PREDICTING THE PHYSICOCHEMICAL PROPERTIES OF PORK BELLY AND THE EFFECT OF COOKING AND STORAGE CONDITIONS ON BACON SENSORY AND CHEMICAL CHARACTERISTICS

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfilment of the Requirements

for the Degree of Doctor of Philosophy

in the Department of Food and Bioproduct Sciences

University of Saskatchewan

Saskatoon

By

Olugbenga Philip Soladoye

2017

© Copyright Olugbenga Philip Soladoye, July 2017. All rights reserved.

PERMISSION TO USE

In presenting this thesis, in partial fulfillment of the requirements for a degree of Doctor of Philosophy from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis, or parts thereof, for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or in part should be addressed to:

Head of the Department of Food and Bioproduct Sciences
University of Saskatchewan
Saskatoon, Saskatchewan
Canada S7N 5A8

ABSTRACT

The first objective of this research was to use a widely varying pig population to create prediction algorithms for dual energy X-ray absorptiometry (DXA) pork carcass compositional estimate and pork belly softness measurement. Further, bellies with compositional extremes were used in bacon production and cooked in two ways to determine the impact of composition, storage days and cooking method on lipid and protein oxidation as well as heterocyclic aromatic amines. A total of 648 pigs, either barrows or gilts, from three sire breeds (Lacombe, Duroc or Iberian boar × Large White * Landrace F1 dams), were provided one of three diets (conventional, canola-based or flaxseed-based feed) *ad libitum* until they reached either ~120 or 140 kg slaughter weight. These variations were intentionally introduced so that the animal population could adequately represent the variation applicable to commercial production. Following slaughter, carcass sides and primal cuts were scanned under DXA equipment. For the second experiment, 198 left side bellies were assigned to belly-flop angle and subjective score measurements to evaluate pork belly softness. The third experiment employed 44 right side bellies which were randomly selected from the treatment extremes (barrows or gilts, Iberian or Lacombe, and control or flaxseed based diet). These 44 bellies were processed into bacon slices which were cooked with either microwave heating or pan frying after 2 or 28 days of refrigerated storage. Regardless of variation in animal population, DXA accurately predicted dissected/chemical fat and lean content of carcass sides and primal cuts ($R^2 > 0.94$, $P < 0.01$; RSD, 0.8 to 2.9%). The multifactorial nature of pork belly softness was confirmed with a stepwise regression model that explained up to 77 and 83% of subjective belly softness score and belly-flop angle measurement, respectively, with both chemical and dimensional factors as the predictors. Employing belly-flop angle measurement in the assessment of pork belly softness would require a correction for belly length. Although microwave cooking of bacon led to a significantly higher increase in protein oxidation ($P < 0.001$), cooking in a frying pan resulted in higher increase in heterocyclic aromatic amines and lipid oxidation in bacon ($P < 0.001$). Storage days and belly composition did not affect the production of these chemical compounds ($P > 0.05$). The cooking treatments and storage days also had minimal effects on bacon sensory attributes. Overall, the present study established mathematical models to improve DXA estimate of pork carcasses and enhance pork belly softness assessments. The results could also inform public health recommendations regarding choice of cooking method for bacon.

ACKNOWLEDGEMENTS

Now the words of Eleanor Roosevelt resonate more with me, that "the future belongs to those who believe in the beauty of their dreams". Although I couldn't see the whole staircase, I took the first step anyways, relishing the support of great mentors and friends that nature has blessed me with. First, I would like to whole-heartedly express my appreciation to my incredible supervisors; Drs. Manuel Juarez and Phyllis Shand for their unparalleled support, understanding, patience, guidance and advice during the entire period of my doctoral studies. You believed in what I can be, you gave me a chance and never doubted my ability for once! You are an integral part of this success story and your names are written with gold on the table of my heart. I would also like to express my profound gratitude to Dr. Jennifer Aalhus whose motherly predisposition was a great encouragement to me. Your great advice, support, kindness and grace have helped me this far. More than a million thanks. The unparalleled encouragement and advice of my advisory committee; Drs. Nicholas Low, Robert Tyler, Michael Nickerson and Denise Beaulieu will never be forgotten and I will forever be grateful for all your kind support. The immense contribution of my external examiner; Dr. Jason Apple is also greatly acknowledged.

The generous funding and support from the Canadian Beef Grading Agency, Alberta Livestock and Meat Agency Ltd. (ALMA), University of Saskatchewan Devolved Scholarship and the Natural Sciences and Engineering Research Council of Canada (NSERC) have made this dream a reality. I am eternally grateful for your support. You have not only made a man, you have made a generation! I would also want to acknowledge all the technical staff of Lacombe Research and Development Centre for their great help in the course of my research. You all hold a special place in my heart. I will never forget the incredible support of my family and friends who stood by me through the thick and thin of this program. My parents, Mr and Mrs Nathaniel and Grace Soladoye, all my siblings and my friends; Ayodele Olagunju, Jeremy Sealock, Duncan Henry, Rhona Thacker, Dapo Fawehinmi, Ayoola Jinadu, Emmanuel Leonard, Peter Maduakor, Ola Amusa, Seun Oluwajana and Omotola Olaniyan, to name a few. All your smiles and tolerances were healing balm for me especially when I felt broken and alone. Thank you so much.

"Now unto HIM who is able to do exceeding abundantly above all that we may think or ask of HIM according to the power that dwell him us." To this only true God, my allegiance I forever pledge!

DEDICATION

To all those who dare to dream despite their dreads

TABLE OF CONTENTS

PERMISSION TO USE ... i
ABSTRACT ... ii
ACKNOWLEDGEMENTS ... iii
DEDICATION ... iv
TABLE OF CONTENTS ... v
LIST OF TABLES ... x
LIST OF FIGURES ... xii
LIST OF ABBREVIATIONS ... xiii
1 **INTRODUCTION** ... 1
1.1 Objectives ... 3
1.2 Hypotheses ... 3
2 **LITERATURE REVIEW** ... 5
2.1 Bacon and its recent consumption trend ... 5
2.1.1 History of bacon ... 5
2.1.2 Recent consumption trends ... 6
2.2 Bacon processing ... 11
2.2.1 Raw material selection and sorting ... 11
2.2.2 Curing methods ... 12
2.2.2.1 Pump curing ... 12
2.2.2.2 Dry curing ... 13
2.2.3 Smoking and thermal treatment ... 13
2.2.4 Slicing and packaging ... 14
2.3 Indices of pork belly and bacon quality ... 16
2.3.1 Pork belly structure ... 16
2.3.2 Pork belly softness defects and possible impact on bacon quality ... 16
2.3.3 Pork belly and bacon quality indices ... 18
2.3.4 Pork belly and bacon quality assessment ... 20
2.3.4.1 Non-invasive methodology to evaluate belly softness and composition ... 20
2.3.4.1.1 Pork belly softness and its non-invasive evaluation ... 20

2.3.4.1.2 Pork belly composition and its non-invasive evaluation 20
2.3.4.1.3 Dual energy X-ray absorptiometry technology in compositional assessment. 21
2.3.4.2 Invasive methodology to evaluate belly softness and composition 24
2.3.4.2.1 Invasive methodology to evaluate belly softness 24
2.3.4.2.2 Invasive methodology to evaluate belly composition 24
2.3.4.3 Bacon quality evaluation 25
2.3.5 Factors affecting pork belly and bacon quality 25
2.3.5.1 Pre-slaughter factors 26
2.3.5.1.1 Genotype/breed effects 26
2.3.5.1.2 Sex effects 27
2.3.5.1.3 Dietary effects 28
2.3.5.1.3.1 Protein and amino acid derivative sources 28
2.3.5.1.3.2 Carbohydrate sources 29
2.3.5.1.3.3 Fat sources 30
2.3.5.1.3.4 Vitamins and mineral sources 31
2.3.5.1.3.5 Growth promotants 32
2.3.5.1.4 Age and slaughter weight 34
2.3.5.1.5 Pre-slaughter environment 34
2.3.5.2 Post-slaughter or processing factors on pork belly and bacon quality 35
2.3.5.2.1 Ageing and storage time 35
2.3.5.2.2 Frozen storage 36
2.4 Effect of cooking methods on bacon and processed meat quality 36
2.4.1 Common cooking methods in bacon processing and their underlying mechanisms 36
2.4.2 Effect of cooking methods on production of toxic compounds in processed meat 39
2.4.3 Lipid oxidation in processed meat 41
2.4.3.1 Indication and analysis of lipid oxidation in meat systems 41
2.4.3.2 Effects of cooking/heat treatment on production of lipid oxidation in meat 43
2.4.3.3 Pathogenesis of lipid oxidation products 43
2.4.4 Protein oxidation in processed meat 48
2.4.4.1 Indication and analysis of protein oxidation in meat systems 49
2.4.4.2 Effect of cooking/heat treatment on protein oxidation 56
2.4.4.3 Effects of protein oxidation on nutritional value of meat and human health 59

2.4.4.3.1 Loss of essential amino acids 61
2.4.4.3.2 Reduced digestibility 62
2.4.4.3.3 Reduced bioavailability 63
2.4.4.3.4 Increased cytotoxicity and mutagenicity 64
2.4.5 Heterocyclic aromatic amines content in processed meat 66
2.4.5.1 Physical and chemical factors crucial in heterocyclic amine formation in meat 72
2.4.5.2 Occurrence of heterocyclic aromatic amine in meat products 73
2.4.5.3 Health implication of heterocyclic amine consumption in human 73
3 ACCURACY OF DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) IN ASSESSING CARCASS COMPOSITION FROM DIFFERENT PIG POPULATIONS ... 76
3.1 Abstract 76
3.2 Introduction 77
3.3 Materials and methods 79
3.3.1 Animal management and experimental design 79
3.2.2 Animal slaughter 79
3.2.3 Carcass scanning and cut out 81
3.2.4 Proximate analysis 81
3.2.5 Statistical analysis 83
3.3 Results 83
3.3.1 Descriptive statistics of variable characteristics 83
3.3.2 DXA prediction of chemical and dissected fat and lean 85
3.4 Discussion 96
3.4.1 Descriptive statistics 96
3.4.2 DXA prediction of fat and lean 96
3.4.4 DXA underestimation of fat and overestimation of lean 98
3.4.5 Effects of variation in population on DXA prediction 100
3.5. Implication for research and industrial applications 101
3.6. Conclusions 102
3.7 Connection to the next study 104
4 COMPOSITIONAL AND DIMENSIONAL FACTORS INFLUENCING PORK BELLY FIRMNESS 105
4.1 Abstract 105
4.2 Introduction 106

4.3 Materials and methods ... 107
4.3.1 Animal managements and slaughter ... 107
4.3.2 Pork belly selection, fabrication and firmness measurement ... 107
4.3.3 Chemical analysis ... 111
4.3.4 Statistical analysis ... 112
4.4 Results and Discussions ... 112
4.4.1 Description of pork belly characteristics ... 112
4.4.2 Pearson correlation coefficients among belly softness parameters ... 115
4.4.3 Predictors of pork belly softness ... 119
4.4.4 Estimating the impact of chemical and physical factors in pork belly softness measurement ... 119
4.6 Conclusions ... 125
4.7 Connection to the next study ... 126
5 **INFLUENCE OF COOKING METHODS AND STORAGE TIME ON LIPID AND PROTEIN OXIDATION AND HETEROCYCLIC AROMATIC AMINES PRODUCTION IN BACON** ... 127
5.1 Abstract ... 127
5.2 Introduction ... 128
5.3 Materials and methods ... 129
5.3.1 Animal management, sample selection and bacon production ... 129
5.3.2 Bacon cooking and treatments ... 130
5.3.3 Sample preparation ... 132
5.3.4 Protein carbonylation analysis ... 132
5.3.4.1 Synthesis of authentic AAS and GGS standards ... 132
5.3.4.2 Preparation of bacon sample for PROTOX HPLC-FLD analysis ... 132
5.3.5 Lipid oxidation ... 134
5.3.6 Heterocyclic aromatic amine analysis ... 134
5.3.6.1 HAA Standards preparation ... 134
5.3.6.2 HAA Sample Extraction and HPLC analysis ... 135
5.3.7 Sensory evaluation ... 136
5.3.8 Statistical analysis ... 136
5.4 Results and discussion ... 136
5.4.1 Effects of cooking method and storage time on protein carbonylation in bacon ... 139

5.4.2 Effect of cooking methods and storage times on lipid oxidation 142

5.4.3 Effect of cooking methods and storage times on heterocyclic amine production 143

5.4.4 Relationships between bacon composition, lipid and protein oxidation and HAA formation 146

5.4.5 Effects of cooking method and storage time on sensory attributes of bacon 151

5.5 Conclusions 155

6 **GENERAL DISCUSSION** 156

7 **OVERALL CONCLUSIONS AND FUTURE STUDIES** 167

8 **REFERENCES**.. 170

9 **APPENDICES** 215

LIST OF TABLES

Table 2.1: Select amino acid residues and their oxidation products 51
Table 2.2: Protein oxidation products in some processed meats... 57
Table 2.3: Selected heterocyclic aromatic amines and their properties[1] 69
Table 3.1: Ingredient composition and nutrient content of animal diets..................................... 80
Table 3.2: Description of the overall dependent and independent parameters before classification based on inherent factors 84
Table 3.3: Carcass side descriptive statistics and prediction equations 86
Table 3.4: Ham descriptive statistics and prediction equations 87
Table 3.5: Loin descriptive statistics and prediction equations 88
Table 3.6: Shoulder descriptive statistics and prediction equations.. 89
Table 3.7: Belly descriptive statistics and prediction equations 90
Table 3.8: Test of slope heterogeneity for carcass sides and primal cut predictions 93
Table 4.1: Summary of compositional and dimensional factors within the pig population 114
Table 4.2: Pearson correlation between dimensional parameters, proximate analysis and the belly softness measures 117
Table 4.3: Pearson correlation between selected fatty acid profiles and belly softness measures 118
Table 4.4: Stepwise regression model for subjective belly score measurements....................... 121
Table 4.5: Stepwise regression model for belly-flop angle measurements............................... 122
Table 4.6: Stepwise regression model for belly-flop angle measurements corrected for length 124
Table 5.1: Characteristics of pork bellies and the control bacon 137
Table 5.2: Change in protein oxidation, lipid oxidation and heterocyclic amine with cooking methods 140
Table 5.3: Change in protein oxidation, lipid oxidation and heterocyclic amine with storage days 142
Table 5.4: Pearson correlation among bacon chemical characteristics and pork belly composition 147
Table 5.5: Effect of animal breed, sex and diet on bacon attributes 150

Table 5.6: Effect of cooking methods on sensory perception of bacon 153

Table 6.1: Effect of animal breed, sex and diet on pork belly attributes 166

LIST OF FIGURES

Figure 2.1: Bacon price increase in Canada and the USA over a 21-year period......................... 7
Figure 2.2: Chilled bacon dollar sales in the United States from a 15-year period. 8
Figure 2.3: In-home consumption of pork in the United States in 2009..................................... 10
Figure 2.4: Industrial sliced bacon process flow chart 15
Figure 2.5: Mid-sagittal plane anatomical description of pork belly; sagittal view 17
Figure 2.6: Mechanism of protein oxidation.. 52
Figure 2.7: a) Lysine formation of AAS with their derivatization mechanisms.......................... 54
Figure 2.7: b) Arginine and proline formation of GGS with their derivatization mechanisms. .. 55
Figure 2.8: Types of heterocyclic aromatic amines with the general groups that characterise each type 68
Figure 3.1: Carcass sides and selected primal cuts during DXA scanning…...............................74
Figure 3.2: Carcass side DXA fat data based on the three sire breeds 94
Figure 3.3: Carcass side DXA lean data based on the three sire breeds 95
Figure 4.1: Component for assessing belly-flop angle measurements with the firmest to the softest belly in the research shown.. 108
Figure 4.2: Measurement locations along the pork belly.. 110
Figure 5.1: Bacon cooking methods a) arranged in Baconwave^{TM} and ready to be cooked in the microwave b) ready to be cooked on a pre-heated skillet 131
Figure 5.2: Values of total and individual HAA in the bacon as they respond to different cooking methods 145
Figure 5.3: Principal component analysis for chemical compounds in cooked bacon……….. 149
Figure 5.4: Effects of cooking methods and storage days on initial crispiness. 152

LIST OF ABBREVIATIONS

AAA	α-aminoadipic acid
AAS	α-aminoadipic semialdehyde
ABA	p-aminobenzoic acid
ACR	Acrolein
BCH	Breeding company hybrid
BIA	Bioimpedance analysis
BSA	Bovine serum albumin
CAT	Computer-assisted tomography
CCAC	Canadian Council on Animal Care
CFIA	Canadian Food Inspection Agency
CFI	Canadian Food Innovators
CLA	Conjugated linoleic acid
CPI	Canadian Pork International
CV	Coefficient of variation
DDGS	Dried distiller grain with solubles
DFD	Dark, firm and dry
DM	Dry matter
DNPH	2,4-dinitrophenylhydrazine
DPA	Dual photon absorptiometry
DTBN	5’5-dithiobis (2-nitrobenzoate)
DXA	Dual Energy X-ray Absorptiometry
EFSA	European Food Safety Authority
ES	Electrical stimulation
FA	Fatty acids

FLD	Fluorescent detector
FSIS	United States Department of Agriculture-Food Safety Inspection Service
GGS	γ-glutamyl semialdehyde
HAA	Heterocyclic aromatic hydrocarbon
HAL	Halothane gene
HNE	Hydroxynonenal
HP	High protein
IARC	International Agency for Research on Cancer
IBD	Inflammatory bowel disease
IC	Immunological castration
IQ	Imidazo quinolone
IQx	Imidazo quinoxaline
IV	Iodine value
LOOH	Lipid hydroperoxide
LP	Low protein
MDA	Malondialdehyde
MES	2-(N-morpholino) ethanesulfonic acid
MRI	Magnetic Resonance Imaging
MUFA	Monounsaturated fatty acids
NIRS	Near Infrared Spectroscopy
NNPC	National Pork Producer Council
NOC	N-nitroso compounds
OEHHA	Office of Environmental Health Hazard Assessment
PAH	Polycyclic aromatic hydrocarbon
PC	Physical castration
PFPH	Pentafluorophenyl hydrazine
PROTOX	Protein oxidation
PSE	Pale, soft and exudative

PSS	Porcine Stress Syndrome
PSt	Porcine somatotropin
PUFA	Polyunsaturated fatty acids
RAC	Ractopamine
RMSE	Root mean square error
RN^-	Rendement Napole gene
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSD	Residual standard deviation
RTE	Ready-to-eat
SFA	Saturated fatty acids
SW	Slaughter weight
TBARS	Thiobarbituric acid reactive substances
TOBEC	Total body electrical conductivity
UFA	Unsaturated fatty acids
WHC	Water-holding capacity
WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

Efficiently providing safe, appetizing and wholesome food to a growing world population may be regarded as the ultimate objective behind scientific explorations, industrial strategies and political programs in agriculture. As consumers' concerns in the western world grow regarding the impact of what they eat on their overall well-being, coupled with increasing scrutiny and interest of regulatory agencies and health authorities on dietary impact on human health, the meat industry faces numerous challenges. These include more than responding to the consumers' negative perception of meat consumption but also the task of balancing production and profitability against consumer demands and regulatory standards.

The bacon industry is a thriving industry in North America. By definition, bacon is a cured belly of a swine (hog) carcass (FSIS, 2011). Despite the continued increase in the price of bacon in recent years, consumer demand has not waned, defying years of higher prices and fragile economy. This is partly because, the consumption of bacon has transformed over the years from the traditional breakfast entrée to a condiment for different dishes, including sandwiches and salads. Its success, which may also be due to consumer's appreciation of this product, is however against a backdrop of consumer's concerns about their health as a result of their diet. Quality expectations may vary among various stakeholders along the bacon production chain and more often than not, consumers' and processors' quality perceptions differ.

Poor fat quality in terms of composition and texture, which may be due to a high proportion of unsaturated fatty acids, has been acknowledged to be responsible for bacon processing difficulty and low slice yield, as well as the poor shelf stability and sensory perceptions of the packaged product (Browne et al., 2013). On the other hand, fatter bellies could result in higher bacon slicing yield (Person et al., 2005). It is however, important that the satisfaction of the various quality perceptions is balanced to enhance market sustainability and profitability for processors alongside safety and quality for consumers.

Recently, the International Agency for Research on Cancer (IARC) classified red and processed meat as "*probably carcinogenic to human*" (class 2A) and "*carcinogenic to human*" (class 1), respectively (Bouvard et al., 2015; Oostindjer et al., 2014). Although this classification was largely based on epidemiological studies and was less convincing in terms of the mechanism of the pathogenesis, as well as the specific chemical components in the meat which may be responsible for these carcinogenic properties, it is possible that the interaction between the composition of the meat and the subsequent processing it is subjected to, are crucial in the production of these carcinogenic compounds. During meat heat processing steps, several carcinogenic compounds could be produced, which might be responsible for the IARC observations. These include N-nitroso-compounds (NOC), polycyclic aromatic hydrocarbon (PAH), lipid oxidation products and heterocyclic aromatic amines (HAA). Recent studies are also highlighting the possible carcinogenic nature of oxidized proteins (Estévez & Luna, 2016). As most of these compounds have been observed to be influenced by the cooking methods, cooking time and cooking temperature (Bouvard et al., 2015; Estévez, 2011; Kizil, Oz, & Besler, 2011; Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008; Sugimura, Wakabayashi, Nakagama, & Nagao, 2004), the consumer households' choice of processing or cooking method as well as modification of their existing cooking style may affect the extent that these carcinogenic compounds are produced in meat and its products.

Given that the composition of pork belly is also very important in the consideration of processors' profitability and consumers' health, it seems necessary to explore efficient technology for fast and accurate decision making in the industry to precisely select good quality bellies for subsequent processing and guaranteed productivity and profitability. Although pork belly prices have increased over the years to compete in value with other expensive primal cuts (e.g. loins), their methods of quality classification are still largely rudimentary. Going by visual cues, Mandigo (2000) has pointed out that the proportion of lean-to-fat in the pork belly is the most crucial factor in the consumers' selection of bacon and that the leaner bellies eventually produce a higher percentage of good quality slices compared to their fatty counterparts. To find the most objective measure to assess this parameter in the bacon industry, will contribute immensely to fairness in the pricing system among the farmers, the processors and the consumers. Employing non-invasive methodology for example, dual energy X-ray absorptiometry (DXA) could offer great improvement in this aspect.

A major quality defect associated with pork belly and subsequent bacon yield, sliceability and shelf stability is the pork belly softness. This defect has been reported to have resulted due to producers' effort to produce lean hogs that satisfy consumer demands over the years (Person et al., 2005; Trusell et al., 2011). The assessment of pork belly softness has, however, not been very successful in the pork industry as it is a complex trait affected by many inherent and interacting factors including the animal's diet, breed, sex and slaughter weights among others. The possibility of exploring several important factors that could influence this defect will improve its assessment in the pork industry for sorting, belly classification and further processing. Given the importance of balancing processors' profitability, consumer palatability and health demands, the present research, therefore, aims to focus on various aspects of pork belly and bacon quality measures that will affect both the consumers' satisfaction and producers' profitability in the bacon industry.

1.1 Objectives

Based on the aforementioned, this research project focused on the following four objectives:

a) To determine the accuracy of DXA in predicting pork belly composition prior to processing into bacon.
b) To explore both physical and chemical factors associated with pork belly softness and develop a multiple regression model that could select the most crucial of these factors.
c) To examine the extent to which storage days (2 or 28 days) and cooking methods (microwave or frying) affect protein and lipid oxidation and heterocyclic aromatic amines formation in vacuum packaged bacon.
d) To examine the overall effects of these treatments (cooking methods and storage days) including level of protein and lipid oxidation, on sensory quality of cooked bacon.

1.2 Hypotheses

In order to realise the objectives of this project, the following hypotheses were formulated, that:

1) Dual energy X-ray absorptiometry can predict lean and fat content of pork bellies with up to 90% accuracy.
2) A multivariate regression analysis explaining up to 80% of belly firmness could be developed using both physical and chemical factors of the measures derived from the pork belly.

3) Extended storage days and subsequent frying pan or microwave cooking of bacon will have additive effects on protein and lipid oxidation, and frying pan cooking will contribute more to these than microwave cooking.
4) Storage days and cooking methods will affect bacon sensory characteristics. Higher levels of lipid and protein oxidation in bacon will negatively impact sensory traits.
5) Extended storage days and subsequent frying pan or microwave cooking will have additive effects on heterocyclic aromatic amines production in bacon and higher level of these compounds will be produced in frying pan than in microwave cooking.
6) Higher levels of polyunsaturated fatty acids in pork bellies will result in higher lipid and protein oxidation as well as heterocyclic aromatic amines in bacon.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacon and its recent consumption trend[1]

2.1.1 History of bacon

The history of bacon could be as old as that of pig domestication itself which dates to about 7000 BC in the Middle East. However, the early form of bacon can be traced to around 1500 BC when the ancient Chinese cured pork bellies with salt (FSIS, 2011). It has been speculated that the Greeks and Romans brought the knowledge of curing and bacon processing with them from their Middle Eastern conquest. The food historians reported a type of bacon called *petaso* which was eaten by the early Roman (NAMI, 2015). In the 1500's, European farmers could not afford to buy pork often, hence it was a sign of affluence if a household could afford to 'bring home the bacon', a term that is used to mean 'earning a living' till today (FSIS, 2011). This term "bring home the bacon" was first used in a church in England around the 12th century where a man who could swear before God and the congregation that he had not disagreed or clashed with his wife within the space of a year and a day was rewarded with a side of bacon. This man became highly reverenced by his entire municipal (Bule, 2016). The etymology of the word "bacon" could date back to the 12th century and is believed to have originated from the French word "*bako*", prehistoric Germanic word "*bakkon*" and the Old Teutonic word "*backe*" all of which refer to the "back of a pig" (Bule, 2016).

---

[1] A version of this section (pages 6-11 and 16-38) has been published and extracts were used with permission from:

Soladoye, P. O., Shand, P. J., Aalhus, J. L., Gariépy, C., & Juárez, M. (2015). Review: Pork belly quality, bacon properties and recent consumer trends. *Canadian Journal of Animal Science*, *95* (3), 325-340.

O. P. Soladoye compiled data, analysed and interpreted information, drafted, revised and finalised manuscript.

The English acquired the word in the 16th century when the word "bacoun" or "bacon" was used to refer to any type of pork. Not until the 17th century was the word "bacon" used to refer exclusively to the salted and smoked belly that is now recognized as bacon. It seems, however, that the present day "bacon" encompasses more than just a category of cured and processed pork bellies that end up in strips. Innovative ideas in response to recent consumption shift have seen bacon being made from other animal species, such as turkey and beef and, in fact, from other parts of pork itself like jowl or loin among others. Bacon has different names from different regions- *Spek* in the Netherlands; *Speck* in Germany; *Tocino or Tocineta* in Spain; *Pancetta* in Italy and *Bacon or Lard* in France just to mention a few. In the United States and Canada, however, the product marketed as bacon must be from pork bellies; otherwise, the portion of the pig where the bacon comes from must be stated in the package (e.g. jowl bacon). Back bacon made from lean pork loin is another common bacon type in North America usually called 'Canadian bacon' in the United States. However, for the present study, bacon will subsequently refer to the sliced bacon from cured belly of hogs.

Hernando Cortez's introduction of hogs to New Mexico and Sir Walter Raleigh's introduction of sows to Jamestown Colony, both in the 17th century, gave rise to the American pork industries (The National Provisioner, 2008). Although the first packaged sliced bacon in North America was in 1924 by Oscar Meyer, bacon commercialisation has a more ancient history in Europe with the first large scale bacon curing industry set up in 1770's by John Harris in Caine, Wiltshire (The National Provisioner, 2008). John Harris is considered the forefather of large scale industrial bacon manufacturing (Bule, 2016).

2.1.2 Recent consumption trends

Despite the continued increase in bacon prices in recent years, consumer demands for this food commodity have not waned (Figure 2.1 and 2.2). This could be attributed to the consumers' appreciation of the product's flavour as well as the more recent shift in the way bacon is used in several dishes worldwide.

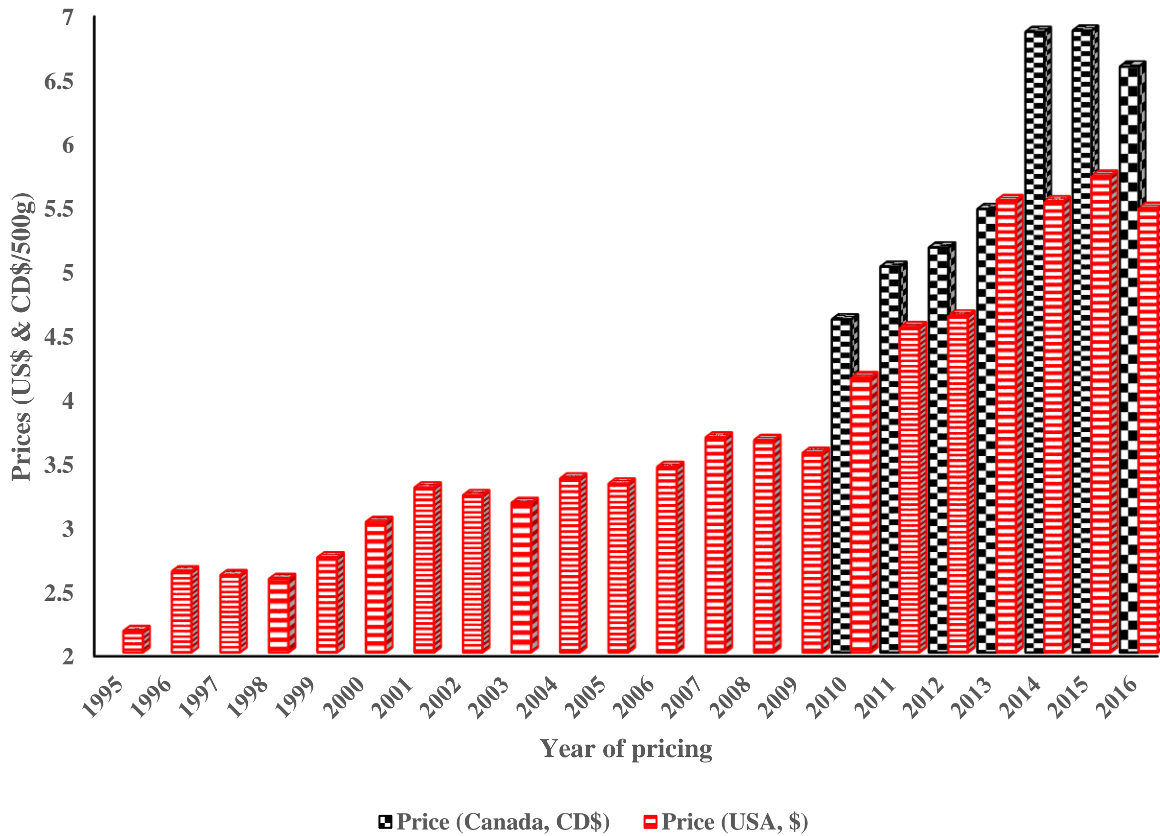


Figure 2.1: Bacon price increase in Canada and the USA over a 21-year period. *Data assessed from Statistics Canada and statistica: http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/econ155a-eng.htm, https://www.statista.com/statistics/236811/retail-price-of-sliced-bacon-in-the-united-states/ (Retrieved on 14th Dec. 2016).*

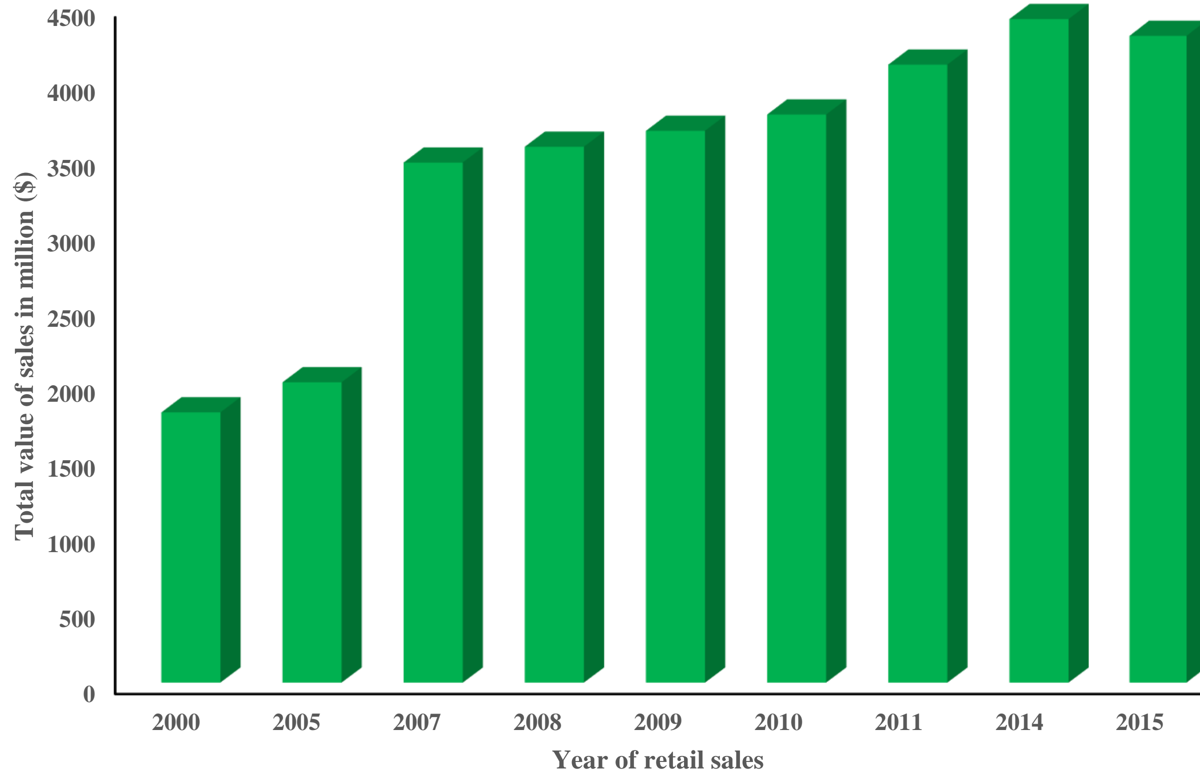
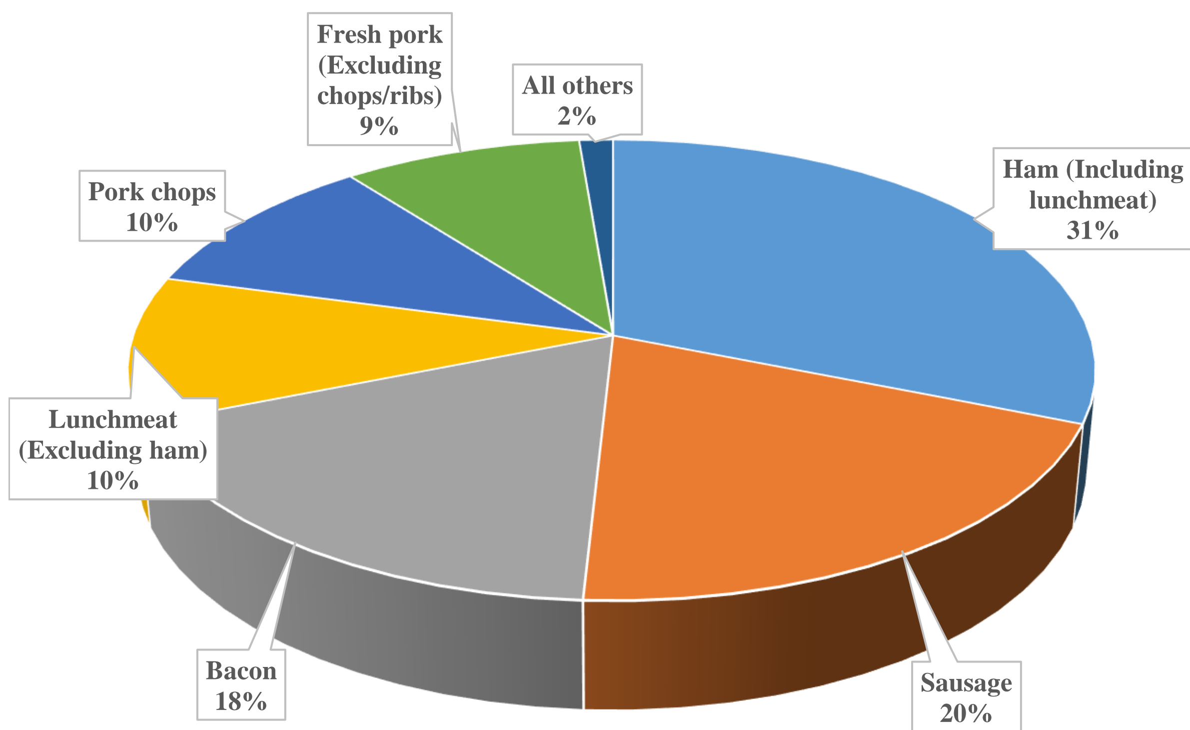


Figure 2.2: Bacon dollar sales in the United States for a 15-year period. *Data compiled from pork checkoff (https://www.porkretail.org/filelibrary/Retail/BaconTrends.pdf), Meatingplace magazine (http://meatingplace.com/Archives/Archives, March 2016) and International Market Bureau (http://www.agr.gc.ca/resources/prod/Internet-Internet/MISB-DGSIM/ATS-SEA/PDF/6124-eng.pdf). (Retrieved on 15th Dec. 2016).*

While bacon is gaining market share in retail outlets, about 69% of bacon is also sold in foodservice outlets (Pellegrini, 2013), and 62% of restaurants were reported to carry bacon on their menu in the United States (National Pork Board, 2008).

According to Canadian market indicator reports published in 2012 by the International Market Bureau, bacon and pork/beef sausages are some of the most popular chilled processed meats in the United States (International Market Bureau, 2012). In fact, bacon was third to ham (31.1%) and sausage (19.8%) in the US in-home pork consumption in 2009 (Figure 2.3). With up to 79% of the pork consumed in the home being in a processed form, bacon represents about 18.1% of this with an average American consuming up to 17.9 lbs (8.12 kg) of bacon per year (Pork Checkoff, 2009, 2016). More than 53% of all homes in the United States reported keeping bacon in hand at all times, 59% of this bacon is consumed in the course of the week while 41% is consumed in the weekends. Although bacon is consumed all through the day, 57% is consumed during breakfast while 29, 12 and 2% is eaten at lunch, dinner and snack time respectively (Pork Checkoff, 2016). Although no data could be accessed for the Canadian market, similar trends would be expected considering the Canadian consumers' appreciation of the product.

Generally, of the various cooking methods available to prepare bacon domestically, consumers prefer both stove top frying pan and microwave (45 and 15%, respectively) compared to other cooking methods (National Pork Board, 2008) although most commercial precooked bacon will employ the latter. With this expanding market and increasing usage, bacon processing including the animal production stage, needs more painstaking quality control processes to ensure consumers' demands and health concerns are considered. It is important that scientifically proven cooking recommendations, that can ensure good sensory attributes of bacon while consumer health is not significantly affected, should be explored and established.



- Ham (Including lunchmeat)
- Sausage
- Bacon
- Lunchmeat (Excluding ham)
- Pork chops
- Fresh pork (Excluding chops/ribs)
- All others

Figure 2.3: In-home consumption of pork in the United States in 2009. *Adapted from Pork Checkoff (Pork Checkoff, 2009). Retrieved on 16th Dec. 2016.*

2.2 Bacon processing

Bacon processing generally follows some basic steps with slight variations with spices and curing ingredient mixtures that differentiate brands, as well as curing methods based on the scale of production. The following briefly describes the basic operations in commercial bacon processing.

2.2.1 Raw material selection and sorting

Although recent scientific improvements have resulted in a much leaner pork from hogs, the composition of pork bellies is still 45 to 65% fat and this varies depending on sex, genetics of the animal or dietary treatments, among other factors (Mandigo, 2002; Smith, West, & Carpenter, 1975). Based on technological requirements and consumer demands for leaner meat, pork processors would prefer to sort pork bellies based on thickness and fat percentages but, presently, weight seems to take precedence. Proper sorting is essential because the bellies are pumped with curing solution at fixed percentage and poor sorting will lead to inconsistent product and poor product quality (The National Provisioner, 2008). Knipe and Beld (2014) confirmed that sorting bellies by thickness results in more uniform bacon than sorting by weight. Researchers have also shown that PSE (pale, soft, exudative) bellies, although take up curing brine more readily, also lose more weight during subsequent maturation (Taylor, Dant, & French, 1973). Similarly, bellies with high fat content have been reported to take up less curing pickle compared to lean ones (Boler et al., 2012; Stiffler, Chant, Kinsman, & Kotula, 1975). Following sorting, pork bellies are skinned removing about 10% of the original pork belly weight. Then, adequate trimming of bellies to individual industrial specifications is done to get rid of spare ribs, residual hair roots, mammary glands and flank ends (The National Provisioner, 2008), which leaves only about 65 to 85% of the original pork belly weight for subsequent curing (Knipe & Beld, 2014). Although fairly thick bellies may be in demand by the processors due to high processing yield, consumers have been found to discriminate against bacon from these bellies due to their high fat content and inferior taste (Person et al., 2005). This makes it important for bacon industries to develop a rapid and accurate tool to classify pork bellies based on their fat or lean content to enhance processing and ensure consumer acceptability.

2.2.2 Curing methods

There are basically three methods of curing bacon: pump/injection, dry and immersion curing. Pump curing allows liquid curing ingredients to be injected directly into the pork belly to accelerate the curing process and enhance bulkiness. This is done either by a stitch or spray needle type machine. Dry curing involves applying premeasured dry cure mixture onto the belly surface and allowing it to cure for a number of days. The immersion method, on the other hand, entails immersing bellies in curing solution for two to three days after which bellies are left to hang until they are cured (FSIS, 2011). However, the first two are the most widely used and will be discussed in this review.

2.2.2.1 Pump curing

Pump curing is widely used for mass production of bacon. The pork belly is injected with a liquid brine mixture (pickle) usually made up of water, salt, sugar (sucrose), nitrite, sodium erythorbate and/or ascorbate, and phosphate. Liquid smoke may also be used if a convection oven will be employed (Mandigo, 2000). Spices and flavourings may also be added to bacon which may be the major factor that differentiates brands. Each of the ingredients in the brine mixture has a specific function. Alkaline phosphates, not more than 0.3 to 0.4% in the finished product, is usually recommended in the United States (FSIS, 2011), whereas 0.5% is the maximum permitted level (in the form of sodium phosphate dibasic) in Canada (CFIA, 2013). Phosphate usually helps with moisture retention during bacon processing and cooking; however, higher levels (> 0.5%) may result in a "soapy" flavour, a sensory defect in bacon. Sodium ascorbate or sodium erythorbate is a cure accelerator and colour stabilizer, with a limit of 550 mg/kg according to the United State Department of Agriculture (FSIS, 2011). In the United States, pumped bacon must include sodium ascorbate or erythorbate to limit the production of nitrosamines during cooking. Nitrite, with a permitted limit of not more than 120 mg/kg, helps with inhibiting bacteria, flavour setting and colour enhancement. This limit applies both in Canada and the United States (CFIA, 2013; FSIS, 2011). Salt (1.5 to 2.0%) and sugar (0.75 to 1.0%) depending on processor preference both help as flavour enhancers and also as microbial inhibitors, with the sugar helping to moderate the taste intensity of the salt in the product (Rocha, 2011). The order to which ingredients are added to water and dissolved is crucial in pickle formulation. Necessarily, phosphate is adequately dissolved first followed by ascorbates, then salt, sugar and other flavouring, whereas nitrites come last. This order

is important to avoid subsequent precipitation of ingredients that could form sludge at the base of the mixing tank (Knipe & Beld, 2014).

The brine mixture is most appropriately held at 4°C (40°F) to avoid food safety issues, and bellies are completely thawed (if previously frozen) and kept at the same temperature before pickle injection. The pumping level of bellies is usually around 112 to 115% (FSIS, 2011; Person et al., 2005) of the belly's green (fresh, pre-pumped) weight (i.e., 12 to 15% brine pick-up), after which it is held for some predetermined time period (typically from 1 to 5 hours) for cure equalization before being heated to avoid inconsistent colour development and streak marks during the smoking sessions. Vacuum tumbling (at -1 to 4°C) could also be applied to bellies for even ingredient distribution prior to smoking.

2.2.2.2 Dry curing

This method employs a premeasured amount of cure mixture (which includes but is not limited to salt, sodium nitrate, granulated sugar and sodium erythorbate) which is rubbed into the belly surface in such a way that the whole surface is covered. Compared to 120 mg/kg limit of nitrite permitted for pumped bacon, up to 200 mg/kg is allowed for dried cured bacon (FSIS, 2011). Following the surface rubbing, the bacon is held for, usually 7 days per inch (2.5 cm) of belly thickness before being smoked for even distribution of cure (Knipe & Beld, 2014). Due to this long processing and labour investment, dry-cured bacon is usually more expensive than the pumped bacon.

2.2.3 Smoking and thermal treatment

Mass-produced injected bacon slabs are usually heat processed in conventional ovens for about 2 to 6 hours, whereas traditional smoking may take longer times. The smoke flavour is obtained directly from smouldering wood chips in the traditional smoking process, whereas liquid smoke extract may replace this in the conventional method. Aside from the typical smoke flavour that smoke impacts to bacon, it also adds aroma, colour and serves as a means of preservation, which comes as a result of the heating, drying and the chemical components from the smoke (e.g., acetic acid, formaldehyde, and creosote among others; The National Provisioner, 2008). Proper hanging of the bellies on bacon combs prior to transfer for smoking has also been pointed out as an important step to ensure more regular belly shape that will subsequently aid high slicing yield.

For this purpose, bacon combs are generally recommended to be pushed in from the lean side of the belly and this can increase slicing yield up to 8% compared to bacon combs pushed in from the fat side. The target temperature for bacon during smoking ranges between 52 to 53°C (Knipe & Beld, 2014). A wider temperature range between 48°C and 55°C has been reported to be safe (Canadian Food Innovators, 2016) and the lower ranges (50 to 52°C) have also been suggested to preclude the release of proline from the connective tissue collagen by the action of enzymes within the pork belly, thereby minimizing the subsequent production of nitrosamine during bacon frying (Ned, 1975). Total smokehouse schedule is dependent on belly size, smokehouse air velocity, smokehouse temperature and internal belly temperature. Overall, the cooking loss following the smoking step may range between 5 and 12%, ensuring compliance with USDA regulation that "weight of cured pork bellies ready for slicing and labelling as bacon shall not exceed the weight of the fresh, uncured pork bellies" since almost the same mass of water from the curing brine infused in the bellies is also lost during smoking (FSIS, 2011).

2.2.4 Slicing and packaging

Prior to slicing, it is recommended that bacon slabs be chilled and tempered. Bacon slabs are rapidly cooled to 4 to 5°C and then slowly chilled to around -6.5°C to allow for proper fat setting. Too rapid chilling at this point can result in the formation of ice crystals in the cooked bacon (Knipe & Beld, 2014). Just before pressing, bacon slabs are held in a tempering cooler until an internal temperature of -3.5 to -5.5°C (26 to 28°F), which aids in shape retention during pressing and also facilitates slicing (Rocha, 2011). Thermally processed and chilled bacon is later pressed hydraulically into rectangular shaped bacon of width between 9.5 and 11 inches (24 to 28 cm) with varying length depending on the extent of trim. Usually, bacon is sliced and vacuum packaged as thin (> 17 strips per pound), regular (7 to 16 slices per pound) or thick (4 to 6 slices per pound) slices (Knipe & Beld, 2014). Figure 2.4 is a schematic representation of the process involved in industrial bacon production.

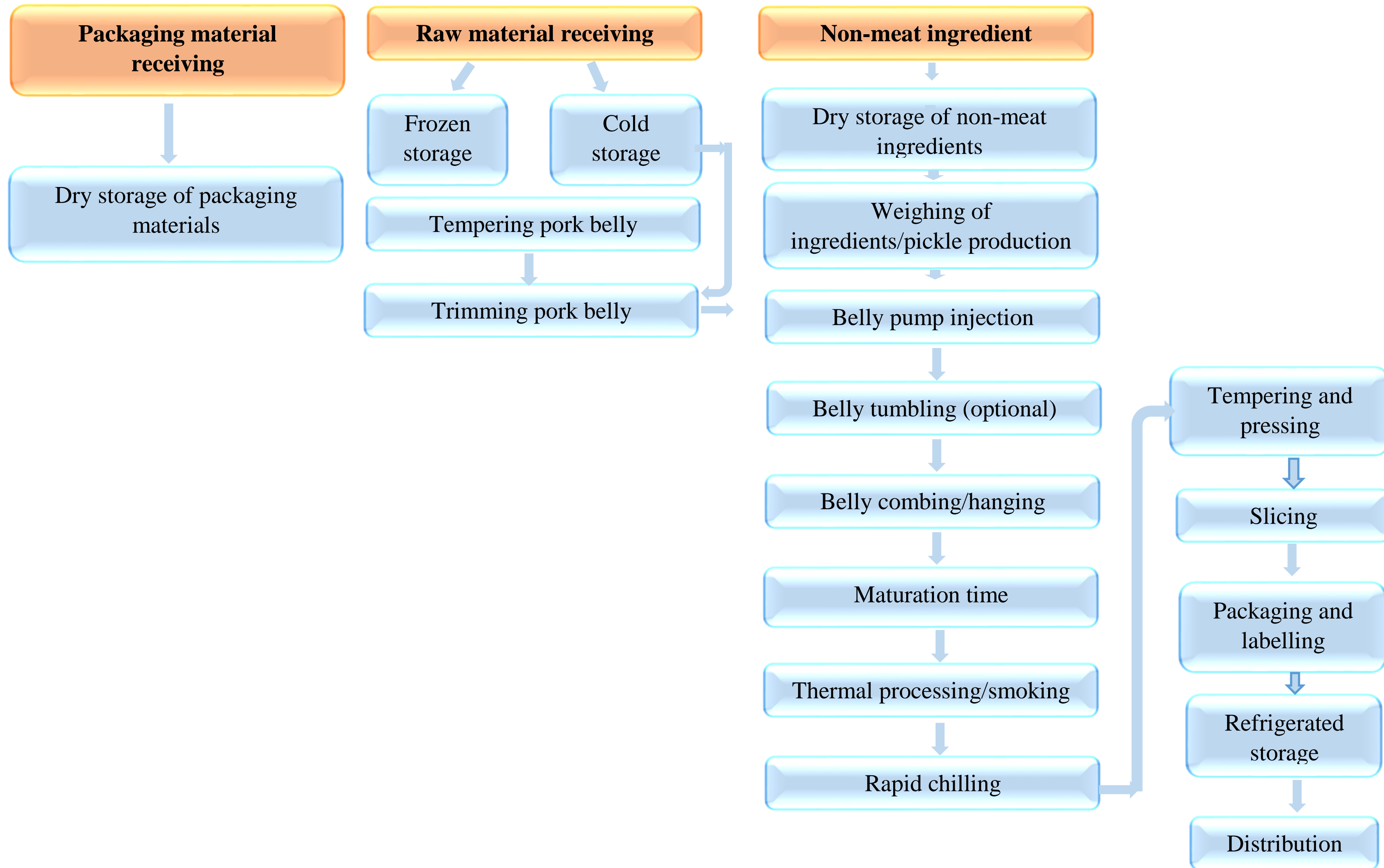


Figure 2.4: Industrial sliced bacon process flow chart. *Adapted from Mandigo (2009) and Rocha (2011).*

2.3 Indices of pork belly and bacon quality

2.3.1 Pork belly structure

As previously stated, consumers' desire for leaner meat has driven the reduction in the fat content of pork belly from 74% (Smith et al., 1975) to today's 45 to 55% (Scramlin et al., 2008), with a corresponding increase in the percentage of unsaturated fatty acids (Trusell et al., 2011). The belly is one of the primal sections obtained from pig carcasses, usually cut from between the second and the third rib to just few inches above the hip bone. The fat content is mainly found in the subcutaneous and intermuscular fat layers. The thin *cutaneous trunci* muscle is found between these fat layers, spanning from the shoulder to the flank end of the belly. The *latissimus dorsi* is another major muscle in the pork belly. It is a wide, triangular muscle that originates at the lumbar and thoracic vertebrae and ends at the humerus, largely contributing to the total lean content of the belly. Other important muscles which can be apparent in belly cuts depending on meat cutting specifications and consistency include: *serratus ventralis*, diaphragm, *teres major*, *triceps brachii*-long head, *intercostal externi* and *obliquus abdominis interni*, among others (Figure 2.5). The total percentage of these muscles determines the lean-to-fat ratio of the pork belly and may affect consumer acceptability of the product (Stiffler et al., 1975). The softness of the two fat layers based on their composition can result in processing difficulties, fat separation in packaged products and sensory issues (Shackelford et al., 1990). Hence, factors that will improve pork belly quality should focus on all these anatomical parts to ensure a profitable product for producers and an appealing and desirable product for consumers.

2.3.2 Pork belly softness defects and possible impact on bacon quality

Pork belly softness is a major quality defect that has been reported to reduce processors' and packers' profitability because of its overall effect on fabrication efficiency, bacon shelf stability, sensory quality and bacon slicing yield. Generally, softer bellies may lead to oily appearance and poor slice definition in bacon retail package, fat and lean separation, reduced slicing efficiency

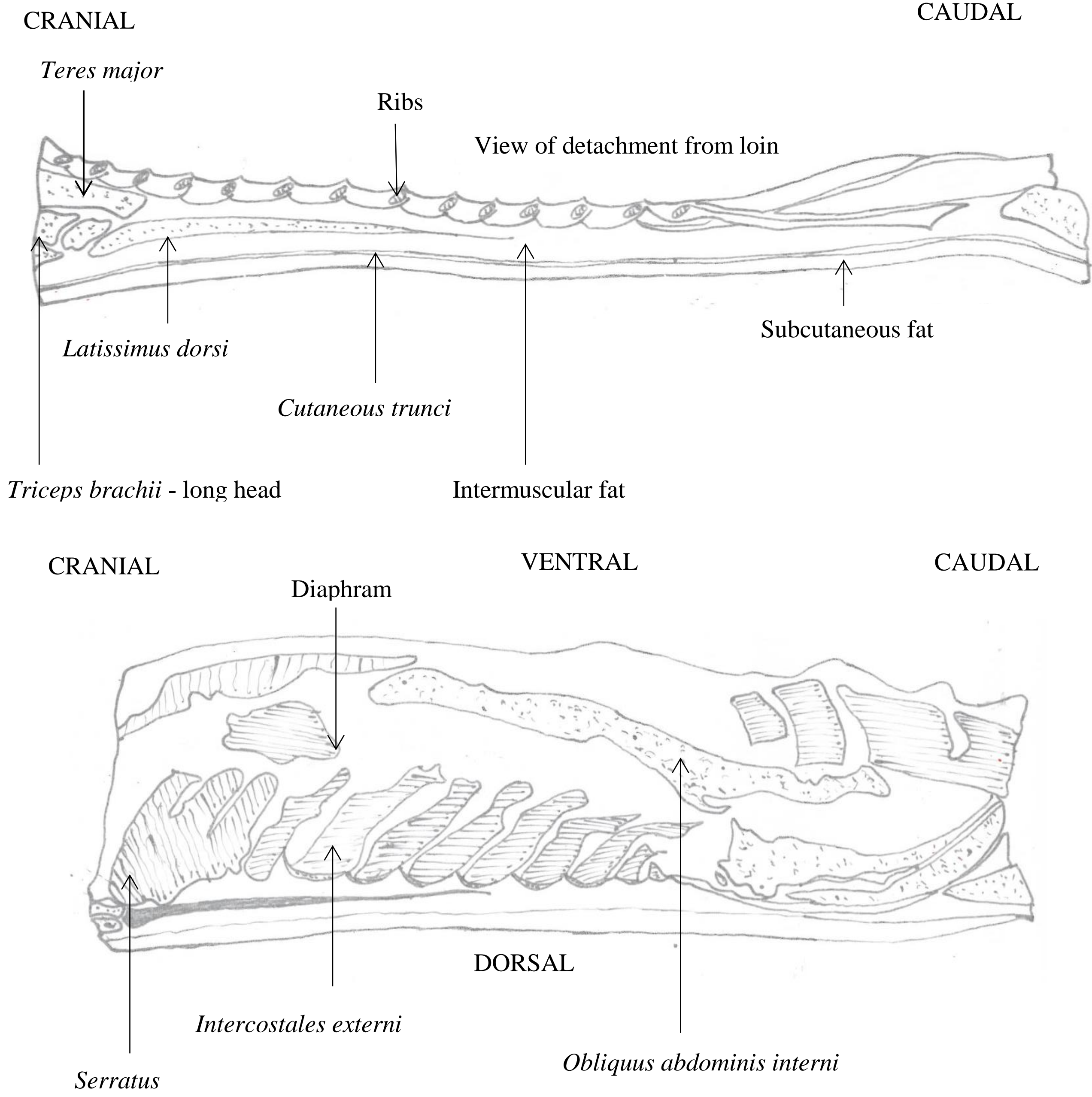


Figure 2.5: Mid-sagittal plane anatomical description of pork belly; sagittal view (above, ribs on and skin side down) and dorsal view (below, spare ribs off, skin side down).

and yield of bacon slabs, and reduced product shelf life due to poor oxidative stability (Benz et al., 2010; Correa, Gariépy, Marcoux, & Faucitano, 2008; Larsen, Wiegand, Parrish, Swan, & Sparks, 2009; Rentfrow, Sauber, Allee, & Berg, 2003; Shackelford et al., 1990).

Genetic and dietary factors are the major factors that influence pork belly softness (Fiego, Santoro, Macchioni, & De Leonibus, 2005; Larsen et al., 2009). The inclusion of DDGS (dried distiller grains with solubles), corn oil, flaxseed oil or soy bean oil among other plant based oils in a pig's diet could result in reduced pork belly fat quality and belly softness (Whitney, Shurson, Johnston, Wulf, & Shanks, 2006; Widmer, McGinnis, Wulf, & Stein, 2008). Furthermore, leaner genetic lines of pigs may have leaner bellies and also have higher deposition of unsaturated fat compared with pigs of fatter genetic predisposition (Larsen et al., 2009). Most of these factors will be discussed in detail in subsequent sections.

In contrast to conventional beliefs, Rentfrow et al. (2003) concluded that softer bellies did not result in poorer sliceability but the softest bellies yielded the highest slice percentage. Furthermore, many other researchers could not find any negative implication of belly softness on bacon sliceability and sensory attributes (Larsen et al., 2009; McClelland, Rentfrow, Cromwell, Lindemann, & Azain, 2012). Thus, from the various literature considered, a softer belly may not necessarily translate into poorer slice yields for bacon, it may, however, hinder the ability of pork producers and processors to meet export standards and may also affect other processing and sensory characteristics (Carr et al., 2005). Because the unsaturated fatty acid profiles in bacon may be important for health reasons, adequate control of dietary and genetic factors as well as environmental factors during the animal production stage is necessary to avoid detrimental effects on pork belly and bacon quality.

2.3.3 Pork belly and bacon quality indices

Based on bacon quality expectations, the definition of pork belly quality may be perceived differently by stakeholders along the bacon production chain. Mandigo (2000) stated that from the producers' perspective, "quality equals weight" as this basically forms the present industrial criterion for pork belly selection and payment. In addition, processors prefer fairly thick bellies, as these have been shown to give a higher processing yield than thinner ones, and higher profitability potential (Person et al., 2005). On the other hand, considering consumers' preference for leaner bacon with not less than 30% lean and less saturated fat (Trusell et al., 2011; Vonada et

al., 2000) there seems to be opposing quality requirements for pork bellies along this commodity value chain.

Brewer et al. (1995) commented that while consumers are attracted to nutrition claims, taste and convenience rank higher in priority for their food purchasing decisions. For instance, crispy, palatable and less distorted bacon which may be more acceptable to consumers would result from firmer fat (Shackelford et al., 1990), which corresponds to thick, less lean belly. As such, producers are drawn into this dilemma and faced with the challenge of ensuring consumer demands (lean yet crispy, palatable and less distorted) are met while their own profits are not compromised. In other words, consumers are more interested in the final product quality while the pork belly technological qualities and subsequent profitability are more important for processors.

The quality parameters that may be important for processors include belly dimensional parameters (i.e., size, thickness, shape or weight) and firmness (Seman, Barron, & Matzinger, 2013; Trusell et al., 2011; Whitney et al., 2006). Other pork belly quality parameters important for bacon processors are absence of hair roots, residual bones and mammary gland residues. For bacon slabs, prior to being processed to bacon slices, processing yield (including pump, cook, curing and smoke yield) and subsequently slice yield are the major parameters of interest (McClelland et al., 2012; Person et al., 2005; Robles, 2004). At the other end of the production chain, the important quality parameters for consumers are those that affect the final product, including nutritional composition (total fat, fatty acid profile, salt and nitrite content) and sensory attributes (flavour, colour, texture, etc.) (Person et al., 2005). Lean-to-fat ratio or muscle distribution score may also be among the quality attributes important to consumers (Person et al., 2005; Stiffler et al., 1975).

Most national recommendations, including those from United States, Canada and Europe, have suggested that saturated fatty acids (SFA), the ratio of n-6/n-3 fatty acids should be kept low, whereas polyunsaturated fatty acids (PUFA) and the PUFA/SFA ratio should increase for health reasons (Mapiye et al., 2012). Levels of > 0.4 and < 4 have been recommended by the UK department of health for PUFA/SFA and n-6/n-3 fatty acid ratio, respectively (Wood et al., 2004a; Wood et al., 2004b), whereas the average contribution of SFA and total fat to dietary energy should be below 10 and 35%, respectively (Kenneth, 1994). This can be considered both a challenge and an opportunity to produce pork in general and bacon in particular. Among other parameters that should be considered along the meat production chain are lipid and protein oxidation, as these can

generate significant quality defects, which may have negative implications on sensory attributes, as well as industrial profitability due to spoilage losses and consumer rejections.

2.3.4 Pork belly and bacon quality assessment

2.3.4.1 Non-invasive methodology to evaluate belly softness and composition

Both objective and subjective means have been explored in the literature for evaluating pork belly quality. There are several quality parameters that are important, as previously discussed; however, this review will focus only on those that affect technological efficiency during bacon processing and how these attributes can be assessed.

