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

By

Angela Araba Bondzewaa Parry-Hanson

Submitted in partial fulfilment of the requirements for the degree
MSc Food Science

In the

Department of Food Science
Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

March 2006

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.

Angela Araba Bondzewaa Parry-Hanson

Dedication

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

ACKNOWLEDGEMENTS

My greatest acknowledgement goes to my Lord for granting me the grace and strength to complete my Masters program and for supplying all my needs throughout the course of my study.

I would like to sincerely thank:

My supervisor, Prof. Elna M. Buys for your patience, encouragement and supervision throughout the course of my study.

My co-supervisor, Prof Amanda Minnaar for your kind words and guidance throughout this study.

Alan Hall, Laboratory of Microscopy and Microanalysis, for your time, patience and assistance with the transmission electron microscopy of *Clostridium perfringens* ATCC 13124 spores.

Enzymes SA for providing the papain for this study.

The staff members and postgraduate students of the Department of Food Science for your encouragement and assistance during the course of my study.

My mother, Irene Parry-Hanson, for being my pillar of support; my sister, Freda Parry-Hanson, for your constant encouragement; and my friends, Eunice Ubomba-Jaswa, Kweku K. Arthur, Komeine Nantanga and Isaac Owusu Boakye for always being there for me.

International Atomic Energy Agency for funding this project.

ABSTRACT

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

By Angela Parry-Hanson

Supervisor: Prof. E. M. Buys
Co-supervisor: Prof. A. Minnaar
Department: Food Science
Degree: MSc Food Science

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

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Tripe is the stomach tissue of ruminants, mainly cattle, sheep and goats (Giaccone, Civera and Parisi, 1994), used for human consumption. Tripe is a common and inexpensive source of food that is rich in protein and calcium (Zarkadas, Karatzas and Zardakas, 1996; Anderson, 1989).

Tripe originated as the poor man's dish in many indigenous cultures since it came from the cheapest cuts of meat. Over the years, it has evolved into a well known delicacy in South Africa among all age groups across the socioeconomic ladder.

In South Africa, fresh tripe can be obtained at abattoirs. Tripe is also distributed frozen to selected retail shops. Prior to packaging, tripe is roughly washed in 21˚C water and may be kept at ambient temperatures for 4 h before cold storage (National Department Agriculture, 2000; Erasmus, 1997). This allows for proliferation of contaminating bacteria. Bleaching, scalding and scraping of tripe are rare as it removes the original flavour of tripe, which is a preference characteristic especially for the traditional consumers (van den Heever, 1977).

1.1 PROBLEM STATEMENT

Bovine tripe is not optimally utilized in South Africa for a number of different reasons: It is not easily accessible to consumers since it is only available at abattoirs and selected retailers; it requires long cooking times (Flynn and Fox, 1981) to tenderize the collagen and elastin fibres; and raw tripe is highly perishable due to the presence of gut microflora and autolytic enzymes (van den Heever, 1977), therefore causing economic losses to suppliers when demand is low. Since tripe is still enjoyed by all age groups especially among the African populations in South Africa, it is anticipated that if made available as a chilled Ready-To-Eat (RTE) product, it will be well patronized.

2.1 THE RUMINANT STOMACH

The ruminant stomach is subdivided into four compartments based on the feeding habits of ruminants (Hofmann, 1973). The subdivisions give rise to the four stomach sacs consisting of the rumen, reticulum, omasum and abomasums. These subdivisions are intended to delay passage of food through the gut (Hofmann, 1973). The rumen and reticulum are commonly used for food. Sometimes the ruminant stomachs, together with the intestines are collectively referred to as tripe.

The rumen extends from the diaphragm to the pelvis of the ruminant (Frandson and Spurgeon, 1992). This is shown in Fig. 2.1. The rumen is the first stomach. Its lining varies in thickness and is often associated with a layer of fat. The rumen also contains numerous aerobic and anaerobic bacteria that aid in the fermentation of feed (Hungate, 1966).

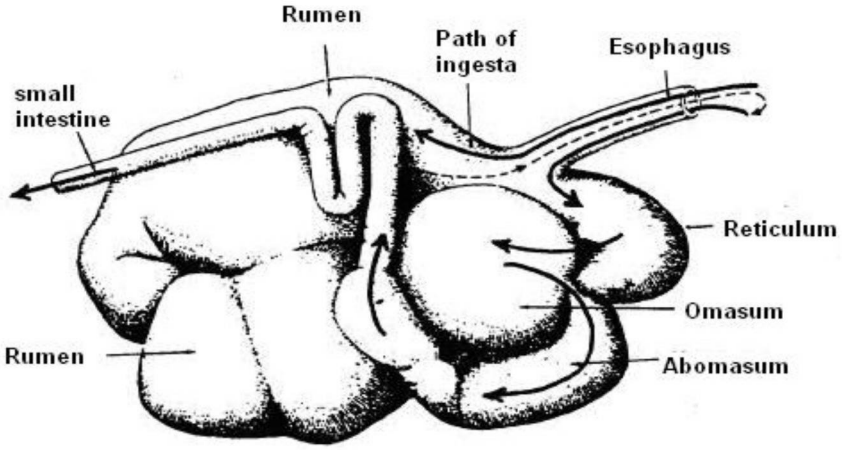

Figure 2.1: The four compartments of the ruminant stomach (Jurgens, 1996)

