal shock and the ability of cells to repair the injury. Moreover, selected heat shock treatments caused severe destruction in cell wall (i.e., rupture, irregular and leakage of cell contents), while heat shocked cells after incubating at 37°C for overnight in enriched medium became similar to that of normal one. The enhanced of heat resistance of the three strains should be thought-about in case of planning effective thermal processes to confirm the microbiological food safety.

**Key Words:** Heat shock, Acid shock, thermotolerance, *S. aureus*, *S. typhimurium*, *L. monocytogenes*, Scanning Electron Microscope (SEM), heat shock proteins and acid shock proteins.

#### **ACKNOWLEDGEMENT**

All praises and thanks are due to ALLAH, who blessed me with kind professors and colleagues, and gave me the support to produce this Thesis.

I would like to express my deepest appreciation and sincere love to my major professor, Prof. Dr. Mohamed Farag Khallaf, Prof. of Food Science and Technology, Department of Food Science Technology, Faculty of Agriculture, Ain Shams University. I would like to call him my second father. He was a good example of the guidance, advice and support throughout my graduate program. Thanks to you Dr. Mohamed Farag for everything, for the valuable advice and guidance that put me on the right track. Thanks for your continuous efforts to make me better. Thanks for your patience with me as long as he helped me.

Also, I do not have to deny the privilege of my dear Prof. Dr. Amal Ahmed Hassan, Prof. of Food Science and Technology, Department of Food Science Technology, Faculty of Agriculture, Ain Shams University for her continuous support and access to academic thinking during my research. Her enthusiasm in research and invaluable advices kept me motivated and focused. She has contributed significantly to my arrival at this stage. I would like to thank her very much for all efforts and advice. She did not stop at any time for providing me help and she had a lot of difficulties to show and reach this Thesis to perfection. Thanks Prof. Dr. Amal for being such a motivation in shaping the future for me.

Last but not least, I would like to extend my sincere thanks and appreciation to Dr. Medhat Al-Bayoumi, the distinguished and respected man, Director of the Color Laboratory. He provided me with necessary tools to conduct my research and laboratory experiments. Thank you for your contribution to this work.

I am eternally grateful to my mother; she has sacrificed so much for me. Thank you for your endless love, support and encouragement throughout my life. I would also like to thank my father, for helping and supporting me in all stages of my life. He has never let me down and he has always been beside me.

## **CONTENTS**

	Page
LIST OF TABLES	
LIST OF FIGURES	
LIST OF ABBERVIATION	
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	7
2.1 Pathogenic microorganisms	7
2.1.1 Salmonella	7
2.1.2 Listeria	8
2.1.3 Staphylococcus	9
2.2 Stress	10
2.2.1 Kind of stress	12
2.2.1.1 Heat	12
2.2.1.2 Cold	13
2.2.3 Acid	14
2.2.4 Osmotic	16
2.2.5 Salt	17
2.3 Heat shock proteins or stress proteins	19
2.3.1 Definition	19
2.3.2 Classification	21
2.3.3 Localization	21
2.3.4 Function	22
2.3.5 Regulation	24
2.4 Cross protection	27
2.5 Stress response and thermotolerance	28
2.6 Heat shock and increasing D-value	33
2.7 Scanning electron microscopy (SEM)	38
2.8 Adaptive response of pathogenic bacteria to heat and its impact on	38
food safety	

3. MATERIALS AND METHODS	40
3.1 Materials	40
3.1.1 Strains	40
3.1.2 Food samples	40
3.1.2.1 Orange juice	40
3.1.2.2 Minced meat	40
3.1.3 Media	40
3.1.3.1 XLD agar	40
3.1.3.2 Baird-Parker Agar Base	41
3.1.3.3 ALOA (Agar Listeria Ottaviani Agosti)	42
3.1.3.4 Nutrient Agar	43
3.1.3.5 Buffered peptone water	43
3.1.4 Chemicals	44
3.2 Methods	45
3.2.1 Bacterial strains and bacterial conditions	45
3.2.3 Preparation of cell suspension in food system	46
3.2.3.1 Meat inoculation	46
3.2.3.2 Juice inoculation	46
3.3 Treatments	46
3.3.1 Sublethal heat shock treatment	46
3.3.1.1 In model system	46
3.3.1.2 In food system	47
3.3.1.2.1 Orange juice	47
3.3.1.2.2 Minced meat	47
3.3.2 Determination of viability	48
3.3.3 Determination of D-values	48
3.3.4 Calculation of heat shock ratio	49
3.3.5 Acid adaptation, acid shock and control treatment	49
3.3.5.1 Acid challenge study	49
3.3.5.2 Determination of viability	49
3.3.6 Protein extraction and electrophoretic analysis	50

