THE ANTIMICROBIAL EFFECTS OF PARA-HYDROXYBENZYL ISOTHIOCYANATE ON *ESCHERICHIA COLI* O157:H7 IN BEAKER SAUSAGE AND THE SENSORY INFLUENCE OF DEHEATED YELLOW MUSTARD ON DRY-FERMENTED SAUSAGE

By

Shuliu Li

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

Department of Food Science

Faculty of Agricultural and Food Science

The University of Manitoba

Winnipeg, Manitoba

Abstract

Para-hydroxybenzyl isothiocyanate (p-HBIT) formed in yellow mustard following hydrolysis of the glucosinolate, sinalbin, is a natural antimicrobial agent. p-HBIT is not dependably available commercially, and a small amount was synthesized for use in beaker sausage fermentations. For these trials stabilized p-HBIT was used in dry sausage meat batter to reduce the viability of inoculated *Escherichia coli* O157:H7. A >4 log CFU/g reduction of *E. coli* O157:H7 was achieved in the beaker sausage containing p-HBIT. For sensory evaluation of fermented sausages containing ≤ 4% (w/w) yellow mustard powder, consumer preference tests were done. Deodorized (deheated) yellow mustard was added at 1,2,3 and 4% (w/w) to dry-fermented sausage and it was found that 3% and 4% mustard negatively affected the flavour, texture and overall acceptability of the fermented sausage. Dry-fermented sausage containing 1% and 2% mustard had a slight change in flavour, texture and overall acceptability.

Acknowledgments

I would like to thank my parents for their steady support and understanding provided for so many years. I thank my colleagues in the Department of Food Science who were patient with my stupid questions and helped and taught me to do my study. I would also like to thank all my friends who have tolerated my horrible temper and bad attitude over all these years. I especially thank Niloufar who has taught me and guided me about the research world. I sincerely appreciate the funding of this project that was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) and Mustard 21. I am also thankful for Donna and Namita's assistance for the reason that I don't think I could have effectively finished the sensory analysis and microbiology laboratory work without their help.

Most of all I need to thank Dr. Holley. You are the one who provided me the precious opportunity to learn and develop myself. You are also the one who let me grow up and be a stronger and better person. I finally know what I want to do and the person I want to be in my future because of the time I spent here with you.

And to Dr. Aliani and Dr. Arntfield, you are always nice and helpful professors no matter how busy you are.

Organization of the thesis

The thesis is organized to include two manuscripts (Chapter 3 and 4) as indicated below. These chapters were standardized for presentation in the thesis format.

Chapter 1 gives an overall introduction to the thesis; Chapter 2 is a comprehensive literature review.

Chapter 3 explains the antimicrobial effect of stabilized para-hydroxybenzyl isothiocyanate on *Escherichia coli* O157:H7 in beaker sausages.

Chapter 4 shows the sensory effect of deheated yellow mustard on dry-fermented sausage.

Chapter 5 gives a general discussion and conclusion to the thesis.

Chapter 6 shows the future work that may be of value to improve the eating quality of dry-fermented sausage.

Table of Contents

List of Tables

List of Figure

Chapter 1

Introduction

Fermented meat products have been prepared in many countries for centuries. They have been popular not only because of their long shelf-life (> 6 months) but also because of their unique flavour and texture. Nowadays, there are many different fermented meat products available that have specific sensory characteristics, often reflecting regional preferences in consumers' expectation. However, food safety is the most important determinant of food quality. Dry-cured fermented meat products have not been commonly associated with food-borne illness due to their low pH, low water activity and curing agents plus spices added during processing. Though, food-borne illnesses associated with dry-cured fermented sausage have been reported recently (Tilden et al., 1996; Williams et al., 2000; MacDonald et al., 2004).Risks associated with consumption of fermented meat products arise through contaminated raw materials and are particularly acute if inappropriate manufacturing procedures are followed. Since these products are not cooked, an organism like *Escherichia coli* O157:H7, which can survive modern and traditional methods of fermented sausage, is of particular concern. Mustard has been shown to have natural bactericidal activity toward many food-borne pathogens. There are two major groups of mustard (*Sinapis alba,* yellow, and *Brassica juncea,* brown), grown and used in North America. Its unique bactericidal activities develop through a series of chemical reactions, which also produce the hot flavour and pungent odour. Two important compounds, allyl isothiocyanate, AIT (in brown mustard) and *para*-hydroxybenzyl isothiocyanate, p-HBIT (in yellow mustard) have been studied for their bactericidal activities in fermented meat products. Graumann and Holley (2008) added deheated or non-deheated yellow mustard powder to dry-fermented sausage to

evaluate their bactericidal activity against *E. coli* O157:H7. They found that 6% (w/w) deodorized yellow mustard powder gave a suitable and rapid reduction of *E. coli* O157:H7 in less than one month. In addition, a texture difference was noticed in the mustard-containing salami (Graumann and Holley, 2008). Also, Luciano and Holley (2011) combined autoclaved (deodorized) and non-deheated (hot) yellow mustard powder and used it in dry-fermented sausage in order to assess its antibacterial ability against *E. coli* O157:H7. Results suggested that the autoclaved yellow mustard powder was a more effective bactericidal agent than deheated and non-deheated yellow mustard powder alone (Luciano and Holley, 2011). Further, Nilson and Holley (2012) found that deodorized (autoclaved) yellow mustard powder at 4% and 6% (w/w) successfully controlled the viability of *E. coli* O157:H7 on dry cured Westphalian ham. Also, no colour difference between control and mustard treated dry-cured ham was evident (Nilson and Holley, 2012).

Although data show that mustard powder is an effective natural antimicrobial for the control of *E. coli* O157:H7 viability in dry cured fermented sausage, there has been no systematic evaluation of the sensory effects that may occur following its use in these products. Therefore, a sensory study on fermented meat products following mustard powder addition would be of value. There are three main types of sensory analysis methods: descriptive, discriminative and affective. Previous sensory studies found that different starter cultures could affect sensory characteristics of dry-cured sausage during ripening and drying (Ansorena, Gimeno, Astiasaran & Bello, 2001; Berdagué et al.,1993; Montel, Masson & Talon, 1998; Erkkilä et al.,2001). Most of the studies focused on instrumental analysis, where gas chromatography and mass spectrometry were used to

identify the volatiles formed during fermentation (Olesen, Meyer & Stahnke, 2004; Ordóñez et al., 1999; Sondergaard and Stahnke, 2002; Tjener, Stahnke, Andersen & Martinussen, 2004). Further, some studies combined instrumental and discriminative sensory methods using trained panels in order to identify the flavour and texture attributes of dry-fermented sausage (Ordonez, Hierro, Bruna & de la Hoz, 1999; Marco, Navarro, Flores, 2007). The affective sensory method was used with dry-fermented sausage by Muthukumarasamy and Holley (2006). They analyzed the sensory quality of dry-fermented sausage containing *Lactobacillus reuteri* using a consumer panel and did not find significant sensory changes in sausage treated with this probiotic when microencapsulated. Also, Calvo, Garcia & Selgas (2008) analyzed the sensory quality of dry-fermented sausage containing tomato peel with a high level of lycopene. Using a consumer-based sensory method, dry-fermented sausage with tomato peel showed good sensory quality. Kumar and Tanwar (2010) demonstrated that chicken nuggets with 1.5 % (w/w) ground mustard had a higher score for texture than the control. A desirable change in flavour and colour of the chicken nuggets was indicated at both the 1.5% and 2% concentration of mustard meal. However, there has been no study done on the sensory evaluation of dry-fermented sausage with mustard powder added. The value of affective sensory evaluation is that it not only provides more information on how consumers appreciate the products, but also reflects the market view toward the tested products.

Deodorized ground yellow mustard is an excellent emulsifier, binder and thickener in cooked processed meat products, and it is a cost-effective protein source with neutral flavour. Historically, it has not been used in uncooked fermented meats, but it is a highly potent bactericidal agent in these products. The objectives of this thesis were to

evaluate the bactericidal properties of stabilized p-HBIT against *E. coli* O157:H7, and to better understand the sensory quality of dry-fermented sausage with different levels of yellow mustard powder added. A consumer-based sensory evaluation was conducted following the addition of ≤ 4% yellow mustard powder to dry-fermented sausage to determine the threshold concentration of mustard which was detectable.

Chapter 2

Literature Review

2.1 The Role of Mustard

Mustard is a unique spice that has been recognized and widely used in food for centuries in China and European countries. There are many different botanical species of mustard. Three main mustard species: *Sinapis alba* (white or yellow), *Brassica juncea* (brown or oriental) and *Brassica nigra* (black) are identifiable by seed colour. The yellow or white and brown or oriental mustards have been most popularly used in many countries; whereas black mustard is not commonly used alone in the food industry, particularly in the North America market. Black mustard has the strongest pungent hot flavour, while brown mustard has a relatively milder flavour. Yellow/white mustard has the smoothest and least hot flavour. The European market prefers the stronger and aromatic flavour of black mustard.

