The Effect of Cranberry Pomace Extracts on the Growth of Meat Starter Cultures and the Subsequent Effect on Dry Fermented Sausage Safety and Quality Characteristics

by

Tsun Yin Alex Lau

A Thesis
presented to
The University of Guelph

In partial fulfilment of requirements
for the degree of
Master of Science
in
Food Science

Guelph, Ontario, Canada
© Tsun Yin Alex Lau, April, 2019
The Effect of Cranberry Pomace Extracts on the Growth of Meat Starter Cultures and the Subsequent Effect on Dry Fermented Sausage Safety and Quality Characteristics

Tsun Yin Alex Lau
University of Guelph, 2019

Advisors:
Dr. Shai Barbut
Dr. S. Balamurugan

The effect of cranberry pomace extracts on the growth of meat starter cultures, inactivation of Salmonella enterica serovars, and physicochemical characteristics associated with dry fermented sausages (DFS) were studied. Species belonging to genus Lactobacillus, and Pediococcus demonstrated concentration-dependent growth stimulation and better growth performance than Staphylococcus spp. at equal CE levels in the growth assay study. At higher CE levels (i.e. ≥ 1.00% wt/vol), all starter cultures showed reduced growth which could mainly be related to the increased acidity and phenolic content. All pathogens studied showed a higher susceptibility towards CE than the starter cultures. DFS containing a five-strain cocktail of S. enterica serovars at 6.5-log CFU/g, with the addition of different levels of CP (control, 0%; low, 0.55%; medium, 1.70%; high, 2.25% wt/wt), or liquid lactic acid were manufactured, and subjected to conventional fermentation and drying conditions. Addition of low CP levels not only resulted in effective pathogen load reduction, but also yielded DFS with physicochemical, color, and textural characteristics most similar to conventional DFS.
ACKNOWLEDGEMENT

First, I would like to thank and express my gratitude, first and foremost, to my advisors, Dr. Shai Barbut and Dr. S. Balamurugan for their guidance, continual support and patience throughout my two-years study. My master study would not have been possible without you two. Thank you Dr. Barbut for all the advice and knowledge you have given during my study, and the opportunity to help with the meat processing course. Thank you Dr. Bala for guiding me into an independent researcher and being patience whenever I ran into difficult situations. I have learnt many things from both of my advisors and I am very grateful that I have the opportunity to pursue my MSc degree under their supervision. I would also like to thank Dr. LaPointe as my committee member for her advice and ideas.

Second, I would like to thank all the members of my lab: Phil, Laura, Madison and Mubashira. Thank you for your help on those long sausage setup and sampling days. I enjoyed every lab gathering we had and it has been my honor to work with your guys. I would also like to thank everyone at Agriculture Agri-Food Canada (AAFC) who have given me assistance and technical support.

Lastly, I want to thank my parents and all my friends, who have never doubted me over the past two years. Thanks to my parents for their encouragement, unconditional support and love throughout the two years. Without them, I would have not decided to pursue my interest in the field of food science. Thank you for always being there whenever I need.
# Table of Contents

ABSTRACT .........................................................................................................................ii
ACKNOWLEDGEMENT .........................................................................................................iii
TABLE OF CONTENTS ...........................................................................................................iv
LIST OF TABLES ...................................................................................................................vii
LIST OF FIGURES ...............................................................................................................viii
CHAPTER 1 INTRODUCTION .................................................................................................1

CHAPTER 2 LITERATURE REVIEW ......................................................................................3

2.1 Introduction to Cranberry .............................................................................................3

2.2 Bioactive Phenolic Compounds in Cranberry .............................................................4

2.2.1 Flavanols ..................................................................................................................4

2.2.2 Phenolic Acids ..........................................................................................................5

2.2.3 Anthocyanins ...........................................................................................................6

2.2.4 Proanthocyanidins (PACs) .....................................................................................8

2.2.5 Flavonols ..................................................................................................................9

2.3 Health Implications of Cranberry ...............................................................................10

2.3.1 Antidegenerative .....................................................................................................10

2.3.2 Anticancer ...............................................................................................................11

2.3.3 Anti-inflammatory .................................................................................................12

2.3.4 Cardiovascular Protection ......................................................................................12

2.3.5 Urinary Tract Health ...............................................................................................13

2.3.5 Antioxidation ...........................................................................................................14

2.4 Antimicrobial Properties of Cranberry Against Human Foodborne Pathogens ..........14

2.4.1 Antimicrobial Mechanisms - Cranberry Phenolic Compounds .............................17

2.5 Effect of Cranberry Phenolic Compounds on Lactic Acid Bacteria ............................19

2.6 Introduction to Cranberry Pomace ..............................................................................21

2.6.1 Cranberry Pomace ..................................................................................................21

2.6.2 Cranberry Pomace Composition ............................................................................22

2.6.3 Application of Cranberry Pomace in Food Systems ...............................................24

2.7 Introduction to Dry Fermented Sausages (DFS) .........................................................25

2.7.1 Dry Fermented Sausage Manufacturing ................................................................25
2.7.2 Dry Fermented Sausage Starter Cultures .......................................................... 26
  2.7.2.1 Lactic Acid Bacteria (LAB) ................................................................. 28
  2.7.2.2 Gram-Positive Catalase-Positive Cocci (GCC+) .................................... 30
2.7.3 Dry Fermented Sausage Associated Risks ...................................................... 31
  2.7.3.1 *Escherichia coli* O157:H7 ................................................................. 31
  2.7.3.2 *Salmonella* ....................................................................................... 32
2.8 Conclusion ............................................................................................................. 34
2.9 References ............................................................................................................ 36

CHAPTER 3 THE EFFECT OF CRANBERRY POMACE ETHANOL EXTRACT ON
THE GROWTH OF MEAT STARTER CULTURES, *ESCHERICHIA COLI* O157:H7,
*Salmonella enteritidis*, AND *LISTERIA MONOCYTOGENES* .................. 44
ABSTRACT .............................................................................................................. 44
3.1 Introduction ......................................................................................................... 46
3.2 Material and Methods ...................................................................................... 49
  3.2.1 Cranberry Pomace 80% Ethanol Extract (CE) Powder ......................... 49
  3.2.2 Bacteria and Growth Condition ............................................................... 49
  3.2.3 Bioscreen Measurements ...................................................................... 50
  3.2.4 pH Effect Analysis ................................................................................ 51
  3.2.5 Statistical Analysis ................................................................................ 51
3.3 Results and Discussion .................................................................................... 53
  3.3.1 Effect of CE on the Growth of Meat Starter Cultures ............................ 53
    3.3.1.1 *Lactobacillus* spp. ......................................................................... 53
    3.3.1.2 *Pediococcus* spp. ......................................................................... 56
    3.3.1.3 *Staphylococcus* spp. .................................................................. 57
  3.3.2 Antimicrobial Activity of CE Against Foodborne Pathogens ............... 58
3.4 Conclusion ......................................................................................................... 61
3.5 References ........................................................................................................ 63
3.6 Tables and Figures ............................................................................................ 66

CHAPTER 4 EFFECT OF CRANBERRY POMACE ON THE INACTIVATION OF
*Salmonella enterica* Serovars AND PHYSICOCHEMICAL PROPERTIES
DURING DRY FERMENTED SAUSAGE MANUFACTURING ................................... 73
ABSTRACT .............................................................................................................. 73
4.1 Introduction ....................................................................................................... 75
4.2 Material and Methods........................................................................................................... 77
  4.2.1 Cranberry Pomace Powder ............................................................................................. 77
  4.2.2 Bacteria and Growth Condition ...................................................................................... 78
  4.2.3 Dry Fermented Sausage Production .............................................................................. 79
  4.2.4 Microbiological Analysis .................................................................................................. 81
  4.2.5 Physicochemical Analysis ................................................................................................ 82
  4.2.6 Color Measurement ......................................................................................................... 82
  4.2.7 Textural Analysis ............................................................................................................ 83
  4.2.8 Statistical Analysis .......................................................................................................... 84
4.3 Results and Discussion ........................................................................................................ 85
  4.3.1 Effect of CP on Changes in pH, \(a_w\) and Moisture Protein Ratio ...................................... 85
  4.3.2 Effect of CP on Moisture, Protein, and Fat level .............................................................. 86
  4.3.3 Effect of CP on \(S.\) enterica serovars Inactivation .......................................................... 87
  4.3.4 Effect of CP on Meat Starter Cultures ............................................................................ 89
    4.3.4.1. \textit{Lactobacillus} spp. and \textit{Pediococcus} spp. ......................................................... 89
    4.3.4.2. \textit{Staphylococcus} spp. ......................................................................................... 90
  4.3.5 Effect of CP on Color Parameters .................................................................................... 92
  4.3.6 Effect of CP on Textural Parameters .............................................................................. 93
4.4 Conclusion ............................................................................................................................ 94
4.5 References ............................................................................................................................ 95
4.6 Tables and Figures ................................................................................................................ 99

CHAPTER 5 CONCLUSION AND FUTURE WORK ...................................................................... 111
APPENDIX BACTERIA GROWTH CURVES .................................................................................. 114
LIST OF TABLES

CHAPTER 2

Table 2.1 Cranberry Pomace Proximate Composition.

Table 2.2 Examples of starter cultures species used for dry fermented sausages manufacturing (Adapted from Laranjo et al., 2017).

CHAPTER 3

Table 3.1 Species, strain, and source of the bacteria used in the study.

Table 3.2 Effect of cranberry pomace 80% ethanol extract (CE) on the growth of *Lactobacillus* spp. at selected concentrations under aerobic condition.

Table 3.3 Effect of cranberry pomace 80% ethanol extract (CE) on the growth of *Lactobacillus* spp. at selected concentrations under anaerobic condition.

Table 3.4 Effect of cranberry pomace 80% ethanol extract (CE) on the growth of *Pediococcus* spp. at selected concentrations.

Table 3.5 Effect of cranberry pomace 80% ethanol extract (CE) on the growth of *Staphylococcus* spp. at selected concentrations.

CHAPTER 4

Table 4.1 Serotypes and sources of isolate of the five strain *S. enterica* serovars cocktail used in the study.

Table 4.2 Formulation of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid (% values are based on wt/wt).

Table 4.3 Log reduction number of *S. enterica* serovars in dry fermented sausages (DFS) added with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and drying stages.

Table 4.4 Effect of CP at varying levels on the textural properties of dry fermented sausages (DFS) during fermentation and dry stages.
LIST OF FIGURES

CHAPTER 2

Fig. 2.1 Chemical structure of epicatechin, a type of monomeric flavan-3-ol (Adapted from Blumberg et al., 2013).

Fig. 2.2 General chemical structure of hydroxycinnamic acids (top) and hydroxybenzoic acids (bottom) (Adapted from Blumberg et al., 2013).

Fig. 2.3 Chemical structure of some anthocyanins. R in the structure indicates a point of variation within anthocyanins, and these variations are defined in the table (Adapted from Blumberg et al., 2013).

Fig. 2.4 Chemical structure of A-type PAC (left) and B-type PAC (right) (Adapted from Blumberg et al., 2013).

Fig. 2.5 Chemical structure of flavonol 3-hydroxyflavone backbone. R in the structure indicates a point of variation within flavonols, and these variations are defined in the table (Adapted from Blumberg et al., 2013).

Fig. 2.6 Possible interactions of polyphenols with cell wall components and plasma membrane of Gram-negative and Gram-positive bacteria (Adapted from Papuc et al., 2017).

CHAPTER 3

Figure 3.1 Effect of cranberry pomace extract (CE) at varying concentrations (wt/vol) and acidic modified dextrose-free TSB (mTSB) on the growth of E. coli O157: H7 (A), S. Enteritidis, and L. monocytogenes (C). Growth of selected starter cultures at 0.75% wt/vol CE were also shown. pH 4.5 mTSB has a pH value equivalent to 1.00% wt/vol CE in mTSB. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation.

CHAPTER 4

Fig 4.1 Changes in pH, aw and Moisture Protein ratio (MPR) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ♦: high CP, 2.25%; ✷: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.
Fig 4.2 Changes in moisture, protein and fat level of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; ×: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.

Fig 4.3 Changes in Lactobacillus spp. (A), Pediococcus spp. (B), and Staphylococcus spp. (C) cell count in dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; ×: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.

Fig 4.4 Changes in exterior lightness L*(A), chroma C* (B), and hue angle h° value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%). Error bars denote ± one standard deviation.

Fig 4.5 Changes in interior lightness L*(A), chroma C* (B), and hue angle h° value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%). Error bars denote ± one standard deviation.

APPENDIX

Figure 1. Effect of CE at varying concentrations (wt/vol) and pH 5.0 modified dextrose-free MRS (mMRS) on the growth of L. sakei FAS 4 (A), L. curvatus FUA 3015 (B), L. plantarum FUA 3073 (C), and L. plantarum UM131L (D) in mMRS under aerobic condition. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.0 mMRS has a pH equivalent to 1.0% wt/v CE in mMRS.

Figure 2. Effect of CE at varying concentrations (wt/vol) on the growth of L. sakei FAS 4 (A), L. curvatus FUA 3015 (B), L. plantarum FUA 3073 (C), and L. plantarum UM131L (D) in modified dextrose-free MRS (mMRS) under anaerobic condition. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation.

Figure 3. Effect of CE at varying concentrations (wt/vol) and pH 5.0 modified dextrose-free MRS (mMRS) on the growth of P. acidilactici UM 104P (A), P. acidilactici FUA 3072 (B), P. pentosaceus UM 116P (C), and P. pentosaceus FUA 3071 (D) in mMRS. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.0 mMRS has a pH equivalent to 1.0% wt/v CE in mMRS.
**Figure 4.** Effect of CE at varying concentrations (wt/vol) and pH 5.4 modified dextrose-free TSB (mTSB) on the growth of *S. carnosus* BBU 001 (A), and *S. xylosus* FUA 3211 (B) in mTSB. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.4 mTSB has a pH equivalent to 0.5% wt/v CE in mTSB.
CHAPTER 1 INTRODUCTION

Cranberry is a rich source of bioactive phenolics that hold excellent antimicrobial activity against prevalent foodborne pathogens such as *E. coli* O157:H7, *Salmonella*, *L. Monocytogenes* etc. Several studies have shown that cranberry phenolics can effectively inhibit pathogenic bacteria without affecting the growth and viability of beneficial microorganisms including lactic acid bacteria (LAB). For these reasons, there is growing interest of using cranberry-derived products as natural alternatives for synthetic preservatives and antibiotics.

Cranberry pomace (CP) an underutilized solid residue from the of the cranberry processing or juicing industry. It contains a high level of dietary fiber, bioactive vitamins and phenolic compounds, such as flavonols, anthocyanins, proanthocyanidins (PACs) etc., that have been shown to exhibit multiple biological health implications. Nonetheless, researches on the application of CP as a functional value-added resource for food models are rare.

Starter cultures for meat fermentation represent a huge segment of beneficial microorganisms commonly used for food production. The use of well-selected strains can greatly improve fermentation predictability and consistency. Species belonging to the genus *Lactobacillus*, *Pediococcus* and *Staphylococcus* are the most commonly used species for meat fermentation. They are not only essential to the microbial, physical and biochemical reactions that occur during meat fermentation and drying but are also important to the final products’ microbiological safety and sensorial characteristics.

Dry fermented sausages (DFS) are a category of ready-to-eat meat products that are manufactured from a mixture of ground meat, animal fat, water, salt, curing salt, spices, and starter cultures (Barbut, 2015). They are not only characterized by its distinctive aroma, texture, and flavor, but are also known for their extended shelf-life. DFS are traditionally manufactured without
thermal processing and chiefly replies on a combination of acidification by lactic acid bacteria fermentation, controlled drying (low aw) and curing agents (e.g. nitrite and nitrates) to achieve preservation. However, consumption of raw DFS have been associated with several pathogens outbreaks and it was later discovered that current hurdles are not sufficient to guarantee adequate pathogen inactivation when present in high levels.

Thus, the main objectives of this study were: i) to examine the effect of CP 80% ethanol extract (CE), a concentrated extract derived from CP, on the growth of commonly used meat fermentation starter cultures, namely *Lactobacillus* spp., *Pediococcus* spp., and *Staphylococcus* spp.; ii) to validate the antimicrobial activity of CE on *E. coli* O157:H7, *S. Enteritidis*, and *L. monocytogenes*; iii) to examine industrial practicality and effect of CP incorporation, as a functional ingredient at varying levels, on *S. enterica* serovars inactivation, starter culture population, and physicochemical properties (i.e. color, texture, composition) associated with DFS.
CHAPTER 2 LITERATURE REVIEW

2.1 Introduction to Cranberry

Cranberry has emerged as one of the super functional foods in the past decades due to their broad nutrient content, strong antioxidant properties and potential health implications. More than 90% of the total world cranberry production is produced in the northeastern part of North America and Canada. In fact, American cranberry was ranked the fifth highest selling plant in North America and annual sales exceed $15 million (Blumenthal et al., 2006). In Canada, cranberries are mainly grown in Quebec and British Columbia (64 and 30%, respectively), with a total gross production of 161,368 tons on 7,369 hectares and an estimated farm gate value of more than CAD $11 million by 2015 (Statistics Canada, 2016). They are commonly found in wooded shrubs that grow in acidic (pH 4-5), infertile and nutrient deficient soil conditions. As a result of their harsh growth conditions, cranberries are abundant in polyphenols which serves as an ecological self-defensive mechanism against microbial and rot infections, oxidative stress, and ionizing radiations.

Cranberry is mainly composed of 80-88% water and 10% carbohydrate, with the remaining being flavonoids, organic acids, minerals and vitamins (Siciliano, 1996). Cranberry has been well-recognized as a rich source of polyphenols, flavonoids, dietary fiber, vitamin A, B, C, calcium, iron, folate, magnesium and manganese (Nile and Park, 2014). However, due to their intrinsic tartness and astringency, cranberries are rarely consumed fresh and are mainly processed as sweetened juices (60%), sweetened dry fruits, and various types of culinary sauce and puree concentrates (Zuo et al. 2002; Vattem et al., 2005).
2.2 Bioactive Phenolic Compounds in Cranberry

2.2.1 Flavanols

Flavanols, known as flavan-3-ols specifically, are complex polyphenol molecules containing more than one aromatic ring. They are characterized by a C₆-C₃-C₆ structure with a hydroxyl group (-OH) in position 3 of the 3-carbon bridge (Fig 2.1). Different constituent units, linkage, degree of hydroxylation, methylation and esterification with phenolic acid gives rise to different variations in flavanol structure and subsequently different biochemical and physical characteristics. Common flavan-3-ols include catechin, catechin gallate (CG), epicatechin, epicatechin gallate (ECG), epigallocatechin gallate (EGCG) and gallocatechin gallate (GCG). Epicatechin and their gallic acid conjugates were reported to be the main phenolic compounds in tea (Yang et al., 2000). Cranberry flavan-3-ols exist as monomers, oligomers, and polymers, with total monomeric and dimeric flavan-3-ol concentrations ranging from 7-33 mg/100g (Gu et al., 2004; Wang and Zuo, 2011). Approximately 85% of the total flavan-3-ols in cranberry are present as oligomers and polymers, which are also referred to as condensed/non-hydrolysable tannins or proanthocyanidins (PACs) (Gu et al., 2004; White et al., 2011). Prior et al. (2001) reported epicatechin was the main constitutional flavan-3-ol unit in cranberry PACs. In fact, the epicatechin to catechin ratio in cranberry PACs varies from species to species and degree of maturation. Numerous studies have suggested flavan-3-ols possess positive health benefits associated with anti-inflammation, anticarcinogen, antimutagen, antiviral, antibacterial, vasodilation, reduction in oxidative stress, cardiovascular disease prevention and inhibition of proliferation of human tumor cell lines.
Fig. 2.1 Chemical structure of epicatechin, a type of monomeric flavan-3-ol (Adapted from Blumberg et al., 2013).

