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The Efficacy of Lactic Acid and Steam Vacuum Applications in Reducing Microbial Load and Prolonging the Shelf Life of Beer Carcases

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The efficacy of lactic acid and steam vacuum applications in reducing microbial load and prolonging the shelf life of beef carcases.

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ABSTRACT

Many foodborne outbreaks occur globally each year compromising consumer confidence and highlighting the need for continued improvements with regards to product safety. Therefore, intervention decontamination applications are currently gaining a lot of attention within the beef industry as an additional measure to improve the microbiological condition of finished products. Many studies have shown decontamination methods to be effective, however variations within the published literature makes scientific comparison difficult. This aim of this study is to determine the efficacy of a 2-3% lactic acid solution and steam vacuum technologies as intervention methods in reducing the microbial load and extending the shelf life of beef carcases. The decontamination methods were applied at the end of the slaughter line upon completion of carcase dressing. Samples were taken of carcases before and after treatment and microbiologically analysed. A shelf life durability study was conducted over a 9-day period on carcases treated with lactic acid, steam vacuum and untreated carcases (control). A lactic acid solution applied at 37ºC reduced ACC, E.coli and Enterobacteriaceae counts by 0.5-2.5 log. Steam vacuum reduced the aforementioned bacterial species by 0.2-1.5 log. Treated carcases chilled and stored at 0-2ºC reported a prolonged shelf-life in comparison to the control. Therefore, the use of these decontamination methods can reduce the microbial surface load improving the quality and shelf-life of the product.

DECLARATION

I hereby certify this material, which I now submit in partial fulfilment of the requirement for the award of M.Sc. in Food Safety Management, is entirely my own work and have not been taken from the work of others and to the extent such work has been cited and acknowledged within the text of my own work.

This thesis was prepared according to the guidelines for dissertation production in the M.Sc. Food Safety Management and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the Institute's guidelines for ethics in research.

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Signed: _____

Candidate

Date: _____

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ABBRREVIATIONS

ACC	Aerobic Colony Count
BPW	Buffered Peptone Water
CCP	Critical Control Point
CFU	Colony-forming unit
CT-SMAC	Sorbitol MacConkey Agar
DAFM	Department of Agriculture, Food and Marine
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPS	Exopolysaccharide
EU	European Union
FBO	Food Business Operator
FSIS	Food Safety and Inspection Services
GMP	Good Manufacturing Practices
GRAS	Generally Regarded as Safe
HACCP	Hazard Analysis and Critical Control Points
INAB	Irish National Accreditation Board
ISO	International Organization for Standardization
MRD	Maximum Recovery Diluent

MTSB	Modified Tryptone Soya Broth
PBST	Phosphate Buffered Saline
PCA	Plate Count Agar
STEC	Shiga toxin-producing Escherichia coli
TBX	Tryptone Bile Glucuronide Agar
USDA	United States Department of Agriculture
VRBGA	Violet Red Bile Glucose Agar
WHO	World Health Organisation

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SECTION 1.0: LITERATURE REVIEW

1.0 Introduction

Food business operators (FBO) are obliged under Community law to ensure products placed on the market are safe, wholesome and pose no risk to consumer health. Since the implementation of the Hygiene Package, the primary objective of legislation is to ensure food safety and a high level of protection of consumer health is achieved and maintained with the secondary aim of facilitating trade (O'Rourke, 2005). However, many foodborne outbreaks occur globally each year compromising consumer confidence and highlighting the need for continued improvements with regards to product safety. Recent figures published by the European Food Safety Authority (EFSA) reports that in 2016, a total of 4,786 foodborne outbreaks were reported. Campylobacter was the most reported zoonosis contributing to 246,307 reported human illnesses. There were also 94,530 confirmed cases of Salmonellosis, 6,378 cases of Shiga toxin-producing Escherichia coli (STEC) and 2,536 cases of Listeriosis (EFSA & ESDC, 2017).

Increased consumer awareness and concerns regarding foodborne illnesses have accelerated efforts to reduce microbial contamination of products.

FBOs are the key link between the primary producer and the final consumer and therefore play a vital role in preventing foodborne outbreaks and minimising these figures.

Although the muscle of a healthy animal is sterile, it can become contaminated with both spoilage and pathogenic microorganisms throughout the production process with the potential of posing a serious risk to both the meat industry and public health. *Pseudomonas, Acinetobacter, Enterobacter, Lactobacillus, Brochothrix Thermosphacta, Moraxella, Leuconostoc* and *Proteus* are spoilage bacteria of concern (Woraprayote et al., 2016). Spoilage bacteria usually do not pose a health risk when present. However, during consumption at high concentrations they can cause gastrointestinal problems. Where significant growth of spoilage bacteria has occurred, the proteins and lipids present

in the meat undergo degradation, impacting on the sensory characteristics of the meat product such as appearance, flavour and texture.

Pathogenic microorganisms of concern relating to meat products are *Salmonella spp.*, enterohemorrhagic *Escherichia coli* 0157:H7, *Listeria monocytogenes, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Staphylococcus aureus, Bacillus cereus* and *Yersinia enterocolitica.* (Woraprayote et al., 2016; Kotula & Kotula, 2000).

As meat products are generally foods that do not undergo a kill step at the end of processing, FBOs must ensure that all necessary measures are taken during the production process to reduce or eliminate microbiological risks posed to the products.

Global demands mean that food safety is now being placed at the forefront of the production process ensuring finished products do not pose a microbiological risk to consumer health and have the ability maintain viability for the duration of the shelf life. To achieve this, it is important to understand the phenomenon of bacterial attachment and invasion, factors affecting microbial survival and growth, microbiological contamination risks within the production process and potential measures to reduce microbiological contamination.

1.1 Microbial attachment to the meat surface

Bacterial attachment to meat is believed to occur in two stages: reversible attachment and permanent irreversible attachment (Dickson & Anderson, 1992; Firstenberg-Eden, 1981). Reversible bacterial attachment involves Van der Walls interactions, electrostatic forces and hydrophobic interactions and more active adhesion occurring later through irreversible attachment results from the anchoring of appendages and/or the production of extracellular polymers (Oliveira, 1992). Early research on bacterial attachments showed that physical forces (intrinsic and extrinsic factors) such regulated the initial reversible attachment (Houdt & Michiels, 2010).

Initial bacterial attachment to meat surfaces have been reported to occur within the first minute of contact (Butler et al, 1979). The level of attachment is believed to be influenced by the numbers present in cell concentration of a suspended media (Butler et al., 1979; Dickson,1991). The increase in bacterial attachment was directly proportional to the increase in inoculum indicating that large numbers saturate attachment sites while small numbers unable occupy all attachment sites.

While permanent irreversible attachment is regulated by the bacterial production of extracellular polysaccharides, a time-dependent process (Firstenberg-Eden, 1981).

Adhesion can be active or passive, influenced by cell motility. Butler et al. (1979) found that motile, gram-negative bacterial attachment was greater than non-motile gram-positive bacterial attachment.

Many studies have shown that irreversible attachment occurs between 20 minutes to 4 hours at temperatures ranging from 4-20ºC (Chmielewski & Frank, 2003). Once irreversible attachment has occurred and bacterial cells proliferate, surface colonisation occurs with the formation of bacterial microcolonies (biofilm) bound by an extracellular matrix known as glycocalyx (Delaquis et al., 1992). These colonies can fuse together over time to form a continuous slime layer on the meat surface, signifying advanced food spoilage. This stage would typically occur in meat products when a cell concentration has reached approximately 10^{9}cfu/cm^2.

Irreversible bacterial attachment on the surface of carcases is believed to require a contact time of 20 minutes and so Butler et al. (1979) concluded that attachment did not impact on the anti-microbial effects of hot water or organic acid treatments applied slaughterhouse level for microbiological reduction.

In a study conducted by Dickson (1990), the transfer of Listeria monocytogenes or Salmonella typhimurium decreased when the inoculum had time to absorb into the tissue

prior to coming into contacted with the second tissue. The composition of the initial meat surface was a contributory factor in the extent of bacterial attachment having a significant transfer from fat tissue with a contact time of less than one minute. Lean adipose tissues required longer contact times for greater attachment. Understanding this concept is of practical importance as carcases can be in close contact with one another during the slaughtering and chilling process, posing a potential cross-contamination risk.

1.1.1 Biofilm formation

Biofilm formation can occur as a direct result of irreversible attachment and provides protection for the microbe from hostile environments and acts as a nutrient catcher (Poulsen, 1999; Chmielewski & Frank,2003). It can also be determined by the nature of the attachment surface, the inherent characteristics of the bacterial cell and environmental stresses (Houdt & Michiels, 2010). Both spoilage and pathogenic microorganisms can form biofilms under suitable conditions. Some bacteria have a higher tendency for forming biofilms such as Enterobacter, Pseudomonas, Staphylococcus, Bacillus, Flavbacterium and Alcaligenes. One defining characteristic of microbial biofilms is the formation of an exopolysaccharide (EPS) matrix (Poulsen, 1999). Biofilm formation increases the risk of cross-contamination in the production process, impacting negatively on shelf life and foodborne disease transmission.

1.2 Factors affecting microbial growth

The chemical, physical and biological properties of food type can promote or inhibit the growth of specific micro-organisms. Therefore, it is important to understand the inherent properties beef carcases (intrinsic factors) and environmental influences (extrinsic factors) that dictate the level of survival and growth of microorganisms.

Meat products, particularly fresh meat have inherent intrinsic factors that are favourable for supporting microbial growth including, high water activity value, abundance of

proteins and essential nutrients and pH level (FSAI, 2010). However, extrinsic factors have the greatest influence on the contamination level detected on carcases.

1.3 Potential sources of microbiological contamination in the beef industry

1.3.1 Cattle as a source of contamination

The exposure risk starts at farm level through geographic and seasoning effects, sampling and isolation methodology, age of animals and farming and husbandry hygiene practices (FSAI, 2010). Both Salmonella and STEC are naturally occurring pathogens present within the gastrointestinal tract of cattle and are also shed in the faecal matter of carriers (FSAI, 2013). It has been reported that high levels of E. coli 0157 are shed by carrier animals during the summer months (Ogden et al, 2004). Young cattle aged between 3-24 months are the most dominant reservoir for E. coli 0157 (EFSA, 2009). Animals outside this age bracket are believed to be less likely to excrete the pathogen. The prevalence of the pathogen in faecal matter in livestock varied from 0-48.8%. The number of E. coli 0157:H7 micro-organisms being shed in faecal matter of individual animals is important in the context of environmental, hide and carcase contamination (FSAI, 2010). Salmonella is also naturally prevalent within nature and can be found within the intestinal tract of both domesticated and wild animals, which result in multiple potential Salmonella infection sources (Carrasco, et al., 2012). The main transmission routes of Salmonella are contaminated meat products with faecal matter.

Sterile meat carcases can become contaminated during carcases dressing with STEC, Salmonella and also Staphylococcus aureus, Listeria monocytogenes, Campylobacter and Clostridium perfringens being reported (Tompkin et al., 2001). Carcases are reported to typically contain between 1.0 to 4.0 log microorganisms (James & James, 1998). However, when the meat is minced, the microorganisms present of the surface become mixed throughout the product, increasing the surface area of contamination (FSAI, 2010).

Therefore, reducing the risk of microbiological contamination to the carcase surface is paramount in ensuring safety and quality of final packed product, particularly minced meat products.

1.3.2 Hide hygiene

The term hide refers to the skin of cattle as removed at slaughterhouse (Ford et al., 2012). Cattle hides can contain a high microbial load and have been identified as a primary source of microbiological cross contamination during dressing. (Loretz et al., 2011). Microbial hide contamination can range from 10^4 to 10^9 cfu/cm^2 depending on the site of the carcase sampled. The bacterial counts obtained from carcases after hide removal have been correlated with the load present on the hide (Loretz et al, 2011). The visual cleanliness of cattle presented for slaughter have been also correlated to hide and carcase contamination through microbiological testing (Serraino et al., 2012). Increasing visible contamination load on the hides of live animals were associated with higher microbial aerobic colony counts, Enterobacteriaceae and generic E. coli on both hide and carcase. Potential sources of hide contamination are faeces, feedstuff, water and soil harbouring pathogenic micro-organisms such as E. coli 0157:H7, Campylobacter spp., Salmonella spp., and Listeria mono (Serraino et al., 2012).

It was reported that the prevalence of E. coli 0157:H7 on hides in abattoirs was higher than that of faecal and carcase prevalence with a reported increase of <4.5% in these matrices to 7.3-22.2% prevalence of hides (EFSA, 2009). Therefore, the hide is likely to be a more important pathogen contamination risk than faeces.

It is imperative that the necessary steps are taken at slaughterhouse level to ensure compliance with the condition outlined in Regulation EC 853/2004 ensuring that animals must be clean. Controls currently in place with regards to hide hygiene are the categorisation of animals being presented for slaughter based on visible hide hygiene

through the implementation of a Clean Livestock Policy and on-line hide clipping. However, Small et al. (2005) reported that no reductions in aerobic bacteria and may facilitate the formation of dust and potential spread of bacteria. Baird et al. (2006) reported bacterial reductions on clipped hides using physical and chemical treatments. However, chemical treatments are not used commercially for the treatment of hides at slaughterhouse level in Ireland.

1.3.3 Understanding the process flow

Figure 1 represents a typical example of a process flow within an abattoir. There are many operational steps involved in the loosing of the hide away from the carcase, facilitating hide removal and the sealing of the rectum (bunging) and oesophagus (rodding) to minimise the risk of rupturing or puncturing the intestinal tract, a significant source of enteric bacteria, during the evisceration process.

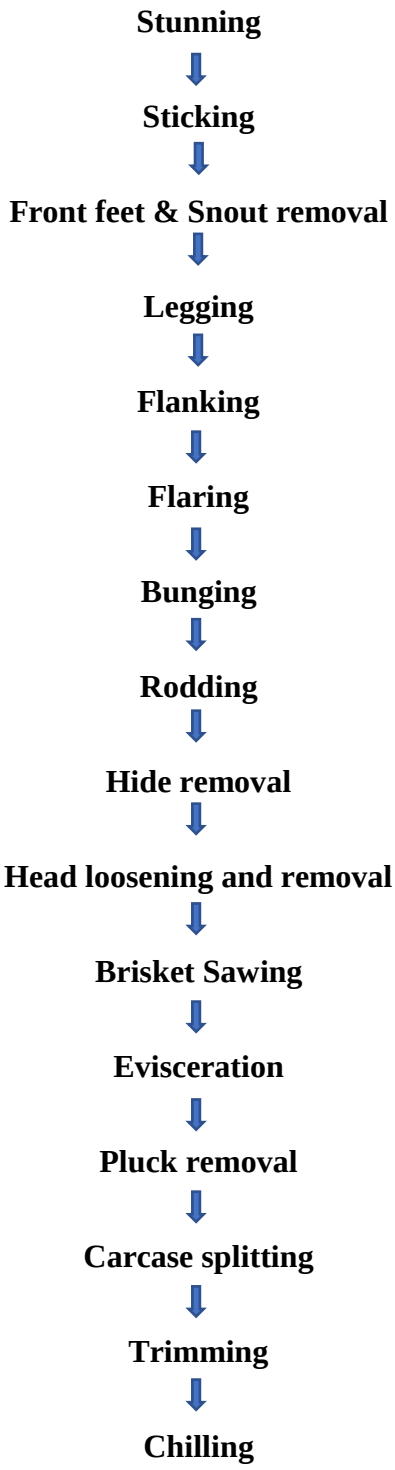

Figure 1. 1: Typical process flow for beef carcase dressing

1.3.4 Cross Contamination

The term cross contamination has been defined by Perez-Rodriguez et al. (2008) as "a general term which refers to the transfer, direct or indirect, of bacteria or virus from a contamination product to a non-contaminated product." The World Health Organisation

(WHO) (1992) reported that 25% of foodborne outbreaks are closely related to cross-contamination issues. Sources of contamination include poor hygiene practices, contamination by food handlers, contaminated equipment, poor process flow/ design or inadequate storage, inappropriate line speed and direct carcase to carcase contact (Huffman,2002). But generally, contamination occurs in slaughterhouses because of poor operational hygiene and handling (Bakhtiary et al., 2016).

In an abattoir, cross-contamination during dressing can result in the transfer of both spoilage and pathogenic bacteria to the carcases surface through faecal matter originating from the hide or gut spillages; hide removal or evisceration (figure 1.1) (FSAI,2010).

Bell (1997) reported that contact between carcases and an operative's unrinsed hands could introduce microbiological contamination consistent with hide to carcase contact. Hand rinsing between carcases removed 90% of the hide-derived bacterial load.

Rinsing and sterilising knives and equipment between carcases in water <82ºC significantly reduced the cross-contamination risk of knives making external incisions, cutting though the hide. (Bell, 1997).

Inadequate cleaning of the slaughter line and food contact equipment and surfaces could lead to the formation of biofilms, increasing the risk of cross-contamination (Houdt & Michiels, 2010).

The best strategy for minimising microbiological contamination of beef carcases is based on the implementation applications that aim to:

- Reduce the sources, levels and transfer of contamination on the animal and in the production process
- Reduce contamination present on animals before slaughter
- Minimise microbial transfer to carcase surfaces

- Reduce the prevalence of pathogens and bacterial load through the implementation of decontamination intervention technologies (Sofos, 2009, Sofos & Geornaras, 2010)

1.4 Intervention methods for carcase surface decontamination

The responsibility falls on the FBO to assess intervention methods as part of their HACCP hazard analysis and risk assessment. Microbiological sampling of beef carcases is required prior chilling as stipulated in legislation (EC) 2073/2005 with any subsequent steps not allowing for a decrease in the microbial quality resulting potential negative impacts on public health.

Decontamination technologies are currently gaining a lot of attention as effective intervention methods for reducing the microbiological contamination present on beef carcases. However, it is important to understand that intervention methods for decontamination cause a relative reduction in the microbial load, not complete elimination and its effectiveness is dependent on the type and initial level of microbial contamination (Hugas & Tsigarida, 2008). It has been stressed through both legislation and research papers that intervention methods should be an additional measure and not compensation for the primary focus of good plant design, effective cleaning programmes, good hygiene practices, implementation of an effective HACCP plan and appropriate speed lines (Sofos & Smith 1998, Hugas & Tsigirida 2008, Loretz et al. 2010, 2011).

When applied correctly, decontamination technologies can effectively reduce the overall microbiological count present on a beef carcase by 1-3 log cfu/cm^2 and a reduction in the prevalence of pathogens (Sofos & Smith, 1998).