Canada is the largest exporter of mustard seed in the world, producing about 35% of the mustard traded internationally and it exports nearly 50% of its production to other countries (AAFC, 2009a).Three major forms of mustard seed are available and used in food: whole, ground and flour. The whole mustard seed has mostly been used in pickling as spice. In India and China, whole mustard seed also has been used as a seasoning for enhancing the hot flavour of food. In fact, the mustard seed itself is without any flavour. It releases its unique pungent and hot flavour through a series of hydrolytic reactions in the presence of moisture. Ground mustard is produced by crushing the whole mustard seed. It is used in the processed meats industry because of its high protein content (about

23%~30%)(Cui and Eskin, 1998).Further, the seed bran contains a natural mucilaginous substance, which is a linear acidic polysaccharide and it thus plays an important role as an emulsifier, thickener and binder in cooked processed meat(Cui and Eskin, 1998). However, ground mustard can only be used in cooked meat products after it has been thermally treated (deheated) in the absence of moisture to destroy endogenous myrosinase, an enzyme responsible for conversion of glucosinolates in the mustard to isothiocyanates (which are responsible for the pungency and odour of mustard). In the early 1990s, only 1% ground mustard was allowed to be added to processed meat products. Nowadays, a maximum of 5% ground mustard can be used in processed meat products, although meat products are still required to declare "mustard" in the ingredients list (Cui and Eskin, 1998). In addition, it should be noted that myrosinase in non-deheated mustard may react with meat proteins and produce off-flavour (Tainter and Grenis, 1993). Mustard flour is a fine powder produced by milling the seed. In North America, mustard seeds are usually de-oiled before milling (Cui and Eskin, 1998). Thermally-treated mustard flour has the least pungency and is a good emulsifier capable of replacing egg yolk in many food products. Deheated mustard flour is also used to improve the quality of processed cheese and enhance the appearance of fried food products. It has been commonly used as prepared mustard paste and as an ingredient in mayonnaise, barbecue sauces, marinades, and salad dressings. Mustard oil is highly pungent and is uncommonly used in North America, but it has been used as a cooking oil in India for years. The oil accounts for about 29%~36% of the mustard seed (Cui and Eskin, 1998). Because of its extremely strong pungency, mustard oil is not appreciated as a food or flavour enhancer in the world market. However, due to its lubricity, mustard oil

has been studied for use as a biodiesel additive and for improvement of airplane engine performance. In addition, a product derived from mustard seed and known as Mustard Organic Soil Stabilizer was used as a natural fertilizer to improve soils, and thus boost plant growth (AAFC, 2011).

2.1.1 Research on mustard and its natural antimicrobial components para-hydroxybenzyl isothiocyanate and allyl isothiocyanate

Mustard is not only popular in foods because of its spiciness, it also has been studied because of other functional characteristics. Its broad range of antibacterial activity has been studied for a long time. Yellow mustard seed is also considered and used as a valuable protein source(Table 2.1).It is also a good source of essential amino acids(Table 2.2),contains a high level of dietary fibre and is rich in antioxidant compounds after being thermally treated(Cui and Eskin, 1998). Therefore, it has potential to be used in many food products.

Table2.1 Chemical properties of mustard seed and flour

Mustard	Oil content (%)	Protein (%)	Ash (%)	Water (%)	Fiber (%)	Isothiocyanate (%)	
						AIT	p-HBIT
Yellow seed	29	30	4	6	9	None	2.3
Brown seed	32	26	4	6	7	0.8	None
Black seed	36	23	4	6	6	0.78	None
Yellow flour	30	35	4	6	3.5	None	None
Brown	40	35	4	6	3.5	0.95	None

flour							
Black flour	42	30	4	6	3.5	0.90	None

Adapted from Functional foods (pp. 235-245), by Cui, W. and Eskin, N.A.M. (1998). Boca Raton, FL: biochemical and processing aspects. CRC Press.

Table 2.2The amino acids in yellow mustard meal (% of dried meal) compared to sunflower, sesame, cotton seed and soybean.

Amino Acids	Mustard	Sunflower	Sesame	Cotton seed	soybean
Leucine	2.7	2.5	3.4	2.5	3.8
Arginine	2.1	3.7	4.7	4.5	3.8
Lysine	2.0	1.4	1.7	1.3	3.2
Valine	1.8	2.2	2.4	0.7	2.7
Threonine	1.7	1.3	1.4	1.6	2.0
Phenylalanine	1.5	2.0	2.2	1.3	2.7
Isoleucines	1.4	1.6	2.1	1.6	2.6
Tyrosine	1.3	none	2.0	0.7	2.0
Methionine	0.9	0.6	1.4	0.6	0.7
Cystine	1.0	0.9	1.1	1.1	1.2
Histidine	0.8	0.7	0.8	0.6	0.8
Tryptophan	0.4	0.6	0.8	0.5	0.6

Adapted from Oil Crops of the World. (pp, 192-195) by Bell, J.M. (1989). New York: McGraw Hill Publishing Company.

As with other cruciferous plants, mustard contains the flavour precursor compounds, glucosinolates. In addition, mustard seeds contain the enzyme myrosinase which can react with glucosinolates in a moist environment and produce allyl

isothiocyanate (AIT, oriental mustard) and p-hydroxy benzyl isothiocyanate (p-HBIT, yellow mustard), respectively. Both AIT and p-HBIT have been shown to be bactericidal against food-borne pathogens. AIT effectively inhibited the growth *of E. coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes* and *Staphylococcus aureus* (Delaquis and Sholberg, 1997; Kyung and Fleming, 1997; Isshiki, Tokuoka, Mori & Chiba, 1992; Lin, Jeongmok, Du & Wei, 2000; Rhee, Dougherty & Kang, 2003). *E. coli*, S. *aureus*, *Campylobacter jejuni*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, *Shigella boydii*, *L. monocytogenes* and *Clostridium perfringens*were inhibited by p-HBIT in a separate study (Ekanayake et al., 2006).Moreover, AIT and p-HBIT exhibited antifungal activity in several studies (Delaquis and Sholberg, 1997; Mayton, 1996; Shofran, Purrington, Breidt & Fleming 1998; Kyung and Fleming, 1997). In addition, AIT and p-HBIT have been studied for their ability to control the weed population in fields (Beekhuis, 1975; Teasdale and Taylorson, 1986). The anticarcinogenic properties of mustard, AIT and p-HBIT were examined in a large number of studies. However, their medical value still remains somewhat uncertain, although mustard extracts have been used to treat snake poisoning, rheumatism and provide muscle relaxation since ancient times. The problem related to AIT and p-HBIT, in particular p-HBIT, is their extreme instability in the presence of moisture where p-HBIT is degraded in few minutes. They account for 0.8%~2.3% of the mustard seed weight (Table 2.1). Previous work has been done to stabilize p-HBIT and it was used on different food samples as an antimicrobial (Ekanayake et al., 2006). However, its instability and pungency have limited work with this compound. Saleemi, Janitha, Wanasundara & Shahidi (1993) evaluated the effect of low-pungency ground mustard seed (deactivated myrosinase) on the quality changes in

ground pork. Different levels of ground mustard seed (0.5, 1, 1.5 and 2%) were added to ground cured and uncured pork and cooked. The cooking loss was remarkably reduced in both types of cured and uncured pork when blended with mustard seed. Additional mustard seed did not negatively influence the colour of either cured or uncured pork and showed high antioxidant activity, which might essentially extend meat shelf-life. Defatted and deheated yellow mustard flour had antioxidant activity that was due to the presence of phenolic compounds (Shahidi, Wanasundara & Amarowicz, 1994). The latter authors suggested that mustard be used to prevent oxidation and rancidity in food. Ildiko et al. (2006) used radio frequency-based heat treatment to deactivate the endogenous myrosinase in yellow/white mustard and found that the emulsification activity of mustard and its the nutritional value were unaffected.

2.1.2 Safety issues associated with mustard

Mustard has recently been identified as a major food allergen in Canada. Health Canada made the decision after examining 42 scientific studies which showed that mustard was allergenic. Health Canada also explained that allergy cases associated with mustard consumption have occurred with both children and adults in Canada (Health Canada, 2009). Moreover, the Commission of European Communities (2007) recognized mustard as an allergen and requested it be identified on food labels. The International Union of Immunological Societies (2009) also found that mustard caused allergy issues in food. Mustard contains irritants that provoke non-immunoglobulin E-mediated mast cell (IgE) de-granulation, which caused the response (Niinimaki, Bjorksten, Puukka, Tolonen & Hannuksela, 1995). However, real allergic reactions caused by mustard were due to its thermally stable protein,2S albumin(Menendez-Arias, Moneo, Dominguez

&Rodriguez, 1988). The 2S albumin is not hydrolyzed by myrosinase and is stable at high temperature. Therefore, the protein might be present in food ingredients, as well as processed and pre-packed food containing mustard (Health Canada, 2009). Moreover, Health Canada (2009) indicated that people who are allergic to one type of mustard were possibly sensitive to other types of mustard. Since mustard can cause severe allergic reactions that include anaphylaxis, the Canadian Food Inspection Agency is adding mustard and its derivatives as allergens in the new regulations on food allergens coming into force in August 2012.

2.2 Safety Issues with Dry-fermented Sausage

Dry-fermented sausage has been popular for a long time, and has been an artisanal product, although recipes presently involve starter culture use in modern sausage processing plants. Regional and ethnic variations in dry sausage manufacture are numerous worldwide. European countries are still the leading producers and have the highest consumption of dry-fermented sausage. Dry-fermented sausage can be categorized on the basis of moisture content as dry (35% moisture), or semi-dry (50% moisture) and the former are shelf-stable at room temperature (pH<4.6, water activity <0.92). They can also be classified on the basis of recipe origin as southern or northern European style, which is based on the processing procedures, sausage diameter, weight and type of meat used. In North America, most of the dry sausage sold in the food market is of the northern European style, which has lower pH and water activity, and involves a smoking process. Therefore, it has generally been considered to be safe as well as flavourful. Agriculture and Agri-food Canada reported that about 65% and 25% of pork and beef produced, respectively, were sold to meat processing plants to manufacture ham,

sausage, bacon and other meat products (Health Canada, 2002). Moreover, Statistics Canada estimated that cured, prepared and cooked meat products represented 6% of total yearly food expenditures per person and 30% of meat sales per year (Health Canada, 2002). However, dry-fermented sausage(a ready-to-eat meat product) has been recently involved in several food-borne illness outbreaks in the U.S and Canada, where *E. coli* O157:H7 was found in the finished dry-fermented sausage (Tilden et al., 1996; Williams et al., 2000; MacDonald et al., 2004). This bacterium can cause serious problems including bloody diarrhea, urinary tract infection, meningitis and even the haemolytic uremic syndrome, which can be fatal, especially in the elderly and children. It is critical to control or eliminate *E. coli* O157:H7 from dry-fermented sausage during its manufacture, and thus ensure product safety.