2.2.2 Phenolic acid

Phenolic acids such as hydroxybenzoic acid, hydroxycinnamic acid represent the biggest phenolic fraction in cranberries (Fig 2.2). Zuo et al. (2002) reported cranberries contain approximately 1 g/kg of phenolic acids, with very high contents of benzoic acid. Hydroxybenzoic acid generally has a chemical structure of C₆-C₁ derived from the hydroxylation of benzoic acid. Additional methylation or hydroxylation of the benzene ring creates a diverse range of hydroxybenzoic acids with unique structure and individuality. Zuo et al. (2002), Zhang and Zuo (2004) and Wang and Zuo (2011) reported the hydroxybenzoic acid content in fresh cranberries can be as high as 474–557 mg/100 g. Vanillic acid and p-hydroxybenzoic acids were found out to be the predominant types of hydroxybenzoic acid in cranberries.

<table>
<thead>
<tr>
<th>Phenolic Acid</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>OCH₃</td>
<td>OCH₃</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>
Fig. 2.2 General chemical structure of hydroxycinnamic acids (top) and hydroxybenzoic acids (bottom) (Adapted from Blumberg et al., 2013).

Hydrolysable tannins, which contribute to pigmentation and sensorial astringency, were found to be a polymeric compound of glycosylated monomeric hydroxybenzoic acids, such as gallic acid. In addition, ellagic acids, which are dimers of gallic acids, are present in high concentrations in many berries including strawberries, blackberries and raspberries, but only traceable amount in cranberries.

Hydroxycinnamic acids are phenolic acids that naturally occur in conjugated forms, with p-coumaric, sinapic, caffeic and ferulic acids being the four predominant types of hydroxycinnamic acid found in cranberries (Macheix et al., 1990). Zuo et al. (2002) reported cranberry hydroxycinnamic concentrations in fresh cranberries range from around 8.8 to 25 mg/100g.

2.2.3 Anthocyanins

Anthocyanins are water-soluble glycosidic compounds derived from anthocyanidins. They are the main pigment compounds that are responsible for the red hue of cranberry. Anthocyanins are anthocyanidins with sugar moieties attached to position 3 of the 3-carbon bridge connecting ring A and B (Fig 2.3). They are responsible for the appealing red color of cranberries and corresponding
processed products, as well as the potential health implications on human health. Cranberries contain a significant level of anthocyanins and their content increases with their degree of maturation. Pappas and Schaich (2009) reported fresh cranberry anthocyanin concentrations ranging from 13.6 to 171 mg/100g. Anthocyanins possess strong antioxidant capacity and are the most abundant antioxidant phenolic compounds in cranberries (Wang et al., 1997). Cranberries contain 13 kinds of anthocyanins, with 3-O-galactosides and 3-O-arabinosides of cyanidin and peonidin being the major anthocyanins (Pappas and Schaich, 2009; Côté et al., 2010; Ross et al., 2017). Similarly, Prior et al. (2001) reported cyanidin-3-O-glycosides comprise of close to 55% of the total cranberry anthocyanins.

![Chemical structure of some anthocyanins](image)

**Fig. 2.3** Chemical structure of some anthocyanins. R in the structure indicates a point of variation within anthocyanins, and these variations are defined in the table (Adapted from Blumberg et al., 2013).

<table>
<thead>
<tr>
<th>Anthocyanin</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peonidin</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OCH₃</td>
<td>OCH₃</td>
</tr>
<tr>
<td>Delphindin</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Petunidin</td>
<td>OCH₃</td>
<td>OH</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

Both intrinsic and extrinsic factors including chemical structure, pH, temperature, light, oxygen, metal ion complexation, intramolecular and intermolecular associations (e.g. tannin stabilization) can hugely alter the color, solubility, reactivity, and stability of anthocyanins. Thus, processing conditions and other food matrix components play a big role in the bioaccessibility, bioavailability, and bioactivity of anthocyanins in our diet. It is estimated that approximately 30 – 40% of the cranberry anthocyanins are lost during multi-step processing via thermal degradation,
activation of endogenous enzymes such as polyphenol oxidase and peroxidase and the removal of polyphenol-rich cranberry skins and seeds (Blumberg et al., 2013).

2.2.4 Proanthocyanidins (PACs)

Proanthocyanidins (PACs) are high molecular weight polymers of flavan-3-ols such as catechin, epicatechin, and their gallic acid esters. They are commonly referred to as condensed tannins or non-hydrolysable tannins. Cranberries typically contain about 133-367 mg/100g of total PACs (Gu et al., 2004; Grace et al., 2012). PACs have a high affinity of forming polyphenol-protein complexes. As a result, through the formation of complexes with salivary protein, PACs are responsible for the astringent character delivered from certain fruits and beverages (e.g. wine, tea).

![Chemical structure of A-type PAC (left) and B-type PAC (right)](Adapted from Blumberg et al., 2013).

Two types of PACs are present in cranberries, A-type PACs and B-type PACs (Fig 2.4). Foo et al. (2000) and Feliciano et al. (2012) suggested A-type PACs account for around 51–91% of total PACs in cranberries. A-type PACs are characterized by a single C-C bond between C₄ of
the upper unit and C₈ or C₆ of the lower unit, with an additional ether bond between C₂ of the upper unit and the hydroxyl group at C₇ of the lower unit. In contrast, B-type PACs do not possess the additional ether linkage. As a result of their difference in chemical structure, their biological properties vary significantly. Howell et al. (2005) reported A-type PACs were more effective than B-type PACs in inhibiting the adherence and colonization of pathogenic bacteria to urological cell surfaces. In fact, A-type PACs were considered the major cranberry bioactive component responsible for human urinary tract health (Howell et al., 1998; Howell et al., 2001).

2.2.5 Flavonols

Flavonols are a class of polyphenol that are characterized by their 3-hydroxyflavone backbone (Fig 2.5). Flavonols naturally present in foods are o-glycosides, with D-glucose being the predominant sugar moiety. Fresh cranberries have been considered as the richest fruit source for dietary flavonols, with concentrations ranging from 20-40 mg/100g (Harnly et al., 2006; Pappas and Schaich, 2009). Cranberry flavonols mainly exist in glycosides of quercetin and myricetin. Flavonols in plants are the main biological antioxidants that serve to protect the plant from reactive oxygen species (ROS) and various infections. As a result, their potential roles in human oxidative stress protection are widely studied. Quercetins neutralize ROS via donating a proton and self-stabilizing the quercetin radicals generated through resonance. As a result of its strong proton donating and radical neutralizing affinity, quecertin and its glycosides are widely studied in human health clinical trials for they potential in oxidative stress protection. Several medical reports have pointed out the chemopreventive ability of plant flavonols in suppressing the proliferation of cancer and tumor cells in vitro (Choi et al., 2001; Lee et al., 2004).
2.3 Health Implications of Cranberry Bioactives

The health benefits associated with cranberry varieties and their corresponding extracts have been of interest for decades. Numerous studies have explored the health implications provided by cranberry phenolic compounds, especially flavonols and anthocyanins, and their potential roles in the chemoprevention of diseases associated with oxidative stress and inflammation, including cardiovascular, degenerative illnesses, cancers and other chronic diseases both in vivo and in vitro. (Manach et al., 2004; Konczak and Zhang, 2004; McKay and Blumberg, 2007; Blumberg et al., 2013).

2.3.1 Antineurodegenerative

Oxidative stress is generally considered as a risk factor in the development of many neurodegenerative diseases. Neto et al. (2005) found out that rat brain neurons treated with whole cranberry extracts experienced 43% less necrosis induced by oxidative stress compared to controls. The presence of cranberries, anthocyanins, and flavonols (e.g. quercetins) were able to slow down
ROS accumulation and cell death in an effective manner (40% at 100 µg/mL), and provide positive effects on neurodegeneration prevention.

2.3.2 Anticancer

An evident amount of research has shown that cranberry exhibits cancer-fighting activity as a result of its abundance in phenolic phytochemicals. Cranberry derivatives were able to slow down cancer cell line proliferation, reduce cell density and viability in numerous in vitro medical trials (Kandil et al., 2002; Ferguson et al., 2004; Weh et al., 2016). Cranberry flavonols, specifically myricetin quercetins, are well-known for their superior anticancer property and were suggested to be the major phenolic fraction responsible for its anticancer activity. Multiple medical studies have pointed out that cranberry quercetins strongly inhibited the proliferation of human breast, colon, liver, bladder, and leukemia cancer cell lines in vitro via anti-inflammatory actions (Murphy et al., 2003; He and Liu, 2006; Vu et al., 2012; Prasin et al., 2016). Cranberry PACs, which protects human urinary tract from uropathogenic infections, have also been reported to exert inhibitory activity against human cancer cell proliferation isolated from lung, colon, and leukemia (Seeram et al., 2004; Neto et al., 2006). Although there is little knowledge regarding the effect and action of cranberry anthocyanins against human tumor cell proliferation, it is believed they may play a critical role in carcinogenesis inhibition and oxidative stress development. Ursolic acid and its ester derivatives in cranberry peels have also been reported to inhibit proliferation of human liver and breast cancer cells in vitro (He and Liu, 2006; Kondo, 2011).
2.3.3 Anti-inflammatory

Cranberry phenolic compounds have been shown to retard the development of oxidative stresses, radical species and inflammations in human endothelial cells in vitro. For instance, Kowalska and Olejnik (2016) reported that lyophilized cranberry extracts were able to lower the secretion of pro-inflammatory cytokines and down-regulated pro-inflammatory gene expressions in 3T3-L1 adipocytes that results in obesity and metabolic-related illness. Youdim et al. (2002) found that both intracellular H$_2$O$_2$-induced damage and cell membrane fatty acid oxidation were suppressed in treatments supplemented with cranberry anthocyanins and/or hydroxycinnamic acids. Human cardiovascular inflammation mediators such as interleukin (IL)-8, intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) also showed reduction and down-regulation after cranberry anthocyanin treatments.

2.3.4 Cardiovascular protection

Cranberries also offer tremendous beneficial implications to cardiovascular health. For instance, polyphenols in cranberry juice may promote vasodilatation and lessen the formation of blood clots commonly associated with the decreased flexibility of arterial walls in diabetes and hypertension patients (Reed, 2002; Blumberg et al., 2013, Khoo and Falk, 2014). Daily consumption of low-calorie cranberry juice had also been found to improve the plasma high-density lipoprotein (HDL) level in overweight individuals (Ruel et al., 2006; Chew et al., 2018). A clinical trial carried out by Vinson (2003) studied the cholesterol-lowering effect of cranberry juice in 19 high cholesterol individuals. No overall change in plasma cholesterol level was observed but plasma antioxidant capacity and HDL level was increased by 121% and 10% respectively at the end of the study. Such marked increase in biological markers is equivalent to
about a 40% reduction in the development of cardiovascular diseases. Similarly, Basu et al. (2011) reported that consumption of low-energy cranberry juice can reduce lipid oxidation development and increase plasma antioxidant capacity in women with metabolic syndrome.

2.3.5 Urinary Tract Health

Numerous studies had illustrated the significance of cranberry phenolics, especially A-type PACs in promoting urinary tract health \textit{in vivo} and \textit{in vitro}. These clinical data revealed that cranberry PACs hold strong uropathogenic bacterial anti-adhesion activity and are responsible for preventing uropathogenic bacteria colonization (Howell et al., 2005; Krueger et al., 2013). In an animal model study carried out by Howell et al. (2001), mice fed with drinking water containing isolated cranberry PACs demonstrated bacterial anti-adhesion activity in their urine. Ibrahim et al. (2008) similarly reported that cranberry extracts were capable of inhibiting \textit{E. coli} O157:H7 adhesion in urinary tract infected rats. In another study by Howell et al. (2005) found that urine samples from human subjects solely fed with cranberry juice cocktail exhibited \textit{E. coli} anti-adhesion activity up to 8 h after consumption. The study also confirmed that cranberry acidity does not necessarily contribute to uropathogen anti-adhesion as similar anti-adhesion phenomenon was noticed in pH neutralized urine samples. Nonetheless, the high acidity of cranberry remains critical in inhibiting bacteria growth. Similar inhibitory activity was reported for blueberry juice PACs by Ofek et al. (1996), and therefore, it is desirable to increase daily consumption of cranberry or other berry varieties in order to maintain urinary tract health.
2.3.6 Antioxidation

Cranberry has also been demonstrated to slow down low-density lipoprotein (LDL) oxidation, neutralize ROS and improve plasma antioxidant capacity in various clinical trials (Duthie et al., 2006; Basu et al., 2011). Ruel et al. (2005) similarly reported a reduction in plasma LDL and an increase in antioxidant capacity in healthy individuals after consumption of cranberry juice for 2 weeks. Furthermore, a clinical study (Pedersen et al., 2000) conducted on 9 healthy subjects between the ages of 23 and 41 revealed that the consumption of cranberry juice significantly increased (p < 0.05) the plasma antioxidant capacity, ascorbic acid content and total phenol content.

2.4 Antimicrobial Properties of Cranberry Against Human Foodborne Pathogens

Cranberry is a rich source of bioactive phenolics and organic acids that display excellent antimicrobial activity against human foodborne pathogens, and thereby hold the potential for being used as an alternative for synthetic preservatives and antibiotics (Puupponen-Pimia et al., 2001; Puupponen-Pimia et al., 2005; Nohynek et al., 2006; Wu et al., 2008; Wu et al., 2009; Côté et al., 2011; Caillet et al., 2012; Lacombe et al., 2013). For instance, a study carried out by Caillet et al. (2012) examined the antimicrobial properties of 30 High Performance Liquid Chromatography (HPLC) purified fractions obtained from various cranberry products (i.e. cranberry juice, press cake) against common foodborne pathogens, including *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes*. Results showed that cranberry components possess good antimicrobial capacities; four fractions rich in apolar phenolic compounds effectively inhibited all bacteria with minimum inhibitory concentration (MIC) below 10 mg phenol/mL, and 25 fractions completely inhibited microbial growth with MICs below 100
mg phenol/well. Wen et al. (2003) also found that phenolic acids in cranberries hold antimicrobial effect against *L. monocytogenes*, and the effectiveness is pH-dependent. Furthermore, Wu et al. (2008) reported a 3 - 8-log CFU/ml reduction in *E. coli* O157:H7, *L. monocytogenes*, *S. Typhimurium*, and *S. aureus* inoculated in Brain Heart Infusion (BHI) broth containing 100µl/ml cranberry concentrate after 5 days. All four pathogens were also reduced to an undetectable level in the presence of distilled water containing 100µl/ml cranberry concentrates after 24 h. More importantly, their pH effect analysis indicated that at the same pH level, cranberry concentrate showed greater antibacterial effects than the acidic solution. This hypothesized that both cranberry acidity and phenolics play a significant role in pathogen inactivation.

Furthermore, it was suggested that bacteria with different structural makeup hold different sensitivity towards cranberry components. Puupponen-Pimiä et al. (2005) found that Gram-negative pathogens being more resistant to cranberry phenolics than Gram-positive pathogens, and could perhaps be linked to their difference in cell wall structure and membrane permeability. Nohynek et al. (2006) suggested that lipopolysaccharides (LPS) mainly found in Gram-negative bacteria are able to hinder polyphenols from binding to the peptidoglycan layers of these microorganisms, hence impart higher resistance. Similarly, Wu et al. (2008) and Lacombe et al. (2013) reported that Gram-positive *L. monocytogenes* being more susceptible to phenolics of cranberry origin than Gram-negative *E. coli* and *Salmonella* as characterized by their lower minimum inhibitory concentration (MIC). Caillet et al. (2012) also reported a higher sensitivity and lower MIC of *L. monocytogenes* towards 20 HPLC purified cranberry fractions. However, the actual mechanisms that cause such difference in susceptibility remain poorly understood.

Nonetheless, the majority of the studies investigating the antimicrobial activity of cranberry and its extracts were *in vitro*. Research that involves food systems where other food matrix
components and/or processing parameters (e.g. time, temperature) are taken into account are lacking.

Wu et al. (2009) examined the antimicrobial effect of cranberry concentrates at different concentrations (2.5%, 5.0%, and 7.5% w/w) in commercial ground beef patties inoculated with 6-log CFU/g *E.coli* O157:H7. They found that *E.coli* O157:H7 was reduced by 0.4 - 2.4-log CFU/g after 5 days, and the degree of inactivation was directly correlated to the incorporation level. As for product quality, sensory evaluation results suggested that cranberry concentrates at a level of 5% or below would not negatively affect the sensorial characteristics (e.g. color, taste, odor) of ground beef patties.

Similarly, Ingham et al. (2006) found that cranberry juice (15% v/v) effectively reduced the numbers of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* by > 5-log CFU/ml in unpasteurized apple cider. The enriched product also obtained a positive score for its acceptability.

Furthermore, Xi et al. (2012) reported a significant inhibition of *L. monocytogenes* in naturally-cured frankfurters supplemented with cranberry powder. Application of commercial cranberry powder at 3% w/w effectively reduced post-cooking inoculated *L. monocytogenes* by 5.3 log CFU/g in vacuum packed frankfurters stored at 4°C after 7 weeks. However, researchers also noticed a darker purplish color, lower firmness, and lower consumer acceptability scores in frankfurters supplemented with > 1% cranberry powder. In their case, it is possible that such adverse changes in color and texture were a result of the nature of finely comminuted products and the high acidity from the cranberry powder. In short, application of cranberry concentrates at proper level may serve as an additional food safety hurdle without altering acceptability and other sensorial characteristics. However, more research is needed to identify how cranberry compounds
can be added to food systems as well as optimal levels as their impact on products’ quality seems to vary among food models.

2.4.1 Antimicrobial Mechanisms - Cranberry Phenolic Compounds

It has long been hypothesized that the high acidity of cranberry is responsible for its antimicrobial properties as it creates a sublethal osmotic stress that causes irreversible damage to bacteria cell membrane (Wen et al., 2003; Lin et al., 2004; Wu et al. 2008). In fact, it was later suggested that other bioactive phenolic compounds are also responsible for the inactivation of pathogenic bacteria. Specifically, it was suggested that cranberry phenolic compounds can destabilize bacteria cell wall, depolarize bacteria cell membrane and induce subsequent cell lysis, as well as structural disintegration. Possible interactions of cranberry polyphenols with cell wall components and the plasma membrane of Gram-negative and Gram-positive bacteria are shown in Fig 2.6.

For instance, Vattem et al. (2005) found that phenolics with partial hydrophobicity could efficiently embed in the bacterial membrane-water interface and alter membrane surface charge, thereby impairing essential cell membrane functions and transport processes. Nohynek et al. (2006) reported that PACs were able to disintegrate membrane LPS, weaken bacteria outer membrane, alter outer membrane fluidity, and induce subsequent cell disruption. Johnson et al. (2008) similarly found that PACs are capable of destabilizing bacteria outer membrane via chelating metal ion-mediated membrane components and complexing membrane protein. Wu et al. (2008) showed that pH neutralized cranberry phenolic concentrates were able to induce bacteria cell wall and cell membrane destabilization, and subsequently cause cell lysis as was viewed by transmission electron microscope (TEM) analysis. They suggested that the damaged areas also
facilitate leakage of cellular contents and allowed certain low molecular phenolic compounds to enter the cell and disrupt genetic expressions responsible for encoding outer membrane proteins and cellular metabolisms. Lacombe et al. (2010) also noticed an outer membrane weakening effect of *E. coli* O157:H7 by pH neutralized cranberry phenolics and anthocyanins as a consequence of localized outer membrane disintegration; by using TEM. Other common berry extracts such as low bush blueberry phenolic extracts showed similar destabilization mechanisms as well (Lacombe et al., 2012).

**Fig 2.6** Possible interactions of cranberry polyphenols with cell wall components and plasma membrane of Gram-negative and Gram-positive bacteria (Adapted from Papuc et al., 2017).