The reticulum, also known as honeycomb tripe, precedes the rumen and lies next to the heart (Hungate, 1966). Although it is a small sac, it has a large opening that leads to the rumen, a much larger compartment. The *rumino-reticular* fold separates the reticulum

CHAPTER 3: RESEARCH

Research chapter is presented in the format of article submitted to Food Microbiology.

The objective of this study was to determine the effect of gamma irradiation at a target dose of 9 kGy on the general bacterial quality and *Clostridium perfringens* ATCC 13124 spores inoculated on RTE bovine tripe, and to determine the shelf life of RTE tripe stored at 5 and 15 °C for 14 days. The initial intention was to inoculate RTE bovine tripe and process it as a *sous-vide* product in an anaerobic environment (vacuum packaged). This is shown in the experimental design for Phase 1 in Fig. 3.1.

However, *C. perfringens* was not detected in inoculated samples after boiling and throughout storage. Gas analysis of RTE bovine tripe with Gaspace2 (Oxfordshire, UK) indicated the presence of oxygen in the packs. Although *C. perfringens* is generally considered anaerobic/ microaerophilic, experience with *C. perfringens* in this research showed that *C. perfringens* ATCC 13124 is an obligate anaerobe. Since *C. perfringens* ATCC 13124 is an obligate anaerobe, it was inhibited in the *sous-vide* RTE bovine tripe because the package was aerobic.

Consequently, the processing of RTE tripe was changed in Phase 2, where raw tripe was boiled in a pot, rapidly chilled and inoculated, prior to vacuum packaging, and heat shrinking of the vacuum packs. The experimental design for Phase 2 is shown in Fig. 3.2. This change in processing of RTE bovine tripe was done to eliminate the presence of oxygen in the vacuum packs so as to create an anaerobic environment, to study the effect of gamma irradiation on *C. perfringens* inoculated on boiled tripe. Consequently, trhe contribution of boiling was not studied in Phase 2. Since a shelf life study (14days) was conducted in Phase 1, the shelf life study in Phase 2 was shortened to 7 days because it was not the main focus area of Phase 2 study.

CHAPTER 4: GENERAL DISCUSSION

The main objective of this study was to determine the effect of boiling for 1 h and irradiation at a target dose of 9 kGy on the bacterial quality and safety of RTE bovine tripe. inoculated with *C. perfringens* ATCC 13124 spores, and stored at 5 and 15 ˚C for 14 days. In order to maintain sensory properties of tripe, mild preservation treatments had to be used (Del Torre *et al*., 2004). For this reason, the following hurdles were employed in the development of RTE bovine tripe: papain treatment, boiling, vacuum packaging, gamma irradiation and chilled storage.

4.1 METHODOLOGY

Bovine tripe is a highly perishable product with a shelf life of 24 h at chilled temperatures (Giaccone *et al.,* 1994) due to the high levels microorganisms and autolytic enzymes present in the gut of ruminants. Since rough washing is the preferred method for cleaning of tripe in South Africa, the initial bacterial numbers remained high prior to boiling. Nonetheless, the long cooking kills many of the resident bacteria on tripe. Tripe requires long cooking time because it contains collagen and elastin fibres (Zarkadas *et al.,* 1996), which gives tripe a tough texture.

However, since RTE bovine tripe is a minimally processed product, cooking time was reduced to 1 h in order to maintain the desirable sensory attributes of tripe. In order to successfully reduce cooking time, washed bovine tripe was tenderised with concentrated papain (5 %) for 2 h at room temperature. Papain is a proteolytic plant enzyme used as a meat tenderizer (Lui and Tang, 2001). Papain tenderizes tripe by breaking the peptide bonds that bind collagen and elastin fibres.

Due to the reduced cooking time, it was expected that the initial high microbiological flora of tripe would persist after cooking. This was the case in this study with APC and ASC remaining high after boiling for 1 h. This can be explained by the principles of heat inactivation kinetics as affected by the initial microbiological numbers on tripe.

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

Boiling alone for 1 h is insufficient to reduce APC and ASC, which occur in very high numbers on fresh bovine tripe. These aerobic bacteria and aerobic spores show heat resistance and they proliferate rapidly to cause early spoilage of 0 kGy RTE bovine tripe.