2.2.1 Studies on the inhibition of pathogens in dry-fermented sausage: *E. coli* O157:H7

Using the bacteriocins produced by probiotic bacteria, *E. coli* O157:H7 viability was reduced in several studies (Erkkila et al., 2002; Tyopponen, Petaja & Mattila-Sandholm 2003; Muthukumarasamy and Holley, 2007). Also, Al-Nabulsi and Holley (2007) used microencapsulated bovine lactoferrin in dry sausage and effectively inhibited *E. coli* O157:H7. Moreover, Graumann and Holley (2008) microencapsulated AIT and used it in dry-fermented sausage to eliminate *E. coli* O157:H7. Non-deheated yellow mustard (hot) powder was another alternative that has been tested on ground beef (Nadarajah, Han & Holley, 2005). Hot yellow mustard was added to meat at 5 and 10% (w/w) and caused a 3 log CFU/g reduction of *E. coli* O157:H7 at day 18 and 12, respectively. Graumann and Holley (2008) used 2,4, 6% (w/w) hot yellow mustard and 6%

cold yellow mustard powder in dry-fermented sausage, inoculated with *E. coli* O157:H7. At day 36 and 24, a 5 log CFU/g reduction of *E. coli* O157:H7 was achieved in the salami treated with 4 and 6% hot yellow mustard, respectively. Moreover, dry-fermented sausage treated with 6% cold yellow mustard achieved>5 log CFU/g reduction of *E. coli* O157:H7 in 18 days. Luciano, Belland & Holley. (2011) added 6% antoclaved (deactivated myrosinase) yellow mustard to dry-fermented sausage and achieved >5 log CFU/g reduction of *E. coli* O157:H7 in 18 days; whereas, 6% hot (non-deheated) or cold (deheated) yellow mustard yielded a >5 log CFU/g reduction of *E. coli* O157:H7 in 31 and 38 days, respectively (Luciano, Belland & Holley., 2011). Moreover, Luciano and Holley (2010) studied how mustard without myrosinase could be bactericidal. They found that *E. coli* O157:H7 synthesized a myrosinase-like enzyme that hydrolyzed the sinalbin in mustard to produce p-HBIT, which eliminated *E. coli* O157:H7. Luciano and Holley (2010) demonstrated that some starter culture bacteria used in fermentation processes also showed myrosinase-like activity, hydrolysing sinalbin and sinigrin to variable degrees.

2.3 The Importance of Sensory Evaluation

Sensory evaluation has attracted a lot of attention in recent years. It has become a more systematic and scientific research discipline in the second half of the 20th century (Lawless and Heymann, 1998). It has grown along with the increasing awareness of the importance of product quality control in the food industry. Nowadays, it is a comprehensive and important method to measure human perception of product quality and thus is used to improve final product quality. Sensory evaluation now plays an essential role in marketing decision-making, especially in the food industry. It is an

influential tool for optimization of product quality and thus helps companies establish reputation and be competitive. It not only identifies the key components that contribute to preferred quality, but provides information about consumer satisfaction, which is the key driver for quality control. In addition, it ultimately links product quality issues with consumer satisfaction, purchase intent and customer loyalty. Stone and Sidel (1993) defined sensory evaluation as "a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing". It was explained that proper sensory evaluation describes the method to be used for preparing samples to be served and the control of the environment necessary for sensory studies. Sensory studies collect a wide variety of data, which must be properly analyzed in order to draw meaningful conclusions. Food quality considers safety, as well as sensory characteristics and nutrition. Tuorila and Pangborn (1988) stated that sensory evaluation was the priority factor that determined food acceptance. Physical, microbiological and chemical analyses are three main methods that are used in food quality studies. Sensory analysis requires that food safety and nutrition issues be considered first, and is a unique measurement that encompasses food physical, microbiological and chemical properties. Sensory evaluation has not only been successfully applied in the food industry, it has also caught the attention of food science and nutrition investigators. It has been used to evaluate the effects of raw material, packaging, storage, food additives, processing conditions, and nutrient supplementation on food sensory quality. Thus, it has contributed to a more comprehensive and better understanding of food quality.

2.3.1 Methods used in sensory evaluation

Three methods used in sensory evaluation include: discriminative, descriptive and affective types. The discriminatory method asks questions about the perceived differences between products. The descriptive method focuses on measuring and identifying the main sensory characteristics of particular food products that contribute to special sensory stimuli. Affective studies are the most popular type and are used in both food business and food science studies. They provide clear information about product acceptance and preference. Additionally, they are the easiest and most cost-effective method for both business people and food scientists to use. Discriminatory tests represent15% of the types of sensory evaluation tests done annually by industry (Stone and Sidel, 2004). In contrast, descriptive methods represented 45% of tests and affective tests represented 40% of those done (Stone and Sidel, 2004). The discrimination method includes three main tests: paired-comparisons, duo-trio and triangle tests. It helps to tell the difference between products, and then proceeds further with descriptive sensory analysis to identify or describe the flavour profile of food. Descriptive analysis is the most sophisticated method that is used in sensory evaluation. It focuses particularly on identifying the volatiles and aroma compounds that contribute to the different taste and smell of food. Descriptive analysis also precisely describes the flavour and aroma profile of food by using highly-trained panellists to examine flavour, do free-choice profiling, and random diagnostic descriptive analysis. Product experts are often involved and spectrum analysis is also done. However, descriptive methods cannot predict product acceptance or customer satisfaction. With the affective method, use of a nine-point hedonic scale enables quantification of panelist responses to multiple characteristics of

the samples. It has been extensively used in food science and by industry to measure how well the food liked by consumers. However, few studies have compared the discriminative, descriptive and affective methods for their sensitivity and reliability. Therefore, in choosing a method for sensory evaluation it is most important to understand the purpose of the evaluation. Sensory evaluation of traditional meat products is complex. A traditional meat product generally refers to a product made from a recipe that has been used for hundreds of years. It is difficult to conduct a sensory analysis of traditional meat products because the production conditions are often poorly controlled, although they are monitored by artisanal craftsmen and adjusted as experience dictates, yet processing conditions influence product flavour formation, in addition to the raw materials and ingredients used during manufacture. Due to the increasing demand for safer, healthier and flavourful meat products, it is of value to characterize the sensory properties of traditional meat products. The products included in the present study are traditional but are less artisanal, produced according to controlled standardized procedures.

2.4 Quality Control and Improvement of Dry-fermented Sausage

2.4.1 Dry-fermented sausage groups

Due to the different meat processing technologies involved, two main groups of fermented meat products: the northern and southern European (Mediterranean) type sausages are recognized. The northern European type sausages are mainly formulated with beef, pork and pork fat, have a relatively short ripening time (about one month) and involve rapid acidulation to ≤ pH 5.3. Fermentation and drying steps are separated by smoking to develop the flavour of final products and prevent mould growth. Southern European type sausages contain mainly pork, have longer ripening times and have a

higher pH value (>pH5). Since fermentation is slower it involves a longer drying period during manufacture. Of this group, Hungarian sausage is the only Mediterranean sausage that involves a smoking step (Demeyer and Stahnke, 2002).

2.4.2 Quality control of dry-fermented sausage

Quality control during manufacture determines the final overall properties of products, as well the competitive success of products in the market. Various types of dry-fermented sausages have different moisture content, physical, chemical and microbiological properties. In addition, different casing size, drying periods, smoking methods, and storage conditions influence the character and quality of final products. These products require that high quality raw meat be used in their production to ensure that the fermentation will consistently achieve pH 5.3 within a defined time period, which is temperature dependent.

Different ingredients and packaging materials have been examined for their ability to maintain the quality of fermented meat products during storage. The sensory impact of the lipid fraction of dry-fermented sausages has a significant influence on the sensory quality of these products. Lipid oxidation can change the flavour, colour, taste and even structure of food products (Dransfield, 2008).Some studies indicated that fermented sausage with a higher level of fat was more appreciated (Papadima and Bloukas, 1999); however, concerns regarding safety and healthfulness were raised (Andres, Cava & Ruiz, 2002).Therefore, vacuum packaging and anti-oxidant compounds were used to prevent oxidative degradation from occurring in fermented sausage, which extended the sausage shelf-life. Some studies have indicated that undesirable quality changes in vacuum-packed sausage can occur during storage due to lipid oxidation

(Rubio, Martinez, Garcia-Cachan, Roira & Jaime 2008; Valencia, Ansorena & Astiasarán, 2006). During5-month storage of vacuum-packed sausage, lipid oxidation and hydrolysis, as well as changes in sensory properties were examined using high performance size exclusion chromatography (Summo, Caponio, Paradiso, Pasqualone & Gomes, 2009).It was found that rapid lipid oxidation occurred at the beginning of storage, and it became less rapid later during the final storage stage. A relationship between sensory response and lipid hydrolysis and oxidation was also suggested. Results indicated that sausages had a significantly lower score for overall acceptability and had an undesirable sour taste when stored for a short period (1 month), while sausage stored for the longer period (5 months) had a relatively better taste and characteristic flavour. Later, Summo, Caponio, Pasqualone, & Gome (2010) demonstrated the protective effects of vacuum packaging on dry-fermented sausage during storage, but indicated that a large amount of volatiles with low perception thresholds were produced by lipid autoxidation even in the absent of oxygen. In addition, volatiles formed due to the added spice ingredients rapidly decreased during storage. In other work, Ansorena and Astiasaran (2004) added olive oil with the antioxidants: butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA)to dry-fermented sausage and effectively controlled the lipid oxidation during storage without vacuum packaging. In addition, they concluded that when sausages were vacuum-packed as uncut whole pieces, they had the least volatiles formed by lipid oxidation in during 5 months of refrigerated storage. Rubio, Martinez, Garcia-Cachan, Roira & Jaime (2008) used two packaging methods: vacuum packaging and 20%CO_2 with 80%$N_2$for storage of dry-fermented sausage(salchichon), which was produced with added monounsaturated and polyunsaturated fatty acids, refrigerated and stored for 7 months. Sausage nutritional

value was significantly increased; however lipid oxidation during storage rapidly increased in both vacuum- and modified atmosphere-packed sausage. Additionally, vacuum and modified atmosphere packaging had limited effects on sausage colour and control of lipid oxidation during long-term storage. Smoking has been used in sausage processing from ancient times. It is a popular processing step in northern European type sausages, while it is rarely used in Mediterranean sausages. It prolongs the sausage shelf-life and creates a unique smoked flavour in the product. Martuscelli et al. (2009) applied different levels of smoking during dry cured ham production and found that intense smoking treatment (three day smoking) significantly reduced the amount of free amino acids, which were considered by others an important flavour precursors in these fermented meat products (Cordoba et al., 1994).In contrast, Martuscelli et al. (2009) concluded that the reduction of free amino acids had little effect on flavour development in dry cured ham.

