Combined boiling and irradiation treatment on the shelf life and safety of Ready-To-Eat bovine tripe

By

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Declaration

I declare that the dissertation herewith submitted for the degree MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other university or institution of higher education.

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Dedication

I dedicate this dissertation to my parents, Hector and Irene Parry-Hanson, and to my siblings, Freda, Michael and Jocelyn Parry-Hanson.

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ABSTRACT

COMBINED BOILING AND IRRADIATION TREATMENT ON THE SHELF LIFE AND SAFETY OF READY-TO-EAT BOVINE TRIPE

By Angela Parry-Hanson

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Bovine tripe is not optimally used in South Africa because it is highly perishable, it is not easily accessible and it requires long cooking time. For, these reasons, ready-to-eat (RTE) technology was used to process tripe. This study was thus undertaken to determine the effect of vacuum packaging and boiling in combination with gamma irradiation at a target dose of 9 kGy on the microbiological safety, with respect to inoculated *Clostridium perfringens* ATCC 13124 spores, and microbiological quality, with respect to aerobic plate counts (APC) and aerobic spore counts (ASC), of RTE bovine tripe during storage at 5 and 15 °C for 14 days. Irradiation dosage of 9 kGy was chosen as an appropriate dosage to eliminate bacterial spores of *C. perfringens* and aerobic spores that were present on inoculated tripe. Also, transmission electron microscopy (TEM) was conducted on *C. perfringens* ATCC 13124 spores to determine whether boiling and gamma irradiation has a synergistic effect on *C. perfringens* spore structure.

In order to maintain sensory properties of tripe, mild preservation treatments were used in the processing of RTE tripe. For this reason, the following hurdles were employed in the processing of RTE tripe: boiling, vacuum packaging, gamma irradiation and chilled storage. Prior to boiling, rough washed tripe was tenderized with papain to reduce the cooking time of raw bovine tripe.

In Phase 1, the fresh tripe was processed as a *sous-vide* RTE product. The washed and papain treated tripe was inoculated in vacuum bags, sealed, boiled in the vacuum bags and gamma irradiated at a target dose of 9 kGy (10±1 °C). Despite vacuum packaging the raw tripe prior to boiling and irradiation, aerobic conditions prevailed due to the presence of residual oxygen in the RTE tripe packs. This resulted in inhibition of *C. perfringens* after boiling. The fresh tripe had a high microbiological load of 8.6 $\log_{10}$ cfu/g for both APC and ASC and 4.5 $\log_{10}$ cfu/g CC due to the high levels of microorganisms naturally present in the ruminant stomach. Although boiling significantly reduced APC and ASC, their levels (6.3 $\log_{10}$ cfu/g for APC and 6.1 $\log_{10}$ cfu/g for ASC) remained high after boiling probably due to the presence of heat resistant spores. Although irradiation significantly reduced APC and ASC on *sous-vide* RTE tripe, aerobic bacteria and aerobic spores showed high resistance to gamma irradiation, with *ca* 4 $\log_{10}$ cfu/g bacteria surviving on irradiated RTE tripe. Storage at 5 °C inhibited increase of APC and ASC on both irradiated and control RTE tripe samples, thus extending the shelf life of RTE tripe to at least 14 days. However, rapid growth occurred in 0 kGy RTE tripe stored at 15 °C. By day 7, APC and ASC on 0 kGy samples stored at 15 °C had exceeded 7 $\log_{10}$ cfu/g and was thus considered spoiled. Low APC and ASC were maintained in irradiated samples stored at 15 °C throughout 14 days of storage. Therefore, in an aerobic environment, irradiation of RTE tripe and storage at 5 °C is required to reduce microbiological counts and extend the shelf life of RTE tripe to at least 14 days.

In Phase 2, tripe was boiled prior to inoculation with 7 $\log_{10}$ cfu/g *C. perfringens* ATCC 13124 spores, vacuum packaged, irradiated at a target dose of 9 kGy (10±1 ºC) and stored for 7 days at 5 and 15 ºC. The change in processing of RTE tripe in Phase 2 was done to eliminate the residual oxygen in Phase 1 to create appropriate growth conditions for inoculated *C. perfringens* spores. Gamma irradiation significantly reduced *C. perfringens* and APC below detection (detection limit of 1 $\log_{10}$ cfu/g) throughout 7 days of storage at 5 and 15 °C. However, aerobic bacteria re-emerged on irradiated samples during storage at 5 and 15 °C due to repair of irradiation injury.