2.3.4.1.1 Pork belly softness and its non-invasive evaluation

Soft fat bellies have been reported to be indicative of increased dietary PUFA, leading to lower sliceability and bacon yield (Larsen et al, 2009). About US$97 million in unrealized revenue for pork packers in the US annually was reported due to this defect and other related issues (e.g. thin belly) (Person et al., 2005). The methods that have been employed in assessing belly firmness include: visual appraisal using either 4, 5 or 6 point scales (Weber et al., 2006); finger testing (Maw, Fowler, Hamilton, & Petchey, 2003); and the belly-flop test using either a suspended round bar (Uttaro & Zawadski, 2010) or a v-shaped smoke house stick (Whitney et al., 2006). Rentfrow et al. (2003) also proposed the belly-flex method to assess fresh pork belly firmness. Another widely employed objective measure of fat firmness is the Durometer reading. This is usually equipped with a probe and the higher its value, the firmer the fat and, hence, better technological/bacon processing quality (Seman et al., 2013). The compression test using the Instron Universal Testing Machine, in which severed pork belly cores are compressed to 50% of their specific thickness, has also found a wide acceptance in the laboratory environment for fat firmness analysis (Apple et al., 2011).

2.3.4.1.2 Pork belly composition and its non-invasive evaluation

Belly chemical composition is another important pork belly quality attribute that influences consumers' product acceptability as well as processing. Its determination using non-invasive methodology have been widely explored. Electromagnetic (EM) scanning is an effective and reliable method for estimating pork carcass composition (Berg, Forrest, & Fisher, 1994). However,

this procedure may not work well with cuts with more external and internal fat (e.g., pork belly), as the EM field penetration is slowed down resulting in equations of reduced predictive accuracy. Tholen, Baulain, Henning, and Schellander (2003) focused on validating three methods usually used in assessing pork belly composition in Germany (breed specific regression equation, digital imaging and AutoFOM). Of the three methods examined, the breed specific regression equation (Gruber formula), which contains different carcass characteristics gave a value closest to the reference method (Magnetic Resonance Imaging; MRI). Accordingly, MRI has been identified as a convenient way to measure muscle and fat content in pig bellies with a high degree of accuracy (Tholen et al., 2003). In fact, other authors have claimed that MRI can replace total dissection as a reference technique to determine carcass lean content if adapted to commercial settings (Mitchell, Scholz, Wang, & Song, 2001).

Yi and Chen (2003) proposed a simple, specific gravity method for predicting lean-to-fat tissue ratio of pork belly, and concluded that this method can explain up to 96.3% of the variation in pork belly fat content. Computer-assisted tomography (CAT) is another method that has been successfully employed to predict full body composition (Vester-Christensen et al., 2009). The use of the Hennessey grading probe for primal composition prediction has not proven successful, probably due to the multiple layers of fat and lean in pork bellies (Uttaro & Zawadski, 2010).

In addition to these technologies that predict total belly composition, other technologies may be useful to predict the fatty acid composition in meat. According to Prieto, Roehe, Lavín, Batten, and Andrés (2009), near-infrared spectroscopy (NIRS) has considerable potential in predicting meat chemical properties, especially pork fatty acid composition (Pérez-Marín, De Pedro Sanz, Guerrero-Ginel, & Garrido-Varo, 2009). Trusell et al. (2011) showed a strong positive relationship between the proportion of 16:0, 18:0 and all SFA with belly firmness and a strong negative correlation was also observed with 18:2n6, 18:3n3 and total PUFA content. Other authors have positively correlated palmitoleic (16:1), oleic (18:1) (Madsen, Jakobsen, & Mortesen, 1992), and primary SFA (especially 18:0) with fat firmness (Davenel, Riaublanc, Marchal, & Gandemer, 1999) and 18:2n6 has been negatively correlated with fat firmness (Prescott & Wood, 1998).

2.3.4.1.3 Dual energy X-ray absorptiometry technology in compositional assessment

Although other advanced technologies have been applied for predicting belly composition non-invasively (such as MRI and CAT), Marcoux, Bernier and Pomar (2003) stated that DXA

holds better promise in assessing carcass composition due to its relatively cheaper, faster and easier to use nature compared to the other technologies. Its rapid characterization of meat composition alongside its non-destructive nature may enable processors to inform consumers of the fat/lean content of their fresh meats at the point of sale (Brienne, Denoyelle, Baussart, & Daudin, 2001). Dual energy X-ray absorptiometry estimates the body composition on the basic principle that as X-rays pass through tissues, they encounter attenuated intensity in proportion to the tissue mass. Because DXA makes use of two different energy peaks, it has the capability to rapidly quantify lean mass, fat mass and bone mineral mass, as well as density alongside the total body mass (Matthew & Alec, 2012).

Some shortcomings of DXA have been highlighted (Roubenoff, Kehayias, Dawson-Hughes, & Heymsfield, 1993), including the need for adequate calibration specific to each research application (Brienne et al., 2001; Brunton, Bayley, & Atkinson, 1993; Mitchell, Scholz, Pursel, & Evock-Clover, 1998); yet, DXA offers great promise for accurate, reliable and fast assessment of body composition analysis. It has also found a wide practical application in human medicine, food science, pharmacology, animal science and nutrition (Matthew & Alec, 2012).

Brunton et al. (1993) compared DXA estimates of body composition with chemically assessed whole carcass composition, and concluded that DXA may not be an appropriate tool for body composition assessment of small animals (e.g., piglets) as some components are underestimated while some are overestimated by DXA. Attenuation error may result due to fractional crossover of the high and low energy beams at thickness outside the range of 10 to 20 cm (Brunton et al., 1993). However, Brienne et al. (2001) have suggested a thickness range between 4 and 10 cm for accurate DXA measurement, whereas Lukaski, Marchello, Hall, Schafer, and Siders (1999) have reported a range between 15 and 28 cm where DXA fat measurement is independent of body thickness. It has also been reported that DXA may overestimate the fat percentage in pigs containing more than 20% fat and underestimate the percentage fat in pigs with below 20% fat (Mitchell, Scholz, & Conway, 1998). Several other factors have been pointed out which may affect DXA estimates of body composition. The age of the animal may be a factor due to increased hydration in lean mass and incomplete bone calcification in young animals, both of which may lead to poor DXA estimates (Brunton et al., 1993; Mitchell, Scholz, & Conway, 1998; Roubenoff et al., 1993). This has led some authors (Brienne et al., 2001) to recommend that

calibration should be specific with regards to weight range, animal age, make of instrument and the version of running software.

Dual energy X-ray absorptiometry estimates of body or carcass composition may also vary among various equipment brands (major players include Lunar, Hologic, Norland and Diagnostic Medical System) due to their different hardware components, method of X-ray generation, detectors and scan acquisition techniques. For example, Lunar equipment uses cerium K-edge filters which yield 38 and 70 KeV energy peaks whereas Norland equipment employs samarium K-edge filters that yield 46.8 and 80 KeV energy peaks at their incident ray (Lösel, Kremer, Albrecht, & Scholz, 2010). The variation between technology/generation of equipment (pencil vs fan beam technology), as well as software version, may contribute to variation in accuracy among DXA equipment estimates (Koo, Hammami, Shypailo, & Ellis, 2004). Aside from these inherent flaws in DXA technology, the variation in animal populations, including species, breed, age, sex, or diet of animal which may affect tissue thickness (Jebb, Goldberg, Jennings, & Elia, 1995; Laskey, Lyttle, Flaxman, & Barber, 1992) or compositional variation (Brunton et al., 1993; Roubenoff et al., 1993) could also influence DXA estimates of body or carcass composition.

Depending on the anatomical complexity of fat and lean distribution in animal body parts or carcass primal cuts, DXA estimates may be affected differently. This is because DXA assumptions regarding distribution of soft tissue below and above the bone as well as the calibration procedure that assigned R-value to certain elemental component or tissue could pose some limitation in the accuracy of its estimation. Due to the complexity in anatomical arrangement of tissue in some pixels that include small proportions of some other larger elements (tissue), DXA may disregard the smaller elements because their average absorption coefficient (R-value) is closer to that of the predominant element (Roubenoff et al., 1993). This error may even increase with increasing pixel size and this may vary among DXA equipment (Lösel et al., 2010). Similarly, the uniform or pure phantoms employed to derive these R-values employed in DXA algorithms may not necessarily be the same in biological tissues and may even vary with breed. Additionally, although some studies have not found any impact of tissue thickness on DXA compositional scans (Lukaski et al., 1999), others have reported that thickness of tissue may impact the accuracy of DXA predictions (Gotfredsen, Bæksgaard, & Hilsted, 1997; Jebb et al., 1995; Laskey et al., 1992). All these factors may influence the reduction in accuracy and efficiency of DXA compositional

estimates. It will, therefore, be interesting to observe the impact of a wide variation in a swine animal population on the DXA estimate.

2.3.4.2 Invasive methodology to evaluate belly softness and composition

2.3.4.2.1 Invasive methodology to evaluate belly softness

Iodine value (IV) has been widely used as an indication of belly firmness. It measures the unsaturation of the fat by reaction with iodine (Seman et al., 2013). Hence, the greater the proportion of unsaturated fatty acids, the higher the IV and, consequently, softer bellies. According to Benz et al. (2010), acceptable IV range from 70 to 75 g/100 g of fat, but some US packing plants have set their maximum IV at 73 g/100 g. The appropriateness of this measurement has been widely criticized for its destructive and time consuming nature, as well as the difficulty in deciding a unique site of sampling for analysis on pig carcass (Trusell et al., 2011). In fact, research has shown that firmness and compositional gradients exist within fresh pork bellies themselves. This makes the method non-representative of the overall pork bellies or the whole carcass. Furthermore, fat samples may have the same IV but could be structurally different (Gatlin, See, & Odle, 2005). In fact, Kyle, Bohrer, Schroeder, Matulis and Boler (2014) observed similar IV from bellies of different softness perceptions. Recently, Seman et al. (2013) employed the use of Fourier transformed infrared technology in predicting IV and, hence, belly firmness and quality. Although IV can now be assessed non-invasively, its limitation in belly softness predictability remains. Thus, only about 14% of variation in belly firmness was reported to be due to IV while about 33% was due to belly thickness (Whitney et al., 2006). There may be other factors that contribute to belly softness in addition to IV and belly thickness. A full assessment of belly composition, including fat-to-lean ratio, fatty acid profile, belly dimensional measurements and proximate composition to understand their influence on overall belly firmness and technological conformity would be of great value to appropriately target necessary control strategies. The speed and accuracy at which this could be done will determine its adaptability to industrial settings.

2.3.4.2.2 Invasive methodology to evaluate belly composition

Traditionally in the industry and research, assessment of carcass composition has been done either chemically or by manual dissection (Marcoux, Faucitano, & Pomar, 2005). These processes are not only destructive and environmentally unfriendly, they are also expensive, tedious

and prone to the personnel's subjectivity and fatigue. Although manual carcass dissection has its inherent shortcomings, it still remains the gold standard to which other procedures are compared especially, when done by trained butchers (Bernau et al., 2015). The possibility of a method to avoid the subjectivity and fatigue of manual dissection while retaining its robustness and accuracy will position such a technique as a gold standard.

2.3.4.3 Bacon quality evaluation

Although there are no standardized grading systems for bacon, manufacturers most often class their bacon into either grade 1 or 2 depending on its uniformity, leanness and consumer appeal. Besides compositional traits (proximate analyses, lipid composition), several experimental procedures have classified bacon quality based on fat shattering, slice distortion and shrink following cooking (Mandigo, 2000; McClelland et al., 2012). A 5-point scale for sensory and visual evaluation has also been proposed to assess fattiness, pinkness, leanness, flavour, crispiness, saltiness and visual appearance of bacon (Person et al., 2005). More recently in the literature, digital imaging systems have been employed to assess the bacon slice composition in terms of total lean and fat area. The digital images are usually analysed with specific software to measure the total lean and fat area (Kyle et al., 2014; Scramlin et al., 2008; Tavárez et al., 2014). Aside from these, there is no available standardized bacon quality measurement technique referred to in the scientific literature, although Mandigo (2009) has highlighted some possible defects of bacon to include shattered, two-toned, wrinkled, severe "S" folding, vertical splits and pickle pockets, among others. Although these have not been standardized for bacon assessment, they hold promise for future assessments and standardization.

2.3.5 Factors affecting pork belly and bacon quality

Animal genotype, environmental and dietary factors seem to be the greatest factors that affect and can be used to manipulate pork belly quality. The following section will discuss the literature regarding the effects of breed, sex, growth promoter, diet, age or weight at slaughter and carcass processing on the pork belly and subsequent bacon quality.

2.3.5.1 Pre-slaughter factors

2.3.5.1.1 Genotype/breed effects

Various studies have shown that breed or genotype significantly affect some technological and sensory qualities of pork (Bahelka, Oravcová, Hanusová, & Demo, 2011; Bertol et al., 2013; Ellis, McKeith, & Miller, 1999). Ellis et al. (1999) showed that breed and genetic line affected pork eating quality with the utilization of Duroc terminal sire lines as a common practice for improving this sensory quality. In fact, Schinckel, Mills, Weber, and Eggert (2002) and Hermesch (2008) reported significant genetic associations between pork belly traits and some swine performance and carcass traits. This implies that selecting for those traits in pigs may affect pork belly qualities. More specifically to bacon, Mandigo (2000) reported that genetic line exerted significant effects on bacon processing parameters (including pump percentage, smoke yield, bacon length and total yield) and proximate composition. Fiego et al. (2005) also confirmed a significant genotype effect between fatty acid composition of two breeds (Landrace × Large White and commercial hybrid), which could have important implications on pork belly and bacon quality.

It seems consistent from the scientific literature that pigs with a genetic predisposition for less subcutaneous fat may also be expected to produce bellies with more unsaturated fatty acids, which is likely due to less *de novo* fatty acid synthesis and greater uptake of dietary fatty acids (Correa et al., 2008). Aside from this, pig genotypes that support larger quantity of adipose tissue will result in lower water content and higher lipids (Fiego et al., 2005), which may translate in a firmer belly for bacon production. Contrary to other results, Cisneros, Ellis, McKeith, McCaw, and Fernando (1996) in a study comparing two crossbred genotype (breeding company hybrid (BCH) and Hampshire × [Yorkshire × Duroc] (HYD)) reported that genotype does not seem to affect the curing yield of bellies. However, Mandigo (2000) and Robles (2004) showed that breed affected bacon pump percent and bacon length. As previously stated, although highly fatty bellies have been confirmed to pick up less curing solution compared to lean bellies (Boler et al., 2012; Jabaay, Forrest, Aberle, Courtenay, & Judge, 1976; Stiffler et al., 1975), genetic lines producing fatty bellies may produce bacon of less pumped yield, but a tendency for higher slice yield, total yield and bacon length may result due to higher fat content and lower pumped weight loss (Boler et al., 2012). Several other researchers have shown that yield of green or cured belly is negatively correlated to its lean content (Kemp, Moody, & Fox, 1969; McMillin, Judge, Forrest, Anderson,

& Aberle, 1977). Hence, leaner breeds may produce bellies with lower overall yield although higher consumer acceptability may result (Jabaay et al., 1976); as such, premiums could be charged for this product, making up for producer's loss due to low processing yield.

Some major genes are among the genetic components affecting quantitative traits in meat quality attributes. These include the halothane and Rendement Napole (RN^-) gene, which affect meat quality due to muscle pH modification. The halothane sensitive (HAL) gene is linked to porcine stress syndrome (PSS) and subsequent pale soft and exudative (PSE) meat, which results in poorer muscle colour and inferior water-holding capacity. The few studies that have observed the effects of this gene on bacon quality have reported a reduced bacon yield, lower moisture content, higher processing/cooking loss and paler colour compared to control (Fisher, Mellett, & Hoffman, 2000; Taylor et al., 1973); however, no significant effects on sensory evaluation (flavour and texture) were observed (Taylor et al., 1973). Similarly, the RN^- gene affects meat quality attributes due to increased glycogen potential of the muscle, resulting in abnormally low pH, paler pork and reduced water-holding capacity (Ellis et al., 1999). This gene may also have negative impacts on belly processing yield and bacon quality. However, the effects of these genes on belly and bacon quality have not been extensively reported in the literature and more research on this subject will enhance the present body of knowledge.

2.3.5.1.2 Sex effects

Animal sex has been identified as affecting lean and fat content, as well as the fatty acid composition of pork belly. Regardless of feed components, barrows usually have a higher fat deposition, which leads to higher backfat than gilts (Benz et al., 2010), and the higher the fat deposition, the less the degree of unsaturation (Wood & Riley, 1982), especially if *de novo* lipogenesis is not inhibited by dietary components. Generally, the order of fatness of pigs based on sex will be barrows (castrated males) > gilts (female) ≥ immunocastrates > boars (intact males). As such, barrows usually have lower PUFA, UFA (unsaturated fatty acids):SFA, PUFA:SFA and higher SFA compared to gilts (Benz et al., 2010; Lonergan, Sebranek, Prusa, & Miller, 1992) as well as immunocastrates and boars. Greater challenges may, therefore, be encountered for bacon processing with bellies from gilts due to fat softness and reduced product shelf life compared to barrows or bellies with firmer fat (Xu et al., 2010). While some studies (Cisneros et al., 1996) have suggested that sex effects do not have an impact on curing or overall yield of bacon, Fredeen

(1980) and Robles (2004) reported that, due to their higher fat content, barrows had greater bacon slicing and total yields compared to gilts.

The effect of physical (PC) and immunological (IC) castration of barrows on various pork belly and bacon characteristics has been recently reported (Asmus et al., 2014; Boler et al., 2012; Kyle et al., 2014; Tavárez et al., 2014). Bellies from IC barrows have generally been reported to be leaner, thinner and with softer, more unsaturated fat (Boler et al., 2012; Tavárez et al., 2014) which may result in lower bacon slicing yield (Kyle et al., 2014). Most of these authors observed lower cooked yield, trimmed cooked weight, sliced weight and overall slice yield percentage (Kyle et al., 2014; Tavárez et al., 2014) in IC compared to PC barrows raised under the same conditions and exposure period, whereas gilts and PC barrow do not differ in slice yield percentage despite gilt bellies having softer fat with a higher unsaturated fatty acid content than PC barrows. In terms of composition, moisture content and lean-to-fat ratio seem to be higher, and lipid content lower, in IC compared to PC barrows, confirming that increased lean content may be related to reduced slicing yield (Kyle et al., 2014).

2.3.5.1.3 Dietary effects

Although genetics and animal management, among other pre-slaughter factors, largely affect the quality of pork meat, more focus has been given to nutritional modification to balance the possible negative influence of animal genotype during pre-slaughter management.

2.3.5.1.3.1 Protein and amino acid derivative sources

The effect of dietary protein content on pork quality is largely dependent on the quantity (level) and quality of the protein, the latter assessed by the lysine content, among other limiting amino acids, as well as the digestibility and bioavailability of the protein sources. Although the effects of protein levels on pork belly and bacon quality have not been reported in the literature, research has shown that high protein (HP) diets lead to increased carcass lean meat content and reduced fat deposition in pork (Karlsson et al., 1993), which signifies a higher lean-to-fat ratio and could enhance consumer acceptability of bacon. This result, however, contradicts an earlier report of Crampton, Ashton, and Lloyd (1954), who found that restricting feed intake and reducing protein content in feeds from 16 to 13% in finishing pigs resulted in reduced fat deposition and, as such, increased bacon quality in terms of increased lean content only. This discrepancy may be

due to variation in the level of essential amino acids in the protein employed in the two studies among other components of the feed and other experimental variations which could have interacted with the reported observations. Similarly, dietary supplementation with L-carnitine (synthesized from lysine and methionine) (Apple et al., 2011; Waylan et al., 2003) and betaine (a trimethyl derivative of the amino acid glycine and a by-product of sugar beet) (Cromwell et al., 1999; Matthews, Southern, Higbie, Persica, & Bidner, 2001b; Pettigrew & Esnaola, 2001) has been reported to enhance body protein accretion resulting in, increased pork carcass leanness.

2.3.5.1.3.2 Carbohydrate sources

Dietary carbohydrate is one of the major sources of energy for swine growth, maintenance and production. Among the major carbohydrate sources for swine are various grains, including barley, corn, sorghum, wheat, and starch supplements (Rosenvold et al., 2001). According to Schinckel et al. (2002), dietary carbohydrate is a major contributor to the triglycerides in the adipose tissue as the fatty acids employed in the formation of triglycerides or phospholipids are synthesized *de novo* from dietary carbohydrate and, as such, are a reflection of the adipose triglyceride composition until their effect is diluted by dietary fat inclusion. Furthermore, Bee (2002) affirmed that dietary energy levels significantly affect the extent to which tissue concentration of fatty acids are altered from dietary fat of different degree of unsaturation. Varying the types of grain included in the diet may not necessarily produce any significant effects on meat quality traits (Lampe, Baas, & Mabry, 2006), as only their contribution to glycolytic potential or the energy reserve of the muscle seems to be relevant (Bee, 2002). Henckel, Karlsson, Jensen, Oksbjerg, and Petersen (2002) have indicated that a glycogen reserve below 53 µmol/g of wet tissue is optimum for improvement in pork quality, and this phenomenon has been explored in feed withdrawal prior to slaughter in swine production (Bertol et al., 2013; Pettigrew & Esnaola, 2001). The variation in dietary energy (dietary carbohydrate) level did not affect protein deposition but increased fat deposition and lipogenic enzyme activities (Bee, Messikommer, & Gebert, 1999). Although no literature has specifically dealt with the effect of dietary carbohydrate on pork belly or bacon quality, high energy diets have been found to result in reduced lean percentage and increased subcutaneous fat in pig carcasses (Robinson & Lewis, 1964). Generally, high levels of dietary carbohydrate increase fat deposition in adipose tissue and this may affect pork belly and bacon alike.

Increased SFA and MUFA, as well as reduced PUFA, have also characterized the fatty acid profile of the adipose tissue of pigs fed high-energy diets (Bee, 2002; Bee et al., 1999). Aside from these, feeding low-energy diets helps to minimize the detrimental effects of rapid *post-mortem* glycolysis and effectively reduces the rate of pH decline and improves fresh pork water-holding capacity and colour attributes (Apple, 2007). Not only does increased energy intake result in reduced water-holding capacity, it also seems to result in reduced meat tenderness, as perceived by a corresponding reduced activity of µ-calpain and increased calpastatin (Pettigrew & Esnaola, 2001; Rosenvold et al., 2001). Swine diets with carbohydrate energy levels above that required for maintenance, especially during the finishing period, must therefore be regulated and controlled to avoid detrimental effects on pork and pork belly quality. Further research is however warranted to specifically assess the effect of different dietary carbohydrate levels on pork belly and bacon quality.

2.3.5.1.3.3 Fat sources

In monogastrics, dietary fatty acids are absorbed relatively unchanged, allowing for the manipulation of fatty acid composition of adipose tissue (Dugan, Aalhus, & Kramer, 2004). As pork belly has a high fat content, most of the nutritional interventions have focused on improving the fatty acid profile, as this appears to be a major factor affecting belly quality and, consequently, bacon technological and sensory attributes. Many dietary fat sources have been employed in nutritional interventions to improve pork belly and bacon quality. As pigs are omnivores, animal fat sources are commonly used in swine nutrition. Beef tallow supplementation generally leads to an increase in the concentration of SFA, leading to firmer bellies (Apple et al., 2007) and lower off-flavour intensity (Browne et al., 2013). On the other hand, yellow grease and choice white grease have been observed to yield softer bellies (Browne et al., 2013; Jackson et al., 2009), which may be due to an increased degree of unsaturation in pork fat. However, no effect has been reported on bacon yield, colour and sensory attributes. Poultry fat, like many other highly unsaturated dietary fat sources in swine production, has been associated with unacceptably soft bellies (Cannon et al., 1996).

Plant fat sources vary in composition and their effects on belly and bacon quality can be very different. In general, high levels of dietary plant fat sources lead to decreased SFA levels in pork fat. Compared to animal fat sources, plant sources are high in oleic and linoleic acids, which

are correlated with belly softness and other technological issues, as well as low consumer acceptability (Schinckel et al., 2002; Shackelford et al., 1990). Inclusion of DDGS in pig diets has increased in recent years due to the high price of grain and increased availability of this relatively cheap by-product from ethanol production. Research has shown that generally, with increasing concentration of corn DDGS in the diet, SFA tend to decrease while PUFA and IV increase (McClelland et al., 2012), resulting in increased belly softness and decreased belly thickness. Beltranena et al. (2011) reported that for every 7.5% inclusion of wheat DDGS in swine diets, a corresponding 0.2% reduction in belly yield, with an accompanying increase in PUFA and IV, was observed. This observation may vary depending on the composition and the percent of the fat in the DDGS (Widmer, et al., 2008). According to Tavárez et al. (2014), regardless of their positive effect on some quality parameters, such as pump uptake, trimmed and pump weight, DDGS feeding strategies alone may have only minimal effects on bacon slicing yield and composition and this may also vary depending on the sex of the animal. Although immunological castration (IC) appears to reduce bacon slice yield, withdrawal of DDGS from IC barrows' diet improved bacon slice yield, normalizing the effect of IC from these bellies (Tavárez et al., 2014). In their report on fresh bellies, these authors demonstrated that inclusion of DDGS in pigs' diet up to 30% had no effects on belly-flop and thickness compared to control, although length of exposure and interaction with other inherent factors may influence these effects. As such, feeding pigs with not more than 20 to 30% DDGS (Whitney et al., 2006) or employing a DDGS withdrawal strategy (Tavárez et al., 2014) may present no detrimental effects on most pork belly or bacon quality traits provided adequate consideration is given to the nutritional requirements of the animal in question.

Dietary inclusion of specific fatty acids, such as conjugated linoleic acid (CLA), has been observed to increase the SFA content, belly leanness and firmness (Larsen et al., 2009; Weber et al., 2006). The mechanism includes the induction of adipocyte apoptosis and stimulation of *de novo* lipogenesis (Weber et al., 2006), leading to increasing the SFA content in bellies from CLA fed pigs.

2.3.5.1.3.4 Vitamins and mineral sources

Several vitamins and minerals have been evaluated as dietary ingredients to enhance growth of pigs and improve pork quality. Dietary inclusion of vitamin E reduced lipid oxidation in pork and improved meat colour (Guo et al., 2006; Lahucky et al., 2007). Even with an observed

trend for increased fatty acid unsaturation in pork with α-tocopherol acetate inclusion in swine diet, lipid oxidation was still observed to be reduced (Guo et al., 2006). This may present a working production system to ensure pork bellies of healthy fat profile are produced with no negative implication on fatty acid oxidation and shelf stability. Similarly, feeding elevated amounts of vitamin D_3 improved pork colour, increased muscle pH and reduced drip loss (Lahucky et al., 2007; Wiegand et al., 2002; Wilborn, Kerth, Owsley, Jones, & Frobish, 2004).

Magnesium, selenium, chromium, manganese and iron all have positive effects on pork quality although their effects may vary from experiment to experiment (Apple, 2007; Ellis et al., 1999). However, studies focusing directly on the effect of mineral supplements on pork belly quality are scarce. Magnesium salt supplementation results in improved colour (lower L*), improved water-holding capacity and higher ultimate pH (D'Souza, Warner, Dunshea, & Leury, 1999; D'Souza, Warner, Leury, & Dunshea, 1998) in pork muscles, and was shown to reduce stress sensitivity in pigs. Chromium supplementation in swine diets decreased PUFA content in belly fat, leading to increased firmness (Jackson et al., 2009), whereas high levels of copper were reported to increase fatty acid unsaturation (Pettigrew & Esnaola, 2001), which may raise issues with soft bellies, poor technological quality, and possibly inferior sensory properties of bacon. It, however, must be proven if some of these effects of vitamins and minerals observed in the aforementioned studies in various muscles also affect pork bellies as the deposition of these supplements may vary from muscle to muscle.

2.3.5.1.3.5 Growth promotants

Ractopamine (RAC) is a beta-adrenergic agonist and porcine somatotropin (pSt) is a naturally occurring peptide hormone, and both have been found to enhance protein accretion in pigs and decreases fatty acid synthesis in adipose tissue. Ractopamine enhances lipolysis while *de novo* lipogenesis is depressed through the action of β-adrenergic receptors on adipose tissue, which influences cellular metabolism via a signalling cascade or increased apoptosis in adipose tissue (Dunshea, D'Souza, Pethick, Harper, & Warner, 2005). On the other hand, pSt simply redirects nutrients toward increased muscle growth, thereby restricting adipose tissue growth (Dunshea et al., 2005). Because a decrease in total fat is usually associated with increased percentages of PUFA and thinner bellies, growth promotants could potentially have negative effects on pork belly technological quality, but the increased leanness may satisfy consumers' demand. According to

the literature, although RAC did not seem to negatively affect some belly quality traits (e.g., fat colour and belly weight), bellies tended to be softer with a corresponding increase in PUFA (Apple et al., 2007). In fact, Scramlin et al. (2008) observed a significant increase in belly yield with a positive effect on bacon quality (in terms of lean content) from pigs fed up to 5 ppm RAC even with no changes in belly thickness, pump uptake and belly-flop. The experiment of Kyle et al. (2014) also supported this trend when they found that RAC addition helped to ameliorate some negative effects of immunological castration on commercial slicing yield in pork belly regardless of the observed increase in fat unsaturation. Similarly, Leick et al. (2010) observed no significant effect of RAC on pork belly quality, including thickness, width, trimmed weight, length, firmness and fat colour. Additionally, bacon cook loss was unaffected by RAC supplementation and the effect on fatty acid profile was minimal (Leick et al., 2010).

Although only a few studies have examined the effect of pSt specifically on pork belly cuts, many reports have demonstrated its efficacy in reducing fat deposition through inhibition of the lipogenic action of insulin and enhancing muscle growth through complex mechanism involving insulin-like growth factor I. This is evident in higher protein and moisture content in the muscle tissue of pSt-treated pigs (Bidanel et al., 1991; Dunshea et al., 2005; Fabry et al., 1991; Lonergan et al., 1992; Nanke, Sebranek, Prusa, & Miller, 1993). Technologically, pSt has been reported to reduce smokehouse yield and increase pump yield in pork bellies; however, these did not have any effect on overall processing yield compared to control (Nanke et al., 1993). The objective colour measurement (*L* and *b* values) was not affected by pSt treatment (Dunshea et al., 2005; Nanke et al., 1993); however, *a* value (redness) decreased in bacon from pSt-treated pigs. Pork bellies also have a tendency to be softer or even thinner in pSt-treated pigs but this did not affect bacon sliceability or other processing properties (Nanke et al., 1993). The softer nature of bellies from pSt treated pigs has been suggested to be the result of its proximate compositional change (higher moisture and protein) rather than altered fatty acid profile (Lonergan et al., 1992; Nanke et al., 1993) as the later does not seem to vary significantly. Although more studies are required to validate this beneficial effect of pSt and RAC, the available literature suggests that these growth promotants could help balance both consumers' need of healthy and leaner product with the producers' technological expectations.

2.3.5.1.4 Age and slaughter weight

Higher slaughter weights (>100 kg), which largely corresponds to increased age, result in increased belly fat deposition and a decreased lean content (Apple, Maxwell, Galloway, Hamilton, & Yancey, 2009; Correa et al., 2008). Increased slaughter weight may also affect the fatty acid profile of belly fat, as an increase in fat deposition results in higher percentages of SFA and MUFA, as well as reduction in PUFA content (Fiego et al., 2005; Virgili et al., 2003; Wood & Riley, 1982) and hence, lower iodine value. However, some studies did not observe an effect of slaughter weight on pork belly fatty acid composition (Correa et al., 2008), which may be due to the different genetic backgrounds of the pigs. Increased slaughter weight may also affect overall chemical composition of the belly, as this has been linked to reduced water content and increased lipid content in back fat, which may increase the overall yield of cured belly due to higher curing gain (Virgili et al., 2003; Wood & Riley, 1982). However, the possibility that pigs of heavier slaughter weights might be prone to greater drip losses has also been proposed (Cisneros et al., 1996), as slower cooling rates in heavier carcasses may increase the incidence of PSE, resulting in commercial losses due to carcass shrink and drip loss. Furthermore, a substantial proportional increase in pork belly weight, with a corresponding increase in trimmed belly percentage, has also been reported with increased slaughter weight. This may increase the producers' profitability (Landgraf et al., 2006), provided the increase is not based solely on the fat content, which may result in consumer rejection.

2.3.5.1.5 Pre-slaughter environment

No studies have specifically looked at the influence of the pre-slaughter environment on pork belly quality. In general, however, pre-slaughter factors (e.g., transport, handling) that increase stress immediately prior to slaughter can result in a higher incidence of PSE, which is likely to influence belly quality as well (Taylor et al., 1973). Pre-slaughter conditions of extended stress, which result in lower glycogen stores in the muscle, can reduce the rate/extent of glycolysis *post-mortem* resulting in higher ultimate pH meat. Although dark, firm and dry (DFD) meat is less encountered in swine than in cattle, the higher water-holding capacity and meat firmness associated with this quality defect may be beneficial to bacon quality and profitability as this may result in firm, well cured belly with higher slicing yield.

2.3.5.2 Post-slaughter or processing factors on pork belly and bacon quality

2.3.5.2.1 Ageing and storage time

Ageing is an inherent process following *rigor mortis* that involves the actions of muscle enzymes. Many researchers have observed its effects in various pork muscles (Channon, Kerr, & Walker, 2004) but little attention has focused on pork belly. It remains to be assessed if ageing conditions and duration will have any significance on pork belly/bacon quality. Apart from improving pork tenderness, ageing was reported to enhance pork cooked flavour, improve blooming potential/colour stability and water-holding capacity attributes in *longissimus thoracis et lumborum* (Juárez et al., 2009). Aside from these quality attributes, because ageing involves protein degradation, the possibility of increased free amino acids and peptide fractions could contribute to overall flavour and increased bioactive peptides, which could have some health benefits, should not be overlooked.

Aside from the positive effects of ageing on meat, in the course of the storage period of either raw or processed meat, oxidative processes may set in. Generally, the oxidative stability of meat is largely dependent on the balance between its inherent prooxidative and antioxidative factors. In addition, the balance between the endogenous and exogenous factors in the meat play a significant role in its oxidative stability. This balance may change during the storage period of the muscle and influence its overall oxidative stability. The susceptibility of meat to oxidation varies among meat from different species and muscles from the same animal (Min, Nam, Cordray, & Ahn, 2008). This depends to a large extent on the fatty acid composition of the muscle as well as the inherent prooxidants (e.g. heme pigment). Whereas lipid and protein oxidation have been previously reported to increase in meat with days in refrigerated storage (Cheng et al., 2011; Ganhão, Morcuende, & Estévez, 2010b; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998), and even under frozen storage (Soyer, Özalp, Dalmış, & Bilgin, 2010), the effect of storage time on the production of heterocyclic aromatic amine has been scarcely reported in the literature. Given that the mechanism that enhances lipid oxidation has similarly been reported to enhance the formation of HAA (Jägerstad, Skog, Arvidsson, & Solyakov, 1998), and because the presence of antioxidants which retard protein and lipid oxidation has also been found to limit the production of HAA (Janoszka, 2010; Kaur, 2008), it may be reasonable to suggest that the effect of storage

days on lipid and protein oxidation may be similar in HAA. The present study will explore this aspect.

2.3.5.2.2 Frozen storage

Freezing bellies prior to the curing process is a common procedure in bacon processing (Robles, 2004). Possible detrimental implications ranging from textural to functional defects may arise during freezing, and these are dependent on the speed and duration of the freezing process as well as the freezing and thawing cycles (Leygonie, Britz, & Hoffman, 2012). The rate of ice crystal growth within the muscle cell may affect meat water-holding capacity, texture and surface colour, with slow freezing resulting in the production of large ice crystals which may disrupt the structural integrity of muscle cells in the pork belly resulting in higher drip loss on thawing with corresponding loss of flavour, colour change and consequently high economic loss. A more rapid and constant freezing process may have milder consequences (due to production of smaller ice crystals) on pork belly quality. A higher slicing yield has been reported for fresh bellies compared to previously-frozen bellies, whereas other processing characteristics (e.g. smokehouse yield) and total yield were unaffected during a 15 day frozen storage period (Robles, 2004). Furthermore, protein and lipid oxidation, as well as protein denaturation, continue during frozen storage, with potential consequences on final product acceptability.

2.4 Effect of cooking methods on bacon and processed meat quality

2.4.1 Common cooking methods in bacon processing and their underlying mechanisms

Both conventional and microwave cooking are typically employed in foodservice and consumer households. As previously mentioned, up to 45% of household bacon is fried while about 15% is prepared using a microwave oven. On the other hand, virtually all foodservice bacon, and up to 10% of the bacon sold in the supermarkets, is precooked using microwave heating (Ahmed & Ramaswamy, 2007). Conventional cooking methods, such as stove tops and conventional ovens, essentially employ heat transmission to food through convection, conduction and radiation of heat from the surface of the material. The process usually involves energy transfer through thermal gradients where neighbouring atoms convey thermal energy derived from an external heat source to each other until there is a constant temperature throughout the body of the food (Venkatesh & Raghavan, 2004). Microwave cooking, in contrast, delivers energy directly to

the food material via molecular interaction with electromagnetic field, and this cooking method has found a wide application in the food industry and consumer households due to its rapid cooking process and reduced cost (Datta & Rakesh, 2013; Venkatesh & Raghavan, 2004). Other authors have attributed the attractiveness of this technology to its volumetric heating, rapid increase in temperature, controllable heat application and easy clean-up (Ahmed & Ramaswamy, 2007).

Microwaves are electromagnetic energies in the frequency band of 300 MHz to 300 GHz (with wavelength from 3 mm to 3 m), bounded by radio frequencies towards its low frequency and by infra-red at its higher frequency end (Yarmand & Rad, 2011). To avoid interferences with radio frequencies for telecommunications, industrial and household applications of microwaves have largely been restricted to 915 ± 25 and 2,450 ± 50 MHz, with their penetration depths ranging from 8 to 22 cm and 3 to 8 cm, respectively. Other microwave frequency bands, including 5,800 ± 75 and 24,125 ± 125 MHz, are among those allocated by the United States Federal Communication Commission for industrial, scientific and medical use (Venkatesh & Raghavan, 2004), whereas other frequencies (including 433.92, 896, and 2,375 MHz) are used in other countries (Ahmed & Ramaswamy, 2007). The mechanisms involved in microwave heating include both dipole rotation of polar molecules and ionic polarization of ionic components of the food material. Polar molecules attempt to align themselves with the microwave electric field at the same rate as the microwave frequency. This results in internal molecular friction that generates rapid heating (Ahmed & Ramaswamy, 2007). Venkatesh and Raghavan (2004) have also reported that non-polar molecules that are asymmetrically charged may also behave as dipoles in an electric field environment. Similarly, dissolved ions migrate towards oppositely charged regions in an oscillating electric field. This results in multiple collisions and disruption of hydrogen bonds which ultimately leads to heat generation (Ahmed & Ramaswamy, 2007).

Although several oven factors (including the position of food inside the oven, its size and geometry, power output and power cycling, along with turntables or mode stirrer) could influence the heating efficiency of microwave cooking, food factors usually play a very significant role (Datta & Rakesh, 2013). Among the food factors that may influence microwave heating are the dielectric and thermal properties of the food, as well as the food's volume, mass and shape (Datta & Rakesh, 2013). The dielectric properties of a food material is its electrical properties in the context of microwave and radio frequency which give a measure of how the food interacts with the electromagnetic energy and this generally impacts whether the microwaves are reflected,

transmitted or absorbed (Venkatesh & Raghavan, 2004). These dielectric properties are mainly a function of the food's dielectric constant (ε′) and the dielectric loss factor (or lossiness; ε″). While the former is a measure of the food's ability to store electric energy, the latter expresses its ability to dissipate the stored energy in the form of heat. Thus, from a simplistic point of view and subject to the food's moisture content, temperature and prevailing microwave frequency, any food substance with high ε′ and high ε″ would also be able to efficiently absorb microwave energy and dissipate it as heat. Water has a very high ε′ and ε″, and, as such, high moisture in food may consequently result in high energy absorption (Ahmed & Ramaswamy, 2007). Dielectric properties of food also vary with temperature (Venkatesh & Raghavan, 2004). Because both temperature and moisture content may change during food processing, the dielectric properties, as well as the heating behaviour of the food, are transient during the heating process. Similarly, the overall thermal properties of the food (thermal conductivity and heat capacity), regardless of the food dielectric properties, will also influence its heating characteristics. Food with high thermal conductivity will rapidly dissipate heat during microwave heating. Although higher fat content may result in lower ε′ and ε″ in food, the fat's lower specific heat capacity compared to that of water may enhance its overall heating efficiency and uniformity (Picouet, Fernández, Serra, Sunol, & Arnau, 2007).

Although ionic conduction is recognised as one microwave heating mechanism, at the frequencies applied in domestic microwave ovens (2,450 MHz), increasing ion concentration in solution has been reported to result in lower heat dissipation (Anwar et al., 2015). This further highlights the importance of prevailing microwave frequencies in the food's dielectric properties and its subsequent heating behaviour. Aside from this, the shape and thickness of food play an important role in the microwave distribution and heating behaviour of food. The closer the thickness of the food to the microwave wavelength, the better the heating rate and uniformity. As such, bacon slices will heat faster than pork roast. Similarly, a food of spherical or cylindrical shape will heat more evenly than a square shaped food (Ahmed & Ramaswamy, 2007).

Even though microwave cooking possesses many advantages compared to conventional cooking, it is limited in that the actual temperature profile of microwave heating in food may be difficult to determine due to its uneven heating pattern and incidences of cold spots. Also, the changes in the dielectric properties of food during thermal processing could affect the heating pattern qualitatively and may make it difficult to predict the degradation kinetics of food quality,

sensory and nutrient retention (Ahmed & Ramaswamy, 2007). For example, although microwave cooking does not give meat the typical browned, crusty surface due to the short cooking time as well as the cool ambient temperature of the surrounding air in the microwave oven during cooking, it generally results in greater cook losses than in meat cooked in conventional oven (Baldwin, 1978; Yarmand & Rad, 2011). Moreover, because microwave ovens do not produce ionizing radiations, they do not leave radioactive residues in food components, but their effect in producing other hazardous components, like products of lipid or protein oxidation as well as other carcinogenic compounds, may be an interesting subject to explore because several studies have shown that lipid and protein oxidation (Gatellier, Kondjoyan, Portanguen, & Santé-Lhoutellier, 2010; Haak et al., 2006), as well as heterocyclic amine formation increased with cooking (Gibis, 2016).

2.4.2 Effect of cooking methods on production of toxic compounds in processed meat

Considering the underlining mechanisms of various cooking methods, their impacts on the production of certain detrimental compounds in processed meat may vary widely. Traditionally in bacon, the most widely assessed carcinogenic compounds are N-nitrosamines. N-nitrosamines are formed by the reaction of organic amines and their derivatives with appropriate nitrosating species, the most stable nitrosamines being formed from secondary amines (Park, Seo, Lee, & Kwon, 2015). Nitrosamines derived from primary amines breakdown rapidly but tertiary amines can barely form nitrosamine (Park et al., 2015). Nitrite ions and nitrous acid are, in themselves, an inefficient nitrosating agent; yet, under acidic conditions (optimally between pH 3 and 4), these species are converted through a series of reactions into nitrous anhydride (dinitrogen trioxide, N_2O_3), which is believed to be the primary reactive agent for nitrosamine formation in meat (Pegg & Shahidi, 2000). While N-nitrosamine can either be volatile (e.g. *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodiethylamine (NDEA), and *N*-nitrosopiperidine (NPIP)) or non-volatile (e.g. *N*-nitrosoproline (NPRO), *N*-nitrosohydroxyproline (NHPRO), *N*-nitroso-thiazolidine-4-carboxylic acid (NTCA) and *N*-nitroso-2-methyl-thiazolidine 4-carboxylic acid (NMTCA), and *N*-nitrososarcosine (NSAR)) compounds, the former have been generally regarded as a more potent carcinogens than the latter and, as such, have been more widely quantified in processed meat (Herrmann, Granby, & Duedahl-Olesen, 2015). From the volatile group, both NDMA and NDEA have been placed in group 2A (probably carcinogenic to human),

whereas NPIP, NPYR and NMOR are placed in group 2B (possibly carcinogenic to human) by the WHO- International Agency for Research on Cancer (IARC, 2017). Similarly, the European Union categorized NDMA and NDEA in category 1B (presumed to have carcinogenic potential for humans) while the US Environmental Protection Agency classified both of these compounds in category B2 (probable human carcinogen) (Selin, 2011). Of the non-volatile group, only NSAR has been classified into group 2B, whereas most of others, including NPRO and NHPRO, are classified into group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 2017).

N-nitrosamine content could vary depending on several factors, including cooking methods, temperature, time, food moisture content and fat composition (Park et al., 2015). Miller, Billedeau, and Miller (1989) reported a higher total nitrosamine (NPYR + NDMA) content in bacon cooked in a frying pan than those cooked with microwave (~11 vs ~5 ng/g). Further, the same study showed that higher cooking temperatures and time, produced greater nitrosamines concentrations. In fact, Pensabene, Fiddler, Gates, Fagan, and Wasserman (1974) have shown that at temperatures lower than 100°C, no NPYR was formed in bacon cooked for up to 104 min; however, when the same bacon was cooked for 4 min at 204°C, NPYR was produced to about 17 µg/kg. Several other studies have also reported similar higher nitrosamine content in fried bacon (Lehotay, Sapozhnikova, Han, & Johnston, 2015; Pensabene et al., 1974; Österdahl & Alriksson, 1990) and fried dry cured sausage (Li, Wang, Xu, & Zhou, 2012) compared to their microwaved counterparts. Furthermore, greater amounts of nitrosamines have been detected in the fat portion of fried bacon compared to the lean portion (Mottram, Patterson, Edwards, & Gough, 1977). This, according to Herrmann et al. (2015), could be due to the fact that most precursors of nitrosamine are more soluble in the lipid than the aqueous phase of meat. More so, other reports has shown that NPYR levels in bacon correlated well with the degree of unsaturation of the adipose tissue (Gray, Skrypec, Mandagere, Booren, & Pearson, 1983). This emphasizes the importance of meat composition in the generation of some of these carcinogens during meat cooking. Among other compounds that could be produced during bacon processing are cholesterol oxidation products (COPs), polycyclic aromatic hydrocarbon (PAH), lipid and protein oxidation, and heterocyclic aromatic amines (HAA). However, the scope of the present thesis will only cover the impact of selected cooking methods on lipid and protein oxidation and heterocyclic aromatic amines and these will be the focus of the subsequent sections.

2.4.3 Lipid oxidation in processed meat[2]

Lipids are crucial components of meat as they contribute to quality attributes such as flavour profile, nutrition and calorific density. In fact, several studies have demonstrated the ability of fat components to activate chemosensory organs (taste buds) and trigger taste signalling cascade which may qualify fat as the sixth primary taste quality (Gilbertson & Khan, 2014; Keast & Costanzo, 2015; Running, Craig, & Mattes, 2015). As important as lipids are in food sensory and nutritional quality; however, their susceptibility to oxidation during storage and cooking (especially their UFA components) constitutes not only sensory defects but also health concerns. This is because lipid oxidation products have been strongly associated with the pathogenesis of various disorders, including cardiovascular and inflammatory diseases, gastropathic conditions, mutagenicity, genotoxicity and teratogenicity (Albert, Cameron-Smith, Hofman, & Cutfield, 2013; Márquez-Ruiz, Garcia-Martinez, & Holgado, 2008). As several food processing methods (irradiation, comminution, cooking etc.) have been identified as contributing to increased lipid oxidation, the impacts of the ingestion of oxidized dietary lipid are largely unknown. Although their consumption may not represent acute toxicity (Esterbauer, 1993), chronic exposure to oxidized lipids may present carcinogenic or atherogenic risk (Albert et al., 2013).

2.4.3.1 Indication and analysis of lipid oxidation in meat systems

Lipid peroxidation can proceed through: photoxidation (singlet oxygen and photosensitizer); enzymatic oxidation (e.g., cyclooxygenase and lipoxygenase); or autoxidation (free radical mediated) (McClements & Decker, 2008). The pathway of lipid oxidation has generally been described to follow initiation, propagation and termination, as well as decomposition and β-scission reaction steps. These steps can produce a series of important and complex oxidation products that may affect not only the organoleptic properties of the food but also may contribute to the aetiology of some disease conditions. Generally, primary lipid oxidation products are those that are produced during the initiation and propagation steps of lipid oxidation.

---

[2] A version of this section (2.4.3) is adapted and used with permission from the book chapter published as:

Estévez, M., Li, Z., Soladoye, O. P., & Van-Hecke, T. (2017). Health risks of food oxidation. In F. Toldrá (Ed.), *Advances in Food and Nutrition Research* (pp. 45-81). London: Academic Press.

O. P. Soladoye drafted, compiled data, revised and finalized this section in the book chapter.

Although lipid hydroperoxides are the major products at this stage of the reaction, some other species, including other conjugated dienes (Ahotupa & Vasankari, 1999) and fatty acyl radicals (e.g. peroxyl and alkyl radicals), are also produced in this step and may be involved in some disease pathogenesis. Secondary lipid oxidation products are compounds that arise from the decomposition of lipid hydroperoxides (LOOH) following the formation of lipid alkoxyl radical ($LO^{\bullet}$) that instigate the β-scission reaction. The secondary lipid oxidation products (e.g., malondialdehyde and hexanal) mainly constitute the organoleptic defect in food, in addition to their health implications on consumers (McClements & Decker, 2008).

Aside from sensory evaluation, another way to assess lipid oxidation in food is by quantifying either their primary or secondary oxidation products. Although the primary lipid oxidation products can be quantified and used as markers of lipid oxidation in meat, their instability due to constant degradation to secondary products of lipid oxidation make them an inefficient marker in food systems (Estévez, Li, Soladoye, & Van-Hecke, 2017). Conjugated dienes can be measured in foods at an absorption maximum of 234 nm and a 2.5×10^4 $M^{-1}cm^{-1}$ molar extinction coefficient. Similarly, conjugated trienes can be measured at 270 nm in foods containing lipids. Methods that measure lipid hydroperoxides depend on their ability to oxidize an indicator compound. For instance, hydroperoxide promotes the conversion of iodide into iodine. This iodine is then back-titrated with sodium thiosulfite to produce iodide that is measured with starch indicator (McClements & Decker, 2008). Ferrous ion may also be used in the place of iodide with the resulting ferric ion being detected with specific chromophore, such as thiocyanate or xylenol orange (Shantha & Decker, 1994).

Secondary products of lipid oxidation are a more stable marker of lipid peroxidation in foods. These include both non-volatile and volatile compounds that are largely implicated in off-flavours and off-aromas in rancid foods. These methods include analysis of volatiles with gas chromatography (using static or dynamic headspace, solid-phase microextraction (SPME) or direct injection to measure hexanal, propanal, etc.), measurement of carbonyls (by reacting lipids with 2,4-dinitrophenylhydrazine to form corresponding hydrazones that absorb light at 430 to 460 nm) and analysis of malondialdehyde using the thiobarbituric acid (TBA) method (McClements & Decker, 2008). The later method involves the reaction between TBA and carbonyls to form red, fluorescent adducts under acidic environment. The MDA-TBA adduct absorbs strongly at ~532 nm which can be quantified spectrophotometrically.

2.4.3.2 Effects of cooking/heat treatment on production of lipid oxidation in meat

Aside from all its positive effects, including taste and flavour enhancement, microorganism destruction, shelf life improvement and improved digestibility (Broncano, Petrón, Parra, & Timón, 2009), a major negative implication of meat cooking is the increased lipid oxidation products. Similar to protein oxidation, this increase in lipid oxidation with heat treatment could be due to increased free radical production, disruption of muscle structure and a decrease in antioxidant protection (Gatellier, Kondjoyan, Portanguen, & Santé-Lhoutellier, 2010). Hoac, Daun, Trafikowska, Zackrisson, and Åkesson (2006) reported a diminished activity of glutathione peroxidase activity in meat with increasing heating temperature. In fact, other studies showed an increased level of free iron in meat during cooking (Rhee, Ziprin, & Ordonez, 1987), which can serve as a prooxidant in meat systems (Lund, Heinonen, Baron, & Estévez, 2011). Other studies have further demonstrated that high temperature decreases the activation energy for oxidation and also breaks down hydroperoxides to free radicals, which enhance the propagation of lipid peroxidation (Haak et al., 2006). Accordingly, many researchers have reported an increased lipid oxidation in meat with heat treatment (Conchillo, Ansorena, & Astiasarán, 2005; Juntachote, Berghofer, Siebenhandl, & Bauer, 2007; Juárez et al., 2010; Traore et al., 2012). Although most of these studies have assessed the effect of cooking temperature and time on this increase in lipid peroxidation, only a few have considered the impact of different cooking methods or the underlining mechanism of these cooking methods.

2.4.3.3 Pathogenesis of lipid oxidation products

As mentioned previously, lipid hydroperoxides (LOOH) are prominent non-radical intermediates derived from peroxidation of unsaturated phospholipids, glycolipids and cholesterol through reactions induced by activated species, such as hydroxyl radicals, lipid peroxyls and alkyl radicals, singlet oxygen and peroxynitrite (Girotti, 1998). Being more polar, while fairly stable and of longer lifetime (at moderate reaction conditions) compared to their free radical precursors and parent lipids (Ayala, Muñoz, & Argüelles, 2014), LOOH can migrate from point of origin to more sensitive sites to initiate membrane perturbation (Girotti, 1998). Shahidi and Zhong (2010) pointed out that ingested lipid hydroperoxides could lead to lipid membrane peroxidation, cell damage and oxidative stress, which may subsequently result in altered membrane fluidity, impaired transport and cell signalling, all of which may be important in certain disease mechanisms.

Although controversies exist in the literature about the absorption of LOOH from the GI tract, many studies have reported its absorption through the gut (Albert et al., 2013; Ursini et al., 1998) and incorporation into chylomicron (Staprans, Rapp, Pan, Kim, & Feingold, 1994), low density lipoprotein, LDL (Ahotupa, Suomela, Vuorimaa, & Vasankari, 2010) and very low density lipoprotein (Suomela, Ahotupa, Sjövall, Kurvinen, & Kallio, 2004). The *in vivo* peroxidation of the PUFA component of LDL has been shown to play a role in atherosclerotic process (Chisolm & Steinberg, 2000; Penumetcha, Khan, & Parthasarathy, 2000) and the ingested LOOH from dietary sources have also been reported to be transported in LDL (Ahotupa et al., 2010). The additive effect of these routes may further enhance the activation of scavenger receptors of macrophages, leading to foam cell formation, a marker of atherosclerosis (Grootveld et al., 2001). In fact, the incorporation of dietary LOOH with the PUFA component of LDL may enhance lipid peroxidation process, as Albert et al. (2013) stated that LOOH hastens oxidation of other fatty acids to create further lipid peroxides in an expansive chain reaction. Previous reports (Girotti, 1998) had also shown that LOOH undergoes one electron reduction to alkyl radical intermediates, which can perpetuate an expansive chain reaction in terms of direct H-abstraction and initiation of chain peroxidation, β-scission with aldehyde generation, and rearrangement and oxygenation to produce epoxyallylic radical; all of which will enhance LOOH atherogenic nature.

The acute toxicity of LOOH in rat intestinal mucosa has been reported with significant impacts on its integrity and functionality (Kanazawa, Ashida, Minamoto, Danno, & Natake, 1988). These authors observed that orally administered LOOH injured the membrane of intestinal microvilli as well as decreased the enzyme activities in jejunum and ileum. Other authors have also reported the tumor-promoting effect of LOOH as they stimulate cell proliferation in the colon (Bull, Nigro, & Marnett, 1988; Earles, Bronstein, Winner, & Bull, 1991). Angeli et al. (2011) showed the possible genotoxicity of linoleic acid hydroperoxide in the presence of heme iron at concentrations found in the human diet. These studies strongly suggest the possible impact of dietary LOOH in certain cancer aetiologies especially colon cancer in humans consuming diets high in fat and red meat. Even if lipid hydroperoxides are not absorbed as suggested by some authors (Márquez-Ruiz et al., 2008), its isomeric forms could still result in gastrophatic conditions in humans (Grootveld et al., 2001).

Among the many different aldehydes formed as secondary product of lipid peroxidation which can exert biological effects relevant to the pathobiology of oxidant injury, malondialdehyde

(MDA), 4-hydrohynonenal (HNE) and acrolein (ACR) have been the most widely studied (Uchida, 2000; Zarkovic, Cipak, Jaganjac, Borovic, & Zarkovic, 2013). In the past decades, more focus has been given to the biological roles of endogenously produced MDA and other α, β-unsaturated aldehydes, whereas the role of these aldehydes absorbed from dietary sources has been scarcely investigated. Studies, have, however confirmed that these aldehydes are absorbed unaltered after digestion and could reach systemic circulation (Goicoechea et al., 2008; Grootveld et al., 1998). Generally, MDA is believed to be the most mutagenic of these, whereas HNE is the most toxic (Esterbauer, Schaur, & Zollner, 1990).

Reactivity of MDA is mainly due to its electrophilicity, which makes it strongly reactive towards nucleophiles such as basic amino acids and thiol groups (Ayala et al., 2014; Ishii et al., 2008). Because MDA reactivity is pH dependent, it displays low reactivity at low physiological or neutral pH, but, still has the capability to interact with proteins and DNA to form several adducts, alter the functions of cell and other macro molecules, and this process may form the backdrop for several disease conditions (Grimsrud, Xie, Griffin, & Bernlohr, 2008). Zarkovic et al. (2013) have identified several proteins that are known to be modified by MDA and, as such, may exhibit altered functions in biological systems. Malondialdehyde was reported to react with primary amines to generate N^{ε}-(2-propenal) lysine and produce lysine-lysine crosslinks with 1-amino-3-iminopropene and pyridyl-dihydropyridine type bridges, the phenomenon that could be implicated in atherogenesis (Palinski et al., 1994; Uchida, 2000).

Studies have also shown that MDA is an important contributor to DNA damage and mutations (Niedernhofer, Daniels, Rouzer, Greene, & Marnett, 2003; VanderVeen, Hashim, Shyr, & Marnett, 2003). Malondialdehyde can react with several nucleosides to form adduct to deoxyguanosine and deoxyadenosine, generating pyrimido [1,2-α] purine -10(3H-) one (M1G or M1dG). This may result in sequence dependent frame-shift mutations (Niedernhofer et al., 2003), strand breaks (Vöhringer, Becker, Krieger, Jacobi, & Witte, 1998), cell cycle arrest (Ji, Rouzer, Marnett, & Pietenpol, 1998) and induction of apoptosis (Willis, Klassen, Carlson, Brouse, & Thiele, 2004), all of which may contribute to cancer and other genetic diseases.

Acetaldehyde, which is a product of MDA metabolism, may also react with MDA under oxidative stress to produce malondialdehyde acetaldehyde (MAA) adducts. Malondialdehyde acetaldehyde can react covalently with protein to form MAA-protein adducts, which can be pro-inflammatory and pro-fibrogenic as well as capable of inducing strong immune responses (Tuma,

2002; Willis, Klassen, Tuma, Sorrell, & Thiele, 2002). However, it remains to be assessed if diet-borne MDA will exhibit or contribute to these biological effects *in vivo* in human subjects.

The most researched of the α, β-unsaturated aldehyde is 4-hydroxy-2-nonenal (HNE), which is a product of n-6 polyunsaturated fatty acid (e.g., linoleic and arachidonic acid) (Esterbauer, 1993). Similar to MDA production, HNE may also be produced either by enzymatic or non-enzymatic process. The enzymatic pathway mainly employs lipoxygenases (either 15 LOX-1 or 15 LOX-2) to transform linoleic acid to 13-hydroperoxy octadecadienoic acid (13-HPODE) and arachidonic acid to 15-hydroperoxyeicosatetraenoic acid (15-HPETE), the main precursors of HNE (Riahi, Cohen, Shamni, & Sasson, 2010; Schneider, Tallman, Porter, & Brash, 2001). Ayala et al. (2014) highlighted the non-enzymatic route of formation of HNE through a free radical mechanism involving the formation of hydroperoxides, alkoxyl radicals, epoxides and fatty acyl crosslinks.

Although HNE has been found to be involved in cell signalling and apoptosis, modulation of cell growth, and differentiation at moderate concentrations (Ayala et al., 2014; Cipak et al., 2006), it has also been considered a secondary messenger of free radicals due to their ability to bind major biomolecules, especially proteins, modifying their structure and functions (Zarkovic et al., 2013). The three main functional groups (the aldehyde group, the C=C double bond and the hydroxyl groups) of HNE allow them to participate in chemical reactions with other molecules either alone or in sequence (Pizzimenti et al., 2013). 4-hydroxy-2-nonenal is a highly electrophilic molecule that can easily react with glutathione, proteins and DNA at higher concentration. It can form adducts with three different amino acyl side chains (Cys, His and Lys residue) via a Michael addition either to the thiol (-SH) or the amino ($-NH_2$) group (Pizzimenti et al., 2013). In addition, HNE can modify protein structure through Schiff base formation (Aldini et al., 2006). Several proteins have been shown to be covalently modified by HNE, some of which have been listed in a recent review (Zarkovic et al., 2013). The preference for amino acid modification by HNE is in the order of Cys >> His > Lys; however, Cys residue are not always the preferential target because protein tertiary structure can condition their accessibility (Dalleau, Baradat, Guéraud, & Huc, 2013; Sayre, Lin, Yuan, Zhu, & Tang, 2006). Reports have shown the acute toxicity of HNE with mammalian cells with cell death within an hour. This cytotoxicity has been largely implicated on several factors including: rapid depletion of glutathione, decrease in protein thiols, onset of peroxidation, disturbance of calcium homeostasis, inhibition of DNA, RNA and protein synthesis,

inhibition of respiration and glycolysis and lactate release among other morphological changes (Esterbauer et al., 1990). All these could subsequently result in gene expression inhibition and its role in promoting the development and progression of different pathological conditions. Its involvement in the pathogenesis of diabetes mellitus (Jaganjac, Tirosh, Cohen, Sasson, & Zarkovic, 2013), neurodegenerative diseases (Perluigi, Coccia, & Butterfield, 2012; Reed, 2011), aging (Zimniak, 2011) and Alzheimer's disease (Butterfield, Reed, & Sultana, 2011) has been widely reported, and most of these disease conditions could be linked to some modification of proteins and DNA by these lipid peroxidation products.