2.5 Sensory Characteristics of Dry-fermented Sausage

2.5.1 Appearance/colour

Although appearance and colour are sometimes discussed separately in studies, both represent the first impression of food safety and quality that determine consumer purchase decisions. Dry-fermented sausage colour is related to the formation of nitrosomyoglobin. Nitrite/nitrate added to the sausage produce nitrogen oxide, which reacts with myoglobin and then forms nitrosylated myoglobin and produces the pinkish colour. Also, sausages made with lactic acid bacteria (LAB) are acidic and this accelerates the formation of nitrogen oxide. Other factors related to sausage

appearance/colour include the occurrence of unsaturated fat globules, which yield holes in meat slices, and discolouration caused by undesirable bacterial contamination.

2.5.2 Texture

The development of sausage texture occurs when salt soluble meat proteins coagulate in a network that entraps fat and forms a gel at ≤ pH 5.3 as a result of lactic acid formation by the LAB. In addition, drying helps to increase sausage hardness and final texture by reducing the water activity. Verplaetse, Van Hoye & Demeyer (1990) suggested that smaller average fat particle size (approximately 1.5mm) produced softer final sausage texture. In addition, meat batter temperature, fat temperature and the length of time used for salt addition during chopping significantly affected meat binding and structure (Van't Hooft, 1999).

2.5.3 Flavour and aroma

A relatively large number of sensory studies focused on identifying and describing the characteristic flavour compounds and aroma developed in sausages. Different starter cultures (LAB used in sausage manufacture and those naturally developed in sausage), fat characteristics and contents, fat replacement materials and characteristic volatile compounds, significantly influenced the sensory properties of dry-fermented sausage. However, more studies have focused on understanding the flavour changes and chemistry of cooked meat products. The flavour characteristics of cooked meat products are mostly derived from thermal processing, whereas, the volatiles in uncooked fermented meat products are produced by a series of complex reactions during fermentation and drying. The raw sausage batter has little or no aroma, but it contains a large amount of flavour precursors that produce the characteristic flavour and aroma of

sausage following microbial activity, chemical reactions and endogenous enzyme action during fermentation and drying. Microbial degradation of fatty acids, utilization of amino acids and carbohydrate fermentation were considered as the major reactions that contributed to the cured flavour in dry-fermented sausage. Smoking gave a special aroma to northern European type sausages due to the 2-furfurylmercaptan and guaiacol which were produced (Stahnke, 2000).

Carbohydrate fermentation

Carbohydrate fermentation caused by LAB produces lactic and acetic acid, which play an important role in the taste of dry-fermented sausage, especially northern European type sausages (Schmidt and Berger, 1998; Stahnke, Sunesen & De Smedt, 1999). The main volatiles derived from carbohydrate fermentation in fermented sausage were ethanol, acetone, butyric acid, acetaldehyde, acetoin, diacetyl and 2,3-butandiol, 2-propanol (Stahnke, Sunesen & De Smedt 1999).Also, sugar added during sausage manufacture is also fermented and produces ketones (mainly diacetyl and acetoin), which are volatile compounds with creamy aroma (Van Opstaele and Dirinck, 1999). In addition, staphylococci especially *Staphylococcus saprophyticus* and *Staphylococcus warneri* produce ketones as well (Berdague ́, Monteil, Montel & Talon, 1993).

Lipid degradation

Fatty acids produced through lipolysis are considered precursors of volatiles that contribute to characteristic flavour in sausage. Fatty acids form alkanes, alkenes, aldehydes, alcohols, ketones and acid during oxidation. Beside alkanes and alkenes, other volatiles have a significant effect on the sensory characteristics of sausage. Mediterranean

sausage contained a higher level of 6-10 carbon straight-chain aldehydes (Schmidt and Berger, 1998; Van Opstaele and Dirinck, 1999). These volatiles are described as rancid, metallic and grassy (Dainty and Blom, 1995). In southern European type sausages, higher amounts of ketones (2-pentanone to 2-nonanone) were identified and described as cheesy and fruity (Schmidt and Berger, 1998; Van Opstaele and Dirinck, 1999; Stahnke, Sunesen & De Smedt, 1999). Also, high levels of 6-8 carbon alcohols were found in southern European type sausages and described as grassy and fruity (Schmidt and Berger, 1998; Van Opstaele and Dirinck, 1999).Olivare, Navarro & Flores (2009) found that a higher amount of volatiles released after lipid oxidation came from apparently lean tissue during the processing of dry-fermented sausage. They suggested that flavour development during sausage processing was mainly due to the intramuscular fat and protein. Olivare, Navarro, Flores (2011) added 10, 20 and 30% (w/w) of fat to dry-fermented sausage and studied the aromatic changes during ripening. They characterized the key volatiles in sausage using gas chromatography and olfactometry. Higher lipolysis and lipid oxidation was observed in sausages with higher levels of fat. Also, a larger amount of volatiles derived from lipid were found in sausages with higher fat; whereas, volatiles released during bacterial metabolism were found in the sausage with lower fat content. Hexanal, 2,4-nonadienal, ethyl butanoate, 2-nonenal and 1-octen-3-ol were found in the sausages containing 20 and 30% fat and these contributed to mushroom, grassy, fruity flavour and aroma. In addition, high fat (30%) sausages with longer ripening were more appreciated by consumers.

Peptides and amino acids

Peptide and amino acids are not volatile compounds. However, a certain concentration of free amino acids and peptides in sausage contributed to a bittersweet, astringent and beefy taste (Dierickx, 1991). Amino acids and peptides were found to contribute to flavour development by Henriksen and Stahnke (1997). In contrast, addition of amino acids and peptides were found to have no effect on further enhancing flavour (Naes., Holck, Axelsson, Andersen & Blom, 1995; Diaz, Fernandez, Garcia de Fernando, de la Hoz & Ordonez 1997). Branched-chain amino acids (leucine, isoleucine and valine) can be broken down to alcohols, acids and aldehydes. Methyl aldehydes, methyl acids and methyl alcohols were responsible for the fruity and cheesy flavour in dry-fermented sausage (Stahnke, 1995; Montel, Reitz, Talon Berdague & Rousset 1996). Also, phenyl acetaldehyde and benzaldehyde found in dry-fermented sausage had a floral, nutty almond flavour and aroma (Montel, Masson & Talon, 1998). In addition, catabolites of branched-chain amino acids involved in the Strecker reaction were identified (Barbieri et al., 1992; Hinrichsen and Andersen, 1994).

Starter cultures used in fermentations affected the release of volatiles associated with the breakdown of amino acids (Montel, Reitz, Talon, Berdague & Rousset, 1996; Berdague ´, Monteil, Montel & Talon, 1993). Dry-fermented sausage with *Staphylococcus carnosus* added contained larger amounts of 3-methyl butanol, 3-methyl butanoic acid and 3-methyl butanal, which mainly contributed to the cured meat taste (Stahnke, 1995). However, sausage fermented with *Staphylococcus warneri* and *Staphylococcus saprophyticus* had a relatively low concentration of 3-methyl butanal (Stahnke, 1995). Hinrichsen and Andersen (1994) also confirmed that *S. carnosus* produced larger amounts of 3-methyl butanol, 3-methyl butanoic acid and 3-methyl butanal in the

laboratory. In contrast, several LAB (*Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus curvatus* and *Pediococcus acidilactici*) had a low aromatic potential and had little effect on the flavour of dry-fermented sausage (Larrouture, Ardaillon, Pepin & Montel, 2000).In related work, Herranz, de la Hoz, Hierro, Fernandez & Ordonez (2005) added free amino acids to dry-fermented sausage to improve its eating quality, and demonstrated that the a mixture of valine, leucine and isoleucine significantly enhanced sausage flavour and improved sausage quality.

Esters

Ethyl esters are the major esters that exist in sausage and they contribute to fruity flavour in these products. However, in German sausages, esters contributed to buttery and sour notes with less spicy and fruity flavour (Schmidt and Berger, 1998; Van Opstaele and Dirinck, 1999). In French, Spanish and Italian sausages, esters present caused sweet, fruity, mild buttery and pungent flavours (Schmidt and Berger, 1998; Van Opstaele and Dirinck, 1999).

Starter culture

Starter cultures play an important role in sausage manufacture. Starter cultures are characterized as LAB and flavouring microorganisms (Demeyer and Stahnke, 2002). Lactic acid bacteria contribute to the acidification, whereas flavouring microorganisms are usually associated with reducing the nitrate. Jessen (1995) indicated that *Pediococcus* and *Lactobacillus* are responsible for the acidification that occurs in fermented sausage, and *Staphylococcus*, *Kocuria*, *Debaryomyces* and *Penicillium* are flavouring microorganisms. Lactic acid bacteria produce lactic acid, and thus contribute to the acidic

taste of sausage. They can also produce acetic acid, ethanol, formate and acetoin (Jessen, 1995). The application of different starter cultures can affect the sensory characteristics of the dry-fermented sausage. Bacteria that have the flavouring capability are members of the family *Micrococcaceae* (Montel, Reitz, Talon, Berdague & Rousset, 1996; Stahnke et al., 2002). Also, different strains formed different volatiles in sausages during production (Masson, Hinrichsen, Talon & Montel, 1999; Stahnke, 1995; Sondergaard and Stahnke, 2002). *Staphylococcus xylosus*, *Staphylococcus carnosus* and *Kocuria varians* are commonly used as starter cultures (Jessen, 1995). The nitrate reduction caused by *Micrococcaceae* positively affected colour development in sausages (Talon, Walter &Montel, 2000). In addition, *Micrococcaceae* used as starter cultures might prevent lipid oxidation through catalase production and its destruction of hydrogen peroxide (Talon, Walter & Montel, 2000). There are limited studies focused on the sensory effect of yeast on the dry-fermented sausage. *Debaryomyces hansenii* mainly found in naturally fermented sausage was identified as a flavour contributor (Encinas, Lopez-Diaz, Garcia-Lopez, Otero & Moreno, 2000). However, some studies suggested that *D. hansenii* did not produce a large amount of aromatic compounds when compared to *Candida utilis* (Olesen and Stahnke, 2000).The effect of fungi on the sensory development of dry-fermented sausage is still unclear. Some southern European type sausages are fermented with surface mould. Sunesen and Stahnke (2003) found that *Penicillium nalgiovense*, *Penicillium chrysogenum* and *Penicillium camemberti* contributed to the different flavour and taste of sausage, but they did not discover how the mould affected flavour development.

