

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

يَسْأَلُكَ عَنِ الرُّوحِ قُلُوبُ الرُّوحِ مِنْ أَمْرِ رَبِّي
وَمَا أُوتِيتُمْ مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدق الله العظيم

(سورة الإسراء الآية 85)

DEDICATION

TO THE SOUL OF MY LATE PARENTS
TO MY FAMILY MEMBERS,
MY HUSBAND AND
MY LOVELY SISTERS
WITH LOVE ...

MEISON

ACKNOWLEDGEMENT

I am grateful to ALMIGHTY ALLAH, for giving me the ability to conduct this research.

My thanks and appreciation for my supervisor Professor Ahmed Elawad El faki for his invaluable supervision and patience.

I am gratefully indebted to my cosupervisor Professor Suleiman Elsanousi for his help and supervision.

My thanks extended to the staff members of the Microbiology Lab in Sudanese Standard and Metrology Organization.

Thanks and appreciations are extended to Professor Mona Agab, Awad Sucrab and Dr Omer Abdalla for their invaluable assistance.

Abstract

The aim of this study was to design a medium for the true bacterial load in frozen meat. The study was carried out in the period from June to October 2009. The injured bacteria on frozen meat like burger, showed a remarkable reduction in their viable count number. This in turn will lead to underestimation of the true bacterial load. Consequently, this will lead to passing food as fit for human consumption while it is not passable. XLD medium was used for the growth of *Salmonella sp* shocked cells. The suggested media consisted of sodium pyruvate at the concentrations 0.5, 1.0 and 1.5%. Vitamin B₆ and B₁₂ in the concentrations of 1, 2 and 3ml were added singly or simultaneously with sodium pyruvate. *Salmonella* was subjected to shock at -20,-30 and -40°C for one hour and then inoculated in the different media. The loss in viability with B₆ was 4.8% at -20°C, 9.7% at -30°C and 26.3% at -40°C. The loss in viability with B₁₂ was 0.98% at -20°C, 2.5% at -30°C and 14.8% at -40°C. The loss in viability with sodium pyruvate was 2.0% at -20°C, 25.2% at -30°C and 26.8% at -40°C. When sodium pyruvate was added in 0.5% recovery was 93.9%, in 1% recovery was 94.0% and in 1.5% recovery was 92.9%. When sodium pyruvate was added in 0.5% with B₆ (1ml) recovery was 97.5% while B₁₂ (1ml) was 99.6% an improvement of recovery was noticed. It is suggested that when counting bacteria in frozen foods e.g. burger medium containing sodium pyruvate and B₁₂ should be included.

ملخص الأطروحة

الهدف من هذه الدراسة لإيجاد وسط غذائى للحمل البكتيرى الحقيقى فى اللحوم المجمدة .كانت الدراسة فى الفترة من يونيو إلى أكتوبر2009. البكتريا المجرحة الموجودة فى البيرقر المجدد أظهرت إنخفاضاً واضحاً فى العد للبكتريا وهذا يوضح الحمل البكتيرى الحقيقى والذى يؤدى حتماً لظهورالغذاء على انة صالح لإستخدام اللآدمى لكن حقيقة هو غير ذلك. وقد اقتضت خطة البحث على تحضيرالأوساط الإنتقائية لنمو البكتريا المجرحة بالتجميد (السالمونيلا) بحيث تحتوى الأوساط على تراكيز مختلفة(10.5 1.5%) من مضاف ،بيروفات الصوديوم. كما تم إضافة فيتامينات ب₆وب₁₂ بتراكيز (3،2،1مل) إما منفردة أو مضافة مع بيروفات الصوديوم . تم تعريض ميكروب السالمونيلا لصدمة باردة عند درجات حرارة - 20°م و - 30°م و - 40°م لمدة ساعة ثم زرعت فى الأوساط المختلفة وقد كانت نتائج الفقد فى عدد البكتريا كالتأتى: الفقد قبل اضافة فيتامين ب₆ 4.8% (- 20°م)، 9.67% (-30°م) (26.3% (- 40°م) . الفقد قبل إضافة فيتامين ب₁₂ 0.98% (-20°م) ، 2.5% (-30°م) ، 14.8% (- 40°م) .الفقد قبل إضافة بيروفات الصوديوم 2.0% (- 20°م) ، 25.2% (-30°م) 26.8% (- 40°م) . عند إضافة البيروفات بتركيز 0.5% كان الإسترجاع للفقد بنسبة 93.8% ،(1%) 94.0% (1.5%) 92.9% . عند إضافة البيروفات بتركيز 0.5% مع 1مل من ب₆ الإستعادة كانت بنسبة 97.2% بينما كانت 99.6% عند إضافة 0.5% من البيروفات مع 1مل من ب₁₂ وجد تحسن ملحوظ فى الإستعادة عليه نوصى بأنه عند عد البكتريا فى الأغذية المجمدة مثل البيرقر يضاف البيروفات وب₁₂ للوسط.