Apart from cell membrane destabilization, it was proposed that certain cranberry phenolic compounds could sequester metal ions essential for bacteria growth and metabolism. Guo et al. (2007) examined the metal ion chelating properties of common cranberry phenolic compounds and found that flavonols, especially quercetins, hold strong iron-binding affinity that may help
inhibit bacteria growth by sequestering free iron ions that are crucial for bacterial infection; or by inhibiting critical functions of the cell membrane such as iron-mediated channels. Scalbert (1991) also pointed out that tannins can not only limit the growth of bacteria through metal ion complexation but can also inhibit extracellular microbial enzymes, deprive essential metabolic substrates or hinder cellular oxidative phosphorylation.

In short, it is believed that both molecular and physical damages caused by cranberry phenolics are involved in cell disruption. However, the exact mechanism for individual phenolic fractions remains unclear and debatable.

2.5 Effect of Cranberry on the Growth of Lactic Acid Bacteria (LAB)

Lactic Acid Bacteria (LAB) belonging to the genus *Lactobacillus* are the most widely used LAB in food production and fermentation. It was found that these beneficial bacteria hold higher tolerance to cranberry extracts than pathogenic bacteria. For instance, Puupponen-Pimiä et al. (2001) found that the growth of beneficial bacteria belonging to the genus *Lactobacillus*, such as *L. plantarum*, *L. rhamnosus*, and *L. reuteri*, was not affected by the presence of up to 7.0 mg/well Finnish cranberry extracts. Flavonols, phenolic acids, and anthocyanins standards also displayed lower antimicrobial activity against *Lactobacilli*. Similarly, Lacombe et al. (2013) reported that the growth of *L. rhamnosus* was not significantly inhibited (*P > 0.05*) by the presence of major cranberry fractions, including sugars and organic acids, monomeric phenols, anthocyanins plus PACs, anthocyanins, and PACs. In addition, Sánchez-Patán et al., (2012) reported that up to 1 mg/ml cranberry extracts has no effect on the max OD$_{600}$ of *L. plantarum*. However, the exact mechanisms remained unclear.
While no studies to date investigated the growth-stimulatory role of cranberry on LAB, a few recent studies reported a stimulatory effect of extracts of other berry origins on LAB. Yang et al. (2014) studied the effect of 10% blackberry (*Rubus fruticosus*) juice on the growth of three different strains of *Lactobacillus*, including *L. casei*, *L. plantarum*, and *L. rhamnosus*, and three foodborne pathogens including *L. monocytogenes*, *S. Typhimurium*, and *E. coli* O157:H7. While the growth of pathogens were inhibited significantly, all three *Lactobacillus* exhibited a 1 – 4 log CFU/ml increase in population, suggesting a stimulatory effect of blackberry juice on LAB species. Researchers suggested that *Lactobacillus* spp. were able to utilize blackberry juice carbohydrates and/or phenolic compounds as metabolic substrates.

Molan et al. (2009) also reported that water-soluble blueberry (*Vaccinium asheii Reade*) extracts could enhance the growth of *L. rhamnosus* and *Bifidobacterium breve* inoculated in MRS broth and human fecal specimen. Addition of blueberry extracts to *L. rhamnosus* and *B. breve* at concentrations of 10% and 25% (v/v) resulted in a 2 – 3 log CFU/ml and 1 – 1.5 log CFU/ml increase in cell population, respectively, after 48 h. They suggested that certain blueberry extract compounds such as sugars, fibers, and polyphenols can be utilized by the probiotic cultures as growth substrates; or can reduce the oxidative stress in the growth media and subsequently provide a more favorable environment for bacteria growth.

While carbohydrates are primarily consumed by LAB as growth substrates, couple mechanisms have been hypothesized for the stimulatory actions of phenolic compounds. The first possibility is the ability of LAB to utilize polyphenols as substrates. It has been found that certain *Lactobacillus* spp. are capable of metabolizing polyphenols, such as monomeric flavan-3-ols, PACs and phenolic acids, during growth by means of galloylesterase, decarboxylase and benzyl alcohol dehydrogenase activities, therefore supplying additional energy for growth (Rodriguez et
al., 2008; Tabasco et al., 2011; Sánchez-Patán et al., 2012). Similarly, Vivas et al., (1997) found a growth-stimulatory role of gallic acid and anthocyanins on LAB growing cells. It is possible that LAB are able to cleave anthocyanins and their sugar moieties as an additional carbohydrate source. Another explanation can be linked to the influence of CE polyphenols on cellular metabolisms. Alberto et al. (2001) and (2004) suggested that polyphenols may positively enhance/up-regulate bacteria metabolisms thus improve nutrient utilization. Nonetheless, the stimulatory action of phenolic compounds, especially those of cranberry origin, are poorly characterized and rarely documented.

2.6 Introduction to Cranberry Pomace

2.6.1 Cranberry Pomace

Cranberry pomace (CP) is the main by-product of the cranberry processing or juicing industry. It accounts for approximately 20% of the cranberry and is composed of the pulp, seed, skin and other fruit structures of cranberry. As a large amount of moisture and soluble solids are removed during fresh cranberry pressing, CP and its subsequent extracts are often considered excellent sources of vitamins, dietary fibers, and other cranberry bioactive phenolic compounds such as phenolic acids, flavonols, PACs and anthocyanins (White et al., 2010; Harrison et al., 2013; Ross et al., 2017). These constituent phenolic compounds, which have been reported to exhibit a wide range of potential biological health implications including antioxidant, antimicrobial, antiviral, anticancer and anti-inflammation, hold the potential of being used as a natural resource for food and pharmaceutical applications. However, due to its acidic nature and low protein content, the potential use of CP, as a value-added resource, has rarely been explored and hence often treated as solid fruit wastes for landfill after processing (Vattem and Shetty, 2002).
For this reason, researching the potential use of CP in food models would not only allow the development of low-cost functional ingredients rich in natural bioactive compounds but would also allow maximum utilization of valuable fruit wastes.

### 2.6.2 Cranberry Pomace Composition

To date, as CP it is still considered a relatively novel resource, reports characterizing the chemical composition of CP are quite limited. Table 2.1 shows the data from some of these sources.

<table>
<thead>
<tr>
<th>Crude component</th>
<th>White et al. 2010</th>
<th>Ross et al. 2017</th>
<th>Park and Zhao 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>2.2</td>
<td>4.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Fat</td>
<td>12</td>
<td>5.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>79.6</td>
<td>88.8</td>
<td>85.8</td>
</tr>
<tr>
<td>Insoluble fiber</td>
<td>65.5</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Soluble fiber</td>
<td>6.7</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>Ash</td>
<td>1.1</td>
<td>1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

n.d. denote not determined

As for bioactive phenolics, Ross et al. (2017) reported a total phenolic content of 24.87 mg gallic acid eq./g and 54.35 mg gallic acid eq./g in organic CP and CP 80% ethanol extract, respectively. White et al. (2010) reported a total polyphenolic content of 0.6% in dried CP. Viskelis et al. (2009) similarly reported a total polyphenol content of approximately 0.79-0.90% in cranberry press cakes produced from 4 different American cranberry varieties. In fact, it is important to point out that the variation in phenolic content can be related to numerous reasons such as cranberry variety, season of harvest, preparation method, and chemical composition.
CP is a rich source of complex polymeric polyphenols, particularly condensed tannins or PACs. The tannin content in organic CP and CP 80% ethanol extract (CE) was reported to be 88% (21.86 mg gallic acid eq./g) and 88.5% of the total phenolic content (48.09 mg gallic acid eq./g), respectively (Ross et al., 2017). Harrison et al. (2013) also reported a similar tannin content in pilot-scale extracted CP.

Anthocyanins, which are the main pigments responsible for the redness of cranberry during maturation, are often retained in the pomace and its derived extracts at a high level after pressing. Ross et al. (2017) quantified and analyzed the individual anthocyanins present in Canadian organic CP and CE using HPLC. The total anthocyanin content was reported to be 4.75 and 9.27 mg/g dry matter, respectively, with Peonidin-3-galactose (29.71-33.24%), Cyanidin-3-galactose (22.13-25.31%), Cyanidin-3-arabinose (15.80-17.90%) and Peonidin-3-arabinose (12.34-14.26%) being the main anthocyanins that account for >10% of the total anthocyanins in both extracts. Harrison et al. (2013) also observed a similar trend where CP extracts had a total anthocyanin concentration ranging from 9.87-19.24 mg/g, depending on the ethanol soaking time.

As for natural flavonols, such as quercetin and its glycosides, Ross et al. (2017) reported a flavonol level of 3.08 and 11.74 mg quercetin eq./g dry matter in organic CP and CE respectively. Their reported value for CP extract are in agreement with the values provided by Harrison et al. (2013), where CP ethanol extract flavonol level ranged from 8.76 to 11.24 mg/g dry powder.
2.6.3 Application of Cranberry Pomace in Food Systems

As being a rich source of bioactive phenolic compounds and dietary fiber, CP holds the potential of being utilized as a natural food additive for antioxidant, antimicrobial, colorant, flavoring, and bioactive supplementation purposes. However, to the best of our knowledge, studies examining the potential application of CP as a functional ingredient in food models are rather scarce. For instance, Mildner-Szkudlarz et al. (2016) evaluated the quality of American-style muffins incorporated with CP (10, 20% w/w) under various baking conditions. Enriched samples baked at 240 °C for 15 min resulted in a moister texture than the control, also with incomplete starch gelatinization. Samples baked at 140 °C for 30 min showed a harder texture as a result of distorted protein matrix and starch granules. Intermediate baking at 180 °C for 20 min yielded muffins with the best texture, microstructure, and optimal bioactive compound retention; indicating that the practicality of using CP as a functional food additive is highly affected by processing conditions and other food matrix components.

A study carried out by Gniewosz and Stobnicka (2018) examined the practicality of using CP as an antimicrobial agent in minced pork products. Samples initially inoculated with approximately 5 – 6-log CFU/g *E.coli* O26, *S. Enteritidis*, *L. monocytogenes*, and *S. aureus* showed a log reduction of 4, 4, 2.55, 2.7 log CFU/g, respectively, two days after adding 2.5% water cranberry pomace extract (w-PACE). The appearance of the minced pork was not affected while odor was improved by the addition of 2.5% w-PACE as it probably served as an antioxidant.

Similarly, Tamkutė et al. (2019) reported a significant growth inhibition of *L. monocytogenes* in pork slurry, hamburgers, and cooked ham supplemented with 2% CP during refrigerated storage. While control products showed marked increase in *L. monocytogenes* counts, CP supplemented products showed no significant elevation in pathogen population during 16-40
days of refrigerated storage. The authors also reported an improved oxidative stability and comparable sensorial qualities when compared to the control products.

2.7 Introduction to Dry Fermented Sausage

2.7.1 Dry Fermented Sausage Manufacturing

Dry fermented sausages (DFS) produced from animal tissues, are characterized by their distinctive aroma, texture and flavor, as well as their extended shelf-life. They are commonly manufactured by mixing ground lean meat, animal fat, water, salt, curing salt, spices, and lactic acid bacteria (LAB) (Barbut, 2015). The sausage batter is then stuffed into natural or artificial casings of varying diameters and subjected to fermentation and drying under natural or controlled conditions of relative humidity (RH) and temperature. DFS such as Genoa salami, dry salami, and pepperoni typically have a $a_w \leq 0.9$, $\text{pH} \leq 5.3$, firm texture, and are not heat treated above 28 °C. During fermentation, LAB convert the sugar to lactic acid which leads to a pH decrease from around 5.8 to 5.3–4.6, depending on the amount of fermentable sugars, sausage recipe, and manufacturing conditions (Barbuti and Parolari, 2002). This drop in pH is notably important to the microbiological safety, taste and texture of non-thermally processed DFS. Another group of bacteria, namely *Staphylococcus* spp., will contribute to flavor, color and aroma development as well as nitrite and nitrate reduction through a wide range of biochemical reactions (Laranjo et al., 2016, 2017). The products are later dried under controlled RH and temperature until desired $a_w$ and moisture level (i.e. 25 – 40% for DFS) is achieved. Smoke can further be applied to DFS to inhibit the growth of surface bacteria and molds, delay lipid oxidation, and add desirable smoky flavor and color. DFS are considered as one of the oldest forms of meat preservation and are traditionally manufactured without the use of thermal treatments, thus their preservation mainly
relies on a combination of food safety hurdles including acidification (LAB fermentation), controlled drying (low $a_w$), addition of chemical preservatives and curing agents (Lücke, 1998). Two types of salts are commonly added to DFS, namely, regular salt (NaCl) and curing salt (NaNO$_2$). NaCl contributes to the microbiological safety and shelf-life of DFS by binding water (reduce $a_w$) and hence preventing the growth of unwanted microorganisms. NaCl also plays an important role in the development of sausage texture. Specifically, it facilitates solubilization of meat myofibrillar proteins (e.g. actin, myosins), and by that improves texture via increasing binding properties of these proteins. It also stabilizes meat batter by forming a meat protein film around fat particles (Barbut, 2015). The main function of nitrite is to inhibit the growth of pathogenic bacteria, particularly neurotoxin-producing Clostridium botulinum. Nitrite is also involved in the development of the red curing color, flavor development, and acts as an antioxidant.

### 2.7.2 Dry Fermented Sausage Starter Cultures

DFS can be manufactured with or without adding starter cultures. Naturally-fermented products mainly rely on indigenous meat microflora to achieve acidification and flavor development. Such artisan products are often considered superior to controlled-fermented products applied with industrial starter cultures, in terms of sensorial qualities, as they often show distinctive characteristics from the raw materials and traditional practices (Neffe-Skocińska et al., 2016). While no external cultures are required for fermentation, the use of meat natural microflora is often considered risky and can sometimes led to poor quality products from a manufacturer perspective as it is difficult to ensure the natural microflora is contamination-free (e.g. biogenic amine producers, pathogens) and present at sufficient load for successful fermentation as well as deliver consistent flavor and aroma characteristics (Neffe-Skocińska et al., 2016). For this reason, starter
cultures composed of various strains of LAB and *Staphylococci* are selected, isolated from the best natural fermentations, studied and employed by the meat industry. The strains selected as starter, or protective cultures typically possess favorable technologically functions and/or bacteriocin production capabilities. Meat starter cultures offer numerous advantages including: i) they are well characterized and known for quality and quantity; ii) they can greatly increase product microbiological safety by outcompeting undesirable microorganisms and ensure rapid pH decrease; iii) certain cultures possess accelerated acid production properties hence can reduce the overall manufacturing time; iv) they reduce quality characteristic variabilities (e.g. flavour, aroma) as long as controlled conditions for fermentation and drying are available; v) the use of certain microorganisms can deliver DFS unique organoleptic characteristics (Laranjo et al., 2017). For all of these reasons, the addition of starter cultures has become a common practices in fermented meat products. Table 2.2 summarized some of the commonly used microorganisms in starter cultures and their functions. Most typical commercial meat starter cultures are combined cultures of 2 - 3 strains of LAB and curing bacteria, mainly *Lactobacillus* spp., *Pediococcus* spp., and *Staphylococcus* spp., and are incorporated to the meat batter as an ingredient at levels around 5-8 log CFU/g (Laranjo et al., 2017). These bacteria are responsible for the microbial, physical and biochemical reactions that occur during meat fermentation and drying, such as acidification, biochemical conversions and degradations, and aroma and flavor development.
Table 2.2 Examples of starter cultures species used for dry fermented sausages manufacturing (Adapted from Laranjo et al., 2017)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Genus</th>
<th>Species</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactic Acid Bacteria</strong></td>
<td><em>Lactobacillus</em></td>
<td><em>L. plantarum</em></td>
<td>Acid production</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. pentosum</em></td>
<td>Acid production</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. sakei</em></td>
<td>Acid production</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>L. curvatus</em></td>
<td>Acid production</td>
</tr>
<tr>
<td></td>
<td><em>Pediococcus</em></td>
<td><em>P. acidilactici</em></td>
<td>Acid production/fast fermenting</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P. pentosaceus</em></td>
<td>Acid production/fast fermenting</td>
</tr>
<tr>
<td><strong>Curing Bacteria</strong></td>
<td><em>Kocuria (Micrococcus)</em></td>
<td><em>K. varians</em></td>
<td>Color and flavor</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus</em></td>
<td><em>S. xylosus</em></td>
<td>Curing, color and flavor development</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. carnosus</em></td>
<td>Curing, color and flavor development</td>
</tr>
</tbody>
</table>

2.7.2.1 Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming cocci or bacilli and are the most important groups of microorganisms used in DFS manufacturing. There are numerous LAB species available for use in sausage fermentations, with species belonging to the genus *Lactobacillus* and *Pediococcus* being the most widely researched and actively used. *Lactobacillus* spp. are mainly isolated today from DFS of south European origin, with *L. sakei* and *L. curvatus* being the most dominant species (Rantsiou et al., 2004, 2005; Leroy et al., 2006). In fact, *L. sakei* has been reported to be more competitive than other species and represents 50 -75% of all LAB isolates from artisan fermented sausages (Leroy et al., 2006). Moreover, Dossmann et al. (1996) showed that *L. sakei*, in general, has a shorter adaption phase, a higher maximum growth rate, and a higher cell density than other lactobacilli. Other lactobacilli, including *L. plantarum*, *L. rhamnosus*, *L. pentosus*, have also been identified in minor levels and have gained considerable interest lately due to their probiotic nature and unique technological features (Papamanoli et al.,
On the other hand, *Pediococcus* spp., typically *P. acidilactici* and *P. pentosaceous*, are more commonly used in North European and US style fast fermented sausages with very short production times where a rapid fermentation onset and fast drop in pH are required (i.e. achieve pH <5.3 within 30 h as a minimum) to ensure efficient inhibition of unwanted bacteria (Leroy et al., 2006).

The main function of both *Lactobacillus* spp. and *Pediococcus* spp. is rapid and steady meat batter acidification via the production of lactic acids through sugar fermentation metabolisms. This pH drop is particularly significant as it favors i) product safety and shelf-life by inactivating pathogens and spoilage microorganisms, ii) biochemical conditions to deliver new sensory properties to the end-product, and iii) product integrity and textural development as it induces meat protein coagulation, water expulsion, as well as subsequent formation of a continuous three-dimensional protein matrix (i.e. increase in final product stability, firmness, chewiness, and cohesiveness). However, the primary contribution of LAB to flavor seems to be limited to the development of a tangy and acidic notes via organic acid production (e.g. lactic acid, acetic acid) as in general most of them do not possess high degrees of proteolytic and/or lipolytic activities, as it is the case for *Staphylococcus* spp. (Leroy et al., 2006). In fact, certain novel LAB strains have been selected as meat starter cultures due to their distinctive biochemical features such as fast lactic acid production; growth at different temperatures, salt concentrations, and pH values; moderate proteolytic and lipolytic enzymatic activities; good compatibility with other combined starter cultures. Moreover, several LAB strains, such as the pediocin PA-1, and MCH14 pediocin-producing *P. acidilactici* strain, have been reported as bacteriocin producers and can be utilized to provide bio-protection against undesirable microorganisms (Lücke, 2000; Nieto-Lozano et al., 2010; Zacharof and Lovitt, 2012).
2.7.2.2 Gram-Positive Catalase-Positive Cocci (GCC+)

The use of *Staphylococcus* spp., mainly non-pathogenic Gram-positive, catalase-positive cocci *Staphylococcus* (GCC+), are also commonplace as they contribute to developing unique product characteristic, while ensuring consistency. *S. xylosus* and *S. carnosus* are the most popular Staphylococcal species for meat starter cultures and are often used either separately or in conjunction with LAB; depending on the recipe and sausage style. Specifically, *Staphylococcus* spp. features a wide range of proteolytic and lipolytic enzymes that modulate the development of end-product characteristic flavor and aroma, particularly through the conversion and catabolism of amino acids, peptides, and free fatty acids (Laranjo et al., 2017; Sánchez Mainar et al., 2017). For instance, strains of *S. xylosus* have been associated with the development of a round-aroma via the generation of 3-methyl-1-butanol, diacetyl, 2-butanone, acetoin, benzaldehyde, acetophenone, and methyl-branched ketones, while *S. carnosus* was found to be mainly linked to “sausage aroma” development through the conversion of branched-chain amino acids into methyl-branched aldehydes (e.g. 3-methyl-butanal, 2-methyl-butanal, and 2-methyl-propanal) and their acids (Søndergaard and Stahnke, 2002; Larrouture-Thiveyrat and Montel, 2003). Others have reported that the flavor-developing potential of *Staphylococcus* spp. is exceptionally important in low-salt and low-fat DFS recipes (Lorenzo et al., 2012; Laranjo et al., 2016, 2017). Furthermore, the use of *Staphylococcus* spp. can enhance DFS curing color development, consistency and stabilization through a range of nitrate/nitrite reductase activity (Sánchez Mainar and Leroy, 2015). Their catalase activity has also been reported to play a significant role in peroxide compound decomposition, controlling unsaturated fatty acid oxidation, and reducing off-flavor (Barrière et al., 2001; Sánchez Mainar et al., 2017). Overall the use of well-selected strains of *Staphylococci*
that generate a specific or wide range of flavor and aroma components can potentially enhance overall product quality and uniqueness.