Soybean proteins and mustard proteins

Soybean proteins have also been recognized as an excellent protein source, water binding agent, gel stabilizer and fat emulsifier in cooked processed meat products (Kinsella, 1976). Due to the higher price of muscle proteins, soybean proteins are added to improve texture and decrease the cost of processed meat products(Campo del, Gallego, Berregi & Casado, 1998; Abdel-Aziz, Esmail, Hussein & Janssen, 1997; Barai, Nayak, Singhal & Kulkarni,1992).There are various forms of soybean protein products which have different levels of protein, and thus have different water binding capacity (from 5 times to 10 times their weight in water)(Soy Protein Council,1987; Garcia, Torre, Marina & Laborda, 1997; Lusas & Riaz, 1995). In addition, soybean proteins provide essential amino acids that are easily absorbed by humans (Barai, Nayak, Singhal & Kulkarni, 1992; Soy Protein Council, 1987; Garcia, Torre, Marina & Laborda, 1997). However, soybean proteins can contribute to an undesirable bean flavour and taste in some meat products (Ho, Wilson & Sebranek,1997).In contrast, a soybean protein isolate moderately improved the colour, texture and taste of buffalo meat emulsion sausage (Ahmad, Rizawi & Srivastava, 2009). Soybean proteins are allowed to be added to processed meat products at levels between 0.5% and 3.5% in the U.S (Soy Protein Council, 1987); also, soy protein isolates can be found in fermented meat products for texture improvement. In addition, using soybean proteins in processed meat products requires an adequate amount of water to achieve optimal hydration. When soybean proteins are hydrated insufficiently, it may cause loss of meat texture, emulsion instability and low gel strength in the final products. Soybean protein is able to gel like muscle proteins but must be cooked to gel. When comparing soybean proteins to deheated mustard, (which are both recognized as an excellent vegetable protein sources) it appears that mustard is a less attractive ingredient

because of anti-nutritional compounds (glucosinolates, phenolics, and phytates) in mustard seed (Dijkstra, Loinnemann & van Boekel, 2003; Naczk, Wanasundara & Shahidi, 1992).Diosady, Xu & Chen (2005) successfully isolated the high quality yellow mustard protein by using a membrane-based process. In addition, Marnoch and Diosady (2006) effectively isolated and recovered 81% of useful proteins from brown mustard seed and used the material as a binder in bologna and wieners, and compared sensory differences with soy proteins. They concluded that the mustard proteins had no effect on texture and flavour properties; however, the meat colour was darker than in products made with soy protein.

Chapter 3

The Antimicrobial Influence of Stabilized para-hydroxybenzyl Isothiocyanate on *Escherichia coli* O157:H7 in Beaker Sausages

3.1 Abstract

Para-hydroxybenzyl isothiocyanate, p-HBIT mainly derived from the yellow mustard glucosinolate, sinalbin, by myrosinase action is a natural antimicrobial agent. Although it is unstable in the presence of moisture, it is potently lethal toward *Escherichia coli* O157:H7. A beaker sausage experiment was used to simulate dry sausage fermentation. Meat batter was inoculated with *E. coli*O157:H7 and p-HBIT stabilized in maltodextrin was added at 103 and 206mg/kg (equivalent to the addition of 2% and 4% mustard powder). It was found that *E. coli* O157:H7 was reduced >4 log CFU/g at day 6 in p-HBIT-treated beaker sausages. *Pediococcus pentosaceus* and *Staphylococcus carnosus* were more resistant to p-HBIT, but were reduced nearly 3 log CFU/g at day 6 in both treatments. The higher level of p-HBIT was more bactericidal against *E. coli* O157:H7. The antimicrobial effect of p-HBIT on *E. coli* O157:H7 was confirmed and the method used to stabilize p-HBIT was also practical for use in sausage batters. However, further study is required for a better understanding of the other effects of p-HBIT in sausages.

3.2 Introduction

Mustard has been used in the food industry in different forms as a condiment, flavour enhancer, protein source, emulsifier and water binder. It also has been studied for years for its anticarcinogenic properties in medical research. Recently, mustard has become popular in food science research due to its potential antimicrobial action. There are two major botanical groups of mustard *Brassica juncea* (brown/oriental mustard) and *Sinapis alba* (yellow/white mustard), most commonly used in food. These both showed excellent bactericidal activity against food-borne pathogens. The antimicrobial effects of mustard are due to a series of chemical reactions. Sinigrin and sinalbin are the two main glucosinolates in brown and yellow mustard, respectively, and are not antimicrobial unless hydrolyzed. Myrosinase, an endogenous enzyme in mustard, in the presence of moisture at neutral pH will catalyze the hydrolysis of sinigrin and sinalbin to form isothiocyanates, which have bactericidal effects on many bacteria. Allyl isothiocyanate (AIT) and *para*-hydroxybenzyl isothiocyanate (p-HBIT) are the main hydrolysis products of sinigrin and sinalbin, respectively, and are the natural antimicrobial compounds that also contribute to the unique pungency of mustard. Isshiki, Tokuoka, Mori & Chiba (1992) demonstrated the bactericidal activity of AIT against *Bacillus cereus*, *Bacillus subtilis*, *Salmonella* Enteritidis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Vibrio parahaemolyticus*. Also, Delaquis and Sholberg (1997) examined the antimicrobial effects of AIT on *Pseudomonas corrugata*. In addition, Lin, Jeongmok, Du & Wei (2000) found that AIT effectively inhibited the growth of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The bactericidal effect of AIT on *E. coli*O157:H7 was further confirmed by Park, Taormina & Beuchat (2000) and

Nadarajah, Han &Holley (2005). Ekanayake et al. (2006) demonstrated the antimicrobial capacity of p-HBIT against *Campylobacter jejuni, Shigella boydii, Clostridium perfringens, E. coli, S. aureus, P. aeruginosa, S.* Enteritidis, and *L. monocytogenes*. Moreover, Kyung and Fleming (1996) evaluated the minimum inhibitory concentration (ppm) of sinigrin hydrolysis products on different bacterial pathogens and lactic acid bacteria (LAB). AIT showed the strongest antimicrobial action against bacterial pathogens, whereas LAB were more resistant (Kyung and Fleming, 1996). Moreover, Luciano and Holley (2010) confirmed that *E. coli* O157:H7 was less resistant to p-HBIT than *Pediococcus pentosaceus* and *Staphylococcus carnosus*, and that it produced a myrosinase-like enzyme that hydrolyzed sinalbin to form p-HBIT, and killed itself.

Although AIT and p-HBIT have advantageous antimicrobial properties, their instability and unappreciated sharp taste limit their use in food manufacture. AIT is very unstable in aqueous media at high temperature and high pH, and will degrade in a short period (Ohta, Takatani & Kawakishi 1995). Some methods have been developed to stabilize AIT, and permit its use in food materials. Chacon, Buffo & Holley (2006) encapsulated AIT and used it in refrigerated, nitrogen packed, finely chopped beef, which was inoculated with *E. coli* O157:H7. Liu and Yang (2010) used medium chain triglyceride/ soybean oil in an oil-in-water emulsion to stabilize AIT and evaluated its bactericidal effects on *E. coli* O157:H7, *S. enterica, S. aureus, L. monocytogenes* and *V. parahaemolyticus*. p-HBIT is highly sensitive and unstable in the presence of moisture. It has a highly electrophilic center, and therefore it is easily hydrolyzed to more stable products (4-hydroxybenzyl alcohol, cyanide ion, and 4-hydroxybenzyl cyanide), which have no antimicrobial activity. Ekanayake et al. (2006) used maltodextrin (nonsweet

polysaccharide) and glycerin as a matrix to stabilize p-HBIT in an oil form, which was extracted from yellow mustard. The approach was further evaluated by Choubdar, Li & Holley (2010).

Another limitation of the application of AIT and p-HBIT in the food industry is that mustard seed only contains 0.8%~2.3% of AIT or p-HBIT. In addition, during extraction from mustard essential oil losses will occur, further reducing yield. The instability of p-HBIT limits its commercial availability. In their purified forms, AIT and p-HBIT are not generally used in food. However, in one recent study, white/ yellow mustard essential oil was produced commercially during a solvent-based process, and its p-HBIT content was further purified and confirmed using simple reverse phase HPLC (Ekanayake, Zoutendam, Strife, Fu & Jayatilake, 2012). Further, the authors suggested that the white mustard essential oil might be used as a novel preservative in food.