TEM of boiled and irradiated *C. perfringens* ATCC 13124 spores showed that boiling alone caused reduction of spore material possibly due to initiation of germination. Gamma irradiation alone caused elongation of *C. perfringens* spores indicating that although germination occurred, outgrowth was inhibited. Boiling and gamma irradiation had a synergistic effect on *C. perfringens* spores as indicated by complete loss *C. perfringens* spore material.

However, due to the high levels of aerobic spores and aerobic bacteria that persisted on irradiated RTE tripe in Phase 1, *sous-vide* processing of RTE tripe is not recommended because it might be unsafe for consumption when pathogenic *Bacillus cereus* spores are present. Irradiated RTE bovine tripe in an anaerobic environment (Phase 2), proved to be safe for consumption throughout 7 days of storage regardless of the storage temperature.

TABLE OF CONTENTS

LIST OF TABLES .... *I*
LIST OF FIGURES .... *II*
CHAPTER 1: INTRODUCTION *1*
1.1 PROBLEM STATEMENT 1
CHAPTER 2: LITERATURE REVIEW *3*
2.1 THE RUMINANT STOMACH 3
2.2 TENDERNESS OF TRIPE 4
2.3 BACTERIAL QUALITY OF TRIPE 5
2.3.1 Effect of intrinsic factors on the bacterial ecology of tripe 7
2.3.2 Effect of extrinsic factors on the bacterial ecology of tripe 8
2.3.3 Implicit factors 9
2.3.4 Bacterial attachment to meat 10
2.4 *CLOSTRIDIUM PERFRINGENS* 11
2.5 SPORE RESISTANCE AND DORMANCY 12
2.5.1 The bacterial spore structure 12
2.5.2 Parameters contributing to spore resistance 14
2.5.2.1 *Genetic composition of spore former* 14
2.5.2.3 *Spore coat* 15
2.5.2.4 *Inner spore membrane* 15
2.5.2.5 *Repair of macromolecular damage* 16
2.5.2.6 *Spore core dehydration* 16
2.5.2.7 *Spore core mineralisation and dipicolinate accumulation* 16
2.6 BACTERIAL SPORULATION AND GERMINATION 17
2.6.1 Sporulation 17
2.6.2 Germination 18
2.7 HEAT RESISTANCE OF BACTERIAL CELLS AND SPORES 20
2.7.1 Mechanism of heat injury 21
2.7.2 Repair of heat injury 23
2.8 IRRADIATION OF MEAT 23
2.8.1 Resistance of bacterial cells and spores to gamma irradiation 25

2.8.2 Repair of ionizing irradiation injury 29

2.9 IRRADIATION IN COMBINATION WITH OTHER PRESERVATION TREATMENTS 31

2.10 OBJECTIVES AND HYPOTHESES 33

2.10.1 Hypotheses.. 33

2.10.2 Objectives 34

CHAPTER 3: RESEARCH *35*

3.1 EFFECT OF BOILING AND GAMMA IRRADIATION ON THE MICROBIOLOGICAL QUALITY OF READY-TO-EAT BOVINE TRIPE 38

3.1.1 Introduction 39

3.1.2 Materials and Methods 40

3.1.2.1 *Raw materials* 40

3.1.2.2 *Preparation of RTE bovine tripe samples* 40

3.1.2.3 *Gamma irradiation treatment*.. 42

3.1.2.4 *Post irradiation storage*... 43

3.1.2.5 *Bacterialogical analyses of bovine tripe* 43

3.1.2.6 *Statistical analyses* 44

3.1.3 Results 45

3.1.3.1 *Bacterial quality of fresh bovine tripe* 45

3.1.3.2 *Bacterial quality of gamma irradiated RTE bovine tripe* 45

3.1.4 Discussion... 50

3.1.5 Conclusions 53

3.2 THE EFFECT OF GAMMA IRRADIATION ON SURVIVAL OF *Clostridium perfringens* ATCC 13124 SPORES ON RTE BOVINE TRIPE 54

3.2.2 Materials and Methods 56

3.2.2.1 *Raw materials* 56

3.2.2.2 *Preparation of RTE tripe samples* 56

3.2.2.3 *Transmission electron microscopy of* C. perfringens *ATCC 13124 spores* 57

3.2.2.4 *Statistical analyses* 58

3.2.3 Results 58

3.2.3.1 *Bacterial quality of fresh bovine tripe* 58

3.2.3.2 *Bacterial quality of RTE bovine tripe* 59

3.2.3.3 *Effect of boiling and gamma irradiation on spore structure of* Clostridium perfringens *ATCC 13124 spores* 64