2.7.3 Dry Fermented Sausages Associated Risks

2.7.3.1 *Escherichia coli* O157:H7

*Escherichia coli* O157:H7 (*E. coli* O157:H7) is a rod shaped, Gram-negative, Shiga-toxin producing serotype of *E. coli* (STEC). It is commonly associated with cattle, pigs, and sheep as they are natural healthy carriers of the bacteria. An estimated 10-25% of healthy cattle have been reported to be healthy carriers of STEC. Approximately 265,000 foodborne illness cases, 3,600 hospitalizations and 30 deaths are associated with STEC in the United States every year (CDC, 2019). Around 474 cases of *E. coli* O157:H7-specific infections are also reported in Canada every year (Government of Canada, 2017) About 10-100 cells of STEC is sufficient to cause infection, and symptoms typically appear around 12 – 72 h after exposure (CDC, 2019). Common symptoms include abdominal cramps, minor fever and severe hemorrhagic diarrhea. About 2-7% infected individuals with also develop hemolytic uremic syndrome (HUS), which is a class of acute kidney failure (CDC, 2019). This risk is higher in high-risk groups including children, the elderly, and immunocompromised individuals.

Illnesses cause by *E. coli* O157:H7 can be associated with the consumption of contaminated water and raw food, including fresh produces, raw milk, and under-cooked meat products. As being heat liable microorganisms, *E. coli* O157:H7 in meat products can be destroyed effectively via the use of sufficient heat. However, this poses a significant food safety issue for DFS which are commonly consumed raw, and non-thermally processed in order to retain organoleptic characteristics. Several outbreaks of *E. coli* O157:H7 has been linked to the consumption of DFS
(Williams et al., 2000; MacDonald et al., 2004; Conedera et al., 2007; Sartz et al., 2008). For these reasons, regulatory protocols have been developed by health and government agencies in order to help manufacturers to control \textit{E. coli} O157:H7 in DFS that are dependent on final product pH and \( a_w \) (Health Canada, 2000; CFIA, 2018). Briefly, producers were given five options to control \textit{E. coli} O157:H7 in DFS products, including i) the incorporation of a Health Canada recognized heat process during DFS manufacturing, where the minimal internal temperature of the product must not be lower than 54.4 - 62.8 °C at 121 min to 4 min, respectively; ii) the use of a manufacturing process (i.e. fermentation, heating, drying) that has been scientifically validated to achieve a 5-log reduction of \textit{E. coli} O157:H7; iii) the use of a scientifically validated alternative manufacturing process (i.e. High pressure processing) that has been assessed by Health Canada and proven to inactivate \textit{E. coli} O157:H7 by 5-log; iv) the implementation of a Hazard Analysis and Critical Control Points (HACCP) system at the manufacturing site and a scientifically validated manufacturing process that can achieve a minimum of 2-log reduction; v) the implementation of mandatory end-product microbiological testing on each production lot; sampled product lots have to be held until they were proven to be negative from \textit{E. coli} O157:H7. In fact, Health Canada has put these options in place not only for \textit{E. coli} O157:H7 but also for \textit{S. enterica}, as both pathogens are capable of surviving under standard DFS production conditions.

\textbf{2.7.3.1 \textit{Salmonella}}

Pathogenic \textit{Salmonella} is a Gram-negative, rod-shaped, non-spore-forming bacteria that can be found naturally in the gut, milk and eggs of healthy carriers including chickens, cows and pigs. There are two species of \textit{Salmonella}, namely \textit{Salmonella bongori} and \textit{Salmonella enterica}. \textit{S. enterica} is further classified into six subspecies with over 2,600 serotypes, with \textit{S. Typhimurium}}
and *S.* Enteritidis being the most prevalent (CDC, 2019). In fact, *S. enterica* is the leading cause of foodborne illness and deaths in North America and the second most common foodborne disease in Europe (Hald, 2013). Approximately 1 million foodborne illness cases, 23,000 hospitalizations and 450 deaths are associated with *Salmonella* in the United States annually (CDC, 2019). Salmonellosis is the symptomatic infection caused by *Salmonella* and its infectious dose is estimated to be 1,000 - 100,000 cells. It occurs typically around 12-72 h after exposure, accompanied with symptoms including fever, diarrhea, abdominal cramps, and vomiting. Symptoms can be mild in healthy adults and last from 2-7 days, but in certain high-risk groups, such as infants, children, pregnant women, the elderly, and immunocompromised individuals, *Salmonella* infection can be fatal.

*Salmonella* are typically spread by ingestion of contaminated and undercooked meat, polluted water, and excretions of infected individuals and animals. Hence, it is important to ensure food is cooked to sufficient level (i.e. 63 -74°C) in order to inactivate the pathogen (CDC, 2019). However, as indicated before, this poses food safety concerns for non-thermally processed traditional meat products, such as DFS, which are typically consumed raw. DFS are traditionally manufactured without heating and, as indicated before, their preservation chiefly relies on a combination of low pH, low aw, and addition of certain preservatives. In fact, despite being traditionally regarded as safe and self-stable, several outbreaks associated with *S. enterica* serovars had been linked to the consumption of sliced DFS, and it was later discovered that the combined effect of low aw and low pH was insufficient to remove *S. enterica* serovars when present at high initial levels (Health Canada, 2000; Bremer et al., 2004; Moore, 2004; Hwang et al., 2009) For instance, an outbreak of *S. Typhimurium* involving 63 patients in Lazio Region of Italy was found to be associated with the consumption of raw traditional pork salami (Luzzi et al., 2007). Another
similar outbreak of *S. Typhimurium* was associated with the consumption of raw salami in Lombardia Region of Italy (Pontello et al., 1998). Raw fermented sausages contaminated with *S. Goldcoast*, an uncommon serotype, was also identified as the cause of an outbreak in Germany back in 2001 (Bremer et al., 2001). More importantly, given that *S. enterica* poses a significant food safety risk for un-cooked meat products, validation studies that examine the inactivation of *S. enterica* serovars during DFS manufacturing are not so common.

### 2.8 Conclusion

To summarize, there is emerging interest in utilizing CP and its extracts as natural ingredients to deliver a broad range of functional properties in food products. It is evident that components in cranberry-derived extracts can effectively inhibit foodborne pathogens without negatively affecting the viability of fermentative bacteria. However, studies exploring their practicality, functionality, effectiveness, and optimal incorporation level (i.e. as an additive in different food systems for antimicrobial purpose) are limited. To the best of our knowledge, there are no studies to date examining the effect of berry extracts, specifically of cranberry origin, on the growth of meat fermentation microorganisms such as bacteria belonging to the genus *Lactobacillus, Pediococcus*, and *Staphylococcus*. As previous studies have shown that similar berry-derived extracts (e.g. blackberry, blueberry) can significantly promote the growth of LAB, it would be worthwhile to explore the potential growth stimulatory role of cranberry extracts and their interactions with beneficial bacteria commonly used in food production, notably starter cultures, both *in vitro* and in food systems. Exploring the use of bioactive-rich CP as a value-added ingredient would not only address current food safety concerns associated with fermented meat products, but would also allow the development of economical functional ingredients for meat
product applications, and maximize utilization of valuable fruit resources. Bioactives in CP may also convey health benefits and therefore worth further research.
2.9 References


Tamkutė, L., Gil, B.M., Carballdo, J.R., Pukalskien, M., Venskutonis, P.R., 2019. Effect of cranberry pomace extracts isolated by pressurized ethanol and water on the inhibition of food pathogenic / spoilage bacteria and the quality of pork products. Food Res Int. 120, 38–51.
White, B.L., Howard, L.R., Prior, R.L. 2011 Impact of different stages of juice processing on the anthocyanin, flavonol, and procyanidin contents of cranberries. J. Agric Food Chem. 59, 4692–4698.


CHAPTER 3
THE EFFECT OF CRANBERRY POMACE ETHANOL EXTRACT ON THE GROWTH OF MEAT STARTER CULTURES, ESCHERICHIA COLI O157:H7, SALMONELLA ENTERITIDIS, AND LISTERIA MONOCYTOGENES

ABSTRACT

The effect of cranberry pomace extract (CE), a 80% ethanol extract of cranberry pomace (CP), on the growth of commonly used meat fermentation starter cultures, and selected foodborne pathogens were studied. Ten meat starter culture strains, belonging to genus Lactobacillus, Pediococcus, and Staphylococcus, and three common foodborne pathogens, Escherichia coli O157:H7, Salmonella Enteritidis, and Listeria monocytogenes were grown in either dextrose-free De Man, Rogosa and Sharpe broth, or dextrose-free tryptic soy broth, respectively. Six micro-filtered CE concentrations (0.125, 0.25, 0.50, 0.75, 1.00, and 1.25% wt/vol CE) in 80% vol/vol methanol were used. Changes in bacterial growth at 37 °C was monitored for 48 h by measuring absorbance at 600 nm (OD600); using Bioscreen C. Concentration-dependent growth stimulation was observed for all starter cultures studied. Lactobacillus spp. and Pediococcus spp. demonstrated maximum stimulatory concentration (MSC) at 0.5 - 1.00% wt/vol CE. Unlike Lactobacillus spp. and Pediococcus spp., Staphylococcus spp. was more sensitive to CE with complete growth inhibition observed at concentration of 0.50% wt/vol or higher CE. Reduced growth at higher CE levels could be the combined effect of inherited acidity and increased phenolic content. Gram-positive pathogen was found to be more susceptible to CE than Gram-negative pathogens, with E. coli O157:H7, S. Enteritidis, and L. monocytogenes demonstrated complete inhibition at concentrations of 1.0, 0.75, and 0.5% wt/vol CE, respectively. All pathogens studied showed a higher sensitivity towards CE than the starter cultures. Gram-positive pathogen was found to be
more susceptible to CE than Gram-negative pathogens. Findings suggested that CE can potentially be used as a natural antimicrobial against foodborne pathogens and growth promoter for certain meat starter cultures.
3.1 Introduction

Meat fermentation represents one of the oldest forms of meat preservation and processing technique. Naturally-fermented meat products mainly utilize the indigenous microbiota present in raw materials to achieve acidification and flavor development (Santos et al., 1998). The use of natural starter culture can convey unique and distinctive product characteristics; however, they often vary in load and composition and hence may result in final products with inconsistent qualities (Moretti et al., 2004; Neffe-Skocińska et al., 2016). Therefore, since the beginning of the past century, certain strains showing biochemical features have been selected and isolated from the best artisanal fermentations. These strains have been studied under defined conditions and employed as commercial starter cultures in fermented meat products.

Microorganisms primarily involved in typical commercial starter cultures include species of lactic acid bacteria (LAB) and Staphylococcus. Among LAB of different genera, species belonging to the genus Lactobacillus and Pediococcus are the most widely researched studied. Lactobacillus spp. are mainly isolated from dry fermented sausages (DFS) of south European origin, with L. sakei and L. curvatus being the most dominant species (Rantsiou et al., 2004, 2005; Leroy et al., 2006). Other lactobacilli such as L. plantarum has also been identified lately in novel fermented products. Unlike species belonging to the genus Lactobacillus, Pediococcus spp., notably P. acidilactici and P. pentosaceus, are more commonly used in North European and US style fast fermented sausages, with very short production times to ensure accelerated drop in pH (i.e. achieve pH < 5.3 within 30 h as minimum) (Leroy et al., 2006). In fact, Lactobacillus spp. and Pediococcus spp. are the most important microorganisms in meat fermentation and their primary function is rapid lactic acid production through sugar fermentation, which in turn favors the development of end-product flavor, texture, and microbiological stability (Laranjo et al., 2017).
The use of *Staphylococcus* spp., mainly non-pathogenic Gram-positive, coagulase-negative *S. carnosus* and *S. xylosus*, is also commonplace in meat starter cultures. They contribute to the development of rounded product characteristics by means of color formation and stabilization, limiting lipid oxidation, as well as aroma generation via a range of endogenous enzymatic activities such as nitrate reductase, catalase, protease, and lipase (Talon et al., 1999; Simonová et al., 2006; Laranjo et al., 2017). For these reasons, the viability and growth of starter cultures are not only essential to products’ quality, consistency and safety but also the development of desirable flavor, aroma, and textural characteristics.

Cranberries are rich in organic acids and bioactive phenolics, such as anthocyanins, flavonols, proanthocyanidins (PACs), that have demonstrated antimicrobial activity against foodborne pathogens, including *E. coli* O157:H7, *Salmonella, Staphylococcus aureus, Listeria monocytogenes, Campylobacter jejuni, Helicobacter pylori*, etc. (Puupponen-Pimia et al. 2001; Puupponen-Pimia et al. 2005; Nohynek et al., 2006; Wu et al. 2008; Wu et al. 2009; Lacombe et al., 2010; Côté et al., 2011; Caillet et al., 2012). In fact, recent studies have shown that beneficial microorganisms such as LAB are less sensitive to cranberry phenolics than pathogenic bacteria. For instance, Puupponen-Pimiä et al. (2001) demonstrated that Finnish cranberry extract up to 7.0 mg/well had no effect on the growth of *L. reuteri* and *L. rhamnosus*. Sánchez-Patán et al. (2012) also reported that cranberry extracts up to 1 mg/ml had no influence on the max OD$_{600}$ of *L. plantarum*. Similarly, Lacombe et al. (2013) reported a higher resistance of *L. rhamnosus* towards cranberry monomeric phenolic acids, anthocyanins, and PACs. These evidence strongly suggest that cranberry and its extracts can be used in food systems to control foodborne pathogens without affecting the viability of beneficial bacteria.
Cranberry pomace (CP) is the main solid residue of the cranberry juicing industry and accounts for approximately 20% of the fruit. It is chiefly composed of polyphenol-rich pulps, seeds, and peels of cranberry, and hence often considered as a good source of vitamins, dietary fibers, and other bioactive phenolic compounds such as phenolic acids, anthocyanins, flavonols, and PACs (White et al., 2010; Harrison et al., 2013). In addition, Ross et al. (2017) reported that the phenolic content and antioxidant capacity of crude CP can further be increased by 2-3 times through 80% ethanol extractions. Nevertheless, due to its low protein content and acidic nature, the potential use of CP as a value-added resource was rarely explored and often disposed at landfills as fruit wastes (Vattem and Shetty, 2002, Harrison et al., 2013). For this reason, there is need to explore CP and/or its extracts as a natural and economic resource for different value-added applications.

To date there is enough evidence that supports the antimicrobial activity of cranberry; however, researches that examine the possible growth-stimulatory role of cranberry extracts on beneficial bacteria are rare. As cranberry pomace holds huge potential of being a multi-functional food ingredient, so it would be worthwhile to understand its growth-promotion potential and interactions with beneficial bacteria commonly used for food fermentation, such as meat starter cultures. Thus, the objective of this study was to examine the effect of CP 80% ethanol extract (CE) on the growth of commonly used meat fermentation starter cultures, notably *Lactobacillus spp.*, *Pediococcus spp.*, and *Staphylococcus spp.*
3.2 Material and Method

3.2.1 Cranberry Pomace 80% Ethanol Extract Powder

CE used in the study was kindly provided by Dr. Moussa Diarra (Guelph Research and Development Centre (GRDC), Agriculture and Agri-Food Canada (AAFC), Guelph, ON, Canada) and was stored in a dark environment at -20 °C until use. Physical properties and chemical composition of the CE were previously characterized by that group (Ross et al., 2017). Briefly, the CE used has a total phenolic content, pH, and sugar content of 54.35 ± 0.85 mg gallic acid eq./g, 2.28 ± 0.01, and 77.77 ± 0.52% dry basis, respectively.

Prior to experimental use, CE was dissolved in 80% v/v methanol, thoroughly vortexed and sterilized through a 0.45 µm cellulose membrane filter (Nalgene, Rochester, NY, USA), yielding a sterile CE stock solution of known concentration (128 mg/ml). The stock solution was then stored at -20 °C until use.

3.2.2 Bacteria and Growth Condition

Bacteria, strain and their source are listed in Table 1. Strains were stored individually in Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) containing glycerol in a 1:1 volume ratio, and maintained at -80 °C. Each strain was streaked on Tryptic Soy Agar (TSA; Becton, Dickinson and Company) or De Man, Rogosa and Sharpe Agar (MRSA; Becton, Dickinson and Company) and incubate for 48 h at 37 °C to produced inoculated colonies. Prior to experimental use, a single colony was used to inoculate TSB or De Man, Rogosa and Sharpe Broth (MRS; Becton, Dickinson and Company), and incubated aerobically at 37 °C and 120 rpm for 24 h. For *Lactobacillus* species, another set of MRS was incubated anaerobically at 37 °C and 120 rpm for 24 h for anaerobic experimental use (BBL® Gas Pak Anaerobic System Envelopes,
Cells were harvested by centrifugation at 4000 rpm and 4 °C for 10 min, and the pelleted cells were washed twice, and re-suspended in 0.01M Phosphate Buffer Saline (PBS; Sigma Chemical Co., St. Louis, MO, USA). The optical density (OD) of the bacterial suspension was adjusted to an absorbance correspond to 0.05 MacFarland Standard at 600 nm using a cell density spectrophotometer (Cell density meter Model 40, Fisher Scientific, Hampton, NH, USA) before used for Bioscreen measurement.

3.2.3 Bioscreen Measurements

The effects of CE on the tested bacteria were tested in Bioscreen honeycomb 100-well microtiter plates (Oy Growth Curves Ab Ltd, Helsinki, Finland). A modified dextrose-free MRS broth (mMRS) or modified dextrose-free TSB (mTSB) was used as the media for Bioscreen assay in order to study the effect of CE as the primary growth substrate under limited carbohydrate conditions. Prior to the Bioscreen assay, CE stock solution (128 mg/ml) was further diluted to different concentrations using either mMRS or mTSB, mixed thoroughly and stored on ice before use. Bioscreen growth assay was performed as follows: 100 µl of washed cell suspensions (Section 2.2) was aseptically added to microtiter plate wells in a biological safety cabinet. 200 µl of diluted CE at various concentrations was then aseptically added into microtiter wells containing the bacterial suspension, resulting in a final well volume of 300 µl, and six different final CE concentrations (0.125, 0.25, 0.5, 0.75, 1.00, and 1.25% wt/vol CE). Uninoculated wells with CE plus sterile PBS were used to test the sterility of the solutions and obtain background absorbance; Inoculated wells added with TSB or MRS served as positive control.