Due to a number of recent food-borne illness outbreaks caused by *E. coli* O157:H7 in fermented meat products (Tilden et al., 1996; Williams et al., 2000; MacDonald et al., 2004), the risk associated with consumption of dry-fermented sausage has been highlighted. This has stimulated work to develop a natural antimicrobial solution for the problem. The use of deheated (cold) and non-deheated (hot) yellow mustard in dry-fermented uncooked meat products showed that yellow mustard powder was potently bactericidal against *E. coli* O157:H7. Microencapsulated AIT was used in dry-fermented sausage and achieved significant reductions in *E. coli* O157:H7 viability (Graumann and Holley, 2008). Non-deheated yellow mustard powder added to dry-fermented sausage at 4 and 6% (w/w) achieved a 5 log CFU/g reduction of *E. coli* O157:H7 after 36 and 24 days, respectively. Salami with 6% deheated (cold) yellow

mustard yielded a>5log CFU/g reduction of *E. coli* O157:H7 after 18 days (Graumann and Holley, 2008). It was also demonstrated by Luciano, Belland & Holley. (2011) that 6% autoclaved (cold, deactivated myrosinase) yellow mustard successfully reduced *E. coli* O157:H7 by 5log CFU/g in salami. However, 2% (w/w) yellow mustard powder was not sufficient to eliminate 5 log CFU/g *E.coli* O157:H7 within 30 days (Graumann and Holley, 2008).

However, there has been no work on the use of stabilized p-HBIT in dry-fermented sausage to evaluate its bactericidal activity against *E. coli* O157:H7. Therefore, the aim of the present study was to use stabilized p-HBIT in a beaker sausage model system to evaluate the bactericidal properties of p-HBIT against *E. coli* O157:H7.This would also provide an opportunity to obtain more information about the potential value of p-HBIT stabilization.

3.3 Material and Methods

3.3.1 Preparation of starter culture and *Escherichia coli* O157:H7

Five strains of *E. coli* O157:H7 (02:0628, 02:0627, 00:0351, 02:0304 and 02:1840 (non-pathogenic, human isolates)) were provided by Rafiq Ahmed, National Microbiology Laboratory, Public Health Agency, Canadian Centre for Human and Animal Health, Winnipeg, MB, Canada. Five strain *E. coli* O157:H7 mixtures were prepared for the inoculation of the dry-fermented sausage batter. *Staphylococcus carnosus* UM123M and *Pediococcus pentosaceus* UM121P were isolated from a commercial starter culture mixture, Lactacel 115 (Microlife Technics, Sarasota, FL, USA). *S. carnosus* and *E. coli* O157:H7 were revitalized from frozen stock cultures and

incubated in Tryptic Soy Broth, TSB (Oxoid, Unipath, Nepean, ON, Canada), whereas *P. pentosaceus* was recovered in deMan Rogosa Sharpe broth, MRS (Oxoid). Cultures were transferred twice in broth incubated at 35 ℃ for 24 h. Then overnight cultures were transferred to fresh broth and incubated at 35 ℃ for 18 h. Cultures were centrifuged (Sorvall Instruments RC-5C; DuPont, Newton, CT, USA) at 2,991 ×g and 4 ℃ for 10 min and then washed with 0.1% (w/ v) peptone water and centrifuged again. The supernatant was discarded once again and the cultures were resuspended using 10mL 0.1% peptone water. Starter cultures were mixed and a total of 10 mL inoculum was prepared. Five strains of *E. coli* O157:H7 were combined in equal volumes and5mL of *E. coli* O157:H7 mixtures were used as inocula for each treatment.

3.3.2 Chemicals

Methanol (HPLC grade), ethyl acetate (HPLC grade), anhydrous sodium sulphate and maltodextrin (dextrose equivalent 4 to 7) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ground yellow mustard seed powder (non-deheated) was supplied by Newly Weds Foods Co., Edmonton, AB, Canada.

3.3.3 Preparation of stabilized p-HBIT

The method of p-HBIT stabilization used was developed during previous studies (Ekanayake et al., 2006; Choubdar, Li & Holley, 2010). Thirty grams of non-deheated yellow mustard powder was weighed, dissolved in 450mL distilled water and stirred for 15 min. A total of 300mL ethyl acetate (reagent grade 99.5%) was used to extract the crude oil from yellow mustard. Since the mixture formed an emulsion, it was centrifuged at 169 ×g for 2 min (Sorvall Instruments RC-5C). The organic phase was separated and dried over anhydrous sodium sulphate. The solvent was removed by rotary evaporation at

35~40 ℃ for 10 to 15 min. The crude oil obtained from the extracted yellow mustard powder was blended with maltodextrin in an approximate ratio of 1:10.

3.3.4 Sausage ingredients

Lean pork trim, pork back fat and lean beef trim were purchased from a local retail butcher (Miller's Meat, Winnipeg, MB, Canada). The sausage ingredients included: pork back fat (18.3%), lean pork trim (63.4%) and beef (18.3%), salt (2.91% w/w; HyGrade, Sifto Canada Corp., Mississauga, ON, Canada), D-glucose (0.06% w/w; Sigma Chemical Co.), Cervelat spice mixture codeC719 (0.44% w/w; Wiberg Corp., Oakville, ON, Canada), and pickle cure concentrate (0.31% w/w; Canada Compound Corp., Winnipeg, MB, Canada) with 6.25% (w/w) sodium nitrite/nitrate and sodium erythorbate (0.05% w/w; Canada Compound Corp).

3.3.5 Sausage model system preparation

The sausage model system was comprised of 3 treatments: control (without stabilized p-HBIT), the equivalent of p-HBIT extractable from 2% (w/w) mustard powder and the equivalent of p-HBIT extractable from 4%(w/w) mustard powder and stabilized in the oil form in maltodextrin. A total of 525 g of sausage batter were prepared for each treatment. Pork back fat, lean pork trim and beef were added and chopped in decreasing order of fatness in a pre-chilled (1 to 2 ℃) rotating bowl chopper (Titane 40, Dadaux, Bersaillin, France). *P. pentosaceus* and *S. carnosus* starter cultures and *E. coli* O157:H7 mixtures were separately added to yield approximately 7 log CFU/g, and chopped for 30 sec. The rest of the cure ingredients were then added and finely chopped for another 1 min. Stabilized p-HBIT was added near the end of the chopping. The sausage batter was aseptically weighed (25 g each) and added to 50mL sterilized beakers, covered with

autoclaved aluminum foil and incubated at 26℃ for 5 days. The incubation temperature was changed to 16℃ at the sixth day. No drying or smoking was performed.

3.3.6 The concentration of p-HBIT

In previous studies, 2% and 4% of yellow mustard in 7.5kg sausage batter have shown a 5 log CFU/g reduction of *E. coli* O157:H7 at 42 and 28 days (Graumann and Holley, 2008). Mustard seeds contain approximately 2.3% to 2.5% p-HBIT, and thus 2% (150g) and 4% (300g) yellow mustard powder in 7.5kg sausage batter would ideally produce 3.45g ~3.75g and 6.9g~7.5g of p-HBIT respectively. Moreover, the minimum bactericidal concentration (MIC) of p-HBIT *in vitro* was shown to be a 0.59mM and 4% deheated yellow mustard reduced the *E. coli* O157:H7 population >6 log CFU/g (Luciano and Holley, 2011). As well, a concentration of 60-120mg/L p-HBIT resulted in a 2-4 log reduction in microbial count, and 360mg/L p-HBIT resulted in a >6 log reduction in microbial count (Ekanayake et al. 2006). Therefore, two sausage treatments at different concentrations of p-HBIT; 150mg, equivalent to 2% mustard powder, and 300mg, equivalent to 4% mustard powder were conducted with p-HBIT stabilized in maltodextrin. The quantification of p-HBIT was performed by supercritical fluid chromatography analysis.

3.3.7 Supercritical fluid chromatography (SFC) analysis

The crude oil equivalent of 2% and 4% mustard powder was purified by flash column chromatography using hexane and ethyl acetate as the solvent in the ratio of 8:1. p-HBIT was then quantified using supercritical fluid chromatography (SFC). SFC analysis was done using a Thar Technologies Inc. model I6-1, Super Pure discovery series unit (Pittsburgh, PA, U.S). Thar Si column (250 × 4.6mm I.D. 5µ particles) was

used to identify and quantify p-HBIT stabilized in maltodextrin. A 2 mL/min flow rate and 200 bar column back pressure was required for the column. Carbon dioxide with 4% methanol was used as the mobile phase for the first 5 min isocratic run. The mobile phase was changed to 15% methanol with carbon dioxide linearly for a second 5 min isocratic run, and was continued for another 4 min isocratic run. The mobile phase was returned to 4% methanol with carbon dioxide for a 9 min isocratic run and equilibrated for 3 min. The column temperature was 40℃ and p-HBIT was identified at 227nm following injection of a 5 µL sample.

3.3.8 Microbiological analysis

Inoculated meat batter was tested at day 0 for microbial and physico-chemical properties. Beaker sausages were sampled and tested daily for 6 days. A 10g sample was aseptically removed from the centre of the beaker sausage, placed into a stomacher bag, and 90mL 0.1% peptone water was added. Samples were homogenized by stomaching for 1 min. Aliquots were serially diluted from 10^{-1} to 10^{-4}. Samples were plated with an Autoplate 4000 Spiral Plater (Spiral Biotech, Bethesda, MD, USA). *P. pentosaceus* was recovered on MRS agar (Oxoid) and *S. carnosus* was recovered on mannitol salt agar, MSA (Oxoid). *E. coli* O157:H7 was cultured on sorbitol MacConkey agar containing cefixime and tellurite supplement (Difco, Sparks, MD, USA, with cefixime tellurite supplement, CTSMAC, Oxoid Ltd). All plates were incubated at 35℃ for 24h.

3.3.9 Statistical analyses

One-way analysis of variance (ANOVA) was performed using SAS (windows 9.0) software (Statistical Analysis System). Treatments were compared using Tukey's test to

find statistical differences ($p<0.05$). All data reported are the average from a minimum of three experiments conducted in duplicate.