Decontamination methods used as intervention technologies can be divided into three main groups: physical, chemical and biological. Each type of method has its own advantages and disadvantages. Different methods can also be used in combination with one another. Some intervention methods would be regarded as traditional such as carcase

washing while others are emerging with research ongoing such as bacteriophages or lysins of phages (Hugas & Tsigarida, 2008).

1.5 Regulatory requirements

Due to many food crises that have occurred over many decades in conjunction with international trade, both community and national legislation and subsequent decision-making practices have been made based on the concept of risk analysis (Hugas & Tsigarida, 2008). Risk analysis consists of three interlinking components; risk assessment, risk management and risk communication. The main objective of risk analysis is to ensure the highest level of consumer production and to facilitate international trade.

The use of specific decontamination technologies to improve the microbiological condition of the carcases must comply with the requirement outlined in the relevant legislation.

1.5.1 European Legislation

EU regulation (EC) 853/2004, specifically Article 3(2), stipulates that the use of any other substance other than potable water to remove surface contamination from carcases is prohibited. Approval of other substances must undergo scientific risk assessment by the European Food Safety Authority (EFSA) which must be subsequently endorsed by the European Parliament. EFSA's risk assessment and scientific opinion has greatly influenced the development of new legislation for the use of certain chemical washes.

Subsequently, the use of lactic acid as a surface decontaminant for beef carcases to reduce microbiological activity is addressed under European legislation (EU) No 101/2013. It outlines the conditions whereby lactic acid can be used to treat beef carcases at a concentration of 2-5% in a potable water solution at temperatures not exceeding 55ºC.

The use of lactic acid on beef carcases must be supported by an effective HACCP system and comply with the food Hygiene Package legislation. Legislation also outlines the need

for good hygiene practices to be implemented and maintained. Therefore, lactic acid treatment is only permitted where is there is no evidence of faecal contamination present on the carcase and GMPs have been maintained. The use of lactic acid as a surface decontaminant within the EU meat industry is only approved for its application on intact carcases, half carcases or quarters of meat from bovine animals at abattoir level.

Regulation (EC) No 1333/2008 stipulates the regulatory requirements for food additives within the European Union. Lactic acid has a food additive registration number of E270 and must comply with legislative requirements for food additives, ensuring the treatment will pose no safety concern (EFSA, 2011). Food additives are referred to as substances that are not consumed as a food itself but are intentionally added to foods where it or its by-products become components of the food (Woraprayote et al., 2016).

Therefore, lactic acid treatment for beef carcases must comply with all the criteria outlined in European Legislation, ensuring it is safe for use, has a technological purpose and must benefit the consumer.

1.5.2 U.S. legislation

In 2002, the USDA issued a Directive concerning the reassessment of HACCP plans in beef slaughter sites. It addresses the requirement for the implementation of a Critical Control Point for zero visible faecal, ingesta and milk contamination on carcases. It also stipulates that if E. coli 0157:H7 is a likely hazard at slaughter, a "validated intervention" method is necessary in the slaughter process and must operate as a CCP (Buege & Ingham, 2003). For the "validated method" to be accepted, there must be sufficient scientific evidence available to show that the intervention method can reduce the likelihood of E. coli 0157:H7 contamination on beef carcases.

The use of organic compounds for microbiological decontamination purposes within the food industry are approved by the United States Food Safety Inspection Service (US-FSIS). There are specific criteria that chemicals must adhere to in order to gain approval:

- They must be generally recognised as safe (GRAS)
- They must not attribute to adulteration
- They must not create issues regarding product labelling
- They must not cause health problems to operators or consumers. (Woraprayote, 2016)

21 Code of Federal Regulations stipulates the approved application of antimicrobial agents for use in meat, poultry and egg products. Lactic acid as an antimicrobial agent has been authorised for the use on a wide range of products from carcases, beef and pork sub-primals and trimmings, beef heads and tongues and poultry carcases, meats, trim, parts and giblets (USDA-FSIS, 2018).

1.5.3 Eligibility requirements for the export of beef to the U.S market

Many third countries have their own eligibility requirement that must be complied to for gaining approval for export to their international market. FBOs eligible for exporting beef and pork products to the United States must demonstrate that their food safety management system is equivalent to the U.S standard, with the ability to supply meat products that are safe, wholesome and unadulterated complying correct labelling and packaging requirements (USDA-FSIS,2017). This is achieved through meeting the following protocols:

- FSIS requirements on sanitation requirements- 9 CFR Part 416
- HACCP regulatory requirements of – 9 CFR Part 417
- Approved beef establishments must comply with FSIS Directive 6420.2 through implementation of a CCP within the slaughterhouse for zero tolerance for visible faecal, ingest and milk contamination along with the necessary on-line decontamination measures to achieve this.

1.6 Physical decontamination technologies

Many physical decontamination technologies have been developed to reduce the bacterial contamination on the surface of beef carcases. Such methods include carcase trimming, carcase washing, steam pasteurisation, steam vacuuming, freezing, electromagnetic and ionising radiation.

1.6.1 Carcase trimming

In the U.S, it is a legal requirement for visible contamination such as faecal, ingesta or milk be removed from the beef carcases by knife trimming (USDA-FSIS, 1996). However, visible contamination may not signify an area that is heavily contaminated with bacteria (Gill & Gill, 2012). Therefore, hand-trimming may only remove bacteria from a small site with little impact on the overall microbiological status of the carcase. There are some conflicting research literature surrounding the effectiveness of trimming to reduce the microbial load. Gorman et al. (1995) reported microbial reductions for trimming of beef briskets, however a substantial amount of contamination remained on the sample. The large variability in the bacterial counts for trimming treatments highlights the risk of cross-contamination during the process when this method is used. While another study conducted by Gill et al (1995) concluded that neither trimming nor carcase washing at a temperature of 40ºC are effective at reducing the initial microbial load present on carcases at industrial level.

It is important to note that studies conducted at laboratory level show a reduction in the microbiological count in relation to trimming as a decontamination method. However, it may not be an accurate reflection on practices at industrial level which show that hand-trimming of carcase wash have little to no effect on the overall microbiological count present on the carcase (Gill et al., 1996).

The efficacy of hand-trimming as a decontamination method may be improved if applied to sites that have a high probability of microbiological contamination, irrespective of the

presence of visible contamination (Gill & Gill, 2012). However, there is currently no scientific evidence available to support this hypothesis.

1.6.2 Water treatments

Washing beef carcases with water is routinely carried out in slaughterhouses for the removal of visible contamination (Hugas & Tsigarida, 2008). In the U.S, water treatments at temperatures greater than 74ºC on beef carcases is widely practiced as endorsed by the USDA-FSIS (1996).

However, the effectiveness of reducing the microbial load by means of carcases washing/spraying is dependent on carcase coverage, the temperature of the water being applied, water pressure and dwell time (Huffman, 2002; Sofos & Smith, 1998).

A hot water spray at a temperature of 95ºC to beef carcases reduced E. coli 0157:H7 and Salmonella typhimurium by 3.7 and 3.8 log respectively, ACC by 2.9 log and coliforms by 3.3 log (Castillo, et al., 1998). Data obtained from this study also showed that spreading of contamination may occur where visible contamination has been washed with water. However, treatment with hot water significantly reduced pathogen count to close to or below the detectable level of 0.5log/cm^2.

A study conducted by Bosilevac et al. (2006) showed that the application of hot water of 74 ºC for 5.5 seconds to beef carcases at pre-evisceration using a hot water wash cabinet showed a reduction in E. coli 0157:H7 prevalence by 81% and a 2.7log reduction on both ACC and Enterobacteriaceae.

Research studies on different tissue types and following different methodology in evaluating the efficacy of water treatments generally showed a bacterial reduction of 1-3 log (Hugas & Tsigarida, 2008).

Cold water (10-15ºC) and warm water (10-40ºC) are effective at removing physical contamination such as blood clots and bone dust, cosmetically improving the appearance of the carcase, however such treatments do not cause reductions in bacterial counts

(Bolten et al., 2001). Not only does water temperature impact on the effectiveness of the decontamination method but also the type of tissue it is being applied to. Eggenberger-Solorzano et al. (2002) found that pork muscle tissue washed with hot water ranging from 65-80ºC resulted in reductions in the Enterobacteriaceae count, while there was no observable effect on the population of pork skin. Therefore, the point during processing where the water treatment is applied to the beef carcases impacts on the efficacy of the decontamination method.

The use of hot carcase washes/sprays may have some disadvantages if the appropriate validated and verified methodology is not applied correctly. High pressures could cause penetration of bacteria into the muscle tissue (Sofos & Smith, 1998). Washing faecal contamination may spread the microbial load across the surface of the carcase or contaminate equipment (Hugas &Tsigarida, 2008; Castillo et al, 1998). Also, increased tissue surface moisture because of water application may promote the growth of bacteria (Hugas & Tsigarida, 2008).

Hot water treatments need to be applied in a manner that will prevent or limit temperature decrease by evaporation (Gill & Gill, 2012). Hot water spray applications can cool quickly due to large surface area relating to droplets (Davey, 1989).

There was also concern within the meat industry on the effect of hot water application on carcase discolouration. Several authors reported that applying hot water treatments of greater than 80ºC did not cause permanent discolouration (Huffman, 2002).

1.6.3 Steam vacuum

Steam vacuum, a combination of physical and thermal treatment, is a variation of steam pasteurisation. Steam is applied to beef carcases followed by vacuuming with the combination effect of removing visible contamination and/or inactivation microbiological contamination present on the carcase surface (Huffman, 2002). Steam vacuum is approved by the USDA-FSIS as a decontamination method and can be used as

an alternative to hand-trimming where contamination at its greatest dimension is less than 2.54cm (USDA-FSIS, 1996).

A typical steam vacuum will include a vacuum to remove visible contamination and a hot water application nozzle, delivering steam to the carcase surface (Huffman, 2002; Dickson & Acuff, 2017). Steam vacuuming is most effective at treating small areas that are highly likely to be contaminated and for the spot treatment of visible contamination (Dickson & Acuff, 2017; Hockreutener et al., 2017). The effectiveness of steam-vacuuming is dependent on several factors including operational practices, steam temperature, application time, the carcase area treated, the level of contamination present on the carcase and the location along the process flow where steam vacuuming is applied (Hockreutener et al., 2017). The main advantage of using steam in comparison to water treatments is that when applied at 100ºC, it has a greater heat capacity (Hugas & Tsigaridia, 2008). Hockreutener et al. (2017) conducted a study of the effectiveness of steam vacuuming on beef carcases at commercial level. A reduction in the mean value of the initial contamination of 0.4-0.9 log cfu/cm^2 was reported.

Kochevar et al. (1997) also reported reductions in ACCs and coliforms of 1.73-2.13 log cfu/cm^2 on carcases where visible contamination was treated with steam vacuum. The microbial reductions noted on treated carcases with no signs of visible contamination ranged between 0.26-0.72 log cfu/cm^2. The reported results were influence by the initial microbial load present on the untreated carcase surface. (Kochevar, et al., 1997).

James et al. (2007) reported steam treatments for up to 20 seconds to poultry carcases reduced numbers of Campylobacter jejuni and E. coli, however damage to the appearance of the carcase occurred. However, one author reported that vacuum treatment with or without hot water application may be ineffective at reducing the microbial load of carcases (Gill, 2009).

The limitation of the effectiveness of steam vacuuming as a spot treatment method could be improved by identifying the carcase surface areas where prevalence of bacteria of interest is high and apply the intervention method to the identified area. Rekow et al. (2011) identified the areas where prevalence of E. coli 0157: H7 was high as the foreshank, the hindshank and the inside rounds. After application of the intervention method, the prevalence of E. coli 0157:H7 was reduced on the foreshanks, hindshanks and inside rounds from 21.7% to 3.1%, 24.2% to 11.5% and from 37.5% to 16.7% respectively (Rekow et al., 2011)..

For steam treatments to work affective as a decontamination method, the carcase surface should be as clean as possible with minimal visible contamination and must be dry (Gill & Gill, 2012). If the surface is not dry, it may prevent the carcase surface from reaching the necessary temperature to allow for rapid inactivation of bacteria.

A study was also conducted on the use of steam vacuum on beef after chilling to enhance the microbiological condition (Bacon et al., 2002). It was concluded that application after carcase chilling did not reduce or eliminate inoculated Salmonella microorganisms. The ineffectiveness of steam vacuuming at this point in the process flow may be attributed to irreversible bacterial attachment.

1.6.4 Other physical decontamination technologies.

Other emerging methods of physical interventions include the use of antimicrobial active packaging to improve product shelf-life. Many different preservatives with antimicrobial properties have been incorporated into packaging including organic acids, chlorine dioxide, plant extracts, lysosome and silver- substituted zeolite (Castellano et al., 2017). Stratakos & Grant (2018) reported a reduction of E. coli counts of 0.6 log after 3 days storage of beef products vacuum packaging in antimicrobial polyethylene terephthalate packaging incorporating silver nanoparticles. The counts remained stable for the duration

of the 7-day storage. This could be improved by adding additional hurdles to improve efficacy on microbiological condition. (Stratakos & Grant, 2018; Stratakos et al., 2015).

Irradiation is a physical treatment where food products are exposed to a defined dose of ionising radiation to inactivate pathogenic and spoilage bacteria (Loaharanu, 2007). EFSA concluded that based on the scientific evidence provided, there was not microbiological risk or immediate toxicological risk to consumers of irradiated food (EFSA, 2011b). However, it may alter sensory characteristics of the product including colour, taste and odour. EFSA recommended that further research is needed. Irradiation along with other emerging technologies including high hydrostatic pressure, nanotechnology, ozone and light pulses require further research and have different degrees of consumer acceptability.

1.6.5 Summary of physical decontamination technologies

Of all the physical decontamination treatments available for use of beef carcases, water-based treatments at the end of the slaughter line were most widely used with efficacy depended on temperature, application pressure, exposure time and initial microbial load. These decontamination treatments cause direct removal of bacteria combined with heat activation (Loretz, et al., 2011). Cold and warm water applications were not as effective which may be attributed to heat inactivation not being achieved. These applications also tend to cause the spread of bacteria to other carcase surface areas.

1.7 Chemical decontamination technologies

1.7.1 Organic Acids

The use of organic acids is the most extensively studied and widely used chemical intervention method within the meat industry as they are inexpensive and effective at improving the microbiological condition of products (Rajkovic et al., 2010). Many studies have involved the treatment of whole carcases, parts of beef carcases or primal cuts with acetic, citric or lactic acid on their own or in combination (Gill & Gill, 2012). Research

studies have also looked at the effectiveness of chemical decontamination methods at different points during the process flow; pre-evisceration, after carcases dressing but prior chilling or after carcase chilling. However, it is widely accepted that organic acids are most effective when applied after hide removal and when the carcase is still warm (Huffman, 2002).

The use of organic acids as an intervention method are believed to be more effective against gram-negative bacteria than gram-positive bacteria (Rajkovic et al., 2010). However, E. coli 0157:H7 shows high acid resistance to organic acid treatment. In a study conducted by Raftari et al. (2009), great log reductions were reported on Staphylococcus aureus than E. coli 0157:H7 signifying a higher susceptibility by the gram-positive bacteria.

Weak organic acids are lipid permeable and can inactivate bacterial cells by penetrating through the cell membrane and disassociating within the internal compartment of the cell (Booth, 1985). This results in a decrease of the intracellular pH which is vital for physiological functions of the cell such as RNA and protein synthesis, DNA replication, ATP synthesis and cell growth. However, further studies have shown that the pH change cannot be the sole attribute for bacterial inactivation. One possibility is that perturbation of the membrane function may also play a role or another is that disassociation causes a high concentration of intracellular anions which may cause an increase in osmolarity and contribute to the cell metabolic perturbation (Hirshfield et al., 2003).

However, there have been concerns raised regarding possible development of bacterial resistance as a result of continued exposure to sub-lethal decontamination methods. The ability for bacterial strains to adapt to acid exposure may facilitate longer survival within the treated food product and allow better chances of survival during movement through the stomach gastric acid barrier (Hirshfield et al., 2003). In a study conducted at

laboratory level, it was reported that selection for acid adaptation was bacterial strain and bactericidal treatment dependent (Rajkovic et al., 2009). Repeated exposure to lactic acid treatment during a 20-cycle exposure test resulted in listeria monocytogenes culture having a higher resistance that the parental strain.

Some research also shows that organic acids such as lactic acid or acetic acid have been ineffective at reducing the microbial load of beef carcases. Gill and Launders (2003) reported that an application of 2% lactic acid on washed beef carcases did not reduce the bacterial counts. Another study reported that beef carcases that underwent hot spray washing followed a 2% lactic acid application did not further improve carcase hygiene than hot water alone (Bosilevac et al., 2006). However, the ineffectiveness noted in these studies may have been influenced by the prior application of water to the carcases which subsequently diluted the concentration of the organic acids to a sublethal level (Gill & Gill, 2012).

1.7.2 Efficacy of Organic Acids

Acetic and lactic acid are common organic acid treatments used in the U.S and Canada as part of an integrated food safety management system to improve the microbiological condition of carcases. Cutter et al. (1997) found that a 2% acetic acid application to beef carcase tissue reduced the level of E. coli 0157:H7 from 7.0 to 2.51 log cfu/cm^2 and from 5.0 to 0.3 log cfu/cm^2. They also reported that the initial bacterial count affects the efficacy of the solution depicted through these results. Anderson et al. (1977) found that the treatment of beef strips with a 3% acetic acid solution reduced bacterial counts by 2.55 log. A laboratory-based study was conducted to determine the efficacy of acetic acid, formic acid and a combination of acetic, formic and propionic acids on microorganisms isolated from beef (Quartey- Papafio et al., 1980). Results obtained showed that all treatments reduced bacterial counts, but reductions were generally less than one log cfu/cm^2. 2% formic acid showed the largest log reduction (1.56 log), followed by 3%

acetic acid (0.89 log). Analysis of samples after a 7-day storage period at 7ºC showed increases in the microbial counts of the treated carcases of between 0.92 to 2.24 log compared to the control with growth of 4.66 log (Quartey-Papafio et al., 1980).

Raftari et al. (2009) conducted spray wash treatments on meat tissues using varying concentrations (1, 1.5 and 2%) of acetic, lactic, formic and propionic acids to evaluate their efficacies in reducing microbial counts of E. coli 0157 and S. aureus. It was concluded from the study that the use of organic acids as surface decontaminants are effective at reducing microbial load. The selected bacteria were sensitive to all applied treatments showing a reduction in bacterial counts.