Acrolein (ACR) is another α, β-unsaturated aldehyde that can also be formed from PUFA lipid peroxidation as well as intracellular enzymatic oxidation of polyamine metabolites (Alarcon, 1970). Acrolein has long been identified to be formed from overheating of frying oil, deriving its name from the acrid smell of overheated oils (Esterbauer et al., 1990). Being by far the strongest electrophile and, as such, the most reactive with nucleophiles, such as the thiol- or amino groups compared to other α, β-unsaturated aldehydes, its consumption in the diet may have significant impacts on human health. Acrolein has been reported to be highly cytotoxic towards mammalian cells with reduced proliferation, which may be due to its ability to damage DNA (Grafström et al., 1988), deplete glutathione (Kehrer & Biswal, 2000), form protein and DNA adducts (Sanchez, Kozekov, Harris, & Lloyd, 2005) and inhibit enzyme functional groups (Cox, Goorha, & Irving, 1988). Studies have also shown it is hepatotoxic (Seiner, LaButti, & Gates, 2007; Srivastava, Watowich, Petrash, Srivastava, & Bhatnagar, 1999) and causes acute gastrointestinal distress and pulmonary congestion and oedema (Esterbauer et al., 1990). Furthermore, elevated plasma concentration of ACR has been detected in patients with chronic renal failure, and ACR-protein adducts have been found to increase in tissues of patients with Alzheimer and Parkinson's disease, as well as atherogenesis and chronic lung diseases (Pizzimenti et al., 2013).

2.4.4 Protein oxidation in processed meat[3]

Over the past decade, there has been an increasing research interest on the implications of protein oxidation (PROTOX) in muscle foods, following many years of medical research on this topic and its possible involvement in several human disease conditions. From the perspective of food science, focus has been given to its effects on meat technological quality and sensory perception, with an oversight of the possible effect of the consumption of PROTOX products in processed meat on human health, as well as its possible contribution to overall oxidative stress and certain health disorders postprandial.

Protein oxidation in muscle foods can be induced directly through reactive oxygen species (ROS) (either free radical or non-free radical) and reactive nitrogen species (RNS) or indirectly by secondary products of oxidative stress (Lund et al., 2011). Many species have been identified as possible precursors of oxidation in proteins, including hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$), peroxyl ($PO^{\cdot}$), and nitric oxide ($NO^{\cdot}$), all of which are free radical species (Stadtman, 1993). Other reactive species, which are not free radicals, are hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and ozone (O_3) (Shacter, 2000; Xiong, 2000). These reactive species are usually produced from external (X-rays, γ-rays, ozone, air pollution, industrial chemicals etc.) or internal (enzyme or metal catalysed systems, metabolic processes etc.) factors, and usually initiate PROTOX in meat system either by modifying the amino acid side chains or attacking the polypeptide backbone of the protein (Lund et al., 2011; Xiong, 2000). The results of this oxidative stress are modifications of the amino acid side chains and fragmentation, aggregation and polymerisation of the protein. These effects result in both biochemical and structural disruption leading to several sensory, technological and nutritional issues in the muscle foods (Estévez, 2011; Lund et al., 2011).

Meat products go through many processing steps, either industrially or in households, which may trigger PROTOX (Estévez, 2011). Recent reports have shown that the occurrence of PROTOX in meat systems may not only affect the sensory or technological attributes of the meat

---

[3] A version of this section (pages 48-68) is published and extracts were used with permission from:

Soladoye, O. P., Juarez, M. L., Aalhus, J. L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, *14* (2), 106-122.

O. P. Soladoye compiled data, interpreted results, drafted, revised and finalised manuscript.

but also the health and safety of the consuming populace (Estévez, 2011). Although interesting advances have been accomplished in this field, the continued need to understand the impact of oxidized proteins in nutrition and health compels food scientists to conduct novel and more challenging studies.

2.4.4.1 Indication and analysis of protein oxidation in meat systems

In order to assess protein oxidation in muscle foods, stable markers must be identified that will demonstrate the occurrence, nature, intensity and consequences of the oxidative damage. However, due to the complexity of the mechanisms, the wide range of free radical species, protein targets and oxidation products, selecting a unique marker that will represent the overall extent of PROTOX in a food system may not be an easy resolution. Reliable evidence has suggested that the mechanism of free radical attack on protein molecules results in protein crosslinking, protein fragmentation and/or modification of amino acid side chains (Lund et al., 2011; Stadtman & Levine, 2003), with each reaction yielding a specific oxidative derivative. However, not all the PROTOX products used as markers of oxidative stress in medical research (Table 2.1) have been applied to food systems. Although not much is known about the fate of proteins in *post-mortem* muscle when exposed to oxidative stress, however, the mechanism of action is believed to proceed via a free radical chain reaction comparable to that of lipid oxidation but with greater pathway complexity and a larger variety of oxidation products (Lund et al., 2011). In a complex matrix, such as meat, the links between lipid peroxidation and PROTOX are still unclear. Due to the earlier onset of lipid oxidation, it has been suggested that lipid peroxide products may have a role in promoting PROTOX (Aalhus & Dugan, 2014). Furthermore, amino acids with reactive side chains are susceptible to reaction with lipid peroxidation products.

The PROTOX chain reactions (Figure 2.6) can be initiated when a ROS (produced either from metal-catalysed cleavages or radiolysis of water) abstracts a hydrogen atom from the protein molecule to produce a carbon-centred radical ($C^{\bullet}$) *(reaction c)* which is converted in the presence of oxygen to an alkyperoxy radical ($COO^{\bullet}$) *(reaction d).* Its subsequent reaction with Fe^{2+} or abstraction of hydrogen atom from another molecule or reaction with the protonated form of superoxide radical can readily yield alkyl peroxide (COOH) *(reaction f, g and h, respectively).* Further reactions with free peroxyl radical ($HO_2^{\bullet}$) or reduced forms of iron (Fe^{2+}) can lead to formation of the alkoxy radical ($CO^{\bullet}$) *(reaction j and k)* and its hydroxyl derivative (COH)

(reaction m and n) (Stadtman & Levine 2003; Lund et al., 2011). However, in the absence of oxygen, two carbon-centred radicals can react with each other to produce carbon-carbon cross linked derivatives (*reaction e)*. Aside from these routes, the alkyl peroxide and alkyl radical derivatives can undergo cleavage reaction by either diamide or α-amidation pathway (*reaction i and l*). Depending on the target and the oxidizing agent, protein oxidation propagates and terminates according to multiple mechanisms, and the consequences include the loss of suphydryl groups, the formation of protein carbonyls, the formation of crosslinks and the modification of aromatic amino acids, among others (Lund et al., 2011; Zhang, Xiao, & Ahn, 2013).

Protein carbonyls have been widely used to assess PROTOX because their generation occurs by many different mechanisms, including the direct oxidation of the side chain of basic amino acids (e.g. lysine, arginine), the oxidative cleavage of the peptide backbone via the α-amidation pathway or the oxidation of glutamyl side chain and the reaction of δ-amino group of an alkaline amino acid with reducing sugar or their oxidation products (Estévez & Luna, 2016). Their detection by the 2,4-dinitrophenylhydrazine (DNPH) method is simple and inexpensive, and it is believed to represent one of the most relevant expressions of the oxidative damage to proteins in biological (Shacter, 2000) and, in food systems (Estévez, 2011). The carbonyl moiety reacts with DNPH to form a 2,4- dinitrophenylhydrazone derivative and the amount of hydrazone formed is quantified spectrophotometrically (Oliver, Ahn, Moerman, Goldstein, & Stadtman, 1987). The amount of carbonyl is usually measured at 370 nm and expressed as nanomol of carbonyl per mg of protein using the adsorption coefficient for the protein hydrazones (21.0 $mM^{-1}cm^{-1}$) (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009). Protein concentration is usually calculated from absorption at 280 nm using bovine serum albumin (BSA) as a standard. Despite its popularity, the drawbacks of this procedure, including the lack of specificity and possible overestimation of carbonyl due to other artefacts, have necessitated better protein carbonyl assessment. A recent paper proposed a simplified and improved procedure to shift the absorbance wavelength from 370 to 450 and, as such, avoid interferences with the remaining free DNPH (Mesquita et al., 2014).

Table 2.1: Select amino acid residues and their oxidation products

Amino acid residue	Process of modification	Product of oxidative modification	References
Arginine	Carbonylation/ metal ion-catalysed oxidation	γ-glutamic semialdehyde	Requena et al. (2001), Stadtman &Levine (2003)
Lysine	Carbonylation/ metal ion-catalysed oxidation	α-aminoadipic semialdehyde	Estevez (2011), Zhang et al. (2013)
Proline	Carbonylation/ metal ion-catalyzed oxidation	Glutamic semialdehyde, 2-pyrolidone, 4-,5 hydroxyproline, pyroglutamic acid	Stadman and Levine (2003)
Cysteine	Glutathiolation/crosslinking/ metal ion-catalysed oxidation	Disulfide, cysteic acid, sulfenic acid, sulfinic acid	Zhang et al. (2013), Shacter, (2000), Lund et al. (2011)
Threonine	Carbonylation/ metal ion-catalysed oxidation	2-amino-3-ketobutyric acid	Taborsky and McCollum (1973), Stadtman and Levine (2003)
Leucine	Hydroxylation	3,4,5-hydroxyleucine	Garrison (1987)
Histidine	Metal ion-catalysed oxidation	Asparagine, aspartic acid, 2-oxohistidine, 3-4-, 5-hydroxyleucine, 4-hydroxyl glutamate	Xiong (2000), Zhang et al. (2013)
Glutamic acid	-[1]	Pyruvate adducts, oxalic acid	Xiong (2000)
Methionine	Sulfoxidation	Methionine sulfoxide, methionine sulfone	Xiong (2000), Zhang et al. (2013)
Phenylalanine	Hydroxylation	2-, 3-, and 4-hyrophenylalanine, 2,3-dihydroxyphenylalanine	Zhang et al. (2013)
Tryptophan	Hydroxylation/nitration	2-,4-,5-,6- and 7-hydroxykynurenine, kynurenine and nitrotryptophan	Zhang et al. (2013), Xiong et al. (2010), Vossen et al. (2013a)
Tyrosine	Metal ion-catalysed oxidation/nitrosylation	3,4-dihydroxyphenylalanine, Tyr-Tyr crosslinks, 3-nitrotyrosine and Tyr-oxygen-Tyr	Xiong (2000), Zhang et al. (2013)
Valine	Hydroxylation	3-hydroxylvaline	Shacter (2000), Garrison (1987)

[1]Not indicated in the study

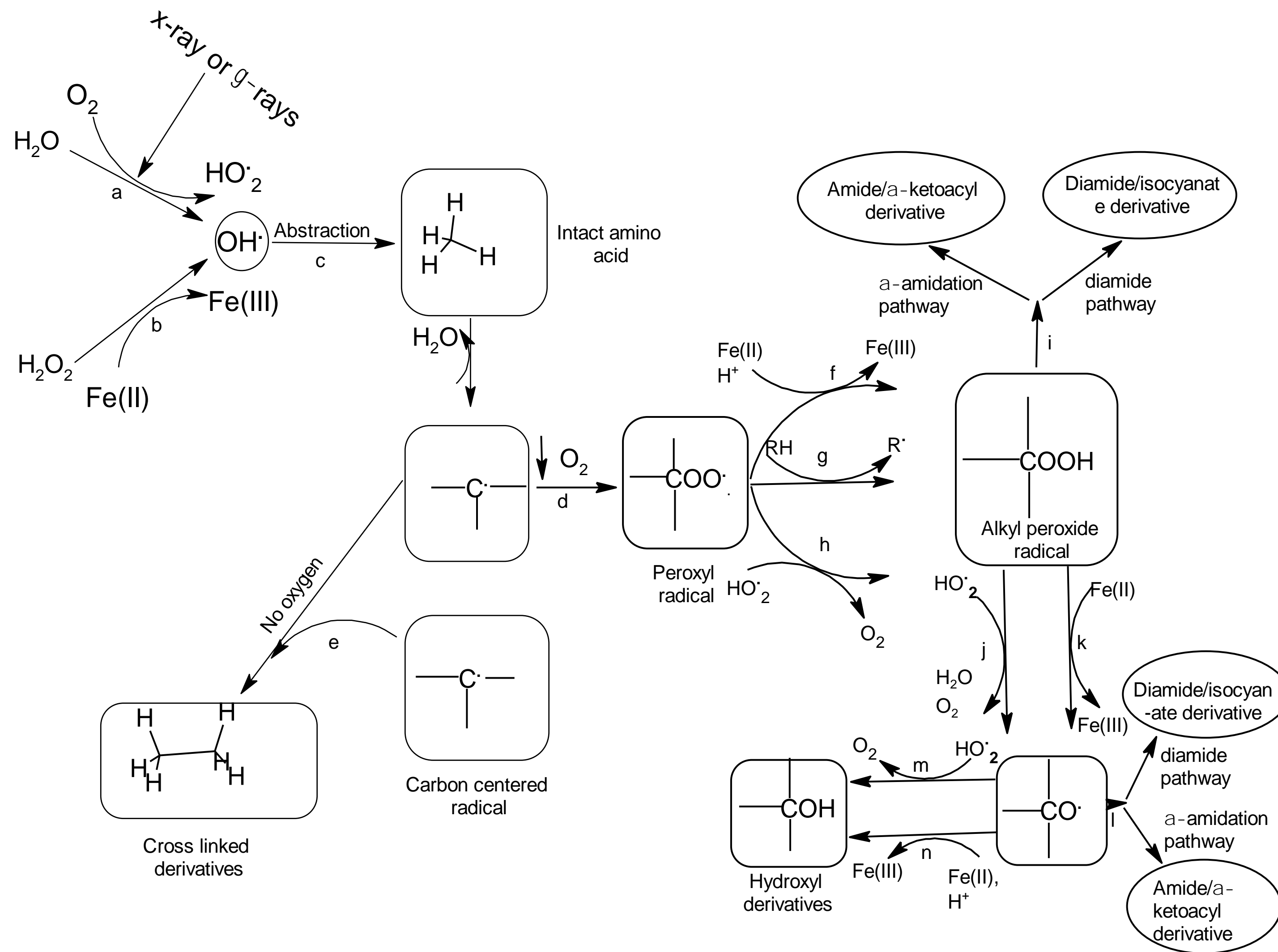


Figure 2.6: Mechanism of protein oxidation. *Adapted from Lund et al. (2011) and Stadtman & Levine (2003).*

Following the discovery of α-aminoadipic semialdehyde (AAS) and γ–glutamyl semialdehyde (GGS) as reliable and specific markers of PROTOX (Daneshvar, Frandsen, Autrupand, & Dragsted, 1997), the analysis of these compounds has been recently applied to meat samples (Estévez, 2011; Timm-Heinrich, Eymard, Baron, Nielsen, & Jacobsen, 2013). As reviewed by Estévez (2011), these compounds are typically formed from the reaction of ROS with alkaline amino acids, and involve a metal-catalysed oxidative deamination mechanism. Recently, AAS and GGS have also been proven to be formed in meat proteins as a result of a Maillard-type mechanism in the presence of reducing sugars (Villaverde & Estévez, 2013). This latter pathway may also involve an oxidative deamination mechanism induced by α-dicarbonyl compounds such as glyoxal and methyl-glyoxal (Akagawa, Sasaki, Kurota, & Suyama, 2005). Because these protein carbonyls are sensitive to acid hydrolysis, a derivatization step is typically done for stabilization. Akagawa et al. (2006) proposed the reductive amination of both semialdehydes in the presence of sodium cyanoborohydride ($NaCNBH_3$) and p-aminobenzoic acid (ABA), and this procedure ensures improved stability of the derivatized carbonyls against acid hydrolysis and even during long periods of cold storage (Figure 2.7 a & b). Once formed in a food matrix, protein carbonyls have been described as active components in additional reactions, which include further oxidation (e.g. AAS into α-aminoadipic acid; AAA), the formation of aldol condensation products and Schiff bases via condensation with secondary amines, and the implication in the formation of Strecker aldehydes, acting as an effective electrophilic agent (Estévez, 2011). The group of reactions implicated in the oxidative degradation of lysine has been recently identified as the carbonylation pathway, and some of the aforementioned products have been detected in protein isolates and muscle foods subjected to storage and processing (Utrera & Estévez, 2012; Utrera & Estévez, 2013a; Utrera & Estévez, 2013b; Utrera, Rodríguez-Carpena, Morcuende, & Estévez, 2012).

Protein bond cleavage is another manifestation of protein oxidation. Free radicals can react with the protein polypeptide backbone at a specific site resulting in its fragmentation. The alkoxyl radicals and the alkyl peroxide derivatives of protein can undergo cleavage either by α-amidation or diamide pathways (Stadtman & Levine, 2003) (Figure 2.6). Furthermore, oxidation of glutamyl and aspartyl residues in proteins can lead to peptide bond cleavage forming N-pyruvyl derivative. Uchida, Kato, and Kawakishi, (1990) have also shown that the oxidation of proline residues in proteins can lead to peptide bond cleavage from which a detectable 4-aminobutyric acid can be derived from 2-pyrrolidone, an immediate derivative of the oxidation process.

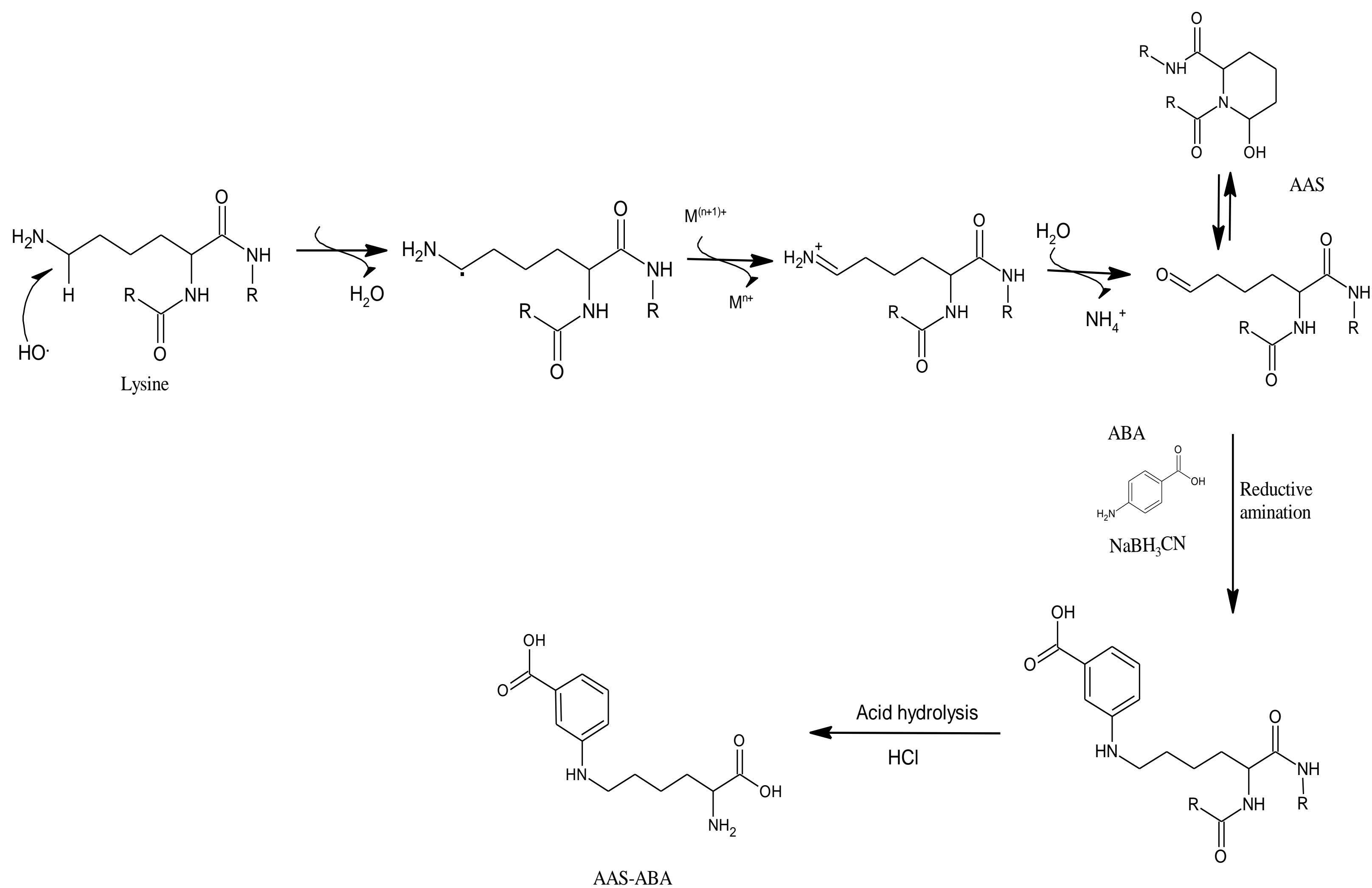


Figure 2.7: a) Lysine formation of AAS with their derivatization mechanisms. *Adapted from Estévez (2011).*

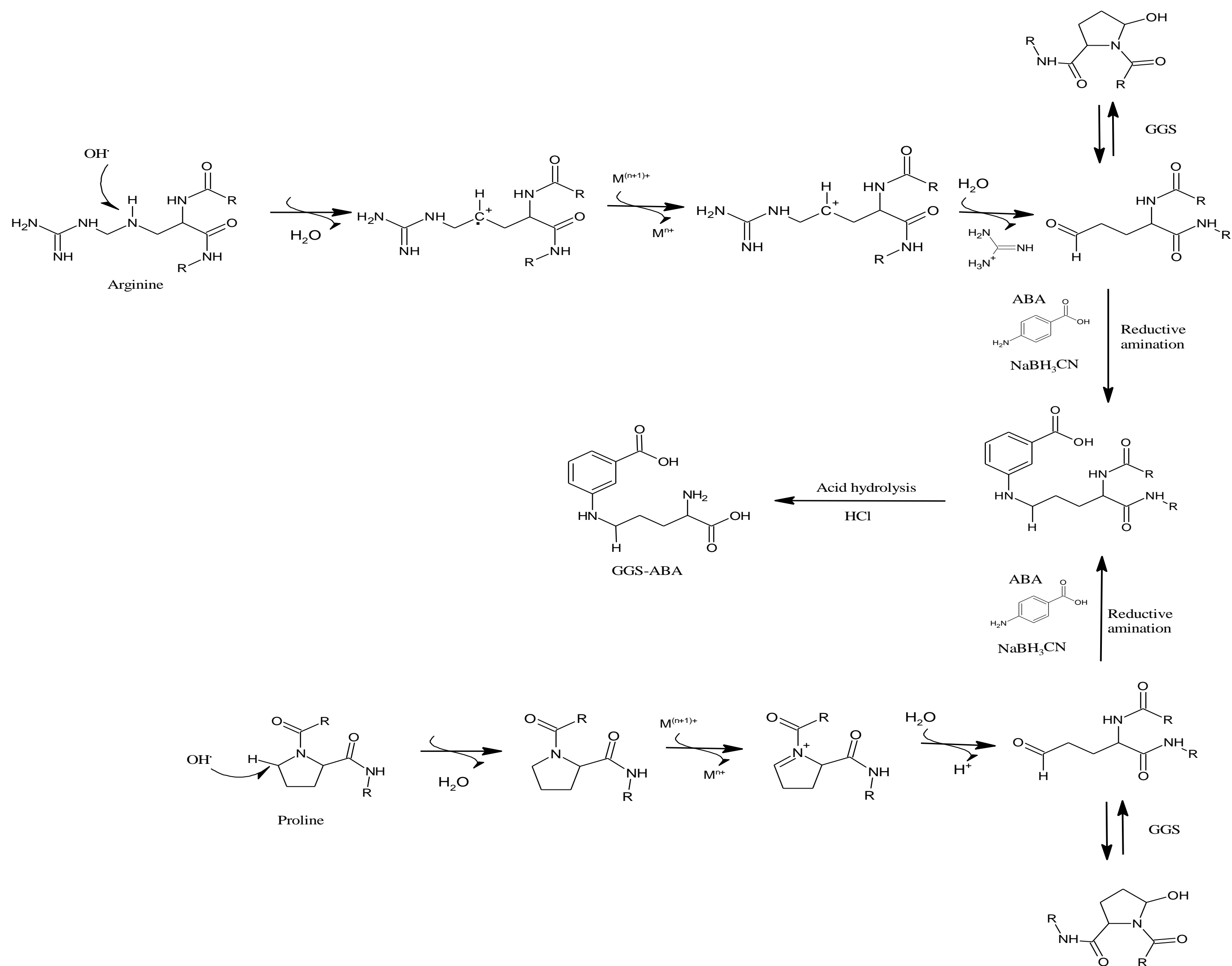


Figure 2.7: b) Arginine and proline formation of GGS with their derivatization mechanisms. *Adapted from Estévez (2011).*

However, most of these reactions have not been well identified in meat samples and the extent to which these mechanisms contribute to fragmentation of meat proteins remains unknown.

Another modification which can be detected as a marker of PROTOX is loss of sulfhydryl groups. Cysteine, like methionine, is a very sensitive amino acid to almost all ROS, and its loss in meat systems may be a reflection of a specific oxidative damage to meat proteins. Ellman reagent or 5'5-dithiobis (2-nitrobenzoate) (DTBN) is used as a derivatization agent to form a disulphide bond with free thiol groups and releases a thiolate ion (TNB dianion) with the assumption that the stoichiometry of protein thiol and TNB formed is in ratio 1 to 1. The TNB dianion is coloured and has a maximal absorbance at 412 nm and this is used to assess the concentration of thiols (Estévez, Morcuende, & Ventanas, 2008). Another protocol has been published with more sensitivity than Ellman's reagent and it is based on a fluorescent maleimide derivative, the ThioGlo-1 (Hawkins, Morgan, & Davies, 2009).

Cross-linking of proteins is another manifestation of protein oxidation and is widely assessed by determination of disulphide bonds, determination of dityrosine bonds and determination of cross-linked myosin heavy chain using SDS-PAGE electrophoresis (Estévez et al., 2008). The formation of disulphide bonds, in particular, has been linked to the action of hypervalent myoglobin, with this mechanism being different from the metal-catalysed mechanism involved in protein carbonylation (Lund et al., 2011). The analysis of tryptophan depletion by spectrofluorometry has also been explored as an expression of PROTOX in myofibrillar protein isolates, meat emulsions and processed muscle foods (Utrera et al., 2012). However, it seems that the detection of specific oxidation markers of tryptophan by food scientists has only been made in tryptophan solutions (Salminen, Estévez, Kivikari, & Heinonen, 2006), while the formation of kynurenine derivatives in muscle foods remains unknown. Although many of these protein oxidation markers and methods have been applied in many fresh and processed meats (Table 2.2), no reference could be located for bacon, a widely-consumed product in the world and especially, North America.

2.4.4.2 Effect of cooking/heat treatment on protein oxidation

Several cooking methods, including steaming, microwave cooking, smoking, grilling and frying, may be employed in meat processing and household meat preparation. In the case of bacon, as previously mentioned, microwave and frying are the consumers' most frequent choice.

Table 2.2: Protein oxidation products in some processed meats.

Processed meat	Processing technology	Oxidation products	Techniques for assessment	References
Beef heart surimi	Mincing and washing	Total carbonyl	2,4, dinitrophenl hydrazine (DNPH)	Srinivasan et al. (1996)
Beef meat	Maturation	Total carbonyl; reduced thiol groups	DNPH; 2,2'dithiobis (5-nitropyridine), DTNP	Martinaud et al. (1997)
Broiler breast meat	Irradiation	Total carbonyl	DNPH method	Rababah et al. (2004)
Beef sausage	Irradiation	Total carbonyl	DNPH method	Badr and Mahmoud (2011)
Pork meat	Nitration	Reduced thiol group; total carbonyl	5,5'-dithiobis (2-nitrobenzoic acid); DNPH method	Van Hecke et al. (2013), Vossen et al. (2013b)
Chicken thigh meat	Irradiation	Total carbonyl	DNPH method	Xiao et al. (2013), Xiao et al. (2011)
Dry cured ham	Dry curing	Total carbonyl	DNPH method	Armenteros et al. (2009), Ventanas et al. (2007)
Dry cured loins	Dry curing	Total carbonyl; specific carbonyl (AAS and GGS)	DNPH method; fluorescence spectroscopy; LC-ESI-MS (Liquid chromatography-electrospray ionization mass)	Armenteros et al. (2009), Ventanas et al. (2006)
Beef meat	Steam cooking, refrigeration storage	Free thiol; aromatic amino acid; total carbonyl; Schiff bases	DTNP method; U.V. spectroscopy; DNPH; fluorescent spectroscopy	Gatellier et al. (2010)
Pork meat	Cooking (boiling)	Total carbonyl; Schiff base; protein aggregate	DNPH method; fluorescent spectroscopy; Raleigh light scattering	Traore et al. (2012)
Beef meat	Cooking (boiling)	Total carbonyl; free thiol group; protein aggregation	DNPH method; Ellaman's method; fluorescent spectroscopy	Santé-Lhoutellier et al. (2008)
Pork muscle	Mincing, cooking, ageing	Total carbonyl; protein aggregation	DNPH method; granulometry measurement	Bax et al. (2012)

Table 2.2 cont'd: Protein oxidation products in some processed meats.

Processed meat	Processing technology	Oxidation products	Techniques for assessment	References
Pork meat patties	Processing	Total carbonyl	DNPH method	Vuorela et al. (2005)
Cooked pork burger patties	Processing, Chilled storage	Total and specific carbonyl (AAS, GGS)	DNPH method and LC-ESI-MS	Ganhão et al. (2010)a,b
Cooked pork burger patties	Processing	Total carbonyl	DNPH method	Salminen et al. (2006)
Dry cured ham	High hydrostatic pressure	Specific carbonyl	LC-ESI-MS	Fuentes et al. (2010)
Dry cured ham	High hydrostatic pressure	Total carbonyl	DNPH method	Cava et al. (2009)
Cantonese sausage	Sausage processing	Loss of tryptophan; total carbonyl; protein aggregation; S-H group		Sun et al. (2011)

Although these may affect PROTOX differently and require continued research, temperature levels and duration of heat treatment have been the major focus in the literature for all the assessed meat products. Cooking has been noted to trigger the generation of ROS, which in turn can increase the tendency for PROTOX (Traore et al., 2012). The antioxidant properties of meat, such as glutathione and catalase activities, have also been observed to drop drastically with heat treatment reducing the natural tendency of meat to resist PROTOX (Hoac et al., 2006; Mei, Crum, & Decker, 1994). Because lipid oxidation produces free radicals (such as alkyl, alcoxyl and peroxyl radicals) which have been observed to initiate PROTOX (Lund et al., 2011), these are proposed to contribute to increased PROTOX during cooking. Heating above 60ºC can trigger oxidative cleavage of the porphyrin ring, resulting in release of heme iron which can lead to increased lipid and PROTOX (Miller, Gomez-Basauri, Smith, Kanner, & Miller, 1994). Although some authors have not observed a direct link between lipid and protein oxidation (Gatellier et al., 2010; Haak et al., 2006; Haak, Raes, Van Dyck, & De Smet, 2008), available evidence suggests a strong link between these two processes.

Various expressions of PROTOX observed during meat cooking include increased surface hydrophobicity and aggregation of meat protein (Chelh, Gatellier, & Santé-Lhoutellier, 2006; Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008), loss of thiol and aromatic groups (Gatellier et al., 2010; Santé-Lhoutellier et al., 2008), and increased carbonylation, carboxylation and Schiff base formation (Gatellier et al., 2010; Promeyrat et al., 2011; Traore et al., 2012). A depletion of heme iron from 10 to 100% has been reported to occur during cooking, with a corresponding increase in non-heme iron (Garcia et al., 1996; Purchas, Busboom, & Wilkinson, 2006). These increased non-heme iron ions are crucial for PROTOX initiation through the Fenton/Fenton-like or the metal catalysed oxidation (MCO) reaction in meat systems (Estévez, 2011). The extent of these effects may be determined by the temperature level and the duration of the treatment (Astruc, Marinova, Labas, Gatellier, & Santé-Lhoutellier, 2007; Gatellier et al., 2010; Traore et al., 2012). Yet, the appropriate temperature and time combination that will ensure safe food in terms of microbial quality and PROTOX development has not been ascertained. Promeyrat et al. (2011) observed increased carbonyl content with heat treatment at 90°C, but not at 45°C, in a myofibril model system without pro-oxidant factors. Similarly, higher amounts of carbonyls were produced at higher time-temperature combinations (120 to 300 s and 123 to 207°C, but not at lower temperature and time) in the study of Gatellier et al. (2010), who also reported greater detrimental effects on protein quality. Time-temperature effects of heating may have to do with a greater impact on the antioxidant defence mechanism coupled with greater release of iron (and free radical production) due to higher protein denaturation in the meat system, resulting in more PROTOX at higher cooking temperature and extended times. Therefore, it needs to be proven if there exists any variation in the level of PROTOX products in meat subjected to different cooking methods, and whether this variation is based solely on the level of temperature and length of time meat is exposed or if other mechanisms of contribute.

2.4.4.3 Effects of protein oxidation on nutritional value of meat and human health

The quality of protein can be described by its ability to achieve certain metabolic functions, which is largely dependent on its constituent amino acids, its peptide sequence (primary structure), the spatial arrangement in its native structure (secondary and tertiary structures), its concentration and its bioavailability (Evenepoel et al., 1998; Xiong, 2000). Recently, the understanding of the biological functions of proteins has expanded beyond mere maintenance of protein body mass into

a wider concept of regulation of body composition and health, cell signalling, glucose homeostasis, gastrointestinal functions and bacterial flora, among others. This new concept, therefore, widens the definition of what protein quality should be (Millward, Layman, Tomé, & Schaafsma, 2008). In order for proteins to fulfil their required activities in the human body, it is important they are in a native chemical and structural state.

The impaired structural and conformational stability caused by oxidative damage to proteins *in vivo* may result in protein-protein interactions (i.e., polymerization/formation of protein aggregate) or scission of the peptide sequence, as well as modification of the amino acid side chains (Stadtman & Levine, 2003; Xiong, 2000). These oxidative modifications are believed to alter protein structure and function, thereby leading to “protein conformational diseases” (Berlett & Stadtman, 1997). The role played by PROTOX *in vivo* on aging and a number of health disorders has been extensively documented (Berlett & Stadtman, 1997). Dalle-Donne, Giustarini, Colombo, Rossi, and Milzani (2003) reviewed the list of diseases associated with carbonylated proteins, and included, among many others, Alzheimer’s disease, chronic renal failure and diabetes. The oxidative damage to proteins is commonly regarded as primary cause of the pathogenesis, whereas the accumulation of protein carbonyls and other PROTOX products are used as markers of the occurrence and extent of the pathological process (Dalle-Donne et al., 2003; Stadtman & Levine, 2003).

The underlying mechanisms by which PROTOX is involved in the aetiology of age-related diseases include physico-chemical modifications leading to impaired functionality and resistance of oxidized proteins to proteolysis (Stadtman & Levine, 2000). Similar mechanisms have been proposed by food scientists to explain the impact of PROTOX on the technological properties of muscle proteins and the overall quality of processed muscle foods (Estévez, 2011; Lund et al., 2011; Zhang et al., 2013). Among the most relevant chemical modifications, protein carbonylation and the formation of protein cross-links have been recurrently identified as the most influential on the loss of muscle protein functionality (Estévez, 2011; Utrera & Estévez, 2012; Xiong, 2000) and the modification of the colour, texture and flavour of meat and processed meat products (Estévez, Ventanas, & Cava, 2005; Ganhão, Morcuende, & Estévez, 2010a; Lund, Hviid, & Skibsted, 2007; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004). Even though it has been proven that PROTOX occurs during handling, processing and storage of muscle foods leading to the accumulation of PROTOX products, the impact of the intake of such dietary oxidized proteins on

nutrition and health is mostly unknown. However, the extent of meat PROTOX with respect to its implication on loss of essential amino acids, reduced digestibility and reduced bioavailability has been proposed based on recent advances in analytical methods. In the following sections, the facts, hypothesis and future perspectives on the influence of muscle food PROTOX on human nutrition and health will be discussed.

2.4.4.3.1 Loss of essential amino acids

Many early studies observed modification of essential amino acids in various food products for animals and humans, resulting in reduced bioavailability of these essential nutrients (Anderson, Ashley, & Jones, 1976; Anderson, Li, Jones, & Bender, 1975; Cuq, Besancon, Chartier, & Cheftel, 1978). Although those studies emphasized the oxidation of sulphur-containing amino acids (methionine, cysteine), the consequences of the oxidative modification on bioavailability is most likely applicable to other amino acids and food systems, because most of the amino acyl side chains are also susceptible to ROS. Protein carbonylation, for instance, results in irreversible modification of the essential amino acids such as lysine, threonine and arginine (Estévez, 2011). Many other expressions of the oxidative damage to amino acid side chains, including hydroxylation of aromatic groups and aliphatic amino acids, nitration of aromatic amino acid residues, nitrosylation of sulfhydryl groups, sulphoxidation of methionine residues, chlorination of aromatic groups and primary amino groups (Stadtman & Levine, 2003), lead to severe chemical changes that may seriously compromise the availability and metabolic activities of the affected amino acids, signifying a reduced nutritional quality of the protein. Park and Xiong (2007) demonstrated a reduction in quantity and quality of essential amino acids in porcine myofibrillar protein isolate following exposure to different oxidizing environments. Ganhão et al. (2010a), Utrera et al. (2012) and Villaverde, Ventanas, and Estévez (2014) reported losses of tryptophan ranging from 30 to 80% during processing of porcine patties and fermented sausages. A depletion of tryptophan up to 80% of the initial concentration was also found in beef subjected to 20 weeks frozen storage (Utrera, Morcuende, & Estévez, 2014a, 2014b). Hęś, Waszkowiak, and Szymandera-Buszka, (2012) observed a loss of lysine and methionine in meatballs subjected to severe oxidative stress. However, to what extent oxidized amino acids are available and useful to humans has been largely unexplored and requires additional research for a more comprehensive assessment of the implication of PROTOX on protein quality of meat. Even though the impact of food PROTOX on

the nutritional value of dietary proteins remains uncertain, this issue may be of particular importance in developing countries where the undernutrition, including insufficient consumption of protein, remains a persistent problem (Schönfeldt & Hall, 2012). Preserving minority and essential amino acids against oxidative modifications in unbalanced low-protein diets should be a primary research area in the future.

2.4.4.3.2 Reduced digestibility

The efficiency of protein digestibility is believed to be a measure of its nutritional value and overall quality (FAO, 2013). Controversy exists about the effects of PROTOX modifications on the susceptibility of meat proteins to proteolytic enzymes and, hence, on their digestibility. However, it seems reasonable to consider that these properties are dependent on the specific conditions under which the proteins are modified oxidatively, as well as the conditions under which the proteins are digested (Xiong, 2000). Biomedical research has observed increased proteolytic susceptibility of protein with oxidative modification in living tissues due to enhanced protein unfolding and corresponding increased accessibility of peptide bonds to proteolytic enzymes (Davies, 2001; Grune, Jung, Merker, & Davies, 2004; Taylor & Davies, 1987). On the other hand, some authors have reported a reduced protein susceptibility to enzymatic proteolysis as a result of increased intermolecular cross-links and formation of aggregates with oxidative modifications (Dizdaroglu, Gajewski, & Simic, 1984). Relatively mild oxidative conditions will yield oxidized protein of higher susceptibility to protease complex, whereas severe protein oxidation will yield otherwise (Davies, 2001; Grune et al., 2004; Xiong, 2000). A mild oxidation will induce slight modifications and partial unfolding of the protein structure enhancing its protease susceptibility, whereas a high oxidative environment will proceed from mere protein unfolding to crosslinking and massive aggregation, as well as modification of protease active sites in protein, all of which result in decreased proteolytic susceptibility and reduced digestibility of meat (Estévez, 2011). Sante-Lhoutellier et al. (2007) reported that low intensity of PROTOX may enhance or not affect protein susceptibility to digestive enzymes, whereas high intensity PROTOX may reduce protein susceptibility to gut proteases. Although less work has been carried out with respect to muscle foods, there is evidence suggesting a decreased susceptibility of protein to digestive enzymes following oxidation (Kamin-Belsky, Brillon, Arav, & Shaklai, 1996; Liu & Xiong, 2000; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006; Sante-Lhoutellier et al., 2007).

This may be due to more insoluble proteins (mostly myofibrillar proteins and its high proportion in meat) employed in meat experiments compared to soluble proteins used in biomedical research (Sante-Lhoutellier et al., 2007). In a semi-automated system designed to mimic gut digestion, Gatellier & Santé-Lhoutellier (2009) reported a reduced myofibrillar digestibility with cooking and attributed this effect to PROTOX-induced changes that occurred during the cooking process. Chen, Zhao, and Sun (2013) recently found that soy protein isolates oxidized *in vitro* by 2,2′-azobis (2-amidinopropane) dihydrochloride underwent a significant loss in the amount of most amino acids, accompanied by decreased susceptibility to proteolysis.

Results from *in vitro* studies may differ from those carried out in animal model systems and these in turn, may not efficiently reflect the occurrence and impact of the intake of oxidized food proteins on the digestibility and nutritional value of muscle foods in humans. Filgueras, Gatellier, Zambiazi, and Santé-Lhoutell (2011) reported that cooking decreased the myofibrillar protein susceptibility to pepsin activity while the proteolysis rate by pancreatic enzymes increased. Bax et al. (2012) reported that cooking meat at temperature above 100 °C for up to 30min, oxidation-related protein aggregation slowed pepsin digestion but improved meat protein overall digestibility in an *in vitro* system. Working with mini-pigs, the same authors (Bax et al., 2013) found out that cooking temperature can modulate the speed of meat protein digestion, without affecting the efficiency of the small intestinal digestion. Rutherfurd, Montoya, and Moughan (2014) reported that PROTOX affected protein digestion in the gastrointestinal tract by i) denaturation of the protein and ii) formation of indigestible peptides. According to these authors the overall effect of oxidation on digestibility as a whole would be the result of the balance of these individual effects.

2.4.4.3.3 Reduced bioavailability

The bioavailability of an amino acid has been defined as the proportion of ingested dietary amino acids that is absorbed in a chemical form suitable for it to be utilized for protein synthesis or metabolism (Boye, Wijesinha-Bettoni, & Burlingame, 2012). If protein digestibility in meat is negatively affected by PROTOX, the bioavailability of the corresponding amino acids to humans may also be compromised. In addition, some modifications that ensue during PROTOX may make some amino acids not adequately available for absorption and protein synthesis. Although this issue has been scarcely covered in the literature, scientific evidence suggests particular oxidation-

driven protein modifications will influence protein and amino acid bioavailability and functionality (Park, Xiong, Alderton, & Ooizumi, 2006). Because particular oxidized amino acids, such as AAS and GGS, are used as reliable markers of oxidative stress and disease in humans (Daneshvar et al., 1997), the intake and potential absorption of such oxidation products from oxidized muscle foods would not reasonably contribute in a positive manner to metabolic functions. Similarly, oxidized forms of sulphur-containing amino acids have been found to display reduced or limited nutritional availability regardless of the true ileal digestibility of proteins (Rutherfurd & Moughan, 2012). Millward et al. (2008) and Boye et al. (2012) have pointed out that processing methods can influence many changes in protein quality, including formation of Maillard compounds, oxidized sulphur amino acids, and cross-linked peptide chain, among others, all of which can limit protein bioavailability. Meat processing and storage has been recently linked to reduced availability of essential amino acids (lysine and methionine) as well as impaired protein digestibility (Hęś et al., 2012).

2.4.4.3.4 Increased cytotoxicity and mutagenicity

In addition to the reduction in nutritional quality and bioavailability of oxidized proteins, it remains to be fully explored whether the consumption of these modified proteins involves an increased risk of developing certain disease conditions. The oxidation of food components during processing/storage and the impact of such oxidation products on particular health issues is an increasing concern among consumers (EFSA, 2010). Studies have shown that consumption of oxidized components in the diet increases oxidation markers in blood and muscle of animals (Engberg, Lauridsen, Jensen, & Jakobsen, 1996; Jensen, Engberg, Jakobsen, Skibsted, & Bertelsen, 1997; Lin et al., 1989; Zhang, Xiao, Lee, & Ahn, 2011). According to these studies, the consumption of oxidized food components will trigger increased oxidative stress in living tissues and that, in turn, could contribute to some disease conditions, either over the long term or the short term. The role played by oxidized dietary molecules in disease pathogenesis is usually linked to the cytotoxicity and mutagenicity potential of these species on the gastrointestinal tract or on internal organs upon absorption (Esterbauer, 1993). Consistently, the effectiveness of dietary antioxidant strategies (i.e. intake of polyphenols) in the reduction of pathological processes reflects the role played by oxidative stress in such disorders and the impact of diet on the *in vivo* oxidation events (Haliwell, 1994; Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005). Although most of

the available studies have focused on the consumption of oxidized lipids (Esterbauer, 1993), recent studies have also highlighted the potential influence of dietary oxidized proteins on particular health risks. Several decades ago, a pioneering study carried out by Youngman, Park, and Ames (1992) reported that protein restriction reduced the accumulation of oxidatively damaged proteins related to aging in rats. Consistent results have been recently reported by Souza et al. (2007), who found increased protein consumption led to increased PROTOX in the frontal cortex with consequences on the anxiety-like behaviour of rats. Unfortunately, the aforementioned studies did not consider the extent of PROTOX in the dietary proteins as a variable under study.

The proven connection, however, between dietary oxidized lipids and PROTOX (Zhang et al., 2011) suggests that not only the quantity but also the quality of food proteins in terms of oxidative damage, may also play a role on oxidative stress and disease *in vivo*. Evenepoel et al. (1998) pointed out that malabsorbed proteins (such as oxidized proteins with reduced digestibility) may be retained in the colon and exposed to bacterial fermentation leading to production of certain metabolites (e.g., phenols and p-cresol) which are mutagenic and could increase the risk of colonic cancer and ulcerative colitis. Under the conditions of the experiments carried out by Bax et al. (2013) in mini-pigs, this effect was not observed as cooking temperature (closely related to the extent of PROTOX) modulated the speed of meat protein digestion, without affecting the efficiency of the small intestinal digestion; consequently, the entry of undigested meat protein residues into the colon was negligible. In the latter study, however, the extent of PROTOX in the dietary proteins was not provided. The implication of oxidized proteins and amino acids in disorders involving cytotoxicity and neurotoxicity is well-documented (Sayre, Perry, & Smith, 2007). Oxidative stress is typically viewed as cytotoxic; yet, the mechanisms that underlie this toxicity are just beginning to be explored in medical research. Hande et al. (2006) reported that hydroxyl radicals can convert L-phenylalanine into m-tyrosine, which has been found to be toxic to cultured cells. The same unnatural isomer of L-tyrosine (m-tyrosine) has also been found to be incorporated into cellular protein, and this misincorporation is toxic to the cell as it can present some pathogenesis for certain disease conditions as well as impair the normal function of such proteins. Because oxidized amino acids may not be further metabolized into other products *in vivo* (Hande et al., 2006; Rodgers, Wang, Fu, & Dean, 2002), it is plausible to hypothesise that certain PROTOX products may be absorbed as ingested and also incorporated into proteins, such as enzymes and structural elements in cells during synthesis, leading to malfunction and disease

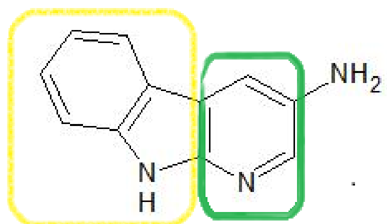
(Estévez & Luna, 2016). In this regard, lysine PROTOX products found in processed muscle foods, such as AAS and AAA, are used as reliable markers of disease in humans (Sell, Strauch, Shen, & Monnier, 2007). Recent findings by Wang et al. (2013) confirmed the absorption and negative biological effects of the lysine oxidation product, amino adipic acid (AAA), which was found as a potential modulator of glucose homeostasis in humans leading to increased risk of diabetes. Thus, AAS and AAA, found in considerable quantities in diverse muscle foods (Estévez, 2011; Utrera & Estévez, 2013b; Utrera et al., 2012), may not only serve as *in vivo* oxidative stress markers, aging and particular diseases, they may also be directly implicated in the pathogenesis of serious physiological disorders upon ingestion. In relation to the cytotoxic effect of oxidized dietary proteins, Li, Wu, Le, and Shi (2013) showed that the intake of oxidized casein caused redox stress in both blood and digestive organs of mice after short-term gavage. In a further study, the same authors reported that dietary oxidized casein induced hepatic and renal injury in mice via impairment of the antioxidant defence system and modification of the expression of fibrosis-related genes (Li, Mo, Le, & Shi, 2014). Along with these adverse biological effects and the visual confirmation of the fibrosis, the authors found increased protein carbonylation and dityrosine, and formation of advanced protein oxidation products in the injured tissues of animals fed with the oxidized protein. While similar mechanisms have not been described for oxidized muscle proteins, it is plausible to consider that oxidized food proteins may have comparable effects.

2.4.5 Heterocyclic aromatic amines content in processed meat

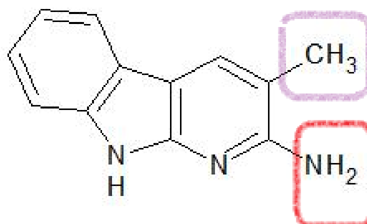
Heterocyclic aromatic amines (HAA) are other potential carcinogens from meat products. Most HAA are formed during the cooking of meat at normal cooking temperature (150 to 300ºC) by condensation of creatinine (a breakdown product of creatine in muscle) with amino acids and monosaccharides (Johansson & Jägerstad, 1994; Jägerstad, Skog, Arvidsson, & Solyakov, 1998). Although there are many HAA in complex mixture in many food systems, individual HAA have their own carcinogenicity; however, the risk of individual consumption of HAA may be difficult to assess due to their possible interactions and coexistence in the human diet. Two groups of HAA have been identified; the IQ type or aminoimidazoazerene HAA and the non-IQ type or aminocarboline HAA groups (Jägerstad et al., 1998; Kizil et al., 2011). According to Gibis (2009), these two groups also correspond to the polar and non-polar HAA and are largely produced under different conditions during cooking regimen. The former group is formed by heat induced non-

enzymatic browning (Maillard reaction) during conventional cooking temperature between 150 and 300°C, with the reaction of creatine or creatinine, amino acid and hexoses (Iwasaki et al., 2010), whereas the non-IQ type are formed by the pyrolytic reaction between amino acids and proteins at higher temperature usually above 300°C (Kizil et al., 2011). The IQ type HAA are usually recognised by the presence of 2-amino-imidazo group in their structure, with a methyl group attached to one of the nitrogen in the imidazole ring. This part of the structure, generally believed to originate from creatine, is linked with either quinoline, quinoxaline or a pyridine moiety (Jägerstad et al., 1998) (Figure 2.8). The 2-aminoimidazo group, as well as the number and position of the methyl group, has been reported to be very essential to HAA mutagenicity/genotoxicity. On the other hand, the non-IQ type group contains an exocyclic amino group and sometimes exocyclic methyl group in their structure which are attached to a pyridine ring that is linked either to an indole or an imidazole moiety (Jägerstad et al., 1998) (Figure 2.8).

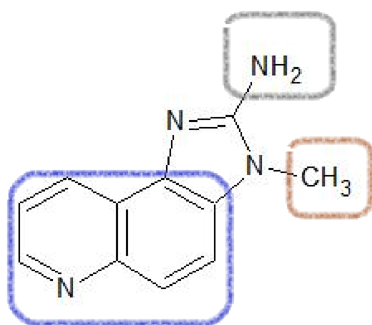
Although up to about 25 different HAA have been identified in different food components (Gibis, 2016) (Table 2.3), the four most researched and characterized have been classified by IARC as either "*probably carcinogenic to human*" (class 2A), which include 2-amino-3-methylimidazo [4,5- *f*] quinoline (IQ), or "*possibly carcinogenic to human*" (class 2B), which include 2-amino-3,4-dimethyl-imidazo[4, 5- *f*] quinoline (MeIQ), 2-amino- 3,8-dimethyl imidazo [4, 5- *f*]quinoxaline (MeIQx) and 2-amino-1- methyl-6-phenylimidazo[4, 5 –*b*] pyridine (PhIP) based on available evidence from epidemiological data and animal studies (IARC, 1993). A similar classification has been conducted by the National Toxicological Program of the United State Department of Health and Human Services, where all these four HAA were classified as "*reasonably anticipated to be human carcinogen"* (National Toxicology Program, 2011). Moreover, several epidemiological studies showed the relationship between fried meat products and risk of colon and other cancers (Schiffman & Felton, 1990). As the dietary intake of HAA has been reported to be a function of cooking method, doneness preference and consumption frequency (Kizil et al., 2011), all these factors come to play in bacon processing and consumption; hence, examining cooking methods in the production of HAA is imperative for consumers` safety and quality assurance.



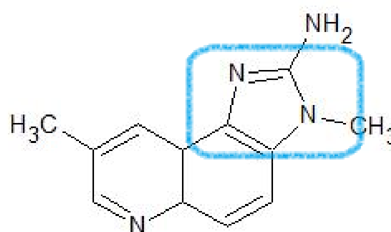
2-Amino-*9H*-pyrido [2,3-*b]*
indol (AαC)



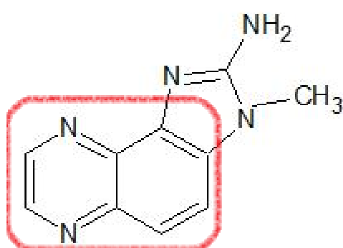
2-Amino-3 methyl-*9H*-pyrido
[2,3-*b]* indol (MeAαC)



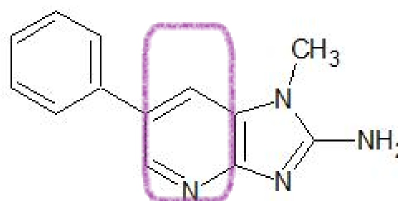
2-Amino-3-methylimidazo
[4,5-*f*] quinoline (IQ)



2-Amino-3,4-dimethylimidazo
[4,5-*f*] quinoline (MeIQ)



2-Amino-3-methylimidazo
[4,5-*f*] quinoxaline (IQx)



2-Amino-1-methyl-6-phenyl-
imidazo [4,5-*b*] pyridine (PhIP)

Figure 2.8: Types of heterocyclic aromatic amines with the general groups that characterise each type. Indole (yellow) and pyridine (green) highlighted in AαC. Exocyclic methyl (purple) and exocyclic amino group (red) highlighted in MeAαC. Quinoline (blue) highlighted in IQ. 2-Aminoimidazo group (sky blue) highlighted in MeIQ. Quinoxaline (red) highlighted in IQx and pyridine (purple) highlighted in PhIP.

Table 2.3: Selected heterocyclic aromatic amines and their properties[1]

Heterocyclic aromatic amine	Abbreviation	Polarity/molecular weight
IQ type (2-Amino-imidazoquinoline)		
2-Amino-3-methylimidazo [4,5-*f*] quinoline	IQ	Polar (192.2 g/mol)
2-Amino-3,4-dimethylimidazo [4,5-*f*] quinoline	MeIQ	Polar (212.3 g/mol)
2-Amino-1-methylimidazo [4,5-*f*] quinoline	Iso-IQ	Polar (192.2 g/mol)
IQ type (2-Amino-imidazoquinoxaline)		
2-Amino-3-methylimidazo [4,5-*f*] quinoxaline	IQx	Polar (199.3 g/mol)
2-Amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline	8-MeIQx	Polar (213.3 g/mol)
2-Amino-3,4-dimethylimidazo [4,5-*f*] quinoxaline	4-MeIQx	Polar (213.3 g/mol)
2-Amino-3,7,8-trimethylimidazo [4,5-*f*] quinoxaline	7,8-DiMeIQx	Polar (227.3 g/mol)
2-Amino-3,4,8-trimethylimidazo [4,5-*f*] quinoxaline	4,8-DiMeIQx	Polar (227.3 g/mol)
2-Amino-3,4,7,8-tetramethylimidazo [4,5-*f*] quinoxaline	TriMeIQx	Polar (241.3 g/mol)

Table 2.3 cont'd: Selected heterocyclic aromatic amines and their properties[1]

Heterocyclic aromatic amine	Abbreviation	Polarity/molecular weight
IQ type (2-Amino-imidazoquinoxaline)		
2-Amino-4-hydroxymethyl-3,8-dimethylimidazo [4,5-*f*] quinoxaline	4-CH_2OH-8-MeIQx	Polar (243.3 g/mol)
2-Amino-1,7-dimethyl-1H-imidazo [4,5-*g*] quinoxaline	7-MeIgQx	Polar (213.2 g/mol)
2-Amino-1,7,9-trimethyl-1H-imidazo [4,5-*g*] quinoxaline	7,9-MeIgQx	Polar (227.3 g/mol)
IQ-type (2-Amino-imidazopyridines)		
2-Amino-1-methyl-6-phenyl-imidazo [4,5-*b*] pyridine	PhIP	Polar (224.3 g/mol)
2-Amino-1-methyl-6-4′hydroxyphenyl-imidazo [4,5-*b*] pyridine	4′OH-PhIP	Polar (240.6 g/mol)
2-Amino-1,6-dimethylphenyl-imidazo [4,5-*b*] pyridine	DMIP	Polar (162.2 g/mol)
2-Amino-1,5,6-trimethylimidazo [4,5-*b*] pyridine	1,5,6 TMIP	Polar (176.2 g/mol)
2-Amino-3,5,6-trimethylimidazo [4,5-*b*] pyridine	3,5,6 TMIP	Polar (176.2 g/mol)
2-Amino-1,6-dimethyl-furo[3,2-e]-imidazo [4,5-*b*] pyridine	IFP	Polar (202.3 g/mol)

Table 2.3 cont'd: Selected heterocyclic aromatic amines and their properties[1]

Heterocyclic aromatic amine	**Abbreviation**	**Polarity/molecular weight**
Non-IQ type (α- ,β- ,δ-carboline)		
2-Amino-*9H*-pyrido [2,3-*b]* indol	AαC	Non-polar (183.2 g/mol)
2-Amino-3 methyl-*9H*-pyrido [2,3-*b]* indol	MeAαC	Non-polar (197.2 g/mol)
1-methyl-*9H*-pyrido [4,3-*b]* indole	Harman	Non-polar (182.2 g/mol)
9H-pyrido [4,3-*b]* indole	Norharman	Non-polar (168.2 g/mol)
3-Amino-1,4-dimethyl-5*H*-pyrido [4,3-*b]* indole	Trp-P-1	Non-polar (211.3 g/mol)
3-Amino-1-dimethyl-5*H*-pyrido [4,3-*b]* indole	Trp-P-2	Non-polar (197.2 g/mol)
2-Amino-6-dimethyldipyrido [1,2-a:3′2′-*d]* imidazole	Glu-P-1	Non-polar (198.3 g/mol)
2-Amino-didipyrido [1,2-a:3′2′-*d]* imidazole	Glu-P-2	Non-polar (184.3 g/mol)

[1]*Tables compiled from Gibis (2016) and Jägerstad et al. (1998).*

2.4.5.1 Physical and chemical factors crucial in heterocyclic amine formation in meat

Apart from the composition of precursors (creatine/creatinine, amino acid and reducing sugar) (Pais, Salmon, Knize, & Felton, 1999), which may vary among meat from different animal species and muscle parts, other physical or chemical variables may equally influence the production of HAA in meat products. Although the effect may vary depending on cooking method, heating temperature and time are among the most important factors that influence the formation of HAA in meat products. Accordingly, no HAA was reported with gentle cooking methods (e.g. stewing, steaming and boiling) which are usually carried out below 120°C (Gibis, 2016; Joshi et al., 2015).

Several studies have reported consistent increases in IQ, MeIQ, MeIQx and 4, 8-DiMeIQx with increasing temperature and time in a ground beef (Ahn & Grün, 2005) and meat flavour model system (Bordas, Moyano, Puignou, & Galceran, 2004). Other studies have shown that increased cook loss may lead to increased HAA formation in cooked meat (Smith, Ameri, and Gadgil 2008; Persson, Sjöholm, and Skog 2003) and this trend has been implicated on the increased transport of water-soluble precursors to the meat surface where the reactions occur. As a result, PSE meat has been reported with higher HAA than normal meat due to poor water-holding capacity (Polak, Došler, Žlender, & Gašperlin, 2009). In fact, attempts to reduce cook loss in meats by the inclusion of various carbohydrates and fibres to beef patties (Persson, Sjöholm, & Skog 2003; Shin, Park, & Park 2003), or marination/enhancement of meat with solution of salt and phosphate (Smith, 2010) have been shown to result in lower formation of HAA. Direct conductive heating system (e.g. pan frying due to higher heat transfer coefficient) will also result in higher HAA than indirect convective or radiation heating (e.g. microwave cooking) (Gibis, 2016). The presence of fat in meat can either result in increased HAA, partly because fat has a low specific heat capacity (Picouet et al., 2007) and hence, results in faster increase in cooking temperature, or it can lead to reduced HAA by diluting the inherent precursors (Knize et al., 1985). More importantly, oxidized fats will result in radicals which enhance HAA formation (Johansson & Jägerstad, 1993). Hence, more susceptible unsaturated fatty acids may result in more HAA formation in the meat. However, the presence of antioxidants, including nitrite, tocopherol (Shin, 2005), ascorbates (Wong, Cheng, & Wang, 2012) among other plant extracts have been reported to result in reduced HAA formation in meat or meat products (Gibis, 2016). Consequently, modifying or controlling these factors can improve the level of HAA in any meat products.

2.4.5.2 Occurrence of heterocyclic aromatic amine in meat products

According to a recent review (Gibis, 2016), the most common HAA in meat are PhIP, MeIQx, 4,8-DiMeIQx, IQ and MeIQ. Although some studies have reported varying values, the level of PhIP found in different meat products has typically ranged between 1 and 70 ng/g, whereas the concentration of MeIQx and 4,8-DiMeIQx has been found to be up to 23 ng/g and 1 ng/g, respectively (Gibis, 2016). Concentration of these compounds also varied in different meat and meat products, and this may be dependent on the cooking method, the cooking time and temperature, concentration of precursors, as well as the presence of water and fat in the raw products (Janoszka, Błaszczyk, Damasiewicz-Bodzek, & Sajewicz, 2009).