Gamma irradiation at a target dose of 9 kGy may considerably improve the microbiological safety and quality of RTE bovine tripe and consequently increase its shelf life to at least 14 days (Phase 1) under aerobic conditions or to at least 7 days (Phase 2) under anaerobic conditions when stored at appropriate refrigeration temperatures (≤ 5 ºC). Gamma irradiation of RTE bovine tripe at a target dose of 9 kGy is sufficient to reduce inoculated *C. perfringens* ATCC 13124 spores to non-detectable levels throughout storage (7 days) in an anaerobic environment irrespective of the temperature at which the irradiated RTE tripe is stored (5 or 15 °C). Gamma irradiation also reduces APC (in an anaerobic environment) to below 2 $\log_{10}$ cfu/g, throughout 7 days of storage regardless of storage temperature. Gamma irradiation at a target dose of 9 kGy is however insufficient to reduce the aerobic spores on RTE bovine tripe to acceptable levels under aerobic conditions. Therefore, it may be unsafe for consumption when *sous-vide* method is used to process RTE bovine tripe.

Transmission electron micrographs show that irradiation inhibition of *C. perfringens* ATCC 13124 spores is manifested in their post-germination system.

Heat sensitisation of aerobic bacteria and aerobic spores to gamma irradiation is minimal in an aerobic environment as evidenced by the relatively high counts of APC and ASC that survive boiling and gamma irradiation. However, these entities loose their ability to proliferate rapidly during storage at both 5 and 15 °C. Therefore, the synergistic effect of boiling in combination with gamma irradiation is probable although it might not be very effective when used on bacterial strains that show high resistance to both heat and irradiation. Boiling prior to gamma irradiation has a synergistic effect on a pure *C. perfringens* ATCC 13124 spore culture as seen by loss of spore material, in the absence

CHAPTER 6: REFERENCES

ADAMS, D. M., 1978. Heat injury of bacterial spores. *Advances in Applied Microbiology* 23, 245-261.

ANDERSON, B. A., 1989. Composition and nutritional value of edible meat by-products. *In*: Edible Meat By-Products. Advances in Meat Research. Volume 5. Edited by A. M. Pearson and T. R. Dutson. Elsevier Science Publishers Ltd. New York. pp. 15-45.

ANDERSSON, A., GRANUM, P. E. AND RONNER, U., 1998. The adhesion of *Bacillus cereus* spores to epithelial cells might be an additional virulence mechanism. *International Journal of Food Microbiology* 39, 93-99.

ANGELOTTI, R., HAL, H. E., FOTER, M. J. AND LEWIS, K. H., 1962. Quantitation of *Clostridium perfringens* in foods. *Applied Microbiology* 10, 193-199.

ARAN, N., 2001. The effect of sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a '*sous-vide*' beef goulash under temperature abuse. *International Journal of Food Microbiology* 63, 117-123.

BEAMAN, T. C., AND GERHARDT, P., 1986. Heat resistance of bacterial spores correlated with protoplast dehydration, mineralisation and thermal adaptation. *Applied and Environmental Microbiology* 52, 1242-1246.

BEAMAN, T. C., KOSHIKAWA, T., PANKRATZ, H. S. AND GERHARDT, P., 1984. Dehydration partitioned within core protoplast accounts for heat resistance of bacterial spores. *FEMS Microbiology Letters* 24, 47-51.

CHAPTER 6: REFERENCES

ADAMS, D. M., 1978. Heat injury of bacterial spores. *Advances in Applied Microbiology* 23, 245-261.

ANDERSON, B. A., 1989. Composition and nutritional value of edible meat by-products. *In*: Edible Meat By-Products. Advances in Meat Research. Volume 5. Edited by A. M. Pearson and T. R. Dutson. Elsevier Science Publishers Ltd. New York. pp. 15-45.

ANDERSSON, A., GRANUM, P. E. AND RONNER, U., 1998. The adhesion of *Bacillus cereus* spores to epithelial cells might be an additional virulence mechanism. *International Journal of Food Microbiology* 39, 93-99.

ANGELOTTI, R., HAL, H. E., FOTER, M. J. AND LEWIS, K. H., 1962. Quantitation of *Clostridium perfringens* in foods. *Applied Microbiology* 10, 193-199.

ARAN, N., 2001. The effect of sodium lactates on growth from spores of *Bacillus cereus* and *Clostridium perfringens* in a '*sous-vide*' beef goulash under temperature abuse. *International Journal of Food Microbiology* 63, 117-123.

BEAMAN, T. C., AND GERHARDT, P., 1986. Heat resistance of bacterial spores correlated with protoplast dehydration, mineralisation and thermal adaptation. *Applied and Environmental Microbiology* 52, 1242-1246.

BEAMAN, T. C., KOSHIKAWA, T., PANKRATZ, H. S. AND GERHARDT, P., 1984. Dehydration partitioned within core protoplast accounts for heat resistance of bacterial spores. *FEMS Microbiology Letters* 24, 47-51.