Changes in bacterial growth were monitored at 37 °C for 48 h by measuring absorbance at 600 nm (OD₆₀₀) using an automated microbiology reader Bioscreen C (Oy Growth Curves Ab
Lactobacillus Ltd). Growth was monitored by recording the OD$_{600}$ readings of each well hourly. Plates were shaken for 20 sec before readings were taken. Growth measurements for *Lactobacillus* species were carried out under both aerobic and anaerobic conditions, while all other species were performed under aerobic conditions only. Bacterial growth under anaerobic conditions was conducted by covering each well with 100 µl sterile mineral oil (BioRad Laboratories, Hercules, CA). Plates remained stationary throughout the 48 h measurement cycle to minimize contact with air. OD$_{600}$ measurements for each bacteria and CE concentration were obtained from a minimum of four microtiter wells per Bioscreen measurement and Bioscreen measurements for each bacteria, and CE concentration were repeated at least three separated times.

### 3.2.4 pH Effect Analysis

Bacterial growth in acidic media (no CE supplementation) was also measured using Bioscreen. mTSB and mMRS were acidified using H$_2$SO$_4$ (Sigma Chemical Co.) and HCl (Fisher Scientific), respectively, to the pH of CE in mTSB or mMRS at different concentrations. The acidified media were microfiltered (0.45 µm cellulose membrane filter, Nalgene) and stored at 4 °C prior to growth assay use. Bioscreen measurement follows the procedures noted above.

### 3.2.5 Statistical Analysis

Bioscreen data were downloaded into Microsoft Office Excel for analysis. Bacteria exponential growth rate and maximum OD$_{600}$ (max OD$_{600}$), at each CE supplementation level, were determined using GrowthRate 3.0 (Hall et al., 2014). Maximum stimulatory concentration (MSC) of each starter culture was defined as the critical CE concentration that yielded the highest max OD$_{600}$ during the 48 h incubation; significant decrease ($P > 0.05$) in max OD$_{600}$ occurred at
concentrations > MSC. Minimum inhibitory concentration (MIC) of pathogens studied was defined as the CE concentration at which no significant ΔOD₆₀₀ was observed after 48 h ($P > 0.05$). Analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test was performed to evaluate the significance of difference in growth kinetic parameters between treatments using R 3.2.3 (R foundation for Statistical Computing, Vienna, Austria) at a confidence level of 95% ($P < 0.05$). All data were reported as mean ± standard deviation.
3.3 Results and Discussion

3.3.1 Effect of CE on the Growth of Meat Starter Cultures

3.3.1.1 Lactobacillus spp.

The effect of CE on the growth kinetics of Lactobacillus spp. (L. plantarum, L. curvatus, and L. sakei,) under aerobic and anaerobic conditions are shown in Tables 3.2 and 3.3, respectively. Growth profile of each species under aerobic and anaerobic conditions are also presented in Appendix I Fig.1 and 2, respectively. All three species showed species-specific growth pattern in the presence of oxygen (Table 3.2; Appendix I Fig.1). In the case of L. plantarum, both strains showed enhanced growth performance at all CE levels when compared to the control. 0.75 and 0.25% wt/vol CE yielding the highest max OD$_{600}$ and growth rate, respectively, among all treatments. Comparable growth kinetics was found between L. plantarum UM131L and FUA3073 (Table 3.2). A similar growth promotion effect was also observed for L. curvatus; growth performance was enhanced in the presence of CE, with 0.75% wt/vol CE supplementation being the MSC. On the other hand, L. sakei demonstrated a lower MSC than the other Lactobacillus species; highest max OD$_{600}$ and growth rate among all treatments was observed at 0.5% wt/vol CE. It is important to point out that, for certain species, the highest growth rate occurred at concentrations < MSC (Table 3.2). This suggests that cells were able to utilize CE as a growth substrate and subsequently attain a higher OD at MSC, yet an extended growth phase is perhaps required due to decreased cell growth metabolisms in the presence of CE components. Other authors have previously reported similar growth promotion effect by extracts of various berry origins on Lactobacillus spp.. For instance, Yang et al. (2014) found that 10% blackberry (Rubus fruticosus) juice supplementation promoted the growth of L. casei, L. plantarum and L. rhamnosus by 1 – 4 log CFU/ml while inhibited the growth of E. coli O157:H7, S. Typhimurium and L.
monocytogenes. Molan et al. (2009) similarly reported a 2 – 3 log CFU/ml increase in L. rhamnosus population after 48 h in the presence of 10% and 25% (vol/vol) water-soluble blueberry (Vaccinium ashei Reade) extracts. However, in both studies, the chemical composition and physical properties of the extracts used were not identified. In short, there are no studies to date examining the effect of CE on the growth of Lactobacillus meat starter cultures. Our results contributed new knowledge to this field.

The stimulation observed in Lactobacillus spp. could mainly be explained by the diverse nutritional options provided by CE; i.e. carbohydrates such as sugars (~78% in the CE used), xyloglucans and oligosaccharides, as well as the potential ability of Lactobacillus spp. to metabolize and/or interact with cranberry phenolic compounds (Demir et al., 2006; Hervert-Hernández et al., 2009; Sánchez-Patán et al., 2012; Yang et al., 2014; Özcan et al., 2017). Specifically, a number of mechanisms may account for the stimulatory effect of phenolic compounds. The first possibility is the ability of Lactobacillus spp. to use polyphenols as substrates. For instance, it has been reported that certain Lactobacillus spp. are capable of metabolizing polyphenols, such as monomeric flavan-3-ols, tannins and phenolic acids, during their growth by means of galloylsterase, decarboxylase and benzyl alcohol dehydrogenase activities, therefore supplying additional energy for growth (Rodríguez et al., 2008; Tabasco et al., 2011; Sánchez-Patán et al., 2012). Vivas et al., (1997) similarly found that gallic acid and anthocyanins positively stimulated LAB growth and were metabolized by growing cells. It is possible that LAB can cleave anthocyanins and use their sugar moieties as an additional carbohydrate source. Moreover, the influence of CE polyphenols on cell metabolisms may be related as polyphenols may positively enhance/up-regulate bacteria metabolisms, thus improve nutrient utilization (Alberto et al., 2001, 2004). Cranberry polyphenols may also serve as an
antioxidant and modulate oxidative stress development, hence provide a more desirable environment for cell growth (Molan et al., 2009). However, the exact mechanisms involved remain unclear and debatable.

To the best of our knowledge, only one recent study has presented data on the metabolism of cranberry phenolic compounds. In particular, *L. plantarum* was capable of degrading (-)-epicatechin-3-O-gallate and phenolic acids, but not A-or B-type PACs (Sánchez-Patán et al., 2012). The presence of glucose was also found to favor the cranberry polyphenol metabolisms of *L. plantarum* in that study; while phenolic compounds alone could not be used as the unique carbon source. Overall, the possible metabolisms of cranberry polyphenols by LAB are unidentified and poorly understood.

Despite having a higher sugar content at CE concentrations > MSC, a significant reduction in max OD<sub>600</sub> and growth rate was observed for all three species (*P* < 0.05; Table 3.2). A longer period of time is also required to reach the same ΔOD<sub>600</sub> (Appendix I Fig. 1). This suggests that the growth-stimulatory role of CE is dosage-dependent. In addition, *L. sakei* was found to be more sensitive to CE components than *L. plantarum* and *L. curvatus* as complete inhibition was observed at 1.0% wt/v CE (Table 3.2). The decreased growth performance, in all species, could mainly be attributed to the bioactive phenolic compounds in CE as solution acidity showed no significant impact on growth kinetics after 48 h in our pH effect analysis (*P* > 0.05; Table 3.2).

Similar dosage-dependent growth-stimulatory effect was observed under anaerobic condition (Table 3.3; Appendix I Fig. 2). However, at the same CE level, all species showed a reduced growth rate and max OD<sub>600</sub> in comparison to aerobic condition. This suggests a better CE utilization and/or metabolism by starter cultures under aerobic environments. While *Lactobacillus* spp. mainly carry out fermentation under anaerobic conditions to generate growth energy, the
presence of oxygen could possibly provide cells more metabolic options to derive energy from CE; or oxygen is necessary for polyphenol metabolisms. In fact, better growth performance and nutrient utilization of Lactobacillus spp. under aerobic conditions has been reported by others (Fu and Matthew et al., 1999; Smetankova et al. 2012). Future research is needed to unravel the possible CE polyphenol metabolisms of Lactobacillus species under both aerobic and anaerobic environments.

3.3.1.2 Pediococcus spp.

The growth curve and kinetics of Pediococcus spp. in the presence of CE are shown in Table 3.4 and Appendix I Fig. 3. The growth of both P. acidilactici strains (UM104P and FUA3072) was positively stimulated in the presence of CE; all supplementation levels yielded a significantly higher max OD$_{600}$ than the control ($P < 0.05$; Table 3.4), with 0.75 and 1.0% wt/v CE being the MSC for P. acidilactici UM104P and FUA3072, respectively. A significant increase ($P < 0.05$) in growth rate was also noticed in the presence of 0.50 – 0.75% wt/v CE. At equal CE levels, P. acidilactici FUA 3072 displayed better growth than UM104P as characterized by its higher max OD$_{600}$ and growth rate (Table 3.4; Appendix I Fig. 3A, B). A similar growth stimulation pattern was observed in the case of P. pentosaceus UM116P and FUA 3071; MSC and highest growth rates were attained at 0.75 – 1.0% and 0.5 – 0.75% wt/v CE, respectively (Table 3.4; Appendix I Fig. 3C, D). Despite showing a lower max OD$_{600}$ than P. pentosaceus FUA3071 at equal CE levels, P. pentosaceus UM116P showed a higher MSC than FUA3071, indicating a lower growth sensitivity towards CE. The difference in growth kinetics between individual strains can perhaps be attributed to variations in specific traits and growth characteristics. It is likely that Pediococcus spp. and Lactobacillus spp. share similar growth-stimulatory mechanisms in the
presence of CE; however, studies looking into the growth metabolisms of *Pediococcus* spp. under various defined conditions and in the presence of polyphenols-rich extracts are lacking. Nonetheless, at concentrations > MSC, all *Pediococcus* spp. showed a marked decrease (*P* < 0.05) in growth rate together with an increase in ΔOD<sub>600</sub> detection time in spite of the increased amount of sugar (Table 3.4; Appendix I Fig. 3). Certain species, notably *P. acidilactici* UM 104P and *P. pentosaceus* FUA 3071, also displayed a significant reduction in max OD<sub>600</sub> (*P* < 0.05; Table 3.4). In other words, the growth-stimulatory effect of CE seems to also be dosage-dependent for both species. The reduced growth rate at >MSC appears to be related to the increased acidity and phenolic level, which possibly caused suboptimal growth under stressful condition. In fact, results from the pH effect analysis indicated that the growth of *Pediococcus* spp. was not affected by solution acidity (*P* > 0.05; Table 3.4). This suggests that the inhibitory effect observed at higher CE levels mainly comes from CE phenolics.

### 3.3.1.3 *Staphylococcus* spp.

The effect of CE on the growth of *Staphylococcus* species (*S. carnosus* and *S. xylosus*) are shown in Table 5 and Appendix I Fig. 4. Among all concentrations, *S. carnosus* displayed the highest max OD<sub>600</sub> in the presence of 0.25% wt/vol CE; OD<sub>600</sub> was approximately 1.7 times higher than the control (*P* < 0.05). However, a significant lower ΔOD<sub>600</sub> (Appendix I Fig. 4A) during exponential growth phase, and an approximately 63% reduction in growth rate was observed when compared to the control. This suggests that although CE can promote *S. carnosus* growth, cells required a longer adaption period to bioactive phenolics or organic acids before achieving the same ΔOD<sub>600</sub>. 0.125% wt/vol CE yielded max OD<sub>600</sub> comparable to 0.25% wt/vol CE, yet demonstrated a higher growth rate. In the case of *S. xylosus*, 0.25% wt/vol CE showed no effect on its growth
max OD$_{600}$ and growth rate was not significantly different from the control and no increase in ΔOD$_{600}$ detection time was observed ($P > 0.05$; Appendix I Fig. 4B). Nonetheless, both *S. carnosus* and *S. xylosus* showed higher sensitivity to CE than other starter cultures as they exhibited complete inhibition starting from 0.50% and higher wt/v CE. This can partially be explained by the inherited low pH of CE since both species demonstrated a reduced max OD$_{600}$ in acidic solutions in comparison to the pH neutral control ($P < 0.05$; Table 3.5). In fact, it has previously been reported that low pH conditions can reduce the viability of acid-sensitive starter cultures including *Micrococcus* spp. and *Staphylococcus* spp. (Lisazo et al., 1999; Rantsiou and Cocolin, 2006). In addition, at the same pH level, CE demonstrated a greater growth-inhibitory effect on both *S. carnosus* and *S. xylosus* than the acidic solution in our pH effect analysis ($P < 0.05$; Table 3.5). This reveals that certain bioactive compounds present in CE, such as anthocyanins, flavonols, PACs, and phenolic acids, might also acted as a growth-limiting factor for *Staphylococcus* spp. rather than just the pH effect. Overall, the complete inhibition, at increasing CE concentrations, was the result of a combined effect of low pH and CE phenolic compounds. A concentration-dependent effect of CE on the growth of these two species may be concluded from our findings. To the best of our knowledge, there are no studies to date examining the effect of berry-derived extracts, especially CE, on the growth of beneficial *Staphylococcus*. Our findings provided new insight to this area.

### 3.3.2 Antimicrobial Activity of CE Against Foodborne Pathogens

Fig 3.1 shows the effect of CE on the growth of three common foodborne pathogens, *E. coli* O157:H7, *S. Enteritidis*, *L. monocytogenes*, and selected starter cultures. As expected, all three pathogens showed a higher susceptibility to CE than *Lactobacillus* species, and the antimicrobial
activity of CE is directly related to the supplementation level. Complete inhibition of *E. coli* O157:H7, *S. Enteritidis*, and *L. monocytogenes* was established at concentrations of 1.0, 0.75, and 0.5% wt/vol CE, respectively, with no significant ΔOD$_{600}$ during the 48 h incubation ($P > 0.05$; Fig. 3.1). At concentrations < MIC, minimal or no inhibitory effect was observed. Recent studies have also reported similar growth inhibition on *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* by extracts or products (i.e. juice, press cake) of cranberry origin (Puupponen-Pimia et al. 2001; Wu et al., 2008; Viskelis et al., 2009; Côté et al., 2013). In fact, the observed inhibition can be attributed to the combined effect of CE inherited acidity (pH $2.28 \pm 0.01$) and bioactive phenolics. Our pH effect analysis showed that CE exhibited greater antimicrobial effect ($p < 0.05$; Fig. 3.1), against all three pathogens, than acidic solutions at the same pH level. Similarly, Wu et al. (2008) reported a greater antibacterial activity of cranberry concentrate solution (total phenolic content: $12.6 \pm 0.2$ g/l) against *E.coli* O157:H7 than acidic solutions at equal pH levels. Lacombe et al., (2010) showed a similar antimicrobial pattern by pH neutral cranberry phenolics and anthocyanins. It is important to point out that at the same CE level, certain starter cultures exhibited their highest max OD$_{600}$ (Fig.3.1). This information suggests that at correct levels, CE may exert a dual positive effect; on the one hand inhibit foodborne pathogens, and on the other hand promote the growth of beneficial bacteria. The lower MIC observed for *L. monocytogenes* indicates a higher susceptibility of Gram-positive foodborne pathogens towards CE than Gram-negative foodborne pathogens, which is in agreement with previous reports (Wu et al., 2008; Cailliet et al., 2012). However, the mechanisms that give Gram-positive LAB advantage over *L. monocytogenes* in the presence of cranberry phenolics have still not been identified. Unlike pathogenic bacteria, most LAB can survive in harsh conditions with low pH, high ethanol concentrations and antimicrobial phenolics (Torres et al., 2007). Lacombe et al. (2013) suggested
that *Lactobacillus* spp. may possess higher levels of metabolic energy-generating/transducing systems or membrane permeability that prevents acid shock from cranberry organic acids. Another possibility is that *Lactobacillus* spp. may possess certain polyphenol catabolic mechanisms that help metabolizing polyphenols and thereby increase tolerance.

Our findings support the theory that both low pH and other bioactive phenolics are responsible for the antimicrobial property of cranberry. Cranberry organic acids (i.e. citric acid, quinic acid, and malic acid) creates an osmotic stress that can cause sublethal damages to bacteria cell membrane components, which consequently impair fundamental membrane functions and/or make the bacteria more susceptible to phenolic antimicrobial compounds in CE (Lin, Labbe, and Shetty, 2004; Wu et al., 2008). On the other hand, most of the cranberry phenolic compounds, including anthocyanins, flavonols, and PACs, act by destabilizing the structural integrity and functional components of bacteria cell wall, membrane, and intracellular matrix. For instance, Vattem et al. (2005) suggested that cranberry phenolics with partial hydrophobicity could efficiently embed to the bacterial membrane-water interface and alter membrane surface charge, thereby impair cell membrane functional components and transport processes. Nohynek et al. (2006) and Johnson et al. (2008) both reported that PACs were able to bind and disintegrate membrane lipopolysaccharides (LPS), weaken bacteria outer membrane, alter membrane permeability/fluidity, and induce subsequent cell lysis. Wu et al. (2008) found that pH neutral cranberry phenolics were able to disintegrate bacteria cell wall, cell membrane and cause cell lysis. They hypothesized that the damaged membrane areas also facilitate leakage of cellular contents and allows certain phenolic compounds to enter the cell and induce cellular metabolism disruptions. Similarly, Lacombe et al., (2010) reported a weakening effect against the outer
membrane structure of \textit{E. coli} O157:H7 as a consequence of localized outer membrane disintegration caused by pH neutral cranberry phenolics and anthocyanins.

It is interesting to note that at CE concentrations <MIC, the growth of all three pathogens was enhanced, and a higher OD$_{600}$ than the control was attained after 48 h (Fig. 1). It is possible that at low concentrations, CE carbohydrates (e.g. glucose, fructose) stimulated the growth of all three pathogens, despite the presence of CE antimicrobial compounds; or CE phenolics at low concentrations acted as antioxidants and modulate oxidative stress, thus favor cell growth and multiplication (Molan et al., 2009). Our results further strengthen the connection between cranberry acidity, phenolic compounds and foodborne pathogen inhibition.