0 1
1
1
1
1
4
3
0
2
2
3
8
9
4
4
4
7
3
)1
)1
)3
)7

4.2.2.4. Determination of D <sub>68</sub> –values after acid shock	108
Part III	
4.3. Staphylococcus aureus	113
4.3.1. Effect of heat stress	113
4.3.1.1. Impact of the sublethal heat stress on development of the	113
thermotolerance	
4.3.1.2 Determination of D <sub>68</sub> –values after heat shock	116
4.3.1.3. SDS – PAGE profile of heat shocked cells	123
4.3.1.4 Scanning Electron Microscopy of bacterial cell wall	129
4.3.2. Effect of pH stress	131
4.3.2.1. Impact of the sublethal pH stress on development of resistance	131
to lethal pH	
4.3.2.2. SDS – PAGE profile of acid shocked cells	133
4.3.2.3. Impact of the sublethal pH stress on development of the	137
thermotolerance	
4.3.2.4. Determination of D <sub>68</sub> -values after acid shock	138
Part IV	
4.4. Application in food system	142
4.4.1 Impact of the sublethal heat stress on development of the	142
thermotolerance and Determination of D <sub>68</sub> -values after heat shock	
4.4.1.1. Minced Meat	142
4.4.1.1.1. Staphylococcus aureus	142
4.4.1.1.2. Salmonella typhimurium	148
4.4.1.1.3. Listeria monocytogenes	154
4.4.1.2. Orange juice	160
4.4.1.2.1. Staphylococcus aureus	160
4.4.1.2.2. Salmonella typhimurium	165
4.4.1.1.3. Listeria monocytogenes	170
5. SUMMARY AND CONCLUSION	175
6. REFERENCE	186
ARABIC SUMMARY	

## LIST OF TABLE

Table No.		Page
1.	Effect of heat shock value and duration time on	
	survival of S. typhimurium at 68°C/15min	53
2.	D-values (min), N, R2 and heat shock ratio (%) at	
	68°C for 15 min of S. typhimurium	60
3.	Changes in protein composition of S. typhimurium in	
	response to heat shock at 47°C.	65
4.	Changes in protein composition of S. typhimurium in	
	response to heat shock at 52°C.	66
5.	Changes in protein composition of S. typhimurium in	
	response to heat shock at 57°C.	67
6.	Changes in protein composition of S. typhimurium in	
	response to heat shock at 60°C.	68
7.	Effect of pH shock time and temperature on survival	
	of S. typhimurium at pH 2.5	72
8.	Changes in protein composition of S. typhimurium in	
	response to acid shock at pH 4.5	76
9.	Changes in protein composition of S. typhimurium in	
	response to acid shock at pH 5.5	77
10.	Effect of pH shock value and duration time	
	on survival of S. typhimurium at 68°C	79
11.	D-values (min) and pH shock ratio (%) of S.	
	typhimurium at 68°C for 15 min	83
12.	Effect of heat shock temperature and time on survival	
	of L. monocytogenes at 68°C/15min	86
13.	D-values (min), $N_{.}$ , $R^{2}$ and heat shock ratio (%) of $L$ .	
	monocytogenes at 68°C for 15 min	88
14.	Changes in protein composition of L. monocytogenes	
	in response to heat shock at 47°C.	96

<b>15.</b>	Changes in protein composition of <i>L. monocytogenes</i>	
	in response to heat shock at 52°C.	97
16.	Changes in protein composition of L. monocytogenes	
	in response to heat shock at 57°C.	98
<b>17.</b>	Changes in protein composition of L. monocytogenes	
	in response to heat shock at 60°C.	99
18.	Effect of pH shock treatment on survival of L.	
	monocytogenes at pH 2.5	103
19.	Changes in protein composition of L. monocytogenes	
	in response to acid shock at pH 4.5	105
20.	Changes in protein composition of L. monocytogenes	
	in response to acid shock at pH 5.5	106
21.	Effect of pH shock treatment on survival of L.	
	monocytogenes at 68°C	108
22.	D-values (min) and pH shock ratio (%) of L.	
	monocytogenes at 68°C for 15 min	112
23.	Effect of heat shock treatment on survival of S. aureus	
	at 68°C/ 15 min	115
24.	D-values (min) and heat shock ratio (%) of S. aureus	
	at 68°C for 15 min	122
25.	Changes in protein composition of S. aureus in	
	response to heat shock at 47°C.	125
26.	Changes in protein composition of S. aureus in	
	response to heat shock at 52°C.	126
27.	Changes in protein composition of S. aureus in	
	response to heat shock at 57°C.	127
28.	Changes in protein composition of S. aureus in	
	response to heat shock at 60°C.	128
29.	Effect of pH shock treatment on survival of S. aureus	
	at pH 2.5	132