LIST OF CONTENTS

2	Dedication	II
3	Acknowledgements	III
4	Abstract	IV
5	Arabic abstract	V
6	List of contents	VI
1	CHAPTER ONE: INTRODUCTION	1
2	CHAPTER TWO: LITERATURE REVIEW	4
2.1	History of burger	4
2.2	Meat contamination	5
2.3	Storing uncooked meat	5
2.3.1	Refrigerating meat	6
2.3.2	Early work on freezing process	6
2.3.2.1	Freezing meat	8
2.3.2.2	Freezing of cooked meat	8
2.3.2.2	Effect of freezing on bacteria	9
2.4	Cold shocked bacteria	11
2.4.1	Effect of cold shock on bacteria	11
2.4.2	Cold shock response	11
2.4.3	Cold shock (Phoenix Phenomenon)	12
2.4.4	Cold shock genes	16
2.5	Definition of stress on bacteria	18
2.5.1	Stress reaction, stress in survival of bacteria	19
2.6	Definition of injury	20
2.6.1	Injury to microorganisms	20
2.6.2	Cause of injury	21
2.6.3	Repair of injured cells	21
2.7	The Pathogenicity of injured cells	22
2.7.1	<i>Escherichia coli</i>	23
2.7.2	<i>Salmonella sp</i>	24

2.7.3	<i>Staphylococcus aureus</i>	25
2.8	The effect of selective media on recovery of injured bacterial cells	25
2.8.1	Pyridoxine	26
2.8.2	Vitamin B ₁₂	26
3	CHAPTER THREE: MATERIALS AND METHODS	27
3.1	Material	27
3.1.1	Recovery media	27
3.2	<i>Salmonella</i> method	27
3.2.1	Biochemical tests	28
3.2.1.1.	TSI Agar	28
3.2.1.2	Urea agar	28
3.2.2	Recovery method	28
3.2.2.1	Before shock	28
3.2.2.2	Aftershock	29
4	CHAPTER FOUR : RESULT AND DISCUSSION	30
4.1	Effect of vitB ₆ on growth of cold shocked <i>Salmonella</i>	30
4.2	Effect of vitB ₁₂ on growth of the cold shocked <i>Salmonella</i>	34
4.3	Effect of sodium pyruvate on growth of cold- shocked <i>Salmonella sp</i>	34
4.4	Effect of sodium pyruvate and vitB ₆ on growth of cold-shocked <i>Salmonella</i>	35
4.5	Effect of sodium pyruvate and vitB ₁₂ on growth of cold-shocked <i>Salmonella</i>	45
5	CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATION	50
5.1	Conclusions	50
5.2	Recommendations	51
6	REFERENCES	52

LIST OF TABLES

Table 1: Effect of addition vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at-20°C	31
---	----

Table 2: Effect of addition of vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at-30°C.....	32
Table 3: Effect of addition of vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at- 40°C.....	33
Table 4: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 20°.....	35
Table 5: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 30°C.....	36
Table 6: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 40°C.....	37
Table 7: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 20°C.....	39
Table 8: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 30°C.....	40
Table 9: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 40°C.....	41
Table 10: Effect of addition of sodium pyruvate and vitB ₆ on growth of <i>Salmonella</i> , cold shocked at-20°C.....	43
Table11: Effect of addition of vitB ₆ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-30°C.....	44
Table 12: Effect of addition of vitB ₆ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-40°C.....	45
Table 13: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-20°C.....	47
Table 14: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-30°C.....	48
Table 15: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked -40°C.....	49

LIST OF FIGURES

Fig 1: Effect of addition vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at-20°C	31
Fig 2: Effect of addition of vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at-30°C.....	32
Fig 3: Effect of addition of vitB ₆ on growth of <i>Salmonella</i> , before and after cold shocked at- 40°C.....	33
Fig 4: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 20°.....	35
Fig 5: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 30°C.....	36
Fig 6: Effect of addition of vitB ₁₂ on growth of <i>Salmonella</i> , before and after cold shocked at- 40°C.....	37
Fig 7: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 20°C.....	39
Fig 8: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 30°C.....	40
Fig 9: Effect of addition of sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at- 40°C.....	41
Fig10: Effect of addition of sodium pyruvate and vitB ₆ on growth of <i>Salmonella</i> , cold shocked at-20°C.....	43
Fig 11: Effect of addition of vitB ₆ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-30°C.....	44
Fig 12: Effect of addition of vitB ₆ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-40°C.....	45
Fig 13: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-20°C.....	47
Fig 14: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked at-30°C.....	48
Fig 15: Effect of addition of vitB ₁₂ and sodium pyruvate on growth of <i>Salmonella</i> , cold shocked -40°C.....	49