\section*{3.4 Conclusion}

To conclude this chapter, the present study examined the growth-stimulatory potential of cranberry on common meat starter cultures. Our findings contribute new and valuable knowledge to this field because the majority of the previous researches about cranberry were centered on its antimicrobial property against pathogenic bacteria, but not its influence on beneficial bacteria use for food production and fermentation. Overall, a CE concentration-dependent stimulatory effect on the growth of meat starter cultures was found. Species belonging to the genus \textit{Lactobacillus} and \textit{Pediococcus} showed MSC at 0.5 - 1.0\% wt/vol CE. \textit{Staphylococcus} spp. showed a higher sensitivity to CE and growth was completely inhibited starting at 0.5\% or higher wt/v CE. Both CE acidity and phenolics were found to be the growth-limiting factors at increasing levels. Moreover, our study validated the excellent antimicrobial activity of CE, higher resistance of beneficial bacteria against polyphenol-rich CE, and the importance of both cranberry acidity and phenolic compounds in controlling foodborne pathogens. \textit{E. coli} O157:H7, \textit{S. Enteritidis}, and \textit{L.
*monocytogenes* demonstrated MIC at concentrations of 1.0, 0.75, and 0.5% wt/vol CE, respectively. These concentrations are particularly significant when studying the use of CE as an antimicrobial additive in food systems in the future.
3.5 References


### 3.6 Tables and Figures

Table 3.1 Species, strain, and source of the bacteria used in the study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Source/Other information</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. sakei</em></td>
<td>FAS4</td>
<td>Frutarom Ltd., Israel</td>
</tr>
<tr>
<td><em>L. curvatus</em></td>
<td>FUA3015</td>
<td>University of Alberta, Edmonton, AB, Canada</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>FUA3073</td>
<td>University of Alberta, Edmonton, AB, Canada</td>
</tr>
<tr>
<td><em>L. plantarum</em></td>
<td>UM131L</td>
<td>University of Manitoba, Winnipeg, NB, Canada</td>
</tr>
<tr>
<td><em>P. acidilactici</em></td>
<td>FUA3072</td>
<td>University of Alberta, Edmonton, AB, Canada</td>
</tr>
<tr>
<td><em>P. acidilactici</em></td>
<td>UM104P</td>
<td>University of Manitoba, Winnipeg, MB, Canada</td>
</tr>
<tr>
<td><em>P. pentosaceus</em></td>
<td>FUA3071</td>
<td>University of Alberta, Edmonton, AB, Canada</td>
</tr>
<tr>
<td><em>P. pentosaceus</em></td>
<td>UM116P</td>
<td>University of Manitoba, Winnipeg, MB, Canada</td>
</tr>
<tr>
<td><em>S. xylosus</em></td>
<td>FUA3211</td>
<td>University of Alberta, Edmonton, AB, Canada</td>
</tr>
<tr>
<td><em>S. carnosus</em></td>
<td>BBU001</td>
<td>Dry fermented sausage isolate, Guelph Research and Development Centre (GRDC), Guelph, ON, Canada</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7</td>
<td>ATCC43890</td>
<td>Human feces; American Type Culture Collection, Manassas, VA, USA</td>
</tr>
<tr>
<td><em>S. enterica</em> Enteritidis</td>
<td>ABB07-SB3071</td>
<td>Broiler chicken isolate; GRDC, Guelph, ON, Canada (Diarra et al., 2014)</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>08-5578 (serotype 1/2a)</td>
<td>Isolated from a human blood specimen associated with a listeriosis outbreaks in Canada in 2008; National Microbiology Laboratory, Winnipeg, MB, Canada</td>
</tr>
</tbody>
</table>
Table 3.2 Effect of cranberry extract (CE) on the growth of *Lactobacillus* spp. at selected concentrations under aerobic condition.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Growth rate (ln OD&lt;sub&gt;600&lt;/sub&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Max OD&lt;sub&gt;600&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. sakei</em> FAS 4</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.28 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.35 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE*</td>
<td>0.37 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE</td>
<td>0.29 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>N.A</td>
<td>N.D</td>
</tr>
<tr>
<td>pH 5.0 mMRS</td>
<td>N.A</td>
<td>0.24 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. curvatus</em> FUA 3015</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.33 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.65 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.51 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.94 ± 0.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.57 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.18 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.24 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.96 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 5.0 mMRS</td>
<td>N.A</td>
<td>0.29 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em> FUA 3073</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.29 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.44 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.41 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.20 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.11 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.63 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 5.0 mMRS</td>
<td>N.A</td>
<td>0.27 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>L. plantarum</em> UM 131L</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.31 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.49 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.41 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.93 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.34 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.16 ± 0.08&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.15 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.91 ± 0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 5.0 mMRS</td>
<td>N.A</td>
<td>0.25 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means ± standard deviation in the same column under the same species followed by different letters are significantly different (*P* < 0.05).
Max OD<sub>600</sub> denote the highest OD<sub>600</sub> attained during the 48 h measurement cycle.
N.D, not determined as no significant ΔOD<sub>600</sub> was observed during the 48 h measurement cycle.
N.A, not available as no significant exponential growth phase was available for growth rate determination.
* denote maximum stimulatory concentration (MSC)
% CE are based on wt/vol.
pH 5.0 modified dextrose-free MRS (mMRS) has a pH value equivalent to 1.0% wt/vol CE in mMRS.
Table 3.3 Effect of cranberry extract (CE) on the growth of *Lactobacillus* spp. at selected concentrations under anaerobic condition.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Growth rate (ln OD$_{600}$ h$^{-1}$)</th>
<th>Max OD$_{600}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. sakei</em> FAS 4</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.43 ± 0.07$^a$</td>
<td>0.43 ± 0.04 $^b$</td>
</tr>
<tr>
<td></td>
<td>0.50% CE*</td>
<td>0.27 ± 0.02$^b$</td>
<td>0.56 ± 0.03 $^c$</td>
</tr>
<tr>
<td></td>
<td>0.75% CE</td>
<td>N.A</td>
<td>0.17 ± 0.10 $^a$</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>N.A</td>
<td>N.D</td>
</tr>
<tr>
<td><em>L. curvatus</em> FUA 3015</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.20 ± 0.02 $^a$</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.55 ± 0.06$^a$</td>
<td>0.45 ± 0.20 $^b$</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.37 ± 0.00$^b$</td>
<td>0.78 ± 0.02 $^c$</td>
</tr>
<tr>
<td></td>
<td>0.75% CE</td>
<td>0.37 ± 0.02$^b$</td>
<td>0.98 ± 0.05 $^d$</td>
</tr>
<tr>
<td></td>
<td>1.00% CE*</td>
<td>0.26 ± 0.04$^c$</td>
<td>1.03 ± 0.02 $^d$</td>
</tr>
<tr>
<td><em>L. plantarum</em> FUA 3073</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>0.18 ± 0.02 $^a$</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.52 ± 0.08$^a$</td>
<td>0.40 ± 0.03 $^b$</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.31 ± 0.04$^b$</td>
<td>0.87 ± 0.05 $^c$</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.22 ± 0.12$^bc$</td>
<td>0.98 ± 0.13 $^c$</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.15 ± 0.02$^c$</td>
<td>0.70 ± 0.05 $^d$</td>
</tr>
<tr>
<td><em>L. plantarum</em> UM 131L</td>
<td>0.00% CE</td>
<td>N.A</td>
<td>N.D</td>
</tr>
<tr>
<td></td>
<td>0.25% CE</td>
<td>0.41 ± 0.10$^a$</td>
<td>0.40 ± 0.05 $^a$</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.25 ± 0.02$^b$</td>
<td>0.65 ± 0.01 $^b$</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.18 ± 0.04$^c$</td>
<td>0.79 ± 0.05 $^c$</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>N.A</td>
<td>0.24 ± 0.20 $^a$</td>
</tr>
</tbody>
</table>

Means ± standard deviation in the same column under the same species followed by different letters are significantly different ($P < 0.05$).
Max OD$_{600}$ denote the highest OD$_{600}$ attained during the 48 h measurement cycle.
N.D, not determined as no significant ΔOD$_{600}$ was observed during the 48 h measurement cycle.
N.A, not available as no significant exponential growth phase was available for growth rate determination.
* denote maximum stimulatory concentration (MSC)
% CE are based on wt/vol.
pH 5.0 modified dextrose-free MRS (mMRS) has a pH value equivalent to 1.0% wt/vol CE in mMRS.
Table 3.4 Effect of cranberry extract (CE) on the growth of *Pediococcus* spp. at selected concentrations

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Growth rate (ln OD&lt;sub&gt;600&lt;/sub&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Max OD&lt;sub&gt;600&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. acidilactici</em> UM 104P</td>
<td>0.00% CE</td>
<td>0.28 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.45 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.35 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.21 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>pH 5.0 mMRS</td>
<td>0.27 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. acidilactici</em> FUA 3072</td>
<td>0.00% CE</td>
<td>0.19 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.43 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.08 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.55 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.17 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.30 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>pH 5.0 mMRS</td>
<td>0.18 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. pentosaceus</em> UM 116P</td>
<td>0.00% CE</td>
<td>0.41 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.60 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.07 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE</td>
<td>0.52 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE*</td>
<td>0.32 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.98 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>pH 5.0 mMRS</td>
<td>0.42 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. pentosaceus</em> FUA 3071</td>
<td>0.00% CE</td>
<td>0.38 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>0.55 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.28 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.75% CE*</td>
<td>0.50 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.35 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1.00% CE</td>
<td>0.29 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>pH 5.0 mMRS</td>
<td>0.40 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± standard deviation in the same column under the same species followed by different letters are significantly different (*P* < 0.05).
Max OD<sub>600</sub> denote the highest OD<sub>600</sub> attained during the 48 h measurement cycle.
% CE are based on wt/vol.
* denote maximum stimulatory concentration (MSC).
pH 5.0 modified dextrose-free MRS (mMRS) has a pH value equivalent to 1.0% wt/vol CE in mMRS.
Table 3.5 Effect of cranberry extract (CE) on the growth of *Staphylococcus* spp. at selected concentrations

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>Growth rate (ln OD&lt;sub&gt;600&lt;/sub&gt; h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Max OD&lt;sub&gt;600&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. carnosus</em> BBU 001</td>
<td>0.00% CE</td>
<td>0.48 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.125% CE</td>
<td>0.38 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE*</td>
<td>0.21 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>N.A</td>
<td>N.D</td>
</tr>
<tr>
<td></td>
<td>pH 5.4 mTSB</td>
<td>0.45 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. xylosus</em> FUA3211</td>
<td>0.00% CE</td>
<td>0.15 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.125% CE</td>
<td>0.40 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.25% CE*</td>
<td>0.20 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.50% CE</td>
<td>N.A</td>
<td>N.D</td>
</tr>
<tr>
<td></td>
<td>pH 5.4 mTSB</td>
<td>0.13 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± standard deviation in the same column under the same species followed by different letters are significantly different (<i>P</i> < 0.05).

Max OD<sub>600</sub> denote the highest OD<sub>600</sub> attained during the 48 h measurement cycle.

N.D, not determined as no significant OD<sub>600</sub> was observed.

N.A, not available as no significant exponential growth phase was available for growth rate determination.

% CE are based on wt/vol.

* denote maximum stimulatory concentration (MSC).

pH 5.4 modified dextrose-free TSB (mTSB) has a pH value equivalent to 0.50% wt/vol CE in mTSB.
Fig 3.1 Effect of cranberry extract (CE) at varying concentrations (wt/vol) and acidic modified dextrose-free TSB (mTSB) on the growth of *E. coli* O157: H7 (A), *S. Enteritidis*, and *L. monocytogenes* (C). Growth of selected starter cultures at 0.75% wt/vol CE were also showed. pH 4.5 mTSB has a pH value equivalent to 1.00% wt/vol CE in mTSB. The curves are drawn from the average of at least three independent experiments. Error bars denote mean ± one standard deviation.
CHAPTER 4

EFFECT OF CRANBERRY POMACE ON THE INACTIVATION OF SALMONELLA ENTERICA SEROVARS AND PHYSICOCHEMICAL PROPERTIES DURING DRY FERMENTED SAUSAGE MANUFACTURING

ABSTRACT

The effect of cranberry pomace (CP) incorporation on S. enterica serovars inactivation, starter culture population, and physicochemical properties of dry fermented sausages (DFS) during manufacturing was studied. DFS containing a five-strain cocktail of S. enterica serovars at 7-log CFU/g, with the addition of different levels of CP (control, 0%; low, 0.55%; medium, 1.70%; high, 2.25% wt/wt) concentrations, or liquid lactic acid (0.33% wt/wt, LA) were manufactured, and subjected to conventional fermentation and drying conditions. A significant ($P < 0.05$) reduction in initial pH was observed in all CP treatments on day 0 as a result of CP native acidity. All treatments except low CP showed a significantly lower pH than the control batch throughout the entire study. $a_w$ was not significantly affected by the level of CP during fermentation. However, medium and high levels CP showed a significantly lower ($P < 0.05$) end-product $a_w$ than the control after drying. Microbiological analysis showed that DFS incorporated with CP exhibited a significantly ($p < 0.05$) faster and greater S. enterica serovars inactivation during the first 5 days; the reduction rate and level were directly correlated to the CP level. Both CP phenolic compounds and natural acidity were found to play a key role in S. enterica serovars inactivation. In the presence of medium and high levels of CP, Staphylococcus spp. growth was suppressed, while Lactobacillus spp. and Pediococcus spp. exhibited a stimulatory effect. All treatments except low CP had no significant effect on product chemical composition, and Moisture Protein ratio (MPR). Low CP level yielded DFS with a slightly higher ($P < 0.05$) moisture content and MPR. Medium
and high CP levels resulted in darker, duller and redder DFS with a softer and more crumbly texture. Findings suggest that low CP levels can be utilized by DFS manufacturers as a natural functional ingredient to further minimize the risk associated with S. enterica serovars during production without altering final product characteristics.
4.1 Introduction

Cranberry has emerged as one of the super foods in the past two decades due to its broad nutrient content and high levels of bioactive phenolic compounds. Specifically, these constituent bioactive phenolics, such as anthocyanins, flavonols, and proanthocyanidins (PACs), have gained considerable interest among the food and pharmaceutical industry as they have been shown to exhibit a wide range of potential biological health benefits including antioxidation, antimicrobial, antiviral, anticancer, and anti-inflammatory implications (Puupponen-Pimia et al., 2001; Manach et al., 2004; Konczak and Zhang, 2004; Howell et al., 2005; McKay and Blumberg, 2007; Côté et al., 2011; Caillet et al., 2012; Nile and Park, 2014). The antimicrobial activity of phenolic compounds of cranberry origin is the most widely recently studied property, as there is growing consumer demand for healthier, natural, and minimally-processed food products free from chemical preservatives. These phenolic constituents have the potential of being utilized as a natural food preservative as they were found to hold excellent antimicrobial activity against foodborne pathogens, such as *E. coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Helicobacter pylori* in vitro, but not against beneficial microorganisms such as lactic acid bacteria (Puupponen-Pimia et al., 2001; Puupponen-Pimia et al., 2005; Nohynek et al., 2006; Wu et al., 2008; Wu et al., 2009; Côté et al., 2011; Caillet et al., 2012; Lacombe et al., 2013).

Cranberry pomace (CP) is the main by-product of cranberry processing and consists of the pulps, seeds, peels and other fruit structures of cranberry. As the polyphenol-rich fractions are not lost and a large amount of moisture and soluble solids are removed during fresh cranberry pressing, CP and its subsequent extracts show high levels of vitamins, dietary fibers, and phenolic compounds (White et al., 2010; Harrison et al., 2013; Ross et al., 2017). Nevertheless, due to its
acidic nature, the potential use of CP as a value-added resource was not fully explored and hence was often treated as solid fruit wastes (Vattem and Shetty, 2002). As being a rich source of bioactive phenolics, researching the potential use of CP in food systems would not only fulfill consumers’ expectation for food product with natural ingredients but would also allow economical utilization of valuable solid fruit waste.

Dry fermented sausages (DFS) are a category of dry meat product are manufactured from a mixture of ground meat, animal fat, water, salt, curing salt, spices, and starter culture containing lactic acid-producing bacteria (Barbut, 2015). The sausage batter is then stuffed into casings and subjected to fermentation plus drying under specific conditions of relative humidity and temperature. DFS represent one of the oldest forms of meat preservation and are traditionally manufactured without the use of thermal treatments, thus their preservation mainly relies on a combination of acidification, controlled drying (low \(a_w\)), addition of chemical preservatives and curing agents such as salt and nitrite. According to Canadian Food Inspection Agency (CFIA, 2018), a shelf-stable DFS should meet one of the following three specifications: a) have a pH of \(\leq 4.6\), regardless of final \(a_w\); b) have an \(a_w\) of \(\leq 0.85\), regardless of final pH; c) have a pH \(\leq 5.3\) at the end of fermentation and an end-product \(a_w\) of \(\leq 0.90\) (CFIA, 2018).

However, despite being traditionally regarded as self-stable, several outbreaks associated with Salmonella enterica serovars had been linked to the consumption of raw DFS and it was later discovered that the combined effect of low \(a_w\) and low pH was insufficient to inactivate S. enterica serovars when present at high level (Health Canada, 2000; Bremer et al., 2004; Moore, 2004; Luzzi et al., 2007; Hwang et al., 2009). As a result, regulatory agencies have developed protocols to help manufacturers to identify options to control S. enterica serovars in DFS that are dependent on final product pH and \(a_w\). One of the options describes the use of a DFS production process that has been
validated to achieve a > 5-log inactivation of *S. enterica* serovars (CFIA, 2018). In fact, it is important to point out that validation studies regarding of *S. enterica* serovars inactivation during DFS manufacturing are limited. For this reason, due to their moderate acidic nature, diverse microbiological background, and food safety concerns, DFS would serve as a suitable food model to investigate the potential use of CP as an value-added ingredient.

Thus, the objectives of this work were to examine the effect of CP incorporation, at varying levels, on *S. enterica* serovars inactivation, meat starter culture growth, and physicochemical properties of DFS. The findings of the study should provide useful knowledge regarding the functional practicality of CP as a natural functional ingredient for DFS, specifically antimicrobial agent.

### 4.2 Materials and Methods

#### 4.2.1 Cranberry Pomace Powder

In the DFS study, CP, a crude cranberry processing residue, instead of CE was used as it offers greater industry practicality, accessibility and lower economic cost. CP used in the study was kindly provided by Dr. Moussa Diarra (Guelph Research and Development Centre (GRDC), Agriculture and Agri-Food Canada (AAFC), Guelph, ON, Canada) and was stored in a dark environment at -20 °C until use. Physical properties and chemical composition of the CP was previously characterized by that group (Ross et al., 2017). Briefly, the CP used in the study has a total phenolic content, pH, and sugar content of 24.87 ± 0.66 mg gallic acid eq./g, 2.74 ± 0.02, and 22.76 ± 0.49 %, respectively.

CP supplementation levels were chosen based on the results from the Bioscreen growth assay (Chapter 3). As phenolic compounds were the fraction of interest, CE concentrations were
converted to their equivalent CP concentrations by multiplying a factor of 2.2; CE contains approximately 2.2 times more phenolic compounds than CP. For instance, 1.70% CP, which has phenolic content equal to 0.75% CE (MIC determined for Salmonella in Chapter 3) was chosen as the medium CP treatment.

4.2.2 Bacteria And Growth Condition

A five-strain cocktail of S. enterica serovars were used to inoculate the raw meat batter in the study. Strain name, serogroup, and source of each isolate are listed in Table 4.1. Strains were stored individually in Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) containing glycerol in a 1:1 volume ratio, and maintained at -80 °C. Prior to experiment use, each strain was streaked on Tryptic Soy Agar (TSA; Becton, Dickinson and Company) and incubate aerobically for 24 h at 37 °C. A single colony was used to inoculate TSB and incubated at 37 °C and 120 rpm for 24 h. An aliquot was transferred to TSB + 1.0% glucose (Sigma Chemical Co., St. Louis, MO, USA) resulting in a final 100-fold dilution and incubated at 37 °C and 120 rpm for 24 h to acid adapt the cultures and ensure high cell growth and density prior to inoculation (Buchanan and Edelson, 1996). Ahead of inoculation, individual strains of the S. enterica serovars cocktail were harvested by centrifugation at 10000 rpm and 4 °C for 10 min, and the pellet collected was washed twice, and re-suspended in sterile distilled water. Each strain was then thoroughly mixed, combined and divided to make a five-strain cocktail containing equal concentrations of each strain, prior to meat batter inoculation at a level of approximately 6.5-log CFU/g in each treatment. All inoculums were stored on ice prior to inoculation.
4.2.3 Dry Fermented Sausage Production

Pork fat, lean pork trims, and lean beef trims were obtained from University of Guelph Meat Abattoir, and grounded through a 3.175 mm (1/8 inch) plate. The ground trims were then thoroughly mixed to form a homogenous mass, portioned into individual 7.5 kg vacuum packed packages, and stored at -20 °C. Meat was then defrost at 4 °C as needed, prior to the experimental trials.