30.	Changes in protein composition of S. aureus in	
	response to acid shock at pH 4.5	135
31.	Changes in protein composition of S. aureus in	
	response to acid shock at pH 5.5	136
32.	Effect of pH shock treatment on survival of S. aureus	
	at 68°C	137
33.	D-values (min) and pH shock ratio (%) of S. aureus at	
	68°C for 15 min	141
34.	Effect of heat shock temperature and time on survival	
	of S. aureus in minced meat at 68°C/15min	143
<b>35.</b>	D-values (min) and heat shock ratio (%) of S. aureus	
	in minced meat at 68°C for 15 min	144
36.	Effect of heat shock treatment on survival of S.	
	typhimurium in minced meat at 68°C/15min	148
<b>37.</b>	D-values (min) and heat shock ratio (%) of S.	
	typhimurium in minced meat at 68°C for 15 min	153
<b>38.</b>	Effect of heat shock treatment on survival of L.	
	monocytogenes in minced meat at 68°C/15min	154
<b>39.</b>	D-values (min) and heat shock ratio (%) of L.	
	monocytogenes in minced meat at 68°C for 15 min	159
40.	Effect of heat shock treatment on survival of <i>S. aureus</i>	
	in fresh orange juice at 68°C/15min	160
41.	D-values (min) and heat shock ratio (%) of S. aureus	
	in fresh orange juice at 68°C for 15 min	164
<b>42.</b>	Effect of heat shock treatment on survival of S.	
	typhimurium in fresh orange juice at 68°C/15min	165
<b>43.</b>	D-values (min) and heat shock ratio (%) of S.	
	typhimurium in fresh orange juice at 68°C for 15 min	169
44.	Effect of heat shock treatment on survival of L.	
	monocytogenes in fresh orange juice at 68°C/15min	170
<b>45.</b>	D-values (min) and heat shock ratio (%) of L.	
	monocytogenes in fresh orange juice at 68°C for 15 min	174

## VIII

## LIST OF FIGURES

Fig.		
No.		Page
1.	Schematic representation of bacterial stress response when	
	exposed to a physical or chemical environment beyond	
	optimum growth range	11
2.	Mechanisms of acid shock response and acid resistance	
	system	16
3.	A graphical exemplification of molecular regulation of heat-	
	shock genes (positive and negative transcriptional	
	regulation) in bacteria	26
4.	Viability of Salmonella typhimurium at 68°C/15 min	53
5.	Schematic representation microbial thermal	
	inactivation curve of <i>S. typhimurium</i> (control cells)	55
6.	Schematic representation of microbial thermal inactivation	
	curves of <i>S. typhimurium</i> that exposed to heat shock at 47°C	
	for (5, 15, 30, 60 min) and sub subsequently subjected to	
	thermal treatment at 68°C for 15 min.	55
7.	Schematic representation of microbial thermal inactivation	
	curves of <i>S. typhimurium</i> that exposed to heat shock at 52°C	
	for (5, 15, 30, 60 min) and subsequently subjected to thermal	
	treatment at 68°C for 15 min.	56
8.	Schematic representation of microbial thermal inactivation	
	curves of <i>S. typhimurium</i> that exposed to heat shock at 57°C	
	for (5, 15, 30, 60 min) and subsequently subjected to thermal	
	treatment at 68°C for 15 min	57
9.	Schematic representation of microbial thermal inactivation	
	curves of <i>S. typhimurium</i> that exposed to heat shock at 60°C	
	for (5, 15, 30, 60 min) and subsequently subjected to thermal	
	treatment at 68°C for 15 min.	59
10	D-values of Salmonella typhimurium at 68°C/15 min	62