3.2.4 Discussion 67

3.2.5 Conclusions 73

CHAPTER 4: GENERAL DISCUSSION *74*

4.1 METHODOLOGY 74

4.2 COMPARISON OF THE MICROBIOLOGICAL QUALITY AND SAFETY OF RTE TRIPE WHEN IRRADIATED AND STORED IN AN AEROBIC AND ANAEROBIC ENVIRONMENT 80

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS *88*

CHAPTER 6: REFERENCES *90*

LIST OF TABLES

TABLE 1 Gamma D_{10} values of selected spoilage and pathogenic bacteria found on meat and sea food 27

TABLE 2 Effect of washing, papain treatment and boiling on the bacterial quality of fresh bovine tripe 44

TABLE 3 P-values for 3-way ANOVA to illustrate the effect of gamma irradiation at 0 and 9 kGy on aerobic plate count of ready-to-eat bovine tripe stored for 14 days at 5 ˚C or 15 ˚C 45

TABLE 4 P-values for 3-way ANOVA to illustrate the effect of gamma-irradiation at 0 and 9 kGy on aerobic spore count of ready-to-eat bovine tripe stored for 14 days at 5 ˚C or 15 ˚C..47

TABLE 5 Effect of rough washing, papain treatment, boiling and inoculation with *Clostridium perfringens* ATCC 13124 spores on the bacterial quality of fresh bovine tripe 57

TABLE 6 P-values for 3-way ANOVA to illustrate the effect of gamma irradiation at 9 kGy on *Clostridium perfringens* ATCC 13124 of ready-to-eat bovine tripe stored for 7 days at 5 ˚C or 15 ˚C 59

TABLE 7 P-values for 3-way ANOVA to illustrate the effect of gamma irradiation at 0 and 9 kGy on aerobic plate count of ready-to-eat bovine tripe stored for 7 days at 5 ˚C or 15 ˚C 61

LIST OF FIGURES

Figure 2.1: The four compartments of the ruminant stomach....3

Figure 2.2: A section of a typical bacterial spore showing its internal structures (Heritage, 2003)....13

Figure 2.3: Changes during sporulation of Bacillus subtilis....18

Figure 3.1: Experimental design for the determination of the effect of boiling and gamma irradiation at a target dose of 9 kGy on the bacterial quality of RTE bovine tripe stored for 14 days at 5 °C and 15 °C....36

Figure 3.2: Experimental design to determine the effect of gamma irradiation at a target dose of 9 kGy on survival of C. *perfringens* ATCC 13124 spores on RTE bovine tripe stored at 5 °C and 15 °C for 7 days....37

Figure 3.3: Overall effect of gamma irradiation on aerobic plate count (APC) on ready-to-eat bovine tripe....46

Figure 3.4: Effect of irradiation at a target dose of 9 kGy on aerobic plate count (APC) of ready-to-eat bovine tripe stored for 14 days at 5 °C and 15 °C 47

Figure 3.5: Overall effect of gamma irradiation on aerobic spore count (ASC) of ready-to-eat bovine tripe....48

Figure 3.6: Effect of irradiation at 9 kGy on aerobic spore count (ASC) of RTE bovine tripe stored for 14 days at 5 °C and 15 °C....49

Figure 3.7: Effect of a target dose of 9 kGy gamma irradiation on survival of *Clostridium perfringens* (CC) of inoculated RTE bovine tripe....59

Figure 3.8: Effect of irradiation at a target dose of 9 kGy on survival of *Clostridium perfringens* ATCC 13124 (CC) on inoculated ready-to-eat bovine tripe stored for 7 days at 5 °C and 15 °C....61

Figure 3.9: Overall effect of gamma irradiation on aerobic plate count (APC) of inoculated ready-to-eat bovine tripe....62

Figure 3.10: Effect of irradiation at a target dose of 9 kGy on aerobic plate count (APC) of inoculated ready-to-eat bovine tripe stored for 7 days at 5 °C and 15 °C....63

Figure 3.11: Untreated *Clostridium perfringens* ATCC 13124 spore....64

Figure 3.12: *Clostridium perfringens* ATCC 13124 spores boiled for 1 h in a water bath 65

Figure 3.13: *Clostridium perfringens* ATCC 13124 spores gamma irradiated at 9 kGy 66

Figure 3.14: *Clostridium perfringens* ATCC 13124 spores boiled for 1 h and gamma irradiated at 9 kGy 67

Figure 4.1: A summary of the effect of *sous-vide* processing and irradiation of RTE tripe on microbiological quality and shelf life....................82

Figure 4.2: A summary of the effect of anaerobic processing and irradiation of RTE tripe in an anaerobic environment on microbiological quality and safety 83