The implementation of an intervention method utilising organic acids needs to be tightly controlled in relation to variables such as application method, pressure, temperature, organic acid concentration, interval time between carcase washing and application to ensure that microbial reduction is achieved (EFSA, 2011a; Signorini et al., 2018). Organic acids have been reported to be more effective when applied at temperatures between 50-55ºC (Acuff, 2005; Pipek et al., 2004).

1.7.3 Lactic Acid

1.7.3.1 Physiological reaction involving lactic acid

Lactate is an endogenous component of carbohydrates and amino acid metabolism and is naturally occurring in many food types such as fruits and fermented products (EFSA, 2011a). Where high energy expenditure in humans occur, skeletal muscles convert glucose to lactic acid under anaerobic conditions which is subsequently released into the bloodstream. The liver then reduces the lactic acid present within the blood back to glucose. Sequentially, any absorbed lactic acid will undergo oxidisation to form water and carbon dioxide. It is also a natural meat component produced during glycolysis at post-mortem (EFSA, 2011a; Pipek et al., 2004).

1.7.3.2 EFSA Risk Assessment outcomes

EFSA conducted a scientific risk assessment on the safety and efficacy of lactic acid for the reduction of microbial contamination on the surface of beef carcases, cuts and trimmings. The study considered treatments of lactic acid solutions at 2% to 5% at temperatures up to 55ºC applied by either misting or spraying (EFSA,2011a). It concluded that lactic acid treatment posed no safety concern where the solution complied with the European requirements for food additives. In accordance with HACCP principles, EFSA also recommended that food business operators verify lactic acid concentration, application temperature and other factors affecting efficacy of the decontamination system and validate the efficacy of microbiological reduction based on the processing conditions applied.

The amount of lactic acid absorption due to lactic acid treatment may be estimated to be approximately 50-190mg/kg bovine meat, corresponding to a daily intake of up to 650 microgram of residual lactic acid/kg body weight/day in a consumer with a high meat intake (EFSA, 2011a). EFSA concluded that based on this scientific evidence, the potential increase in lactic acid based on consumption of treated product would be negligible as lactic acid is an endogenous substance and also given the low level of exposure as a result of treatment.

EFSA guidance document also concluded that any reduction in the microbial load of pathogenic microorganisms because of decontamination treatment is statistically significant in comparison to controls. Risk assessments conducted by EFSA on other microbial species have shown that a reduction of 0.5 $\log_{10}$ unit can reduce risk to consumer health significantly (EFSA ,2010; EFSA, 2011c).

1.7.3.3 Application of lactic acid in the meat industry

There are many published scientific reports showing that the use of lactic acid is an effective intervention method for microbial decontamination due to it bactericidal

properties and residual inhibitory effects, extending the shelf life of the product and enhancing food safety. It also acts by decreasing the pH of the treated carcase. Scientific research has reported that lactic acid treatment prolonged the log phase by one day (Pipek et al, 2004; Rodriguez-Melcon et al., 2017). It is important to understand that variation in the application conditions of lactic acid can impact on the efficacy of the lactic acid treatment system (EFSA, 2011a).

A study conducted by Pipek et al., (2004) evaluated the efficacy of surface decontamination of beef carcases using a spraying system with a 2% solution of lactic acid. It was found to be effective, reducing the surface microbial load by one to three decimal orders of cfu. A comparison study was carried out by Pipek et al. (2004) in relation to the temperature at which the lactic acid solution is applied. It was proved that the effectiveness of lactic acid in reducing the microbial surface count is higher at warmer temperatures (45ºC) compared to a colder temperature of 15ºC.

It was also reported that lactic acid-treated beef carcases had reduced weight losses during chilling and storage of between 0.3-0.6% in comparison to the control treated with water (Pipek et al., 2004). This occurs as a result of changes in the protein structure (denaturation) on the carcase surface and leads to pore closure, reducing the amount of water evaporating from the meat surface.

Signorini et at. (2018) evaluated nine chemical decontamination methods against STEC contamination present on beef carcases. Automated application was more effective than manual as it guaranteed full coverage during application and at suitable volumes. (Signorini, et al., 2018). 2% lactic acid treatment reduced *stx* gene prevalence from 20.7 to 6.1% and *eae* gene prevalence from 16.4% to 3.6%. Similarly, 3% lactic acid treatment reduced *stx* gene prevalence from 72.6% to 43.3% and *eae* gene prevalence from 58% to 26%.

Ransom et al. (2003) reported a 3.3 log reduction of E. coli 0157:H7 presence on beef carcases when treated with a 2% lactic acid solution and a reduction of 1.6 log when treated with 2% acetic acid.

Although a lactic acid concentration of between 2-5% is allowed as per EU legislation, higher concentrations have been reported to cause unwanted discolouration on the surface of carcases (Bolten et al., 2001; Rodriguez-Melcon et al., 2017). Pipek et al. (2005) observed the colour changes when using steam and lactic acid at a concentration of 2%. Reported subtle colour changes were reported after decontamination where the haem pigments slightly changed from the reduced form to the oxy-form. This was attributed to the application of high temperature and a decrease in pH due to denaturation of the surface layers. The surface lightness increased slightly after treatment, but no further changes were reported during storage. Redness was also reduced after treatment but increased on all samples during storage (Pipek et al., 2005).

According to EFSA (2011a) a decontaminating agent is effective when a reduction in microbial load of pathogenic or indicator microorganisms is statistically different when compared to controls. An efficient lactic acid should establish an acid concentration that should fulfil this requirement without adversely impacting on the quality attributes of the product (Rodrigues-Melcon et al., 2017).

1.7.4 Summary of chemical decontamination technologies

Currently in Europe, lactic acid is the only approved decontamination method for the treatment of bovine carcases. Organic acids are effective at reducing the overall microbial load by between one to three log. The bactericidal effects of chemical treatment are believed to be attributed to disruption to the cell membrane, intracellular components and physiological processes. Lactic acid also exhibits residual protection against later microbial growth. Studies conducted at commercial level to determine the efficacy of acetic and lactic acids reported reductions below 1.6 orders of magnitude with results

being influenced by the stage of application on the slaughter line (Loretz et al., 2011). Higher reductions were observed on inoculated carcases ranging between 2-3 orders of magnitude. (Loretz et al., 2011). Consideration needs to be given in relation to the application concentration ensuring microbial reduction is achieved without adversely impacting on the quality attributes of beef carcases.

1.8 Biological decontamination technologies

Some bacteria have the ability to produce bacteriocins, anti-microbial compounds, which exhibit bacteriostatic or lethal effects on other microorganisms (Hugas & Tsigarida, 2008).

Lactoferrin, a natural occurring iron binding protein, has the potential to be an effective antimicrobial. The USDA-FSIS has accepted the use of an "activated lactoferrin" for the use on beef product (Huffman, 2002). Naidu (2002) reported it can be effective when applied as a spray to whole carcases or on primal cuts and have demonstrated efficacy against many different pathogenic micro-organisms.

Ecoshield is a bacteriophage technology commercially available for the reduction and control of E.coli 0157:H7 growth. Stratakos et al. (2018) reported a reduction in E.coli counts by 0.63 log after 24 hours in meat samples stored below 4ºC with an overall reduction of 1.53 log at the end of a 7-day storage test. Bacteriophages are strain specific and tend not to interfere with the natural microflora present (Greer, 2005). They are easy to apply and do not cause unwanted organoleptic changes and are able to survive under commercial production conditions (Hugas & Tsigarida, 2008).

Biological decontamination methods require further research with many studies being conducted at laboratory level. Concerns remain regarding resistance, threshold levels, environmental factors and food barriers (Loretz et al, 2010; Hugas & Tsigirida, 2008).

1.9 Hurdle Technology

The objective of the hurdle concept is to control food safety and spoilage, ensuring that intrinsic and extrinsic factors are investigated and appropriate controls are implemented within the production process to prevent the growth of micro-organisms (Wareing, 2011). The industry is moving away from the idea of controlling one specific factor beyond the tolerance levels of a targeted micro-organism. By implementing small barriers addressing the various factors, the micro-organisms do not have the ability to overcome the small hurdles and subsequent growth is prevented. This concept is similar to the "decimal reduction time", outlining the temperature and time required to reduced 90% of a targeted microbial population within a specific food type, and it is affected by intrinsic and extrinsic factors such as pH, Aw, nutritional content, size of microbial population etc. (Laury et al, 2009).

By applying an intervention method, specific conditions are applied to the surface of the carcases affecting bacterial growth and survival by altering intrinsic and extrinsic factors. Steam application involves the application of temperature greater than 100ºC in activating bacteria. (Hugas & Tsigarida, 2008). The use of lactic acid causes changes to the surface pH and causes residual protection effects. (Rodriguez-Melcon et al., 2017). After slaughter, carcase storage and chilling is another hurdle bacteria would have to overcome (Castellano, et al., 2017). The mesophilic and psychotropic bacteria are selected during the chilling process with inhibition of mesophilic growth, while psychotropic bacterial will predominate. As most pathogenic bacteria are mesophilic, chilled meat undergoing good hygiene practices would not be expected to pose a microbiological risk.

1.10Research Limitations

There has been a large variation in the methodology and sample size (table 1.1) when comparing research literatures which have influence the experimental outcomes. Loretz et al. (2011) highlighted that many studies have been conducted at laboratory level and may not be reflective of the results achieved at commercial level.

Research study	Scale of research (laboratory/slaughterhouse)	Inoculation of carcases (Yes/No)	Total Sample size
Bacon et al.,2000	Slaughterhouse	No	960
Ramson et al., 2003	Laboratory	Yes	132
Bosilevac et al., 2003	Slaughterhouse	No	256
Castillo et al., 1997	Laboratory	Yes	Undefined
Cutter et al., 1997	Laboratory	Yes	Undefined
Gill & Launders, 2003	Slaughterhouse	No	50
Gorman et al., 1995	Laboratory	Yes	9
Hockreutener et al., 2017	Slaughterhouse	No	105
Kochevar et al., 199	Slaughterhouse	No	Undefined
Pipek et al., 2004	Slaughterhouse	No	Undefined
Quartey-Papafio et al., 1980	Laboratory	Yes	Undefined
Rekow et al., 2011	Slaughterhouse	No	Undefined

Table 1. 1: Decontamination studies conducted on carcases.

Greig et al., (2012) reported a lack of large controlled trials and relevancy in intervention published research literature. They noted a lack of methodology reporting with variations in temperature, application and duration, application settings, sampling methodology and reporting of actual results rather than a point estimate.

They also concluded that the industry may possess intervention efficacy data achieved at commercial level and should be encouraged to share this information. This indicates the need for more data to be obtained at slaughterhouse level to allow for a more accurate scientific opinion to be formed.

1.11 Research objectives

The primary objectives of this research study are:

- to implement effective lactic acid and steam vacuum systems at commercial level
- to determine the efficacy of these decontamination technologies as intervention methods in reducing the microbial load present on the surface of beef carcases
- to evaluate their impact on product shelf life.

The secondary aim of this study is to compare the effectiveness of the two intervention methods.

SECTION 2.0: METHODOLOGY

In this section, the system designs, sampling and experimental methodology to be used within the scope of the thesis will be outlined in detail.

2.1 Aims of this section

The aim of this section is to set out the methodology behind the experiments conducted, aimed at answering the research problems of this thesis:

- Is the use of lactic acid and steam vacuuming effective intervention methods for reducing the microbial load present on beef carcases.

- Can the shelf-life of a beef carcase be extended due to the improved microbiological condition of the treated carcases.

2.2 Scope of the experiment design

The scope of this experiment was conducted in an Irish abattoir on beef carcases that had successfully passed final post mortem inspection by the DAFM officials. The selected carcases had undergone typical carcases dressing practices and de-hiding using an automated downward hide puller. Animals sampled differed in age, sex and grade classification.

Microbiological analysis was conducted in an Irish National Accreditation Board (INAB) accredited laboratory under the supervision of the Laboratory Supervisor.

2.3 Choice of experiment design

Quantitative research is required to seek measurable, observable data on variables using statistical, numerical or mathematical techniques.

2.4 Implementation of the Lactic Acid System

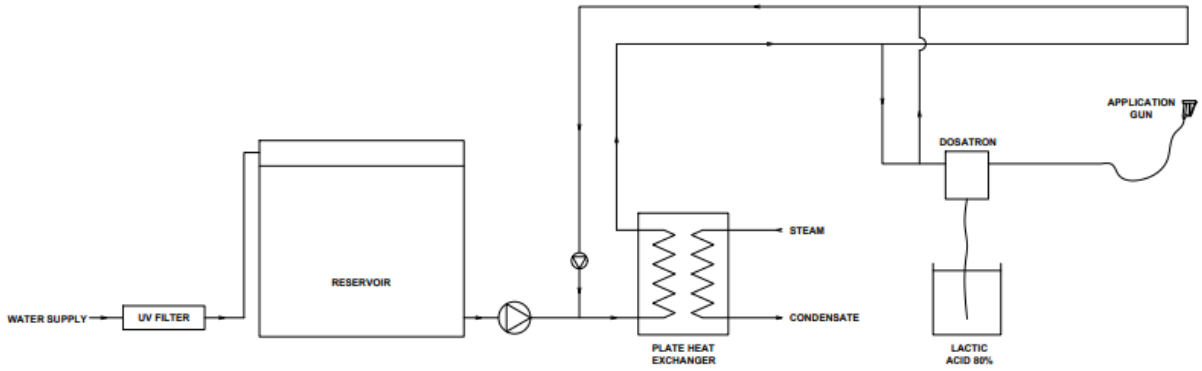

Figure 2. 1: An overview of the Lactic Acid System

Figure 2.1 illustrates the overall operations of the lactic acid system.

The incoming potable water is heated to 50ºC. There is a return system in placed to allow the water to continuously circulate within the pipework ensuring the temperature of the water is maintained. The water is returning to the boiler house at approximately 47ºC. (See Figure 2.2)

Figure 2. 2: Boiler reading.

A Dosatron D3RE-5 Proportioning Pump was implemented to allow a dosage of approximately 2-3% lactic acid concentration to incoming warm potable water (figure 2.3). The lactic acid dosing level is directly proportional to the amount of water passing through the system to the hose applicator. The lactic acid chemical has a concentration of 80%, meaning the proportioning pump is required to be set at approximately 2.5-2.8 (0.02÷0.8=0.025) to ensure the target dose is achieved at all times.

All pipework relating to the Lactic Acid system has been insulated to help maintain and control the temperature of the water (figure 2.3).

Figure 2. 3: The Lactic Acid System

The Lactic Acid solution is applied to beef carcases using a handheld hose. The pressure of the hot water system is set at a pressure of 3 bar with a 4005 nozzle which applies approximately 2.7 litres of solution per minute through a sprinkle angle of 40ºC. The trained operative applies the Lactic Acid solution to the whole carcase ensuring all areas are covered. Lactic acid application was implemented at the end of the slaughter line after carcase dressing but prior chilling.

2.5 Lactic Acid System Controls

2.5.1 Temperature

The temperature of the water is measured at the point of application. A solution sample is obtained from the hose using a sterile sample bottle and the temperature is checked using a calibrated temperature probe. Due to the design of water flow system, the target lactic acid solution temperature at the application point is 35-40ºC.

2.5.2 Lactic Acid Concentration

For the lactic acid system to be effective at reducing the microbial load without causing undesirable effects such as discolouration, the target lactic acid concentration is 2-3%. This is verified using lactic acid quick test kits.

(a) The vial is rinsed with the lactic acid water solution obtained from the point of application.

(b) 1ml of the lactic acid water solution is measured using a small syringe and dispensed into a clean vial.

(c) Add 1 drop of Phenolphthalein indicator to the water solution and swirl the solution in the vial.

(d) Add the Sodium Hydroxide one drop at a time to the vial, swirling after each drop.

(e) Count the number of drops it takes to turn the solution pink. Colour change (clear to pink) occurs with one single drop so care needs to be exercised.

(f) Multiply the number of drops x 0.1= % Lactic Acid e.g. 25drops x 0.1 = 2.5% concentration

2.6 Implementation of the Steam Vacuum system

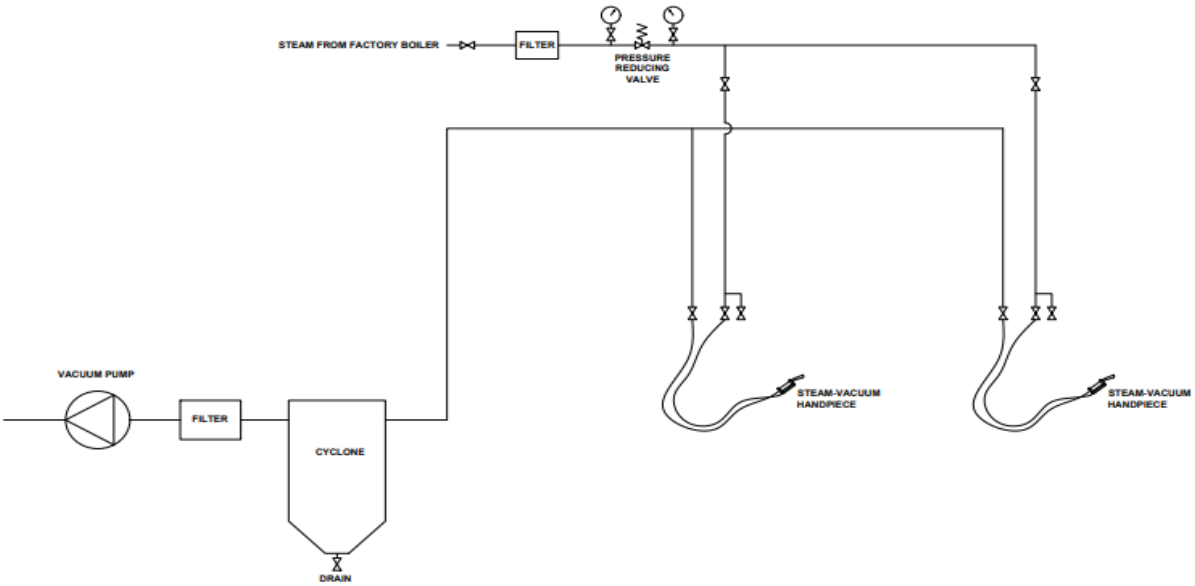

Figure 2. 4: An overview of the steam vacuum system

Figure 2.4 illustrates the overall operations involved in the steam vacuum system.

The system is manufactured using 304 grade stainless steel and non-corrosive material. The handheld device is connected to a vacuum pump and a steam supply.