All DFS were manufactured in a dedicated biosafety level 2 containment pilot plant at GRDC, Guelph, ON, Canada. All equipment were chilled at 4 °C overnight to prevent fat smearing during production. Pre-weighted meat mix for each treatment were loaded to a stand food mixer (KitchenAid®, Mississauga, ON, Canada) and mixed for 30 sec to break down large meat clumps, before adding *S. enterica* serovars inoculum cocktail resulting in an initial inoculation level of approximately 6.5-log CFU/g. Curing salt (Hela Spice Canada Inc., Uxbridge, ON, Canada), salami seasoning (Hela Spice Canada Inc.), starter culture (B-LC-007 SafePro®, Chr Hansen, Denmark), and/or CP powders (GRDC, Guelph, ON, Canada) at selected levels or liquid lactic acid (≥ 90%; Sigma Chemical Co.) were added to the meat batter following the addition of *S. enterica* serovars inoculum. Formulation for each treatment are provide in Table 4.2. Mixing apparatuses (e.g. bowls, paddles) were changed between batches to prevent cross-contamination. The meat batter was mixed for an extra 5 min before being stuffed into moistened pre-cut 33 mm caliber size fibrous cellulose casings (Nalo Fibrous, Kalle Gmbh, Wiesbaden, Germany) at a length of ≥ 100 mm (i.e., at least twice the size of casing diameter as required by CFIA “Meat Hygiene Manual of Procedures” (CFIA 2018)) using a pre-chilled stuffer (LEM dual gear stuffer, West Chester, OH, USA). Each sausage was clipped shut using a pneumatic clipper (Poly-Clip®, Koch equipment,
Sausages were transferred to a programmed fermentation cabinet (Stagionello STG100 MTO, Crotone, Italy) conditioned at 25 ºC and at a relative humidity (RH) of 88%. Temperature was decreased stepwise at 2 ºC every 24 h until it reaches 20 ºC, while RH was reduced to 80% after 24 h and subsequently 2% drop every 24 h. After the fermentation period, the temperature was reduced by 2 ºC every 12 h until a final temperature of 14 ºC and RH of 75% was reached. DFS were then transferred to a drying cabinet fitted with a heatless dryer (Caron Environmental Chamber 6020, Caron Products and Services Inc., Marietta, OH, USA) and dried at 14 ºC and 75% RH for the next 28 days of the study.

Two batches of sausages for each treatment, each with approximately 15 kg of salami meat batter, were manufactured one batch each on two separated days, resulting in a total of 10 batches. Two sausages per batch were randomly selected on day 0, 1, 2, 5, 12, 19, 26 & 33 of the process for different analyses.
4.2.4 Microbiological Analysis

The sampling of DFS for microbial analysis was carried out for all treatments. Briefly, 225 ml of 0.1% sterile peptone water (Difco Peptone Water, Becton, Dickinson and Company) was added to 25g of aseptic composite sausage in a sterile stomacher bag (Filtrabag, VWR Canada, AB, Canada) and homogenized using a stomacher (Stomacher 400 circulator, Seward Laboratory Systems Inc., FL, USA) for 2 min at 230 rpm. The stomached sample was then serially diluted with 0.1% sterile peptone water, and 100 µl of sample was surface plated onto selective agar plates including De Man, Rogosa and Sharpe Agar (MRSA; De Man, Rogosa and Sharpe Agar, Becton, Dickinson and Company), Mannitol Salt Agar (MSA; Mannitol Salt Agar, Becton, Dickinson and Company), and Xylose-Lysine-Tergitol 4 (XLT-4; Xylose-Lysine-Tergitol 4, Becton, Dickinson and Company), and incubated aerobically at 37 °C for 48 h. Another set of MRSA was incubated at 37 °C anaerobically for 48 h. Colony forming units (CFU) were enumerated with a detection limit of 25 - 250 CFU/g. When plates had fewer than 25 colonies the actual plate counts were used for calculation of inactivation and denoted with an “*”. The reduction in *S. enterica* serovars numbers was represented as log reduction, and was calculated using the formula below:

\[
Log \text{ reduction} = \log \frac{N_t}{N_0}
\]

where \(N_t\) is the average CFU/g at time \(t\) and \(N_0\) is the average CFU/g at time zero. Two random sausages per batch were analyzed at each sampling day and final bacterial count were presented as mean log CFU/g ± standard deviation obtained from duplicate plated samples from each sausages.

In order to account for injured *S. enterica* serovars cells, 10 g of composite sample were enriched in Selenite Cystine Broth (SCB; Selenite Cystine Broth, Becton, Dickinson and Company).
and incubated for 24 h at 37°C when no colony growth was noted throughout the 33 days sampling plan. The enriched samples were then spread-plated in duplicate onto XLT-4 agar plates for \textit{S. enterica} serovars detection.

### 4.2.5 Physicochemical Analysis

Approximately 15 g of composite sausage sample was ground into small uniform particles using a food processor (Blixer 2, Robot Coupe U.S.A., Jackson, MS, USA) and analyzed for $a_w$, pH, protein, moisture, and fat content. $a_w$ was analyzed using a Dew point water activity meter 4TE (Aqua Lab, Pullman, WA, USA) calibrated at 25°C, while pH was assessed using a bench-top flat surface pH probe (B10P Benchtop Meter, VWR, Radnor, PA, USA) after a 2-point calibration with buffers at different pH (pH 7.01 and 4.01). The protein content was determined by using a CEM Sprint Rapid protein analyzer (AOAC Method 967.12, 930.33, and 930.29. CEM Corporation, Matthews, NC, USA). The moisture and fat level were analyzed using a Meat Trac Fat and Moisture Analyzer, microwave moisture analyzer and LF-NMR, respectively (AOAC Method 2008.06, CEM Corporation). Two sausages per batch were analyzed at each sampling point and physicochemical results were presented as mean ± standard deviation of the two batches.

### 4.2.6 Color Measurement

Color analysis was conducted on all treatments, except the LA treatment. The color of the DFS samples was evaluated with a Nix™ Pro Color Sensor (Nix™ Pro Color Sensor, Nix sensor Ltd., Hamilton, ON, Canada) based on the CIE L*a*b* color space with a D-65 illuminant source setting, and 2° standard observer angle. The device is controlled wirelessly by any Android or Apple phone or tablet through Bluetooth and has its own light-emitting diode (LED) light source.
located within the concave base of the sensor about 1 cm above the field of view. Both the exterior and interior color of DFS were evaluated. The exterior color of DFS was measured immediately after removing the sausage casing, whereas the interior color was measurements after slicing the sausages into slices of 1 cm thick. Since color redness is highly influenced by color yellowness, the L*a*b* color space was transformed and reported as L*, C*, and h° value, which represents color lightness, chroma, and hue angle of the CIE Lab color space, respectively, by the following equations:

\[ C^* = \sqrt{a^{*2} + b^{*2}} \]

\[ h^\circ = \tan^{-1}\left(\frac{b^{*}}{a^{*}}\right) \]

All exterior and interior color measurements were performed 4 times for each sausage at room temperature and two sausages were analyzed for each batch.

4.2.7 Textural Analysis

Textural Profile Analysis (TPA) was carried out on all treatments, except the LA treatment, on day 1, 2, 5, 12, 19, 26 & 33 of the process. The TPA was performed to evaluate the effect of CP on product textural parameters using a textural analyzer equipped with a 30 kg load cell (Texture Technologies Corp., Model TA.XT2, Scarsdale, NY, USA). Three cylindrical samples from the core of the of each sausage were compressed twice to 50 % of their original height with a cross-head speed of 1.5 mm/s. All textural parameters expect cohesiveness and springiness were modified by dividing the sample cross-section area (cm²), and presented as the following: Hardness (N/cm²) = the maximum force required to compress the sample; Springiness (cm) = the vertical distance the sample recovered after the deformation force is removed; Cohesiveness =
ratio of the area of the second force-displacement curve to the area of the first curve; Gumminess (N/cm²) = force to disintegrate a semi-solid meat sample for swallowing (Hardness x Cohesiveness); Chewiness (N/cm) = work to masticate the sample for swallowing (Springiness x Gumminess). Two sausages were analyzed for each treatment on each sampling day at room temperature, and any samples which exceeded the load cell threshold were discarded.

4.2.8 Statistical Analysis

Microbial counts were transferred to log CFU/g. The value 1 was added to samples where no colony growth was observed in order to accommodate the value of zero CFU/g. Analysis of variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test was carried out to evaluate the difference between the treatments at each sampling point using R 3.2.3 (R foundation for Statistical Computing, Vienna, Austria) at a confidence level of 95% (P < 0.05). All data were reported as mean ± standard deviation.
4.3 Result and discussion

4.3.1 Effect of CP on Changes in pH, aw, and Moisture Protein Ratio

Changes in pH, aw, and Moisture Protein ratio (MPR) of DFS of varying treatments during sausage fermentation and drying are presented in Fig. 4.1 DFS initial pH was significantly affected by CP incorporation and was found to be CP level-dependent ($P < 0.05$; Fig. 4.1A); with high CP treatment showing the lowest initial pH (5.04 ± 0.01) and control showing the highest initial pH (5.65 ± 0.05). It is interesting to point out that in the present study, the initial pH of high CP treatment was similar to the final pH of conventional DFS products with a similar manufacturing process (Balamurugan et al., 2017; De Souza et al., 2018). All DFS exhibited a gradual drop in pH during the fermentation process and can be related to the starter culture activity, which metabolized the sugars, dextrose present in the meat mix into lactic acid. All CP added DFS showed a markedly lower ($P < 0.05$) pH than that of the control by the end of the 5-day fermentation process. Similarly, pH of CP added treatments remained significantly ($P < 0.05$) lower than that of the control during the drying process; the degree of pH reduction was related to CP level. Such dramatic change in pH profile appears to be related to the acidic nature of CP (pH 2.74 ± 0.02). This could explain the relatively higher $S. enterica$ serovar inactivation rate, lower meat starter culture population, and the more crumbly texture observed, which will be addressed in the later sections. According to CFIA (CFIA, 2018), a shelf-stable DFS should have a final pH of ≤ 4.6 or have a pH ≤ 5.3 and aw of ≤ 0.90 at the end of fermentation. In the present work, all DFS reached the regulated pH of ≤ 5.3 after fermentation and attained a final pH of < 4.9 after the drying phase. The liquid lactic acid (LA) treatment resulted in a pH profile similar to that of medium CP treatment throughout the full process and was not statistically different ($P > 0.05$) from it. A slight elevation in pH towards the
end of the drying stage was observed in all treatments and could be attributed to the generation of ammonia and amide compounds as a result of proteolytic activities (Pérez-Alvarez et al., 1999).

The change in $a_w$ was not significantly different ($P > 0.05$) between treatments during fermentation and all treatments achieved the regulated $a_w \leq 0.9$ by the end of the full process (Fig. 4.1B). The $a_w$ level of the treatments ranged from approximately 0.960 on day 0 to 0.710 – 0.750 at the end of the drying period. The final product $a_w$ was not significantly different ($P > 0.05$) between treatments except the medium and higher CP treatments, which showed a considerably lower ($P < 0.05$) final $a_w$. The lower $a_w$ observed could perhaps be related to the additional amount of sugar and dietary fiber provided by the CP. Similar $a_w$ reduction in DFS enriched with fiber has also been reported by others (Garcia et al., 2002; Eim et al. 2008; Fernandez-Lopez et al., 2008).

All DFS reached a shelf-stable MPR of $< 1.9$ (CFIA, 2018) after the 5-day fermentation process and continued to steadily drop to a final MPR of $< 0.90$ after the additional 4-weeks drying (Fig. 4.1C). There was no significant difference ($p > 0.05$) seen between the treatments except the low CP treatment, which showed a final MPR approximately 0.50 higher ($P < 0.05$) than that of the control. The outcome for low CP addition was possibly due to the enhanced moisture retention ability in the presence of dietary fiber-rich CP (Gracia et al., 2002).

4.3.2 Effect of CP on DFS Moisture, Protein, and Fat Level

Overall, all treatments showed a stepwise reduction in moisture content, and increase in protein and fat content throughout the study as a result of drying. The relative percentage of moisture, protein, and fat changed from approximately 54-57% to 19-23%, 16-18% to 26-29%, and 21-23% to 38 to 41%, respectively ($P > 0.05$; Fig 4.2). Such typical changes in DFS chemical compositions during manufacturing are consistent with earlier studies with similar manufacturing
process (Chacon et al., 2006; Graumann and Holley, 2008; Holck et al., 2011; Balamurugan et al., 2017). All treatments, except the low CP level, showed no significant \( P > 0.05 \) effect on product chemical composition when compared to the control. Low CP level yielded DFS with a moisture content approximately 2.04\% higher than the control and is in agreement with Gracia et al. (2002) and Sánchez-Zapata et al. (2013), who also reported a similar 2-4\% higher final water content in DFS with fruit fiber, and tiger nut fiber, respectively. On the other hand, Eim et al. (2008) found that increased level of carrot dietary fiber yielded DFS with a lower moisture and fat content. In fact, it is believed that such changes in moisture content are linked to the type of fiber studied and in any case have minimal impact on DFS organoleptic properties.

4.3.3 Effect of CP on \textit{S. enterica} serovars Inactivation

To the best of our knowledge, there are limited studies to date examining the practicality of cranberry processing by-products as functional antimicrobial agents in a processed meat matrix, especially models that involve other biological factors (e.g. meat starter culture bacteria) that are crucial to the development of end-product structure, organoleptic characteristics, and microbiologically safety. In the present work, DFS added with CP resulted in a significantly \( P < 0.05 \) greater \textit{S. enterica} serovar reduction than the control (Table 3). After the 5-day fermentation stage a mean reduction of 3.62 ± 0.07\,-, 4.91 ± 0.07\,-, 5.73 ± 0.62\,-, > 6.59 ± 0.10\,-, and 4.80 ± 0.30-log of \textit{S. enterica} serovar was observed in the control, low, medium, high CP, and LA treated DFS, respectively. This suggests that both inactivation level and rate are positively related to CP incorporation level as well as sausage acidity. Our findings are in agreement with Gniewosz and Stobnicka (2018), who similarly reported that the application of 2.5\% water-soluble CP extract to minced pork effectively reduced the number of \textit{S. enterica} by 4-log CFU/g after 6 days.
Nonetheless, a non-fermented meat system was employed in their case. Moreover, in our experiment, CP shortened the duration required to achieve a 5-log reduction of *S. enterica* serovar required for validation studies for non-thermally processed meat products (CFIA, 2018). For instance, the regulated inactivation level was achieved by around day 12, 5, 5, and 2 of the manufacturing process for the control, low, medium, and high CP DFS, respectively (Table 3). The progressively greater (*P* < 0.05) reduction observed, in DFS with increasing levels of CP, is likely to be related to the pH-decreasing characteristic of CP, as it has long been hypothesized that the low pH of cranberry plays an important role in foodborne pathogen inhibition via creating an osmotic stress that causes sublethal injury to bacteria membranes (Wen et al., 2003; Lin, Labbe, and Shetty, 2004; Wu et al., 2008; Lacombe et al., 2013). In fact, LA treatment in our work also strengthened the significance of CP-induced acidification on *Salmonella* inactivation when compared to the control. Furthermore, the smaller log reduction value (*P* < 0.05; 4.80 ± 0.09-log CFU/g) than the medium CP treatment observed in the LA treatment by the end of the fermentation process suggests that CP also contain other key bioactive compounds in addition to natural organic acids (i.e. citric acid, quinic acid, malic acid, etc.) that are capable of inhibiting the growth of *S. enterica* serovars; such as anthocyanins, flavonols, PACs, and phenolic acids. Various researchers pointed out that the antimicrobial action of cranberry, against foodborne pathogens, not only comes from its acidity but can also be attributed to certain cell structure destabilization and downregulation actions manifested by bioactive phenolics as well as synergistic effects between the several components (Puupponen-Pimia et al., 2001; Wu et al., 2008; Wu et al., 2009; Lacombe et al., 2010; Côté et al., 2011; Caillet et al., 2012; Lacombe et al., 2013). For instance, Wu et al. (2008) reported that cranberry concentrates hold greater antibacterial activity than acidic solutions, and their transmission electron microscope analysis revealed that pH standardized
cranberry phenolics were able to disintegrate bacteria cell wall, cell membrane and cause subsequent cell death. Lacombe et al. (2010) and (2013) also noticed a similar outer membrane weakening effect against *E. coli* O157:H7 by pH neutral cranberry phenolic and anthocyanin components. Although the antimicrobial activity of CP greatly depends on the cranberry variety, preparation method, and chemical composition, our work demonstrated a pronounced concentration-dependent effect of CP on *S. enterica* serovar inactivation in an acidified meat matrix and further support the idea of the relationship between cranberry natural acidity, phenolic compounds and foodborne pathogen inhibition. It is important to note that the reported days to reach the required 5-log reduction are only a guideline duration as it varies widely between DFS recipes, manufacturing conditions, and CP preparation procedures. Results from the enrichment samples showed that all DFS remained positive for *S. enterica* serovar at the end of the study. In fact, similar observations were reported in other challenge studies involving *E. coli* O157:H7 (Balamurugan et al., 2017; De Souza et al., 2018).

**4.3.4 Effect of CP on Meat Starter Cultures**

**4.3.4.1 Lactobacillus spp. and Pediococcus spp.**

The growth and viability of lactic acid-producing bacteria is essential for DFS as they are responsible for the formation of organic acids, mainly lactic acid, which contributes to the development of product flavor, color, aroma, textural characteristics, as well as microbiological stability. In the present study, the control treatment demonstrated the highest *Lactobacillus* spp. and *Pediococcus* spp. initial population size, whereas the medium and high CP treatments showed a progressive reduction in initial population size and time to reach maximum size, especially as the CP level increased (Fig. 4.2A, B). Overall, the *Lactobacillus* spp. and *Pediococcus* spp.
population of the low CP treatment and the control were not statistically different throughout the study \((P > 0.05)\). The lower initial population size observed for higher CP level treatment could perhaps be related to the relatively lower meat batter pH mentioned previously, as LA treatment also showed a similar trend. Nevertheless, both *Lactobacillus* spp. and *Pediococcus* spp. population in the medium and high CP treatments were able to rebound, and attained a significantly \((P < 0.05)\) higher number than the control after 2 days, and remained higher \((P < 0.05)\) than control throughout the remaining process (Fig. 4.2A, B). Yalınkılıç et al., (2012) also reported a higher lactic acid bacteria count in Turkish-type DFS (Sucuk) produced with 4% orange fiber. In fact, the observed higher population size, after 2-days and onwards, could be attributed to the presence of growth stimulators and/or additional growth substrates (e.g. sugars) from CP. Our results suggest a stimulatory effect of CP on specifically *Lactobacillus* spp. and *Pediococcus* spp. However, initial adaptation to CP natural acidity appears to be required.

4.3.4.2 *Staphylococcus* spp.