In a survey of frequently consumed meat products in the US, most RTE (ready-to-eat) meat products contained lower levels of total HAA (which include IQ, IQx, MeIQ and PhIP; 0.05 to 1.9 ng/g) whereas higher levels were reported in fried and baked meat products (2.34 to 17.91 ng/g), with fried bacon having the highest level (Smith, 2010). In another study, PhIP, MeIQx and 4,8-DiMeIQx were detected in grilled bacon at the level of 0.1 to 53, 0.9 to 18 and below 1 ng/g, respectively, in bacon and around 1.4 to 27 ng/g in bacon fat (Gross et al., 1993) whereas MeIQ was reported in the range of 0.02 ng/g (in pork) and 1.7 ng/g (in well-done bacon) (Lynch, Murray, Gooderham, & Boobis, 1995). In the study by Johansson and Jägerstad (1994) for some selected meat products in the Swedish market, about 3.8 ng/g (moderately done) and 10.5 ng/g (well-done) IQ were found in fried bacon, and the total HAA found in bacon was between 10.2 (moderately done) and 16.7 (well-done) ng/g. This study showed that HAA levels increased with degree of doneness. Meat products across different cultures, cooking methods and animal species have been shown to have a wide range of HAA (Busquets, Bordas, Toribio, Puignou, & Galceran, 2004; Gibis, 2016; Johansson & Jägerstad, 1994; Puangsombat, Gadgil, Houser, Hunt, & Smith, 2012).

2.4.5.3 Health implication of heterocyclic amine consumption in human

The mutagenicity and carcinogenicity of various HAAs have been reported in various studies. Several HAA have been shown to produce both frameshift-type and base pair change-type mutagenicities in S*almonella typhimurium* (employing TA98 and TA100 respectively) (Sugimura et al., 2004). Similar results were reported with mammalian cell lines using *Hprt* gene or the *Ef-2* genes (Thompson et al., 1987). Because the IQ type HAA are stronger mutagens than the non-IQ type compound, they have been shown to have a stronger mutagenic activity than aflatoxin B1 and

benzo(a)pyrene (Sugimura, 1997). Although some HAA have been found to be non-mutagenic on their own, they become co-mutagenic in the presence of another HAA (e.g. harman and norharman) (Wakabayashi, Yahagi, Nagao, & Sugimura, 1982). The carcinogenicity of HAA has also been demonstrated in various animal trials. Target organs for HAA-induced tumours include lungs, liver, ear ducts, skin, mammalian glands, colon and clitoral glands (Sugimura et al., 2004). An IQ dose of between 10 and 20 mg/kg of body weight administered to non-human primate produced carcinoma following several months of exposure (Adamson, Thorgeirsson, & Sugimura, 1996).

Although the HAA levels found in typical human diets may not present significant acute toxicity, it is important to limit their production during meat processing to prevent chronic exposure. A cohort study found that levels of PhIP > 41.40 ng/day posed a relative risk of colorectal adenomas (relative risk: 1.47; 95% CI: 1.13 - 1.93) in high intake compared to low intake groups (Rohrmann, Hermann, & Linseisen, 2009). A similar result was reported in a population-based case-control study where PhIP > 42.3 ng/day (odd risk: 1.81; 95% CI: 1.24 - 2.64), MeIQx > 19.0 ng/day (odd risk: 1.45; 95% CI: 0.99 - 2.12) and DiMeIQx > 3.7 ng/day (odd risk: 1.35; 95% CI: 0.94 - 1.93) were associated with the advent of colorectal adenomas (Barbir et al., 2012). It is important to note that considering the amount of HAA found per gram of meat, the values above amount could possibly accumulate in the diet considering that daily human consumption of meat and meat products may range in several grams in addition to exposures from other environmental sources. For example, the food guide serving per day in Canada for meat is about 75 g and adult men in the age range of 19 and 50 years are recommended to take three times this meat serving daily (Dieticians of Canada, 2012). Inappropriate choice of cooking method and considerable cooking time and temperature may lead to higher exposure of consumers to these carcinogens, even if the recommended guidelines are strictly adhered to.

Consequently, considering the rudimentary nature of pork belly quality assessment and the possibility of variation that could be introduced by several *ante*- and *post-mortem* production factors, the present research will, therefore, explore some tools that could enhance pork belly quality assessment and evaluate how some animal production factors can influence their effectiveness. Furthermore, because factors during animal production (especially dietary treatments), as well as processing parameters (e.g., antioxidant addition or heat treatment) during bacon processing/cooking could have impacts on its oxidative stability and overall chemical

composition, the present study will also focus on the effect of selected cooking methods and storage days on lipid and protein oxidation, as well as HAA in bacon derived from pork bellies from compositionally varying swine population.

CHAPTER 3

ACCURACY OF DUAL ENERGY X-RAY ABSORPTIOMETRY (DXA) IN ASSESSING CARCASS COMPOSITION FROM DIFFERENT PIG POPULATIONS[4]

3.1 Abstract

The accuracy of dual energy X-ray absorptiometry (DXA) in assessing carcass composition from pigs with diverse characteristics was examined. A total of 648 pigs from three different sire breeds, two sexes, two slaughter weights, and three different diets were employed. DXA estimations were used to predict the dissected/chemical yield for lean and fat of carcass sides and primal cuts. The accuracy of the predictions was assessed based on coefficient of determination (R^2) and residual standard deviation (RSD). The linear relationships for dissected fat and lean for all the primal cuts and carcass sides were high ($R^2 > 0.94$, $P < 0.01$), with low RSD (< 1.9%). Relationships between DXA and chemical fat and lean of pork bellies were also high ($R^2 > 0.94$, $P < 0.01$), with RSD < 2.9%. These linear relationships remained high over the full range in variation in the pig population, except for sire breed, where the coefficient of determination decreased when carcasses were classified based on this variable.

[4] Published and used with permission from:

Soladoye, O. P., Campos, Ó. L., Aalhus, J. L., Gariépy, C., Shand, P., & Juárez, M. (2016). Accuracy of dual energy X-ray absorptiometry (DXA) in assessing carcass composition from different pig populations. *Meat Science, 121: 310-316*.

O. P. Soladoye conducted experiments, analysed data, compiled and interpreted results, drafted, revised and finalised manuscript.

3.2 Introduction

Carcass composition and lean meat yield evaluation play an important role in determining carcass market value and, as such, are largely incorporated in most national grading systems. Not only this, carcass composition analyses are fundamental in the evaluation of growth and genetic selection in animal production. Traditionally, to assess the composition of meat animals, either dissection of carcasses into their component parts or chemical determination after carcass grinding is employed (Pearce et al., 2009). Both methods are labour intensive, destructive, expensive and time-consuming, making their application to a fast-paced pork industry unrealistic. Furthermore, the biases associated with personnel's dexterity and fatigue involved in these procedures may also make these methods unattractive to commercial meat industries (Marcoux et al., 2005).

Many technologies have been employed in the past to assess body composition non-invasively (Scholz, Bünger, Kongsro, Baulain, & Mitchell, 2015), some of which have been limited in their application either by cost, accuracy, speed or complexity of operation. These include, but are not limited to bioimpedance analysis (BIA) (Smith, Johnson, & Nagy, 2009), total body electrical conductivity (TOBEC) (Berg, Asfaw, & Ellersieck, 2002), magnetic resonance imaging (MRI) (Tholen et al., 2003), X-ray computed tomography (CT) (Vester-Christensen et al., 2009) and dual photon absorptiometry (DPA) (Barden & Mazess, 1989). A common feature of these non-invasive techniques is their reliance on specific signals which can be of different nature, including electrical (e.g. BIA, TOBEC), X-radiation (e.g. CT, DXA), radio frequency (MRI) or other ionizing electromagnetic waves (DPA) which interact with body tissues at an atomic level (Scholz et al., 2015). Dual energy X-ray absorptiometry (DXA) is a non-invasive, easy to operate, precise and relatively cheap technology that is based on differential attenuation of high- and low-energy X-rays by the major components of animal tissues including bone, lean and fat. The principle of DXA has been widely described in the literature (Brienne et al., 2001; Jebb, 1997; Matthew & Alec, 2012), and it has found application not only in the medical field in the treatment of osteoporosis but also in meat science for predicting the chemical composition of carcasses and meat primal of pigs (Lukaski et al., 1999; Marcoux et al., 2005; Mitchell, Scholz, & Conway, 1998; Mitchell, Scholz, & Pursel, 2003; Suster et al., 2003), cattle (Mitchell, Solomon, & Rumsey, 1997), lamb (Mercier et al., 2006) and poultry (Salas, Ekmay, England, Cerrate, & Coon, 2012). DXA may offer similar accuracy compared to the traditional methods in a very objective way with other additional benefits as previously highlighted. Other authors have emphasized the important

application of DXA in research involving live animals due to its non-invasive nature (Hunter et al., 2011; Pearce et al., 2009). DXA also has great potential as a high throughput method for the study of carcass merit traits, highly needed in fields like genomics (Kogelman et al., 2013; Rothammer et al., 2014), where abundant and accurate phenotypic information is required in order to develop effective marker panels. More traditional research in animal production and carcass quality could also find this technology beneficial as a large number of carcasses can be evaluated without depreciation. More recently, DXA has found wider applications in online meat processing plant where it is used in fat content analysis and product weight assessment, as well as the detection of physical contamination in fresh, frozen, ground and packaged meat operating with speed of operation at about 3,800 kg per hour (e.g. MeatMaster™, FOSS Electric A/S Denmark, Eagles-FA, EAGLES Product Inspection, Tampa, FL, USA and Ishida IX-GA Series X-ray Inspection System, ISHIDA Incorporation).

Although several authors have reported the accuracy of DXA in estimating pork lean and fat, only a few of these have used animals of heterogenous population (Kremer, Fernandez-Figares, Förster, & Scholz, 2012). The accuracy of DXA in variable animal populations is largely unreported. Yet, it is important to know the effect of these variations in animal population on DXA prediction accuracy and if a model developed using a heterogenous population will successfully predict a specific animal population. Considering the increasing variability in pig production systems coupled with more recent efforts in the production sector to develop carcass traits targeted to accommodate niche markets, a robust prediction tool will be valuable for efficient pork marketing.

The present study used three widely different pig genotypes of two different sexes, fed one of three diets and harvested at two different slaughter weights. The study intends to examine the accuracy of DXA in predicting fat and lean in pig carcasses and primal cuts. Specifically, the effect of variation within animal population on the accuracy of DXA prediction will be explored. Prediction equations will also be established for each primal cut and carcass sides in the overall animal population and within their variability clusters.

3.3 Materials and methods

3.3.1 Animal management and experimental design

The treatment design for this study consisted of a 3 × 2 × 3 × 2 factorial (breed combination, gender, diet and slaughter weight, respectively). Commercial dams (42 Large White × Landrace F1; Hypor Inc., Regina, SK, Canada) were inseminated with semen from three different breeds (4 boars per breed): Duroc (Peak Swine Genetics, Leduc, AB, Canada), Lacombe (Peak Swine Genetics Inc.) and Iberian (Semen Cardona, Cardona, Spain). In order to balance the genetic background for the maternal line, the entire design was replicated (72 pen and 216 pigs per replicate) which resulted in a total of 648 pigs. This was done to ensure that each sow was inseminated and produced offspring from each of the three paternal lines. After attaining 70 kg live weight, pigs were selected and transferred into the experimental rooms, and then sorted into pens of three (same breed combination and gender in each pen), and these pens were then assigned to one of three dietary treatments (control, canola, or flaxseed): a conventional Canadian commercial diet (control; 44% wheat, 38% barley, 15% canola meal, 1% soya meal; Masterfeeds, Winnipeg, MB, Canada), a high-oleic diet (canola; 10% ExtraPRO® containing 50% full fat canola and 50% extruded field peas; O&T Farms, Ltd., Regina, SK, Canada), or a high-linolenic diet (flaxseed; 10% LinPRO® containing 50% flaxseed and 50% extruded field peas; O&T Farms, Ltd.), for the last three weeks prior to slaughter. In all cases, diets were formulated (Verus Animal Nutrition, Winnipeg, MB) to meet the nutrient requirement of the pigs (Table 3.1). All experimental procedures were carried out at the Agriculture and Agri-Food Canada Lacombe Research Centre swine unit (Lacombe, AB). Dietary treatments and experimental procedures were approved by the Lacombe Research and Development Centre Animal Care Committee for compliance to the guidelines established by the Canadian Council on Animal Care (2009). Pigs were allowed to feed *ad-libitum,* and were selected for slaughter weekly based on two pre-set slaughter weights (120 or 140 kg).

3.2.2 Animal slaughter

At the designated slaughter weights, pigs were transported to the Agriculture and Agri-Food Canada Lacombe Research and Development Centre, a federally inspected abattoir (1 km away from swine unit).

Table 3.1: Ingredient composition and nutrient content of animal diets

Ingredient, %	Control	Canola	Flaxseed
Wheat	44.0	27.8	17.1
Barley	37.7	43.7	56.4
Canola meal	15.0	15.0	14.0
ExtraPro®[1]	-	10.0	-
LinPro®[2]	-	-	10.0
Calcium carbonate	1.20	1.20	0.95
Soybean meal 46% CP	1.00	1.05	-
Salt	0.43	0.42	0.41
Dicalcium phosphate	0.33	0.25	0.31
Lysine	0.14	0.31	0.38
Threonine	-	0.09	0.13
Methionine	-	-	0.09
Vitamin mix	0.10	0.10	0.10
Mineral mix	0.10	0.10	0.10
Ronozyme	0.02	0.02	0.02
Nutrient Composition	**Control**	**Canola**	**Flaxseed**
Dry matter (%)	87.8	88.5	88.5
ME (Mcal/kg)	3.03	3.13	3.12
Crude protein (%)	15.0	15.0	15.0
Crude fat (%)	2.00	3.94	3.88
Crude fibre (%)	5.00	5.72	5.78
Sodium (%)	0.10	0.19	0.19
Calcium (%)	0.70	0.65	0.65
Phosphorus (%)	0.50	0.50	0.50
Vitamin A (KIU/kg)	8.00	8.00	8.00
Vitamin D (KIU/kg)	1.00	1.00	1.00
Vitamin E (IU/kg)	20.0	20.0	20.0
Zinc (mg/kg)	175.0	175.0	175.0
Copper (mg/kg)	26.8	27.4	26.8
Lysine (%)	0.72	0.97	0.95
Methionine (%)	0.26	0.28	0.37
Threonine (%)	0.53	0.68	0.67
Tryptophan (%)	0.18	0.19	0.18

[1]ExtraPRO® containing 50% full fat canola and 50% extruded field peas

[2]LinPRO® containing 50% flaxseed and 50% extruded field peas

Pigs were electrically stunned, exsanguinated, de-haired and emptied of their gut contents. Carcasses were then split into two halves and the weight of each side was recorded as hot side weight. The carcass sides were placed into the cooler at 2ºC for 24 h.

3.2.3 Carcass scanning and cut out

Following slaughter and carcass chilling, left carcass sides were weighed and scanned with a Lunar iDXA unit (EnCore 2011, version 13.60.033, GE Lunar, General Electric, Madison, WI, USA) using the whole-body scan option on the standard mode (~7 minutes per whole DXA table scan) to estimate DXA fat, lean and bone tissues (Figure 3.1). The DXA unit was calibrated using the automated 6-point calibration and quality assurance system of Lunar iDXA twice a day (before and half way into daily experimental trial) to ensure there were no variations within and between experimental batches. Subsequently, the half carcasses were split into four primal cuts (shoulder, ham, loin and belly) according to the Canadian Pork Buyer's Manual (Canadian Pork International, 1995). The primal cuts were weighed and later scanned separately with the DXA (Figure 3.1). All the primal cuts, except the bellies, were fully dissected by trained personnel to quantify the lean, fat and bone content of each primal cut. The bellies were weighed and scanned untrimmed skin-on, trimmed skin-on, and trimmed skin-off. Note that, only 545 carcasses of the 648 animals produced could be scanned with DXA due to instrument breakdowns as well as intermittent low manpower availability for dissection in the course of the experiment. For the primal cuts, as well, some were used in other studies and were not scanned under DXA.

3.2.4 Proximate analysis

Subsequently, the trimmed without skin bellies were ground twice through two different grinder plates (6.35 and 4.76 mm), with mixing between grindings for subsequent proximate analysis. One hundred grams of the homogenized belly was sub-sampled, placed in a pre-weighed stainless-steel cup and heated in a drying oven at 102ºC for 24 h. The dried samples were weighed to calculate the moisture content in each sample. As pork belly is usually fatty, excess fat resulted from the dried sample. The excess fat was drained and the sample was washed with ethyl ether to remove the remaining adhered fat in the sample. The drained sample was air dried overnight and reweighed to assess the drained fat. The remaining dried sample was then crushed into finer pieces using a portable Robot Coupe Blixir

Figure 3.1: Carcass sides and selected primal cuts during DXA scanning

BX3 (Robot Coupe USA Inc., Ridgeland, MS, USA) for subsequent fat analysis. Soxtec (diethyl ether) extraction procedure, carried out in duplicates, (FOSS TECATOR, 2050 Soxtec, Höganås, Sweden) (Anderson, 2004) was used to completely extract the rest of the fat from the sample. Total fat was calculated as the weight of drained fat plus the analysed fat, while lean represented the sample weight minus moisture and total fat.

3.2.5 Statistical analysis

Statistical analyses were performed using PROC REG of SAS 9.3. The accuracy of the prediction equations was evaluated using the coefficient of determination (R^2), as well as residual standard deviation (RSD), which, as standard output in SAS, is root mean square error (RMSE). DXA lean and fat measurements were used for predicting dissected fat and lean content, respectively, for all the primal cuts, except belly. Bone composition analysis was not carried out with any of the primal cuts in the present study because DXA has been widely reported to poorly predict bone weight in animal carcasses (Marcoux et al., 2003; Marcoux et al., 2005; Mercier et al., 2006). For bellies, the DXA lean and fat measurements were used to predict the chemical lean and fat content, respectively. The percent dissected lean and fat content of the entire carcass sides were estimated from the addition of the ham, loin, butt and picnic. As pork bellies were not manually dissected, the percent composition given by the addition of ham, loin, butt and picnic was extrapolated to the entire weight of the carcass sides. The heterogeneity of slope of regression for each equation was also tested using the PROC ANCOVA of SAS. Significance was declared at $P < 0.05$. Data were subjected to PROC MIXED of SAS with PDIFF option to analyse the difference in LSMEAN of diet, sex, sire breed and slaughter weight, with slaughter batch as the random term. Significant difference was declared at $P < 0.05$.

3.3 Results

3.3.1 Descriptive statistics of variable characteristics

Based on the variation within the pig population employed in the present study, a wide compositional variation was evident in terms of lean and fat content of the carcasses. The coefficient of variation (CV) for dissected/chemical fat for all primal cuts and carcass sides ranged from 19.3 to 23.7%, whereas the dissected/chemical lean for these primal cuts and carcass sides spanned between 10.0 and 20.1% (Table 3.2).

Table 3.2: Description of the overall dependent and independent parameters before classification based on inherent factors

	Fat variables					Lean variables				
Primal cuts/side carcass	N	Dissected Mean ± SD% (y variable)ǂ	DXA Mean ± SD% (x variable)ǂ	Dissected CV (%)ǂ	DXA CV (%)	N	Dissected Mean ± SD% (y variable)ǂ	DXA Mean ± SD% (x variable)ǂ	Dissected CV (%)ǂ	DXA CV (%)
Side	545	35.9±7.01	28.8±6.22	19.5	21.6	545	54.8±6.71	69.2±6.17	12.3	8.9
Ham	350	31.8±6.13	24.7±5.01	19.3	20.2	350	59.8±6.19	72.4±5.16	10.4	7.1
Loin	356	40.5±8.99	32.5±8.32	22.2	25.6	356	48.8±8.18	65.6±8.25	16.8	12.6
Shoulder	392	34.8±5.94	26.2±5.01	17.1	19.1	392	56.2±5.61	71.4±5.00	10.0	7.0
Belly	182	46.1±10.89	43.4±9.30	23.7	21.5	182	53.9±10.89	56.6±9.29	20.2	16.4

ǂ values for bellies were chemically derived not dissection.

Although a wide variation was evident in carcass composition for the carcass sides, the diet did not contribute significantly to the compositional variation, whereas the contribution of gender, sire breed and slaughter weight (SW) were significant ($P < 0.01$) (Table 3.3). Carcasses of barrows had greater fat content than gilts ($P < 0.01$), whereas carcasses from Iberian-sired pigs had the greatest fat percentage, followed by the progeny of Lacombe and then the Duroc sires ($P < 0.01$). Among all the primal cuts examined in this study, the ham had the greatest percentage of lean relative to bone and fat (Table 3.4). The fattest ham contained about 46.3% fat, whereas the leanest had only about 17.8% fat. Similar to the carcass sides, the compositional variation appeared to be largely influenced by the sire breed, gender and SW of the pig. Within diet treatments, all hams appeared to have similar composition with an average of about 32% fat and 60% lean content.

Loin yielded the lowest mean lean content relative to fat and bone (Table 3.5), but the wide compositional variation observed in other primal cuts was still evident with fat content ranging between 20 and 60%. This compositional range was the widest observed of all the primal cuts (except for bellies) considered in this study. Similar to other primal cuts, diet did not significantly contribute to this compositional variation, as loins within the same diet cluster contained an average of 40% fat and 49% lean content. The compositional variation in the shoulder also ranged widely with about 30% separating the shoulders with the greatest and least fat contents (Table 3.6). Again, no significant difference was attributed to dietary treatment, only sire breed, gender and SW were responsible for this compositional variation.

The bellies were the only primal assessed on a chemical basis, but had a wider compositional range (about 47% between the fattest and the leanest pork bellies) compared to other primal cuts (Table 3.7). Similar to other primal cuts, diet did not contribute to the compositional variation observed in the pork bellies. The order of fatness for sire breed type and gender were similar to that reported for the previous primal cuts, and, as expected, bellies from the 140 kg SW group had greater fat content than the 120 kg group (Soladoye, Shand, Aalhus, Gariépy, & Juárez, 2015).

3.3.2 DXA prediction of chemical and dissected fat and lean

Overall, the dissected fat and lean of carcass sides were accurately predicted with low RSD and high R^2 (Table 3.3).

Table 3.3: Carcass side descriptive statistics and prediction equations

Variable	Dissected carcass fat ǂ								Dissected carcass lean ǂ							
	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2
Overall[*]	545	35.9±7.01	20.28	54.19	1.513	1.09x+4.33	4.2	0.95	545	54.8±6.71	37.13	69.10	1.689	1.05x-17.49	3.1	0.94
By Sire Breed																
Duroc	167	29.3±0.55[c]	20.28	38.46	1.416	1.00x+6.20	4.8	0.86	167	61.3±0.57[a]	52.47	69.10	1.356	0.92x-7.83	2.2	0.84
Iberian	170	44.4±0.47[a]	31.05	54.19	1.794	1.10x+4.14	4.1	0.82	170	46.4±0.47[c]	37.13	59.90	2.085	1.01x-15.34	4.4	0.72
Lacombe	208	34.3±0.44[b]	25.0	44.55	1.187	1.09x+4.80	3.4	0.91	208	56.5±0.45[b]	46.67	66.73	1.405	1.02x-16.22	2.5	0.85
By Diet																
Canola	183	36.0±0.37	21.33	54.19	1.591	1.07x+4.93	4.4	0.95	183	54.7±0.37	37.13	68.12	1.733	1.02x-15.37	3.2	0.93
Flaxseed	187	35.9±0.36	22.32	51.21	1.458	1.11x+3.81	4.1	0.96	187	54.9±0.37	39.79	67.34	1.704	1.07x-19.27	2.1	0.94
Control	175	36.1±0.38	20.28	52.57	1.448	1.11x+4.08	4.0	0.96	175	54.6±0.38	38.96	69.10	1.571	1.06x-18.49	2.9	0.95
By Sex																
Gilt	268	34.3±0.35[b]	20.28	52.24	1.539	1.09x+4.19	4.5	0.96	268	56.3±0.36[a]	39.01	69.10	1.707	1.04x-16.89	3.0	0.94
Barrow	277	37.6±0.35[a]	25.55	54.19	1.469	1.09x+4.67	3.9	0.94	277	53.1±0.36[b]	37.13	64.15	1.636	1.04x-17.52	3.1	0.93
By Slaughter Weight																
120	283	35.1±0.35[b]	20.28	51.88	1.436	1.10x+4.11	4.1	0.96	283	55.5±0.36[a]	39.72	69.10	1.608	1.05x-18.41	2.9	0.94
140	262	36.9±0.36[a]	22.32	54.19	1.581	1.09x+4.43	4.4	0.95	262	54.0±0.36[b]	37.13	67.34	1.729	1.05x-17.29	3.2	0.93

ǂ Carcass fat and lean (y variable) were obtained by manual dissection (extrapolated to the entire carcass side weight from the addition of ham, loin, butt and picnic composition without the pork bellies) and DXA estimate (x variable) was used to predict these dissected values. [1] This equation will predict the variable y in carcass composition prediction while variable "x" is the DXA fat or lean estimate in percentage (%). Mean values are LSM ± SE and mean values with different letters in the same column are significantly different at $P < 0.01$. * values for the "overall" were expressed as Mean ± SD. CV (%): coefficient of variation of the equation, RSD: Residual standard deviation.

Table 3.4: Ham descriptive statistics and prediction equations

Variable	Dissected ham fat ǂ								Dissected ham lean ǂ							
	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2
Overall*	350	31.8±6.13	17.77	46.31	0.937	1.21x+1.87	3.0	0.98	350	59.8± 6.19	45.45	72.82	1.039	1.18x-25.92	1.7	0.97
By Sire Breed																
Duroc	98	24.8±0.55^{c}	17.77	32.24	0.826	1.14x+2.70	3.3	0.92	98	66.9±0.51^{a}	59.31	72.82	0.876	1.09x-6.80	1.3	0.89
Iberian	115	39.0±0.42^{a}	26.11	46.31	0.860	1.16x+3.24	2.3	0.94	115	52.3±0.39^{c}	45.45	64.10	0.960	0.94x-1.76	1.8	0.68
Lacombe	137	31.3±0.41^{b}	22.69	39.34	0.895	1.16x+3.59	2.9	0.92	137	60.6±0.38^{b}	52.73	69.10	0.997	1.08x-7.24	1.7	0.79
By Diet																
Canola	112	32.0±0.36	19.15	46.31	0.939	1.22x+1.78	2.9	0.98	112	59.6±0.34	45.45	72.16	1.049	1.17x-12.85	1.8	0.95
Flaxseed	123	31.5±0.34	21.0	42.89	0.902	1.18x+2.52	2.8	0.98	123	60.2±0.32	48.41	71.25	1.015	1.15x-11.10	1.7	0.94
Control	115	31.6±0.37	17.77	44.76	0.981	1.23x+1.32	3.1	0.98	115	59.9±0.35	47.10	72.82	1.052	1.08x-6.79	1.8	0.92
By Sex																
Gilt	175	30.6±0.34^{b}	17.77	45.07	0.989	1.21x+1.77	3.2	0.98	175	61.1±0.32^{a}	47.10	72.82	1.092	1.12x-9.17	1.7	0.95
Barrow	175	32.9±0.33^{a}	22.69	46.31	0.895	1.20x+1.99	2.7	0.97	175	58.7±0.31^{b}	45.45	69.28	0.981	1.16x-11.69	1.7	0.92
By Slaughter Weight																
120	202	30.8±0.32^{b}	17.77	43.55	0.974	1.20x+2.29	3.1	0.97	202	60.8±0.30^{a}	48.39	72.82	1.066	1.13x-10.32	1.8	0.95
140	148	32.7±0.35^{a}	21.05	46.31	0.883	1.23x+1.24	2.8	0.98	148	59.0±0.33^{b}	45.45	71.25	0.932	1.14x-10.40	1.7	0.92

ǂ Carcass fat and lean (y variable) were obtained by manual dissection and DXA estimate (x variable) was used to predict these dissected values. [1] This equation will predict the variable "y" in carcass composition prediction while variable "x" is the DXA fat or lean estimate in percentage (%). Mean values are LSM ± SE and mean values with different letters in the same column are significantly different at $P < 0.01$. * values for the "overall" were expressed as Mean ± SD. CV (%): coefficient of variation, RSD: Residual standard deviation.

Table 3.5: Loin descriptive statistics and prediction equations

Variable	Dissected carcass fat ǂ								Dissected carcass lean ǂ							
	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2
Overall*	356	40.5±8.99	19.90	59.85	1.647	1.07x+5.90	4.1	0.97	356	48.8±8.18	46.61	84.31	1.875	0.97x-14.76	3.9	0.95
By Sire Breed																
Duroc	98	31.3±0.63[c]	19.90	43.48	1.622	0.99x+6.67	5.2	0.90	98	57.2±0.55[a]	60.24	84.31	1.743	0.91x-9.09	3.1	0.86
Iberian	120	51.1±0.50[a]	35.34	59.85	1.410	0.93x+11.4	2.8	0.91	120	39.1±0.44[c]	46.61	68.15	1.530	0.80x-5.17	3.7	0.85
Lacombe	138	38.5±0.48[b]	26.43	48.93	1.324	1.02x+7.92	3.5	0.92	138	50.7±0.43[b]	58.45	79.36	1.665	0.88x-9.37	3.3	0.84
By Diet																
Canola	113	40.4±0.45	22.06	59.85	1.374	1.04x+6.63	3.4	0.98	113	48.9±0.40	46.61	81.50	1.683	0.94x-12.55	3.4	0.96
Flaxseed	125	40.4±0.42	24.18	59.21	1.780	1.08x+5.35	4.4	0.96	125	49.0±0.37	48.17	78.67	1.917	1.00x-16.84	4.0	0.95
Control	118	40.2±0.45	19.90	59.03	1.737	1.07x+5.84	4.3	0.97	118	49.1±0.40	47.60	84.31	1.964	0.96x-14.45	4.0	0.95
By Sex																
Gilt	178	37.8±0.40[b]	19.90	58.58	1.569	1.08x+5.31	4.1	0.97	178	51.2±0.36[a]	48.22	84.31	1.720	0.98x-15.63	3.4	0.96
Barrow	178	42.8±0.40[a]	26.28	59.85	1.700	1.04x+6.90	4.0	0.95	178	46.7±0.36[b]	46.61	78.09	1.997	0.94x-13.14	4.3	0.92
By Slaughter Weight																
120	203	39.0±0.38[b]	19.90	59.39	1.745	1.06x+6.11	4.4	0.96	203	56.2±0.33[a]	46.61	84.31	1.956	0.96x-14.31	4.0	0.94
140	153	41.6±0.42[a]	24.18	59.85	1.501	1.07x+5.63	3.6	0.97	153	55.3±0.35[b]	47.25	78.67	1.667	0.99x-15.56	3.5	0.96

ǂ Carcass fat and lean (y variable) were obtained by manual dissection and DXA estimate (x variable) was used to predict these dissected values. [1] This equation will predict the variable "y" in carcass composition prediction while variable "x" is the DXA estimate fat or lean in percentage (%). Mean values are LSM ± SE and mean values with different letters in the same column are significantly different at $P < 0.01$. * values for the "overall" were expressed as Mean ± SD. CV (%): coefficient of variation, RSD: Residual standard deviation.

Table 3.6: Shoulder descriptive statistics and prediction equations

Variable	Dissected shoulder fat ǂ								Dissected shoulder lean ǂ							
	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2
Overall*	392	34.8±5.94	22.32	51.12	1.405	1.15x+4.55	4.0	0.94	392	56.2±5.61	40.13	67.40	1.432	1.09x-21.46	2.6	0.94
By Sire Breed																
Duroc	140	30.1±0.53[c]	22.32	39.39	1.412	1.03x+6.98	4.7	0.84	140	60.6±0.48[a]	51.83	67.40	1.478	0.97x-12.52	2.4	0.80
Iberian	115	41.7±0.57[a]	31.05	51.12	1.248	1.08x+7.08	3.0	0.88	115	49.7±0.51[c]	40.13	59.90	1.342	0.98x-14.77	2.7	0.83
Lacombe	137	33.9±0.50[b]	25.00	45.16	1.417	1.11x+5.89	4.2	0.87	137	57.1±0.45[b]	46.25	66.73	1.380	1.08x-20.64	2.4	0.87
By Diet																
Canola	137	35.1±0.39	22.86	51.12	1.385	1.16x+4.45	4.0	0.95	137	55.9±0.35	40.13	67.40	1.365	1.10x-22.39	2.4	0.94
Flaxseed	130	35.0±0.39	22.32	47.79	1.425	1.17x+4.06	4.1	0.95	130	56.0±0.36	43.57	67.34	1.523	1.09x-21.55	2.7	0.93
Control	125	35.6±0.41	23.74	49.84	1.413	1.13x+5.40	4.0	0.94	125	55.5±0.37	42.21	66.59	1.402	1.06x-19.60	2.5	0.94
By Sex																
Gilt	200	33.6±0.36[b]	22.32	49.84	1.381	1.16x+4.51	4.2	0.95	200	57.3±0.33[a]	42.21	67.40	1.447	1.08x-20.61	2.5	0.94
Barrow	192	36.8±0.38[a]	25.55	51.12	1.449	1.15x+4.80	4.0	0.92	192	54.2±0.35[b]	40.13	63.78	1.407	1.07x-20.10	2.6	0.92
By Slaughter Weight																
120	209	34.7±0.36[b]	22.86	51.12	1.356	1.13x+4.83	3.9	0.95	209	56.2±0.33[a]	40.13	67.40	1.458	1.08x-20.72	2.6	0.93
140	183	35.7±0.39[a]	22.32	49.84	1.468	1.17x+4.30	4.2	0.94	183	55.3±0.35[b]	42.21	67.34	1.418	1.10x-22.00	2.5	0.94

ǂ Carcass fat and lean (y variable) were obtained by manual dissection and DXA estimate (x variable) was used to predict these dissected values. [1] This equation will predict the variable "y" in carcass composition prediction while variable "x" is the DXA fat or lean estimate in percentage (%). Mean values are LSM ± SE and mean values with different letters in the same column are significantly different at $P < 0.01$. * values for the "overall" were expressed as Mean ± SD. CV (%): coefficient of variation, RSD: Residual standard deviation.

Table 3.7: Belly descriptive statistics and prediction equations

Variable	Chemical belly fat ǂ								Chemical belly lean Ψ							
	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2	N	Mean ± SE (%)	Min (%)	Max (%)	RSD (%)	Equation[1]	CV	R^2
Overall*	182	46.1±0.89	21.91	68.74	2.732	1.13x-2.93	5.9	0.94	182	53.9±0.89	31.26	78.09	2.744	1.13x-10.07	5.1	0.94
By Sire Breed																
Duroc	63	36.7±1.40[c]	21.91	49.47	2.548	1.08x-1.77	7.0	0.89	63	63.3±1.40[a]	50.53	78.09	2.556	1.09x-6.80	4.0	0.89
Iberian	54	58.5±1.25[a]	48.29	68.74	2.831	0.94x-1.76	4.8	0.68	54	41.5±1.25[c]	31.26	51.71	2.833	0.94x-1.76	6.8	0.68
Lacombe	65	44.6±1.28[b]	30.71	55.22	2.757	1.09x-0.98	6.1	0.79	65	55.4±1.28[b]	44.78	69.29	2.773	1.08x-7.24	5.1	0.79
By Diet																
Canola	58	47.1±1.09	21.91	65.36	2.381	1.17x-4.35	5.2	0.96	58	52.9±1.09	34.65	78.09	2.400	1.17x-12.85	4.4	0.95
Flaxseed	65	46.4±1.02	25.18	66.73	2.884	1.15x-3.80	6.2	0.94	65	53.6±1.02	33.27	74.82	2.889	1.15x-11.10	5.4	0.94
Control	59	46.3±1.06	24.87	68.74	2.889	1.08x-0.84	6.3	0.92	59	53.7±1.06	31.26	75.13	2.905	1.08x-6.79	5.4	0.92
By Sex																
Gilt	81	43.9±1.01[b]	21.91	63.89	2.667	1.11x-2.23	6.3	0.95	81	56.1±1.01[a]	36.11	78.09	2.687	1.12x-9.17	4.7	0.95
Barrow	101	49.3±0.93[a]	22.67	68.74	2.813	1.16x-4.32	5.8	0.92	101	50.7±0.93[b]	31.26	77.34	2.819	1.16x-11.69	5.5	0.92
By Slaughter Weight																
120	89	45.4±0.98[b]	21.90	64.52	2.596	1.13x-2.99	5.6	0.95	89	54.6±0.98[a]	35.48	78.09	2.611	1.13x-10.32	4.7	0.95
140	93	47.8±0.99[a]	24.87	68.74	2.900	1.14x-3.26	6.0	0.92	93	52.2±0.99[b]	31.26	75.14	2.911	1.14x-10.40	5.6	0.92

ǂ Chemical belly fat (y variable) determine by soxtec extraction and predicted by DXA fat mass estimates (x variable); Ψ Chemical belly lean (y variable) assessed by the difference between the weight subsampled and fat content. [1] This equation will predict the variable "y" in carcass composition prediction while variable "x" is the DXA fat or lean estimate in percentage (%). CV (%): coefficient of variation, RSD: Residual standard deviation. Mean values are LSM ± SE and mean values with different letters in the same column are significantly different at P < 0.01. * values for the "overall" were expressed as Mean ± SD.

This accuracy persisted even when the fat content in the carcass sides was classified based on diet, gender and SW ($R^2 > 0.94$ and RSD < 1.6%). However, when classified by sire breed, a slight drop in R^2 value was observed, with a higher RSD for the Iberian sire breed (RSD < 1.5% for the other sire breed types). In all cases, the slope of the equation was close to unity, and no variation in the pig population significantly influenced this parameter. The intercepts were also similar in all cases (ranging between 3.8 and 4.9), regardless of variation within the pig population, except in the case of sire breed classification where the slope and intercept appeared to be slightly more variable from breed to breed. This was ascertained by the consistently low standard error of both slope (SEE = 0.01 to 0.03) and intercept (SEE = 0.31 to 1.44) estimate for the regression equations, regardless of variation factors within the pig population (results not shown). Similarly, the dissected lean was well predicted, regardless of variation within the population ($R^2 > 0.93$ and RSD < 1.7%), except in the sire breed classification where the R^2 value dropped to 0.72, with a corresponding increased RSD in the Iberian-sired pigs. Contrary to fat prediction, lean prediction had slopes of all the equations close to unity but the intercepts were in the negative axis, with larger variation within the sire breeds.

DXA also accurately predicted the dissected fat and lean in hams regardless of variation in population (Table 3.4). The R^2 and RSD values were high (> 0.97) and low (< 1.1%), respectively, at all levels of variable classifications for both fat and lean, except for sire breed where the R^2 decreased slightly for both fat (0.92 to 0.94) and lean (0.88 to 0.91) predictions. Overall, ham had the lowest RSD compared to other primal cuts observed in this study. Similar to the overall carcass side prediction, the slopes of the equations were close to unity with limited variation, regardless of variation in the population (SEE of slope = 0.01 to 0.03).

The overall fat and lean in the loin primal were accurately predicted with high R^2 and low RSD values (Table 3.5). Prediction equations for the loin primal were similar for all factors in the population except for sire breed. This difference in predicted equations for loins among sire breeds was even more pronounced in the lean prediction, and also slightly less well fitted to the model compared to fat (Table 3.5). The slope and the intercept were also similar in all cases except in the lean prediction for the sire breed classified groups (consistently low SEE). Similar trends were observed in the shoulder primal (Table 3.6), with a lower R^2 for lean and fat prediction within sire breeds.

For DXA prediction of pork bellies where composition was determined chemically (Table 3.7), the regression equation accounted for up to 94% of the predicted chemical lean and fat, and this value was comparable to those of other primal cuts and carcass sides considered in this study. The intercepts for both fat and lean prediction equations were, however, in the negative axis and they appeared to vary more widely, especially within the sire breed classified groups. Compared to other primal cut predictions, pork belly predictions also had a higher RSD and lower R^2 values, particularly within sire breed (as low as $R^2 = 0.68$ in the Iberian sire breed). Although the slope was close to unity, the intercept varied within and between treatments.

Overall prediction equations for fat and lean content for each varying factor of classification had similar slopes (Table 3.8). Despite numerical differences in the R^2, slope and intercept for sire breed, significant differences in the slopes only tended to be significant in the fat prediction equation for the carcass side and the lean prediction equation for the loin. Slopes were also significantly different for the fat prediction in the loin primal for gender and the lean prediction in the ham primal for slaughter weight. The contributions of each sire breed to the overall regression model was plotted (only for carcass side) (Figure 3.2 and 3.3) and showed that each sire breed was different and contributed to the regression model in unique clusters occupying different regression planes.

Table 3.8: Test of slope heterogeneity for carcass sides and primal cut predictions

Variables	P-value									
	Side		Ham		Shoulder		Loin		Belly	
	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean	Fat	Lean
Sire Breed	0.0505	0.1093	0.9280	0.6755	0.3117	0.1118	0.0935	0.0535	0.3169	0.3251
Diet	0.2033	0.1216	0.1227	0.2588	0.5649	0.4796	0.3425	0.0804	0.2091	0.2120
Gender	0.6825	0.8730	0.5759	0.4630	0.7599	0.6742	0.0258	0.1212	0.3203	0.3225
Slaughter weight	0.5253	0.6688	0.0771	0.0014	0.3307	0.5653	0.6644	0.2245	0.9481	0.9378

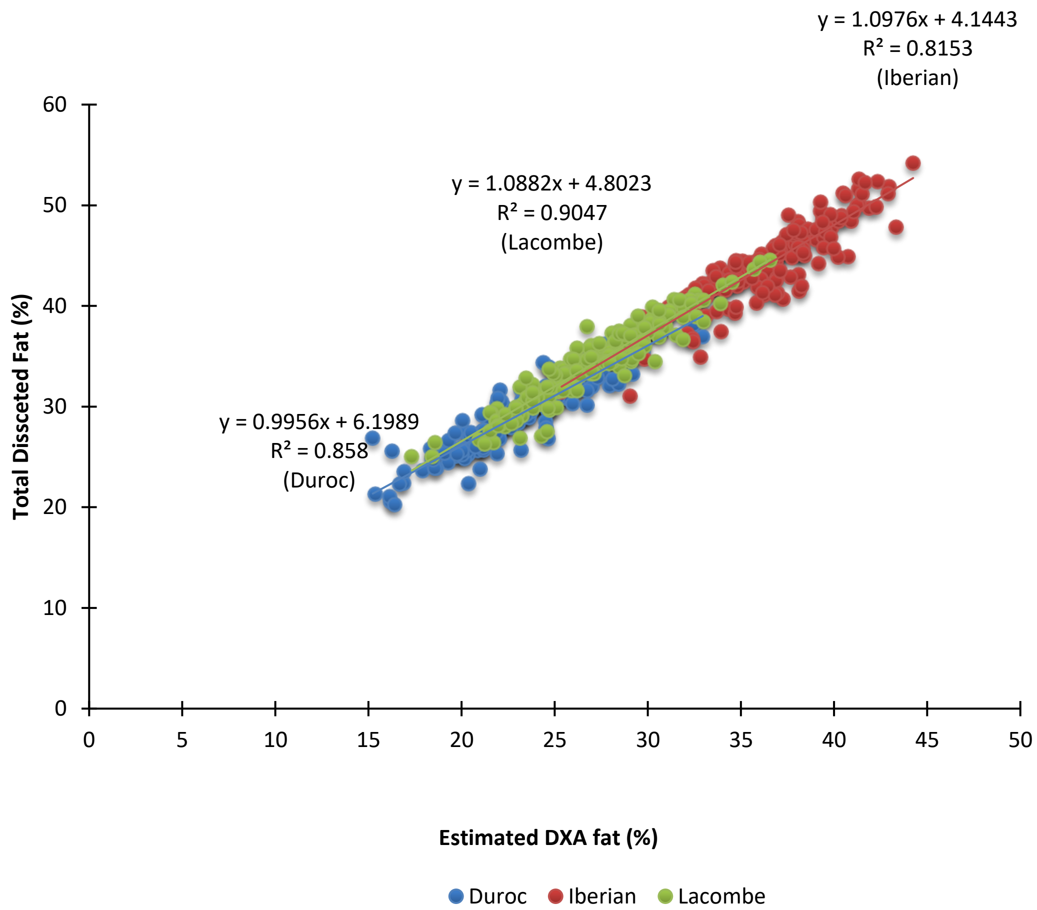


Figure 3.2: Carcass side DXA fat data based on the three sire breeds

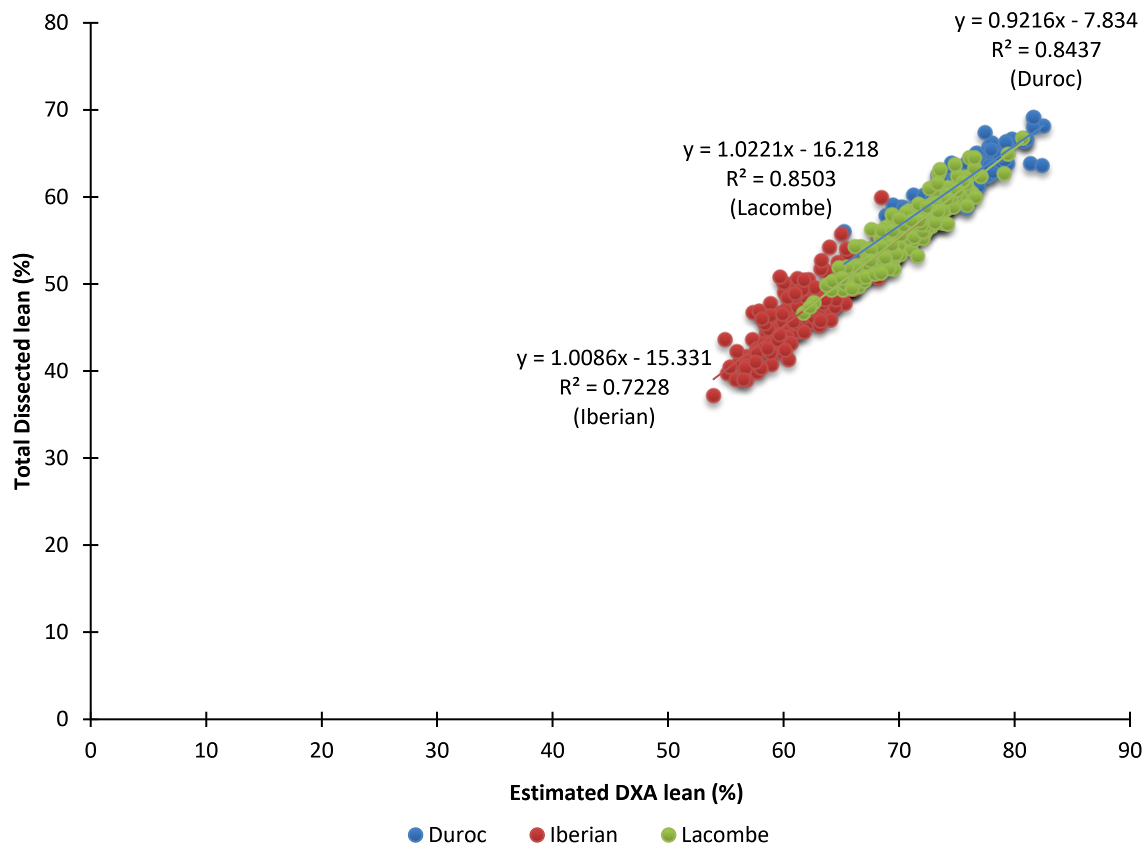


Figure 3.3: Carcass side DXA lean data based on the three sire breeds

3.4 Discussion

3.4.1 Descriptive statistics

The wide range of compositional variation within the pigs in the present study spans a range that could possibly be encountered within the pork market place. Compared to other studies that have assessed the prediction accuracy of DXA in pigs (Marcoux et al., 2003; Marcoux et al., 2005), the present study covers a wider range of fat and lean composition; hence, could have a wider applicability in varying pig populations. Among the primal cuts, the belly had the highest average fat content (46 ± 1%), whereas ham had the lowest (32 ± 6.1%). As such, the ham was the leanest of the primal cuts, with lean content of about 60% and, coincidentally, this cut also had the lowest RSD which may signify that at this fat-to-lean ratio, higher precision can be observed with DXA prediction. The loin primal had the lowest lean content (49%), followed by the belly (54%). In absolute terms by weight, however, the belly had the lowest lean content among all the primal cuts, but because the bone in this cut was removed prior to this calculation, the loin primal had the lowest percentage of lean relative to other primal cuts.

3.4.2 DXA prediction of fat and lean

The fat content of the half carcasses and primal cuts was accurately predicted by the corresponding DXA fat and lean estimate with $R^2 > 0.94$. Assessing the accuracy of fat prediction equations by the R^2 of each primal cuts and carcass sides, the following order was observed: ham > loin > side > shoulder = belly. A similar order was observed with lean prediction, except that the side, shoulder and belly shared a similar value. Moreover, in terms of precision of these equation (RSD value), the order was: ham > shoulder > side > loin > belly, even though the difference between these was not so substantial (Tables 3.3 to 3.7). The superior prediction for ham could be due to the less complex nature of its fat and lean arrangement and the ease in which butchers could separate these components with less error. Nord (1998) had pointed out that the algorithms for estimating the composition of tissue rely largely on the distribution of fat and lean being reasonably close to that of the human body. Out of all the primal cuts observed, the belly appeared to be somewhat less accurately predicted by DXA (highest RSD compared to other primal cuts). However, this was not unexpected, as previous studies have observed a similar trend (Mercier et al., 2006; Mitchell, Scholz, & Conway, 1998). This lower prediction accuracy may be due to not

only the complex anatomical arrangement of belly component but also the thinness of this primal, which have been reported to limit the capacity of the instrument to accurately detect the attenuation of X-rays passing through (Mazess, Barden, Bisek, & Hanson, 1990; Mercier et al., 2006). Nord (1998) indicated that DXA may produce lower precision and accuracy with smaller bodies because its software was developed for the adult human body. Moreover, Mitchell, Scholz and Conway (1998) pointed out that the variation between DXA and chemical measurement of fat may be a function of both fat content and sample size. On the other hand, several studies have attested to the fact that, the degree of X-ray penetration of the body and hence, the subsequent signal for measurement decreases with body thickness in DXA, and this reduces the efficiency at which attenuation can be determined (Nord, 1998; Suster, Leury, Hofmeyr, D'Souza, & Dunshea, 2004). Therefore, there may be size or fat content range where DXA optimally performs in assessing carcass composition, below and above which its efficiency may reduce. In the current study, as previously mentioned, the fat level in the ham primal cuts appeared to be the most optimum with DXA prediction having the highest R^2 and lowest RSD value. Indeed, Lukaski et al. (1999) established that there is no appreciable effect of body thickness on DXA estimation of soft tissue within the thickness range of 16 to 28 cm and within the weight range of 52 to 113 kg similar to the lower slaughter weight classification employed in the present study.

Aside from the lower number of pork bellies used for this prediction compared to the sample size used for the other primal cuts prediction, another factor possibly contributing to the observed lower prediction in belly may be the traditional diethyl ether extraction method which was used as the reference method in this experiment. Some inherent errors may be involved with its multiple-step nature, especially when evaluating high-fat samples. Lean in this experiment was defined as 100 g ground pork belly minus the fat content. Although this may be the closest lean estimation that could be made, it may not completely align with DXA classification of lean based on density because some components which do not strictly fit into either fat or lean might have been included as lean, whereas some fat fractions which could not be completely separated from the lean (e.g. intramuscular fats) might also have been included with lean. Svendsen, Haarbo, Hassager, and Christiansen (1993) reported that DXA fat estimation does not solely consist of adipose tissue, rather it is the sum total of the fatty elements of all the soft tissues. These could have led to some lower precision of the prediction observed in the lean and fat content of the pork

belly in addition to its complex heterogeneous nature which may make it more susceptible to error with DXA estimation.

However, the prediction accuracy for all primal cuts in the present study was higher relative to previous studies which reported a much lower coefficient of determination with dissection (Marcoux et al., 2003; Marcoux et al., 2005) and chemical analysis (Mitchell et al., 2003) as reference methods. The newer fan beam DXA equipment employed in this study, with better and more up-to-date software algorithms, may have also contributed to these better prediction accuracies.

Fat content was predicted as well as lean content by DXA. Previous literature has suggested that the precision of prediction of dissected/chemical fat ranks second to that of dissected/chemical lean in pig carcasses (Marcoux et al., 2005; Mitchell, Scholz, & Conway, 1998; Mitchell, Scholz, Pursel et al., 1998). This discrepancy may be due to the difference in instrument generation, manufacturer, software version or beam technology. Previously, equipment and software differences were thought to influence the accuracy of DXA predictions (Kistorp & Svendsen, 1998; Mercier et al., 2006). Aside from this, as DXA measures were compared to manual dissection, the expertise of the meat processing staff may play a part in adding to the variability. In the present study, the meat processing staff were trained for research dissection, which ensured a greater rigour than in commercial settings.

3.4.4 DXA underestimation of fat and overestimation of lean

DXA consistently underestimated fat in all primal cuts and carcass sides, with the percentage of underestimation at about 5.9% for bellies, 22.2% for ham, 24.7% for shoulder, 19.7% for both the loin and half carcass (when comparing predicted variables with DXA estimates). This is in agreement with previous studies (Marcoux et al., 2003; Marcoux et al., 2005; Mercier et al., 2006; Mitchell, Scholz, & Conway, 1998; Mitchell et al., 2003; Mitchell, Scholz, Pursel, et al., 1998). Mitchell, Scholz and Conway (1998) observed that the difference between DXA and chemical/dissected values tend to be larger in carcasses with less fat. In the present study, hams and shoulders were the leanest primal cuts and appeared to have the largest percent of underestimation. These observed discrepancies have largely been attributed to the inherent errors in DXA technology, including the assumption of constant hydration state of animal tissue (Roubenoff et al., 1993), application of uniformly distributed phantoms in the technology

development as against the heterogeneous nature of animal carcasses (Mazess et al., 1990; Mercier et al., 2006), and the capability and assumption involved with the two energy peaks employed in the soft tissue estimate (Lukaski et al., 1999; Mercier et al., 2006).

DXA was originally calibrated to estimate human body composition (Suster et al., 2004) with uniform phantoms, like acrylic and aluminium (Kelly, Berger, & Richardson, 1998). DXA estimation of pork carcasses may not be expected to exactly correspond with chemical or dissected values. Moreover, DXA usually uses R-value (which is the ratio of mass attenuation coefficient of soft tissue at the low energy relative to that at the higher energy) to determine the fat content of the scanned cuts (Scholz et al., 2015). However, this relationship has been found to be non-linear over the wide range of carcass fatness (Mercier et al., 2006; Mitchell, Scholz, & Conway, 1998; Svendsen et al., 1993) and may constitute some errors in the estimation of fat content of the soft tissue mass, especially in a wide range of animal population. Mitchell, Scholz, Pursel et al. (1998) reported that DXA algorithms would overestimate the percentage of fat in pigs containing more than 20% fat and, at the same time, underestimate the percentage of fat in pigs with less than 20% fat. This is contrary to the results from Suster et al. (2004), who reported that the DXA systematic underestimation applies to carcasses of high fat content and overestimation applies to leaner carcasses. This contrary view may be due to the difference in instrument make and software versions used in the two experiments (Lunar versus Hologic instrument, respectively).

Unlike the dissected/chemical fat, lean composition of primal cuts and carcass side was always overestimated by DXA estimation in the present study. The belly was the least affected, with the percentage of overestimation for lean being around 4.7%, followed by 17.4% for ham, 20.9% for half carcass, 21.3% for shoulder and about 25.6% for loin. This is largely in agreement with previous literature (Mercier et al., 2006) and could be a result of the factors highlighted in the preceding paragraphs. It is also important to note that the fat underestimation or lean overestimation observed in DXA equipment may not be due only to its own inherent error but also to the inaccuracies involved with manual dissections. This can be observed in the lower level of underestimation reported in belly primal (which was assessed using chemical analysis) compared to other primal cuts subjected to manual dissection (5.6 vs >20%). It is expected that chemical analysis will be able to assess intramuscular fat which manual dissection may not be able to quantify and as such may present a better representation of total carcass composition.

3.4.5 Effects of variation in population on DXA prediction

As the interaction of sire breed, sex, diet and slaughter weights were not significant (except occasionally in sire breed × sex interaction), only main effects were reported. Although gender and slaughter weight significantly influenced the fat and lean content observed in all the primal cuts and carcass sides, these factors did not have any impact on the prediction accuracy of their respective equations compared to the equation derived from the overall animal population. This can be observed with the R^2 and RSD values, which did not change drastically among the main effect classified equations and also in comparison with equations derived from the overall animal population (Tables 3.3 to 3.7). Previous studies have reported that gender (Marcoux et al., 2005; Pintauro, Nagy, Duthie, & Goran, 1996) did not have an effect on the prediction accuracy of DXA lean and fat mass and, as such, DXA measurements may not need to be adjusted for this within the pig population.

Previous reports have shown that breed did not have any significant effect on the prediction accuracy of DXA lean and fat mass (Marcoux et al., 2003; Marcoux et al., 2005; Pintauro et al., 1996; Suster et al., 2003); however, in the present study, comparing the sire breed classified equations with the equations derived from the overall pig population for all primal cuts and carcass sides, it can be observed that the coefficient of determination was negatively affected (reduction in R^2) even though no effect was observed for the RSD values and the slope of the sire breed classified equations (Tables 3.3 to 3.7). The reason for these decreases in R^2 values is unclear but could be due to the wide variation in these pigs' genotypic characteristics and phenotypic features. The Iberian crossbreds consistently had the highest fat content in all primal cuts and carcass sides in the present study, followed by Lacombe and Duroc crossbreds. With genotype constituting this wide range of fat-to-lean ratio in the pig population, it seemed clear that each genotype occupied unique fat/lean range and, as such, forms clusters specific to each sire breed (Figures 3.2 and 3.3). When these regression clusters from each genotype are grouped in a single regression equation, the wide variability in the pig population enabled a very high R^2. However, when comparing within genotype, the range within the group was drastically reduced, and this had a large influence on the R^2 value. As such, a more robust and broadly applicable equation may be developed when widely varying genotypes are employed in model development compared to when a narrow range of genotype variation. This may be the case in this study and, as a result, the overall model can be used to correct DXA algorithms for varying carcass measurements.

Diet did not affect the fat or lean components of the carcasses in this pig population nor did it affect the DXA prediction of dissected/ chemical fat and lean in the primal cuts and carcass sides. The test of slope heterogeneity also showed that the regression equations for the factors classified pig populations were generally not significantly different from each other (Table 3.8) and, as such, may not necessarily be adjusted for in the prediction equations. Although some slopes appeared to be significant or showed some trends (e.g., loin fat and ham lean), it stands to be determined if these will have any practical implication within the pig population.

3.5. Implication for research and industrial applications

Estimation of the carcass composition by DXA has also been reported to vary from instrument to instrument (Lösel, Kremer, Albrecht, & Scholz, 2010); currently, Norland (now Swissray), Hologic, Diagnostic Medical System (DMS) and Lunar are the major manufacturers. This variation, which may influence individual estimation, may be due to the underlying beam technologies used by each manufacturer. The DXA unit employed in the present study uses the narrow angle fan beam technology, which is often referred to as third generation DXA technology. This technology allows images to be acquired without magnification (Nord, Homuth, Hanson, & Mazess, 2000). The narrow angle beam uses multiple passes to acquire multiple images which are reconstructed to combine the precision of pencil beam and the speed of wide angle fan beam, the two earlier generations of DXA technology. More recently, the fourth-generation cone or flash beam DXA technologies (e.g. Lexxos scanner) has been developed (Scholz et al., 2015), and this holds a great promise for a wider application of this technology in the meat industry.

Recent advances in the beam technologies and DXA technology seem promising for subsequent online application in the meat industry. Mitchell et al. (2003) have shown that the chain speed of the modern slaughter facilities is approximately 16.6 cm/s whereas an average DXA equipment scans at the rate of 4 to 16 cm/s. Although this DXA speed has improved over the years, subsequent achievement of scan time equivalent to industrial or slaughter house chain speed and the possibility of collating multiple scans from carcass cross sections into a single point could further bring this technology into wider application in carcass composition assessment, especially in fast-paced industrial/slaughter house settings. The equipment may also need to be modified to accommodate the harsh temperature and humidity condition in meat processing facilities and animal slaughter houses.

Furthermore, as animal populations in the pork market vary widely in genetic features, physical characteristic and management strategies, development of a very robust model to correct for the inbuilt DXA algorithms may be crucial. Among all the variations examined in the present study, none but sire breed affected the prediction accuracy of DXA in terms of reduced R^2 values when pigs were classified based on sire breed variation. It remains to be verified if a model developed using a particular breed population could be used to predict another completely different population of pigs with limited body composition estimation errors. However, a model developed with a widely varying breed population, as developed in the present study, may be more robust, accurate and applicable to a larger pig population and processing environment with theoretically little or no error in body composition estimation assessment.

Regardless of these limitations, DXA still predicts carcass composition and live animal body composition to a very high degree of accuracy, and may be applied in the research and industrial environment, especially where compromise could be reached between speed and accuracy. With the present technical progress and operation speed of DXA technology, especially in the aspect of online physical hazard inspection and quality control (% fat analysis) of boxed meat products and ground meat batches, it holds promise not only for the meat processing and animal slaughter plants, but also in performance testing in animal breeding programmes, carcass grading and finally selection or payment of meat-producing animals. Scholz et al. (2015) emphasized the accuracy, as well as the superiority of DXA technology, compared to other non-invasive methodologies (MRI, ultrasound imaging and CT) in terms of its immediate delivery of a whole body composition results without subsequent image manipulations. Bernau et al. (2015) indicated the possibility of using DXA as a tool to update outdated regression equations as a result of altered genetics or genders within animal population for performance testing purposes. However, continued adaptation of this technology to industrial pace and processes, as well as further compositional analysis, is still warranted.

3.6. Conclusions

The third generation DXA technology used in the present study accurately and equally predicted lean and fat composition in pork carcasses. Dual energy X-ray absorptiometry hold great promise, in particular, for pork bellies compositional assessment where chemical analyses and manual dissection are very time consuming. Its estimation could be used to sort high/low fat bellies

accurately for specific market requirements and this could offer financial rewards to pork producers. This information could also be used to provide signals to producers to subsequently improve pork belly composition. Within the limits employed in the present study, wide variation in pig populations did not affect the prediction accuracy of DXA. The only exception was sire breed, possibly due to a reduction in the population range within individual breed, leading to a reduction in the coefficient of determination. In most cases, based on the test of the slope heterogeneity, a more robust model, as developed in the present study, could be widely applied to varying pig populations. The possibility of eradicating the underlying limitations associated with DXA while considering the inherent variation in pig populations will move this technology a step closer to being a gold standard in compositional assessment, and may likely replace the traditional dissection methods presently employed in experimental settings.