The function Jarvis steam vacuum handpiece device is as follows:

(a) Steam exits from the handpiece onto the carcase surface.

(b) A regulated flow and pressure of steam is drawn back into the handpiece by vacuum.

(c) This forms a continually rotating envelope of steam.

(d) This steam action is active across the carcase contact surface and it is movement back up the handpiece that removes visible contamination (faeces, hair, blood, etc) from the surface and reduces microbial counts.

The system uses filtered steam set at 30 pounds per square inch (psi) or 2-bar corresponding to a carcase application temperature of approximately 134ºC. The vacuum pump allows for the movement of physical contamination from the handheld vacuum pump to the receiving cyclone tank, preventing potential cross-contamination.

The application of steam vacuum was implemented at the end of the slaughter line after completion of carcase dressing but prior to chilling. Two handheld steam vacuum units were implemented on the slaughter line to allow for application across a greater carcase surface area with one focussing on the hindquarter and the other focussing on the forequarter of the animal.

2.7 Steam vacuum system Controls

To ensure the steam vacuum system is operating correctly, daily checks are conducted as part of the Preventative Maintenance Programme. The steam pressure is monitored throughout the day to ensure the correct parameters are being met.

2.8 Carcase selection

The carcases sampled were pre-selected prior to slaughter using a random numbering system, allowing the total forecasted slaughter volume and both leading (side 1) and trailing (side 2) carcase sides having an equal chance of selection. This allowed for the selection of a sample set representative of the overall day's kill.

2.9 Shelf life experiment

A shelf life study was performed on beef carcases under the following conditions outlined in table 2.1.

Table 2. 1: Shelf life study conditions.

Treatment application	Sample set	Storage conditions
Untreated (Control)	N=3	Carcase chilling at 0-2ºC
Lactic acid application	N=3	Carcase chilling at 0-2ºC
Steam vacuum application	N=3	Carcase chilling at 0-2ºC

Microbiological analysis was conducted using the swabbing technique outlined in 2.10. Samples were collected for analysis in accordance with the shelf life schedule outlined below (table 2.2):

Table 2. 2: Shelf life sampling schedule

Days from production (P)	P+1	P+2	P+3	P+4	P+5	P+6	P+7	P+8	P+9
Sample collection & analysis	X						X	X	X

2.10 Carcase swabbing method

The microbiological carcase sponge swabbing is carried out in line with the requirements stipulated in Commission Regulation (EC) 2073/2005 on the microbiological criteria of foodstuff and in line with the Teagasc guidelines for Standard Operating Procedure for Microbiological Examination of Carcases by Wet/Dry swabbing (2008).

(a) Swabbing is conducted using sterile abrasive sponge swabs that have been moistened with Maximum Recovery Diluent (MRD) 0.1% peptone and 0.85% NaCl.

(b) The recommended swabbing sites of bovine animals are: neck, brisket, flank, rump (Figure 2.5). Sterile disposable gloves must be worn when swabbing and changed between samples.

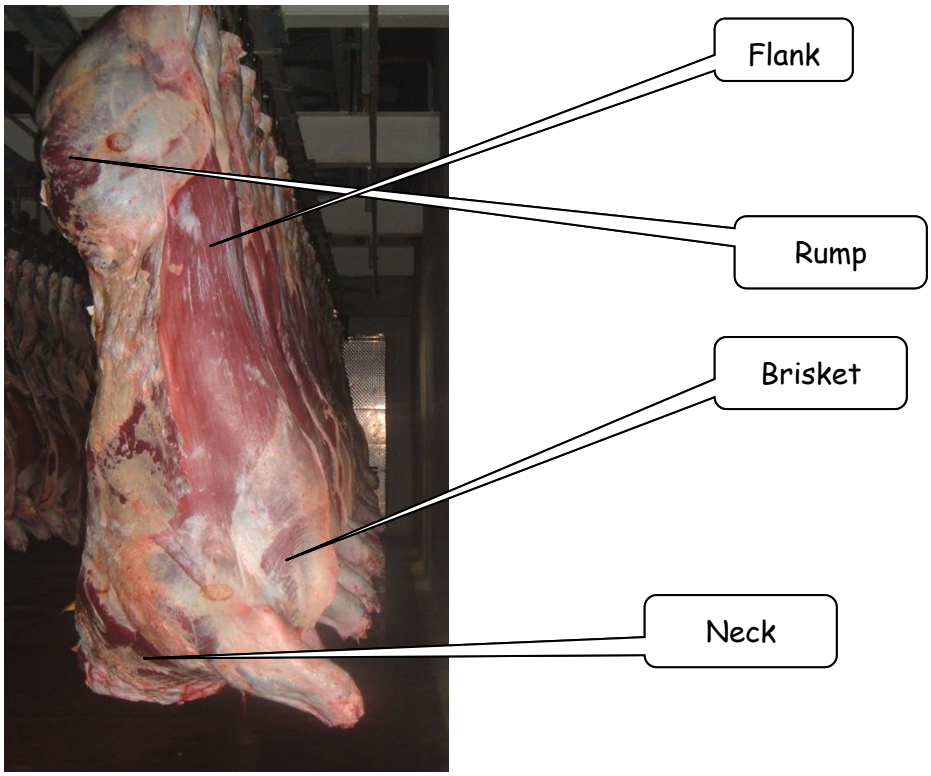

Figure 2. 5: The swabbing sites for beef carcases.

(c) Applying firm pressure, swab the first site horizontally, vertically and diagonally for no less than 20 seconds, covering an area of 100cm^2.

(d) Using the other side of the sponge swab, the second site (brisket) is swabbed using the same technique as outlined above.

(e) This procedure is repeated for swabbing of the remaining two sites; the flank and rump.

(f) All swabs corresponding to an individual carcase are then pooled together in one sampling bag and sealed.

(g) The sealed bag is then marked with the carcase identification number, the slaughter date and other relevant information (pre-lactic acid/ post-lactic acid/ pre-steam/ post-steam).

(h) Samples are kept in an insulated polystyrene box under chilled conditions of between 0-4ºC during storage and transport to the laboratory for analysis.

(i) Samples must reach the laboratory within 24 hours of sampling.

2.10.1 Lactic Acid swabbing

Using the technique outlined in 2.10, 50 carcases were swabbed after carcase dressing but prior to the application of lactic acid. This allowed for the determination of the initial microbial load present on the beef carcase before treatment.

The same carcases were swabbed post-lactic acid treatment but prior chilling, using the same swabbing technique to identify any reductions in the microbial load.

2.10.2 Steam vacuum swabbing

Using the technique outlined in 2.10, 45 carcases were swabbed after carcase dressing but prior to steam vacuum application. This allowed for the determination of the initial microbial load present on the beef carcases prior treatment.

The same carcases were swabbed post-steam vacuum treatment but prior chilling to identity of there was a reduction in the overall microbial load present on the carcases.

2.11 Microbiological testing

2.11.1 Aerobic Colony Count

The Aerobic Colony Count testing was conducted under sterile conditions in accordance with ISO 4833-1:2013.

(a) 10g of sample is weighed out with 90ml diluent added.

(b) Perform serial dilutions with 9ml MRD.

(c) From each dilution performs, pipette 1 ml of the sample into a sterile petri-dish.

(d) Pour with 12-15ml Plate Count Agar (PCA)

(e) Mix inoculum with the media and place the lid on the petri-dish. Allow the media to set and invert petri-dish.

(f) Incubate petri-dishes at 30ºC for 48 hours.

(g) Remove from the incubator and count the colonies present on the plate.

2.11.2 Enterobacteriaceae

Enterobacteriaceae testing was conducted in accordance with ISO 21528-2:2017.

(a) 10g of sample is weighed out and 90ml diluent added.

(b) Conduct serial dilutions using 9ml MRD.

(c) From each serial dilution, pipette 1ml of the sample onto a sterile petri-dish.

(d) Pour 10-15ml Violet Red Bile Glucose Agar (VRBGA) into the petri-dishes.

(e) Mix the inoculum with the media, close the petri-dishes with the lids and allow to set.

(f) Overlay with 5-10ml VRBGA, allow to set and then invert the petri-dishes.

(g) Incubate the samples at 37ºC for 24 hours.

(h) Remove from the incubator and count the typical colonies present on the plate (figure 2.6).

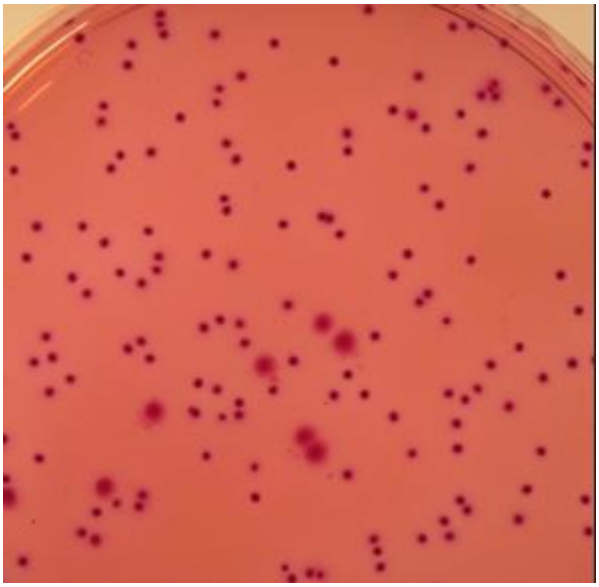

Figure 2. 6: Enterobacteriaceae colonies on VRBGA.

2.11.3 Generic E.coli Testing

E.coli testing was conducted in accordance with the ISO 16649-2 (2001) standards.

(a) 10g of sample is weighed out with 90ml diluent added.

(b) Perform serial dilutions with 9ml MRD.

(c) Pipette 1ml of solution and place into a sterile petri-dish. Repeat this for all serial dilutions prepared.

(d) Pour 12-15ml Tryptone Bile Glucuronide Agar (TBX) into each petri-dish.

(e) Mix inoculum with the media and close petri-dish with the lid.

(f) Allow the media to set and invert the petri-dishes.

(g) Incubate petri-dishes at 44ºC for 24 hours.

(h) Remove from the incubator and count the typical colonies present on the plate (figure 2.7).

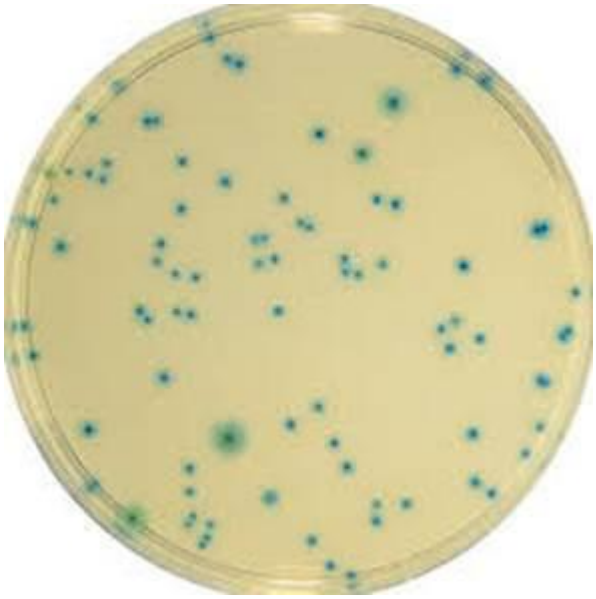

Figure 2. 7: Typical E.coli colonies on TBX.

2.11.4 Salmonella testing

Salmonella testing was conducted based on Solus ELISA.

(a) 25g of sample is weighed out with 225ml of Buffered Peptone Water (BPW) added.

(b) The sample is incubated for 37ºC for 24 hours.

(c) Add 100ul of sample to a sterile test tube containing 10ml RVS.

(d) Incubate the sample at 41.5ºC for 24 hours.

(e) Prepare and load the sample onto the ELISA machine as per kit instructions and allow for the ELISA machine to complete sample testing.

(f) Samples that are considered presumptive are streaked onto petri-dishes containing XLD and BGA.

(g) The petri-dishes are incubated at 37ºC for 24 hours.

(h) Upon removal from the incubator, typical colonies are identified and are streaked onto Nutrient Agar.

(i) These samples are then incubated for 37ºC for 24 hours.

(j) Oxidase and serological tests are performed on pure isolated colonies.

(k) Perform biochemical tests using AP120e.

(l) If all typical criteria for salmonella have been detected (figure 2.8), then Salmonella presence is reported.

Figure 2. 8: Salmonella positive colonies present on XLD.

2.11.5 Pathogenic E.coli 0157:H7 testing

E.coli 0157:H7 testing was conducted in accordance with the ISO 16654 (2001) protocol.

(a) 25g of sample is weighed out with 225ml of Modified Tryptone Soya Broth (MTSB) added to the sample

(b) The sample is incubated at 41.5ºC for 24 hours.

(c) Add 20ul of captivate beads to the sample and then pipette 1ml of the sample into Eppendorf tubes.

(d) Rotate tubes for 30 minutes.

(e) Place the mixed samples on a magnetic rack and perform three washes using Phosphate Buffered Saline (PBST).

(f) Add 50ul of PBST, vortex and streak the 50ul solution onto a petri-dish containing Sorbitol MacConkey Agar (CT-SMAC).

(g) Incubate plates at 37ºC for 24 hours.

(h) Remove from the incubator and streak any typical colonies (figure 2.9) identified onto Nutrient Agar.

(i) Incubate plates at 37ºC for 24 hours.

(j) Remove from the incubator and put any pure isolated colony into Tryptone water and incubate at 37ºC for 24 hours.

(k) Add 0.2ml Kovacs reagent. It a red colour is reported, the sample is considered positive.

(l) From the original pure isolated colony, perform the latex test.

(m) If the sample is tryptone positive and latex positive, then the sample is reported as E.coli 0157:H7 detected.

Figure 2. 9: E.coli 0157:H7 on CT-SMAC

2.12 Statistical Analysis

Microbial counts were converted to $\log_{10}$ cfu/cm^2. Data was analysed statistically using the paired T-Test. The data obtained before and after treatment were compared to establish statistical significance. Significance was determined at the $P<0.05$ level.

$P<0.05$ represents an acceptable level of a 95% confidence interval.

2.13 Experimental limitations

- Sampling was conducted on animals during the months of October and November. This may not accurately reflect the carcases hygiene expected throughout the year. The microbial load would be anticipated to increase further during the winter months when animals are being housed and changes to diet occur or during warm periods with increased shedding.
- Lactic acid is most effective when applied to the carcase at a solution temperature of between 50-55ºC. However, due to the current water system in operation in the abattoir and the relatively low water pressure at application, the optimal temperature currently being achieved at carcase surface application is 35-40ºC.
- It was not possible to contaminate beef carcases within production with inoculated pathogenic bacteria such as Salmonella and E.coli 0157:H7 due to the high microbiological risk it posed to the entire production process. Therefore, we relied on our carcase selection process to identify if any carcases had pathogenic bacteria present on the surface and if the intervention methods were effective at reducing their prevalence.
- Due to insufficient chill storage capacity and production planning, the shelf life study was conducted for a duration of P+9 days. To gain a better understanding of the impact interventions have on shelf life, a longer durability study may be required.

2.14 Conclusion

The purpose of this section was to outline in adequate detail the experimental methodology followed and collection of data to address the research problem.

Section 2.0: Methodology was addressed under the following headings:

- Aims of this section
- Scope of the experiment design
- Choice of experiment design
- Implementation of the Lactic Acid System
- Lactic Acid System Controls
- Implementation of the Steam Vacuum system
- Steam Vacuum Controls
- Shelf life experiment
- Carcase selection
- Carcase swabbing method
- Microbiological testing
- Statistical Analysis
- Experimental limitations
- Conclusion

SECTION: 3.0 RESULTS AND DISCUSSION

This section will compile the microbiological results obtained from the experimental research, analyse the data collected and discuss their relevance in detail.

3.1 Aim of this section

The aim of this section is to analyse the results and to form an unbiased scientific opinion based on the data collected to address the objectives of this research study:

- Does the use of lactic acid treatment and steam vacuum as intervention methods effectively reduce the microbial count present on beef carcases.
- Which decontamination method yields greater microbial reductions.
- Can the shelf life of a beef carcase be extended due to the improved microbiological condition as a result of application of these decontamination methods.

3.2 The effect of intervention methods in reducing the microbial load present on beef carcases

The primary variable directly impacting on sample size showing log reductions for both steam vacuum and lactic acid was the initial microbial load present on a carcase prior treatment. In general, the microbiological results of carcases prior treatment showed the presence of relatively small microbial counts. Many pre-treatment results reported values below the lowest limit of detection for enumeration (<10 cfu/cm^2). This illustrates that good manufacturing and hygiene practices are being utilised on the slaughter line during carcases dressing, complying with requirements stipulated in the Food Hygiene package and Regulation (EC) 2073/2005. All microbiological data collected from the research study is outlined in the attached appendix section. In correlation with the methodology of this research study, all data obtained and analysed were with respect to carcases that had a microbial load present on their surface prior to treatment; a value above the lowest detection limit of the method of enumeration. This means carcases that had ACC,

Enterobacteriaceae or E.coli counts of greater than 10cfu/cm^2 were included in the data analysis.

Microbiological analysis for Salmonella and E.coli 0157:H7 presence was conducted on all carcases swabs for both sample sets prior to treatment. All carcase swabs tested negative for the pathogenic microorganisms. It was not possible to inoculate carcases with the pathogenic strains due to the high risk it posed to the entire production process. However, both treatments were effective at reducing Enterobacteriaceae and E.coli, indicators for these bacterial pathogens.

3.2.1 Lactic Acid Treatment

3.2.1.1 Control of Lactic Acid System variables.

The lactic acid concentration and temperature applications were monitored and controlled during this research study. The condition of both variables applied to the surface of the beef carcases during this study are depicted in Figure 3.1 and Figure 3.2. The results yielded a mean lactic acid concentration of 2.3% with a maximum value 2.8% and a minimum value of 2.0%. (See appendix 3 for full set of results).

The target concentration of 2-3% was achieved and maintained for the duration of the sampling period. Although the concentration allowance according to Regulation (EU) 101/2013 is between 2-5%, the target concentration range for this research study was established based on scientific evidence to ensure microbial reduction was achieved without causing discolouration of the treated carcases. Pipek et al. (2005) observed the colour changes when using lactic acid at a concentration of 2%. It was reported that after decontamination, the haem pigments slightly changed from the reduced form to the oxy-form. However, the colour changes reported were subtle. The surface lightness increased slightly after treatment, but no further changes were reported during storage. Redness was also reduced after treatment but increased on all samples during storage (Pipek, et al., 2005). Applications using higher concentrations of lactic acid have been reported to yield

unacceptable discolouration of the meat surface (Rodriguez- Melcon, et al., 2017). Therefore, it was hypothesised that by maintaining a concentration of between 2-3%, it would facilitate a microbial log reduction without causing undesirable discolouration.