3.4 Results and Discussion

3.4.1Quantity of stabilized p-HBIT in maltodextrin

p-HBIT that was freshly produced and stabilized in the oil form in maltodextrin was quantified by SFC analysis. The standard curve was developed in a previous study (Choubdar, Li & Holley 2010; Fig 3.1). Although ideally 150mg (equivalent to 2% (w/w) mustard) and 300mg (equivalent to 4% (w/w) mustard) of p-HBIT needed to be produced and stabilized, 103mg (from 2% mustard) and 206mg (from 4% mustard) p-HBIT was recovered from 10g and 20g maltodextrin, respectively (Table 3.1). Some other methods have been described and used to stabilize p-HBIT; however, p-HBIT stabilization was still not optimal. Maltodextrin and glycerin are considered chemical stabilizers, and commonly can be used as food additives. The effectiveness of maltodextrin and glycerin in stabilizing p-HBIT were compared and maltodextrin has been shown to yield more significantly stable p-HBIT (data not shown). In addition, maltodextrin is a fine power that mixed easily with the meat batter; whereas glycerin is a viscous liquid that was difficult to mix with dry ingredients in meat.

3.4.2 The antimicrobial effect of stabilized p-HBIT on *P. pentosaceus*

P. pentosaceus numbers were reduced >1log CFU/g in 24 h in both p-HBIT-treated beaker sausages (Table 3.2). A nearly 3 log CFU/g reduction of *P. pentosaceus* was occurred at day 6 in both p-HBIT treatments. *P. pentosaceus* has previously been

reported to be relatively resistant to p-HBIT (Luciano and Holley, 2010), and so its sensitivity here may have been related to the greater length of incubation at 26 ℃.

3.4.3 The antimicrobial effect of stabilized p-HBIT on *S. carnosus*

p-HBIT significantly reduced *S. carnosus* numbers>1log CFU/g in 24 h and reduced *S. carnosus*>2 log CFU/g in 48 h in treated beaker sausages (Table 3.3). *S. carnosus* was virtually stable throughout the experiment from day 3 to day 6, ranging from 4.3 to 4.1 log CFU/g in both p-HBIT treatments. *S. carnosus* numbers were reduced nearly 3 log CFU/g at the end of experiment in p-HBIT treated beaker sausages. The lethality observed here to p-HBIT was greater than expected, and as with *P. pentosaceus* may have been related to the extended incubation at 26 ℃.

3.4.4 The bactericidal effect of stabilized p-HBIT on *E. coli* O157:H7

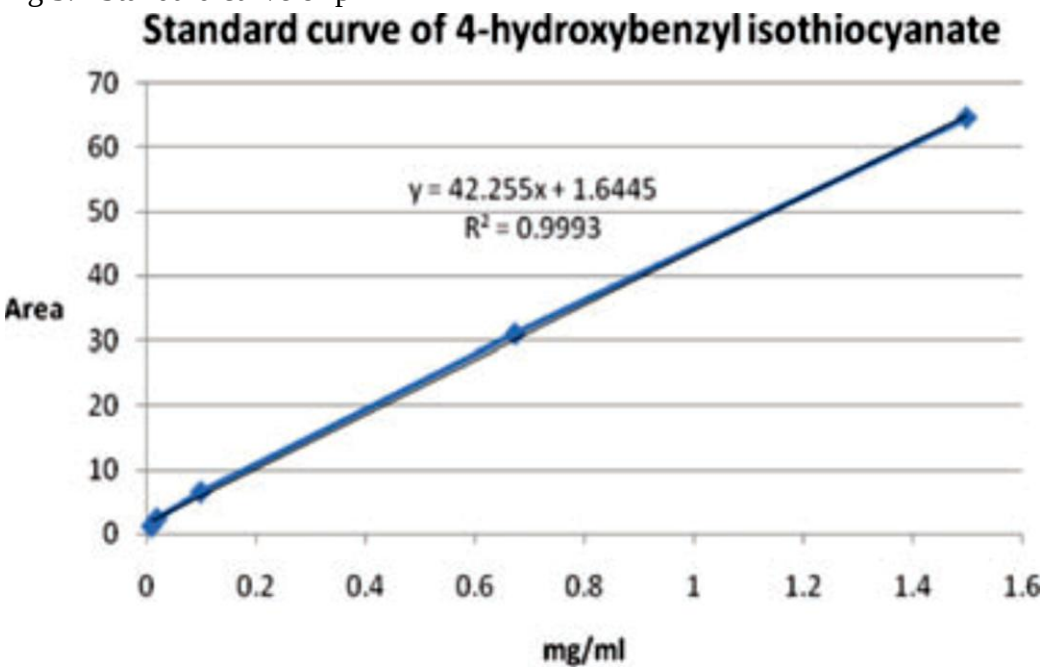
The bactericidal effect of stabilized p-HBIT on *E. coli* O157:H7 is shown in Table 3.4. p-HBIT reduced *E. coli* O157:H7 numbers >2 log CFU/g in 24 h and by almost 3 log CFU/g after 72 h in both p-HBIT beaker sausage treatments. In addition, the higher level of p-HBIT showed a significantly greater reduction of *E. coli* O157:H7 at day 5. A >4 log CFU/g reduction of *E. coli* O157:H7 was achieved in both p-HBIT treatments at the end of the tests at 16 ℃. p-HBIT at the higher level was more effective in reducing *E. coli* O157:H7 viability. Moreover, similar results were observed in dry-fermented sausage containing 4% yellow mustard. At this level mustard eliminated *E. coli* O157:H7 (>5 log CFU/g reduction) in 30 days; whereas 2% yellow mustard reduced *E. coli* O157:H7 numbers by>5 log CFU/g at 42 days (Graumann and Holley, 2008). *E. coli* O157:H7 had the ability to degrade sinalbin to produce p-HBIT in the absence of plant myrosinase, which had been thermally deactivated (Luciano and Holley, 2010). *E. coli* O157:H7 was

the least resistant toward p-HBIT when compared to *S .carnosus* and *P. pentosaceus* (Luciano and Holley, 2010). Therefore, it was not surprising that the higher level of p-HBIT was more effective against *E. coli* O157:H7. Results obtained here are consistent with those of Ekanayake et al. (2006) which were done in laboratory media.

3.5 Conclusion

Results of the simulated dry sausage fermentation contaminated with *E. coli* O157:H7 and treated with stabilized p-HBIT confirmed previous reports of the antimicrobial effects of yellow mustard against *E. coli* O157:H7 in dry-fermented sausage, and established that p-HBIT was likely responsible for the antimicrobial effects of yellow mustard. Results from the present study also confirmed that the stabilization of p-HBIT in maltodextrin was practical and applicable in a food application to effectively control *E. coli* O157:H7 viability. One would normally expect fermentation to reduce *E.coli*O157:H7 viability by <1 log CUF/g. However, further quantification of p-HBIT residues and p-HBIT hydrolysis products would be necessary to better understand p-HBIT action against *E. coli* O157:H7 and the profile of p-HBIT degradation during fermentation. Since p-HBIT may be produced in small amounts from yellow mustard, and is extremely unstable, it may be more practical to use mustard powder instead of applying p-HBIT directly. It is evident that yellow mustard (both hot and cold), a natural antimicrobial agent containing p-HBIT, is likely to have value in dry-fermented sausage manufacture.

Fig 3.1 Standard curve of p-HBIT



Adapted from "Supercritical fluid chromatography of myrosinase reaction products in ground yellow mustard seed oil", by Choubdar, N., Li, S. and Holley, R.A. (2010). J. Food Sci., 75(4), C341-C345.

Table 3.1The concentration of p-HBIT in oil form in maltodextrin

	p-HBIT in 2% mustard	p-HBIT in 4% mustard
Crude oil weight (g)	1.41	2.23
Maltodextrin weight (g)	12.19	23.02
Expected amount of p-HBIT (mg)	150	300
Actual amount of p-HBIT(mg)	103	206

Table 3.2 *Pediococcus pentosaceus* viability during fermentation of beaker sausage inoculated with *E. coli* O157:H7 at 26 ℃ for the first five days and at 16 ℃ for day 6for treatment groups containing p-HBIT extracted from the equivalent of 2% and 4% non-deheated yellow mustard.

Pediococcus pentosaceus (log CFU/g)[a]			
Day	Control (without p-HBIT)	103 mg p-HBIT (derived from 2% non-deheated mustard)	206mg p-HBIT (derived from 4% non-deheated mustard)
0	7.39 ±0.07a	7.21 ±0.04b	6.96 ±0.04c
1	7.01 ±0.18a	5.76 ±0.14b	5.55 ±0.06b
2	7.24 ±0.23a	5.25 ±0.17b	4.80 ±0.16c
3	7.21 ±0.05a	4.29 ±0.12b	4.17 ±0.12b
4	6.81 ±0.13a	4.68 ±0.07b	4.28 ±0.10c
5	6.79 ±0.18a	4.69 ±0.15b	4.51 ±0.71b
6	6.87 ±0.42a	4.34 ±0.23b	4.10 ±0.20b

[a] Value are the mean ±standard deviation of three trials replicated two times. Within a row, means with different letter are significantly different ($p<0.05$).

Table 3.3*Staphylococcus carnosus* viability during fermentation of beaker sausage inoculated with *E. coli* O157:H7 at 26 ℃ for the first five days and at 16 ℃ for day 6 for treatment groups containing p-HBIT extracted from the equivalent of 2% and 4% non-deheated yellow mustard.

	Staphylococcus carnosus (log CFU/g)[a]		
Day	Control (without p-HBIT)	103 mg p-HBIT (derived from 2% non-deheated mustard)	206mg p-HBIT (derived from 4% non-deheated mustard)
0	7.14 ±0.05a	6.88 ±0.03b	6.81 ±0.08b
1	6.97 ±0.04a	5.32 ±0.20b	5.32 ±0.09b
2	6.83 ±0.10a	4.48 ±0.05b	4.28 ±0.09c
3	6.91 ±0.11a	4.39 ±0.05b	4.26 ±0.10b
4	6.74 ±0.27a	4.25 ±0.17b	4.21 ±0.29b
5	6.70 ±0.06a	4.20 ±0.06b	4.17 ±0.09b
6	6.52 ±0.17a	4.19 ±0.14b	4.12 ±0.11b

[a] Value are the mean ± standard deviation of three trials replicated two times. Within a row, means with different letter are significantly different ($p<0.05$).