3.7 Connection to the next study

Carcass composition plays a crucial role, not only in consumers' acceptability of meat and its products but also in many technological attributes that may influence further processing and product classification and marketing. Although traditionally, invasive methodologies have been widely employed for compositional assessments, the advent of more non-invasive options will be advantageous for researchers in evaluating animal growth and genetic selection, as well as for the entire meat industry for carcass classification, grading and assessment.

While DXA offers this promising possibility with its efficiency largely unaffected by variation within the population, pork belly softness, which is a major quality defect in the pork industry, will be contributed to by other factors other than the pork belly's chemical composition. Having the understanding that this attribute is multifactorial in nature, the next study intends to explore beyond chemical attributes, the overall factors that may influence pork belly softness and how the variation within the animal population influences this attribute.

CHAPTER 4

COMPOSITIONAL AND DIMENSIONAL FACTORS INFLUENCING PORK BELLY FIRMNESS[5]

4.1 Abstract

Various dimensional and compositional factors that can influence the perception of pork belly firmness were explored. Bellies from 198 pigs representing widely variable population (three different genotypes, two sexes, two slaughter weights and three different diets) were recovered and belly firmness was assessed using the belly-flop angle and a 5-point scale subjective measurement. Dimensional and compositional factors were recorded on intact and sheet-ribbed bellies. Subjective belly score was negatively correlated with belly-flop angle (r = -0.89). Regression analysis accounted for 77 and 83% of the variability in subjective belly firmness and belly-flop angle measurement, respectively. Belly length, weight and width influenced both measures of belly firmness, but these effects were more important for the belly-flop angle. After correcting flop angle using belly length, the effect of belly weight disappeared and the effect of other traits was more like those observed for subjective scoring. Hence, the effect of belly length should be corrected for if this technique is to be implemented in commercial plants.

---

[5] Published and used with permission from:

Soladoye, O. P., Uttaro, B., Zawadski, S., Dugan, M. E. R., Gariépy, C., Aalhus, J. L., Shand, P., and Juárez, M. (2017). Compositional and dimensional factors influencing pork belly firmness. Meat Science. 129, 54-61.

O. P. Soladoye designed and conducted experiments, analysed data, compiled and interpreted results, drafted, revised and finalised manuscript.

4.2 Introduction

Pork belly softness is associated with increased handling/processing difficulties, reduced slicing and fabrication efficiency, reduced bacon yield, increased fat separation and/or oily unattractive appearance in packaged bacon, as well as reduced product shelf life due to poor oxidative stability (Eggert, Belury, Kempa-Steczko, Mills, & Schinckel, 2001; Soladoye et al., 2015; Trusell et al., 2011). According to Sather, Jones, Robertson, and Zawadski (1995), genetic, dietary or management strategies directed towards increased lean content in pork carcasses may result in belly softness. Such an effect could be explained by an increased proportions of moisture and polyunsaturated fatty acid (PUFA) of the adipose tissue in lean pigs (Trusell et al., 2011).

Pork belly firmness appears to be a multifactorial quality trait. Its perception is contributed to by many interacting factors, with iodine value (IV) being the most reported reference method for assessment despite the fact that it could account for only a relatively low proportion of the variability observed in belly firmness. Indeed, Whitney et al. (2006) reported that belly thickness explained 33% of the variability in belly firmness, compared to the 14% explained by the IV. However, most industrial objective assessments of belly firmness have focused solely on assessing belly or fat firmness (Correa et al., 2008; Davenel et al., 1999) using either calculated or instrumental measurements of IV. Iodine value itself has been widely criticized for its expensive, destructive and time-consuming nature. Furthermore, the compositional gradient along belly length and width (Trusell et al., 2011), as well as among belly fat and lean layers (Apple et al., 2011) makes it difficult to decide on a representative sampling site for IV measurement. All these observations have caused White et al. (2009), among others, to conclude that IV may not be the best measure for assessing pork belly firmness.

Other methods have been employed among different research groups to assess pork belly firmness, including visual firmness scoring (Weber et al., 2006), finger-pressure testing (Maw et al., 2003), compression and puncture test using texture analysers (Trusell et al., 2011) and belly-flop testing (Murray, Robertson, Johns, & Landry, 2002). Belly-flop tests have been used in different variations in the literature based on the shape of the suspending support. This method measures either the distance between the caudal and cranial ends of the suspended belly or the angle of the isosceles triangle formed in the suspended belly either on a round bar (Engel et al., 2001; Jackson et al., 2009; Larsen et al., 2009; Uttaro & Zawadski, 2010) or a V-shaped smokehouse stick (White et al., 2009; Whitney et al., 2006; Widmer et al., 2008). However, part

of the softness variability measured by belly-flop may be due to the influence of belly dimensions rather than actual firmness. Thus, the objective of the current study was to assess the relative contributions of dimensional and compositional factors on pork belly firmness measurements.

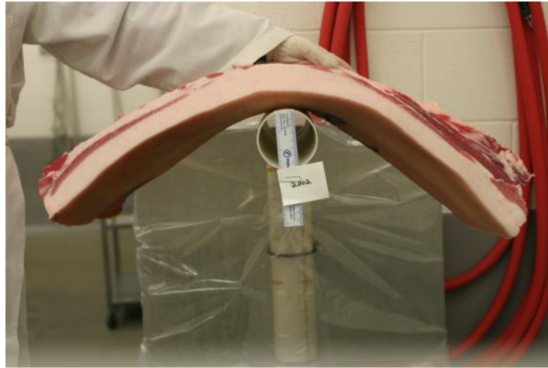
4.3 Materials and methods

4.3.1 Animal managements and slaughter

In order to obtain variability in belly firmness, 198 barrows and gilts, from the mating of Duroc, Lacombe, or Iberian sires to commercial Large White × Landrace dams, were fed one of three diets: 1) control, commercial diet; 2) a high-oleic acid, canola-based diet (10% ExtraPro; O & T Farms Ltd., Regina, SK, Canada); or 3) a high-linolenic acid, flaxseed-based diet (10% LinPro; O & T Farms, Ltd.) for the last three weeks prior to slaughter at either 120 or 140 kg. Details of this pig population and treatments have been recently published (Soladoye et al., 2016). All dietary treatments and experimental procedures were approved by the Lacombe Research and Development Centre Animal Care Committee, Lacombe, Alberta, Canada. Pigs were cared for as outlined under the guidelines established by the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). On reaching the required slaughter weights, pigs were transported only 1 km to the federally-inspected abattoir at the Agriculture and Agri-Food Canada, Lacombe Research and Development Centre. Following conventional slaughter processes, carcasses were chilled overnight at 2ºC.

4.3.2 Pork belly selection, fabrication and firmness measurement

Pork bellies were fabricated from the left carcass sides in accordance with the guidelines of the Canadian Pork Buyer's Manual (Canadian Pork International, 1995), and the weights of the bellies were recorded. The bellies were separated from the shoulder between the second and third ribs, with a straight cut perpendicular to the long axis of the carcass side, and from the hind leg with a cut positioned about 3 cm cranially from the exposed aitch bone and lined up with the first coccyl vertebra (Uttaro & Zawadski, 2010). Each belly was positioned on an adjustable inclined support such that the dorsal plane (the line of severance from the loin) was perpendicular to the table top (Figure 4.1). Images of these sides were taken using a digital camera (Canon A-80, 4 megapixel) with an integrated lens. Following image capture, the ribs were removed from the bellies as a single sheath.



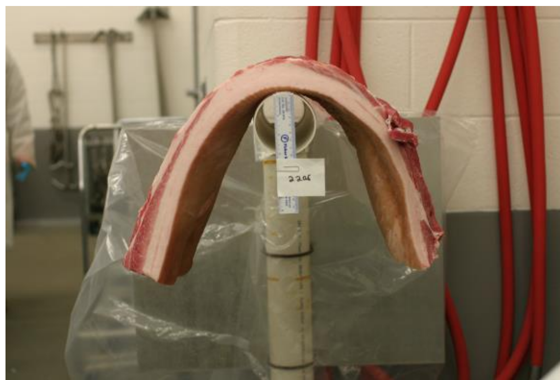


Figure 4.1: Component for assessing belly-flop angle measurements with the firmest to the softest belly in the research shown.

The sheet-ribbed bellies were thereafter suspended skin side down on their central short axis over an 8.3-cm-diameter, round bar, such that both the caudal and the cranial ends could freely fall (Figure 4.1). After 2 minutes of suspension on the bar, images of the bent bellies were again captured. A 5-point visual and tactile response scale, based on commercial practices, was also used to subjectively categorize bellies into one of the following classes: (1) firm fat, no finger depression, almost horizontal (firmest belly class); (2) firm fat, no finger depression, partly floppy; (3) soft spongy fat, finger depression remains, floppy, roll over with resistance; (4) soft spongy fat, finger depression remains, very floppy, roll over easily; and (5) soft spongy fat, finger depression remains, very floppy, roll over easily, oily (softest belly class). Two assessors independently assessed these bellies and classed them into any of the above categories. Bellies were also allowed to be assigned values between these categories.

The acquired belly images were processed using imageJ software (v 1.32j; available at http://rsb.info.nih.gov/ij; developed by Wayne Rasband, National Institute of Health, Bethesda, MD) to collect measurements on the images. The following measurements were taken according to Uttaro and Zawadski (2010), with slight modifications based on personal communications with Uttaro (Figure 4.2): thickness of the side lean (*latissimus dorsi*) at the thickest point (**SLn**); thickness of the side fat (subcutaneous and intermuscular fat, which may include the *cutaneous trunci*) at the shoulder end where the SLn was measured (**SFt**); *cutaneous trunci* at shoulder end (point where SLn was measured) and midpoint (**CuTr1** and **CuTr2,** respectively); belly-side thickness measured from the caudal end of the *latissimus dorsi* either with or without the rib just adjacent to this perpendicular axis (**SThK** and **SThK1,** respectively); thickness of intermuscular fat just below the rib and just in front of the same rib after the tail end of *latissimus dorsi* (**Seam 1** and **Seam 2,** respectively); thickness of subcutaneous fat just above and below *cutaneous trunci* (**Subq1** and **Subq2,** respectively). Belly-flop angle was measured as the angle created at the belly pivot point where each leg of the angle terminated at the outermost corner of the skin. Other measurements included ribbed-belly weight, length and widths at midpoint and at shoulder end. All these measurements constitute the dimensional factors considered in the present study and all these belly fabrications occurred in a cooler at temperature of 7 to 10°C.

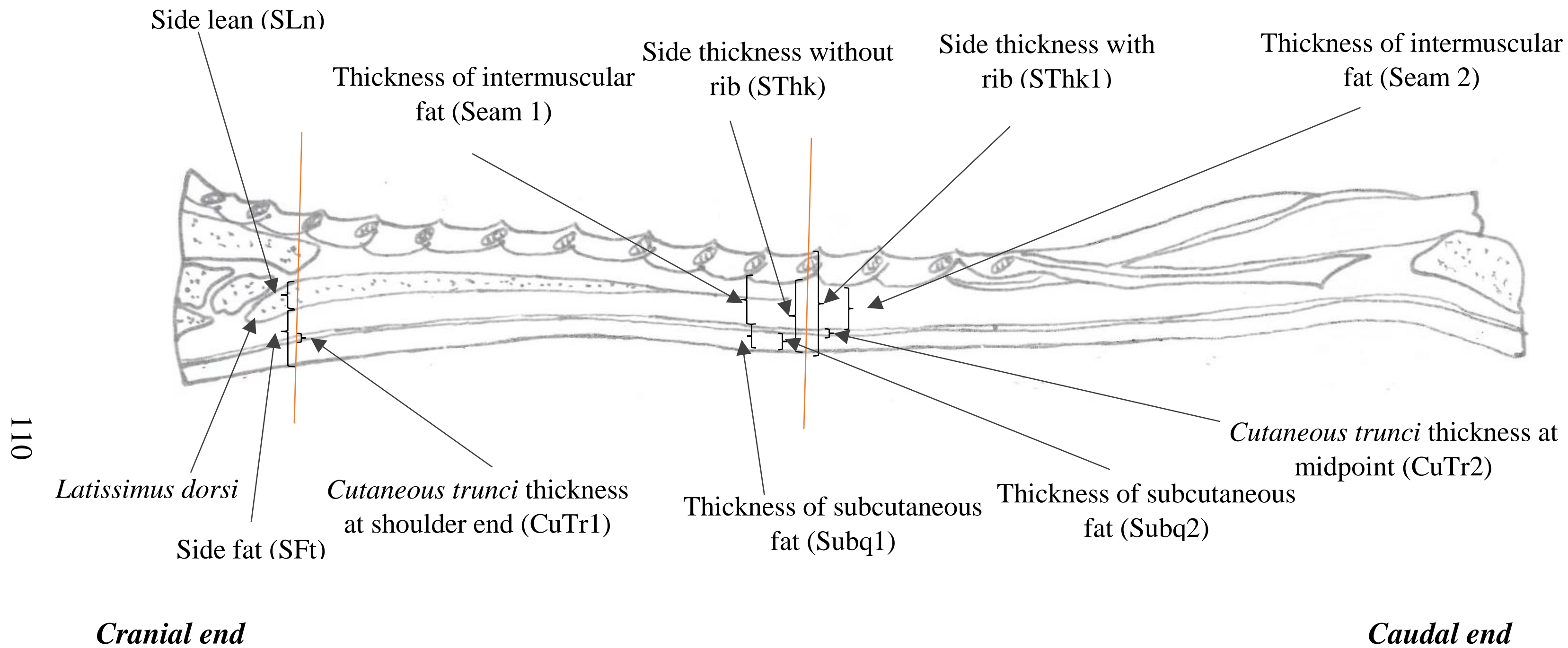


Figure 4.2: Measurement locations along the pork belly

4.3.3 Chemical analysis

Following the belly-flop tests and dimensional assessments, samples of intermuscular fat, subcutaneous fat and *latissimus dorsi* were taken within 15 cm of the cranial end of the belly. Fatty acid profiles of each were determined according to the procedure described by Turner et al (2014). Briefly, lean tissues were comminuted (Robot Coupe BX3, Ridgeland, MS, USA) and 0.75 ± 0.01 g subsampled for fatty acid analysis. Muscle lipids were extracted using 2:1 chloroform : methanol, with a solvent-to-sample ratio of 20:1 (Folch, Lees, & Stanley, 1957). Muscle lipid extracts (1 mL) were dried using a rotoevaporator (P12 Rotavapor, Buchi, Thornhill, ON, Canada) after which they were freeze dried (Genesis 25 SQ EL, VirTis Company, Gardiner, NY, USA) overnight to remove other traces of moisture. Similarly, adipose tissues were subsampled (40 ± 1 mg) and freeze dried overnight. The freeze-dried lean and adipose tissue lipids were dissolved in toluene-containing internal standard (10% of lipid weight, methyl nonadecanoic acid; Nu-Chek Prep Inc. Elysian, MN, USA) and directly methylated with 5% methanolic hydrochloric acid at 80 °C for 1 h. Samples were cooled before deionized water and hexane were added and then 0.88% potassium chloride to separate hexane containing the fatty acid methyl esters. Hexane extracts were transferred and dried over anhydrous sodium sulphate inside amber vials and stored at -20 °C until analysis. Fatty acid methyl esters were analysed by gas chromatography as described previously by (Dugan et al., 2007). Individual fatty acids were identified and IV was calculated as: IV = $([16{:}1] \times 0.95) + ([18{:}1] \times 0.86) + ([18{:}2] \times 1.732) + ([18{:}3] \times 2.616) + ([20{:}1] \times 0.785) + ([22{:}1] \times 0.723)$, where brackets indicate the proportion of a particular FA (AOCS, 1998). The total proportion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA, omega-6 (n-6) and omega-3 (n-3) were also calculated.

For proximate analyses, the whole belly was ground twice through a 3-mm plate (Butcher Boy Meat Grinder Model TCA22, Lasar Manufacturing Co., Los Angeles, CA, USA), and subsampled for measurement of percent fat and moisture according to the methods described by Anderson (2004) and Soladoye et al. (2016), respectively. The lean content in this study was estimated as 100% minus percent fat assuming ribbed bellies were made up of only fat and lean (DM basis).

4.3.4 Statistical analysis

Pearson correlation coefficients among all variables were obtained using the correlation procedure of SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA), whereas multiple regression models among all the dimensional and compositional factors (53 variables altogether) were built using the PROC REG of SAS for both belly-flop angle and belly score measurements. Belly-flop angle measurements were corrected for the variation in belly length using PROC GLM of SAS by calculating the residuals between predicted and actual values when belly length was used to predict belly-flop angle. Following this correction, a new model was built for these corrected values. Collinearity among various parameters was eliminated using the variable inflation factor < 10 to ensure all predictors in the model were not significantly dependent on one another. Both forward and backward stepwise regression procedures were used to select the most precise and least biased models for subsequent predictions. Predictors were only retained in the model when they were significant ($P < 0.05$).

4.4 Results and Discussions

4.4.1 Description of pork belly characteristics

Bellies were selected from carcasses with considerable compositional and phenotypic variation, resulting in a wide range in belly firmness as evidenced by the belly-flop angles (18.5 to 125.3°) and subjective firmness scores (1.5 to 5.0; Table 4.1). As expected, the percentages of lean, fat and moisture also varied widely, as well as the proportions of SFA (32.3 to 46.5%), MUFA (43.0 to 67.2%), PUFA (6.5 to 17.1%), and IV (53.6 to 71.5). These factors could largely explain some physical traits, especially softness, of bellies. According to the National Pork Producer Council (NPPC, 2010), good quality pork fat should normally have < 15% PUFA and > 15% stearic acid (C18:0). Moreover, Renfrow et al. (2003) have also reported that linoleic acid > 14% is associated with soft fat. Although IV values below 70 are preferred for acceptable fat quality, a threshold up to 75 could be permitted depending on the prevalent production system in specific locations (Benz et al., 2010; Madsen et al., 1992). Stearic acid ranged between 8.7 and 17.5%, whereas linoleic acid (C18:2) ranged between 4.8 and 12.3% (Table 4.1). The variation among these physical and chemical factors with breed, sex, diet and slaughter weights are shown in Appendix A2 and A3.

The IV, as well as the proportion of PUFA and PUFA/SFA, varied with belly layers, with an observable increase from the inside (lean layer) to the outside (subcutaneous fat), signifying higher degree of unsaturation from inside to outside (Table 4.1). Previous studies have also reported higher unsaturation in the fatty acid profile of the subcutaneous fat of the belly compared to the lean and the intermuscular fat (Apple et al., 2011; Monziols, Bonneau, Davenel, & Kouba, 2007). The greater concentration of structural lipids (e.g. phospholipid) in the muscle layer, which is not readily affected by dietary fat, contributes to the fatty acid compositional variations of this belly layer (Benz et al., 2010). As a compositional gradient exists along the belly length and width (Trusell et al., 2011), variation in composition and fatty acid profile among the fat and lean layers of belly also exist (Yancey, Apple, Sawyer, Lee, & Woodworth, 2008). Similar to other studies (Apple et al., 2011; Monziols et al., 2007), however, intermuscular fat had the highest proportion of SFA, followed by the *latissimus dorsi* and then the subcutaneous fat, which had the least (Table 4.1). Similar result was also reported by Cook (2015), who also observed the highest level of SFA in intermuscular fat compared to other fat layers. Hence, the intermuscular fat layer may be important to the overall pliability of pork bellies as SFA have been found to be strongly and positively correlated with belly firmness (Trusell et al., 2011).

Table 4.1: Summary of compositional and dimensional factors within the pig population*

	Mean	SD	Minimum	Maximum
Belly weight (kg)	9.2	1.3	6.4	14.7
Ribbed belly weight (kg)	7.6	1.3	5.3	11.2
Length (cm)	70.6	2.6	61.9	77.3
Width (cm)	26.3	1.7	21.5	32.0
Thickness (cm)[1]	6.00	1.1	3.6	8.7
Moisture content (%)	41.3	8.4	24.0	60.1
Fat content (%)	46.0	10.9	21.9	68.7
Lean content (%)	54.0	10.9	31.3	78.1
Flop angle (°)	52.5	26.0	18.5	125.3
Subjective score[2]	3.2	0.9	1.5	5.0
Subcutaneous fat layer				
SFA (%)[3]	36.8	1.7	32.3	42.5
Palmitic acid (%)	23.9	1.0	21.5	26.8
Stearic acid (%)	11.1	1.0	8.7	15.5
MUFA (%)[4]	51.0	1.6	45.8	55.1
PUFA (%)[5]	12.2	1.4	9.4	16.4
n-6 fatty acids (%)	10.2	1.0	8.0	12.7
n-3 fatty acids (%)	2.0	0.7	1.0	4.1
Linoleic acid (%)	9.5	1.0	7.5	12.0
PUFA/SFA	0.33	0.04	0.23	0.47
Iodine value (IV)[6]	64.6	2.5	58.0	71.5
Intermuscular fat layer				
SFA (%)[3]	41.2	2.1	32.9	46.5
Palmitic acid (%)	25.4	1.0	21.6	28.1
Stearic acid (%)	14.0	1.4	9.6	17.5
MUFA (%)[4]	48.0	2.0	43.0	53.0
PUFA (%)[5]	10.8	1.7	7.7	16.3
n-6 fatty acids (%)	8.9	1.1	6.8	13.1
n-3 fatty acids (%)	1.9	0.8	0.9	4.3
Linoleic acid (%)	8.3	1.1	6.3	12.3
PUFA/SFA	0.26	0.10	0.17	0.46
Iodine value (IV)[6]	59.7	2.9	53.0	70.7
Lean layer				
SFA (%)[3]	38.8	1.4	35.1	43.3
Palmitic acid (%)	24.8	0.9	21.9	26.5
Stearic acid (%)	12.2	0.8	10.3	15.2
MUFA (%)[4]	51.3	1.5	47.2	55.0
PUFA (%)[5]	9.9	1.7	6.5	17.1
n-6 fatty acids (%)	8.3	1.3	5.6	14.4
n-3 fatty acids (%)	1.6	0.5	0.8	3.9
Linoleic acid (%)	7.0	1.1	4.8	11.4
PUFA/SFA	0.26	0.10	0.16	0.48
Iodine value (IV)[6]	58.6	2.0	53.6	67.2

[1]Belly thickness before ribs were removed as a single sheath (SThk1 in Figure 4.1). * n=198

[2] 1 = firmest belly to 5 = softest belly.

[3] SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0

[4] MUFA = C16:1cis7 + C16:1cis9 + C18:1cis9 + C18:1cis11 + C18:1cis13 + C20:1cis11

[5] PUFA = C18:2n-6 + C18:2n-3 + C20:2n-6 + C20:3n-6 + C20:3n-3 + C20:4n-6 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3

[6] IV = ([16:1] × 0.95) + ([18:1] × 0.86) + ([18:2] × 1.732) + ([18:3] × 2.616) + ([20:1] × 0.785) + ([22:1] × 0.723), where brackets indicate the proportion of a particular fatty acid.

4.4.2 Pearson correlation coefficients among belly softness parameters

The correlation between belly-flop angle and subjective belly softness score was negative ($r =.-0.89$, $P < 0.01$) in the present study. Significant correlations were also observed between the compositional and dimensional parameters and the objective measure of belly softness. Of the dimensional parameters, those associated with belly lean content/thickness, SLn ($r = -0.59$), CuTr1 ($r = -0.44$) and CuTr2 ($r = -0.31$), were negatively correlated with belly-flop angle (Table 4.2). These results support the generally accepted association between increased belly leanness and increased belly softness (Apple et al., 2007; Wood, Enser, Whittington, Moncrieff, & Kempster, 1989) as belly leanness is related to both increased moisture and PUFA content (Fiego et al., 2005; Sather et al., 1995) and reduced belly thickness (Averette Gatlin, See, Hansen, & Odle, 2003). Other dimensional factors associated with the fat layers of the belly (SFt, SThK, SthK1, Seam 1, Seam 2, Subq1 and Subq2) had moderate to strong positive correlations with belly-flop angle ($r = 0.69$ to 0.76, $P < 0.01$), suggesting that increased belly fat deposition and thickness will enhance belly firmness. Similarly, belly-flop angle measurements were positively correlated ($P < 0.05$) with belly weight and length. Although belly weight and belly length were both positively correlated with each other ($r = 0.60$), the former was more strongly correlated with belly-flop angle measurement than the latter ($r = 0.58$ *vs* 0.14). Surprisingly, width at belly midpoint was more strongly and negatively correlated with belly-flop angle ($r = -0.43$) compared to a similar measurement at the shoulder end of the belly, which was positively, but weakly correlated to the same measure ($r = 0.15$), suggesting that belly width at the midpoint is more likely associated with the objective measurement of belly softness than belly width at the shoulder end. The negative relationship at the midpoint also implies that the leaner and softer a pork belly is, the wider it will be at the belly's midpoint. Similarly, width at midpoint and shoulder end were positively and negatively correlated with lean content ($r = 0.36$ and -0.23, respectively, $P < 0.01$). Moreover, all the fat layer measurements (including the intermuscular and subcutaneous fat) were negatively associated with subjective belly softness, whereas dimensional parameters related to lean were positively associated with the same subjective belly softness (1 = firmest belly to 5 = softest belly).

As expected, moisture content of pork bellies was negatively and positively correlated ($P < 0.01$; Table 4.2) with belly-flop angle and subjective belly softness score measurement ($r = 0.70$ and -0.71, respectively). Fat content was negatively correlated with belly moisture content (r = -

0.99) but positively correlated with belly-flop angle (r = 0.70) and negatively correlated with belly softness score (r = -0.71). These correlations confirmed previous reports demonstrating that increase in fat accumulation results in increased belly firmness (Correa et al., 2008; Marcoux, Pomar, Faucitano, & Brodeur, 2007). Many compositional factors considered in the present study were also significantly correlated with both subjective and objective measures of belly softness (Table 4.3). The SFA content of belly layers was positively correlated with belly-flop angle (r = 0.20 to 0.56), whereas PUFA content of belly layers was negatively correlated with belly-flop angle (r = -0.35 to -0.72), which are much higher correlation values than those reported by (Rentfrow et al., 2003). Palmitic acid (C16:0) content had a higher correlation coefficient with belly softness measurements than stearic acid (C18:0) content, and this difference was greater in the lean layer (Table 4.3). Rentfrow et al. (2003) also reported a similar observation with C16:0 being more highly correlated with measures of belly softness than C18:0. Due to its longer chain and, hence, higher melting point, C18:0 may be expected to have a higher association with belly firmness; yet, the nearly doubled proportion of C16:0 in pork belly layers compared to C18:0 (Table 4.1) may be the major factor influencing the observed association of SFA with belly firmness. Furthermore, linoleic acid (C18:2) showed a strong relationship (r = 0.62 to 0.72) with belly softness measurements compared to IV (r = 0.58 to 0.67), the most commonly used objective measure, and this was consistent in all belly layers. Except in the lean layers, MUFA was not ($P > 0.05$) correlated with belly-flop angle or subjective belly softness (Table 4.3). Although oleic acid (C18:1 cis-9) was previously reported to be negatively associated with soft fat (Miller, Shackelford, Hayden, & Reagan, 1990), the lack of any relationship between MUFA and belly softness in our study may be due to the fairly low range of C18:1cis-9 in this population of pigs (Table 4.1).

Table 4.2: Pearson correlation between dimensional parameters, proximate analysis and the belly softness measures ǂ

Belly softness measures	MC	Fat	WdthSh	Wdt	Lgt	Wgt	Sln	Sft	Ctr1	Sthk	Sthk1	Ctr2	Seam 1	Seam 2	Subq1	Subq2
Belly-flop angle	-0.70	0.70	0.15	-0.43	0.14	0.58	-0.59	0.71	-0.44	0.75	0.76	-0.31	0.67	0.69	0.72	0.70
Subjective belly firmness	0.71	-0.71	-0.12	0.40	-0.24	-0.58	0.53	-0.71	0.43	-0.73	-0.75	0.28	-0.66	-0.70	-0.71	-0.69

ǂ All values were significant at $P < 0.01$. MC: Moisture content; Lgt: length; Wgt: Weight; Fat: Total fat; Wdt; width at midpoint of the belly, WdthSh; Width at shoulder end of the belly, SLn; thickness of *latissimus dorsi* at the thickest point, SFt; thickness of subcutaneous + intermuscular fat at the shoulder end, CuTr 1 &2; *cutaneous trunci* at shoulder end (point where SLn was taken) and mid-point respectively, SThK & SThK1; belly side thickness measured from the caudal end of the *latissimus dorsi* either with or without respectively, the rib just adjacent to this perpendicular axis, Seam 1 & 2; Thickness of intermuscular fat below the rib and just in front of the same rib after the tail end of *Latissimus dorsi* respectively, Subq 1 & 2; Thickness of subcutaneous fat just above and below *Cutaneous trunci* respectively (Refer to Fig 4.2).

Table 4.3: Pearson correlation between selected fatty acid profiles and belly softness measures [Ψ]

Layer	C16	C18	C18:2	IV	MUFA[ǂ]	SFA	PUFA	n-6	n-3	n-6/n-3	PUFA/SFA
					Belly-flop angle measurements						
Lean (*Latissimus dorsi*)	0.56	0.20	-0.62	-0.58	0.27	0.46	-0.62	-0.60	-0.46	0.24	-0.63
Intermuscular fat	0.48	0.32	-0.69	-0.62	0.07	0.45	-0.65	-0.70	-0.38	0.18	-0.66
Subcutaneous fat	0.54	0.32	-0.72	-0.67	0.05	0.50	-0.68	-0.72	-0.35	0.17	-0.71
					Subjective belly firmness score						
Lean (*Latissimus dorsi*)	-0.56	-0.27	0.64	0.61	-0.27	-0.49	0.65	0.62	0.50	-0.26	0.66
Intermuscular fat	-0.43	-0.34	0.72	0.64	-0.10	-0.44	0.68	0.73	0.40	-0.19	0.69
Subcutaneous fat	-0.48	-0.37	0.70	0.67	-0.06	-0.49	0.68	0.70	0.37	-0.19	0.70

[Ψ] All values were significant at $P < 0.01$

[ǂ] Pearson correlation for MUFA in the belly layers for both belly-flop and subjective score measurements are not significant except only in the lean layer.

MUFA here includes summation of c7-16:1, c9-16:1, c9-18:1, c11-18:1, c13-18:1 and c11-20:1, SFA includes summation of c14:0, c16:0, C17:0, c18:0 and c20:0 and PUFA includes summation of c18:2n-6, c18:2n-3, c20:2n-6, c20:3n-6, c20:4n-6, c20:3n-3, c20:5n-3, c22:3n3, c22:5n-3 and c22:6n-3.

4.4.3 Predictors of pork belly softness

Unsaturated fatty acids contribute to pork belly softness (Averette Gatlin, See, Hansen, Sutton, & Odle, 2002; Larsen et al., 2009; Leick et al., 2010; McClelland et al., 2012). However, because fatty acid compositions (Yancey et al., 2008) and other compositional attributes of pork bellies (e.g. moisture content) (Schroder & Rust, 1974b) vary among belly layers and also considering the belly's physical dimensions which may also affect the perception of pork belly softness, a multifactorial approach may provide the most accurate prediction of pork belly firmness/softness. A total of 53 measures of chemical composition and dimensional/physical attributes of the belly were used to predict both objective (flop angle) and subjective belly softness. Subjective belly softness was predicted with 8 unique predictors ($R^2 = 0.77$; Table 4.3) selected in the following order; omega-6 fatty acids of the intermuscular fat, Subq2, subcutaneous fat IV, overall fat content, belly width at midpoint, belly weight, belly length and intramuscular C18:2 content. The first 6 predictors explained up to 74% of the observed variation in subjective pork belly softness, whereas other predictors only marginally contributed to the models' predictability (based on R^2).

For the belly-flop angle prediction (Table 4.5), the final model included 7 regressors with an R^2 of 0.83. Predictors in their order of strength included Subq2, subcutaneous fat IV, width at midpoint, belly weight, belly length, intermuscular C16:0 and SLn. Considering the stepwise progression of the analysis, the first five predictors accounted for about 82% of the whole belly firmness variation.

4.4.4 Estimating the impact of chemical and physical factors in pork belly softness measurement

In the prediction model of the objective belly-flop angle, more dimensional/physical predictors were selected compared to more chemical predictors selected in the subjective belly softness score model (Tables 4.4 and 4.5). In fact, when these 7 selected predictors were separated into dimensional/physical and chemical predictors, belly-flop angle was predicted to about 78 and 48%, respectively. This disproportionate contribution of the dimensional/physical factors in this model confirmed the conclusion of Whitney et al. (2006), who stated that the degree of carcass fatness had a larger impact on belly firmness than its fatty acid composition when assessed with

the belly-flop method. Overall, the impact of the combined dimensional factors was higher than that from the combined chemical predictors in the prediction of the belly-flop angle measure of belly softness. On the other hand, analysing these dimensional and chemical factors separately against subjective belly firmness score resulted in models that could respectively explain about 68 and 70% of the variation, respectively, reflecting a more balanced contribution of both predictor groups. This observation may suggest that the belly-flop angle measurement could be overly influenced by dimensional properties of the belly and may not correctly represent the perception of belly firmness/softness.

Changes in carcass fatty acid profile will affect pork fat firmness (Correa et al., 2008; Davenel et al., 1999). However, this single factor does not totally explain the aggregate perception of pork belly firmness/softness. The fatty acid composition of the intermuscular fat had a larger influence on subjective belly firmness scores, whereas the subcutaneous fat dimension appeared to be the one factor having a larger influence on the belly-flop angle measurement (first selected predictor; Table 4.4 and 4.5). For both measurements, however, the important influence of the fatty acid composition of subcutaneous fat and/or its dimensions is undeniable and would significantly affect the overall pliability of pork bellies.

Although the thickness of the subcutaneous fat layer seemed to be the most influential dimensional variable in belly-flop angle measurements, the thickness of the *latissimus dorsi* also had a small, but significant, impact on objective belly firmness. The large variability in subjective and, especially, objective belly firmness measurements explained by thickness variables indicates that thickness traits, either subcutaneous fat, belly and/or lean thickness as well as belly width, could be considered, together with IV, for classification purposes. Whitney et al. (2006) demonstrated that both IV and belly thickness explained up to 37% of the observed variation in belly firmness. Even higher percentages of the variation in belly firmness have been explained by this combination of variables in the present study (see later section). Overall proximate composition of the belly did not seem to explain much variation in belly-flop angle measurements in the present study (Table 4.5).

Table 4.4: Stepwise regression model for subjective belly score measurements*

Step	Intercept	Intermuscular n-6	Subq 2	Subcutaneous IV	Total fat	Width Midpoint	Belly Weight	Belly length	Linoleic acid Lean	R^2	Cp	RMSE
1	-2.11	0.60								0.549	166	
2	0.83	0.42	-1.07							0.645	93.4	
3	-3.98	0.29	-1.01	0.09						0.677	70.5	
4	-3.03	0.17	-0.69	0.10	-0.02					0.710	46.2	
5	-4.39	0.17	-0.71	0.10	-0.02	0.07				0.724	37.7	
6	-3.82	0.13	-0.47	0.08	-0.01	0.12	-0.19			0.744	23.8	
7	-7.46	0.15	-0.30	0.08	-0.01	0.13	-0.30	0.06		0.761	12.5	
8	-6.94	0.11	-0.28	0.07	-0.01	0.13	-0.27	0.05	0.10	0.768	9.00	0.43

* Predictors all significant at $P < 0.05$; Subq2; thickness of subcutaneous fat just below *cutaneous trunci*. C_p; Mallow's C_p to assess model for overfitting, RMSE; root mean square error for the final model.

Table 4.5: Stepwise regression model for belly-flop angle measurements*

Steps	Intercept	Subq 2	Subcutaneous IV	Width Midpoint	Belly weight	Belly length	C16:0 Intermuscular fat	Sln	R^2	C_p	RMSE
1	-21.70	58.3							0.485	380	
2	320.8	40.0	-4.94						0.651	199	
3	380.1	37.5	-4.40	-3.47					0.696	152	
4	318.2	20.4	-3.27	-5.33	7.86				0.749	94.9	
5	529.6	10.3	-3.13	-6.03	14.8	-3.42			0.815	23.6	
6	404.8	11.0	-2.37	-6.07	14.5	-3.42	3.04		0.824	15.3	
7	412.3	9.11	-2.34	-5.51	13.7	-3.44	3.09	-7.37	0.831	9.40	10.9

* Predictors all significant at $P < 0.05$; Sln thickness of *latissimus dorsi* at the thickest point. Subq2; thickness of subcutaneous fat just below *cutaneous trunci*. C_p; Mallow's C_p to assess model for overfitting, RMSE; root mean square error for the final model.

However, subjective belly firmness scores were influenced by total fat content (Table 4.4). Jabaay et al. (1976) and Schroder and Rust (1974a) suggested that substantial variation in moisture and protein content, as well as an anterior-to-posterior separable lean content gradient, can contribute to the perception of belly softness.

Both subjective (Table 4.4) and objective (Table 4.5) belly firmness values were affected by belly length, width and weight. The influence of these variables was much larger for the belly-flop angle compared to subjective scores. Although length was the trait with the smallest contribution to the model for subjective evaluation, it may possibly explain more variability in belly-flop angle than width or weight. During the subjective test, part of the procedure involves manipulating the belly and, therefore, changes in dimensional traits may affect the evaluator's perception. In the case of the objective belly-flop, the belly is suspended across the midline on a round bar that goes from its dorsal to its ventral edges. Longer bellies will result in additional weight at the extremes of the sample, leading to an increase in bending and, therefore, a decrease in the measured angle. Thus, correcting the belly-flop angle measurements by belly length could influence the position of the belly during the test and potentially improve the accuracy and repeatability of the method.

Correcting the belly-flop angle measurements for belly length resulted in a new prediction equation (Table 4.6). As previously observed, a thickness measure and the subcutaneous fat IV explained most of the variability in the model; however, in this case, SThk1 replaced Subq2 as the first thickness value in the model, and belly width was still the third variable, but belly weight was no longer included in the model. This supports the hypothesis that belly length would influence the results of the test by modifying the weight at the extremes of the sample and, therefore, the final angle of the belly. This length influence might have come into play in the study of Apple et al. (2007), who found that bellies from pigs fed 10 $mg.kg^{-1}$ ractopamine were deemed softer when suspended skin-side down as in our study but then not considered as soft when tested with other methods such as belly compression. Although many studies have employed belly-flop as a measure of belly firmness and have in fact considered belly bends as “the standard measurement for discerning firmness difference” (Trusell et al., 2011), it is important to consider the possible undue influence of belly length on this measurement. The fourth variable was another thickness trait, Subq2, followed by the percentages of C16:0 in the intermuscular fat, C18:2 in the lean layer,

Table 4.6: Stepwise regression model for belly-flop angle measurements corrected for length*

Steps	Intercept	SThK1	Subcutaneous IV	Width midpoint	Subq2	C16:0 Intermuscular	Linoleic acid lean	Lean IV	R^2	C_p	RMSE
1	-105.0	17.5							0.494	145	
2	196.4	12.3	-4.18						0.606	72.9	
3	262.5	11.8	-3.45	-4.19					0.673	31.0	
4	248.4	7.93	-3.31	-3.97	17.4				0.690	22.0	
5	110.6	7.73	-2.44	-4.08	18.1	3.35			0.701	16.5	
6	135.6	6.59	-2.19	-4.11	17.8	2.78	-2.73		0.708	14.2	
7	63.59	6.65	-2.92	-4.22	16.6	3.11	-4.90	2.21	0.716	10.9	14.0

* Predictors all significant at $P < 0.05$; SThK1; belly side thickness measured from the caudal end of the *latissimus dorsi* either with the rib, just adjacent to this perpendicular axis. Subq2; thickness of subcutaneous fat just below *cutaneous trunci*. Cp; mallow's cp, RMSE; root mean square error for the final model.

and the IV in the lean layer. The selection of C16:0 of the intermuscular fat region further highlights the importance of the fatty acid composition of this layer (higher SFA compared to other layers) in the overall pliability of pork belly. In the original regression model, intramuscular fat composition (i.e. fat content from lean) had not been selected for belly-flop angle but was included in the model for subjective belly softness, pointing to the fat that, in addition to the subcutaneous fat composition, intramuscular fat composition of the lean is also important to the belly firmness/softness assessments.

Clearly, combining both the dimensional and the compositional factors improved the prediction of the objective and the subjective measure of belly softness compared to the commonly used IV. Individual IV in the subcutaneous fat, intermuscular fat and the lean layer accounted for 45, 41 and 37%, respectively, of the variation in subjective belly firmness scores. Variation in belly-flop angle was accounted for by subcutaneous fat, intermuscular fat and lean layer IV to about 45, 39 and 33%, respectively. Although this is much larger than the 14% previously reported in the literature (Whitney et al., 2006), it is still much lower than the prediction accuracy determined by combining dimensional and chemical predictors developed in this study (77 to 83%). The present results also confirm that the subcutaneous fat layer of the belly would provide the most accurate result if only IV is to be used to predict pork belly softness.

4.6 Conclusions

The multifactorial nature of pork belly softness ultimately limits its adequate prediction with just a single parameter such as IV. A more comprehensive and accurate prediction will include not only compositional parameters but also physical/dimensional factors. The present study suggests that IV, hence, the degree of unsaturation from other pork belly layers other than subcutaneous fat could also have an impact on overall pork belly firmness. Moreover, belly thickness traits could be used, in combination with IV, to increase accuracy in firmness evaluation. On the other hand, belly-flop angle has the potential to be used as an objective, inexpensive, non-destructive alternative for measuring firmness and for belly classification. However, the belly-flop angle should be corrected for belly length to avoid variations in the angle measurements. Furthermore, the belly-flop methodology would need to be modified to become a rapid, on-line technology prior to implementation in commercial plants.

4.7 Connection to the next study

Considering the importance of these chemical and physical factors on pork belly attributes and given that these factors can be influenced by some inherent production variables, including animal breed, diet and sex among others, the producers could explore these variables to improve the technological attributes of pork bellies as well as other meat cuts. However, because this action may also influence the nutritional properties of the meat product, it is imperative that the impact of these modifications should be assessed in the final meat product to evaluate their impact on consumers' safety and health.

The next objective was to explore the impact of two commonly employed cooking methods for bacon, industrially and in households (microwave and pan frying, respectively), on the production of certain detrimental compounds, including heterocyclic aromatic amines, protein and lipid oxidation. The influence of the compositional variation within the pig population on these compounds, as well as the conventional storage time, will also be explored.

CHAPTER 5

INFLUENCE OF COOKING METHODS AND STORAGE TIME ON LIPID AND PROTEIN OXIDATION AND HETEROCYCLIC AROMATIC AMINES PRODUCTION IN BACON[6]

5.1 Abstract

This study aimed to examine the influence of cooking methods and pre-determined refrigerated storage days on the production of lipid oxidation (TBARS), protein oxidation (PROTOX) and heterocyclic aromatic amines (HAA) in bacon. Forty-four pork bellies selected from pigs varying in breed, sex and diets to introduce variability in composition were processed as bacon. Sliced bacon was stored (4ºC) either for 2 or 28 days, and these storage groups were cooked either with a microwave oven or pan-frying. Microwave heating led to significantly higher PROTOX ($P < 0.001$), whereas pan-frying led to higher levels of HAA and TBARS in bacon ($P < 0.001$). Pan frying increased the saltiness and crispiness of bacon ($P < 0.05$), but other sensory attributes were not affected ($P > 0.05$) by the cooking method and storage time. Similarly, the composition of pork belly did not significantly influence the production of HAA, TBARS and PROTOX produced in bacon during cooking. Overall, microwave cooking had lesser impact on the production of carcinogenic compounds in bacon, with only minor impact on sensory attributes.

---

[6] Published in Food Research International and used with permission as:

Soladoye, O. P., Shand, P., Dugan, M. E. R., Gariépy, C., Aalhus, J. L., Estevez, M., and Juárez, M. (2017). Influence of cooking method and storage time on lipid and protein oxidation and heterocyclic aromatic amine production in bacon. In Press (https://doi.org/10.1016/j.foodres.2017.06.029).

O. P. Soladoye designed experiments and collected most data, analysed data, compiled and interpreted results, drafted, revised and finalised manuscript.

5.2 Introduction

Epidemiological data, as well as animal studies, have highlighted the important role diet plays in cancer development, especially as it relates to meat consumption. The recent classification of red and processed meats, based on hazard identification rather than risk assessment, as "*probably carcinogenic to human*" (class 2A) and "*carcinogenic to human*" (class 1), respectively, by the WHO- International Agency for Research on Cancer (IARC), has reignited consumers' concerns on the possible, or perceived, negative effect of meat consumption on their overall health and well-being (Bouvard et al., 2015). During thermal processing of meat, several carcinogenic compounds may be produced, including N-nitroso-compounds (NOC), polycyclic aromatic hydrocarbons (PAH), lipid oxidation products and heterocyclic aromatic amines (HAA). Dietary intake of oxidized protein and its impacts on human health have been the subject of many recent scientific explorations. As the advent of most of these compounds has been observed to be influenced by the cooking methods, cooking time and cooking temperature, consumers' choice of cooking method, as well as modification of their existing cooking style, may significantly alleviate the extent at which these carcinogenic compounds are produced in meat and its products (Kizil et al., 2011).

Heterocyclic aromatic amines are potent mutagens which play a crucial role in the aetiology of cancer, having been shown to be carcinogenic and genotoxic in long-term animal studies and during DNA repair tests, respectively (Jägerstad et al., 1998). Two groups of HAA have been identified, including the IQ type, or aminoimidazoazerene HAA and the non-IQ type, or aminocarboline HAA groups (Jägerstad et al., 1998; Kizil et al., 2011). According to Gibis (2016), these two groups also correspond to the polar and non-polar HAA and are largely produced under different cooking conditions. The IQ type is formed by heat induced non-enzymatic browning (Maillard reaction) during conventional cooking temperatures between 150 and 300°C involving reaction of creatine or creatinine, amino acids and hexose. The non-IQ type, on the other hand, is formed by the pyrolytic reaction between amino acids and proteins at higher temperature, usually greater than 300°C (Kizil et al., 2011).

Protein oxidation (PROTOX) is the covalent alteration of protein, induced either directly by reactive oxygen species (ROS) or indirectly by reaction with secondary products of oxidative stress (Shacter, 2000). Protein carbonyls have been identified as the main product of radical-mediated protein oxidation and act as markers in numerous pathological conditions. Recent

reviews also highlight the possible carcinogenic nature of oxidized proteins (Estévez & Luna, 2016; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015), whereas other studies have shown that oxidized proteins may contribute to inflammatory bowel diseases (IBD) (Keshavarzian et al., 2003), fibrotic degeneration of liver and kidney (Li, Shi, Le, Ding, & Zhao, 2015) and other age-related diseases such as Alzheimer and cataractogenesis (Berlett & Stadtman, 1997). Thermal treatments have been reported to accelerate oxidative processes not only in lipids but also in proteins due to their increasing effect on free radical production and decreasing effect on the food antioxidant protection (Gatellier et al., 2010).

The understanding that the production of carcinogens is dependent on, and can largely be influenced by, cooking process and methods stipulates that recommendations on ways to limit their production during household or industrial cooking may be the best public health intervention system. The objective of this study was to observe how storage time and two widely employed cooking methods in the industry, food service establishments and households for bacon (pan-frying and microwave cooking) may influence the levels of HAA, PROTOX and lipid oxidation in bacon produced from pork bellies of widely varying chemical composition. A secondary objective was to evaluate the sensory attributes of bacon as affected by storage days and cookery method. This is the first study assessing the level of PROTOX in cooked bacon while comparing different cooking methods.

5.3 Materials and methods

5.3.1 Animal management, sample selection and bacon production

All dietary treatments and experimental procedures were approved by the Animal Care Committee of the Agriculture and Agri-Food Canada-Lacombe Research and Development Centre and pigs were cared for as outlined under the guidelines established by the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). After reaching the required slaughter weights (120 to 140 kg), pigs were electrically stunned and slaughtered according to conventional slaughter process, after which they were split into four primal cuts following the Canadian Pork Buyers Manual's guide (Canadian Pork International, 1995).

Forty-four right-side bellies were selected randomly from a larger experimental population pool (Soladoye et al., 2016), including barrows and gilt from two crossbreeds (Lacombe or Iberian boars mated to Large White × Landrace dams) that were fed either a conventional diet or a diet

containing 10% Extrapro (50% field pea and 50% flax). These pigs were selected with these criteria to create a population with inherent variation in carcass composition. Bellies were sent for processing into bacon slabs in a commercial processing plant in Edmonton, AB, Canada, and were returned to the research facility packaged in polythene-line boxes under refrigeration for subsequent analysis. During processing, bellies were injected with ~12.5% brine containing 9.1% curing salt (~6.25% nitrite in common salt) and ~1% brown sugar. Thermal processing/smoking schedules were as follows: 2 h at 65ºC, 1 h at 70ºC, then 30 min at 80ºC. This was followed by steam cooking at 80ºC until internal temperature of 65ºC.

5.3.2 Bacon cooking and treatments

Within 24 h of receipt, the 44 bacon slabs were sliced manually (Globe gravity slicer, Stamford, Conn, USA) into 2.45-mm-thick slices and vacuum packaged with each package containing six slices. Five packages were collected from each bacon slab. A package was frozen (-30ºC) immediately and this represented the control (uncooked and 0 d storage, which will be subsequently called "control bacon" in this dissertation). Two of the packages were held at 4ºC for 2 d, one for microwave cooking and the other for pan-frying cooking. The last two sets were refrigerated (4ºC) for 28 d, after which they were then cooked by either microwave or pan-frying. Another four corresponding packages were collected and subjected to similar treatment described above (except control treatment) and used for sensory evaluation.

Microwave cooking was done on a Baconwave™ (Emson Inc., NY, USA), a cooking utensil for bacon microwave cooking. The microwave (Amana Radarange, Model CRSW459P, 850 Watts, Newton, Iowa, USA) was set to five minutes to cook bacon. For pan-frying, a Garland Grill (Ed30b, Condo Barr Food Equipment Ltd., Edmonton, AB, Canada) was pre-set to 250ºC and cast-iron frying pans were placed on the grill and equilibrated to the cooking temperature prior to cooking (Figure 5.1). The bacon slices were cooked on one side for 5 min, turned and cooked for 2.5 min and then turned again to cook for a final 1 min. Microwave and pan-frying treatments were previously tested to provide a similar degree of doneness. Bacon slices were cooled for 10 min and wiped with paper towel. The bacon slices were weighed prior to and after the cooking treatments to calculate cook losses.

a)

b)

Figure 5.1: Bacon cooking methods a) arranged on a Baconwave^{TM} and ready to be cooked in the microwave b) ready to be cooked on a pre-heated skillet.

5.3.3 Sample preparation

Following storage days and cooking, bacon slices were comminuted using a robot coupe (Robot Coupe Blixir BX3, Robot Coupe USA Inc.; Ridgeland, MS, USA). These samples were stored frozen at -80°C until further analysis. The samples were further ground frozen prior to chemical analysis using a mortar and pestle to ensure homogenous sampling.

5.3.4 Protein carbonylation analysis

5.3.4.1 Synthesis of authentic AAS and GGS standards

Authentic AAS-ABA and GGS-ABA were respectively synthesized from Nα-acetyl-L-lysine and Nα-acetyl-L-ornithine using lysyl oxidase activity from egg shell membrane following the procedure described by Akagawa et al. (2006). Briefly, Nα-acetyl-L-lysine (188 mg) and Nα-acetyl-L-ornithine (174 mg) were individually incubated with 5 g egg shell membrane in 100 mL of 20 mM sodium phosphate buffer (pH 9.0) at 37°C for 24 h with continuous stirring. Following the egg shell membrane removal by centrifugation, the pH of the solution was adjusted to 6.0 using 1M HCl. The ensuing aldehydes were then reductively aminated with p-aminobenzoic acid (ABA, 2 g as solid) in the presence of sodium cyanoborohydride ($NaBH_3CN$, 1.5 g as solid). The mixture was allowed to react at 37°C for 20 h with constant stirring. One hundred millilitres of 12 M HCl was carefully added to the solution, which was then cooled for about 1 h and then hydrolysed for 10 h at 110°C in a tube. The hydrolysate was evaporated *in vacuo* to dryness at 45°C (Acid Resistant Refrigerated Centrivap centrifugal vacuum concentrator, Labconco, Kansas City, US). The resulting residue was later reconstituted in 100 mL of distilled water, and this served as the standard solution.

5.3.4.2 Preparation of bacon sample for PROTOX HPLC-FLD analysis

Details of sample derivatisation were reported by Utrera et al. (2011). Briefly, 1 g of finely ground bacon was homogenized in 6 M NaCl (1:10 w/v), in 20 mM sodium phosphate buffer pH 6.5, using Polytron homogenizer (model 3100) for 30 s. Two hundred microliter aliquot was dispensed in 2-mL screw-capped Eppendorf tubes and mixed with 1.8 mL cold 10% TCA to precipitate the proteins, which was subsequently followed by centrifugation at 2000 rpm for 30 min. The resulting pellet was again treated with 1.8 mL cold 5% TCA followed by centrifugation at 5000 rpm for 5 min. Pellets were then treated with the following buffer: 0.5 mL of 250 mM 2-

(N-morpholino) ethanesulfonic acid (MES) buffer (pH 6), containing 1% sodium dodecyl sulfate (SDS) and 1mM diethylenetriaminepentaacetic acid (DTPA), 0.5 mL of 50 nM ABA in 250 mM MES buffer (pH 6.0) and 0.25 mL of 100 mM $NaBH_3CN$ in 250 mM MES buffer (pH 6.0), all prepared fresh on day of analysis. The derivatization proceeded for 90 min at 37°C in a pre-set water bath with constant agitation. The derivatization procedure was halted by adding 0.5 mL 50% cold TCA, followed by centrifugation at 5000 rpm for 5 min; thereafter, the pellets were washed twice with 1 mL of 10% TCA and 1 mL of ethanol-diethyl ether (1:1, v/v). This was followed by centrifugation at 5000 rpm for 5 min after each washing step. Protein hydrolysis then ensued for 18 h at 110°C following the addition of 6 M HCl. Final hydrolysates were dried *in vacuo* at 45°C using the vacuum concentrator. The dried hydrolysate was reconstituted in 1000 mL HPLC-grade water and filtered through hydrophilic polypropylene GH polypro (GHP) syringe filters (0.45 µm pore size, Pall Corporation, USA) for HPLC analysis.

Ten microliters of the reconstituted derivatised protein hydrolysates were injected and analysed using Waters Alliance HPLC system (Milford, MA, USA), equipped with COSMOSIL 5C18-AR-II RP-HPLC column (150 × 4.6 mm) and a guard column (10 × 4.6 mm) containing similar packing material. Ten microliters of the reconstituted standard was also injected and subjected to similar condition as the samples. The elution program was based on the low-pressure gradient method of Utrera et al. (2011), with Eluent A (50 mM sodium acetate buffer; pH 5.4) and Eluent B (Acetonitrile), where the concentration of Eluent B varied from 0% (min 0) to 8% (min 20). The flow rate was maintained at 1 mL/min, column temperature at 30°C and a total run time of 30 min. The eluate was monitored with excitation and emission wavelengths of 283 and 350 nm, respectively.

Identification of both AAS-ABA and GGS-ABA in the FLD chromatogram was done by comparing their retention times with those of the standards. These components were manually integrated and their resulting areas were plotted against a known concentration (0.01 to 0.05 mM) of ABA standard curve ($R^2 > 0.9991$). This follows the assumption that the fluorescence emitted by 1 mol of ABA is equivalent to that emitted by 1 mol of AAS-ABA or GGS-ABA. Results were expressed in nmol carbonyl/mg protein, where bacon protein was quantified using a RapidN cube (Elementar, Rhine main, Germany) employing the Dumas combustion method. The protein content of samples ranged between 0.080 and 0.63 g/g. All reagents employed were HPLC grade and purchased from Sigma–Aldrich Company (St. Louis, MO, USA).

5.3.5 Lipid oxidation

Lipid oxidation was measured as thiobarbituric acid reactive substances (TBARS) according to Nielsen, Sørensen, Skibsted, and Bertelsen (1997). Briefly, 5 g of finely ground bacon was homogenized for 30 s in 30 mL of 7.5% TCA solution with a Polytron homogenizer (PT 3100 D, Kinematics, Littau-Luzern, Switzerland). Homogenate was filtered through Whatman #4 filter paper, and 2.5 mL of TBA-solution (20 mM) was added to 2.5 mL of the filtrate in pyrex screw top tubes. Tubes were vortexed and allowed to incubate for 40 min in water bath at 94°C. Total MDA (malondialdehyde) in samples was measured spectrophotometrically at 541 nm and quantified against a standard curve (1,1,3,3-Tetraethoxy-propane, 0 to 20 μM, $R^2 > 0.99$). Results were expressed in mg MD/kg of sample.

5.3.6 Heterocyclic aromatic amine analysis

5.3.6.1 HAA Standards preparation

All HAA standards (purity > 98%) were purchased from Toronto Research Chemicals (North York, ON, Canada): 2-amino-3-methyl-3H imidazo [4,5,f] quinoline (IQ), 2-amino-3-methyl-3H imidazo [4,5,f] quinoxaline (IQx), 2-amino-3,4-dimethyl-3Himidazo [4,5,f] quinolone (MeIQ), 2-amino-3,8-dimethyl-3H imidazo [4,5,f] quinoxaline (MeIQx), 2-amino-3,7,8-trimethyl-3H imidazo [4,5,f] quinoxaline (7,8-DiMeIQx), 2-amino-3,4,8-trimethyl-3H imidazo [4,5,f] quinoxaline (4,8-DiMeIQx), 2-amino-3,4,7,8-tetramethyl-3H imidazo [4,5,f] quinoxaline (TriMeIQx), 3-amino-1-methyl-5H pyrido [4,3-b] indole acetate (Trp-P-2), 3-amino- 1,4-dimethyl-5H pyrido [4,3-b] indole acetate (Trp-P-1), 2-amino- 9H pyrido [2,3-b] indole (A**α**C), 2-amino-3-methyl-9H pyrido [2,3- b] indole (MeA**α**C), 2-amino-6-methydipyrido [1,1-A:3,2] imidazole, hydrochloride hydrate (Glu-P-1), 2-amino dipyrido [1,2-A: 3,2D] imidazole, dichloride (Glu P-2), Norharmane, Harmane and 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP). All solvents employed in this study were HPLC grade. Triethylamine, HCl, NaOH, and ammonium acetate were purchased from Sigma–Aldrich Company (St. Louis, MO, USA). Propyl-sulphonic acid (PRS), Bond-Elut cartridges (500 mg), C-18 cartridges (100 mg), Bond-Elut reservoir, and packing materials (diatomaceous earth) were obtained from Varian (Harbor City, CA, USA). A working combined standard was prepared from respective individual stock solutions. The concentration of individual standard in ng/μL within the combination was as follows: IQ, 0.2; IQx,

0.2; MeIQ, 0.24; MeIQx, 0.24; TriMeIQx, 0.24; 4.8 DiMeIQx, 0.15; 7,8 Di MeIQx, 0.15; Glu P-1, 0.05; Glu P-2, 0.05; Harmane, 0.05; Norharmane, 0.08; Trp-P-1, 0.05; Trp-P-2, 0.1; PhIP, 0.07; AαC, 0.07 and MeAαC, 0.05 (internal standard).

5.3.6.2 HAA Sample Extraction and HPLC analysis

Fifteen different HAA were analysed in each bacon sample, including IQ type (IQx, IQ, MeIQ, MeIQx, 7,8 DiMeIQx, 4,8 DiMeIQx, 4,7,8 TriMeIQx and PhIP) and non-IQ type (Glu P-2, Glu P-1, Norharman, Harman, AαC, Trp P-2 and Trp P-1). Sample extraction was based on Ruan et al. (2014). Briefly, 1 ± 0.05 g of ground cooked bacon or 3.0 ± 0.05 g of raw control was weighed in duplicate into 50 mL centrifuge tubes. Then, 7.5 mL 1 M NaOH and 100 µL of the internal standard were added to samples and samples were left to stand for 20 min at 37°C. Subsequently, 2.75 g diatomaceous earth (Agilent Hydromatrix Bulk Material 198003) and 25 mL ethyl acetate (Fluka 34972) were added and samples were vortexed for 20 min (Multi Pulse Vortexer 099A VB4, Terre Haute, USA). Samples were centrifuged for 5 min at 6900 rpm and extracted by using the PRS cartridge. Heterocyclic amines were eluted from PRS to a C18 cartridge and then eluted from the C18 cartridge into clean tubes by using 1.2 mL methanol:ammonium (9:1 v:v) under low vacuum. Solvents were evaporated from the eluted samples at room temperature under nitrogen and reconstituted with 120 µL trimethylamine phosphate buffer, 0.01M, pH 3.0/Methanol (1:1) for HPLC injection.

High performance liquid chromatography was performed using the Waters Alliance HPLC (Milford, MA, USA) equipped with TSKgel Super-ODS C18 column (4.6 mm × 10 cm with a 2 µm particle size; TOSOH Bioscience, San Francisco, CA, USA). The mobile phase was a gradient of solvent A (0.01 M triethylamine, pH 3.0), solvent B (0.01 M triethylamine, pH 4.0), and solvent C (acetonitrile) at a flow rate of 1 mL/min. The detailed elution program has been previously reported (Ruan et al., 2014). Analytes were detected using UV/FLR and the total running time for the program was 21 min. Quantitative determinations were performed using the standard calibration curves ($R^2 = 0.9896$ to 0.9999). The LOD was ($\sim 1.89 \times 10^{-3}$ ng/g, average of standards) determined using the formula of 3.3*(standard deviation of the y-intercept/slope of calibration curve), whereas the LOQ ($\sim 5.73 \times 10^{-3}$ ng/g, average) was determined using 10*(standard deviation of the y-intercept/slope of calibration curve) (Shrivastava & Gupta, 2011). The recovery

of HAA using the internal standard also ranged between 57.14 and 88.10% similar to what was previously reported in our earlier study in beef (Ruan et al., 2014).

5.3.7 Sensory evaluation

Sensory evaluation of bacon was carried out by a seven-member trained panel. Bacon was blind coded and presented warm to panellists in a well-ventilated, partitioned booth under 124 Iux red lightning. A nine-point scale was employed with 9 representing "extremely intense" and 1 "none" for all attributes (initial and overall crispiness, salt intensity, bacon flavour, smoke flavour, mouth coating, chewiness and off-flavour intensity), except off-flavour intensity, which was in reverse order (Holdstock et al., 2014). Attribute ratings were electronically collected using the Compusense 5 (Release 4.6) computer software (Compusense Inc. Guelph. ON). Apple juice and unsalted crackers were provided to panellists to cleanse their palate of flavour notes between samples.