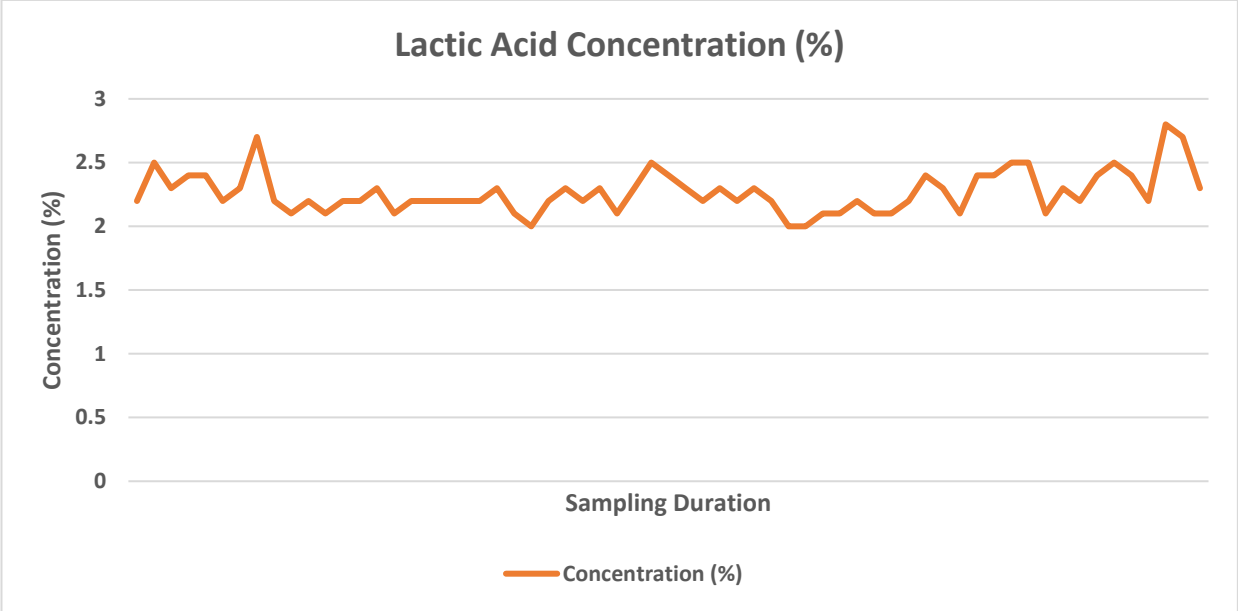

Figure 3. 1: Lactic acid concentrations at the point of application to the carcase surface**.**

When looking at the temperature of the lactic acid solution at the point of application for the duration of this study, the results showed a mean temperature value of 37ºC with a temperature ranging from between 36ºC and 38ºC was achieved (figure 3.2). Scientific evidence reports that applying a lactic acid solution to carcases at a concentration of between 50-55ºC improves the efficacy of the intervention method (Acuff, 2005). Research conducted by Pipek et al. (2004) reported that the efficacy of the lactic acid solution is higher when applied at a warm temperature of 45ºC when compared to a cold solution treatment of 15ºC. In Regulation (EU) 101/2013 the maximum temperature allowance is up to 55ºC.

The target temperature of 36-40ºC was established based on the system design. Although alterations were made to the waterflow system to improve the temperature of the solution, the optimal temperature at application is hindered by the original design of the waterflow

system as discussed in the Section 2.0. However, a microbial log reduction should still be achieved by maintaining a mean temperature of 37 +/- 1ºC.

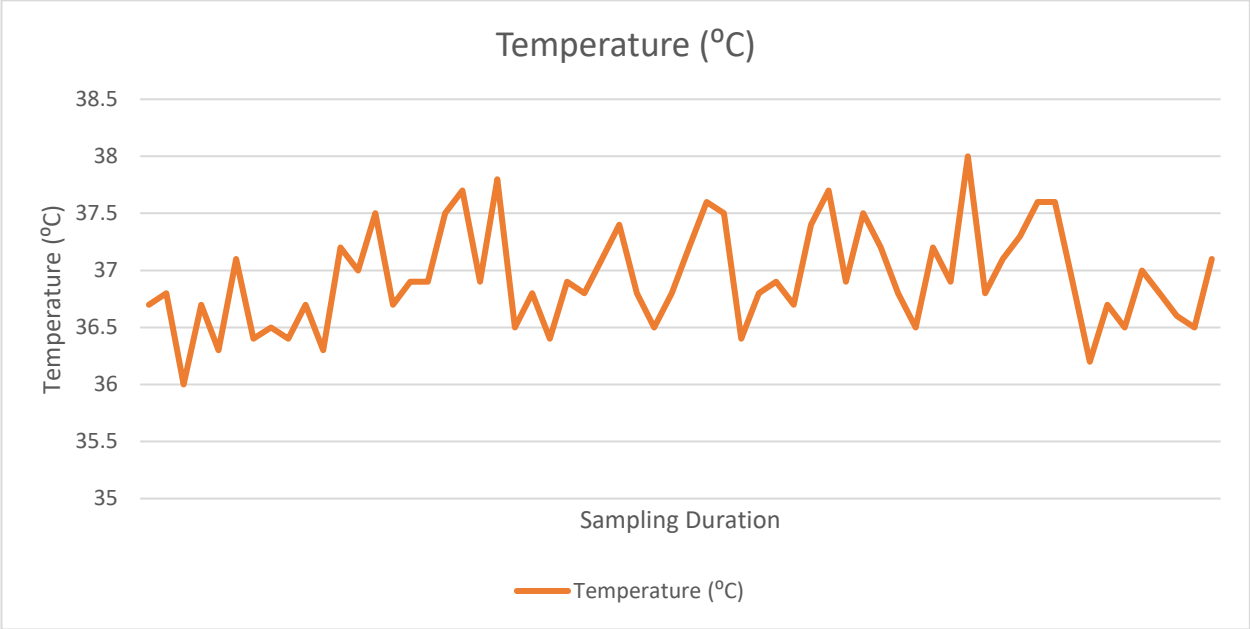

Figure 3. 2: Temperature recordings at the point of application to the carcase surface.

3.2.1.2 Efficacy of the Lactic Acid application in reducing microbiological counts

The efficacy of the lactic acid application in reducing the microbial load present on the surface of carcases is highlighted in table 3.1.

Table 3. 1: Microbiological data on Lactic Acid Treatment

	Lactic Acid Treatment Data				
	Initial sample set size	**Samples showing initial microbial load >10 cfu/cm^2**	**Samples showing log reduction**	**Mean log reduction (cfu/cm^2)**	**Standard deviation**
ACC	50	44	44	1.77	0.65
Enterobacteriaceae	50	15	15	1.39	0.97
E.coli	50	12	12	1.33	0.75

When examining the log cfu/cm^2 reduction in relation to ACC, lactic acid application resulted in a mean reduction of 1.77 with a standard deviation of 0.65. Statistical analysis of this data also reported a P-value of <0.05, suggesting the results obtained from our research study are highly significant.

With regards to Enterobacteriaceae, a mean log cfu/cm^2 reduction of 1.39 with a standard deviation of 0.97 was achieved. Statistical analysis also shows this data to be significant with a P-value <0.05. Similarly, a log reduction was also noted in relation to generic E.coli counts with a mean log cfu/cm^2 result of 1.33 with a standard deviation of 0.75. This data is also significantly different with a P-value <0.05. All data is comprehensively displayed in the attached Appendices.

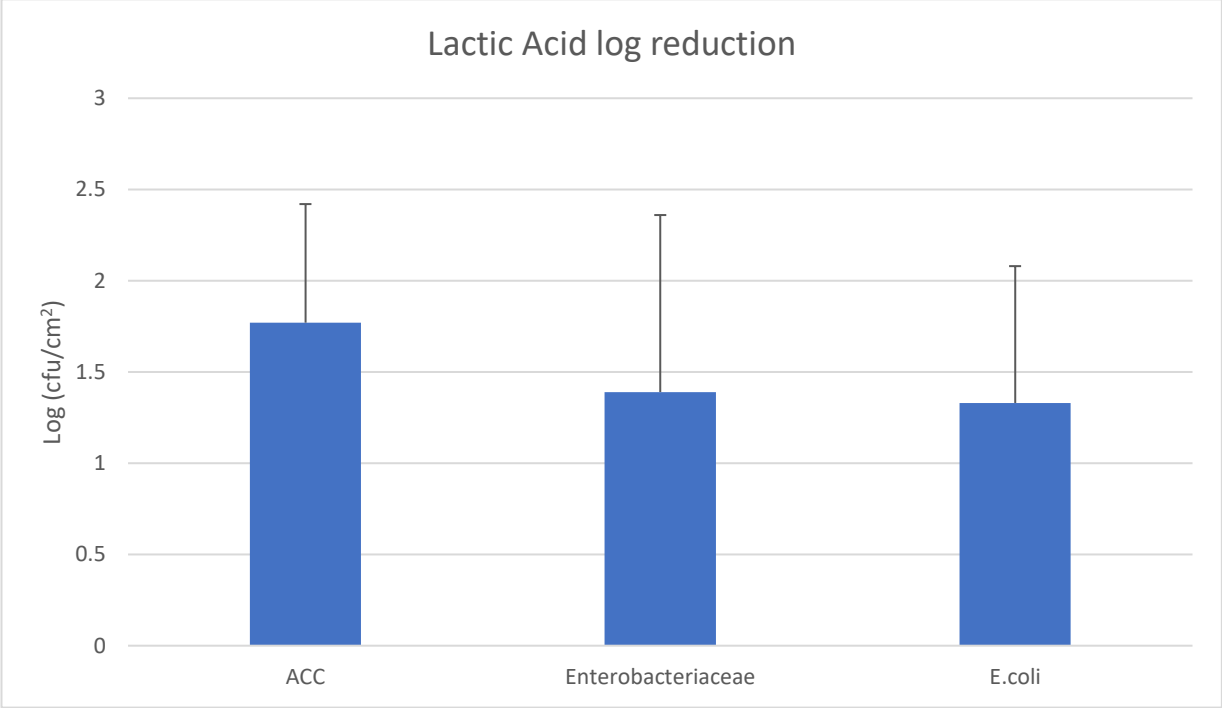

Figure 3. 3: Microbiological reductions for to the use of lactic acid on beef carcases**.**

Overall, the use of lactic acid achieved a log reduction of between 0.5-2.5 for ACC, Enterobacteriaceae and E.coli. The efficacy of the lactic acid treatment in this research study was dependent primarily on the initial microbial count. 44 carcases within the

sample set of 50 showed initial microbial presence greater than 10cfu/cm^2 as depicted in table 3.1. All 44 carcases showed a log reduction when treated with lactic acid.

Scientific literature reports reduction of between 1.0-3.0 log through the use of organic acids as a surface decontaminant. A more accurate log reduction may be achieved by conducting further testing on carcases with a higher initial microbial load present on the surface.

3.2.2 Steam vacuum treatment

Similarly, steam vacuum treatment on the surface of beef carcases also decreased the initial microbial load detected pre-treatment. The efficacy of the steam vacuum application is highlighted in Table 3.2.

Table 3. 2: Microbiological data on Steam vacuum treatment.

	Steam Vacuuming treatment data				
	Initial sample set size	**Samples showing initial microbial load >10 cfu/cm^2**	**Samples showing log reduction**	**Mean log reduction (cfu/cm^2)**	**Standard deviation**
ACC	45	39	39	0.83	0.71
Enterobacteriaceae	45	7	7	0.55	0.28
E.coli	45	0	0	0	0

When looking at the ACC count, steam vacuum resulted in a mean log (cfu/cm^2) reduction of 0.83 with a standard deviation of 0.71. A P-value <0.05 also shows that the results in relation to ACC reduction for steam vacuum treatment is statistically significant.

Similarly, an Enterobacteriaceae mean log reduction of 0.55 with a standard deviation of 0.28 was achieved with steam vacuum application. A P-value<0.05 was obtained for this set of data, showing statistical significance.

In relation to E.coli counts on beef carcases treated with steam vacuum, there was no reduction reported. This was due to the initial E.coli count on all carcase sampled (n=50)

being reported below the detection limit of <10 cfu/cm^2 prior to treatment. 39 carcases out of a total sample set of 45 had an initial microbial load greater than 10cfu/cm^2. All 39 carcases reported a log reduction when treated with steam vacuuming. This highlights its efficacy as a decontamination technology.

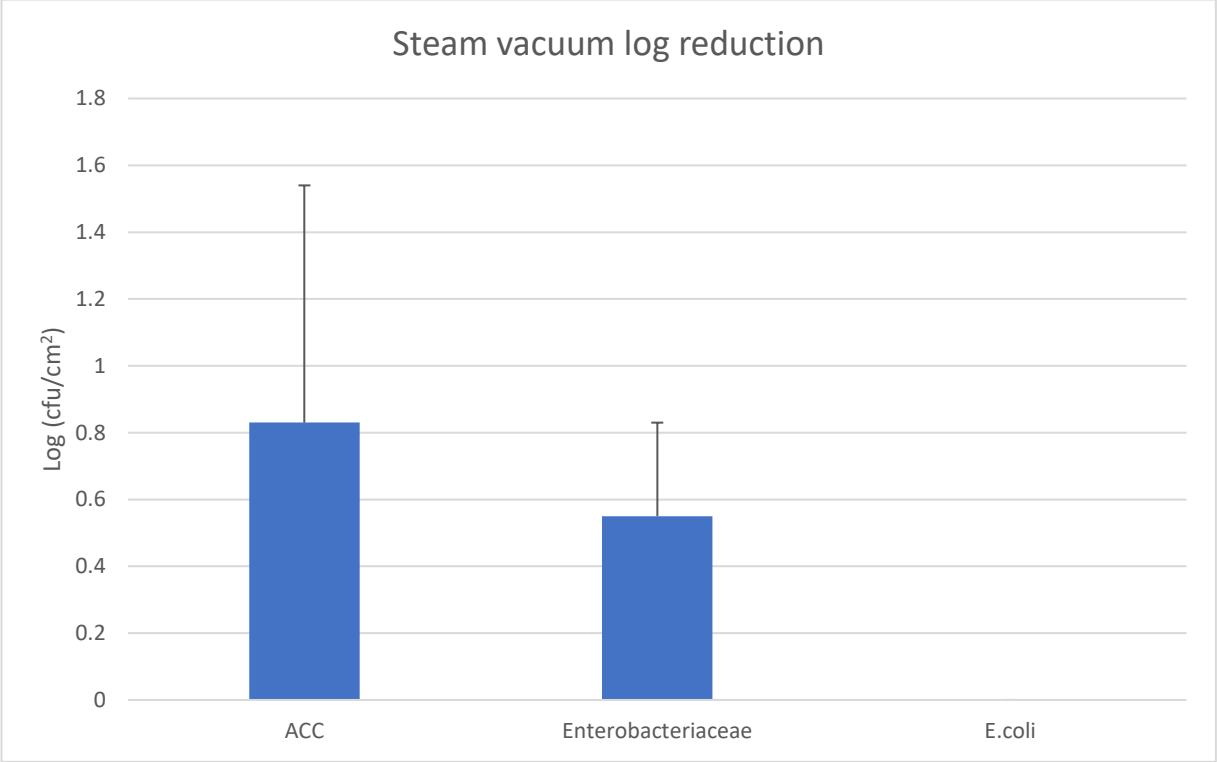

Figure 3. 4: Microbiological reductions for to the use of steam vacuum on beef carcases.

3.3 Comparing the efficacy of Lactic Acid and Steam Vac treatments

The microbiological data obtained for both lactic acid and steam vacuum interventions can be compared in Figure 3.5. Lactic acid treatment achieved a relatively larger ACC mean log reduction than steam vacuum treatment, with a mean reduction difference of 0.94 log cfu/cm^2 when compared to steam vacuum. Lactic acid was also more effective at reducing Enterobacteriaceae counts with a mean log reduction of 1.39 cfu/cm^2 when compared to a reduction of 0.55 cfu/cm^2 achieved by steam vac; a difference of 0.84 log. Lactic acid was also effective at reducing E.coli counts. However, this reduction cannot be compared to steam vacuum treatment as all carcases within the sample set had an initial

E.coli count of <10 cfu/cm^2 prior treatment. Therefore, we were unable to identify the potential mean log reduction for this intervention.

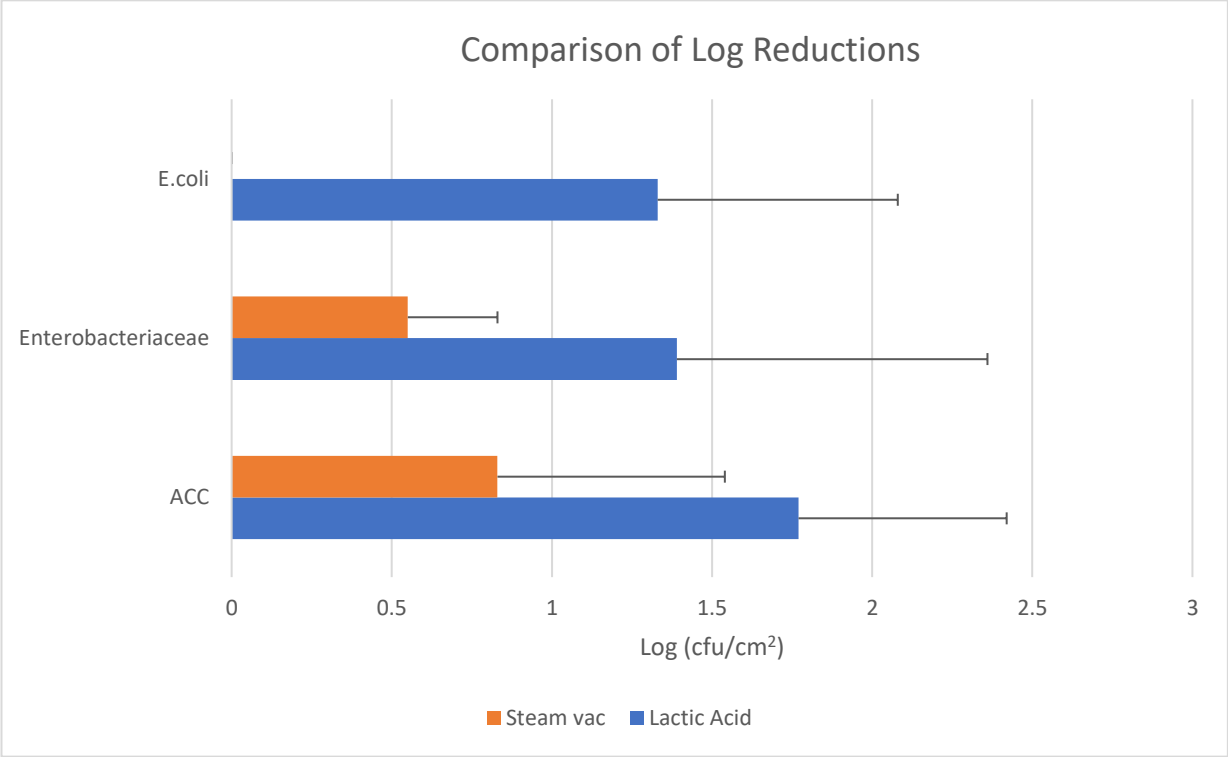

Figure 3. 5: Comparison of microbiological log reductions

There are many variables in relation to lactic acid treatment that need to be investigated and controlled to ensure an effective application is achieved. These include the acid concentration, temperature, pressure, application technique etc Our results show that the implemented lactic acid system as outlined in section 2.4 is adequately controlled and effective at reducing the microbial load on carcases. Lactic acid is also applied to whole carcases, impacting on the overall microbial load. Lactic acid treatment on carcases acts by lowering surface pH and exhibiting residual inhibitory effects when applied which may initially be bactericidal. (Rodriguez- Melcon, et al., 2017).