Changes in the *Staphylococcus* spp. population during DFS manufacturing are presented in Fig. 4.3C. All treatments demonstrated a similar initial population on production day \((P > 0.05)\). Among all treatments, the control showed the fastest increase in cell number and reached the greatest \((P < 0.05)\) population size at around 7.52 ± 0.07-log CFU/g after 2 days of fermentation, and then remained relatively stable until the end of the study. Similarly, low CP level seemed not to have any impact on *Staphylococcus* spp. growth \((P > 0.05)\) throughout the entire study. A significant \((P < 0.05)\) decrease in population was observed in DFS added with medium and high levels of CP after 24 h of fermentation; the number remained significantly \((P < 0.05)\) lower than that of the control until the end of the process. For instance, cell count for the medium and high
CP treatment on day 33 was approximately 0.68- and 0.95-log CFU/g lower than that of the control, respectively. Our results are in agreement with Yalınkılıç et al. (2012), who similarly reported a reduction in Staphylococcus spp. population in Sucuk added with increasing levels (2-4%) of orange fiber. Li et al. (2013) also reported a inhibitory effect on Staphylococcus spp. in DFS added with antimicrobial-rich ground deodorized yellow mustard. Such phenomenon in our work could perhaps be explained by the pH-decreasing effect of CP. It has been previously reported that rapid acidification in DFS could reduce the viability of certain acid-sensitive functional microorganisms such as Micrococcus spp. and Staphylococcus spp. (Lisazo et al., 1999; Rantsiou and Cocolin, 2006; Fernández-López et al., 2007). LA treatment demonstrated a growth profile similar ($P > 0.05$) to that of medium and high CP treatment during the fermentation process, but elevated and remained at a considerably higher ($P < 0.05$) level during the 4-week drying stage. This suggests that the reduction in Staphylococcus spp. population might not solely be caused by the CP acidity, but also a range of phenolic compounds present in CP. Such finding is in line with another work by the authors (Chapter 3), where increasing levels of cranberry bioactive phenolics and acidity were found to be the growth-limiting factors for S. carnosus and S. xylosus. The major functions of these microorganisms consist of limiting lipid oxidation, color formation and stabilization as well as aromatic profile development by means of their nitrate reductase, proteolytic and lipolytic activities (Hammes and Hertel, 1998; Talon et al., 1999; Simonovà et al., 2006). For this reason, the impact on DFS sensorial attributes due to reduced Staphylococcus spp. population should be further researched.
4.3.5 Effect of CP on DFS Color Parameters

Color of DFS is a very important characteristic that influence consumer acceptability and, eventually, associated with products’ quality when purchased. It is important to keep in mind that when attempting to incorporate a new ingredient into a traditional product, consumers might reject the product if it does not retain its original characteristics. The results for both exterior and interior color measurements are shown in Fig. 4.4 and 4.5. A significant ($P < 0.05$) reduction in $L^*$, $C^*$, and $h^o$ value for both exterior and interior color was observed for all CP treatments on day 0; and could mainly be attributed to the presence of purple-red cranberry pomace pigment compounds such as anthocyanins and PACs. Despite the bright red hue of CP alone, the darker purplish-red color observed was probably due to the partial color shift of anthocyanin ions from red to blue under increased pH when added to the meat batter (von Elbe and Swartz, 1996), yielding a dark and purplish mixture of the CP and raw meat. The observed spike in exterior $C^*$ value, and reduction in $h^o$ value in the control after the 5-days fermentation was due to the formation of bright red nitrosylmyoglobin pigment; i.e. between myoglobin and nitric oxide, which essentially turned the DFS from pale yellow to bright red ($P > 0.05$; Fig. 4.4A, B). In fact, such drastic change in color parameters was not observed in CP added DFS as their darker hue might have had masked the brightness and intensity of the newly formed meat color pigments. By the end of the process, all treatments showed a lower $L^*$ value than day 0 as a result of stepwise moisture loss. Both medium and high CP treatments showed a progressively ($P < 0.05$) lower exterior $L^*$, $C^*$ and $h^o$ value than the control during the 4-week drying stage (Fig. 4.4A, B, C); suggesting a darker, duller, as well as redder end-product color. Low CP level yielded a final exterior color slightly darker (lower $L^*$ value; $P < 0.05$) than the control while color dullness and redness was not impacted ($P > 0.05$). End-product interior $C^*$ values were also significantly reduced ($P < 0.05$; Fig 4.5A, B),
while a greater \( h^0 \) value \((P < 0.05; \text{Fig. 4.5C})\) was observed for the medium and high CP treatments; indicating a more yellow final interior. In contrast, low CP level only reduced interior color saturation (lower \( C^* \) value; \( P < 0.05 \)) but not lightness and redness. Our findings suggest that the influence of CP on DFS color characteristics is related to the incorporation level; with low CP treatment showing the most similar color to the control. In any case, such differences in end-product color require future research to validate consumer acceptability.

### 4.3.6 Effect of CP on DFS Textural Parameters

Texture of DFS are considered an important attribute of consumer acceptance and is often employed as a parameter to determine the quality of the finished product (Herrero et al., 2007). Low CP level showed no significant effect on the textural characteristics of DFS throughout the entire manufacturing process \((P > 0.05; \text{Table 4.4})\). Contrarily, a significantly lower hardness, springiness, cohesiveness, gumminess, and chewiness \((P < 0.05)\) were observed for the medium and high CP DFS after 24 h into the fermentation stage, and values remained considerably lower \((P < 0.05)\) than that of the control until the end of the study; indicating that the addition of higher levels of CP impart a softer and more crumbly texture. However, our results show conflicting trends compared to earlier reports examining the effect of fiber or pomace addition to DFS. Calvo et al., (2008), Eim et al. (2008), and Sánchez-Zapata et al. (2013) reported an increase in hardness of DFS enriched with tomato peel, dried carrot pomace, and Tiger nut fiber respectively. The addition of 1.5 - 3.0% peach, orange or apple fiber was also found to decrease DFS firmness only, however not springiness, cohesiveness, gumminess, and chewiness (Gracia et al., 2002). Backers and Noli (1997) suggested that incorporation of dietary fiber can enhance the integrity of meat products by forming a more ordered three-dimensional network, or by reinforcing the existing
meat protein network which can subsequently alter the rheological properties as well as the water-holding capacity of meat emulsions. However, the variation in our case could be due to the difference in type and/or chemical composition of the pomace used. In the present study at which a significant pH-decreasing phenomenon was observed, the softer and more crumbly texture could be attributed to the significant acidification effect seen in the medium and high CP treatments (Fig 4.1). When a meat batter is subjected to instant and drastic pH reduction, rapid protein denaturation and dis-ordered aggregation occurs, causing clumping of ground meat particles, loss of liquid, and irreversible reduction in product integrity (Barbut, 2005). Fernandez-Lopez et al. (2008) and Li et al. (2013) similarly reported a reduction in textural acceptability in DFS supplemented with 2% orange fiber and 3-4 % ground yellow mustard, respectively, as result of their pH-lowering characteristics and accelerated pH reduction. The observed phenomenon in our work could also be the result of meat protein cross-linking disruption and subsequent network weakening in the presence of high levels of dietary fiber from CP (Ktari et al., 2014; Wang et al., 2017).

4.4 Conclusion

To conclude this chapter, it appears that the present study is the first comprehensive research examining the antimicrobial effect of CP in DFS as well as its impact on product quality attributes. The results show that CP has a significant effect on S. enterica serovar inactivation in a fermented meat matrix, and the level and inactivation rate are concentration-dependent. Overall the growth of LAB was stimulated in the presence of medium to high levels of CP while Staphylococcus spp. growth was inversely linked to the CP level. Addition of low levels of CP not only resulted in effective pathogen load reduction, but also yielded DFS with physicochemical, color, and textural characteristics similar to conventional DFS. DFS incorporated with medium
and high CP levels showed a softer, more crumbly texture and a darker, duller, yet redder color. By choosing the appropriate level, CP can be utilized as a natural antimicrobial to further minimize the risk associated with *S. enterica* serovars during DFS manufacturing, without altering final product properties.
4.5 References


### 4.6 Tables and Figures

Table 4.1 Serotypes and sources of isolate of the five strain *S. enterica* serovars cocktail used in the study.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella enterica subsp. enterica</em> serovar Heidelberg ATCC 8326</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td><em>Salmonella enterica subsp. enterica</em> serovar Enteritidis ATCC 13076</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td><em>Salmonella enterica subsp. enterica</em> serovar Berta ATCC 8392</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td><em>Salmonella enterica subsp. enterica</em> serovar Typhimurium ATCC 14028</td>
<td>Pig body fluid/excretion; American Type Culture Collection</td>
</tr>
<tr>
<td><em>Salmonella enterica subsp. enterica</em> serovar Newport ATCC 6962</td>
<td>Isolated from food poisoning fatality; American Type Culture Collection</td>
</tr>
</tbody>
</table>
Table 4.2 Formulation of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid (% values are based on wt/wt).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Salami meat mix&lt;sup&gt;1&lt;/sup&gt;</td>
<td>95.82%</td>
</tr>
<tr>
<td>Salami cure&lt;sup&gt;2&lt;/sup&gt;</td>
<td>3.71%</td>
</tr>
<tr>
<td>German salami seasonings</td>
<td>0.44%</td>
</tr>
<tr>
<td>Starter culture&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.022%</td>
</tr>
<tr>
<td>S. enterica serovars cocktail</td>
<td>6.5-log CFU/g</td>
</tr>
<tr>
<td>Cranberry pomace</td>
<td>0%</td>
</tr>
<tr>
<td>Liquid lactic acid</td>
<td>/</td>
</tr>
</tbody>
</table>

<sup>1</sup>-Salami meat mix contains pork back fat 18.46% wt/wt, lean pork 68.57% wt/wt and lean beef 8.79% wt/wt.
<sup>2</sup>-Salami cure resulted in 2.98% wt/wt salt; 0.654% wt/wt dextrose and corn syrup solid; 0.05% wt/wt sodium ascorbate; 0.0104% wt/wt sodium nitrite; 0.0104% wt/wt sodium nitrate in the batter.
<sup>3</sup>-Starter culture contains *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*, *Lactobacillus sakei*, and *Debaryomyces hansenii*. Added at a level of ~ 1 x 10<sup>7</sup> CFU/g.
<sup>4</sup>-Liquid lactic acid (LA) was added at a pre-determined level to create salami meat batter with an initial pH similar to Medium CP.
Table 4.3 Log reduction number of *S. enterica* serovars in dry fermented sausages (DFS) added with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and drying.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Control</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-2.06 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.23 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.20 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.70 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-2.12 ± 0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>-2.78 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.41 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.63 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-4.68 ± 0.42&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-3.17 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>-3.57 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.78 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-5.55 ± 0.28&lt;sup&gt;c*&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-4.80 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Drying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-4.63 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>19</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>26</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>33</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND/+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± standard deviation in the same row followed by different letters are significantly different (p < 0.05).
Control: 0% CP; Low CP: 0.55%; Medium CP: 1.70%; High CP: 2.25%; LA: 0.33% liquid lactic acid.

ND indicates no colonies detected with a reduction level greater than initial inoculated level.
+ indicates positive results for Selenite Cystine Broth Enrichment.
* *S. enterica* serovars less than 25 CFU per plate.

Mean initial inoculation level in control, low, medium, high, and LA treatment was 6.62 ± 0.06, 6.60 ± 0.08, 6.60 ± 0.04, 6.59 ± 0.13, and 6.58 ± 0.14, respectively.
Table 4.4 Effect of cranberry pomace (CP) at varying levels on the textural properties of dry fermented sausages (DFS) during fermentation and dry stages.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Hardness (N/cm²)</th>
<th>Cohesiveness (ratio)</th>
<th>Springiness (mm)</th>
<th>Gumminess (N/cm²)</th>
<th>Chewiness (N/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>L</td>
<td>M</td>
<td>H</td>
<td>C</td>
</tr>
<tr>
<td>Fermentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.77± 1.52</td>
<td>9.15</td>
<td>5.48</td>
<td>4.94</td>
<td>0.79</td>
</tr>
<tr>
<td>10.01</td>
<td>8.77</td>
<td>7.85</td>
<td>7.21</td>
<td>±0.8</td>
<td>±0.9</td>
</tr>
<tr>
<td>2</td>
<td>±1.23</td>
<td>±1.6</td>
<td>±0.6</td>
<td>±1.4</td>
<td>±0.0</td>
</tr>
<tr>
<td>19.91</td>
<td>21.1</td>
<td>18.6</td>
<td>17.6</td>
<td>±0.7</td>
<td>±0.6</td>
</tr>
<tr>
<td>5</td>
<td>±1.32</td>
<td>±1.5</td>
<td>±1.1</td>
<td>±1.1</td>
<td>±0.0</td>
</tr>
<tr>
<td>Drying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>49.48</td>
<td>57.2</td>
<td>58.3</td>
<td>49.4</td>
<td>0.57</td>
</tr>
<tr>
<td>±10.4</td>
<td>±1.10</td>
<td>±1.1</td>
<td>±1.0</td>
<td>±0.8</td>
<td>±0.0</td>
</tr>
<tr>
<td>1.8</td>
<td>±0.9</td>
<td>±0.9</td>
<td>±0.9</td>
<td>±0.9</td>
<td>±0.9</td>
</tr>
<tr>
<td>±78.67</td>
<td>±85.3</td>
<td>±70.3</td>
<td>±69.4</td>
<td>±0.5</td>
<td>±0.5</td>
</tr>
<tr>
<td>±19</td>
<td>32.5</td>
<td>32.5</td>
<td>32.5</td>
<td>32.5</td>
<td>0.54</td>
</tr>
<tr>
<td>±26</td>
<td>6±7.5</td>
<td>7±8.2</td>
<td>7±8.4</td>
<td>±0.0</td>
<td>±0.0</td>
</tr>
<tr>
<td>±14.4</td>
<td>±13.3</td>
<td>±10.6</td>
<td>±10.6</td>
<td>±0.5</td>
<td>±0.5</td>
</tr>
<tr>
<td>±33</td>
<td>3±7.0</td>
<td>5±7.0</td>
<td>5±7.0</td>
<td>±0.0</td>
<td>±0.0</td>
</tr>
<tr>
<td>±3±7.0</td>
<td>±3±7.0</td>
<td>±3±7.0</td>
<td>±3±7.0</td>
<td>±0.0</td>
<td>±0.0</td>
</tr>
</tbody>
</table>

Means ± standard errors in the same row followed by different letters are significantly different (p < 0.05).
C: control, 0% CP; L: low, 0.55% CP; M: medium, 1.70% CP; H: high, 2.25% CP.
**Fig 4.1** Changes in pH, $a_w$ and Moisture Protein ratio (MPR) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; ×: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.
Fig 4.2 Changes in moisture, protein and fat level of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ◆: high CP, 2.25%; ×: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.
**Fig 4.3** Changes in *Lactobacillus* spp. (A), *Pediococcus* spp. (B), and *Staphylococcus* spp. (C) cell count in dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) or liquid lactic acid during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ●: high CP, 2.25%; ◼: 0.33% liquid lactic acid). Error bars denote ± one standard deviation.
**Fig 4.4** Changes in exterior lightness L*(A), chroma C*(B), and hue angle h° value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ●: high CP, 2.25%). Error bars denote ± one standard deviation.
Changes in interior lightness L*(A), chroma C* (B), and hue angle h° value (C) of dry fermented sausages (DFS) with varying levels of cranberry pomace (CP) during fermentation and the drying stages (●: 0%, control; ■: low CP, 0.55%; ▲: medium CP, 1.70%; ♦: high CP, 2.25%). Error bars denote ± one standard deviation.
CHAPTER 5 CONCLUSION AND FUTURE WORK

The present study focused on the utilization of CP, a valuable fruit industry by-product, as a multi-functional resource for food-related applications. The effect of CP on the growth of both beneficial and pathogenic microorganisms, as well as quality characteristics associated with fermented meat products were examined, and results revealed that at correct levels, CP has minimal influence on product quality attributes and may exert a dual positive effect on the microorganisms present; on the one hand inhibit foodborne pathogens, and on the other hand promote the growth of beneficial bacteria.

More importantly, to the best of our knowledge, Chapter 3 appears to be the first study to date examining the growth-stimulatory potential of extracts of berry origins on common meat starter cultures. In short, a concentration-dependent growth stimulation was observed for all starter cultures. *Lactobacillus* spp. and *Pediococcus* spp. showed maximum OD$_{600}$ in the presence of 0.50 – 0.10% wt/vol CE, and could perhaps be attributed to the carbohydrates, particularly sugars, fibers and xyloglucans, in CE and several possible positive interactions with CE phenolic compounds. *Staphylococcus* spp. overall showed a higher sensitivity towards CE than other starter cultures and complete growth inhibition was observed starting from 0.50% wt/vol CE. Reduced growth performance at higher CE levels (i.e. > 0.75% wt/vol CE) was found to be a result of increased CE inherited acidity and phenolic content. *E. coli* O157:H7, *S. Enteritidis*, and *L. monocytogenes* all demonstrated a higher susceptibility towards CE than the starter cultures; MICs were established at 0.5 –1.0% wt/vol CE. These findings will provide valuable insight and fundamental information for later researches aiming for food product applications (e.g. Chapter 4). Future researches can focus on i) application of cranberry pomace extracts as a multi-functional ingredient in food systems and/or as a growth substrate for starter cultures; ii) characterization of
stimulatory actions of CE components on the growth of meat starter cultures, as well as the mechanism and metabolism pathways involved; iii) changes in cranberry phenolic compounds profile such as composition, bioactivity and bioavailability in the presence of meat starter cultures.

With the findings from Chapter 3, Chapter 4 looked more closely into the application of CP as a functional ingredient in a fermented meat system under industrial processing conditions. CP supplementation resulted in a faster and greater *S. enterica* serovars inactivation during fermentation in comparison to the control; pathogen inactivation was found to be a combined effect of CE acidity and phenolic compounds. It is interesting to point out that while 0.25% wt/vol CE showed no inhibitory effect on *S. Enteritidis* *in vitro* as mentioned in Chapter 3, low CP treatment (0.55% wt/wt), which has a phenolic content equivalent to 0.25% wt/vol CE, showed a pronounced inhibitory effect on *Salmonella* when applied to a meat system. This could perhaps be explained by the markedly lower pH observed in DFS (pH > 5.0) when compared to 0.25% wt/vol CE in nutrient broth (pH 6.05). The considerably higher pH in nutrient broth possibly caused destabilization of cranberry polyphenols, thereby losing their antimicrobial activity. Such information suggests that the antimicrobial properties of cranberry extracts may vary between *in vitro* and *in vivo* models and require additional studies to verify their antimicrobial activity when incorporated to particular food system. The effect of CP on DFS starter cultures agrees with the finding from Chapter 3; *Staphylococcus* spp. growth was limited due to cranberry acidity and phenolic components, while *Lactobacillus* spp. and *Pediococcus* spp. exhibited a growth-stimulatory effect in the presence of medium and high levels of CP. As for quality characteristics, low CP level yielded DFS that are similar to conventional DFS in terms of physicochemical parameters, composition, color, and texture. Medium and high CP levels resulted in darker, duller and redder DFS with a softer and more crumbly texture. In summary, by choosing the appropriate
level, CP can be utilized as a natural antimicrobial additive to further reduce the risk associated with *S. enterica* serovars during DFS manufacturing, without altering final product properties. Future research on product flavor characteristics and consumer acceptability in the presence of CP is recommended. As cranberry phenolic compounds have also been reported as excellent antioxidants against lipid and protein oxidation, it would also be valuable to investigate the antioxidant potential of CP in DFS or other food models. Application of CP in other meat products and acidic food systems such as yogurt-type products can be explored as well. Due to growing consumer interest for natural, organic and chemical-free food products, our findings are particularly significant as CP, a crude underutilized solid fruit waste, can potentially be used as a multi-functional and economical resource for meat product applications.
Fig 1. Effect of CE at varying concentrations (wt/vol) and pH 5.0 modified dextrose-free MRS (mMRS) on the growth of *L. sakei* FAS 4 (A), *L. curvatus* FUA 3015 (B), *L. plantarum* FUA 3073 (C), and *L. plantarum* UM131L (D) in mMRS under aerobic condition. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.0 mMRS has a pH equivalent to 1.0% wt/vol CE in mMRS.
**Fig 2.** Effect of CE at varying concentrations (wt/vol) on the growth of *L. sakei* FAS 4 (A), *L. curvatus* FUA 3015 (B), *L. plantarum* FUA 3073 (C), and *L. plantarum* UM131L (D) in modified dextrose-free MRS (mMRS) under anaerobic condition. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation.
Fig 3. Effect of CE at varying concentrations (wt/vol) and pH 5.0 modified dextrose-free MRS (mMRS) on the growth of *P. acidilactici* UM 104P (A), *P. acidilactici* FUA 3072 (B), *P. pentosaceus* UM 116P (C), and *P. pentosaceus* FUA 3071 (D) in mMRS. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.0 mMRS has a pH equivalent to 1.0% wt/v CE in mMRS.
Fig 4. Effect of CE at varying concentrations (wt/vol) and pH 5.4 modified dextrose-free TSB (mTSB) on the growth of *S. carnosus* BBU 001 (A), and *S. xylosus* FUA 3211 (B) in mTSB. The curves are drawn from the average of at least three independent experiments. Error bars denote ± one standard deviation. pH 5.4 mTSB has a pH equivalent to 0.5% wt/v CE in mTSB.