5.3.8 Statistical analysis

Data were analysed using a factorial design with cooking methods (microwave and frying pan) and storage days (2 and 28 days) as factors. Data for each treatment combination represented the difference between the treatment and the control, except for sensory data, which were analysed non-parametrically (using the PROC NPAR1WAY of SAS). The mixed model procedure in SAS (version 9.3) was used to analyse all data and significant differences were declared at $P < 0.01$. Correlation between chemical composition of bacon and pork bellies within each treatment group as well as the average of all treatments, were also carried out using PROC CORR in SAS; however, only the significant correlations data ($P < 0.05$) for the average values were reported in the present chapter. To examine the possibility of explaining the variation within these samples with fewer parameters, Minitab 17 Statistical Software was used to explore principal components employing various compounds quantified in this study as variables.

5.4 Results and discussion

There was a wide variation in the chemical composition of the pork belly used in this study (Table 5.1; Appendix A1).

Table 5.1: Characteristics of pork bellies and the control bacon (n = 44)[1]

Variable	Mean ± SD	Minimum	Maximum	CV (%)[2]
Belly traits				
Belly weight (kg)	8.64±0.84	6.88	10.34	9.72
Moisture content (%)	37.28±7.18	23.99	53.00	19.26
Fat content (%)	51.17±9.26	30.71	68.74	18.10
Protein content[3] (g/100g)	12.00±0.02	8.00	16.00	16.67
Iodine value[4](g/100g)	64.35±2.32	58.62	68.56	3.61
PUFA[5](%)	12.11±1.35	9.36	14.98	11.15
n-3 (%)	2.09±0.76	1.04	3.37	36.36
n-6 (%)	10.01±0.93	.8.32	12.31	9.29
n-6/n-3 (%)	5.45±1.97	3.30	8.23	36.15
SFA[6](%)	37.04±1.61	34.15	41.31	4.35
PUFA/SFA (%)	0.33±0.05	0.23	0.42	15.15
MUFA[7] (%)	50.86±1.54	47.93	54.49	3.03
Bacon traits				
AAS (nmol/mg protein)[a]	75.13±15.40	40.04	107.33	20.49
GGS (nmol/mg protein)[b]	4.88±1.08	2.73	7.31	22.19
Total PROTOX (nmol/mg protein)[c]	80.01±15.93	45.74	113.33	19.91
TBARS (mg MD/kg)[d]	0.19±0.06	0.09	0.34	29.51
HAA (ng/g)[e]	0.27±0.27	0.05	1.05	100

[1] All fatty acid profiles are those of the subcutaneous fat layer

[2] Coefficient of variation

[3] protein content represents that of the control bacon.

[4] IV = ([16:1] × 0.95) + ([18:1] × 0.86) + ([18:2] × 1.732) + ([18:3] × 2.616) + ([20:1] × 0.785) + ([22:1] × 0.723), where brackets indicate the proportion of a particular fatty acid.

[5] PUFA = C18:2n-6 + C18:2n-3 + C20:2n-6 + C20:3n-6 + C20:3n-3 + C20:4n-6 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3

[6] SFA = C14:0 + C16:0 + C17:0 + C18:0 + C20:0

[7] MUFA = C16:1cis7 + C16:1cis9 + C18:1cis9 + C18:1cis11 + C18:1cis13 + C20:1cis11

[a]AAS; α-Aminoadipic semialdehyde, [b]GGS; γ-glutamic semialdehyde, [c]Total PROTOX; GGS + AAS, [d]TBARS; Thiobarbituric reactive substances, [e]HAA; Heterocyclic aromatic amine.

[a-e] Note: These are values of control bacon samples, 0 storage day and not cooked.

The iodine value (IV), polyunsaturated (PUFA), monounsaturated (MUFA) and saturated fatty acids (SFA) varied widely and may have effects on products' oxidative stability. The National Pork Producer Council indicated that good quality pork fat should have < 15% PUFA, with IV values below 70 g/100 g (Benz et al., 2010). The IV and PUFA of pork bellies reported in the present study were below this limit set for acceptable fat quality. Differences in diets fed, however, resulted in rather large variability in PUFA compared to literature reports (Correa et al., 2008), and the average n-6/n-3 ratio was close to the 4:1 ratio recommended for optimum health (Wood et al., 2004b).

The levels of PROTOX, TBARS and HAA in control bacon prior to cooking treatments were presented in Table 5.1. The amount of PROTOX in the present study were higher compared to previously reported values for other processed meat products in the literature. The concentration of AAS, GGS and total protein carbonyls (Total PROTOX) in assorted muscle foods range from 0.5 to 1 nmol of carbonyls/ mg protein in fresh muscle to around 20 nmol carbonyls/ mg protein in heavily processed meats or cooked and long-term stored liver products (Estévez, 2011; Estévez & Cava, 2006; Utrera et al., 2012; Ventanas, Estevez, Tejeda, & Ruiz, 2006). The high levels of protein carbonyls found in the present study may be related to the nature of the raw material, the formulation, and bacon processing. All the relevant ingredients added to, or excluded from, bacon in the present study have been recently reported to increase protein carbonylation in muscle foods. Salt promotes protein oxidation by facilitating myofibril swelling and exposure of susceptible functional groups to oxidation (Lobo, Ventanas, Morcuende, & Estévez, 2016). Nitrite is an efficient inducer of reactive oxygen and nitrogen species, which can initiate the oxidative deamination of alkaline amino acids leading to carbonylation (Villaverde et al., 2014). These authors further showed that although nitrite promoted protein carbonylation, ascorbate addition led to a significant reduction of the same; thus, the exclusion of ascorbate in bacon in the present study could have contributed to the observed higher level of protein carbonylation. This result may further highlight the importance of including ascorbate in the bacon ingredient mixture as mandated in the United States (FSIS, 2011). Similarly, the exclusion of phosphate from the ingredient mixture of the bacon in the present study, which could have further suppressed oxidation, might have also contributed to the high levels of PROTOX observed following bacon processing. Sugars have also been directly implicated in the formation of carbonyls in meat proteins through a Maillard-mediated mechanism (Villaverde & Estévez, 2013). It is unknown to

which extent smoking would also promote carbonylation but the subsequent thermal processing to 65°C increased the formation of AAS and GGS. The higher concentration of AAS compared to GGS fluorescence in the present study is in agreement with other reports (Estévez, Ollilainen, & Heinonen, 2009), and could reflect a higher abundance of lysine in the bacon sample compared to other basic amino acids, because lysine is the precursor for AAS, whereas GGS is derived from proline and arginine. These high levels of protein oxidation products in bacon need to be taken into consideration due to their potential implication for human nutrition and health.

5.4.1 Effects of cooking method and storage time on protein carbonylation in bacon

In comparison to control samples (Table 5.1), the extent of protein carbonylation increased with cooking ($P < 0.001$) (Table 5.2). Similar effects have been reported with protein carbonyls increasing with increasing cooking temperature and cooking time (Gatellier et al., 2010; Santé-Lhoutellier et al., 2008). During meat cooking, not only are antioxidant defences impaired, free radicals are also produced, leading to protein oxidation (Santé-Lhoutellier et al., 2008). Basic amino acids (lysine, histidine and arginine) are particularly susceptible to attack by free radicals produced during cooking and, as such, easily converted to their carbonyl derivatives. Accordingly, the formation of these carbonyl compounds has been highlighted as one of the most measurable modifications in oxidized proteins, with the formation of α-amino adipic semialdehyde (AAS) and γ-glutamic semialdehyde (GGS) accounting for about 60 to 70% of the total protein carbonyls in oxidized protein (Estévez, 2011; Utrera et al., 2011). Furthermore, the thin nature of bacon slices (2.45 mm) compared to the thickness of other meats products might have also contributed to the high value reported in the present study due to greater heat and other prooxidant interactions. It is worth noting that the majority of the PROTOX produced in bacon during the present study occurred during the smoking/initial thermal processing stage (with total PROTOX in the finished bacon up to about 80.01 nmol/mg protein) and AAS was the carbonyl mostly produced (Table 5.1). This observation may emphasize the need to control PROTOX at an earlier stage during bacon smoking or initial thermal processing in addition to during household cooking or industrial pre-cooking. Aside from the factors previously described which could have largely contributed to the high PROTOX level in the control bacon, the cooking condition during the smoking schedule could also be a major factor.

Table 5.2: Change in protein oxidation, lipid oxidation and heterocyclic amine with cooking methods (n = 44)[1]

	Cooking methods		P value
	Microwave	Frying Pan	
ΔAAS (nmol/mg protein)	43.69±3.19[a]	31.42±3.24[b]	< 0.001
ΔGGS (nmol/mg protein)	7.09±0.30[a]	6.11±0.30[b]	< 0.001
ΔTotal PROTOX (nmol/mg protein)	50.77±3.38[a]	37.20±3.32[b]	< 0.001
ΔTBARS (mg MD/kg)	0.51±0.03[b]	0.74±0.03[a]	< 0.001
ΔHAA (ng/g)	1.84±0.40[b]	5.26±0.41[a]	< 0.001

[1]Values represent difference between treatment and control. AAS; α-Aminoadipic semialdehyde, GGS; γ-glutamic semialdehyde, Total PROTOX; GGS + AAS, TBARS; Thiobarbituric acid reactive substance, HAA; Heterocyclic amine. [a-b] Different superscripts in the same row are significantly different at P < 0.001.

The control bacon in this study has been subjected to smoking schedule at temperature ranging from about 65 to 80°C and the bacon slabs reached internal temperature of about 65°C. This temperature is higher than the typical temperature of 53°C employed as the final internal temperature in processing commercial bacon (using smokehouse temperature range between 42 and 63°C during the smoking schedule) (The National Provisioner, 2008).

The increase in AAS and GGS was significantly greater in microwave cooking compared to frying pan (Table 5.2). Although this has not been reported previously, higher PROTOX from microwave cooking may be related to the cooking mechanisms. Contrary to heating by conduction from a hot surface that characterizes conventional oven cooking or pan-frying, electromagnetic microwaves have frequencies between 0.3 and 300 GHz and wavelengths between 1 nm and 1 mm that can penetrate food and directly excite specific molecules by oscillating ionic molecules (e.g. salt), as well as dipole rotation (water molecules). The overall effects of these movements leads to increases in the kinetic energy of the molecules, and ultimately to increased temperature and cooking rate (Vollmer, 2004). Giving that this mechanism allows for intimate interaction with the

molecules of the food, generation of reactive oxygen species could result due to disruption of cellular compartmentalization. Badiani et al. (2002) reported the highest post-cooking temperature rise with microwave cooking compared to other culinary practices (broiling, boiling and oven cooking) even though it took the shortest time. Similarly, other reports have also emphasized the ability of fat content to enhance and accelerate heating rate (regardless of its low dielectric constant, ε' and dielectric loss factor, ε'') and, as such, increasing the maximum temperature attained possibly due to its lower specific heat and higher boiling point compared to water (Picouet et al., 2007). The overall consequence of these reactions may be responsible for the higher observed PROTOX. Yet, because the same trend was not observed with TBARS and HAA which could also be influenced by increased temperature, the present observation could possibly be due to the non-thermal effect of microwave cooking on proteins rather than the effect of temperature increase (Bohr & Bohr, 2000b). In agreement with present results, Silva, Ferreira, Madruga, and Estévez (2016) recently observed significant effects of cooking procedures on the extent of oxidative damage to meat proteins. They hypothesized, however, that the heat transfer system and formation of Maillard products with antioxidant potential in grilled chicken breast would have diminished the formation of protein carbonyls in this cooking technique compared to some others.

The increase in PROTOX values in bacon stored for 28 days was significantly lower than those stored for 2 days (Table 5.3). This could be explained by the possible breakdown of protein carbonyls with storage. Previous reports have shown that protein carbonyls may diminish during ongoing meat processing and storage as a result of their degradation to yield Schiff base structures and alpha-amino adipic acid, the end product of lysine oxidation (Estévez, 2011). Furthermore, the possible higher drip loss in bacon stored for 28 days compared to those stored for 2 days, may enhance higher Maillard reaction products during cooking, because Maillard reaction has been reported to increase with varying water activity, maximally between 0.6 and 0.7 (BeMiller & Huber, 2008). As confirmed by Silva et al. (2016), these may act as antioxidant for protein oxidation; hence, reduced protein carbonyls with higher storage days. Contrary to the present study, Ganhão et al. (2010b) reported a significant increase in the amount of carbonyl compounds with days in chilled storage (up to 5.8 nmol/mg protein in 12 days at 2ºC). This may be due to the shorter storage days and different packaging method employed in this study. In addition, the higher heme iron content in beef compared to pork, having been identified as an effective prooxidant (Estévez & Heinonen, 2010; Lund et al., 2011), could have constituted a greater degree of

susceptibility. Other studies have also reported similar increases in protein oxidation during chilled storage of several meats (Mercier et al., 1998; Santé-Lhoutellier, Engel, Aubry, & Gatellier, 2008) where the nature of product, storage days and packaging conditions differ from the present study.

Table 5.3: Change in protein oxidation, lipid oxidation and heterocyclic amine with storage days (n = 44)[1]

	Storage days		P value
	2 days	28 days	
ΔAAS (nmol/mg protein)	41.33±3.21[a]	33.78±3.22[b]	0.0002
ΔGGS (nmol/mg protein)	6.74±0.30	6.4572±0.30	0.1509
ΔTotal PROTOX (nmol/mg protein)	47.71±3.28[a]	40.25±3.29[b]	0.0006
ΔTBARS (mg MD/kg)	0.63±0.03	0.62±0.03	0.4334
ΔHAA (ng/g)	3.55±0.40	3.55±0.41	0.9864

[1]Values represent difference between treatment and control. AAS; α-Aminoadipic semialdehyde, GGS; γ-glutamic semialdehyde, Total PROTOX; GGS + AAS, TBARS; Thiobarbituric acid reactive substance, HAA; Heterocyclic amine. [a-b] Different superscripts in the same row are significantly different at $P < 0.001$.

5.4.2 Effect of cooking methods and storage times on lipid oxidation

Similar to protein oxidation, lipid oxidation has been reported to increase with cooking temperature and time in pork (Broncano et al., 2009). Apart from the increase in free radical and the decrease in the antioxidant defence system mentioned previously, Broncano et al. (2009) also emphasized the importance of the heat transfer coefficients of the medium involved in the development of lipid oxidation. Generally, lipid oxidation was greater in cooked bacon, compared to raw, regardless of cooking treatments (Table 5.2). However, contrary to the trend in protein oxidation, pan-frying contributed to a greater increase in lipid oxidation (TBARS) compared to microwave cooking. In contrast, some studies have reported greater lipid oxidation in microwave compared to pan-frying (Domínguez, Gómez, Fonseca, & Lorenzo, 2014), whereas others have

reported similar results compared to the present study (Broncano et al., 2009). This discrepancy maybe due to the nature of the meat involved, as well as the length and temperature of the cooking method. In addition, fat adherence in bacon with pan-frying (bacon cooked in its own fat compared to microwave cooking in the Baconwave^{TM} utensil that allowed for fat drainage) could have also partly contributed to the higher TBARS value in the fried bacon. The average TBARS value in the raw bacon was about 0.19 mg MD/kg (Table 5.1) and increased over three- and four-folds after microwave and pan-frying (0.69 and 0.93 mg MD/kg), respectively. These post cooking levels are close to the generally acceptable sensory detection thresholds level of about 0.5 to 1 mg MD/kg (Ruan, Aalhus, & Juárez, 2014).

The fact that lipid and protein oxidation did not follow similar propensity in their change with different cooking methods may signify that not only temperature and time are important in these oxidative processes, but the underlying mechanism of these cooking methods may be crucial as well (Inchingolo, Cardenia, & Rodriguez-Estrada, 2013; Sánchez-Muniz & Bastida, 2006). It is noteworthy that the correlation between TBARS and protein oxidation markers was significant ($P < 0.01$) with microwave but not with pan-frying ($P > 0.05$). This also may confirm the possibility of difference in cooking mechanism in affecting these processes differently. The storage time of bacon under refrigeration and vacuum packaging did not influence the level of lipid oxidation ($P > 0.05$; Table 5.3), only the effect of cooking method was significant in this study ($P < 0.001$). Aside from the vacuum packaging and refrigeration temperature that could have limited lipid oxidation, the antioxidant nature of nitrite added could have also contributed to the lack of effect observed for storage time. It stands to be proven, however, if longer storage time would overpower these effects.

5.4.3 Effect of cooking methods and storage times on heterocyclic amine production

Overall, the IQ type HAA represented about 72% of the total HAA quantified in bacon, whereas the aminocarboline only represented up to 24% (Figure 5.2). Gross et al. (1993) have reported that the MeIQx, 4,8-DiMeIQx and PhIP, all of which belong to the IQ type HAA, are mainly responsible for the mutagenicity observed in grilled bacon, and they represented the larger portion of the HAA detected compared to the non-IQ type. The values of the IQ type HAA ranged between 0.001 and 18.48 ng/g whereas the non-IQ types were below 0.71 ng/g, with the exception being Trp-P-1 and Trp-P-2, which were up to 5.61 and 2.94 ng/g, respectively, in some bacon

samples. Although there are no established guidelines defining the recommended consumption limit of HAA in meat, the California Environmental Protection Agency defines the following "no significant risk level" (NSRL) values (in µg/day) as : MeIQx, 0.41; MelQ, 0.46; Glu-P-1, 0.1; Glu-P-2 and IQ, 0.5; AαC, 2; MeAαC, 0.6; Trp-P-1, 0.03; and Trp-P-2, 0.2. (OEHHA, 2016). Even though these values may not be easily attainable in regular human diets, per capita exposure level between 50 to 1820 ng/day has been reported in different countries, and these variations in exposure are dependent on the frequency of consumption, preparation method and consumer preference for colour and roasted flavour (Gibis, 2016).

Similar to other reports (Gross et al., 1993; Oz, Kaban, & Kaya, 2010), pan-frying contributed more to increased HAA in bacon than microwave cooking (Table 5.2). This increase in individual HAA (Figure 5.2) could be due to higher heat transfer coefficient in pan-frying than in microwave cooking. Gibis (2016) has reported that direct contact cooking methods (e.g., frying) have better heat transfer than indirect cooking by convection (oven roasting) or radiation (e.g., microwave). The lower heat transfer in microwave cooking, accompanied by higher drip and loss of precursors, could have resulted in its lower HAA. In fact, Felton, Fultz, Dolbeare, and Knize (1994) have shown that microwave pre-treatment can reduce HAA precursors and mutagenic activity in meat. Regardless of storage time, the average total HAA in raw bacon was about 0.31 ng/g (0.05 to 1.98 ng/g) and this value increased to about 5.43 ng/g (0.98 to 19.5 ng/g) in fried bacon but only 2.19 ng/g (0.44 to 8.20 ng/g) in microwaved bacon. No interaction between cooking time and storage days was observed in this study for HAA production and days of storage also did not influence HAA production (Table 5.3).

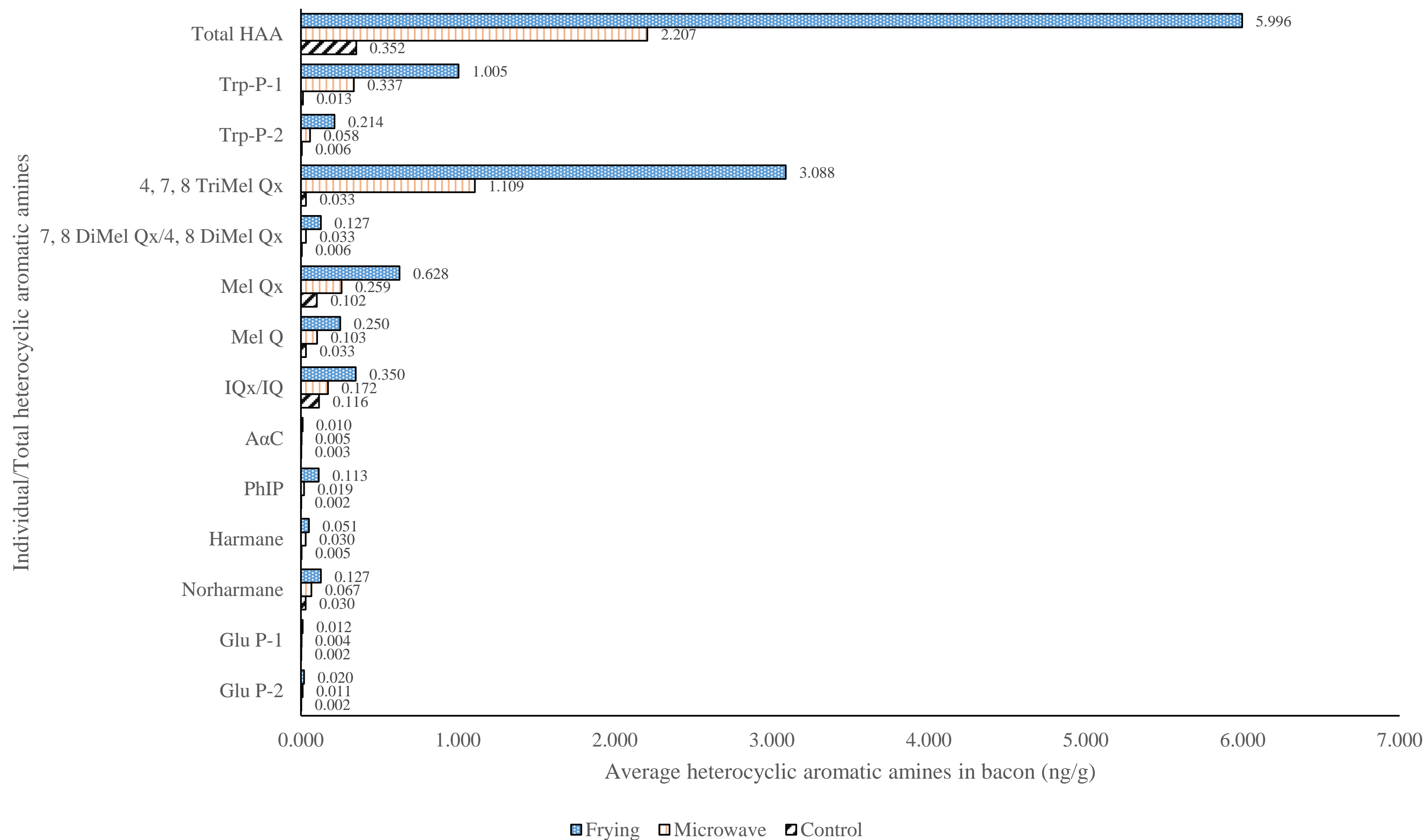


Figure 5.2: Values of total and individual HAA in the bacon as they respond to different cooking methods

5.4.4 Relationships between bacon composition, lipid and protein oxidation and HAA formation

There were significant correlations (albeit sometimes weak) between TBARS, HAA and protein oxidation values in the present study (Table 5.4). The Maillard reaction has been suggested to play a central role in the formation of these chemical compounds. Zamora and Hidalgo (2005) concluded that the Maillard reaction and lipid oxidation are interrelated and share common chemical mechanisms and intermediates. The breakdown products of lipid oxidation (e.g., aldehydes) participate in the Maillard reaction and significantly influence flavour development in meat (Zamora & Hilgado, 2005). Similarly, the relationship between HAA formation and the Maillard reaction has been emphasized. Jägerstad et al. (1998) postulated that the amino-imidazo part of the IQ type HAA molecule is formed by cyclisation and water elimination from creatine, whereas the remaining part (e.g., pyridine and pyrazine) could be formed from Strecker degradation products of Maillard reaction. The same authors proposed that free radical production during fat oxidation might enhance the yield of HAA in meat systems. Several studies have also established a connection between protein carbonylation and Maillard reaction products. Akagawa et al. (2005) have shown that AAS and GGS could be formed in the presence of glucose and Maillard-derived dicarbonyls while reacting with protein-bound amino acids. These semialdehydes have been highlighted as possible carbonyl candidates to interact with free amino groups in the formation of Strecker aldehydes (Villaverde et al., 2014). In fact, the Maillard-mediated pathway has been highlighted as one of three established mechanisms by which protein carbonyls are formed in food systems (Akagawa et al., 2005; Estévez & Luna, 2016). As these reactions are related, this could explain the significant correlation observed among them in the present study (Table 5.4). However, further studies are necessary to explore these relationships and the underlying mechanisms.

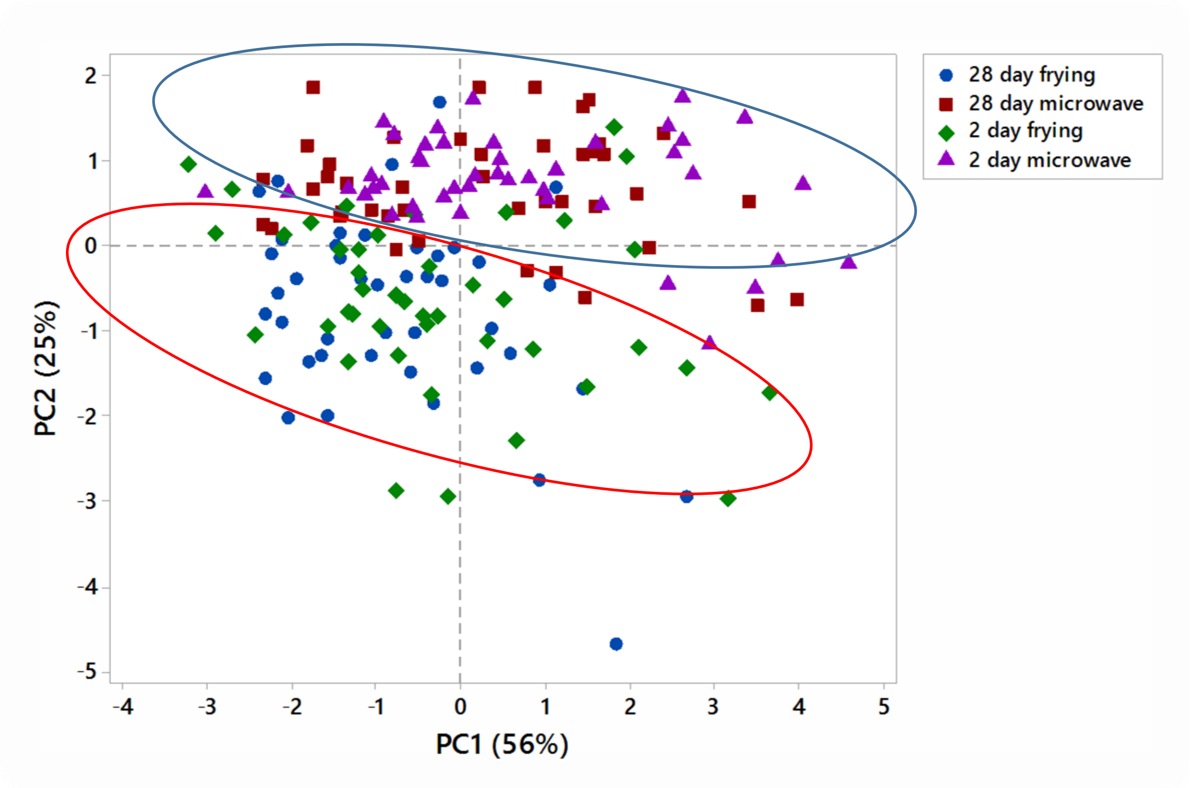
Table 5.4: Pearson correlation among bacon chemical characteristics and pork belly composition

	AAS	GGS	Total PROTOX	HAA	TBARS	IV	ω 6	ω3	ω6/ω3	MUFA	SFA	PUFA	PUFA/SFA	Cook Loss
AAS	1.00	0.86^{**}	1.00^{**}	0.25^{**}	0.43^{**}	0.05	0.04	0.07	-0.05	-0.04	-0.02	0.07	0.07	0.30
GGS		1.00	0.89^{**}	0.37^{**}	0.59^{**}	0.04	0.03	0.04	-0.04	0.02	-0.05	0.04	0.06	0.38
Total PROTOX			1.00	0.27^{**}	0.45^{**}	0.05	0.05	0.07	-0.04	-0.03	-0.03	0.07	0.07	0.30
HAA				1.00	0.45^{**}	-0.05	-0.06	-0.08	-0.06	0.11	-0.04	-0.09	-0.06	0.25
TBARS					1.00	0.10	0.08	0.08	-0.07	-0.02	-0.06	0.10	0.10	-0.05

* significant at $P < 0.05$, **significant at $P < 0.01$. AAS; α-aminoadipic semialdehyde, GGS; γ-glutamic semialdehyde, Total PROTOX; total protein oxidation (GGS + AAS)

Although some authors have reported the possible interaction between lipid and protein oxidation (Estévez et al., 2008; Mercier et al., 1998; Ventanas et al., 2006), others have suggested that the oxidizing environment of these reactions influences the coupling of these two phenomena (Lund et al., 2011; Park, et al., 2006). In the present study, TBARS values were positively correlated with the protein carbonyls, although the relationship was not strong ($r = 0.42$ to 0.59, $P < 0.01$) (Table 5.4). In fact, principal component analysis of all the chemical variables considered in the present study produced two principal components explaining up to 81% of the variation in the dataset (Figure 5.3). This analysis included the 176 observations (microwave × pan-frying treatments) for the 44 pigs included in this study. For each observation, HAA, TBARS and measures of protein oxidation were included in the analysis as variables. The first principal component (PC1), which explains up to 56% of the variation within the dataset, loaded high on measures of protein oxidation, whereas the second principal component (PC2), explaining about 25% of the variation, loaded high on TBARS and HAA. Considering that these two PCs are orthogonal in nature, this may suggest that, under different cooking treatments or processing environments, these two groups of reactions may not follow the same progression. It is worth noting that the PC2 separated these datasets based on cooking methods, confirming the greater contribution of pan-frying to lipid oxidation. Although PC1 did not segregate this dataset, more microwaved bacon was clustered around the positive axis of PC1 than the fried bacon, indicating more protein oxidation in microwaved bacon than fried bacon, as discussed previously.

Furthermore, all the measures of fatty acid unsaturation on raw bellies (IV, n-3, n-6 and PUFA) were not correlated with these chemical compounds from cooked bacon ($P > 0.05$, Table 5.5). This lack of relationship could possibly be explained by the strong effects the processing steps and added ingredients in bacon could have had on the overall oxidation status of the product which, in turn, could have, negated the effects that fatty acid composition could have contributed in the production of these compounds. For example, nitrite, a crucial curing ingredient employed in bacon processing possesses a strong antioxidant activity. Its overall effect during processing may have been sufficient to suppress the minimal susceptibility the fatty acid unsaturation could have in the product. This result further confirms that the variation in pork belly composition may not have significant carry-over effect on the production of lipid oxidation, as well as HAA, in cooked bacon.



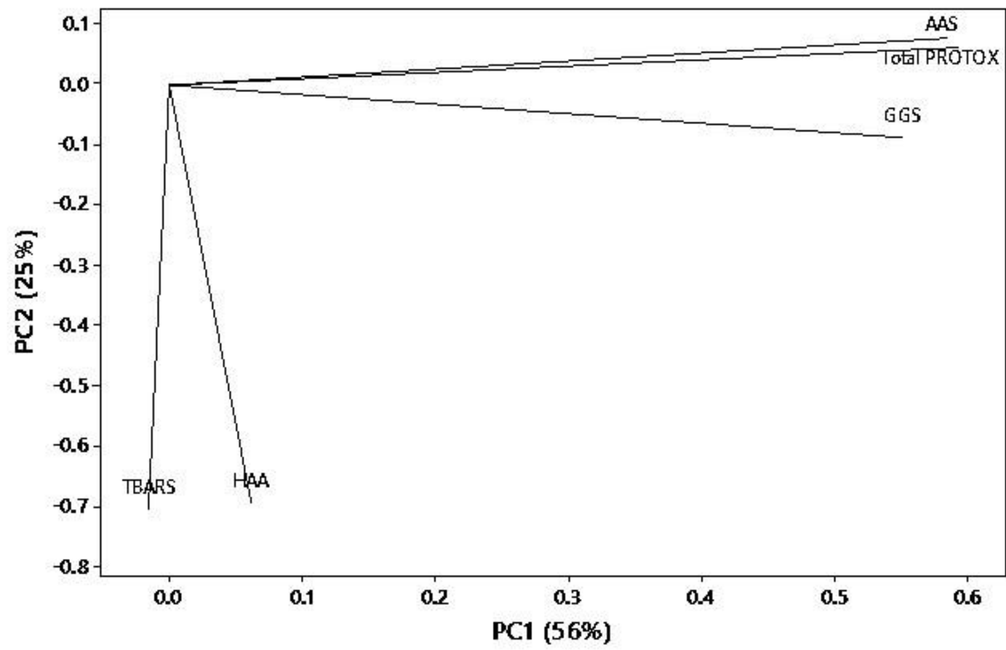


Figure 5.3: Principal component analysis for chemical compounds in cooked bacon: a) score plot for all observations b) loading plot for all variables.

Table 5.5: Effect of animal breed, sex and diet on bacon attributes

	GGS (nmol/mg protein)	AAS (nmol/mg protein)	Total PROTOX (nmol/mg protein)	HAA (ng/g)	TBARS (mg MD/kg)
			Breed		
Lacombe	10.38±0.41	109.74±2.85[a]	120.08±3.21	3.11±0.40	0.72±0.03
Iberian	10.00±0.39	101.89±2.59[b]	111.94±2.91	3.05±0.37	0.66±0.03
			Sex		
Barrow	10.68±0.37	110.64±2.44[a]	121.35±2.74[a]	3.18±0.37	0.65±0.03
Gilt	9.60±0.40	99.16±2.68[b]	108.68±3.00[b]	2.96±0.40	0.73±0.03
			Diet		
Flaxseed	10.20±0.39	106.27±2.77	116.47±3.10	2.81±0.37	0.70±0.03
Control	10.20±0.41	104.58±2.89	114.72±3.23	3.37±0.39	0.70±0.03

[a-b] Different letters in each column indicate significant difference at $P < 0.05$. AAS; α-aminoadipic semialdehyde, GGS; γ-glutamic semialdehyde, Total PROTOX; total protein oxidation (GGS + AAS).

Further, the variation within this pig population (breed, sex and diet), responsible for the varying chemical composition of their respective pork bellies, also did not have any effects on the production of TBARS and HAA ($P > 0.05$; Table 5.5). Yet, PROTOX varied between breed and sex and this may be due to the ability of these factors to influence pork belly brine/ingredient uptake rather than the variation they created in the belly composition. Cook loss was also weakly correlated with all the measures of protein oxidation (Table 5.4), which agrees with the loss of water-holding capacity in meat due to protein modification (Estévez, 2011).

5.4.5 Effects of cooking method and storage time on sensory attributes of bacon

Sensory evaluation was included in this study to understand the impact of cooking method and storage time on bacon palatability. From all the sensory attributes assessed, only initial crispiness was affected by a cooking method × storage day interaction ($P = 0.0046$) (Figure 5.4; Appendix A5). Greater initial crispiness was found in pan-fried rather than in microwaved bacon after 2-day refrigerated storage, but this effect disappeared after 28-day storage. This may suggest a possibility for improved bacon cook texture with increasing days of storage for bacon to be cooked by microwave. Although this may not have a simple direct explanation, it may be due to possible moisture loss in the vacuum package as storage days progressed. Several studies have reported increased drip loss in vacuum packaged meat with vacuum pressure related lower water-holding capacity resulting in drier meat (Marcinkowska-Lesiak et al., 2016; Morales-delaNuez et al., 2009) which could translate into greater bacon crispiness after cooking.

Of the other sensory attributes of bacon assessed in the present study, overall crispiness, salt intensity and chewiness were affected ($P < 0.01$) by cooking method (Table 5.6), whereas storage time did not affect any sensory attributes ($P > 0.05$). Pan-frying enhanced overall crispiness in bacon ($P < 0.01$) compared to microwave cooking, and this could be related to the crust-forming nature of this cooking method. Furthermore, the high evaporative loss in frying, as well as the high cooking temperature employed in this cooking method (250°C), could have contributed to the crust formation and, hence, overall crispiness.

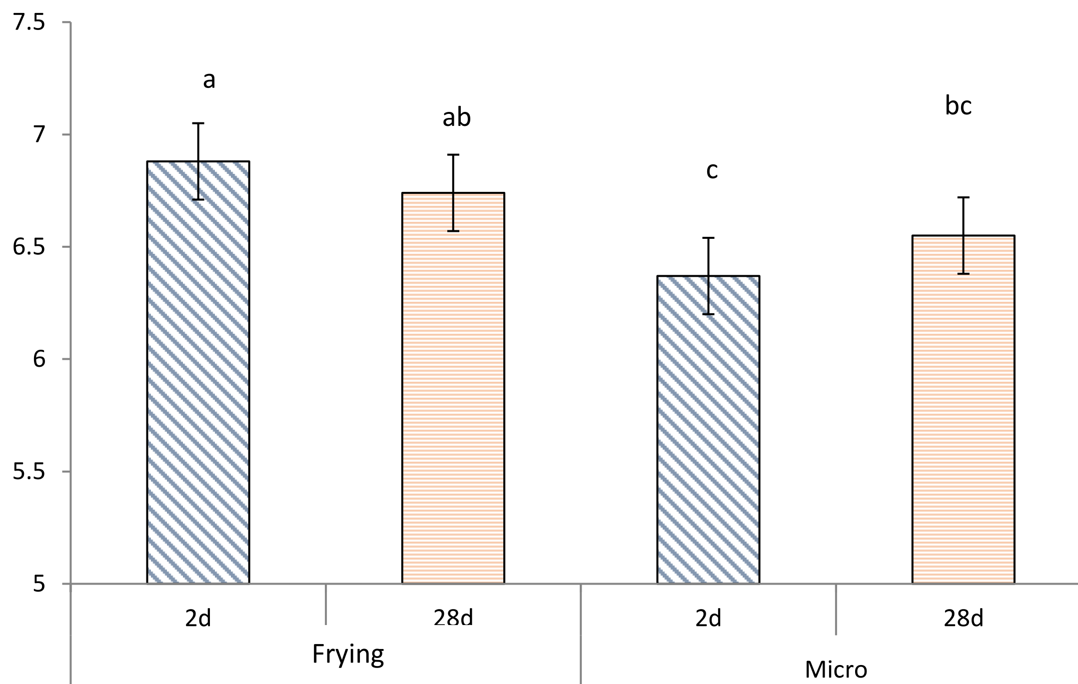


Figure 5.4: Effects of cooking methods and storage days on initial crispiness. Micro = Microwave cooking, Frying = Frying pan cooking. [a-c] Different letters in each bar indicate significant difference at $P < 0.05$.

Table 5.6: Effect of cooking methods on sensory perception of bacon

	Frying Pan	Microwave		P value
	Mean	Mean	SEM	
Initial Crispiness	6.81[a]	6.46[b]	0.16	0.019
Overall Crispiness	6.72[a]	6.28[b]	0.15	0.003
Salt intensity	5.70[a]	5.42[b]	0.18	0.008
Smoke flavour	5.41	5.66	0.20	0.077
Bacon flavour	5.97	5.93	0.19	0.548
Chewiness	5.23[b]	5.50[a]	0.23	0.014
Mouth Coating	3.71	3.57	0.29	0.187
Off-flavor Intensity	8.33	7.92	0.23	0.077

[a-b]Different letters in each row indicate significant difference at $P < 0.05$. A 9-point scale was used with 9=extremely intense and 1=none for all observations except off-flavour intensity in reverse order.

Contrary to other reports where cook loss has been reported to increase in microwave cooking compared to frying, this cook loss has been attributed more to moisture than fat loss (Domínguez et al., 2014; Serrano, Librelotto, Cofrades, Sánchez-Muniz, & Jiménez-Colmenero, 2007); however, the present work showed no difference in cook losses between these two methods (~ 77%; $P > 0.05$). Further exploration on these bacon samples showed that difference in the losses due to fat or water could not be attributed to different cooking methods (results not shown). This could be due to the fatty nature of the thin-sliced bacon compared to leaner and thicker meat steaks employed in the other studies, as well as the varying cooking conditions.

Traditionally, lipid peroxidation has been considered the major form of chemical deterioration that could affect the overall flavour, including both the odour and taste of meat and meat products. The decomposition of lipid hydroperoxides to form secondary products, such as aldehydes, ketones, alcohols and hydrocarbons, has been largely implicated in this quality modification. More recently, the role of protein oxidation in deterioration of quality including texture and water-holding capacity, among other sensory attributes of meat, has been documented (Estévez, 2011; Lund et al., 2011). Protein oxidation may manifest as the modification of amino acid side chains (e.g. protein carbonylation), cleavage of protein peptide bonds or through formation of cross links (e.g. protein polymerization), and these could have significant effects on meat quality deterioration. Apart from variation in moisture content, which can play a role, the higher chewiness observed in microwaved bacon is also hypothesized to be related to the increase in protein oxidation/polymerization. As previously mentioned, microwave cooking can increase the level of protein oxidation in bacon compared to pan-frying. This could have possibly contributed to the observed greater bacon chewiness. Carbonyl groups have been reported to react with non-oxidized amino groups of protein to yield amide bonds, resulting in protein polymerization and aggregate formation that could be perceived in meat product as increased chewiness (Morzel et al., 2006). The involvement of protein carbonyls in such reactions has not been assessed previously and this hypothesis may require future exploration.

Saltiness was also found to increase in pan-fried compared to microwave cooked bacon. Although overall cook losses due to cooking method were not different, the possible high evaporative loss during frying could result in concentration of salt in the bacon during cooking. The overall effect of the salt concentration and crust formation may be perceived as higher salt intensity in bacon by the panellists. The trend for more off-flavour with microwave cooking (P =

0.077; Table 5), which was mostly described by the panellists as “maple”, “sour” and “greasy” (data not shown) may not be of practical significance to consumers. However, because this off-flavour was expected to be higher in pan fried bacon due to its higher TBARS value, the impact of protein oxidation in this perception may also be of interest for future studies.

5.5 Conclusions

Although microwave cooking produced lower lipid oxidation products and HAA compared to pan-frying, it led to greater protein oxidation products which appeared to diminish when bacon was cooked after longer refrigerated storage. The overall effects of storage time and pork belly chemical composition on bacon chemical and sensory characteristics was of minimal practical significance. Generally, cooking methods impacted bacon lipid oxidation, protein oxidation and HAA production as well as bacon sensory traits, and this should be considered when developing cooking recommendations for this popular meat product. The high level of protein oxidation products in bacon also need to be taken into consideration due to their potential implication on human nutrition and health. Further clarification on how the underlying mechanism of these cooking methods may influence the production of these compounds differently is also crucial. The overall impact of protein oxidation on sensory attributes of bacon still needs to be further elucidated.

CHAPTER 6
GENERAL DISCUSSION

Despite the increasing value of pork belly primals, less research focus has been given to its quality assessment and its classification/grading methodology has remained largely rudimentary. Pork bellies accounts for about 9% of live weight and up to 16.7% of chilled carcass weight (Fredeen, Martin, & McAndrews, 1975; Stiffler et al., 1975; USDA, 2017). Thus, pork belly, especially in its cured form, represents a significant economic share of the entire pork carcass (Mandigo, 2000). In most of the world, pork belly is used as the raw material for bacon. The diversified in-home and hotel, restaurant and institutional (HRI) use of bacon has largely contributed to the growing demand for pork bellies, making this primal cut competitive with more traditional pork primals in value (Mandigo, 2001; Soladoye et al., 2015).

In the bacon industry presently, the pork belly pricing system is based on weight, whereas consumers typically prefers to pay premiums for bacon of higher lean content (Person et al., 2005). This suggests that higher prices may be paid by bacon processors for highly fatty pork bellies which typically produce inferior bacon that are discriminated against by consumers. This underscores the need for the bacon industry to review their pricing yardstick for pork bellies to ensure the process is fair for pork producers as well as driven by consumer quality demands. Furthermore, pork belly classification and sorting based on either fat-to-lean ratio or belly softness for different markets and purposes is typically done subjectively by an employee who has the responsibility of promptly sorting the pork bellies at the speed of the conveyor belt transporting them. Errors arise due to the personnel's subjectivity and the dexterity and fatigue inherent in the process.

.

Ante- and post-mortem production factors can play a significant role in pork belly physical and chemical attributes, and these can subsequently influence pork belly technological properties as well as bacon sensory and nutritional quality. Less research has, however, been dedicated to exploring these aspects of research. Consumers' appreciation of this product, even against the backdrop of strict advisories from health organisations to limit their consumption of processed meats, means that studies are necessary to examine various factors (either during pig production or the pork processing stage) that can limit the production of some detrimental chemical compounds that could be responsible for the potential carcinogenicity of these products.

Considering the aforementioned, it appears that multiple gaps exist regarding pork belly research. Given the unique features of the pork belly primal, which include high fat content, thin nature and multiple anatomical layers, direct interpolation of quality behaviours from other primal cuts would not be appropriate in most cases. Thus, the present study attempted to fill some of these research gaps and further elucidate some areas that would enhance pork belly merchandizing, improve consumer acceptability and safety as well as improve processors' profitability. This study has employed some *ante-mortem* factors (including breed, sex, diet and slaughter weight) to create compositional variability within the carcasses of these pigs and, as such, establish a pig population that represent the wide range of variations found in the market place. To fulfil the specific objectives of this study, the first experiment explored a non-invasive technology (DXA) that could be employed in carcass composition assessment particularly for the belly. It was hypothesized that this non-invasive technology would accurately ($R^2 > 0.9$) estimate pork carcass composition regardless of the inherent variation within the pig population. This may position DXA technology for the enhancement of pork belly and carcass classification, sorting or grading. The present research successfully confirmed this hypothesis with a prediction accuracy of $R^2 = 0.94$ and RSD of $< 2.73\%$ for fat and $R^2 = 0.94$ and RSD of $< 2.74\%$ for lean in the pork belly primal.

In view of consumers' discriminatory behaviour against highly fatty bacon among other meat products (Person et al., 2005), the opportunity to accurately and precisely measure carcass composition is crucial for final product quality assurance and consumer acceptability. Traditionally, carcass composition measurement has been done using carcass dissection or chemical analysis. However, these are destructive in nature, as well as subjective and tedious (Marcoux et al., 2005). Therefore, considering their objectivity, reliability, as well as speed and safety during analysis, non-invasive methodologies may be more advantageous for carcass

composition measurements. Most of these non-invasive techniques are unique in that they use electromagnetic frequencies either in the non-ionising range [e.g. sound waves (ultrasound), radio frequency waves (magnetic resonance imaging)] and the ionising range (dual energy X-ray absorptiometry and X-ray computed tomography), which can partially or completely penetrate body or carcass tissues and interact at atomic or molecular levels, resulting in attenuated signals that can be quantitatively analysed by the instrument software (Scholz et al., 2015).

Generally, DXA equally and accurately predicted percent fat and lean content in pork carcasses and primal cuts, which positions this technology as a viable option that can be exploited to enhance sorting of pork belly and other primals as well as their classification and fair pricing in the pork industry. Furthermore, DXA offers opportunity for enhanced performance testing and fast collation of carcass merit traits, highly needed in fields like genomics to develop effective marker panels (Kogelman et al., 2013; Scholz et al., 2015). Dual energy X-ray absorptiometry also holds promise for an enhanced carcass grading system, upgrading lean yield estimate and efficient quality control in the meat processing industry (Aalhus et al., 2014). Because this study has proven that DXA accurately predicts pork belly's lean and fat content, the bacon industry, for instance, can take advantage of this technology in assessing the fat-to-lean ratio of incoming pork belly combos from the various suppliers. This will enhance fairness in the payment system and also improve product consistency and quality. Furthermore, DXA offers a possibility for the overall replacement of the more widely used traditional invasive methodologies for carcass compositional assessment.

As previously discussed, not only will factors like breed, sex, diet and slaughter weight introduce wide compositional variation within the pig population, they may also constitute a major variance in the anatomy and phenotypic features of the pigs. Even though this variation will not impact overall DXA efficiency and accuracy, it will also help in the development of more robust regression models applicable for correcting DXA algorithms in a much wider animal population. In other words, a model built with a narrow animal population may not be very appropriate to predict another animal population that is outside its regression plane. However, before DXA technology finds wider application in the meat industry, it is important that it is developed to accommodate process-line speeds, as well as the humid condition of meat processing plant.

Although the lean-to-fat ratio is an important trait regarding belly quality, pork belly's chemical composition may also have a significant impact on its processing characteristics. The

inclusion of dietary fat in pig's diets may enhance the nutritional benefits derived from the subsequent meat and its products. However, this dietary regimen may obstruct *de novo* fatty acid synthesis (Correa et al., 2008), resulting in higher PUFA in pork carcasses which are associated with pork belly softness. The changes may, subsequently, impact bacon fabrication and increase slicing inefficiency, leading to poor retail appearance and reduced product shelf life (Person et al., 2005; Seman et al., 2013; Shackelford et al., 1990; Trusell et al., 2011). Studies have shown that the fatty acid profile (basically IV), which has been widely used to assess belly softness, may be responsible for only about 14% of the variation in pork belly softness, whereas up to 33% could be explained by belly thickness (Whitney et al., 2006). Moreover, the increasing leanness of pig breeds also contributes to poor technological quality of pork bellies. As previously mentioned, animals with a genetic predisposition for leaner carcasses may produce softer pork bellies. This confirms that both physical and chemical factors are important in adequate assessment of pork belly softness. Because pork belly softness may be affected by many interacting factors, assessing this attribute for the purpose of grading or sorting in the industry and commodity merchandize has been difficult. Considering this, the present study has explored the hypothesis that belly firmness measurements are not only influenced by the chemical attributes of the fat and lean layers of the pork belly, but also by its physical properties. This study sought to derive a multiple regression equation that could explain up to 80% of the variation in pork belly firmness using both physical and chemical factors collected from the pork bellies. The present research successfully developed such proof of concept model.

Following multiple regression model development for the assessment of pork belly softness, both physical and chemical factors were selected and accounted for up to 77 and 83% of the variation in subjective belly softness score and belly-flop angle measurements, respectively. Even though pork belly softness is a major quality defect in the bacon processing industry, no single tool has been established to assess this attribute. The present result confirmed that belly-flop angle is a good tool that could be developed and scaled-up to bacon commercial and processing settings. However, the influence of belly length will need to be considered to ensure reliable results. Furthermore, with the realisation that the physical and chemical attributes are strongly correlated with pork belly firmness and could predict belly softness, the possibility of collecting some of these data through DXA equipment, which could be employed in the multi-

regression for the estimation of pork belly softness, may further enhance the usefulness of this technology.

Despite the possible health benefits of increased unsaturated fatty acids (Simopoulos, 2002) that may be associated with dietary fat inclusion in animal diets and leaner meat, the same fatty acid profile may subject the meat product to oxidative instability. Moreover, cooking time and temperature have been reported to increase products of lipid and protein oxidation (Traore et al., 2012), as well as other carcinogenic compounds, including HAA (Jägerstad et al., 1998) which may pose health risks to humans. Several experimental animal studies and biochemical investigations have shown that not only endogenously produced lipid oxidation products but also those ingested with foods represent significant health risk to humans (Esterbauer, 1993). Although their consumption may not represent acute toxicity (Esterbauer, 1993), chronic exposure may present carcinogenic or atherogenic risk (Albert et al., 2013). Additionally, oxidized proteins have been associated with age-related diseases, including Alzheimer's, Parkinson's, rheumatoid arthritis, diabetes, inflammatory bowel, muscular dystrophy and cataractogenesis (Berlett & Stadtman, 1997; Dalle-Donne et al., 2006). Recent findings point to the possibility that oxidized dietary protein upon ingestion could be involved in the aetiology of many disease conditions (Estévez & Luna, 2016; Keshavarzian et al., 2003; Xie et al., 2014). A recent review has reported the pathogenesis of oxidation products of tyrosine, lysine, as well as some sulphur-containing amino acids (Estévez et al., 2017). Among other compounds that can be classified as process-induced/enhanced are the HAA. These compounds are particularly predominant in heated foods of animal origin. This is because these foods contain abundant levels of the precursors necessary for their production (Jägerstad et al., 1998). The mutagenicity and carcinogenicity of HAA have been widely reported in the literature (Ohgaki et al., 1984; Sugimura et al., 2004). In fact, a recent review has reported that the association between meat and cancer is not in the meat itself but in the ingestion of the various accompanying carcinogens including the HAA(Gibis, 2016).

Most previous studies in this research area focused on the effect of time and temperature of heat treatment on production of these chemical compounds in meat products. The impact of different cooking methods with different underlying mechanisms has been scarcely examined. Furthermore, the typical household practise following purchase of meat products has been to store them in the refrigerator for a period of time prior to preparation for consumption. As the level of oxidative markers in meat products may increase with storage days, assessing the impact of this

practise on the overall level of oxidative and carcinogenic products in bacon post cooking was also crucial. Consequently, the level of oxidative or carcinogenic compounds produced in cooked meat products may be a function of animal production factors as well as household and culinary practises. Hence, the present study explored the hypothesis that pan-frying would lead to higher production of these compounds compared to microwave cooking. The present study partly confirmed this hypothesis, especially with lipid oxidation and HAA. The levels of increased PROTOX was, however, higher in microwave than in fried bacon. There was also significant PROTOX produced during bacon processing which should be explored further.

Microwave cooking has found a wide application both in households and industrial settings due to its convenience of usage, faster cooking time and energy saving benefits. The heating process in microwave cooking is the result of dipolar rotation and ionic conduction. The oscillating microwave field triggers rotational and vibrational energies of dipoles and the dissolved ions will also migrate towards oppositely charged regions in the electric field regions (Venkatesh & Raghavan, 2004). The sum total of these molecular agitations results in frictional generation of heat. As such, moisture and salt content are considered key in the dielectric properties of food and are crucial in overall microwave heating. The higher the moisture content, the higher the dielectric constant and the loss factor of food and the higher it responds to microwave heating (Venkatesh & Raghavan, 2004). However, the temperature profile and heating rate of any food material is not only dependent on the relationship of the food's dielectric properties with moisture, temperature and the prevailing microwave frequency but also on the chemical composition as well as the thermos-physical properties (e.g., thermal conductivity, specific heat, etc.) of all the existing constituents. A recent study (Anwar et al., 2015) showed that the final temperature attained by a solution reduces with increasing salt concentration. This study confirmed the possible implication of food composition on its dielectric heating properties. The study further concluded that in domestic microwaves, the ionic conduction mechanism of heating may not necessarily apply. On the other hand, although fat is a non-ionic and less polar compound which will result in decreased dielectric constant and loss factor of food and, hence, less response to microwave cooking, its low specific heat supports accelerated microwave heating which could result in attainment of high final temperature as well as uniform heating of the food component (Picouet et al., 2007).

Although it appears unequivocal that microwave radiation has thermal effects on food or materials it is exposed to, controversies exist as to whether this effect is solely responsible for the

physical and chemical modification that is observed in foods after-cooking. In the present result, although the thermal effect could have been responsible for the overall increase in PROTOX, HAA and TBARS during pan-frying, the greater increase in PROTOX with microwave cooking could be due to some non-thermal microwave effects not seen in pan-frying (de la Hoz, Diaz-Ortiz, & Moreno, 2005). Several studies have shown significantly higher altered protein conformation and functions due to microwave exposure than conduction heating (Bohr & Bohr, 2000a, 2000b; Copty et al., 2005; George, Bilek, & McKenzie, 2008). These studies support the hypothesis that microwave radiation may have some non-thermal effects (also called "microwave effects") on protein conformation and function through direct interaction of the electromagnetic field with the protein (de la Hoz, Diaz-Ortiz, & Moreno, 2005). Although these non-thermal effects on food materials have not been specifically identified, they may be responsible for the increased PROTOX observed with microwave cooking which was higher than that in pan-frying in the present study. Studies to assess these non-thermal effects may be an interesting subject of future exploration. Also, a detailed assessment of temperatures in different parts of the bacon during heating would be useful.

Even though several studies have suggested a coupling of both protein and lipid oxidation (Mercier et al., 1998; Ventanas et al., 2006), the observation from the present study suggests that this may not always be the case. This confirms reports from other studies that suggest the oxidizing environment of these reactions largely influence the coupling of these two phenomena (Lund et al., 2011; Park et al., 2006). Furthermore, as the relationship between lipid and protein oxidation has been widely assessed by association, not by exploration of the underlining mechanisms, it is possible that these reported relationships are partly due to artefactual compounds that are simultaneously assessed with MDA when using the TBARS method. The reliability of this method to accurately assess lipid peroxidation in meat systems has been challenged (Fenaille, Mottier, Turesky, Ali, & Guy, 2001; Giera, Lingeman, & Niessen, 2012). Considering the harsh derivatisation conditions of this method, the MDA quantified could be overestimated due to this rigorous treatment, as well as derivatization of other species not from lipid peroxidation (Ruan et al., 2014). In an attempt to assess lipid oxidation using a less rigorous MDA derivatisation procedure with pentaflurophenyl hydrazine (PFPH), no significant correlation was observed between the measures of protein oxidation and MDA-PFPH production in this study (Appendix

A4). Overall, to assess the relationship between lipid and protein oxidation, exploring the underlying mechanisms will be more elucidative than mere associative relationship.

Compared to previous studies that have reported the presence of PROTOX in other processed meats, the levels reported in bacon in the present study were many fold greater. As a similar trend was not observed with lipid oxidation, this could signify the presence of a possible protein-specific prooxidant among the ingredients or processes employed in the bacon processing. Of note is the exclusion of sodium erythorbate/ascorbate among the ingredients in the bacon used in the present study. In the United States, sodium erythorbate or ascorbate is a mandatory ingredient in cured meat because it has been found to greatly diminish the formation of nitrosamines by accelerating the reaction of nitrite with the meat protein (FSIS, 2011). Previous reports have also shown that nitrite may promote protein oxidation in meat products in the absence of ascorbate (Villaverde et al., 2014). Although this requires future exploration, it is possible that the high level of PROTOX in the bacon may be due to the exclusion of erythorbate/ascorbate in the ingredient mix and this could further emphasize the importance of adding a reducing agent to cured meat products during processing. As mentioned previously, almost two-thirds of the PROTOX produced in bacon occurred during the smoking/initial thermal processing stage. This could be due to the presence, or absence, of a number of ingredients in the brine that have previously been reported to contribute to protein oxidation in meat products. The initial thermal processing to a higher temperature (65°C internal) than most commercial bacon could also be another reason for this high PROTOX value. This observation may suggest that the control of PROTOX in bacon may be more crucial at the early processing stage than at a later stage of cooking. A detailed look at the smoking process and processing steps would provide much needed information to provide better control of PROTOX production in bacon.

Generally, increasing storage days of meat products results in increasing levels of oxidative products (Cheng et al., 2011; Soyer et al., 2010). However, the choice of packaging methods will have a significant effect on the overall progression of this process (Kang, Kang, Seong, Park, & Cho, 2014; Xiao et al., 2011). Vacuum packaging, as well as other added ingredients in bacon which have antioxidative effects (e.g. nitrite), will limit the oxidative progression during storage days as observed in the present study. Yet, PROTOX products measured in the present study were reduced with storage days. This could be due to subsequent degradation of protein carbonyls to yield Schiff base structures and alpha-amino adipic acid, which is the end product of lysine

oxidation (Utrera et al., 2012). Hence, it is obvious that this observation did not connote a reduction in protein oxidation with storage days, rather a breakdown of one PROTOX marker to some subsequent end products not quantified in the present study.

Although no previous study has implicated protein oxidation in any bacon sensory defect, the increased chewiness in the microwaved bacon in the present study seemed consistent with increased PROTOX. While this still require future exploration, we postulated that this could be due to protein aggregation or folding as a result of the non-thermal effect of microwave radiation (Bohr & Bohr, 2000b). Although this was observed from data collected from trained panellists, it remains to be assessed if untrained sensory panellists would also be able to detect the change in crispiness between these cooking methods. Aside from this, there was no other significant effect of either cooking method or storage days on any bacon sensory attributes. As the average TBARS values in the bacon, regardless of cooking methods and storage days, were below the upper limit of sensory detection threshold of 1 mg MD/kg, no off-flavour was observed in this study. Generally, the production of these chemical compounds did not affect the sensory attributes of the bacon in the present study.

Consistently higher increases in TBARS and HAA ($P < 0.001$), as reported in the present study, may be due to the higher heat transfer coefficient with pan-frying compared to microwave cooking (Gibis, 2016). More importantly, however, could be the adhering fat on bacon that characterises pan-frying given that the bacon was cooked in its own rendered fat. In addition, this may also suggest that the precursors for these products are not a major substrate for the non-thermal effect of microwaves as applicable to proteins. In contrast to microwave cooking, pan-frying is also characterised by crust formation with enhanced Maillard reaction. This could have also contributed to the overall increase of these HAA and the lipid oxidation products, which have been reported to be widely contributed by Maillard reaction products. It is noteworthy that significant, but weak, correlations exist among HAA, TBARS and PROTOX. Although some of these may be due to the artefacts in TBARS quantification previously discussed, the Maillard reaction has been found to be involved in each of these chemical processes. This hypothesis, however, requires future exploration. Surprisingly, there was no correlation between TBARS in bacon and the measure of fatty acid unsaturation in the pork belly. It is possible that the antioxidant effect of nitrite in bacon might have contributed to this observation. In fact, studies have shown that nitrite interferes with TBARS analysis by nitrosation of MDA and this could have influenced the present observation

(Shahidi, Rubin, Diosady, & Wood, 1985). Similarly, Herrick (2014) also found that higher PUFA content did not correspond to higher TBARS value in bacon made from immune- and physically-castrated pigs.

Of the variation within the pigs used, only breed and diet significantly influenced the pork belly and bacon chemical composition (Table 6.1). However, this compositional variation did not influence the production of these compounds (including HAA and lipid oxidation products) (Table 5.5). This may suggest that the level of variation introduced within the pig population in the present study, while influencing chemical composition, did not have any detrimental effect on the production of most of these toxic compounds. Overall, TBARS and HAA remained unaffected in the bacon regardless of the breed, sex or diet of the pig from which the pork belly had been derived.

Although the health impact of dietary PROTOX is still being debated, its impact on digestibility and loss of essential amino acids has been ascertained (Estévez, 2011). With the high increase in PROTOX observed in bacon, especially with no ascorbate addition in the ingredient mixture, health agencies in Canada may need to re-evaluate the regulations that guide the use of ascorbate in bacon. Unlike in the United States where the addition of ascorbate is mandatory in bacon, addition of ascorbate is discretionary in Canada at present. More research is still required to understand how ingredient selection and processing steps in bacon may influence the production of these chemical compounds.