Although, log reductions were achieved, steam vacuum is an intervention method designed for treating small areas of the carcase, inactivating the bacteria at these specific sites. It not a practical whole carcase treatment. The effectiveness of the treatment is

dependent on operational practices, steam temperature, application time, the carcase area treated etc. Two steam vacuum systems were implemented during this research study, increasing the surface area treated which may have contributed to the log reductions reported.

3.4 The impact of invention technology on improving the shelf life of the product

The baseline for the shelf life study was established based on the requirements set out in Regulation (EC) 2073/2005 on the microbiological criterial for foodstuff with the acceptability limit set at 5.0 $\log_{10}$ cfu/cm^2. A durability study was conducted in accordance with FSAI guidance note 18 (2017) over a 9-day period.

The mean $\log_{10}$ cfu/cm^2 value of each sample set of n=3 was calculated for untreated carcases (control), carcases treated with lactic acid and carcases treated with steam vacuum.

Table 3. 3: Results from shelf life durability study.

Sample date	Acceptable Limit (M) ($\log_{10}$ cfu/cm^2)	Control ACC Mean ($\log_{10}$ cfu/cm^2)	Lactic Acid ACC Mean ($\log_{10}$ cfu/cm^2)	Steam vacuum ACC Mean ($\log_{10}$ cfu/cm^2)
P+1	5.00	4.11	1.37	3.50
P+7	5.00	4.21	2.16	4.04
P+8	5.00	5.80	3.98	4.39
P+9	5.00	6.25	4.18	4.40

Based on the results depicted in table 3.3, the Control ACC mean was within the acceptable limit at P+ 7 days with a log value of 4.21. However, at P+8 days, the mean value exceeded the threshold with a reported value of 5.8 log cfu/cm^2. This result was anticipated as the abattoir currently allows for a shelf-life of 7 days from the slaughter date on whole carcases when stored under chilled conditions of 0-2ºC.

When looking at the mean results for carcases that underwent intervention applications, a reduced ACC count was noted for both treatments at the beginning of the study in comparison to the control. The initial mean log cfu/cm^2 of lactic acid treated carcases and steam vacuum treated carcases were 1.37 and 3.50 respectively in comparison to the control microbial load of 4.11.

The difference in the initial microbial load when comparing both decontamination application would be attributed to the variation in the modes of application. Lactic acid application, whether automated or manual, allows for the whole carcases to be treated. In contrast steam vacuum is an effective spot treatment, allowing small surface areas to be effectively decontaminated.

The microbial growth on carcases treated with lactic acid was relatively low between P+1 to P+7 days ranging from 1.37 to 2.16 log. This again highlights the potential initial bactericidal effects and subsequent protection from microbial growth during chilling and storage as a result of residual inhibitory effects (Rodriguez- Melcon, et al., 2017). Research also reported that lactic acid treatment prolonged the log phase of microbial growth by one day. (Rodriguez-Melcon et al., 2017; Pipek et al., 2004).

At P+9 days, all results obtained for treated carcases were below the acceptable threshold of 5.0 log with a lactic acid ACC mean of 4.18 log and a steam vacuum ACC mean of 4.40 log. From examining the graph in figure 3.6, the lines representing lactic acid and steam vacuum applications suggest a reduction in the rate of microbial growth with the lines on the graph appearing to level somewhat. Based on this observation, it is suggested that product shelf life may be further extended beyond P+9 days, however further testing would be required to validate this hypothesis.

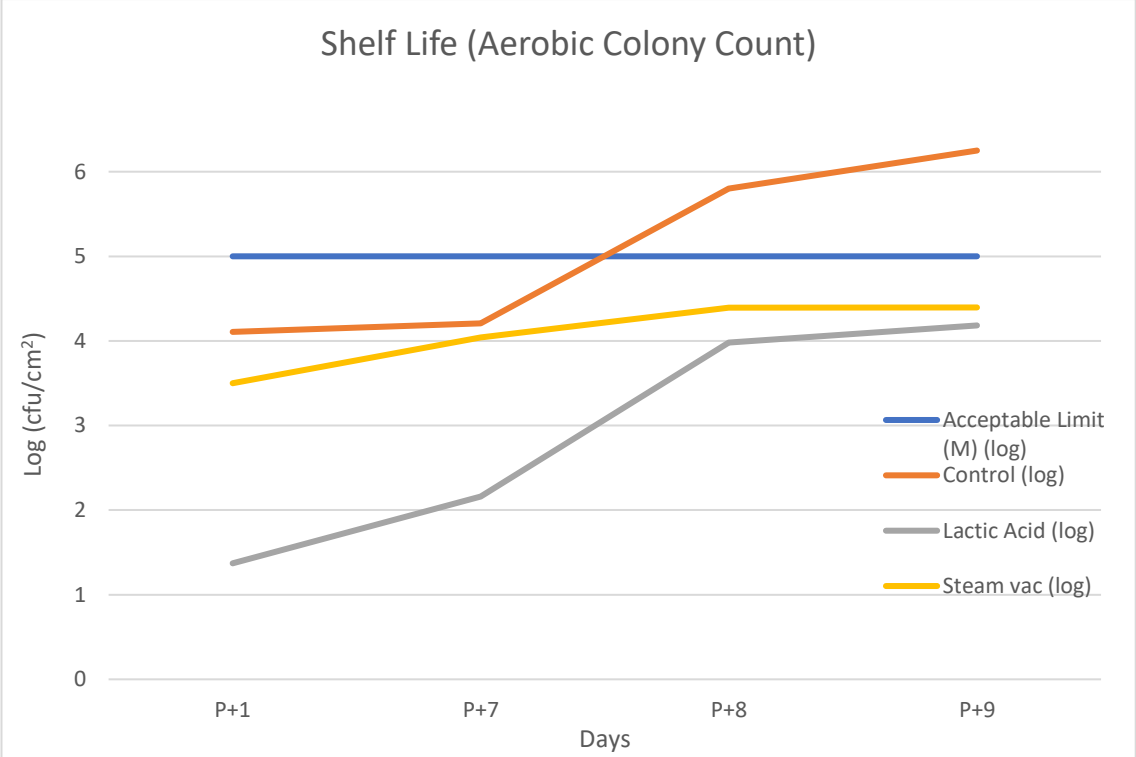

Figure 3. 6: Shelf life results graph

Food processors can manipulate the food product and control product safety and shelf-life by selecting and altering intrinsic and extrinsic factors to act as hurdles for microbial survival and growth (FSAI, 2017). Fresh meat products have many favourable conditions such as a high water content and nutrient availability that make it attractive for microbial growth. By using the decontamination methods as part of an integrated food safety management system and applying the hurdle technologies, improved food safety and shelf life can be achieved.

Hurdle technology exercised during the above durability shelf life was:

- Good hygiene and manufacturing practices throughout the slaughtering process (reflected through data collected during the research study showing compliance with the process hygiene criteria outlined in Regulation (EC) 2073/2005)
- Implementation of HACCP-based procedures as required in Regulation (EC) 852/2004.

- The use of intervention technologies to reduce the microbial load, thus improving the microbiological quality of the carcase.
- Lactic acid application decreases surface pH (Pipek et al, 2004)
- The application of carcases chilling immediately after dressing and storage of carcases at temperatures between 0-2ºC.

By applying the hurdle concept effectively to the production process, food processors can produce food products with extended shelf life that are microbiologically safe, of good quality and commercially viable over the desired period.

The need to achieve a longer shelf life of packaged product means achieving low microbial counts on the carcases through decontamination technologies on the slaughter line. This efficacy of lactic acid treatment appears in many scientific literatures in the context of prolonging the shelf life of meat packaging in oxygen atmosphere (Pipek, et al., 2004).

Our research findings support current scientific literature that surface decontamination using steam and lactic acid reduce microbial counts and prolongs the shelf life of meat (Pipek et al., 2004; Pipek et al., 2005). The shelf life of carcases treated with lactic acid technology or steam vacuum technology prior chilling could be extended to 9 days on the condition that cold chain storage of 0-2ºC is upheld.

SECTION 4.0: CONCLUSIONS/ RECCOMMENDATIONS

4.1 Background

In section 1.0, a comprehensive review of published scientific literature created the basis for this research study. It provided information on potential sources of microbiological contamination, the phenomenon of bacterial attachment, survival and growth, various intervention methods, legislation surrounding their use and hurdle technology. The literature review also highlighted the limitations of scientific data available in relation to decontamination technologies.

The reviewed literature provided vital information to aid in the development and implementation of steam vacuum and lactic acid systems as intervention methods for microbiological surface decontamination. It also allowed for hypothesises to be formed in relation to the objectives of the research study.

The methodology applied during this research study was outlined in section 2.0. This section outlined the implementation and control of the intervention systems, sample selection and collection, shelf life study, microbiological analysis and statistical analysis.

Section 3.0 addressed the results of the experiments and discussed the findings. In this final section, conclusions and recommendation will be made based on the findings of this research study.

4.2 The research problem revisited

The primary aim of this research study was to evaluate the efficacy of lactic acid and steam vacuum treatment in reducing the microbial load naturally present on beef carcases and to establish if the treatments have an impact on prolonging product shelf life.

The secondary aim was to compare the two intervention methods and to determine if one was more effective than the other with regards to the improving the microbiological condition of beef carcases.

4.3 Research study outcomes and recommendations

This study demonstrated that both lactic acid and steam vacuum are effective intervention methods in reducing the microbial load present on beef carcases.

However, the research study was limited by the sample size. Although the data correlates with scientific opinions published in research papers, more microbiological testing may be required to further validated the findings.

Compliance with the Hygiene Package requirements for the implementation of good hygiene and manufacturing practices and an effective food safety management system are reflected in the low microbial counts detected on carcase surfaces prior to treatment. Therefore, our research study shows that lactic acid and steam vacuum treatments were used to complement the current manufacturing and hygiene procedures being applied within the slaughterhouse. Although the invention methods were effective are reducing microbial loads, it was not necessary for all carcases to undergo decontamination treatment, with many bacterial counts being below the level of detection ($<10 cfu/cm^2$) before decontamination. However, as it is impossible to physically identify carcases at the end of the slaughter line based on the level of microbiological contamination present, it would be beneficial to treat all carcases from a commercial point of view to ensure high quality, wholesome and microbiological safe products are being produced at all times. Even a slight reduction in the overall microbial load has been found to greatly reduce the risk of foodborne illness posed to consumers.

It may be useful to further investigate possible strategies that would facilitate the identification of periods where microbial fluctuations may occur; perhaps using historical microbiological data. Decontamination methods would be operating at their optimum during these periods as the initial microbial load would be higher. This would allow for

the accurate identification of intervention efficacy in reducing the microbial load on beef carcases.

The data also shows that lactic acid treatment is more effective at reducing carcase surface microbial load in comparison to steam vacuum treatment. This also supports the widely recognised concept that lactic acid treatment is a whole carcase application where steam vacuum is effective at treating small areas on a carcase. To improve the efficacy of steam vacuum treatment, it may be beneficial to conduct further research into the identification of areas on beef carcases that are prone to a high microbial load presence. This would allow steam vacuum treatment to be effectively applied to these targeted carcase areas, improving the efficacy of the system.

As lactic acid is more effective when applied at higher temperatures, further investigation needs to be conducted into possible ways of modifying the current system to allow for an increase in solution temperature at application. Scientific research has also shown that an automated application approach allows for homogeneity of the organic acid solution across the entire surface of the carcase. The food processor would have to consider whether it would be cost-effective to implement such an application system, taking into consideration current labour costs for manual application and whether lactic acid treatment is to be incorporated into the slaughter line as a permanent step within the process flow.

Concentration is also a variable that influences the efficacy of lactic acid applications and causes undesirable colour changes. Although a log reduction was achieved during this research study using a 2-3% concentration, further investigation could be carried out to establish the upper threshold concentration value that could be applied without causing unacceptable discolouration to the surface of beef carcases. A comparison could then be

conducted on results obtained at 2% versus those at higher concentrations with respect to microbial log reductions achieved.

The use of decontamination methods as part of the slaughterhouse's food safety management system impacted positively on shelf life of beef carcases. This research study shows that carcase shelf life could be extended from P+7 days (untreated carcases) to P+ 9 days, provided they are chilled and stored at temperatures between 0-2ºC.

From the data obtained, it is likely that product shelf life could be extended further beyond P+ 9days. It would be beneficial for further shelf life durability studies to be conducted over a longer period to identify the point where the microbial load of treated carcases exceeds the acceptability baseline.

In conclusion, the study shows that the implementation of two different intervention methods, a steam vacuum system and a 2-3% lactic acid solution application, are effective microbiological surface decontamination technologies for the treatment of beef carcases at the end of the slaughter line. They also have a positive impact on the shelf life of the product when applied as part of the hurdle concept.

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SECTION 6.0: JOURNAL ARTICLE

The efficacy of lactic acid and steam vacuum applications in reducing microbial load and prolonging the shelf life of beef carcases.

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ABSTRACT

The aim of this study is to determine the efficacy of a 2% lactic acid solution and steam vacuum technologies as intervention methods in reducing the microbial load and extending the shelf life of beef carcases. The decontamination methods were applied at the end of the slaughter line prior chilling. Samples were taken on carcases pre and post treatment and microbiologically analysed. A durability shelf life study was conducted over a 9-day period on carcases treated with 2% lactic acid, steam vacuum and untreated carcases (control). A 2% lactic acid solution applied at 37ºC reduced ACC, E.coli and Enterobacteriaceae counts by 0.5-2.5 log. Steam vacuum reduced the aforementioned bacterial species by 0.2-1.5 log. Treated carcases chilled and stored at 0-2ºC reported a prolonged shelf-life in comparison to the control. Thus, the use of these decontamination methods can reduce the microbial surface load improving the quality and shelf-life of the product.

Keywords: beef, decontamination, microbial load, lactic acid, steam vacuum, shelf life

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1.0 Introduction

During the slaughtering process, the surface of beef carcases can become contaminated with both pathogenic and spoilage bacteria during carcases dressing through contact with the hide, gut spillages, operational hygiene practices and direct contact with other carcases on the slaughter line (Huffman, 2002). Invention methods have recently gained much attention as the industry seek to improve product safety and extend shelf life to

ensure growing consumer demands are met. However, decontamination methods should be implemented as an additional process step within the slaughterhouse, complementing good hygiene and manufacturing processes, plant design, appropriate line speeds and implementation of HACCP principles (Sofos & Smith, 1998; Hugas & Tsigridia, 2008).

Regulation (EC) 853/2004, specifically Article 3(2), stipulates the permitted use of potable water to remove surface contamination from carcases. The use of other substances must undergo scientific risk assessment by the European Food Safety Authority (EFSA) and subsequent endorsement by the European Parliament. The use of lactic acid on bovine carcases is addressed under Regulation (EU) 101/2013. It outlines the conditions whereby lactic acid can be used to treat carcases at a concentration of between 2 to 5% in a potable water solution at temperatures not exceeding 55ºC and in line with good manufacturing practices.

Lactic acid treatment results in immediate microbial reduction through penetration of the carcase surface cell membrane and disassociating within the internal compartment of the cell (Booth, 1985). This results in a decrease of the intracellular pH which is vital for physiological functions of the cell such as RNA and protein synthesis, DNA replication, ATP synthesis and cell growth. The residual effect from the lowered pH may initially be bactericidal, but later forms protection against bacterial growth (Rodriguez- Melcon, Alonso-Calleja, Capita, 2017)

The implementation of an intervention method utilising lactic acid would need to be tightly controlled in relation to variables such as application method, pressure, temperature, organic acid concentration, interval time between carcase washing and application to ensure that microbial reduction is achieved (EFSA, 2011; Signorini et al., 2018). There are many published scientific reports supporting the use of lactic acid for

reducing microbial contamination. Pipek, Fila, Jelenikova, Brychta, & Miyahara (2004) reported a reduction of between 1-3 decimal orders of colony forming unit (cfu) on carcases sprayed with a 2% lactic acid solution. Similarly, Signorini et al (2018) and Ramson, Belk, Sofos, Stopforth, Scanga, & Smith (2003) reported a significant reduction in Escherichia coli 0157:H7 counts.

Steam vacuum is an effective carcase surface spot treatment utilising combination of both physical and thermal treatment. Steam is applied to beef carcases followed by vacuuming with the combination effect of removing visible contamination and/or inactivation microbiological contamination present on the carcase surface (Huffman, 2002). The effectiveness of steam-vacuuming is dependent on a number of factors including operational practices, steam temperature, application time, the carcase area treated, the level of contamination present on the carcase and the location along the process flow where steam vacuuming is applied (Hockreutener, Zweifel, Corti, & Stephan, 2017). Microbial reductions of the initial contamination of 0.4-0.9 log cfu/cm^2 and 1.73-2.13 log cfu/cm^2 have been reported (Hockreutener, Zweifel, Corti, & Stephan, 2017; Kochevar, Sofos, Bolin, Reagan, & Smith,1997).