Furthermore, considering the bacon market trend regardless of higher product prices against the backdrop of consumers' increasing scrutiny of their diet based on their concern on its impact on their health and overall wellbeing, more focus must be given by meat industries and researchers to pork belly and bacon quality improvement through the exploration of dietary, genetic, processing and environmental factors among other *ante*- and *post-mortem* factors. This not only enhances meat's technological processing attributes, but also its subsequent nutritional and sensory qualities. Exploration of new, and improvement of already existing, non-invasive technologies for pork belly quality assessment will further help in filling the research gaps for pork belly.

Table 6.1: Effect of animal breed, sex and diet on pork belly attributes

	Moisture (%)	Total Fat (%)	Protein (g/100g) #	IV (g/100g)	n-6 (%)	n-3 (%)	n-6/n-3 (%)	MUFA (%)	SFA (%)	PUFA (%)	PUFA/SFA (%)
						Breed					
Lacombe	42.90±1.06^{a}	43.98±1.39^{b}	12.86±0.004^{a}	66.01±0.38^{a}	10.70±0.14^{a}	2.34±0.16^{a}	5.27±0.40	50.92±0.34	36.05±0.29^{b}	13.03±0.23^{a}	0.36±0.01^{a}
Iberian	32.36±1.00^{b}	57.47±1.30^{a}	10.44±0.004^{b}	62.89±0.35^{b}	9.42±0.14^{b}	1.88±0.15^{b}	5.60±0.43	50.80±0.32	37.90±0.27^{a}	11.30±0.21^{b}	0.30±0.01^{b}
						Sex					
Barrow	35.55±1.43	53.51±1.84	12.00±0.005	64.44±0.48	9.93±0.19	2.21±0.15	5.14±0.40	50.81±0.32	37.05±0.33	12.14±0.28	0.33±0.01
Gilt	39.26±1.53	48.50±1.97	11.19±0.005	64.24±0.51	10.11±0.20	1.96±0.16	5.79±0.43	50.92±0.34	37.02±0.36	12.07±0.30	0.33±0.01
						Diet					
Flaxseed	36.68±1.48	52.00±1.90	11.31±0.005	65.37±0.42^{a}	10.02±0.19	2.75±0.06^{a}	3.67±0.09^{b}	50.29±0.29^{b}	36.94±0.33	12.77±0.24^{a}	0.35±0.01^{a}
Control	37.96±1.58	50.23±2.03	11.87±0.005	63.18±0.45^{b}	10.00±0.21	1.35±0.06^{b}	7.47±0.10^{a}	51.51±0.31^{a}	37.15±0.35	11.34±0.25^{b}	0.31±0.01^{b}

$^{a-b}$Different letters in each column indicate significant difference at $P < 0.05$. # Values for raw bacon

CHAPTER 7

OVERALL CONCLUSIONS AND FUTURE STUDIES

Pork belly has become one of the most valuable cuts within commercial pork carcasses. However, little research is currently available regarding belly and bacon quality. This thesis focussed on three aspects of pork belly and bacon attributes that demanded further scientific evidence: 1) evaluating a non-invasive technology to estimate belly fat and lean content (DXA); 2) understanding the factors contributing to variability in an objective method to measure belly softness (belly-flop); and 3) providing data regarding the impact of storage time and cooking method on bacon oxidative stability and palatability. Because belly and bacon attributes can be affected by production factors, such as breed, diet, gender and slaughter weight of the animal, the present study used animal pig population from a wide range of production systems in order to create variability similar to that found in the market place. The following paragraphs highlight the key findings of the present study. Pertinent areas of future research to expand knowledge in this research field have also been suggested.

The bacon industry has used weight as the main payment measure for pork belly which selects for fatter bellies despite consumers' preference for leaner bacon. The present study confirmed that DXA technology could be developed to accurately assess fat and lean content of pork primal cuts and especially pork belly, regardless of variation within the pig population. The present study demonstrated that a widely varying pig population was more appropriate in building an accurate model for pork carcasses. Further research could explore DXA technology to generate more valuable data from the scanned carcasses rather than mere bone, fat and lean tissue estimates. Some of these could include data (possibly, dimensional factors that have strong association with some chemical attributes) that could be applied in pork belly softness estimates.

The present study has shown that combining both dimensional and compositional factors can successfully predict pork belly softness to between 72 and 83%. Accordingly, dimensional attributes could be combined with chemical composition of selected anatomical layers, to develop an accurate pork belly sorting system to enhance both domestic and international merchandizing. Although belly-flop angle accurately predicted pork belly softness, there is an undue influence of pork belly length in its overall prediction. Hence, in order for this tool to be reliably used in predicting pork belly softness, belly-flop angle measurements should be corrected for length. Although the present results have shown that the physical and chemical properties of all the anatomical layers of pork belly could influence pork belly softness, it was confirmed that, if IV is intended to be used as a single factor in pork belly softness prediction, the outermost subcutaneous layer is the most reliable anatomical position. Future studies are still needed in several areas to: adequately estimate the overall impact of pork belly softness on bacon slice yield, conduct multiple regression analysis to assess individual predictors that may be contributing to bacon slice yield; and develop the concept put forward in this research for objective and automated online pork belly softness assessment, classification and sorting for fair pricing to enhance various domestic and international market requirements.

The present study has elucidated the impact of cooking methods and storage days on the production of some chemical compounds in bacon. Heat treatment increased the production of HAA, lipid oxidation (TBARS) and PROTOX in bacon. Pan-frying led to higher increase in TBARS and HAA whereas microwave cooking resulted in higher increase in PROTOX in bacon. Days in refrigeration storage did not affect the production of HAA or TBARS. Protein carbonyls reduced with bacon storage days, possibly due to degradation of protein carbonyl to a more stable end-product of protein oxidation. The present study showed that variation within pork belly composition did not affect the production of these chemical compounds during bacon cooking. As such, manipulation of production parameters within the limits employed in the present study may not result in any defect in bacon chemical composition. Generally, the cooking treatments and storage days had little or no impact on bacon sensory attributes. Microwave cooking, however, increased bacon chewiness and reduced bacon crispiness compared to frying pan cooking. These properties also corresponded with the increased PROTOX also observed with microwave cooking in the present study.

Further studies are still required to adequately assess the impact of PROTOX on meat textural and other sensory attributes. Although HAA and MDA have been widely recognised for their carcinogenic and mutagenic properties, more studies are needed to fully understand the biological impact of PROTOX on humans. Furthermore, levels of these chemical species that can result in acute or chronic toxicity have not been established. More studies to characterise these chemical compounds and also recognise their toxicity level will greatly enhance public health and safety recommendations. Further explorations on the mechanistic impact of cooking methods on the level of production of these chemical compounds are necessary. The overall impact of thermal processing/smoking step as well as the ingredient inclusion or exclusion in bacon and their corresponding impact on PROTOX formation also require further exploration. Significant but weak relationship among these chemical compounds (HAA, TBARS and PROTOX) were observed in the present study and this was postulated to be mediated by the Maillard reaction. Future study is warranted to examine this relationship and possibly explore a common mechanism that could be exploited to significantly and simultaneous reduce these chemical species in cooked meat products.

CHAPTER 8

REFERENCES

Aalhus, J. L., & Dugan, M. E. R. (2014). Spoilage, factors affecting: oxidative and enzymatic. In C. Devine & M. Dikeman (Eds.), *Encyclopedia of Meat Sciences 2nd Edition* (pp. 394-400). Oxford: Elsevier.

Aalhus, J. L., López-Campos, Ó., Prieto, N., Rodas-González, A., Dugan, M. E. R., Uttaro, B., & Juárez, M. (2014). Review: Canadian beef grading-opportunities to identify carcass and meat quality traits valued by consumers. *Canadian Journal of Animal Science, 94*(4), 545-556.

Adamson, R. H., Thorgeirsson, U. P., & Sugimura, T. (1996). Extrapolation of heterocyclic amine carcinogenesis data from rodents and nonhuman primates to humans. In J.P. Seiler, O. Kroftová, & V. Eybl (Eds.), *Toxicology-From Cells to Man* (pp. 303-318). Berlin, Heidelberg: Springer.

Ahmed, J., & Ramaswamy, H. S. (2007). Microwave pasteurization and sterilization of foods. In M.S. Rahman (Ed.), *Handbook of Food Preservation 2nd Edition* (pp. 691-711). New York: CRC Press.

Ahn, J., & Grün, I. U. (2005). Heterocyclic amines: 1. Kinetics of formation of polar and nonpolar heterocyclic amines as a function of time and temperature. *Journal of Food Science, 70*(2), C173-C179.

Ahotupa, M., Suomela, J.-P., Vuorimaa, T., & Vasankari, T. (2010). Lipoprotein-specific transport of circulating lipid peroxides. *Annals of Medicine, 42*(7), 521-529.

Ahotupa, M., & Vasankari, T. J. (1999). Baseline diene conjugation in LDL lipids: An indicator of circulating oxidized LDL. *Free Radical Biology and Medicine, 27*(11), 1141-1150.

Akagawa, M., Sasaki, D., Ishii, Y., Kurota, Y., Yotsu-Yamashita, M., Uchida, K., & Suyama, K. (2006). New method for the quantitative determination of major protein carbonyls, α-aminoadipic and γ-glutamic semialdehydes: Investigation of the formation mechanism and chemical nature in vitro and in vivo. *Chemical Research in Toxicology, 19*(8), 1059-1065.

Akagawa, M., Sasaki, D., Kurota, Y., & Suyama, K. (2005). Formation of α-aminoadipic and γ-glutamic semialdehydes in proteins by the maillard reaction. *Annals of the New York Academy of Sciences, 1043*(1), 129-134.

Alarcon, R. A. (1970). Acrolein: IV. Evidence for the formation of the cytotoxic aldehyde acrolein from enzymatically oxidized spermine or spermidine. *Archives of Biochemistry and Biophysics, 137*(2), 365-372.

Albert, B. B., Cameron-Smith, D., Hofman, P. L., & Cutfield, W. S. (2013). Oxidation of marine omega-3 supplements and human health. *BioMed Research International, 2013, 1-9*.

Aldini, G., Gamberoni, L., Orioli, M., Beretta, G., Regazzoni, L., Maffei Facino, R. & Carini, M. (2006). Mass spectrometric characterization of covalent modification of human serum albumin by 4-hydroxy-trans-2-nonenal. *Journal of Mass Spectrometry, 41*(9), 1149-1161.

Anderson, G. H., Ashley, D. V. M., & Jones, J. D. (1976). Utilization of l-methionine sulfoxide, l-methionine sulfone and cysteic acid by the weanling rat. *The Journal of Nutrition, 106*(8), 1108-1114.

Anderson, G. H., Li, G. S. K., Jones, J. D., & Bender, F. (1975). Effect of hydrogen peroxide treatment on the nutritional quality of rapeseed flour fed to weanling rats. *The Journal of Nutrition, 105*(3), 317-325.

Anderson, S. (2004). Soxtec: Its Principles and Applications. In D. L Luthria (Ed.), *Oil extraction and analysis: Critical issue and comparative studies* (pp. 11-24). Champaign: AOCS Press.

Angeli, J. P. F., Garcia, C. C. M., Sena, F., Freitas, F. P., Miyamoto, S., Medeiros, M. H. G. & Di Mascio, P. (2011). Lipid hydroperoxide-induced and hemoglobin-enhanced oxidative damage to colon cancer cells. *Free Radical Biology and Medicine, 51*(2), 503-515.

Anwar, J., Shafique, U., Rehman, R., Salman, M., Dar, A., Anzano, J. M., Ashraf, U. & Ashraf, S. (2015). Microwave chemistry: Effect of ions on dielectric heating in microwave ovens. *Arabian Journal of Chemistry, 8*(1), 100-104.

Apple, J. K. (2007). Effects of nutritional modifications on the water-holding capacity of fresh pork: A review. *Journal of Animal Breeding and Genetics, 124(S1)*, 43-58.

Apple, J. K., Maxwell, C. V., Galloway, D. L., Hamilton, C. R., & Yancey, J. W. S. (2009). Interactive effects of dietary fat source and slaughter weight in growing-finishing swine: III. Carcass and fatty acid compositions. *Journal of Animal Science, 87*(4), 1441-1454.

Apple, J. K., Maxwell, C. V., Sawyer, J. T., Kutz, B. R., Rakes, L. K., Davis, M. E. & Armstrong, T. A. (2007). Interactive effect of ractopamine and dietary fat source on quality characteristics of fresh pork bellies. *Journal of Animal Science, 85*(10), 2682-2690.

Apple, J. K., Sawyer, J. T., Maxwell, C. V., Yancey, J. W. S., Frank, J. W., Woodworth, J. C., & Musser, R. E. (2011). Effects of L-carnitine supplementation on quality characteristics of fresh pork bellies from pigs fed 3 levels of corn oil. *Journal of Animal Science, 89*(9), 2878-2891.

Armenteros, M., Heinonen, M., Ollilainen, V., Toldrá, F., & Estévez, M. (2009). Analysis of protein carbonyls in meat products by using the DNPH-method, fluorescence spectroscopy and liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS). *Meat Science, 83*(1), 104-112.

Asmus, M. D., Tavarez, M. A., Tokach, M. D., Dritz, S. S., Schroeder, A. L., Nelssen, J. L. & DeRouchey, J. M. (2014). The effects of immunological castration and corn dried distillers grains with solubles withdrawal on growth performance, carcass characteristics, fatty acid analysis, and iodine value of pork fat depots. *Journal of Animal Science, 92*(5), 2116-2132.

Astruc, T., Marinova, P., Labas, R., Gatellier, P., & Santé-Lhoutellier, V. (2007). Detection and localization of oxidized proteins in muscle cells by fluorescence microscopy. *Journal of Agricultural and Food Chemistry, 55*(23), 9554-9558.

Averette Gatlin, L., See, M. T., Hansen, J. A., & Odle, J. (2003). Hydrogenated dietary fat improves pork quality of pigs from two lean genotypes. *Journal of Animal Science, 81*(8), 1989-1997.

Averette Gatlin, L., See, M. T., Hansen, J. A., Sutton, D., & Odle, J. (2002). The effects of dietary fat sources, levels, and feeding intervals on pork fatty acid composition. *Journal of Animal Science, 80*(6), 1606-1615.

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity, 2014, 1-32*.

Badr, H. M., & Mahmoud, K. A. (2011). Antioxidant activity of carrot juice in gamma irradiated beef sausage during refrigerated and frozen storage. *Food Chemistry, 127*(3), 1119-1130.

Badiani, A., Stipa, S., Bitossi, F., Gatta, P. P., Vignola, G., & Chizzolini, R. (2002). Lipid composition, retention and oxidation in fresh and completely trimmed beef muscles as affected by common culinary practices. *Meat Science, 60*(2), 169-186.

Bahelka, I., Oravcová, M., Hanusová, E., & Demo, P. (2011). The effect of sex and terminal sire line on carcass characteristics of pork belly. *Archiv Tierzucht, 54*(3), 264-270.

Baldwin, R. E. (1978). Microwave cookery for meats. Paper presented at: 31st Annual Reciprocal Meat Conference. University of Connecticut, Storrs, Connecticut, United States.

Barbir, A., Linseisen, J., Hermann, S., Kaaks, R., Teucher, B., Eichholzer, M., & Rohrmann, S. (2012). Effects of phenotypes in heterocyclic aromatic amine (HCA) metabolism–related genes on the association of HCA intake with the risk of colorectal adenomas. *Cancer Causes & Control, 23*(9), 1429-1442.

Barden, H. S., & Mazess, R. B. (1989). Bone densitometry of the appendicular and axial skeleton. *Topics in Geriatric Rehabilitation, 4*(2), 1-12.

Bax, M., Buffière, C., Hafnaoui, N., Gaudichon, C., Savary-Auzeloux, I., Dardevet, D. & Rémond, D. (2013). Effects of meat cooking, and of ingested amount, on protein digestion speed and entry of residual proteins into the colon: A study in minipigs. *PLoS ONE, 8*(4), 1-7.

Bax, M. L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., & Santé-Lhoutellier, V. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: Investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry, 60*(10), 2569-2576.

Bee, G. (2002). Effect of available dietary carbohydrate on glycolytic potential and meat quality of swine muscles. *Canadian Journal of Animal Science, 82*(3), 311-320.

Bee, G., Messikommer, R., & Gebert, S. (1999). Dietary fats and energy levels differently affect tissue lipogenic enzyme activity in finishing pigs. *Lipid/Fett, 101*(9), 336-342.

Beltranena, E., Aalhus, J. L., Dugan, M. E. R., Young, M., Campbell, N., Oryschak, M., & Zijlstra, R. T. (2011). Graded inclusions of wheat distillers dried grains with solubles (DDGS) in hog diets: Growth performance, carcass traits, dissected pork yield, loin and belly quality. Paper presented at: 40th Banff Pork Seminar, Banff, Alberta, Canada.

BeMiller, J. N., & Huber, K. C. (2008). Carbohydrates. In S. Damodaran, L. K. Parkin, & O. R. Fennema (Eds.), *Fennema's Food Chemistry 4th Edition* (pp 83-154). Boca Raton, London, New York, CRS Press: Taylor and Francis Group.

Benz, J. M., Linneen, S. K., Tokach, M. D., Dritz, S. S., Nelssen, J. L., DeRouchey, J. M. & Prusa, K. J. (2010). Effects of dried distillers grains with solubles on carcass fat quality of finishing pigs. *Journal of Animal Science, 88*(11), 3666-3682.

Berg, E. P., Asfaw, A., & Ellersieck, M. R. (2002). Predicting pork carcass and primal lean content from electromagnetic scans. *Meat Science, 60*(2), 133-139.

Berg, E. P., Forrest, J. C., & Fisher, J. E. (1994). Electromagnetic scanning of pork carcasses in an on-line industrial configuration. *Journal of Animal Science, 72*(10), 2642-2652.

Berlett, B. S., & Stadtman, E. R. (1997). Protein oxidation in aging, disease, and oxidative stress. *Journal of Biological Chemistry, 272*(33), 20313-20316.

Bernau, M., Kremer, P. V., Lauterbach, E., Tholen, E., Petersen, B., Pappenberger, E., & Scholz, A. M. (2015). Evaluation of carcass composition of intact boars using linear measurements from performance testing, dissection, dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI). *Meat Science, 104*, 58-66.

Bertol, T. M., Campos, R. M. L. D., Ludke, J. V., Terra, N. N., Figueiredo, E. A. P. D., Coldebella, A. & Lehr, N. M. (2013). Effects of genotype and dietary oil supplementation on performance, carcass traits, pork quality and fatty acid composition of backfat and intramuscular fat. *Meat Science, 93*(3), 507-516.

Bidanel, J. P., Bonneau, M., Pointillart, A., Gruand, J., Mourot, J., & Demade, I. (1991). Effects of exogenous porcine somatotropin (pST) administration on growth performance, carcass traits, and pork meat quality of Meishan, Pietrain, and crossbred gilts. *Journal of Animal Science, 69*(9), 3511-3522.

National Pork Board. (2008). *Bacon trends*. Available at: https://www.porkretail.org/filelibrary/Retail/BaconTrends.pdf. Accessed on 23/12/2016.

Bohr, H., & Bohr, J. (2000a). Microwave enhanced kinetics observed in ORD studies of a protein. *Bioelectromagnetics, 21*(1), 68-72.

Bohr, H., & Bohr, J. (2000b). Microwave-enhanced folding and denaturation of globular proteins. *Physical Review E, 61*(4), 4310-4314.

Boler, D. D., Killefer, J., Meeuwse, D. M., King, V. L., McKeith, F. K., & Dilger, A. C. (2012). Effects of slaughter time post-second injection on carcass cutting yields and bacon characteristics of immunologically castrated male pigs. *Journal of Animal Science, 90*(1), 334-344.

Bordas, M., Moyano, E., Puignou, L., & Galceran, M. T. (2004). Formation and stability of heterocyclic amines in a meat flavour model system: Effect of temperature, time and precursors. *Journal of Chromatography B, 802*(1), 11-17.

Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., Ghissassi, F. E., Benbrahim-Tallaa, L. & Straif, K. (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet Oncology,* 16(16), 1599.

Boye, J., Wijesinha-Bettoni, R., & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition, 108*(S2), S183-S211.

Brewer, M. S., Stites, C. R., McKeith, F. K., Bechtel, P. J., Novakofski, J. E., & Bruggen, K. A. (1995). Belly thickness effects on the proximate composition, processing, and sensory characteristics of bacon. *Journal of Muscle Foods, 6*(3), 283-296.

Brienne, J. P., Denoyelle, C., Baussart, H., & Daudin, J. D. (2001). Assessment of meat fat content using dual energy X-ray absorption. *Meat Science, 57*(3), 235-244.

Broncano, J. M., Petrón, M. J., Parra, V., & Timón, M. L. (2009). Effect of different cooking methods on lipid oxidation and formation of free cholesterol oxidation products (COPs) in Latissimus dorsi muscle of Iberian pigs. *Meat Science, 83*(3), 431-437.

Browne, N. A., Apple, J. K., Maxwell, C. V., Yancey, J. W., Johnson, T. M., Galloway, D. L., & Bass, B. E. (2013). Alternating dietary fat sources for growing-finishing pigs fed dried distillers grains with solubles: II. Fresh belly and bacon quality characteristics1. *Journal of Animal Science, 91*(3), 1509-1521.

Brunton, J. A., Bayley, H. S., & Atkinson, S. A. (1993). Validation and application of dual-energy X-ray absorptiometry to measure bone mass and body composition in small infants. *The American Journal of Clinical Nutrition, 58*(6), 839-845.

Bule, G. (2016). *The history of bacon.* Available at: http://www.englishbreakfastsociety.com/history-of-bacon.html. Accessed on 23/12/2016.

Bull, A. W., Nigro, N. D., & Marnett, L. J. (1988). Structural requirements for stimulation of colonic cell proliferation by oxidized fatty acids. *Cancer Research, 48*(7), 1771-1776.

Busquets, R., Bordas, M., Toribio, F., Puignou, L., & Galceran, M. T. (2004). Occurrence of heterocyclic amines in several home-cooked meat dishes of the Spanish diet. *Journal of Chromatography B, 802*(1), 79-86.

Butterfield, D. A., Reed, T., & Sultana, R. (2011). Roles of 3-nitrotyrosine-and 4-hydroxynonenal-modified brain proteins in the progression and pathogenesis of Alzheimer's disease. *Free Radical Research, 45*(1), 59-72.

Canadian Council on Animal Care. (1993). *Guide to the care and use of experimental animals.* Canadian Council on Animal Care. Ottawa, CA. Available at: http://www.ccac.ca/Documents/Standards/Guidelines/Experimental_Animals_Vol1.pdf. Accessed on 05/05/2015.

Canadian Council on Animal Care. (2009). *The care and use of farm animals in research, teaching and testing.* Canadian Council on Animal Care., Ottawa, CA. Available at: http://www.ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf. Accessed on 05/05/2015.

Canadian Food Innovators. (2016). *Innovative bacon production processes meet food safety standards* [Press release]. Available at: http://canadianfoodinnovators.ca/project/innovative-bacon-production-processes-meet-food-safety-standards. Accessed on 22/12/2016.

Canadian Pork International. (1995). *Canadian Pork Buyers Manual. Ontario, Canada.* Canadian Pork International. Available at http://www.canadapork.com/documents/file/canadian-pork-buyer-s-guide-retail-and-foodservice-end-user-guide-en.pdf. Accessed on 23/12/2016.

Cannon, J. E., Morgan, J. B., McKeith, F. K., Smith, G. C., Sonka, S., Heavner, J., & Meeker, D. L. (1996). Pork chain quality audit survey: Quantification of pork quality characteristics1. *Journal of Muscle Foods, 7*(1), 29-44.

Carr, S. N., Rincker, P. J., Killefer, J., Baker, D. H., Ellis, M., & McKeith, F. K. (2005). Effects of different cereal grains and ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. *Journal of Animal Science, 83*(1), 223-230.

Cava, R., Ladero, L., González, S., Carrasco, A., & Ramírez, M. R. (2009). Effect of pressure and holding time on colour, protein and lipid oxidation of sliced dry-cured Iberian ham and loin during refrigerated storage. *Innovative Food Science & Emerging Technologies, 10*(1), 76-81.

CFIA. (2013). *Annex C: Use of Phosphate Salts and Nitrites in the Preparation of Meat Products*. Canadian Food Inspection Agency. Avaialable at: http://www.inspection.gc.ca/food/meat-and-poultry-products/manual-of-procedures/chapter-4/annex-c/eng/1370525150531/1370525354148. Accessed on 18/12/2016.

Channon, H. A., Kerr, M. G., & Walker, P. J. (2004). Effect of Duroc content, sex and ageing period on meat and eating quality attributes of pork loin. *Meat Science, 66*(4), 881-888.

Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified procedure for myofibril hydrophobicity determination. *Meat Science, 74*(4), 681-683.

Chen, N., Zhao, M., & Sun, W. (2013). Effect of protein oxidation on the in vitro digestibility of soy protein isolate. *Food Chemistry, 141*(3), 3224-3229.

Cheng, A., Wan, F., Xu, T., Du, F., Wang, W., & Zhu, Q. (2011). Effect of irradiation and storage time on lipid oxidation of chilled pork. *Radiation Physics and Chemistry, 80*(3), 475-480.

Chisolm, G. M., & Steinberg, D. (2000). The oxidative modification hypothesis of atherogenesis: an overview. *Free Radical Biology and Medicine, 28*(12), 1815-1826.

Cipak, A., Hasslacher, M., Tehlivets, O., Collinson, E. J., Zivkovic, M., Matijevic, T., Wonisch, W., Waeg, G., Dawes, I.W., Zarkovic, N. & Kohlwein, S.D. (2006). Saccharomyces cerevisiae strain expressing a plant fatty acid desaturase produces polyunsaturated fatty acids and is susceptible to oxidative stress induced by lipid peroxidation. *Free Radical Biology and Medicine, 40*(5), 897-906.

Cisneros, F., Ellis, M., McKeith, F. K., McCaw, J., & Fernando, R. L. (1996). Influence of slaughter weight on growth and carcass characteristics, commercial cutting and curing yields, and meat quality of barrows and gilts from two genotypes. *Journal of Animal Science, 74*(5), 925-933.

Conchillo, A., Ansorena, D., & Astiasarán, I. (2005). Intensity of lipid oxidation and formation of cholesterol oxidation products during frozen storage of raw and cooked chicken. *Journal of the Science of Food and Agriculture, 85*(1), 141-146.

Cook, D. (2015). Effects of amino acid supplementation of reduced crude protein (RCP) diets formulated on a NE basis on belly fatty acid deposition in swine. Paper presented at: *ADSA-ASAS 2015 Midwest Meeting*, Des Moines, Iowa, United States.

Copty, A., Sakran, F., Popov, O., Ziblat, R., Danieli, T., Golosovsky, M., & Davidov, D. (2005). Probing of the microwave radiation effect on the green fluorescent protein luminescence in solution. *Synthetic Metals, 155*(2), 422-425.

Correa, J. A., Gariépy, C., Marcoux, M., & Faucitano, L. (2008). Effects of growth rate, sex and slaughter weight on fat characteristics of pork bellies. *Meat Science, 80*(2), 550-554.

Cox, R., Goorha, S., & Irving, C. C. (1988). Inhibition of DNA methylase activity by acrolein. *Carcinogenesis, 9*(3), 463-465.

Crampton, E. W., Ashton, G. C., & Lloyd, L. E. (1954). The effect of restricting feed intake of market hogs during the finishing period on the quality of the bacon carcass. *Journal of Animal Science, 13*(2), 321-326.

Cromwell, G. L., Lindemann, M. D., Randolph, J. R., Monegue, H. J., Laurent, K. M., & Parker, G. R. (1999). Efficacy of betaine as a carcass modifer in finishing pigs fed normal and reduced energy diets. *Journal of Animal Science, 77*(S1), 179.

Cuq, J. L., Besancon, P., Chartier, L., & Cheftel, C. (1978). Oxidation of methionine residues of food proteins and nutritional availability of protein-bound methionine sulphoxide. *Food Chemistry, 3*(2), 85-102.

D'Souza, D. N., Warner, R. D., Dunshea, F. R., & Leury, B. J. (1999). Comparison of different dietary magnesium supplements on pork quality. *Meat Science, 51*(3), 221-225.

D'Souza, D. N., Warner, R. D., Leury, B. J., & Dunshea, F. R. (1998). The effect of dietary magnesium aspartate supplementation on pork quality. *Journal of Animal Science, 76*(1), 104-109.

Dalleau, S., Baradat, M., Guéraud, F., & Huc, L. (2013). Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance. *Cell Death & Differentiation, 20*(12), 1615-1630.

Dalle-Donne, I., Giustarini, D., Colombo, R., Rossi, R., & Milzani, A. (2003). Protein carbonylation in human diseases. *Trends in Molecular Medicine, 9*(4), 169-176.

Dalle-Donne, I., Aldini, G., Carini, M., Colombo, R., Rossi, R., & Milzani, A. (2006). Protein carbonylation, cellular dysfunction, and disease progression. *Journal of Cellular and Molecular Medicine, 10*(2), 389-406.

Daneshvar, B., Frandsen, H., Autrupand, H., & Dragsted, L. O. (1997). γ-Glutamyl semialdehyde and 2-amino-adipic semialdehyde: Biomarkers of oxidative damage to proteins. *Biomarkers, 2*(2), 117-123.

Datta, A. K., & Rakesh, V. (2013). Principles of microwave combination heating. *Comprehensive Reviews in Food Science and Food Safety, 12*(1), 24-39.

Davenel, A., Riaublanc, A., Marchal, P., & Gandemer, G. (1999). Quality of pig adipose tissue: relationship between solid fat content and lipid composition. *Meat Science, 51*(1), 73-79.

Davies, K. J. A. (2001). Degradation of oxidized proteins by the 20S proteasome. *Biochimie, 83*(3–4), 301-310.

de la Hoz, A., Diaz-Ortiz, A., & Moreno, A. (2005). Microwaves in organic synthesis: Thermal and non-thermal microwave effects. *Chemical Society Reviews, 34*(2), 164-178.

Dizdaroglu, M., Gajewski, E., & Simic, M. G. (1984). Enzymatic digestibility of peptides cross-linked by ionizing radiation. *International Journal of Radiation Biology, 45*(3), 283-295.

Dieticians of Canada (2012). *Making the most of meat and alternatives*. Available at: https://www.dietitians.ca/Downloads/Factsheets/Making-Most-of-Meats-and-Alternatives.aspx. Accessed on 22/07/2017.

Domínguez, R., Gómez, M., Fonseca, S., & Lorenzo, J. M. (2014). Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. *Meat Science, 97*(2), 223-230.

Dugan, M. E. R., Aalhus, J. L., & Kramer, J. K. G. (2004). Conjugated linoleic acid pork research. *The American Journal of Clinical Nutrition, 79*(6), 1212S-1216S.

Dugan, M. E. R., Kramer, J. K. G., Robertson, W. M., Meadus, W. J., Aldai, N., & Rolland, D. C. (2007). Comparing subcutaneous adipose tissue in beef and muskox with emphasis on trans 18:1 and conjugated linoleic acids. *Lipids, 42*(6), 509-518.

Dunshea, F. R., D'Souza, D. N., Pethick, D. W., Harper, G. S., & Warner, R. D. (2005). Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat Science, 71*(1), 8-38.

Earles, S. M., Bronstein, J. C., Winner, D. L., & Bull, A. W. (1991). Metabolism of oxidized linoleic acid: characterization of 13-hydroxyoctadecadienoic acid dehydrogenase activity from rat colonic tissue. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1081*(2), 174-180.

Eggert, J. M., Belury, M. A., Kempa-Steczko, A., Mills, S. E., & Schinckel, A. P. (2001). Effects of conjugated linoleic acid on the belly firmness and fatty acid composition of genetically lean pigs. *Journal of Animal Science, 79*(11), 2866-2872.

Ellis, M., McKeith, F. K., & Miller, K. D. (1999). The effects of genetic and nutritional factors on pork quality-review. *Asian-Australasian Journal of Animal Sciences, 12*(2), 261-270.

Engberg, R. M., Lauridsen, C., Jensen, S. K., & Jakobsen, K. (1996). Inclusion of oxidized vegetable oil in broiler diets. its influence on nutrient balance and on the antioxidative status of broilers. *Poultry Science, 75*(8), 1003-1011.

Engel, J. J., Smith, J. W., Unruh, J. A., Goodband, R. D., O'Quinn, P. R., Tokach, M. D., & Nelssen, J. L. (2001). Effects of choice white grease or poultry fat on growth performance, carcass leanness, and meat quality characteristics of growing-finishing pigs. *Journal of Animal Science, 79*(6), 1491-1501.

Esterbauer, H. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *The American Journal of Clinical Nutrition, 57*(5), 779S-785S.

Esterbauer, H., Schaur, R. J. R., & Zollner, H. (1990). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology & Medicine, 11*(1), 81-128.

Estévez. (2011). Protein carbonyls in meat systems: A review. *Meat Science, 89*(3), 259-279.

Estévez, M., & Cava, R. (2006). Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: Contradictory effects in different types of frankfurters. *Meat Science, 72*(2), 348-355.

Estévez, M., & Heinonen, M. (2010). Effect of phenolic compounds on the formation of α-aminoadipic and γ-glutamic semialdehydes from myofibrillar proteins oxidized by copper, iron, and myoglobin. *Journal of Agricultural and Food Chemistry, 58*(7), 4448-4455.

Estévez, M., Li, Z., Soladoye, O. P., & Van-Hecke, T. (2017). Health risks of food oxidation. In F. Toldrá (Ed.), *Advances in Food and Nutrition Research* (pp. 45-81). London: Academic Press.

Estévez, M., & Luna, C. (2016). Dietary protein oxidation: A silent threat to human health? *Critical Reviews in Food Science and Nutrition*. http://dx.doi.org/10.1080/10408398.2016.1165182.

Estévez, M., Morcuende, D., & Ventanas, S. (2008). Determination of oxidation. In L. M. Nollet & F. Toldra (Eds.), *Handbook of Processed Meat and Poultry Analysis* (pp. 141-162). Boca Raton: CRC Press.

Estévez, M., Ollilainen, V., & Heinonen, M. (2009). Analysis of protein oxidation markers α-Aminoadipic and γ-Glutamic semialdehydes in food proteins using liquid chromatography (LC)− electrospray ionization (ESI)− multistage tandem mass spectrometry (MS). *Journal of Agricultural and Food Chemistry, 57*(9), 3901-3910.

Estévez, M., Ventanas, S., & Cava, R. (2005). Protein oxidation in frankfurters with increasing levels of added rosemary essential oil: Effect on color and texture deterioration. *Journal of Food Science, 70*(7), c427-c432.

Evenepoel, Claus, Geypens, Maes, Hiele, Rutgeerts, & Ghoos. (1998). Evidence for impaired assimilation and increased colonic fermentation of protein, related to gastric acid suppression therapy. *Alimentary Pharmacology & Therapeutics, 12*(10), 1011-1019.

Fabry, J., Demeyer, D., Thielemans, M. F., Deroanne, C., Van de Voorde, G., Deroover, E., & Dalrymple, R. H. (1991). Evaluation of recombinant porcine somatotropin on growth performance, carcass characteristics, meat quality, and muscle biochemical properties of Belgian Landrace pigs. *Journal of Animal Science, 69*(10), 4007-4018.

Fang, W., Sun, J., Li, Z. L., Le, G., & Shi, Y. (2012). Effect of oxidated food protein on mice gut floraand redox state. *Chinese Journal of Microecology, 24*, 193-196.

FAO. (2013). *Dietary protein quality evaluation in human nutrition*. Available at: http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf. Accessed on 27/12/2016.

Felton, J. S., Fultz, E., Dolbeare, F. A., & Knize, M. G. (1994). Effect of microwave preteatments on heterocyclic aromatic amines mutagenic/carcinogens in fried meat patties. *Food Chemistry and Toxicology, 32*(10), 897-903.

Fenaille, F., Mottier, P., Turesky, R. J., Ali, S., & Guy, P. A. (2001). Comparison of analytical techniques to quantify malondialdehyde in milk powders. *Journal of Chromatography A, 921*(2), 237-245.

Fiego, D. P. L., Santoro, P., Macchioni, P., & De Leonibus, E. (2005). Influence of genetic type, live weight at slaughter and carcass fatness on fatty acid composition of subcutaneous adipose tissue of raw ham in the heavy pig. *Meat Science, 69*(1), 107-114.

Filgueras, R. S., Gatellier, P., Zambiazi, R. C., & Santé-Lhoutellier, V. (2011). Effect of frozen storage duration and cooking on physical and oxidative changes in *M. Gastrocnemius pars interna* and *M. Iliofiburalis* of *rhea americana*. *Meat Science, 88*(4), 645-651.

Fisher, P., Mellett, F. D., & Hoffman, L. C. (2000). Halothane genotype and pork quality. 2. Cured meat products of three halothane genotypes. *Meat Science, 54*(2), 107-111.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry, 226*(1), 497-509.

Fredeen, H. T. (1980). Yields and dimensions of pork bellies in relation to carcass measurements. *Journal of Animal Science, 51*(1), 59-68.

Fredeen, H. T., Martin, A. H., & McAndrews, J. G. (1975). Criteria of belly bacon desirability. iii. within-belly variance in chemical and physical characteristics. *Canadian Journal of Animal Science, 55*(4), 661-672.

FSIS. (2011). *Bacon and food safety*. Food Safety and Inspection Service, United State Department of Agriculture. Available at: http://www.fsis.usda.gov/wps/wcm/connect/98dfaf58-75f3-4535-a0fa-589ca077cc76/Bacon_and_Food_Safety.pdf?MOD=AJPERES. Accessed on 27/12/2016.

Fuentes, V., Ventanas, J., Morcuende, D., Estévez, M., & Ventanas, S. (2010). Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure. *Meat Science, 85*(3), 506-514.

Ganhão, R., Morcuende, D., & Estévez, M. (2010a). Tryptophan depletion and formation of α-aminoadipic and γ-glutamic semialdehydes in porcine burger patties with added phenolic-rich fruit extracts. *Journal of Agricultural and Food Chemistry, 58*(6), 3541-3548.

Ganhão, R., Morcuende, D., & Estévez, M. (2010b). Protein oxidation in emulsified cooked burger patties with added fruit extracts: Influence on colour and texture deterioration during chill storage. *Meat Science, 85*(3), 402-409.

Garcia, M. N., Martinez-Torres, C., Leets, I., Tropper, E., Ramirez, J., & Layrisse, M. (1996). Heat treatment on heme iron and iron-containing proteins in meat: Iron absorption in humans from diets containing cooked meat fractions. *The Journal of Nutritional Biochemistry, 7*(1), 49-54.

Garrison, W. M. (1987). Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chemical Reviews, 87*(2), 381-398.

Gatellier, P., Kondjoyan, A., Portanguen, S., & Santé-Lhoutellier, V. (2010). Effect of cooking on protein oxidation in n-3 polyunsaturated fatty acids enriched beef. Implication on nutritional quality. *Meat Science, 85*(4), 645-650.

Gatellier, P., & Santé-Lhoutellier, V. (2009). Digestion study of proteins from cooked meat using an enzymatic microreactor. *Meat Science, 81*(2), 405-409.

Gatlin, L. A., See, M. T., & Odle, J. (2005). Effects of chemical hydrogenation of supplemental fat on relative apparent lipid digestibility in finishing swine. *Journal of Animal Science, 83*(8), 1890-1898.

George, D. F., Bilek, M. M., & McKenzie, D. R. (2008). Non-thermal effects in the microwave induced unfolding of proteins observed by chaperone binding. *Bioelectromagnetics, 29*(4), 324-330.

Gibis, M. (2009). Optimized HPLC method for analysis of polar and nonpolar heterocyclic amines in cooked meat products. *Journal of AOAC International, 92*(3), 715-724.

Gibis, M. (2016). Heterocyclic aromatic amines in cooked meat products: Causes, formation, occurrence, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety, 15*(2), 269-302.

Giera, M., Lingeman, H., & Niessen, W. M. A. (2012). Recent advancements in the LC- and GC-based analysis of malondialdehyde (MDA): A brief overview. *Chromatographia, 75*(9), 433-440.

Gilbertson, T. A., & Khan, N. A. (2014). Cell signaling mechanisms of oro-gustatory detection of dietary fat: advances and challenges. *Progress in Lipid Research, 53*, 82-92.

Girotti, A. W. (1998). Lipid hydroperoxide generation, turnover, and effector action in biological systems. *Journal of Lipid Research, 39*(8), 1529-1542.

Goicoechea, E. n., Van Twillert, K., Duits, M., Brandon, E. D. F. A., Kootstra, P. R., Blokland, M. H., & Guillén, M. a. D. (2008). Use of an in vitro digestion model to study the bioaccessibility of 4-hydroxy-2-nonenal and related aldehydes present in oxidized oils rich in omega-6 acyl groups. *Journal of Agricultural and Food Chemistry, 56*(18), 8475-8483.

Gotfredsen, A., Bæksgaard, L., & Hilsted, J. (1997). Body composition analysis by DEXA by using dynamically changing samarium filtration. *Journal of Applied Physiology, 82*(4), 1200-1209.

Grafström, R. C., Dypbukt, J. M., Willey, J. C., Sundqvist, K., Edman, C., Atzori, L., Harris, C.C. (1988). Pathobiological effects of acrolein in cultured human bronchial epithelial cells. *Cancer Research, 48*(7), 1717-1721.

Gray, J. I., Skrypec, D. J., Mandagere, A. K., Booren, A. M., & Pearson, A. M. (1983). Further factors influencing N-nitrosamine formation in bacon. *IARC Scientific Publications, 57*(57), 301-309.

Grootveld, M., Atherton, M. D., Sheerin, A. N., Hawkes, J., Blake, D. R., Richens, T. E. & Claxson, A. W. (1998). In vivo absorption, metabolism, and urinary excretion of alpha, beta-unsaturated aldehydes in experimental animals: Relevance to the development of cardiovascular diseases by the dietary ingestion of thermally stressed polyunsaturate-rich culinary oils. *Journal of Clinical Investigation, 101*(6), 1210.

Grootveld, M., Silwood, C. J. L., Addis, P., Claxson, A., Serra, B. B., & Viana, M. (2001). Health effects of oxidized heated oils. *Foodservice Research International, 13*(1), 41-55.

Gross, G. A., Turesky, R. J., Fay, L. B., Stillwell, W. G., Skipper, P. L., & Tannenbaum, S. R. (1993). Heterocyclic aromatic amine formation in grilled bacon, beef and fish and in grill scrapings. *Carcinogenesis, 14*(11), 2313-2318.

Grimsrud, P. A., Xie, H., Griffin, T. J., & Bernlohr, D. A. (2008). Oxidative stress and covalent modification of protein with bioactive aldehydes. *Journal of Biological Chemistry, 283*(32), 21837-21841.

Grune, T., Jung, T., Merker, K., & Davies, K. J. A. (2004). Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during

oxidative stress, aging, and disease. *The International Journal of Biochemistry & Cell Biology, 36*(12), 2519-2530.

Guo, Q., Richert, B. T., Burgess, J. R., Webel, D. M., Orr, D. E., Blair, M. & Gerrard, D. E. (2006). Effect of dietary vitamin E supplementation and feeding period on pork quality. *Journal of Animal Science, 84*(11), 3071-3078.

Haak, L., Raes, K., Smet, K., Claeys, E., Paelinck, H., & De Smet, S. (2006). Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. *Meat Science, 74*(3), 476-486.

Haak, L., Raes, K., Van Dyck, S., & De Smet, S. (2008). Effect of dietary rosemary and α-tocopheryl acetate on the oxidative stability of raw and cooked pork following oxidized linseed oil administration. *Meat Science, 78*(3), 239-247.

Haliwell, B. (1994). Free radicals antioxidants and human disease: Curiosity, cause or consequence. *Lancet, 344*(7), 21-724.

Hande, G., Nuran, E., Suneetha, M., Subramaniam, P., Hilmi, O., & Jay, W. H. (2006). Misincorporation of free m-tyrosine into cellular proteins: A potential cytotoxic mechanism for oxidized amino acids. *Biochemical Journal, 395*(2), 277–284.

Hawkins, C. L., Morgan, P. E., & Davies, M. J. (2009). Quantification of protein modification by oxidants. *Free Radical Biology and Medicine, 46*(8), 965-988.

Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N., & Petersen, J. S. (2002). Metabolic conditions in porcine longissimus muscle immediately pre-slaughter and its influence on peri- and post mortem energy metabolism. *Meat Science, 62*(2), 145-155.

Hermesch, S. (2008). Genetic relationships between composition of pork bellies and performance, carcase and meat quality traits. *Animal, 2*(8), 1178-1185.

Herrick, R. T. (2014). Evaluating lipid oxidation and sensory characteristics in bacon from immunologically castrated barrows. (Doctoral dissertation), University of Illinois, Urbana-Champaign. Available at https://www.ideals.illinois.edu/handle/2142/50753. Accessed on 03/01/2017.

Herrmann, S. S., Granby, K., & Duedahl-Olesen, L. (2015). Formation and mitigation of N-nitrosamines in nitrite preserved cooked sausages. *Food Chemistry, 174*, 516-526.

Hoac, T., Daun, C., Trafikowska, U., Zackrisson, J., & Åkesson, B. (2006). Influence of heat treatment on lipid oxidation and glutathione peroxidase activity in chicken and duck meat. *Innovative Food Science & Emerging Technologies, 7*(1–2), 88-93.

Holdstock, J., Aalhus, J. L., Uttaro, B. A., López-Campos, Ó., Larsen, I. L., & Bruce, H. L. (2014). The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. *Meat Science, 98*(4), 842-849.

Hunter, T. E., Suster, D., Dunshea, F. R., Cummins, L. J., Egan, A. R., & Leury, B. J. (2011). Dual energy X-ray absorptiometry (DXA) can be used to predict live animal and whole carcass composition of sheep. *Small Ruminant Research, 100*(2–3), 143-152.

Hęś, M., Waszkowiak, K., & Szymandera-Buszka, K. (2012). The effect of iodine salts on lipid oxidation and changes in nutritive value of protein in stored processed meats. *Meat Science, 92*(2), 139-143.

IARC. (1993). *IARC monographs on the evaluation of carcinogenic risks to humans. Some natural occurring substances: Food items and constituents, heterocyclic amines and mycotoxins.* Available at: https://monographs.iarc.fr/ENG/Monographs/vol56/mono56.pdf. Accessed on 02/01/2017.

IARC. (2017). *IARC monographs of the evaluation of carcinogenic risks to human: List of classification*. Available at: http://monographs.iarc.fr/ENG/Classification/latest_classif.php. Accessed on 02/01/2017.

Inchingolo, R., Cardenia, V., & Rodriguez-Estrada, M. T. (2013). The effects of microwave heating on edible oils and lipid-containing food. *Lipid Technology, 25*(3), 59-61.

International Market Bureau. (2012). *Market Indication Report 2012: Processed meats in the United States*. Agriculture and Agri-Food Canada, Ottawa. Available at http://www.agr.gc.ca/resources/prod/Internet-Internet/MISB-DGSIM/ATS-SEA/PDF/6124-eng.pdf. Accessed on 24/12/2016.

Ishii, T., Ito, S., Kumazawa, S., Sakurai, T., Yamaguchi, S., Mori, T., Nakayama, T. & Uchida, K. (2008). Site-specific modification of positively-charged surfaces on human serum albumin by malondialdehyde. *Biochemical and Biophysical Research Communications, 371*(1), 28-32.

Iwasaki, M., Kataoka, H., Ishihara, J., Takachi, R., Hamada, G. S., Sharma, S. & Tsugane, S. (2010). Heterocyclic amines content of meat and fish cooked by Brazilian methods. *Journal of Food Composition and Analysis, 23*(1), 61-69.

Jabaay, R. W., Forrest, J. C., Aberle, E. D., Courtenay, H. V., & Judge, M. D. (1976). Bacon quality criteria and associated carcass traits. *Journal of Food Science, 41*(2), 431-437.

Jackson, A. R., Powell, S., Johnston, S. L., Matthews, J. O., Bidner, T. D., Valdez, F. R., & Southern, L. L. (2009). The effect of chromium as chromium propionate on growth performance, carcass traits, meat quality, and the fatty acid profile of fat from pigs fed no supplemented dietary fat, choice white grease, or tallow. *Journal of Animal Science, 87*(12), 4032-4041.

Jaganjac, M., Tirosh, O., Cohen, G., Sasson, S., & Zarkovic, N. (2013). Reactive aldehydes–second messengers of free radicals in diabetes mellitus. *Free Radical Research, 47*(S1), 39-48.

Janoszka, B. (2010). Heterocyclic amines and azaarenes in pan-fried meat and its gravy fried without additives and in the presence of onion and garlic. *Food Chemistry, 120*(2), 463-473.

Janoszka, B., Błaszczyk, U., Damasiewicz-Bodzek, A., & Sajewicz, M. (2009). Analysis of heterocyclic amines (HAs) in pan-fried pork meat and its gravy by liquid chromatography with diode array detection. *Food Chemistry, 113*(4), 1188-1196.

Jebb, S. A. (1997). Measurement of soft tissue composition by dual energy X-ray absorptiometry. *British Journal of Nutrition, 77*(2), 151-163.

Jebb, S. A., Goldberg, G. R., Jennings, G., & Elia, M. (1995). Dual-energy X-ray absorptiometry measurements of body composition: Effects of depth and tissue thickness, including comparisons with direct analysis. *Clinical Science, 88*(3), 319-324.

Jensen, C., Engberg, R., Jakobsen, K., Skibsted, L. H., & Bertelsen, G. (1997). Influence of the oxidative quality of dietary oil on broiler meat storage stability. *Meat Science, 47*(3–4), 211-222.

Ji, C., Rouzer, C. A., Marnett, L. J., & Pietenpol, J. A. (1998). Induction of cell cycle arrest by the endogenous product of lipid peroxidation, malondialdehyde. *Carcinogenesis, 19*(7), 1275-1283.

Johansson, M., & Jägerstad, M. (1993). Influence of oxidized deep-frying fat and iron on the formation of food mutagens in a model system. *Food and Chemical Toxicology, 31*(12), 971-979.

Johansson, M. A. E., & Jägerstad, M. (1994). Occurrence of mutagenic/carcinogenic heterocyclic amines in meat and fish products, including pan residues, prepared under domestic conditions. *Carcinogenesis, 15*(8), 1511-1518.

Joshi, A. D., Kim, A., Lewinger, J. P., Ulrich, C. M., Potter, J. D., Cotterchio, M. & Stern, M. C. (2015). Meat intake, cooking methods, dietary carcinogens, and colorectal cancer risk: Findings from the colorectal cancer family registry. *Cancer Medicine, 4*(6), 936-952.

Juntachote, T., Berghofer, E., Siebenhandl, S., & Bauer, F. (2007). The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. *LWT - Food Science and Technology, 40*(2), 324-330.

Juárez, M., Caine, W. R., Larsen, I. L., Robertson, W. M., Dugan, M. E. R., & Aalhus, J. L. (2009). Enhancing pork loin quality attributes through genotype, chilling method and ageing time. *Meat Science, 83*(3), 447-453.

Juárez, M., Failla, S., Ficco, A., Peña, F., Avilés, C., & Polvillo, O. (2010). Buffalo meat composition as affected by different cooking methods. *Food and Bioproducts Processing, 88*(2–3), 145-148.

Jägerstad, M., Skog, K., Arvidsson, P., & Solyakov, A. (1998). Chemistry, formation and occurrence of genotoxic heterocyclic amines identified in model systems and cooked foods. *Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 207*(6), 419-427.

Kamin-Belsky, N., Brillon, A. A., Arav, R., & Shaklai, N. (1996). Degradation of myosin by enzymes of the digestive system: comparison between native and oxidatively cross-linked protein. *Journal of Agricultural and Food Chemistry, 44*(7), 1641-1646.

Kanazawa, K., Ashida, H., Minamoto, S., Danno, G., & Natake, M. (1988). The effects of orally administered linoleic acid and its autoxidation products on intestinal mucosa in rat. *Journal of Nutritional Science and Vitaminology, 34*(4), 363-373.

Kang, S. M., Kang, G., Seong, P., Park, B., & Cho, S. (2014). Effect of packaging method on the lipid oxidation, protein oxidation, and color in aged top round from hanwoo (Korean native cattle) during refrigerated storage. *Korean Journal for Food Science of Animal Resources, 34*(3), 273-279.

Kaur, I. P. (2008). Antimutagenicity of curcumin and related compounds against genotoxic heterocyclic amines from cooked food: The structural requirement. *Food Chemistry, 111*(3), 573-579.

Karlsson, A., Enfält, A. C., Essén-Gustavsson, B., Lundström, K., Rydhmer, L., & Stern, S. (1993). Muscle histochemical and biochemical properties in relation to meat quality during selection for increased lean tissue growth rate in pigs. *Journal of Animal Science, 71*(4), 930-938.

Keast, R. S. J., & Costanzo, A. (2015). Is fat the sixth taste primary? Evidence and implications. *Flavour, 4*(5), 1-7.

Kehrer, J. P., & Biswal, S. S. (2000). The molecular effects of acrolein. *Toxicological Sciences, 57*(1), 6-15.

Kelly, T. L., Berger, N., & Richardson, T. L. (1998). DXA body composition: Theory and practice. *Applied Radiation and Isotopes, 49*(5–6), 511-513.

Kemp, J. D., Moody, W. G., & Fox, J. D. (1969). Effect of fatness and fresh pork quality on yield and quality of bacon and yield of ham. *Journal of Animal Science, 28*(5), 612-614.

Kenneth, C. (1994). *Nutritional aspects of cardiovascular disease: Report of the cardiovascular review group committee on medical aspects of food policy*. Reports of health and social subjects, Volume 46 (pp 1-186). United Kingdom: HMSO.

Keshavarzian, A., Banan, A., Farhadi, A., Komanduri, S., Mutlu, E., Zhang, Y., & Fields, J. Z. (2003). Increases in free radicals and cytoskeletal protein oxidation and nitration in the colon of patients with inflammatory bowel disease. *Gut, 52*(5), 720-728.

Kistorp, C. N., & Svendsen, O. L. (1998). Body composition results by DXA differ with manufacturer, instrument generation and software version. *Applied Radiation and Isotopes, 49*(5–6), 515-516.

Kizil, M., Oz, F., & Besler, H. T. (2011). A review on the formation of carcinogenic/mutagenic heterocyclic aromatic amines. *Journal of Food Process Technology, 2*(5), 1-5.

Kogelman, L. J. A., Kadarmideen, H. N., Mark, T., Karlskov-Mortensen, P., Bruun, C. S., Cirera, S. & Fredholm, M. (2013). An F2 pig resource population as a model for genetic studies of obesity and obesity-related diseases in humans: Design and genetic parameters. *Frontiers in Genetics, 4(*29), 1-14.

Koo, W. W. K., Hammami, M., Shypailo, R. J., & Ellis, K. J. (2004). Bone and body composition measurements of small subjects: discrepancies from software for fan-beam dual energy X-ray absorptiometry. *Journal of the American College of Nutrition, 23*(6), 647-650.

Kremer, P. V., Fernandez-Figares, I., Förster, M., & Scholz, A. M. (2012). In vivo body composition in autochthonous and conventional pig breeding groups by dual energy X-ray absorptiometry and magnetic resonance imaging under special consideration of Cerdo Iberico. *Animal, 6*(12), 2041-2047.

Knipe, C.L & Beld, J. (2014). Bacon Production (Part B). In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences 2nd Edition* (pp. 53-77). Oxford: Elsevier.

Knize, M. G., Andresen, B. D., Healy, S. K., Shen, N. H., Lewis, P. R., Bjeldanes, L. F., Hatch, F.T., & Felton, J. S. (1985). Effects of temperature, patty thickness and fat content on the production of mutagens in fried ground beef. *Food and Chemical Toxicology, 23*(12), 1035-1040.

Kyle, J. M., Bohrer, B. M., Schroeder, A. L., Matulis, R. J., & Boler, D. D. (2014). Effects of immunological castration (Improvest) on further processed belly characteristics and commercial bacon slicing yields of finishing pigs. *Journal of Animal Science, 92*(9), 4223-4233.

Lahucky, R., Bahelka, I., Kuechenmeister, U., Vasickova, K., Nuernberg, K., Ender, K., & Nuernberg, G. (2007). Effects of dietary supplementation of vitamins D3 and E on quality characteristics of pigs and longissimus muscle antioxidative capacity. *Meat Science, 77*(2), 264-268.

Lampe, J. F., Baas, T. J., & Mabry, J. W. (2006). Comparison of grain sources for swine diets and their effect on meat and fat quality traits. *Journal of Animal Science, 84*(4), 1022-1029.

Landgraf, S., Susenbeth, A., Knap, P. W., Looft, H., Plastow, G. S., Kalm, E., & Roeh, R. (2006). Developments of carcass cuts, organs, body tissues and chemical body composition during growth of pigs. *Animal Science, 82*, 889-899.

Larsen, S. T., Wiegand, B. R., Parrish, F. C., Swan, J. E., & Sparks, J. C. (2009). Dietary conjugated linoleic acid changes belly and bacon quality from pigs fed varied lipid sources. *Journal of Animal Science, 87*(1), 285-295.

Laskey, M. A., Lyttle, K. D., Flaxman, M. E., & Barber, R. W. (1992). The influence of tissue depth and composition on the performance of the Lunar dual-energy X-ray absorptiometer whole-body scanning mode. *European Journal of Clinical Nutrition, 46*(1), 39-45.

Lehotay, S. J., Sapozhnikova, Y., Han, L., & Johnston, J. J. (2015). Analysis of nitrosamines in cooked bacon by QuEChERS sample preparation and gas chromatography–tandem mass spectrometry with backflushing. *Journal of Agricultural and Food Chemistry, 63*(47), 10341-10351.

Leick, C. M., Puls, C. L., Ellis, M., Killefer, J., Carr, T. R., Scramlin, S. M. & McKeith, F. K. (2010). Effect of distillers dried grains with solubles and ractopamine (paylean) on quality and shelf-life of fresh pork and bacon. *Journal of Animal Science, 88*(8), 2751-2766.

Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat: Review. *Meat Science, 91*(2), 93-98.

Li, L., Wang, P., Xu, X., & Zhou, G. (2012). Influence of various cooking methods on the concentrations of volatile N-nitrosamines and biogenic amines in dry-cured sausages. *Journal of Food Science, 77*(5), C560-C565.

Li, Z. L., Mo, L., Le, G., & Shi, Y. (2014). Oxidized casein impairs antioxidant defense system and induces hepatic and renal injury in mice. *Food and Chemical Toxicology, 64*, 86-93.

Li, Z. L., Shi, Y., Le, G., Ding, Y., & Zhao, Q. (2015). 24-week exposure to oxidized tyrosine induces hepatic fibrosis involving activation of the mapk/tgf-β1 signaling pathway in sprague-dawley rats model. *Oxidative Medicine and Cellular Longevity, 2016,* 1-13.

Li, Z. L., Wu, L., Le, G., & Shi, Y. (2013). Effect of oxidized casein on the oxidative damage of blood and digestive organs in mice. *Acta Nutrimenta Sinica, 35*(1), 39-43.

Lin, C. F., Asghar, A., Gray, J. I., Buckley, D. J., Booren, A. M., Crackel, R. L., & Flegal, C. J. (1989). Effects of oxidised dietary oil and antioxidant supplementation on broiler growth and meat stability. *British Poultry Science, 30*(4), 855-864.

Lobo, F., Ventanas, S., Morcuende, D., & Estévez, M. (2016). Underlying chemical mechanisms of the contradictory effects of NaCl reduction on the redox-state of meat proteins in fermented sausages. *LWT-Food Science and Technology, 69*, 110-116.

Lonergan, S. M., Sebranek, J. G., Prusa, K. J., & Miller, L. F. (1992). Porcine somatotropin (PST) administration to growing pigs: Effects on adipose tissue composition and processed product characteristics. *Journal of Food Science, 57*(2), 312-317.

Lukaski, H. C., Marchello, M. J., Hall, C. B., Schafer, D. M., & Siders, W. A. (1999). Soft tissue composition of pigs measured with dual X-ray absorptiometry: Comparison with chemical analyses and effects of carcass thicknesses. *Nutrition, 15*(9), 697-703.

Lund, M. N., Heinonen, M., Baron, C. P., & Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition & Food Research, 55*(1), 83-95.

Lund, M. N., Hviid, M. S., & Skibsted, L. H. (2007). The combined effect of antioxidants and modified atmosphere packaging on protein and lipid oxidation in beef patties during chill storage. *Meat Science, 76*(2), 226-233.

Lund, M. N., Luxford, C., Skibsted, L. H., & Davies, M. J. (2008). Oxidation of myosin by haem proteins generates myosin radicals and protein cross-links. *Biochemical Journal, 410*(3), 565-574.

Lynch, A. M., Murray, S., Gooderham, N. J., & Boobis, A. R. (1995). Exposure to and activation of dietary heterocyclic amines in humans. *Critical Reviews in Oncology/Hematology, 21*(1–3), 19-31.

Lösel, D., Kremer, P., Albrecht, E., & Scholz, A. M. (2010). Comparison of a GE Lunar DPX-IQ and a Norland XR-26 dual energy X-ray absorptiometry scanner for body composition measurements in pigs–in vivo. *Archives Animal Breeding, 53(2)*, 162-175.

Madsen, A., Jakobsen, K., & Mortesen, H. P. (1992). Influence of dietary fat on carcass fat quality in pigs: A review. *Acta Agriculture Scandinavia, 42(4)*, 220-225.

Mandigo, R. (2001). Bacon quality evaluation methods. Paper presented at: 54th American Meat Science Association, Reciprocal Meat Conference, Indianapolis, Indiana, United States.

Mandigo, R. W. (2000). *Quality Lean Growth Modeling-Bacon Quality Assessment – NPB #97-1999*. Available at: http://www.pork.org/FileLibrary/ResearchDocuments/97-1999-Mandigo.pdf. Accessed on 23/12/2016.

Mandigo, R. W. (2002). *A new look at belly and bacon values*. Available at: http://nationalhogfarmer.com/mag/farming_new_look_belly. Accessed on 23/12/2016.

Mandigo, R. W. (2009). *Bacon processing today.* Paper presented at: 62nd American Meat Science Association Reciprocal Meat Conference, Savoy, United States.

Mapiye, C., Aldai, N., Turner, T. D., Aalhus, J. L., Rolland, D. C., Kramer, J. K. G., & Dugan, M. E. R. (2012). The labile lipid fraction of meat: From perceived disease and waste to health and opportunity. *Meat Science, 92*(3), 210-220.