However, many of the research studies have been laboratory based and may not accurately reflect reductions achieved commercially. Therefore, the aim of this study is to determine the efficacy of a 2% lactic acid solution and steam vacuum in reducing the microbial load present naturally on beef carcases after dressing and to establish if the use of these intervention methods will positively impact on product shelf life.

2.0 Materials and Methods

2.1 Sample selection

Carcases were randomly selected prior slaughter, allowing the total forecasted laughter volume and both the leading (side 1) and trailing (side 2) sides having an equal chance of selection.

2.2 Chemical treatment

A 2% lactic acid solution was applied to the carcases at a temperature of 37ºC. The treatment was applied to the whole carcase manually using a handheld hose. The pressure of the lactic acid treatment system is set at a pressure of 3 bar with a 4005 nozzle, applying approximately 2.7 litres of solution per minute through a sprinkle angle of 40ºC.

2.3 Steam vacuum treatment

Steam vacuum treatment was applied manually to the carcase surface using a handheld device that is connected to a vacuum pump and a steam supply. The system uses filtered steam set at 30 pounds per square inch (psi) or 2-bar corresponding to a carcase application temperature of approximately 134ºC.

2.4 Carcase swabbing

Microbiological swabbing was carried out using sterile abrasive sponge swabs. Four areas were swabbed; neck, brisket, flank, rump. Each site was swabbed horizontally, vertically and diagonally using firm pressure for no less than 20 seconds, covering an area of 100cm^2. Swabs from each animal were pooled together for analysis. Samples were collected from carcases before and after treatment using this method.

2.5 Shelf life study

Carcases treated with lactic acid, steam vacuum and untreated carcases (control) chilled and stored at temperatures between 0-2ºC and swabbed using the methodology outlined in 2.4 at P+1, P+7, P+8 and P+9 days.

2.6 Microbiological Analysis

Aerobic Colony Count (ACC) testing was conducted under sterile conditions in accordance with ISO 4833-1:2013. Enterobacteriaceae testing was conducted in accordance with ISO 21528-2:2017. E.coli testing was conducted in accordance with the ISO 16649-2 (2001) standards.

2.7 Statistical Analysis

Microbial counts were converted to $\log_{10}$ cfu/cm^2. Data was analysed statistically using the paired T-Test. The data obtained before and after treatment were compared for statistical significance. Significance was determined at the $P<0.05$ level.

3.0 Results and Discussion

All data obtained and analysed are with respect to carcases that had a microbial load present on their surface prior to treatment; a value above the lowest detection limit of the method of enumeration; <10 cfu/cm^2.

3.1 The efficacy of the intervention methods in reducing microbial load

3.1.1 Lactic Acid treatment

Table 1: Microbiological data on Lactic Acid Treatment

	Lactic Acid Treatment Data				
	Initial sample set size	Samples showing initial microbial load >10 cfu/cm^2	Samples showing log reduction	Mean log reduction (cfu/cm^2)	Standard deviation
ACC	50	44	44	1.77	0.65
Enterobacteriaceae	50	15	15	1.39	0.97
E.coli	50	12	12	1.33	0.75

Lactic acid application resulted in an ACC mean reduction of 1.77 with a standard deviation of 0.65. Statistical analysis of this data also reported a P-value of <0.05

suggesting the results obtained is highly significant. With regards to Enterobacteriaceae, a mean log cfu/cm^2 reduction of 1.39 with a standard deviation of 0.97 was achieved. Statistical analysis also shows this data to be significantly with a P-value <0.05. Similarly, a log reduction was also noted in relation to generic E.coli counts with a mean log cfu/cm^2 result of 1.33 with a standard deviation of 0.75. This data is also significantly different with a P-value <0.05.

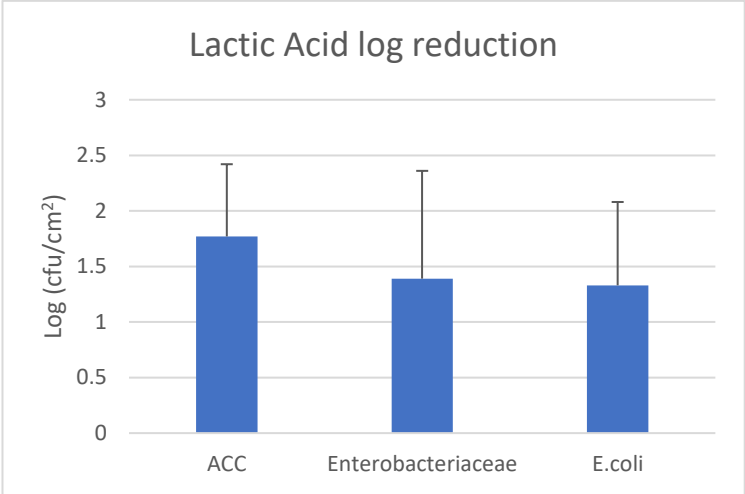

Figure 1: Microbiological reductions in relation to the use of lactic acid on beef carcases.

3.1.2 Steam vacuum treatment

Table 2: Microbiological data on Steam vacuum treatment.

	Steam Vacuuming treatment data				
	Initial sample set size	**Samples showing initial microbial load >10 cfu/cm^2**	**Samples showing log reduction**	**Mean log reduction (cfu/cm^2)**	**Standard deviation**
ACC	45	39	39	0.83	0.71
Enterobacteriaceae	45	7	7	0.55	0.28
E.coli	45	0	0	0	0

Steam vacuum resulted in a ACC mean log (cfu/cm^2) reduction of 0.83 with a standard deviation of 0.71. A P-value <0.05 also shows that the results in relation to ACC reduction for steam vacuum treatment is statistically significant. Enterobacteriaceae mean log cfu/cm^2 reduction of 0.55 was achieved with a standard deviation of 0.28. A P-value<0.05 shows the data is statistically significance. No reduction in E.coli counts were observed as the initial E.coli count on all carcase sampled (n=50) were <10 cfu/cm^2 prior to treatment.

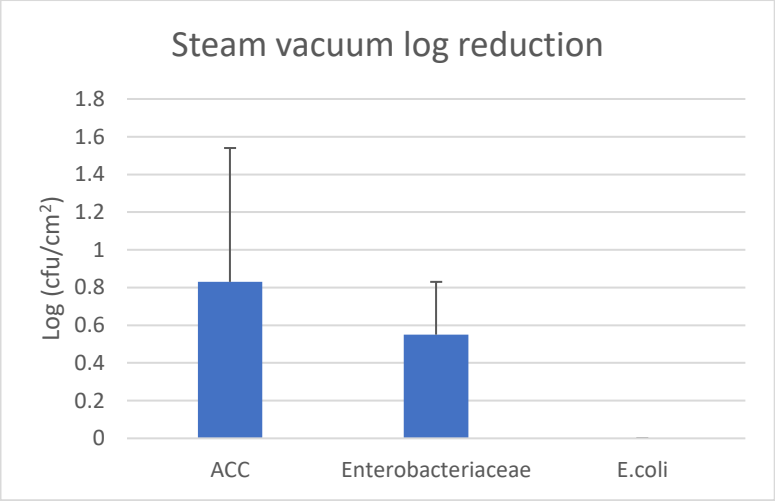

Figure 2: Microbiological reductions in relation to the use of steam vacuum on beef carcases.

The primary variable within the research study directly impacting on sample size showing log reductions was the initial microbial load present on the carcase prior treatment. In general, the microbiological results of carcases prior treatment showed the presence of relatively small microbial counts. Many pre-treatment results reported below the lowest limit of detection for enumeration (<10 cfu/cm^2). This illustrates good manufacturing and hygiene practices are being utilised on the slaughter line during carcases dressing.

3.2 Comparing the efficacy of Lactic Acid and Steam Vac treatments

Lactic acid treatment achieved a relatively larger ACC mean log reduction than steam vacuum treatment. Lactic acid application resulted in a mean difference of 0.94 log cfu/cm^2 ACC reduction when compared to steam vacuum. Lactic acid was also more effective at reducing Enterobacteriaceae counts with a mean log reduction of 1.39 cfu/cm^2 when compared to a reduction of 0.55 cfu/cm^2 achieved by steam vac; a difference of 0.84 log. Lactic acid was also effective at reducing E.coli counts. However, this reduction cannot be compared to steam vacuum treatment as all carcases within the sample set had an initial E.coli count of <10 cfu/cm^2 prior treatment.

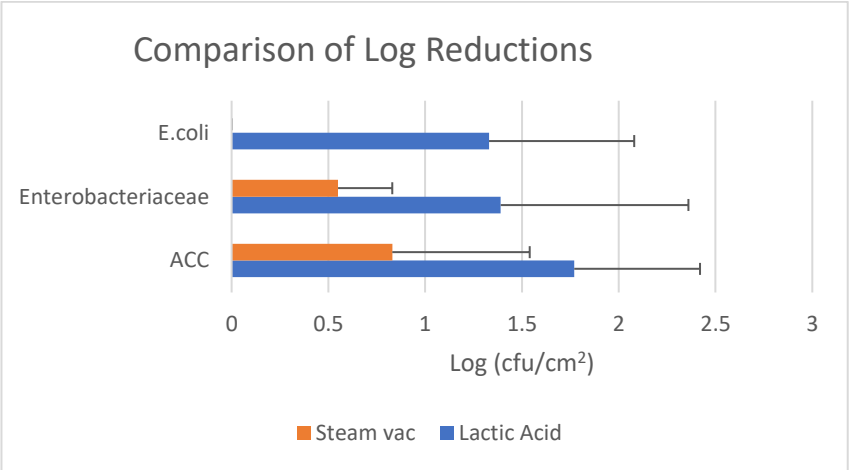

Figure 3: Comparison of microbiological log reductions achieved through intervention methods.

Lactic acid application yielded a higher log reduction than steam vacuum application. Lactic acid can be applied to whole carcases, impacting on the overall microbial load and acts by lowering surface pH and exhibiting residual inhibitory effects when applied which may initially be bactericidal (Rodriguez-Melcon, Alonso-Calleja, Capita, 2017). Although, log reductions were achieved, steam vacuum is an intervention method designed for treating small areas of the carcase. It works by applying high temperatures to the carcase surface and physically removing visible contamination. As steam vacuum is both a physical and thermal treatment, there is no residual effect associated with its use.

3.3 Effects of decontamination technologies on shelf life

The baseline for the shelf life study was established based on the requirements set out in Regulation (EC) 2073/2005 on the microbiological criterial for foodstuff with the acceptability limit set at 5.0 $\log_{10}$ cfu/cm^2.

Table 3: Results from shelf life durability study.

Sample date	Acceptable Limit (M) (log cfu/cm^2)	Control ACC Mean (log cfu/cm^2)	Lactic Acid ACC Mean (log cfu/cm^2)	Steam vacuum ACC Mean (log cfu/cm^2)
P+1	5.00	4.11	1.37	3.50
P+7	5.00	4.21	2.16	4.04
P+8	5.00	5.80	3.98	4.39
P+9	5.00	6.25	4.18	4.40

The control ACC mean was within the acceptable limit at P+ 7 days with a log value of 4.21. However, at P+8 days, the mean value exceeded the threshold with a reported value of 5.8 log cfu/cm^2. The initial mean log cfu/cm^2 of lactic acid treated carcases and steam vacuum treated carcases were 1.37 and 3.50 respectively in comparison to the control microbial load of 4.11. At P+9 days, all results obtained for treated carcases were below the acceptable threshold of 5.0 log with a lactic acid ACC mean of 4.18 log and a steam vacuum ACC mean of 4.40 log. From examining the graph, the lines representing lactic acid and steam vacuum applications suggest a reduction in the rate of microbial growth with the lines on the graph appearing to level somewhat. Based on this observation, it is suggested that product shelf life may be further extended beyond P+9 days, however further testing would be required to validate this hypothesis.

However, based on the scientific data obtained, the shelf life of carcases treated with lactic acid technology or steam vacuum technology prior chilling could be extended to 9 days on the condition that cold chain storage of 0-2ºC is upheld.

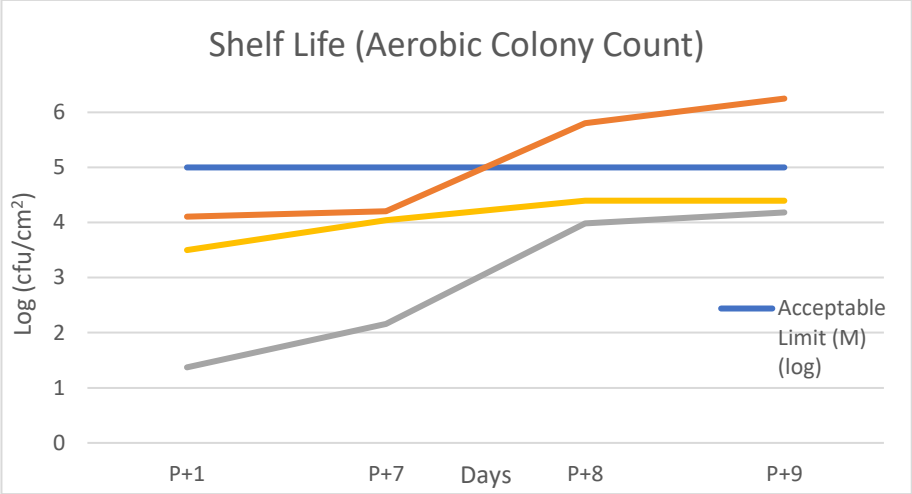

Figure 4: Shelf life results graph

4.0 Conclusion

This study demonstrated that both lactic acid and steam vacuum treatments are effective intervention methods in reducing the microbial load present on beef carcases. The data also shows that lactic acid treatment is more effective at reducing carcase surface microbial load in comparison to steam vacuum treatment. This also supports the fundamental concept that lactic acid treatment is a whole carcase application where steam vacuum is an effective spot treatment decontamination technology.

However, the use of either decontamination treatments as part of an integrated food safety management system has the ability to improve the microbiological condition, extending the shelf life of meat products.

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SECTION 7.0: APPENDICES

Appendix 1: Lactic Acid- Analysis of data (Pre-treatment V. Post-treatment)

Lactic Acid	ACC log diff	Enterobacteriaceae log diff	E.coli log diff
	1.59	3.71	3.49
	0.15	1.00	1.00
	2.39	2.79	1.00
	1.74	2.08	1.78
	1.92	1.00	1.00
	2.18	1.60	1.00
	2.66	2.46	1.48
	1.67	1.78	1.48
	0.99	1.00	1.00
	1.79	1.00	1.00
	1.91	0.60	0.60
	1.98	0.30	1.18
	1.51	0.95	
	1.09	0.30	
	1.59	0.30	
	1.65		
	2.11		
	1.00		
	2.20		
	1.66		
	1.01		
	3.45		
	2.46		
	0.90		
	1.63		
	1.79		
	2.40		
	3.08		
	1.04		
	1.89		
	1.96		
	1.15		
	0.90		
	3.18		
	1.91		
	1.87		
	1.40		
	1.87		
	1.82		

	1.83		
	1.51		
	2.18		
	2.08		
	0.90		
Sample total	**44**	**15**	**12**
Mean	**1.77**	**1.39**	**1.33**
Standard Deviation	**0.65**	**0.97**	**0.75**
P-Value	**0.00**	**0.00**	**0.00**

Appendix 2: Lactic Acid- Aerobic colony count data

Sample Number	Aerobic Colony Count (Pre-lactic) (cfu/cm^2)	Aerobic Colony Count (Post lactic) (cfu/cm^2)	Log Reduction (cfu/cm^2)
1	5.63	4.04	1.59
2	4.69	4.54	0.15
3	5.64	3.26	2.39
4	5.60	3.86	1.74
5	5.38	3.46	1.92
6	5.08	2.90	2.18
7	4.96	2.30	2.66
8	4.94	3.28	1.67
9	4.81	3.82	0.99
10	4.79	3.00	1.79
11	2.91	1.00	1.91
12	2.98	1.00	1.98
13	2.51	1.00	1.51
14	3.20	2.11	1.09
15	2.59	1.00	1.59
16	4.30	2.65	1.65
17	4.11	2.00	2.11
18	2.00	1.00	1.00
19	3.20	1.00	2.20
20	1.00	1.00	0.00
21	2.66	1.00	1.66
22	4.00	2.99	1.01
23	4.45	1.00	3.45
24	3.46	1.00	2.46

25	1.90	1.00	0.90
26	2.63	1.00	1.63
27	1.00	1.00	0.00
28	2.79	1.00	1.79
29	3.40	1.00	2.40
30	1.00	1.00	0.00
31	4.08	1.00	3.08
32	2.04	1.00	1.04
33	2.89	1.00	1.89
34	2.96	1.00	1.96
35	2.15	1.00	1.15
36	1.90	1.00	0.90
37	1.00	1.00	0.00
38	4.18	1.00	3.18
39	2.91	1.00	1.91
40	2.87	1.00	1.87
41	2.40	1.00	1.40
42	2.87	1.00	1.87
43	1.00	1.00	0.00
44	2.82	1.00	1.82
45	2.83	1.00	1.83
46	1.00	1.00	0.00
47	2.51	1.00	1.51
48	3.18	1.00	2.18
49	3.08	1.00	2.08
50	1.90	1.00	0.90

Appendix 3: Lactic Acid- Concentration and Temperature results applied during Sampling

Sampling Duration	Concentration (%)	Temperature (°C)
Week 1 Day 1	2.2	36.7
	2.5	36.8
	2.3	36
	2.4	36.7
	2.4	36.3
	2.2	37.1
	2.3	36.4
Week 1 Day 2	2.7	36.5
	2.2	36.4
	2.1	36.7
	2.2	36.3
	2.1	37.2
	2.2	37
	2.2	37.5
	2.3	36.7
Week 2 Day 1	2.1	36.9
	2.2	36.9
	2.2	37.5
	2.2	37.7
	2.2	36.9
	2.2	37.8
Week 2 Day 2	2.3	36.5
	2.1	36.8
	2	36.4
	2.2	36.9
	2.3	36.8
	2.2	37.1
Week 3 Day 1	2.3	37.4
	2.1	36.8
	2.3	36.5
	2.5	36.8
	2.4	37.2
	2.3	37.6
	2.2	37.5
	2.3	36.4
Week 3 Day 2	2.2	36.8
	2.3	36.9
	2.2	36.7
	2	37.4
	2	37.7
	2.1	36.9
Week 4 Day 1	2.1	37.5

	2.2	37.2
	2.1	36.8
	2.1	36.5
	2.2	37.2
	2.4	36.9
	2.3	38
Week 4 Day 1	2.1	36.8
	2.4	37.1
	2.4	37.3
	2.5	37.6
	2.5	37.6
	2.1	36.9
	2.3	36.2
Week 5 Day 1	2.2	36.7
	2.4	36.5
	2.5	37
	2.4	36.8
	2.2	36.6
	2.8	36.5
	2.7	37.1

Appendix 4: Lactic Acid- E. coli data

Sample Number	E. coli (Pre-lactic) (cfu/cm^2)	E. coli (Post lactic) (cfu/cm^2)	Log Reduction (cfu/cm^2)
1	4.49	1.00	3.49
2	2.00	1.00	1.00
3	2.00	1.00	1.00
4	2.78	1.00	1.78
5	2.00	1.00	1.00
6	2.00	1.00	1.00
7	2.48	1.00	1.48
8	2.48	1.00	1.48
9	2.00	1.00	1.00
10	2.00	1.00	1.00
11	1.00	1.00	0.00
12	1.00	1.00	0.00
13	1.00	1.00	0.00
14	1.00	1.00	0.00
15	1.00	1.00	0.00
16	1.00	1.00	0.00
17	1.00	1.00	0.00
18	1.00	1.00	0.00
19	1.00	1.00	0.00
20	1.00	1.00	0.00
21	1.00	1.00	0.00
22	1.00	1.00	0.00
23	1.60	1.00	0.60
24	2.18	1.00	1.18

25	1.00	1.00	0.00
26	1.00	1.00	0.00
27	1.00	1.00	0.00
28	1.00	1.00	0.00
29	1.00	1.00	0.00
30	1.00	1.00	0.00
31	1.00	1.00	0.00
32	1.00	1.00	0.00
33	1.00	1.00	0.00
34	1.00	1.00	0.00
35	1.00	1.00	0.00
36	1.00	1.00	0.00
37	1.00	1.00	0.00
38	1.00	1.00	0.00
39	1.00	1.00	0.00
40	1.00	1.00	0.00
41	1.00	1.00	0.00
42	1.00	1.00	0.00
43	1.00	1.00	0.00
44	1.00	1.00	0.00
45	1.00	1.00	0.00
46	1.00	1.00	0.00
47	1.00	1.00	0.00
48	1.00	1.00	0.00
49	1.00	1.00	0.00
50	1.00	1.00	0.00

Appendix 5: Lactic Acid- Enterobacteriaceae data

Sample Number	Enterobacteriaceae (Pre-lactic) (cfu/cm^2)	Enterobacteriaceae (Post-lactic) (cfu/cm^2)	Log Reduction (cfu/cm^2)
1	4.71	1.00	3.71
2	2.00	1.00	1.00
3	3.79	1.00	2.79
4	3.08	1.00	2.08
5	2.00	1.00	1.00
6	2.60	1.00	1.60
7	3.46	1.00	2.46
8	2.78	1.00	1.78
9	2.00	1.00	1.00
10	2.00	1.00	1.00
11	1.00	1.00	0.00
12	1.00	1.00	0.00
13	1.00	1.00	0.00
14	1.00	1.00	0.00
15	1.00	1.00	0.00
16	1.60	1.00	0.60
17	1.00	1.00	0.00
18	1.00	1.00	0.00
19	1.00	1.00	0.00
20	1.00	1.00	0.00
21	1.00	1.00	0.00
22	1.00	1.00	0.00
23	1.30	1.00	0.30

24	1.95	1.00	0.95
25	1.00	1.00	0.00
26	1.00	1.00	0.00
27	1.00	1.00	0.00
28	1.00	1.00	0.00
29	1.00	1.00	0.00
30	1.00	1.00	0.00
31	1.00	1.00	0.00
32	1.00	1.00	0.00
33	1.00	1.00	0.00
34	1.30	1.00	0.30
35	1.30	1.00	0.30
36	1.00	1.00	0.00
37	1.00	1.00	0.00
38	1.00	1.00	0.00
39	1.00	1.00	0.00
40	1.00	1.00	0.00
41	1.00	1.00	0.00
42	1.00	1.00	0.00
43	1.00	1.00	0.00
44	1.00	1.00	0.00
45	1.00	1.00	0.00
46	1.00	1.00	0.00
47	1.00	1.00	0.00
48	1.00	1.00	0.00
49	1.00	1.00	0.00
50	1.00	1.00	0.00

Appendix 6: Lactic Acid- Pathogenic prevalence data

Sample Number	Salmonella detection (Pre-lactic) cfu/cm^2	E. coli 0157:H7 (Pre-lactic) cfu/cm^2
1	Not detected	Not detected
2	Not detected	Not detected
3	Not detected	Not detected
4	Not detected	Not detected
5	Not detected	Not detected
6	Not detected	Not detected
7	Not detected	Not detected
8	Not detected	Not detected
9	Not detected	Not detected
10	Not detected	Not detected
11	Not detected	Not detected
12	Not detected	Not detected
13	Not detected	Not detected
14	Not detected	Not detected
15	Not detected	Not detected
16	Not detected	Not detected
17	Not detected	Not detected
18	Not detected	Not detected
19	Not detected	Not detected
20	Not detected	Not detected
21	Not detected	Not detected
22	Not detected	Not detected
23	Not detected	Not detected
24	Not detected	Not detected
25	Not detected	Not detected
26	Not detected	Not detected
27	Not detected	Not detected
28	Not detected	Not detected
29	Not detected	Not detected
30	Not detected	Not detected
31	Not detected	Not detected
32	Not detected	Not detected
33	Not detected	Not detected
34	Not detected	Not detected
35	Not detected	Not detected
36	Not detected	Not detected
37	Not detected	Not detected
38	Not detected	Not detected
39	Not detected	Not detected
40	Not detected	Not detected

41	Not detected	Not detected
42	Not detected	Not detected
43	Not detected	Not detected
44	Not detected	Not detected
45	Not detected	Not detected
46	Not detected	Not detected
47	Not detected	Not detected
48	Not detected	Not detected
49	Not detected	Not detected
50	Not detected	Not detected

Appendix 7: Microbiological Test Method Summary

Test Type	Test Organism	Presumptive/ Confirmed	Test Method	Method Ref	Method Summary	Method based on	EC Regulation Compliance	INAB Accredited
Food Enumeration	TVC 48hr	N/A	Pour Plate	SP048	30°C Pour plate using PCA, 48hr incubation	ISO 4833-1: 2013	Yes	Yes
Food Enumeration	Enterobacteriaceae	Presumptive	Pour Plate	SP033	VRBGA pour plate 37°C for 24 hrs	ISO 21528-2:2017	Yes	Yes
Food Enumeration	E. coli	Presumptive	Procedure A – plate count	SP049	TBX pour plate 44°C for 24hrs	ISO 16649 – 2: 2001	Yes	Yes
Pathogen	Salmonella spp	Presumptive	SOLUS Optima ELISA method	SP102	Pre-enrichment in BPW (37°C for 16-20 hrs), selective enrichment in SOLUS RVS (41.5°C for 24 hrs), reading on ELISA	SOLUS Salmonella ELISA test (AFNOR Approved)	Validated as Equivalent	Yes
Pathogen	Salmonella spp	Confirmed	Confirmation	SP178	Serology, Oxidase, API 20E	ISO 6579-1: 2017	Yes	Yes

Pathogen	E. coli 0157	Presumptive	Dynabead method	SP041	Pre enrichment in MTSB (24hrs at 41.5°C), Immuno separation with Dynabead anti E. Coli 0157 and streaking onto CT SMAC (37°C for 18-24hrs).	ISO 16654: 2001/A1:2017	N/A	Yes
Pathogen	E. coli 0157	Confirmed	Confirmation	SP041	Presumptive colonies are sub cultured onto NA (24 hrs for 37°C). Indole test (24 hrs for 37°C) and Latex agglutination kit used to confirm	ISO 16654: 2001/A1:2017	N/A	Yes
Miscellaneous	Sample preparation	N/A	All	SP139	Preparation of food samples (10g or 25g) into a liquid form that can be further diluted as required for standard plate counts and other methods, diluents used include MRD, BPW	BS EN ISO 6887 Parts 1 - 5	N/A	No

					or specific diluents as required by the product.			

Appendix 8: Shelf life -Microbiological data

Control	P+ 1 days			P+ 7 days			P+ 8 days			P+9 days		
	ACC	Enteros	E. coli	ACC	Enteros	E. coli	ACC	Enteros	E. coli	ACC	Enteros	E. coli
Carcase 1	4.46	2.00	2.00	4.51	2.00	2.00	7.56	2.00	2.00	6.46	2.00	2.00
Carcase 2	4.48	2.00	2.00	3.81	2.00	2.00	5.36	2.00	2.00	5.48	2.00	2.00
Carcase 3	3.38	2.00	2.00	4.30	2.00	2.00	5.84	2.00	2.00	5.46	2.00	2.00
	4.11	**2.00**	**2.00**	**4.21**	**2.00**	**2.00**	**5.80**	**2.00**	**2.00**	**6.25**	**2.00**	**2.00**

Steam application	P+1 days			P+7 days			P+ 8days			P+9days		
	ACC	Enteros	E. coli	ACC	Enteros	E. coli	ACC	Enteros	E. coli	ACC	Enteros	E. coli
Carcase 1	4.49	1.00	1.00	4.70	2.00	2.00	3.99	2.00	2.00	4.26	2.00	2.00
Carcase 2	3.00	1.00	1.00	3.70	2.00	2.00	4.67	2.00	2.00	4.15	2.00	2.00
Carcase 3	3.00	1.00	2.00	3.72	2.00	2.00	4.52	2.00	2.00	4.79	2.00	2.00
	3.50	**1.00**	**1.33**	**4.04**	**2.00**	**2.00**	**4.39**	**2.00**	**2.00**	**4.40**	**2.00**	**2.00**

Lactic Acid	P+1 days			P+ 7 days			P+ 8 days			P+ 9 days		
	ACC	**Enteros**	**E. coli**	**ACC**	**Enteros**	**E. coli**	**ACC**	**Enteros**	**E. coli**	**ACC**	**Enteros**	**E. coli**
Carcase 1	1.00	1.00	1.00	2.48	2.00	2.00	4.04	2.00	2.00	4.51	2.00	2.00
Carcase 2	1.00	1.00	1.00	2.00	2.00	2.00	3.67	2.00	2.00	3.90	2.00	2.00
Carcase 3	2.11	1.00	1.00	2.00	2.00	2.00	4.23	2.00	2.00	4.15	2.00	2.00
	1.37	**1.00**	**1.00**	**2.16**	**2.00**	**2.00**	**3.98**	**2.00**	**2.00**	**4.18**	**2.00**	**2.00**

Appendix 9: Steam vacuum - Analysis of data (Pre-treatment V. Post-treatment)

Steam vac	ACC log diff	Enterobacteriaceae log diff
	0.98	0.48
	0.30	0.30
	0.30	0.48
	0.50	0.30
	1.99	0.70
	3.73	0.48
	0.08	1.11
	0.30	
	0.70	
	0.05	
	1.47	
	0.78	
	1.04	
	0.38	
	0.15	
	0.38	
	0.61	
	1.62	
	0.40	
	0.85	
	0.60	
	0.35	
	1.15	
	0.22	
	1.11	
	2.04	
	1.49	
	0.51	
	1.56	
	0.09	
	0.82	
	1.34	
	1.23	
	0.90	
	0.30	
	0.37	
	0.60	
	0.90	
	0.30	
Sample total	**39**	**7**
Mean	**0.83**	**0.55**

Standard deviation	**0.71**	**0.28**
P-value	**0.00**	**0**

Appendix 10: Steam Vacuum treatment- Aerobic colony count data

Sample Number	Aerobic Colony Count (Pre-steam) (cfu/cm^2)	Aerobic Colony Count (Post steam) (cfu/cm^2)	Log reduction (cfu/cm^2)
1	2.28	1.30	0.98
2	1.60	1.30	0.30
3	1.90	1.60	0.30
4	5.23	4.73	0.50
5	5.65	3.66	1.99
6	5.58	1.85	3.73
7	2.57	2.49	0.08
8	2.00	1.70	0.30
9	6.48	5.78	0.70
10	5.57	5.52	0.05
11	2.77	1.30	1.47
12	1.78	1.00	0.78
13	2.04	1.00	1.04
14	2.78	2.40	0.38
15	2.45	2.30	0.15
16	2.08	1.70	0.38
17	2.69	2.08	0.61
18	2.62	1.00	1.62
19	2.00	1.60	0.40
20	2.54	1.70	0.85
21	1.60	1.00	0.60
22	2.43	2.08	0.35
23	2.15	1.00	1.15
24	2.93	2.72	0.22

25	2.89	1.78	1.11
26	3.04	1.00	2.04
27	2.49	1.00	1.49
28	2.41	1.90	0.51
29	1.00	1.00	0.00
30	2.56	1.00	1.56
31	1.00	1.00	0.00
32	2.69	2.60	0.09
33	3.56	2.74	0.82
34	2.34	1.00	1.34
35	2.53	1.30	1.23
36	1.90	1.00	0.90
37	1.30	1.00	0.30
38	1.85	1.48	0.37
39	1.00	1.00	0.00
40	1.00	1.00	0.00
41	1.00	1.00	0.00
42	1.60	1.00	0.60
43	1.90	1.00	0.90
44	1.00	1.00	0.00
45	1.30	1.00	0.30

Appendix 11: Steam vacuum treatment- E. coli data

Sample Number	E. coli (Pre-steam) (cfu/cm^2)	E. coli (Post steam) (cfu/cm^2)	Log Reduction (cfu/cm^2)
1	1.00	1.00	0.00
2	1.00	1.00	0.00
3	1.00	1.00	0.00
4	1.00	1.00	0.00
5	1.00	1.00	0.00
6	1.00	1.00	0.00
7	1.00	1.00	0.00
8	1.00	1.00	0.00
9	1.00	1.00	0.00
10	1.00	1.00	0.00
11	1.00	1.00	0.00
12	1.00	1.00	0.00
13	1.00	1.00	0.00
14	1.00	1.00	0.00
15	1.00	1.00	0.00
16	1.00	1.00	0.00
17	1.00	1.00	0.00
18	1.00	1.00	0.00
19	1.00	1.00	0.00
20	1.00	1.00	0.00
21	1.00	1.00	0.00
22	1.00	1.00	0.00
23	1.00	1.00	0.00
24	1.00	1.00	0.00

25	1.00	1.00	0.00
26	1.00	1.00	0.00
27	1.00	1.00	0.00
28	1.00	1.00	0.00
29	1.00	1.00	0.00
30	1.00	1.00	0.00
31	1.00	1.00	0.00
32	1.00	1.00	0.00
33	1.00	1.00	0.00
34	1.00	1.00	0.00
35	1.00	1.00	0.00
36	1.00	1.00	0.00
37	1.00	1.00	0.00
38	1.00	1.00	0.00
39	1.00	1.00	0.00
40	1.00	1.00	0.00
41	1.00	1.00	0.00
42	1.00	1.00	0.00
43	1.00	1.00	0.00
44	1.00	1.00	0.00
45	1.00	1.00	0.00

Appendix 12: Steam vacuum treatment: Enterobacteriaceae data

Sample Number	Enterobacteriaceae (Pre-steam) (cfu/cm^2)	Enterobacteriaceae (Post steam) (cfu/cm^2)	Log Reduction (cfu/cm^2)
1	1.00	1.00	0.00
2	1.00	1.00	0.00
3	1.00	1.00	0.00
4	1.00	1.00	0.00
5	1.00	1.00	0.00
6	1.78	1.30	0.48
7	1.00	1.00	0.00
8	1.00	1.00	0.00
9	1.00	1.00	0.00
10	1.00	1.00	0.00
11	1.00	1.00	0.00
12	1.00	1.00	0.00
13	1.00	1.00	0.00
14	1.00	1.00	0.00
15	1.00	1.00	0.00
16	1.00	1.00	0.00
17	1.30	1.00	0.30
18	1.00	1.00	0.00
19	1.00	1.00	0.00
20	1.00	1.00	0.00
21	1.48	1.00	0.48
22	1.00	1.00	0.00
23	1.00	1.00	0.00

24	1.00	1.00	0.00
25	1.30	1.00	0.30
26	1.00	1.00	0.00
27	1.00	1.00	0.00
28	1.00	1.00	0.00
29	1.00	1.00	0.00
30	1.00	1.00	0.00
31	1.70	1.00	0.70
32	1.48	1.00	0.48
33	2.11	1.00	1.11
34	1.00	1.00	0.00
35	1.00	1.00	0.00
36	1.00	1.00	0.00
37	1.00	1.00	0.00
38	1.00	1.00	0.00
39	1.00	1.00	0.00
40	1.00	1.00	0.00
41	1.00	1.00	0.00
42	1.00	1.00	0.00
43	1.00	1.00	0.00
44	1.00	1.00	0.00
45	1.00	1.00	0.00

Appendix 13: Steam vacuum -Pathogenic prevalence data

Sample Number	Salmonella detection (Pre-steam)	E. coli detection (Pre-steam)
1	Not detected	Not detected
2	Not detected	Not detected
3	Not detected	Not detected
4	Not detected	Not detected
5	Not detected	Not detected
6	Not detected	Not detected
7	Not detected	Not detected
8	Not detected	Not detected
9	Not detected	Not detected
10	Not detected	Not detected
11	Not detected	Not detected
12	Not detected	Not detected
13	Not detected	Not detected
14	Not detected	Not detected
15	Not detected	Not detected
16	Not detected	Not detected
17	Not detected	Not detected
18	Not detected	Not detected
19	Not detected	Not detected
20	Not detected	Not detected
21	Not detected	Not detected
22	Not detected	Not detected
23	Not detected	Not detected
24	Not detected	Not detected
25	Not detected	Not detected
26	Not detected	Not detected
27	Not detected	Not detected
28	Not detected	Not detected
29	Not detected	Not detected
30	Not detected	Not detected
31	Not detected	Not detected
32	Not detected	Not detected
33	Not detected	Not detected
34	Not detected	Not detected
35	Not detected	Not detected
36	Not detected	Not detected
37	Not detected	Not detected
38	Not detected	Not detected
39	Not detected	Not detected
40	Not detected	Not detected
41	Not detected	Not detected
42	Not detected	Not detected

43	Not detected	Not detected
44	Not detected	Not detected
45	Not detected	Not detected