Marcinkowska-Lesiak, M., Zdanowska-Sąsiadek, Ż., Stelmasiak, A., Damaziak, K., Michalczuk, M., Poławska, E. & Wierzbicka, A. (2016). Effect of packaging method and cold-storage time on chicken meat quality. *CyTA-Journal of Food, 14*(1), 41-46.

Marcoux, M., Bernier, J. F., & Pomar, C. (2003). Estimation of Canadian and European lean yields and composition of pig carcasses by dual-energy X-ray absorptiometry. *Meat Science, 63*(3), 359-365.

Marcoux, M., Faucitano, L., & Pomar, C. (2005). The accuracy of predicting carcass composition of three different pig genetic lines by dual-energy X-ray absorptiometry. *Meat Science, 70*(4), 655-663.

Marcoux, M., Pomar, C., Faucitano, L., & Brodeur, C. (2007). The relationship between different pork carcass lean yield definitions and the market carcass value. *Meat Science, 75*(1), 94-102.

Martinaud, A., Mercier, Y., Marinova, P., Tassy, C., Gatellier, P., & Renerre, M. (1997). Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *Journal of Agricultural and Food Chemistry, 45*(7), 2481-2487.

Matthew, D. S., & Alec, J. T. (2012). Use of dual-energy X-ray absorptiometry (DXA) with non-human vertebrates: Application, challenges, and practical considerations for research and clinical practice. *Bird's-Eye View of Veterinary Medicine, 6, 99-116.*

Matthews, J. O., Southern, L. L., Bidner, T. D., & Persica, M. A. (2001a). Effects of betaine, pen space, and slaughter handling method on growth performance, carcass traits, and pork quality of finishing barrows. *Journal of Animal Science, 79*(4), 967-974.

Matthews, J. O., Southern, L. L., Higbie, A. D., Persica, M. A., & Bidner, T. D. (2001b). Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs. *Journal of Animal Science, 79*(3), 722-728.

Maw, S. J., Fowler, V. R., Hamilton, M., & Petchey, A. M. (2003). Physical characteristics of pig fat and their relation to fatty acid composition. *Meat Science, 63*(2), 185-190.

Mazess, R. B., Barden, H. S., Bisek, J. P., & Hanson, J. (1990). Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *The American Journal of Clinical Nutrition, 51*(6), 1106-1112.

McClelland, K. M., Rentfrow, G., Cromwell, G. L., Lindemann, M. D., & Azain, M. J. (2012). Effects of corn distillers dried grains with solubles on quality traits of pork. *Journal of Animal Science, 90*(11), 4148-4156.

McClements, J., & Decker, A. E. (2008). Lipids. In S. Damodaran, K. L. Parkin, & O. R. Fennema (Eds.), *Fennema's Food Chemistry 4th Edition* (pp. 155-216). Boca Raton, London, New York: CRC press.

McMillin, K. W., Judge, M. D., Forrest, J. C., Anderson, V. L., & Aberle, E. D. (1977). Relationship of bacon quality to pork carcass traits. *Journal of Animal Science, 45*(5), 1023-1031.

Mei, L., Crum, A. D., & Decker, E. A. (1994). Development of lipid oxidation and inactivation of antioxidant enzymes in cooked pork and beef. *Journal of Food Lipids, 1*(4), 273-283.

Mercier, J., Pomar, C., Marcoux, M., Goulet, F., Thériault, M., & Castonguay, F. W. (2006). The use of dual-energy X-ray absorptiometry to estimate the dissected composition of lamb carcasses. *Meat Science, 73*(2), 249-257.

Mercier, Y., Gatellier, P., Viau, M., Remignon, H., & Renerre, M. (1998). Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Science, 48*(3), 301-318.

Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2, 4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Analytical Biochemistry, 458*, 69-71.

Miller, D. K., Gomez-Basauri, J. V., Smith, V. L., Kanner, J., & Miller, D. D. (1994). Dietary iron in swine rations affects nonheme iron and TBARS in pork skeletal muscles. *Journal of Food Science, 59*(4), 747-750.

Miller, M. F., Shackelford, S. D., Hayden, K. D., & Reagan, J. O. (1990). Determination of the alteration in fatty acid profiles, sensory characteristics and carcass traits of swine fed elevated levels of monounsaturated fats in the diet. *Journal of Animal Science, 68*(6), 1624-1631.

Miller, B. J., Billedeau, S. M., & Miller, D. W. (1989). Formation of N-nitrosamines in microwaved versus skillet-fried bacon containing nitrite. *Food and Chemical Toxicology, 27*(5), 295-299.

Millward, D. J., Layman, D. K., Tomé, D., & Schaafsma, G. (2008). Protein quality assessment: Impact of expanding understanding of protein and amino acid needs for optimal health. *The American Journal of Clinical Nutrition, 87*(5), 1576S-1581S.

Minitab 17 Statistical Software (2010). [Computer software]. State College, PA: Minitab, Inc.

Mitchell, A. D., Scholz, A. M., & Conway, J. M. (1998). Body composition analysis of small pigs by dual-energy X-ray absorptiometry. *Journal of Animal Science, 76*(9), 2392-2398.

Mitchell, A. D., Scholz, A. M., & Pursel, V. G. (2003). Prediction of pork carcass composition based on cross-sectional region analysis of dual energy X-ray absorptiometry (DXA) scans. *Meat Science, 63*(2), 265-271.

Mitchell, A. D., Scholz, A. M., Pursel, V. G., & Evock-Clover, C. M. (1998). Composition analysis of pork carcasses by dual-energy X-ray absorptiometry. *Journal of Animal Science, 76*(8), 2104-2114.

Mitchell, A. D., Scholz, A. M., Wang, P. C., & Song, H. (2001). Body composition analysis of the pig by magnetic resonance imaging. *Journal of Animal Science*, *79*(7), 1800–1813.

Mitchell, A. D., Solomon, M. B., & Rumsey, T. S. (1997). Composition analysis of beef rib sections by dual-energy X-ray absorptiometry. *Meat Science, 47*(1–2), 115-124.

Monziols, M., Bonneau, M., Davenel, A., & Kouba, M. (2007). Comparison of the lipid content and fatty acid composition of intermuscular and subcutaneous adipose tissues in pig carcasses. *Meat Science, 76*(1), 54-60.

Morales-delaNuez, A., Moreno-Indias, I., Falcón, A., Argüello, A., Sánchez-Macias, D., Capote, J., & Castro, N. (2009). Effects of various packaging systems on the quality characteristic of goat meat. *Asian-Australasian Journal of Animal Sciences, 22*(3), 428-432.

Morzel, M., Gatellier, P., Sayd, T., Renerre, M., & Laville, E. (2006). Chemical oxidation decreases proteolytic susceptibility of skeletal muscle myofibrillar proteins. *Meat Science, 73*(3), 536-543.

Mottram, D. S., Patterson, R. L. S., Edwards, R. A., & Gough, T. A. (1977). The preferential formation of volatile N-nitrosamines in the fat of fried bacon. *Journal of the Science of Food and Agriculture, 28*(11), 1025-1029.

Murray, A., Robertson, W., Johns, M., & Landry, S. (2002). Sources of variation in belly softness in pigs. Paper presented at: 48th International Congress of Meat Science and Technology, Rome, Italy.

Márquez-Ruiz, G., Garcia-Martinez, M. C., & Holgado, F. (2008). Changes and effects of dietary oxidized lipids in the gastrointestinal tract. *Lipid Insights, 2*, 11-19.

NAMI. (2015). *The ultimate guide to bacon*. North American Meat Institute. Available at: https://www.meatinstitute.org/index.php?ht=a/GetDocumentAction/i/111466. Accessed on 11/12/2016.

Nanke, K. E., Sebranek, J. G., Prusa, K. J., & Miller, L. F. (1993). Effects of porcine somatotropin (PST) administration on the fat/lean content and processing properties of pork bellies. *Meat Science, 35*(3), 341-353.

National Toxicology Program. (2011). *Reports on carcinogens*. Available at: http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf. Accessed on 12/12/2016.

Ned, D. H. (1975). Process for manufacturing bacon. In Google Patents. Available at: http://www.google.ca/patents/US3901981. Accessed on 23/12/2016.

Niedernhofer, L. J., Daniels, J. S., Rouzer, C. A., Greene, R. E., & Marnett, L. J. (2003). Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *Journal of Biological Chemistry, 278*(33), 31426-31433.

Nielsen, J. H., Sørensen, B., Skibsted, L. H., & Bertelsen, G. (1997). Oxidation in pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Science, 46*(2), 191-197.

Nord, R. H. (1998). DXA body composition properties: Inherent in the physics or specific to scanner type? *Applied Radiation and Isotopes, 49*(5–6), 517-518.

Nord, R. H., Homuth, J. R., Hanson, J. A., & Mazess, R. B. (2000). Evaluation of a new DXA fan-beam instrument for measuring body composition. *Annals of the New York Academy of Sciences, 904*(1), 118-125.

NPPC. (2010). *Pork composition and quality assessment procedures*. Des Moines, IA: National Pork Producers Council.

OEHHA. (2016). *Proposition 65 No Significant Risk Levels (NSRLs) for Carcinogens and Maximum Allowable Dose Levels (MADLs) for Chemicals Causing Reproductive Toxicity*. Office of environmental health hazard assessment, CA, United States. Available at: http://oehha.ca.gov/proposition-65/general-info/current-proposition-65-no-significant-risk-levels-nsrls-maximum. Accessed on 05/01/2017.

Ohgaki, H., Kusama, K., Matsukura, N., Morino, K., Hasegawa, H., Sato, S. & Sugimura, T. (1984). Carcinogenicity in mice of a mutagenic compound, 2-amino-3-methylimidazo [4, 5-f] quinoline, from broiled sardine, cooked beef and beef extract. *Carcinogenesis, 5*(7), 921-924.

Oliver, C. N., Ahn, B. W., Moerman, E. J., Goldstein, S., & Stadtman, E. R. (1987). Age-related changes in oxidized proteins. *Journal of Biological Chemistry, 262*(12), 5488-5491.

Oostindjer, M., Alexander, J., Amdam, G. V., Andersen, G., Bryan, N. S., Chen, D. & Egelandsdal, B. (2014). The role of red and processed meat in colorectal cancer development: a perspective. *Meat Science, 97*(4), 583-596.

Österdahl, B. G., & Alriksson, E. (1990). Volatile nitrosamines in microwave-cooked bacon. *Food Additives & Contaminants, 7*(1), 51-54.

Oz, F., Kaban, G., & Kaya, M. (2010). Heterocyclic aromatic amine contents of beef and lamb chops cooked by different methods to varying levels. *Journal of Animal and Veterinary Advances, 9*(10), 1436-1440.

Pais, P., Salmon, C. P., Knize, M. G., & Felton, J. S. (1999). Formation of mutagenic/carcinogenic heterocyclic amines in dry-heated model systems, meats, and meat drippings. *Journal of Agricultural and Food Chemistry, 47*(3), 1098-1108.

Palinski, W., Ord, V. A., Plump, A. S., Breslow, J. L., Steinberg, D., & Witztum, J. L. (1994). ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arteriosclerosis, Thrombosis, and Vascular Biology, 14*(4), 605-616.

Park, D., & Xiong, Y. L. (2007). Oxidative modification of amino acids in porcine myofibrillar protein isolates exposed to three oxidizing systems. *Food Chemistry, 103*(2), 607-616.

Park, D., Xiong, Y. L., Alderton, A. L., & Ooizumi, T. (2006). Biochemical changes in myofibrillar protein isolates exposed to three oxidizing systems. *Journal of Agricultural and Food Chemistry, 54*(12), 4445-4451.

Park, J. E, Seo, J. E., Lee, J. Y., & Kwon, H. (2015). Distribution of seven N-nitrosamines in food. *Toxicological Research, 31*(3), 279.

Pearce, K. L., Ferguson, M., Gardner, G., Smith, N., Greef, J., & Pethick, D. W. (2009). Dual X-ray absorptiometry accurately predicts carcass composition from live sheep and chemical composition of live and dead sheep. *Meat Science, 81*(1), 285-293.

Pegg, R. B., & Shahidi, F. (2000). *Nitrite curing of meat: The N-nitrosamine problem and nitrite alternatives.* Trumbull, Connecticut: John Wiley & Sons.

Pellegrini, M. (2013). The 2013: Bacon report. *The National Provisioner, 227,* 23-30.

Penumetcha, M., Khan, N., & Parthasarathy, S. (2000). Dietary oxidized fatty acids: An atherogenic risk? *Journal of Lipid Research, 41*(9), 1473-1480.

Pensabene, J. W., Fiddler, W., Gates, R. A., Fagan, J. C., & Wasserman, A. E. (1974). Effect of frying and other cooking conditions on nitrosopyrrolidine formation in bacon. *Journal of Food Science, 39*(2), 314-316.

Perluigi, M., Coccia, R., & Butterfield, D. A. (2012). 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: A toxic combination illuminated by redox proteomics studies. *Antioxidants & Redox Signaling, 17*(11), 1590-1609.

Person, R. C., McKenna, D. R., Griffin, D. B., McKeith, F. K., Scanga, J. A., Belk, K. E. & Savell, J. W. (2005). Benchmarking value in the pork supply chain: Processing characteristics and consumer evaluations of pork bellies of different thicknesses when manufactured into bacon. *Meat Science, 70*(1), 121-131.

Persson, E., Sjöholm, I., & Skog, K. (2003). Effect of high water-holding capacity on the formation of heterocyclic amines in fried beefburgers. *Journal of Agricultural and Food Chemistry, 51*(15), 4472-4477.

Pettigrew, J. E., & Esnaola, M. A. (2001). Swine nutrition and pork quality: A review. *Journal of Animal Science, 79*(E-Suppl), E316-E342.

Picouet, P. A., Fernández, A., Serra, X., Sunol, J. J., & Arnau, J. (2007). Microwave heating of cooked pork patties as a function of fat content. *Journal of Food Science, 72*(2), E57-E63.

Pintauro, S. J., Nagy, T. R., Duthie, C. M., & Goran, M. I. (1996). Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. *The American Journal of Clinical Nutrition, 63*(3), 293-298.

Pizzimenti, S., Ciamporcero, E. S., Daga, M., Pettazzoni, P., Arcaro, A., Cetrangolo, G., Minelli, R., Dianzani, C., Lepore, A., Gentile, F. & Barrera, G. (2013). Interaction of aldehydes derived from lipid peroxidation and membrane proteins. *Frontiers in Physiology, 4*(242), 1-17.

Pork Checkoff, P. (2009). *Quick fact: The Pork Industry at a Glance*. Available at: http://porkgateway.org/wp-content/uploads/2015/07/quick-facts-book1.pdf. Accessed on 23/12/2016.

Pork Checkoff, P. (2016). Bits about bacon. *America's pork producer and pork checkoff.* Available at: http://www.pork.org/pork-quick-facts/home/pork-bytes/bits-about-bacon/. Accessed on 26/12/2016.

Prescott, N. J., & Wood, J. D. (1998). The influence of dietary linoleic acid on backfat firmness in bacon weight pigs. *Animal production, 46*, 502 (Abstract).

Prieto, N., Roehe, R., Lavín, P., Batten, G., & Andrés, S. (2009). Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science, 83*(2), 175-186.

Promeyrat, A., Le Louët, L., Kondjoyan, A., Astruc, T., Santé-Lhoutellier, V., Gatellier, P., & Daudin, J. D. (2011). Combined effect of meat composition and heating parameters on the physicochemical state of proteins. *Procedia Food Science, 1*(0), 1118-1125.

Purchas, R. W., Busboom, J. R., & Wilkinson, B. H. P. (2006). Changes in the forms of iron and in concentrations of taurine, carnosine, coenzyme Q10, and creatine in beef longissimus muscle with cooking and simulated stomach and duodenal digestion. *Meat Science, 74*(3), 443-449.

Pérez-Marín, D., De Pedro Sanz, E., Guerrero-Ginel, J. E., & Garrido-Varo, A. (2009). A feasibility study on the use of near-infrared spectroscopy for prediction of the fatty acid profile in live Iberian pigs and carcasses. *Meat Science, 83*(4), 627-633.

Rababah, T., Hettiarachchy, N., Horax, R., Eswaranandam, S., Mauromoustakos, A., Dickson, J., & Niebuhr, S. (2004). Effect of electron beam irradiation and storage at 5°C on thiobarbituric acid reactive substances and carbonyl contents in chicken breast meat infused with antioxidants and selected plant extracts. *Journal of Agricultural and Food Chemistry, 52*(26), 8236-8241.

Reed, T. T. (2011). Lipid peroxidation and neurodegenerative disease. *Free Radical Biology and Medicine, 51*(7), 1302-1319.

Rentfrow, G., Sauber, T. E., Allee, G. L., & Berg, E. P. (2003). The influence of diets containing either conventional corn, conventional corn with choice white grease, high oil corn, or high oil high oleic corn on belly/bacon quality. *Meat Science, 64*(4), 459-466.

Requena, J. R., Chao, C.-C., Levine, R. L., & Stadtman, E. R. (2001). Glutamic and aminoadipic semialdehydes are the main carbonyl products of metal-catalyzed oxidation of proteins. *Proceedings of the National Academy of Sciences, 98*(1), 69-74.

Rhee, K. S., Ziprin, Y. A., & Ordonez, G. (1987). Catalysis of lipid oxidation in raw and cooked beef by metmyoglobin-H_2O_2, nonheme iron, and enzyme systems. *Journal of Agricultural and Food Chemistry, 35*(6), 1013-1017.

Riahi, Y., Cohen, G., Shamni, O., & Sasson, S. (2010). Signaling and cytotoxic functions of 4-hydroxyalkenals. *American Journal of Physiology-Endocrinology and Metabolism, 299*(6), E879-E886.

Robinson, D. W., & Lewis, D. (1964). Protein and energy nutrition of the bacon pig. II. The effect of varying the protein and energy levels in diets of 'finishing' pigs. *The Journal of Agricultural Science, 63*(02), 185-190.

Robles, C. C. (2004). The effect of fresh and frozen bellies on bacon processing characteristics and bacon quality. (Master Desertation), University of Nebraska - Lincoln. Available at: http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1018&context=animalscidiss (Paper 17). Accessed on 26/05/2013.

Rocha, A. E. (2011). *Basic Manufacturing of Bacon.* Available at: http://www.nassaufoods.com/index.php?content=basicmanufacturingofbacon. Accessed on 26/05/2013.

Rodgers, K. J., Wang, H., Fu, S., & Dean, R. T. (2002). Biosynthetic incorporation of oxidized amino acids into proteins and their cellular proteolysis. *Free Radical Biology and Medicine, 32*(8), 766-775.

Rohrmann, S., Hermann, S., & Linseisen, J. (2009). Heterocyclic aromatic amine intake increases colorectal adenoma risk: Findings from a prospective European cohort study. *The American Journal of Clinical Nutrition, 89*(5), 1418-1424.

Rosenvold, K., Petersen, J. S., Lwerke, H. N., Jensen, S. K., Therkildsen, M., Karlsson, A. H. & Andersen, H. J. (2001). Muscle glycogen stores and meat quality as affected by strategic finishing feeding of slaughter pigs. *Journal of Animal Science, 79*(2), 382-391.

Rothammer, S., Kremer, P. V., Bernau, M., Fernandez-Figares, I., Pfister-Schär, J., Medugorac, I., & Scholz, A. M. (2014). Genome-wide QTL mapping of nine body composition and bone mineral density traits in pigs. *Genetics Selection Evolution, 46*(68), 1-11.

Roubenoff, R., Kehayias, J. J., Dawson-Hughes, B., & Heymsfield, S. B. (1993). Use of dual-energy X-ray absorptiometry in body-composition studies: Not yet a "gold standard". *The American Journal of Clinical Nutrition, 58*(5), 589-591.

Rowe, L. J., Maddock, K. R., Lonergan, S. M., & Huff-Lonergan, E. (2004). Influence of early postmortem protein oxidation on beef quality. *Journal of Animal Science, 82*(3), 785-793.

Ruan, E. D., Aalhus, J., & Juárez, M. (2014). A rapid, sensitive and solvent-less method for determination of malonaldehyde in meat by stir bar sorptive extraction coupled thermal desorption and gas chromatography/mass spectrometry with in situ derivatization. *Rapid Communications in Mass Spectrometry, 28*(24), 2723-2728.

Ruan, E. D., Juárez, M., Thacker, R., Yang, X., Dugan, M. E. R., & Aalhus, J. L. (2014). Dietary vitamin E effects on the formation of heterocyclic amines in grilled lean beef. *Meat Science, 96*(2), 849-853.

Running, C. A., Craig, B. A., & Mattes, R. D. (2015). Oleogustus: The unique taste of fat. *Chemical Senses*, *40*(7), 507-516.

Rutherfurd, S. M., Montoya, C. A., & Moughan, P. J. (2014). Effect of oxidation of dietary proteins with performic acid on true ileal amino acid digestibility as determined in the growing rat. *Journal of Agricultural and Food Chemistry, 62*(3), 699-707.

Rutherfurd, S. M., & Moughan, P. J. (2012). Available versus digestible dietary amino acids. *British Journal of Nutrition, 108*(S2), S298-S305.

Salas, C., Ekmay, R. D., England, J., Cerrate, S., & Coon, C. N. (2012). Determination of chicken body composition measured by dual energy X-ray absorptiometry. *International Journal of Poultry Science, 11*(7), 462-468.

Salminen, H., Estévez, M., Kivikari, R., & Heinonen, M. (2006). Inhibition of protein and lipid oxidation by rapeseed, camelina and soy meal in cooked pork meat patties. *European Food Research and Technology, 223*(4), 461-468.

Sanchez, A. M., Kozekov, I. D., Harris, T. M., & Lloyd, R. S. (2005). Formation of inter-and intrastrand imine type DNA-DNA cross-links through secondary reactions of aldehydic adducts. *Chemical Research in Toxicology, 18*(11), 1683-1690.

Sante-Lhoutellier, V., Aubry, L., & Gatellier, P. (2007). Effect of oxidation on in vitro digestibility of skeletal muscle myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 55*(13), 5343-5348.

Santé-Lhoutellier, V., Astruc, T., Marinova, P., Greve, E., & Gatellier, P. (2008). Effect of meat cooking on physicochemical state and in vitro digestibility of myofibrillar proteins. *Journal of Agricultural and Food Chemistry, 56*(4), 1488-1494.

Santé-Lhoutellier, V., Engel, E., Aubry, L., & Gatellier, P. (2008). Effect of animal (lamb) diet and meat storage on myofibrillar protein oxidation and in vitro digestibility. *Meat Science, 79*(4), 777-783.

SAS Institute Inc. (2011). Base SAS® 9.3 Procedures Guide. Cary, NC: SAS Institute Inc.

Sather, A. P., Jones, S. D. M., Robertson, W. M., & Zawadski, S. (1995). Sex effects on fat hardness meter readings of market weight pigs. *Canadian Journal of Animal Science, 75*(4), 509-515.

Sayre, L. M., Lin, D., Yuan, Q., Zhu, X., & Tang, X. (2006). Protein adducts generated from products of lipid oxidation: Focus on HNE and one. *Drug Metabolism Reviews, 38*(4), 651-675.

Sayre, L. M., Perry, G., & Smith, M. A. (2007). Oxidative stress and neurotoxicity. *Chemical Research in Toxicology, 21*(1), 172-188.

Scalbert, A., Manach, C., Morand, C., Rémésy, C., & Jiménez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*(4), 287-306.

Schiffman, M. H., & Felton, J. S. (1990). Re: “Fried foods and the risk of colon cancer”. *American Journal of Epidemiology, 131*(2), 376-378.

Schinckel, A. P., Mills, S. E., Weber, T. E., & Eggert, J. M. (2002). A review of genetic and nutritional factors affecting fat quality and belly firmness. Paper presented at: National Swine Improvement Federation, Nashville, Tennessee.

Scholz, A. M., Bünger, L., Kongsro, J., Baulain, U., & Mitchell, A. D. (2015). Non-invasive methods for the determination of body and carcass composition in livestock: dual-energy X-ray absorptiometry, computed tomography, magnetic resonance imaging and ultrasound: Invited review. *Animal, 9*(07), 1250-1264.

Schneider, C., Tallman, K. A., Porter, N. A., & Brash, A. R. (2001). Two distinct pathways of formation of 4-hydroxynonenal mechanisms of nonenzymatic transformation of the 9-and 13-hydroperoxides of linoleic acid to 4-hydroxyalkenals. *Journal of Biological Chemistry, 276*(24), 20831-20838.

Schroder, B. G., & Rust, R. E. (1974a). Composition of pork bellies. I. Specific gravity as a predictor of chemical composition. *Journal of Animal Science, 39*(6), 1032-1036.

Schroder, B. G., & Rust, R. E. (1974b). Composition of pork bellies. II. Compositional variation between and within animals and the relationship of various carcass measurements with the chemical components of the belly.1. *Journal of Animal Science, 39*(6), 1037-1044.

Schönfeldt, H. C., & Hall, N. G. (2012). Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition, 108*(S2), S69-S76.

Scramlin, S. M., Carr, S. N., Parks, C. W., Fernandez-Dueñas, D. M., Leick, C. M., McKeith, F. K., & Killefer, J. (2008). Effect of ractopamine level, gender, and duration of ractopamine on belly and bacon quality traits. *Meat Science, 80*(4), 1218-1221.

Seiner, D. R., LaButti, J. N., & Gates, K. S. (2007). Kinetics and mechanism of protein tyrosine phosphatase 1B inactivation by acrolein. *Chemical Research in Toxicology, 20*(9), 1315-1320.

Sell, D. R., Strauch, C. M., Shen, W., & Monnier, V. M. (2007). 2-Aminoadipic acid is a marker of protein carbonyl oxidation in the aging human skin: effects of diabetes, renal failure and sepsis. *Biochemical Journal, 404*(2), 269-277.

Selin, N. E. (2011). *Environmental guidelines and regulations for nitramines: A policy summary.* Massachusetts Institute of Technology (MIT), Cambridge, 21. Available at: http://www.tcmda.com/Global/Aminrapporter/MIT%20nitramine_report_final_July2011.pdf. Accessed on 27/01/2017.

Seman., D. L., Barron., W. N. G., & Matzinger., M. (2013). Evaluating the ability to measure pork fat quality for the production of commercial bacon. *Meat Science, 94*(2), 262-266.

Serrano, A., Librelotto, J., Cofrades, S., Sánchez-Muniz, F. J., & Jiménez-Colmenero, F. (2007). Composition and physicochemical characteristics of restructured beef steaks containing walnuts as affected by cooking method. *Meat Science, 77*(3), 304-313.

Shackelford, S. D., Miller, M. F., Haydon, K. D., Lovegren, N. V., Lyon, C. E., & Reagan, J. O. (1990). Acceptability of bacon as influenced by the feeding of elevated levels of monounsaturated fats to growing-finishing swine. *Journal of Food Science, 55*(3), 621-624.

Shacter, E. (2000). Quantification and significance of protein oxidation in biological samples. *Drug Metabolism Reviews, 32*(3-4), 307-326.

Shahidi, F., Rubin, L. J., Diosady, L. L., & Wood, D. F. (1985). Effect of sulfanilamide on the TBA values of cured meats. *Journal of Food Science, 50*(1), 274-275.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews, 39*(11), 4067-4079.

Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of perorlride values of food lipids. *Journal of AOAC International, 77*(2), 421-424.

Shin, H. S. (2005). Influence of food ingredients on the formation of heterocyclic aromatic amine in cooked pork patties. *Food Science and Biotechnology,* 14(5), 572-575..

Shin, H. S., Park, H., & Park, D. (2003). Influence of different oligosaccharides and inulin on heterocyclic aromatic amine formation and overall mutagenicity in fried ground beef patties. *Journal of Agricultural and Food Chemistry, 51*(23), 6726-6730.

Silva, F. A. P., Ferreira, V. C. S., Madruga, M. S., & Estévez, M. (2016). Effect of the cooking method (grilling, roasting, frying and sous-vide) on the oxidation of thiols, tryptophan, alkaline amino acids and protein cross-linking in jerky chicken. *Journal of Food Science and Technology, 53*(8), 3137-3146.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & pharmacotherapy, 56*(8), 365-379.

Smith, J. S. (2010). Assessment of human exposure to heterocyclic amines (HCAs) from cooked meat products – *NPB# 08-177*. Available at: http://research.pork.org/FileLibrary/ResearchDocuments/08-177-SMITH-KState.pdf. Accessed on 23/12/2016.

Smith, D. L., Johnson, M. S., & Nagy, T. R. (2009). Precision and accuracy of bioimpedance spectroscopy for determination of in vivo body composition in rats. *International Journal of Body Composition Research, 7*(1), 21-26.

Smith, G. C., West, R. L., & Carpenter, Z. L. (1975). Factors affecting desirability of bacon and commercially-processed pork bellies. *Journal of Animal Science, 41*(1), 54-65.

Soladoye, O. P., Campos, Ó. L., Aalhus, J. L., Gariépy, C., Shand, P., & Juárez, M. (2016). Accuracy of dual energy X-ray absorptiometry (DXA) in assessing carcass composition from different pig populations. *Meat Science, 121,* 310-316.

Soladoye, O. P., Juárez, M. L., Aalhus, J. L., Shand, P., & Estévez, M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety, 14*(2), 106-122.

Soladoye, P. O., Shand, P. J., Aalhus, J. L., Gariépy, C., & Juárez, M. (2015). Review: Pork belly quality, bacon properties and recent consumer trends. *Canadian Journal of Animal Science, 95*(3), 325-340.

Souza, C. G., Moreira, J. D., Siqueira, I. R., Pereira, A. G., Rieger, D. K., Souza, D. O. & Perry, M. L. S. (2007). Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. *Life Sciences, 81*(3), 198-203.

Soyer, A., Özalp, B., Dalmış, Ü., & Bilgin, V. (2010). Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chemistry, 120*(4), 1025-1030.

Spiteller, G. (2006). Peroxyl radicals: inductors of neurodegenerative and other inflammatory diseases. Their origin and how they transform cholesterol, phospholipids, plasmalogens, polyunsaturated fatty acids, sugars, and proteins into deleterious products. *Free Radical Biology and Medicine, 41*(3), 362-387.

Srinivasan, S., Xiong, Y. L., & Decker, E. A. (1996). Inhibition of protein and lipid oxidation in beef heart surimi-like material by antioxidants and combinations of pH, NaCl, and buffer type in the washing media. *Journal of Agricultural and Food Chemistry, 44*(1), 119-125.

Srivastava, S., Watowich, S. J., Petrash, J. M., Srivastava, S. K., & Bhatnagar, A. (1999). Structural and kinetic determinants of aldehyde reduction by aldose reductase. *Biochemistry, 38*(1), 42-54.

Shrivastava, A., & Gupta, V. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21-21.

Stadtman, E. R. (1993). Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. *Annual Review of Biochemistry, 62*(1), 797-821.

Stadtman, E. R., & Levine, R. L. (2000). Protein oxidation. *Annals of the New York Academy of Sciences, 899*(1), 191-208.

Stadtman, E. R., & Levine, R. L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids, 25*(3-4), 207-218.

Staprans, I., Rapp, J. H., Pan, X. M., Kim, K. Y., & Feingold, K. R. (1994). Oxidized lipids in the diet are a source of oxidized lipid in chylomicrons of human serum. *Arteriosclerosis, Thrombosis, and Vascular Biology, 14*(12), 1900-1905.

Stiffler, D. M., Chant, J. L., Kinsman, D. M., & Kotula, A. W. (1975). Indices of leanness in commercial bacons. *Journal of Animal Science, 41*(6), 1611-1617.

Sugimura, T. (1997). Overview of carcinogenic heterocyclic amines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 376*(1), 211-219.

Sugimura, T., Wakabayashi, K., Nakagama, H., & Nagao, M. (2004). Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Science, 95*(4), 290-299.

Sun, W., Cui, C., Zhao, M., Zhao, Q., & Yang, B. (2011). Effects of composition and oxidation of proteins on their solubility, aggregation and proteolytic susceptibility during processing of Cantonese sausage. *Food Chemistry, 124(1),* 336–341.

Suomela, J. P., Ahotupa, M., Sjövall, O., Kurvinen, J. P., & Kallio, H. (2004). Diet and lipoprotein oxidation: analysis of oxidized triacylglycerols in pig lipoproteins. *Lipids, 39*(7), 639-647.

Suster, D., Leury, B. J., Hofmeyr, C. D., D'Souza, D. N., & Dunshea, F. R. (2004). The accuracy of dual energy X-ray absorptiometry (DXA), weight, and P2 back fat to predict half-carcass and primal-cut composition in pigs within and across research experiments. *Australian Journal of Agricultural Research, 55*(9), 973-982.

Suster, D., Leury, B. J., Ostrowska, E., Butler, K. L., Kerton, D. J., Wark, J. D., & Dunshea, F. R. (2003). Accuracy of dual energy X-ray absorptiometry (DXA), weight and P2 back fat to predict whole body and carcass composition in pigs within and across experiments. *Livestock Production Science, 84*(3), 231-242.

Svendsen, O. L., Haarbo, J., Hassager, C., & Christiansen, C. (1993). Accuracy of measurements of body composition by dual-energy X-ray absorptiometry in vivo. *The American Journal of Clinical Nutrition, 57*(5), 605-608.

Sánchez-Muniz, F. J., & Bastida, S. (2006). Effect of frying and thermal oxidation on olive oil and food quality. In J. L. Quiles, M. C. Ramírez-Tortosa, & P. Yaqoob (Eds.). *Olive Oil and Health* (pp. 74–108). Wallingford, United Kingdom: CABI Publishing.

Taborsky, G., & McCollum, K. (1973). Oxidative modification of proteins in the presence of ferrous ion and air: Effect of ionic constituents of the reaction medium on the nature of the oxidation products. *Biochemistry, 12*(7), 1341-1348.

Tavárez, M. A., Bohrer, B. M., Asmus, M. D., Schroeder, A. L., Matulis, R. J., Boler, D. D., & Dilger, A. C. (2014). Effects of immunological castration and distiller's dried grains with solubles on carcass cutability and commercial bacon slicing yields of barrows slaughtered at two time points. *Journal of Animal Science, 92*(7), 3149-3160.

Taylor, A., & Davies, K. J. A. (1987). Protein oxidation and loss of protease activity may lead to cataract formation in the aged lens. *Free Radical Biology and Medicine, 3*(6), 371-377.

Taylor, A. A., Dant, S. J., & French, J. W. L. (1973). Processing Wiltshire bacon from PSE-prone pigs. *Journal of Food Technology, 8(2)*, 167-174.

The National Provisioner, (2008). Bacon Technology 101. *Bacon Technology Journal*. Available at: http://www.provisioneronline.com/ext/resources/march2011images/Tech_Journals/PO-Bacon-Tech-Journal-08.pdf. Accessed on 26/05/2013.

Tholen, E., Baulain, U., Henning, M. D., & Schellander, K. (2003). Comparison of different methods to assess the composition of pig bellies in progeny testing. *Journal of Animal Science, 81*(5), 1177-1184.

Thompson, L. H., Tucker, J. D., Stewart, S. A., Christensen, M. L., Salazar, E. P., Carrano, A. V., & Felton, J. S. (1987). Genotoxicity of compounds from cooked beef in repair-deficient CHO cells versus Salmonella mutagenicity. *Mutagenesis, 2*(6), 483-487.

Timm-Heinrich, M., Eymard, S., Baron, C. P., Nielsen, H. H., & Jacobsen, C. (2013). Oxidative changes during ice storage of rainbow trout (Oncorhynchus mykiss) fed different ratios of marine and vegetable feed ingredients. *Food Chemistry, 136*(3–4), 1220-1230.

Traore, S., Aubry, L., Gatellier, P., Przybylski, W., Jaworska, D., Kajak-Siemaszko, K., & Santé-Lhoutellier, V. (2012). Effect of heat treatment on protein oxidation in pig meat. *Meat Science, 91*(1), 14-21.

Trusell, K. A., Apple, J. K., Yancey, J. W. S., Johnson, T. M., Galloway, D. L., & Stackhouse, R. J. (2011). Compositional and instrumental firmness variations within fresh pork bellies. *Meat Science, 88*(3), 472-480.

Tuma, D. J. (2002). Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radical Biology and Medicine, 32*(4), 303-308.

Turner, T. D., Mapiye, C., Aalhus, J. L., Beaulieu, A. D., Patience, J. F., Zijlstra, R. T., & Dugan, M. E. R. (2014). Flaxseed fed pork: n-3 fatty acid enrichment and contribution to dietary recommendations. *Meat Science, 96*(1), 541-547.

Uchida, K. (2000). Role of reactive aldehyde in cardiovascular diseases. *Free Radical Biology and Medicine, 28*(12), 1685-1696.

Uchida, K., Kato, Y., & Kawakishi, S. (1990). A novel mechanism for oxidative cleavage of prolyl peptides induced by the hydroxyl radical. *Biochemical and Biophysical Research Communications, 169*(1), 265-271.

Ursini, F., Zamburlini, A., Cazzolato, G., Maiorino, M., Bon, G. B., & Sevanian, A. (1998). Postprandial plasma lipid hydroperoxides: A possible link between diet and atherosclerosis. *Free Radical Biology and Medicine, 25*(2), 250-252.

USDA, 2017. USDA Estimated Composite Pork Carcass Cutout – An Overview. United States Department of Agriculture, Agricultural Marketing Service. Available at

https://www.ams.usda.gov/sites/default/files/media/National%20Daily%20Carlot%20Pork%20Overview%20PDF.pdf. Accessed on 04/06/2017.

Utrera, M., & Estévez, M. (2012). Oxidation of myofibrillar proteins and impaired functionality: Underlying mechanisms of the carbonylation pathway. *Journal of Agricultural and Food Chemistry, 60*(32), 8002-8011.

Utrera, M., & Estévez, M. (2013a). Impact of trolox, quercetin, genistein and gallic acid on the oxidative damage to myofibrillar proteins: The carbonylation pathway. *Food Chemistry, 141*(4), 4000-4009.

Utrera, M., & Estévez, M. (2013b). Oxidative damage to poultry, pork, and beef during frozen storage through the analysis of novel protein oxidation markers. *Journal of Agricultural and Food Chemistry, 61*(33), 7987-7993.

Utrera, M., Morcuende, D., & Estévez, M. (2014a). Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties. *LWT-Food Science and Technology, 56*(1), 62-68.

Utrera, M., Morcuende, D., & Estévez, M. (2014b). Temperature of frozen storage affects the nature and consequences of protein oxidation in beef patties. *Meat Science, 96*(3), 1250-1257.

Utrera, M., Morcuende, D., Rodríguez-Carpena, J.-G., & Estévez, M. (2011). Fluorescent HPLC for the detection of specific protein oxidation carbonyls – α-aminoadipic and γ-glutamic semialdehydes in meat systems. *Meat Science, 89*(4), 500-506.

Utrera, M., Rodríguez-Carpena, J.-G., Morcuende, D., & Estévez, M. (2012). Formation of lysine-derived oxidation products and loss of tryptophan during processing of porcine patties with added avocado byproducts. *Journal of Agricultural and Food Chemistry, 60*(15), 3917-3926.

Uttaro, B., & Zawadski, S. (2010). Prediction of pork belly fatness from the intact primal cut. *Food Control, 21*(10), 1394-1401.

Van Hecke, T., Vanden Bussche, J., Vanhaecke, L., Vossen, E., & De Smet, S. (2013). The effect of nitrite-curing of chicken, pork and beef on oxidation and noc-specific DNA adduct formation during in vitro digestion. Paper presented at: 59th International Congress of Meat Science and Technology, Izmir, Turkey.

VanderVeen, L. A., Hashim, M. F., Shyr, Y., & Marnett, L. J. (2003). Induction of frameshift and base pair substitution mutations by the major DNA adduct of the endogenous carcinogen malondialdehyde. *Proceedings of the National Academy of Sciences, 100*(24), 14247-14252.

Venkatesh, M. S., & Raghavan, G. S. V. (2004). An overview of microwave processing and dielectric properties of agri-food materials. *Biosystems Engineering, 88*(1), 1-18.

Ventanas, S., Estevez, M., Tejeda, J. F., & Ruiz, J. (2006). Protein and lipid oxidation in Longissimus dorsi and dry cured loin from Iberian pigs as affected by crossbreeding and diet. *Meat Science, 72*(4), 647-655.

Ventanas, S., Ventanas, J., Tovar, J., García, C., & Estévez, M. (2007). Extensive feeding versus oleic acid and tocopherol enriched mixed diets for the production of Iberian dry-cured hams: Effect on chemical composition, oxidative status and sensory traits. *Meat Science, 77*(2), 246-256.

Vester-Christensen, M., Erbou, S. G. H., Hansen, M. F., Olsen, E. V., Christensen, L. B., Hviid & M., Larsen, R. (2009). Virtual dissection of pig carcasses. *Meat Science, 81*(4), 699-704.

Villaverde, A., & Estévez, M. (2013). Carbonylation of myofibrillar proteins through the Maillard pathway: effect of reducing sugars and reaction temperature. *Journal of Agricultural and Food Chemistry, 61*(12), 3140-3147.

Villaverde, A., Parra, V., & Estévez, M. (2014). Oxidative and nitrosative stress induced in myofibrillar proteins by a hydroxyl-radical-generating system: Impact of nitrite and ascorbate. *Journal of Agricultural and Food Chemistry, 62*(10), 2158-2164.

Villaverde, A., Ventanas, J., & Estévez, M. (2014). Nitrite promotes protein carbonylation and Strecker aldehyde formation in experimental fermented sausages: Are both events connected? *Meat Science, 98*(4), 665-672.

Virgili, R., Degni, M., Schivazappa, C., Faeti, V., Poletti, E., Marchetto, G., Mordenti, A. (2003). Effect of age at slaughter on carcass traits and meat quality of Italian heavy pigs. *Journal of Animal Science, 81*(10), 2448-2456.

Vollmer, M. (2004). Physics of the microwave oven. *Physics Education, 39*(1), 74.

Vonada, M. L., Bidner, B. S., Belk, K. E., McKeith, F. K., Lloyd, W. R., O'Connor, M. E., & Smith, G. C. (2000). Quantification of pork belly and boston butt quality attribute preferences of South Korean customers. *Journal of Animal Science, 78*(10), 2608-2614.

Vossen, E., Gevaert, K., & De Smet, S. (2013a). Effect of sodium nitrite on protein oxidation in pork myofibrillar proteins. Paper presented at: 59th International Congress of Meat Science and Technology, Izmir, Turkey.

Vossen, E., Gevaert, K., & De Smet, S. (2013b). Effect of sodium nitrite on protein oxidation in pork myofibrillar proteins Paper presented at: 59th International Congress of Meat Science and Technology, Izmir, Turkey.

Vuorela, S., Salminen, H., Makela, M., Kivikari, R., Karonen, M., & Heinonen, M. (2005). Effect of Plant phenolics on protein and lipid oxidation in cooked pork meat patties. *Journal of Agricultural and Food Chemistry, 53*(22), 8492-8497.

Vöhringer, M. L., Becker, T. W., Krieger, G., Jacobi, H., & Witte, I. (1998). Synergistic DNA damaging effects of malondialdehyde/Cu (II) in PM2 DNA and in human fibroblasts. *Toxicology Letters, 94*(3), 159-166.

Wakabayashi, K., Yahagi, T., Nagao, M., & Sugimura, T. (1982). Comutagenic effect of norharman with aminopyridine derivatives. *Mutation Research Letters, 105*(4), 205-210.

Wang, T. J., Ngo, D., Psychogios, N., Dejam, A., Larson, M. G., Vasan, R. S. & Rhee, E. P. (2013). 2-Aminoadipic acid is a biomarker for diabetes risk. *The Journal of Clinical Investigation, 123*(10), 4309-4317.

Waylan, A. T., O'Quinn, P. R., Goodband, R. D., Unruh, J. A., Nelssen, J. L., Woodworth, J. C., & Tokach, M. D. (2003). Effects of dietary additions of modified tall oil, chromium nicotinate, and L-carnitine on growth performance, carcass characteristics, and bacon characteristics of growing-finishing pigs. *Canadian Journal of Animal Science, 83*(3), 459-467.

Weber, T. E., Richert, B. T., Belury, M. A., Gu, Y., Enright, K., & Schinckel, A. P. (2006). Evaluation of the effects of dietary fat, conjugated linoleic acid, and ractopamine on growth performance, pork quality, and fatty acid profiles in genetically lean gilts. *Journal of Animal Science, 84*(3), 720-732.

White, H. M., Richert, B. T., Radcliffe, J. S., Schinckel, A. P., Burgess, J. R., Koser, S. L. & Latour, M. A. (2009). Feeding conjugated linoleic acid partially recovers carcass quality

in pigs fed dried corn distillers grains with solubles. *Journal of Animal Science, 87*(1), 157-166.

Whitney, M. H., Shurson, G. C., Johnston, L. J., Wulf, D. M., & Shanks, B. C. (2006). Growth performance and carcass characteristics of grower-finisher pigs fed high-quality corn distillers dried grain with solubles originating from a modern Midwestern ethanol plant. *Journal of Animal Science, 84*(12), 3356-3363.

Widmer, M. R., McGinnis, L. M., Wulf, D. M., & Stein, H. H. (2008). Effects of feeding distillers dried grains with solubles, high-protein distillers dried grains, and corn germ to growing-finishing pigs on pig performance, carcass quality, and the palatability of pork. *Journal of Animal Science, 86*(8), 1819-1831.

Wiegand, B. R., Sparks, J. C., Beitz, D. C., Parrish, F. C., Horst, R. L., Trenkle, A. H., & Ewan, R. C. (2002). Short-term feeding of vitamin D3 improves color but does not change tenderness of pork-loin chops. *Journal of Animal Science, 80*(8), 2116-2121.

Wilborn, B. S., Kerth, C. R., Owsley, W. F., Jones, W. R., & Frobish, L. T. (2004). Improving pork quality by feeding supranutritional concentrations of vitamin D3. *Journal of Animal Science, 82*(1), 218-224.

Willis, M. S., Klassen, L. W., Carlson, D. L., Brouse, C. F., & Thiele, G. M. (2004). Malondialdehyde–acetaldehyde haptenated protein binds macrophage scavenger receptor (s) and induces lysosomal damage. *International Immunopharmacology, 4*(7), 885-899.

Willis, M. S., Klassen, L. W., Tuma, D. J., Sorrell, M. F., & Thiele, G. M. (2002). Adduction of soluble proteins with malondialdehyde-acetaldehyde (MAA) induces antibody production and enhances T-cell proliferation. *Alcoholism: Clinical and Experimental Research, 26*(1), 94-106.

Wood, J. D., Enser, M., Whittington, F. M., Moncrieff, C. B., & Kempster, A. J. (1989). Backfat composition in pigs: differences between fat thickness groups and sexes. *Livestock Production Science, 22*(3), 351-362.

Wood, J. D., Nute, G. R., Richardson, R. I., Whittington, F. M., Southwood, O., Plastow, G. & Chang, K. C. (2004a). Effects of breed, diet and muscle on fat deposition and eating quality in pigs. *Meat Science, 67*(4), 651-667.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Enser, M. (2004b). Effects of fatty acids on meat quality: a review. *Meat Science, 66*(1), 21-32.

Wood, J. D., & Riley, J. E. (1982). Comparison of boars and castrates for bacon production. 1. Growth data, and carcass and joint composition. *Animal Science, 35*(01), 55-63.

Wong, D., Cheng, K.-W., & Wang, M. (2012). Inhibition of heterocyclic amine formation by water-soluble vitamins in Maillard reaction model systems and beef patties. *Food Chemistry, 133*(3), 760-766.

Xiao, S., Zhang, W. G., Lee, E. J., & Ahn, D. (2013). *Effects of Diet, Packaging and Irradiation on Protein Oxidation, Lipid Oxidation of Raw Broiler Thigh Meat*. Available at: http://www.ans.iastate.edu/report/air/2013pdf/R2761.pdf. Accessed on 23/05/2014.

Xiao, S., Zhang, W. G., Lee, E. J., Ma, C. W., & Ahn, D. U. (2011). Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage. *Poultry Science, 90*(6), 1348-1357.

Xie, F., Sun, S., Xu, A., Zheng, S., Xue, M., Wu, P. & Bai, L. (2014). Advanced oxidation protein products induce intestine epithelial cell death through a redox-dependent, c-jun N-terminal kinase and poly (ADP-ribose) polymerase-1-mediated pathway. *Cell Death & Disease, 5*(e1006), 1-12.

Xiong, Y. L. (2000). Protein oxidation and implications for muscle food quality. In E. Decker, C. Faustman, & J. L. B. Clemente (Eds.). *Antioxidant in Muscle Foods* (pp. 85-111). Chichester: Wiley.

Xu, G., Baidoo, S. K., Johnston, L. J., Bibus, D., Cannon, J. E., & Shurson, G. C. (2010). Effects of feeding diets containing increasing content of corn distillers dried grains with solubles to grower-finisher pigs on growth performance, carcass composition, and pork fat quality. *Journal of Animal Science, 88*(4), 1398-1410.

Yancey, J. W. S., Apple, J. K., Sawyer, J. T., Lee, M. S., & Woodworth, J. C. (2008). Comparisons of fatty acid in pork belly primary and secondary lean, and seam and subcutaneous fat. *Journal of Animal Science, E-Supplement 2*, 39.

Yarmand, M., & Rad, A. H. (2011). Microwave processing of meat. In U. Chandra (Ed.), *Microwave Heating* (pp. 1-30). Rijeka: InTech.

Yi, Y. H., & Chen, T. C. (2003). Prediction of lean to fat tissue ratio of pork belly by specific gravity. *Journal of Food Engineering, 58*(3), 295-297.

Youngman, L. D., Park, J. Y., & Ames, B. N. (1992). Protein oxidation associated with aging is reduced by dietary restriction of protein or calories. *Proceedings of the National Academy of Sciences, 89*(19), 9112-9116.

Zamora, R., & Hidalgo, F. J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Critical Reviews in Food Science and Nutrition, 45*(1), 49-59.

Zarkovic, N., Cipak, A., Jaganjac, M., Borovic, S., & Zarkovic, K. (2013). Pathophysiological relevance of aldehydic protein modifications. *Journal of Proteomics, 92*, 239-247.

Zhang, W., Xiao, S., & Ahn, D. U. (2013). Protein oxidation: Basic principles and implications for meat quality. *Critical Reviews in Food Science and Nutrition, 53*(11), 1191-1201.

Zhang, W., Xiao, S., Lee, E. J., & Ahn, D. U. (2011). *Effects of Dietary Oxidation on the Quality of Broiler Breast Meat*. Available at: http://www.ans.iastate.edu/report/air/2011pdf/R2624.pdf. Accessed on 23/12/2016.

Zimniak, P. (2011). Relationship of electrophilic stress to aging. *Free Radical Biology and Medicine, 51*(6), 1087-1105.

APPENDICES

Appendix A1: Fatty acid profile summary for the pork bellies employed in this study

		Intermuscular fat				Subcutaneous fat				Lean layer			
	N	***Min***	***Max***	***Mean***	***Std Dev***	***Min***	***Max***	***Mean***	***Std Dev***	***Min***	***Max***	***Mean***	***Std Dev***
IV	198	53.01	70.7	59.75	2.94	58.02	71.51	64.60	2.48	53.6	67.16	58.62	1.97
MUFA	198	42.97	53.02	47.98	1.95	45.84	55.06	50.98	1.61	47.19	55.00	51.27	1.48
SFA	198	32.86	46.53	41.23	2.07	32.31	42.47	36.828	1.73	35.10	43.30	38.81	1.38
PUFA	198	7.68	16.25	10.8	1.65	9.36	16.36	12.216	1.41	6.45	17.10	9.91	1.66
n/6	198	6.75	13.06	8.86	1.10	8.03	12.71	10.17	0.99	5.62	14.39	8.30	1.31
n/3	198	0.87	4.34	1.93	0.79	0.99	4.08	2.03	0.69	0.75	3.87	1.62	0.54
n-6/n-3	198	2.36	9.06	5.26	1.83	2.51	8.88	5.52	1.68	2.87	8.69	5.54	1.46
PUFA/SFA	198	0.17	0.46	0.26	0.05	0.23	0.47	0.33	0.05	0.16	0.48	0.26	0.05

IV; iodine value, MUFA: monounsaturated fatty acid, SFA; saturated fatty acid, PUFA; polyunsaturated fatty acid; n/6; omega 6 PUFA, n/3; omega 3 PUFA.

Appendix A2: Effect of breed, gender, diet and slaughter weights on belly softness measures and other compositional measurements

	Belly-flop angle (°)	Belly score measurement	Fat content (%)	C16 (%)ǂ	C18 (%)ǂ	C18:2 (%)ǂ	Moisture content (%)	Iodine Value (%) ǂ	SFA (%) ǂ	PUFA (%)ǂ
					Breed					
Iberian	78.35±2.04^{a}	2.48±0.07^{c}	58.32±0.74^{a}	24.07±0.12^{a}	11.21±0.13	8.81±0.09^{c}	31.55±0.57^{c}	63.38±0.24^{c}	37.15±0.22^{a}	11.26±0.12^{c}
Duroc	47.28±1.78^{b}	3.43±0.06^{b}	36.46±0.65^{c}	24.12±0.11^{a}	11.00±0.12	9.69±0.08^{b}	48.62±0.50^{a}	64.76±0.21^{b}	36.93±0.20^{a}	12.38±0.10^{b}
Lacombe	35.41±1.85^{c}	3.69±0.07^{a}	44.46±0.67^{b}	23.30±0.11^{b}	10.98±0.12	9.95±0.09^{a}	42.53±0.52^{b}	65.65±0.22^{a}	36.15±0.20^{b}	12.83±0.11^{a}
P value	<.0001	<.0001	<.0001	<.0001	0.3961	<.0001	<.0001	<.0001	0.0022	<.0001
					Gender					
Barrow	58.79±2.5^{a}	2.98±0.05^{b}	48.97±0.52^{a}	24.00±0.08^{a}	10.98±0.09	9.21±0.07^{b}	38.90±0.40^{b}	64.25±0.17^{b}	36.87±0.16	11.87±0.08^{b}
Gilt	48.57±2.8^{b}	3.43±0.06^{a}	43.85±0.59^{b}	23.66±0.10^{b}	11.14±0.11	9.76±0.08^{a}	42.89±0.46^{a}	64.95±0.20^{a}	36.62±0.18	12.45±0.10^{a}
P value	<.0001	<.0001	<.0001	0.0078	0.2631	<.0001	<.0001	0.0068	0.3116	<.0001
					Diet					
Canola	48.55±1.89^{b}	3.34±0.07^{a}	46.82±0.69	23.56±0.11^{b}	10.92±0.13	9.61±0.09^{a}	40.51±0.53	65.93±0.22^{b}	36.33±0.21^{b}	12.22±0.11^{b}
Flaxseed	51.97±1.83^{b}	3.30±0.06^{a}	46.31±0.66	23.78±0.11^{b}	11.08±0.12	9.53±0.08^{ab}	41.06±0.51	65.79±0.22^{a}	36.69±0.20^{ab}	12.96±0.11^{a}
Control	60.52±1.96^{a}	2.96±0.07^{b}	46.11±0.71	24.16±0.11^{a}	11.19±0.13	9.31±0.09^{b}	41.13±0.55	63.08±0.293^{c}	37.22±0.22^{a}	11.29±0.11^{c}
P value	<.0001	0.0001	0.7585	0.0011	0.3162	0.0466	0.6688	<.0001	0.0125	<.0001
					Slaughter weight					
120	47.02±1.59^{b}	3.42±0.05^{a}	44.88±0.58^{b}	23.76±0.09	10.92±0.11	9.69±0.07^{a}	42.07±0.42^{a}	65.10±0.18^{a}	36.56±0.16	12.48±0.09^{a}
140	60.34±1.50^{a}	2.98±0.06^{b}	47.94±0.54^{a}	23.90±0.09	11.20±0.11	9.28±0.07^{b}	39.73±0.45^{b}	64.10±0.19^{b}	36.93±0.18	11.83±0.09^{b}
P value	<.0001	<.0001	0.0002	0.3057	0.0546	<.0001	0.0002	0.0173	0.1176	<.0001

ǂ These values are only for the subcutaneous fatty acid profile. Values are expressed as mean ± SEM. Values within the same column with different letters are significantly different at $P < 0.01$.

Appendix A3: Effect of breed, gender, diet and slaughter weights on selected physical factors

Variable	Belly length	Belly weight	Belly width	ShTk1	ShTk	Sln	Subq2	Seam 1	Sft
				Breed					
Iberian	71.23±0.29^{a}	9.93±0.11^{a}	25.1±0.20^{b}	7.08±0.08^{a}	5.68±0.07^{a}	1.63±0.03^{c}	1.57±0.03^{a}	3.60±0.06^{a}	4.01±0.06^{a}
Duroc	69.46±0.30^{b}	8.85±0.09^{b}	26.5±0.18^{a}	5.40±0.07^{c}	3.85±0.06^{c}	2.36±0.03^{a}	1.16±0.02^{b}	2.23±0.05^{c}	2.70±0.05^{c}
Lacombe	71.23±0.30^{a}	8.80±0.10^{b}	27.0±0.18^{a}	5.62±0.07^{b}	4.23±0.06^{b}	2.22±0.03^{b}	1.11±0.03^{b}	2.71±0.05^{b}	3.08±0.06^{b}
P value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
				Sex					
Barrow	70.77±0.21	9.33±0.08^{a}	26.2±0.16	6.38±0.05^{a}	4.94±0.05^{a}	2.04±0.03	1.42±0.02^{a}	3.06±0.04^{a}	3.48±0.04^{a}
Gilt	70.50±0.23	9.06±0.09^{b}	26.5±0.18	5.69±0.06^{b}	4.23±0.06^{b}	2.10±0.03	1.14±0.02^{b}	2.63±0.05^{b}	3.05±0.05^{b}
P value	0.4102	0.0235	0.1931	<.0001	<.0001	0.0722	<.0001	<.0001	<.0001
				Diet					
Canola	70.32±0.27^{b}	9.11±0.10	26.5±0.21^{a}	5.95±0.07	4.53±0.07	2.10±0.03	1.26±0.02	2.80±0.06	3.26±0.06
Flaxseed	70.20±0.26^{b}	9.17±0.10	26.5±0.20^{a}	6.13±0.07	4.67±0.06	2.06±0.03	1.29±0.02	2.93±0.05	3.32±0.05
Control	71.39±0.28^{a}	9.31±0.11	25.9±0.21^{b}	6.02±0.07	4.56±0.07	2.06±0.03	1.29±0.03	2.81±0.06	3.21±0.06
P value	0.0038	0.3868	0.0567	0.1716	0.2938	0.5923	0.7273	0.2080	0.3278
				Slaughter weight					
120	69.38±0.21^{b}	8.33±0.08^{b}	25.7±0.16^{b}	5.63±0.06^{b}	4.24±0.05^{b}	2.07±0.02	1.19±0.02^{b}	2.61±0.04^{b}	3.04±0.04^{b}
140	71.90±0.23^{a}	10.06±0.09^{a}	26.8±0.16^{a}	6.44±0.06^{a}	4.93±0.06^{a}	2.08±0.02	1.37±0.02^{a}	3.08±0.05^{a}	3.49±0.05^{a}
P value	<.0001	<.0001	<.0001	<.0001	<.0001	0.7474	<.0001	<.0001	<.0001

Values are expressed as mean ± SEM. Values within the same column with different letters are significantly different at P < 0.0

Appendix A4: Pearson correlation between belly attributes and bacon characteristics

	AAS	GGS	Total PROTOX	HAA	TBARS	MDA	IV	ω 6	ω3	ω6/ω3	MUFA	SFA	PUFA	PUFA/SFA
AAS	1.00	0.86**	1.00**	0.25**	0.42**	0.05	0.05	0.04	0.07	-0.05	-0.04	-0.02	0.07	0.07
GGS		1.00	0.89**	0.37**	0.59**	0.18	0.04	0.03	0.04	-0.04	0.02	-0.05	0.04	0.06
Total PROTOX			1.00	0.27**	0.45**	0.05	0.05	0.05	0.07	-0.04	-0.03	-0.03	0.07	0.07
HAA				1.00	0.45**	0.50**	-0.05	-0.06	-0.08	-0.06	0.11	-0.04	-0.09	-0.06
TBARS					1.00	0.40**	0.10	0.08	0.08	-0.07	-0.02	-0.06	0.10	0.10
MDA						1.00	0.15*	0.11	0.16*	-0.14*	-0.06	-0.08	0.16*	0.16*
IV							1.00	0.72**	0.67**	-0.50**	0.09	-0.82**	0.88**	0.97**
ω6								1.00	0.27**	-0.02	-0.22**	-0.49**	0.84**	0.84**
ω3									1.00	-0.95**	-0.42**	-0.23**	0.75**	0.68**
ω6/ω3										1.00	0.38**	0.10	-0.55**	-0.48**
MUFA											1.00	-0.64**	-0.39**	-0.12
SFA												1.00	-0.47**	-0.69**
PUFA													1.00	0.96**
PUFA/SFA														1.00

* significant at $P < 0.05$, **significant at $p < 0.01$. AAS; α-aminoadipic semialdehyde, GGS; γ-glutamic semialdehyde, Total PROTOX; total protein oxidation (GGS + AAS)

Appendix A5: Interaction of cook method by storage day with sensory attributes

	Cook Method								
	Frying pan				Microwave				
	Storage days				Storage days				
	2		28		2		28		
Variable	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P value
Initial Crispiness	6.88[a]	0.17	6.74[ab]	0.17	6.37[c]	0.17	6.55[bc]	0.17	0.0460
Overall Crispiness	6.76	0.16	6.67	0.16	6.25	0.16	6.31	0.16	0.3424
Saltiness	5.71	0.18	5.69	0.18	5.45	0.18	5.39	0.18	0.6417
Smoke	5.42	0.21	5.41	0.21	5.67	0.21	5.65	0.21	0.8474
Bacon	5.93	0.20	6.00	0.20	5.90	0.20	5.95	0.20	0.8082
Chewiness	5.26	0.23	5.19	0.23	5.57	0.23	5.44	0.23	0.6303
Mouth Coating	3.68	0.29	3.73	0.29	3.59	0.29	3.54	0.29	0.3816
Off-flavour Intensity	8.31	0.23	8.36	0.23	7.78	0.23	8.06	0.23	0.0780

[a-b]Different letters in each row indicate significant difference at $P < 0.05$. A 9-point scale was used with 9=extremely intense and 1=none for all observations except off-flavour intensity in reverse order.