Table 3.4Viability of inoculated *Escherichia coli* O157:H7 during beaker sausage fermentation incubated at 26 ℃ for the first five days and at 16 ℃ for day 6 containing p-HBIT extracted from the equivalent of 2% and 4% non-deheated yellow mustard.

	Escherichia coli O157:H7 (log CFU/g)[a]		
Day	Control (without p-HBIT)	103mg p-HBIT (derived from 2% non-deheated mustard)	206mg p-HBIT (derived from 4% non-deheated mustard)
0	7.14 ±0.02a	6.95 ±0.05b	6.79 ±0.03c
1	7.46 ±0.23a	4.95 ±0.11b	4.91 ±0.15b
2	7.03 ±0.08a	4.30 ±0.12b	4.32 ±0.13b
3	6.76 ±0.15a	3.84 ±0.21b	3.82 ±0.06b
4	6.99 ±0.33a	3.52 ±0.26b	2.72 ±0.18c
5	6.85 ±0.08a	3.14 ±0.31b	2.40 ±0.29c
6	6.75 ±0.24a	2.88 ±0.30b	2.05 ±0.25c

[a] Value are the mean ± standard deviation of three trials replicated two times. Within a row, means with different letter are significantly different ($p<0.05$).

Chapter 4

Sensory Evaluation of Dry-fermented Sausage Containing Ground Deheated Yellow Mustard

4.1 Abstract

Ground deheated yellow mustard is used as a binder and meat protein substitute in cooked processed meat products. It has a huge potential to be used in uncooked processed meat products because of its natural antimicrobial properties. In this study, ground deheated yellow mustard was added to dry-fermented sausage during manufacture at 1%, 2%, 3% and 4% (w/w) and analyzed for its effect on sausage appearance, colour, flavour, texture and overall acceptability by conducting 9-point hedonic sensory tests. The 3% and 4% mustard-treated sausages had significantly lower consumer scores on all sensory attributes as well as overall acceptability. The 4% mustard sausage had the lowest consumer scores and was significantly different from the other treatments. The results suggested that a large amount of ground deheated yellow mustard negatively affected sausage sensory properties. However, the appearance and colour of 3% and 4% mustard-treated sausages was liked slightly, whereas flavour, texture and overall acceptability were less acceptable. The untreated control (without mustard) and 1% mustard-treated sausages had similar sensory properties and were the most appreciated. The 2% mustard-treated sausages were given “like moderately” and “like slightly” descriptors.

4.2 Introduction

Dry-fermented sausages are “high-end” classic ready-to-eat meat products that are manufactured with curing, fermentation, smoking, but are not cooked. Provided required pH reductions and the minimum water activity value are achieved according to regulatory requirements, they are shelf-stable at room temperature and have a refrigerated shelf-life of over 6 months. It is unusual to find reports of food-borne illness outbreaks being caused by the consumption of dry-fermented sausages. Most of the dry-fermented sausages have relatively low pH and water activity, and been considered as safe products. However, in 1994, a food-borne illness outbreak associated with *Escherichia coli* O157:H7 was caused by the consumption of dry salami, which resulted in 17 people being sickened (Tilden et al., 1996).Another outbreak happened in 1998and involved 39 cases of *E. coli* O157:H7 illness caused by the consumption of Genoa salami, and in 1999 143 cases of food-borne illness caused by *E. coli* O157:H7 were associated with the consumption of dry Hungarian and Cervelat salami (Williams et al., 2000; MacDonald et al., 2004). As a result, food inspection agencies in Canada and the U.S (CFIA, 1999; Reed, 1995) required that during production of dry-fermented sausages a ≥5log CFU/g reduction of viable *E. coli* O157:H7 must occur. However, current manufacturing methods can only achieve a 2 log reduction, and so product safety is reliant upon the absence of the pathogen from raw materials. Currently raw material and end product testing are required for all products not cooked.

Two main types of mustard (*Brassica juncea* and *Sinapis alba*) are used in North America in food. Both *Brassica juncea* (brown or oriental mustard) and *Sinapis alba* (yellow or white mustard) can produce an intense pungent aroma and hot flavour through

a series of hydrolysis reactions (glucosinolate degradation) in the presence of myrosinase and moisture. The volatiles produced from brown and yellow/ white mustard are known as allyl isothiocyanate (AIT) and *para*-hydroxybenzyl isothiocyanate (p-HBIT), respectively. The pure compounds have intense pungent and hot biting flavour, which can cause some people irritation, sometimes resulting in burning and swollen tissue around the mouth area. As a result, these compounds are undesirable and of limited use in ready-to-eat products and products that are uncooked. However in the processed meat industry, mustard flour is used for its high protein content. It can be used in cooked sausage or if treated with heat to destroy myrosinase, it can be added to fresh sausage. Otherwise, myrosinase might react with the meat protein or glucosinolates and produce off flavours (Tainter and Grenis, 1993). Moreover, mustard with mild flavour and texture are more popular in the North American market; whereas the European market prefers stronger flavoured mustard. In addition, the thermally-treated yellow mustard powder (known as cold or deodorized flour) is an excellent emulsifier, binder, stabilizer and thickener without an unpleasant pungent or hot flavour. Further, it is an economic replacement for part of the meat protein in cooked cured meat products. AIT and p-HBIT in mustard also have been studied for their unique antimicrobial properties in foods. Graumann and Holley (2008)added 2,4, and 6% hot mustard powder and 6% cold mustard powder to dry-fermented sausage and evaluated the bactericidal activity of the treatment against the survival of *E. coli* O157:H7. The results showed that the 6% cold and hot mustard powder rapidly reduced *E. coli* O157:H7 numbers (≥5 log CFU/g) in 6 and 24 days, respectively. Also, 4% hot mustard effectively reduced numbers of viable *E. coli* O157:H7 (≥5 log CFU/g) in about 30 days. Luciano, Belland & Holley. (2011) showed

that 6% hot, cold and autoclaved yellow mustard powder eliminated *E. coli* O157:H7 in 31, 38 and 18 days, respectively. Moreover, Luciano and Holley (2010) found that *E. coli* O157:H7 showed myrosinase-like activity in the sausages with mustard that hydrolyzed sinalbin (the major glycosidic compound found in yellow/ white mustard) in the presence of moisture to produce p-HBIT, and thus the organism killed itself.

Although mustard powder (both cold and hot) has such unique antimicrobial ability, it also has the potential to affect product flavour or texture. Therefore, sensory evaluation of mustard-containing sausages was investigated. It is notable that there are few sensory studies where the eating quality of dry-fermented sausage treated with mustard powder were examined. Most of the recent fermented sausage sensory studies focused on fat replacement (with olive oil or inulin)(Mendoza, Garcia, Casas & Selgas, 2001; Bloukas, Paneras & Fournitzis, 1997).In addition, a large number of sensory studies focused on instrumental analysis of the contribution of volatile compounds to flavour and aroma development during sausage ripening(Ansorena, Gimeno, Astiasaran & Bello, 2001; Olesen, Meyer & Stahnke, 2004),but there is limited information on consumer acceptability and appreciation of dry-fermented sausages. This is perhaps related to the difficulty in recruiting the large number of panellists needed to make a satisfactory assessment of consumer acceptability. In one study, Kumar and Tanwar (2010) found that chicken nuggets containing 1.5 % (w/w) ground mustard scored higher than controls in terms of texture, and with both 1.5 and 2% mustard, significantly higher scores for flavour and colour were observed. However, this was a sensory study of mustard powder added to a cooked food. The use of deheated yellow mustard powder in dry-fermented sausage manufacture might not only reduce the safety risk associated with

E. coli O157:H7, but if it were to be an effective antimicrobial at concentrations that were undetectable organoleptically, it would be an immediate commercial solution to the bacterial pathogen problem. Therefore, to better understand the effect of deheated yellow mustard powder on the sensory quality of dry-fermented sausage, an untrained consumer-based sensory analysis of dry-fermented sausage treated with 1, 2, 3 and 4% (w/w)deheated yellow mustard powder was undertaken.

4.3 Material and Methods

4.3.1 Preparation of starter cultures for production of dry sausage

Staphylococcus carnosus UM123M and *Pediococcus pentosaceus* UM121P isolated from the commercial starter culture mixture Lactacel 115 (Microlife Technics, Sarasota, FL, USA)were revitalized from frozen stock cultures and incubated in Tryptic Soy Broth, TSB(Oxoid, Unipath, Nepean, ON, Canada) and deMan Rogasa Sharpe broth, MRS (Oxoid), respectively. Cultures were transferred twice in broth incubated at 35 ℃ for 24 h. Then cultures were transferred to fresh broth and incubated overnight at 35 ℃ for 18 h. Cultures were centrifuged (Sorvall Instruments RC-5C; DuPont, Newton, CT, USA) at 4,225 ×g and 4 ℃ for 10 min. The supernatant was discarded and the bacterial pellet was washed with 0.1% (w/v) peptone water and centrifuged again. The supernatant was discarded and the cultures were resuspended using 50mL 0.1% peptone water. The two bacteria were combined in equal volumes and 100mL of the starter culture mixtures were used for each treatment.

4.3.2 Dry-fermented sausage manufacture

The dry-fermented sausage production involved 5 treatments: control (without mustard powder), 1%, 2%, 3% and 4% deheated (inactive myrosinase) mustard powder (G.S.Dunn Ltd., Hamilton, ON, Canada). A total of 10.5 kg salami batter was manufactured for each treatment and these were replicated 4 times. Lean pork trim, pork back fat and lean beef trim were purchased from a local butcher (Miller's Meat, Winnipeg, MB, Canada). Materials were cut into 8cm cubes and kept frozen at -18℃ prior use. The pork and beef meat were tempered overnight at 5℃ before sausage production. Pork back fat (18.3%), lean pork trim (63.4%) and beef (18.3%) were added in decreasing order of fatness to a pre-chilled (1 to 2℃) rotating bowl chopper (Titane 40, Dadaux, Bersaillin, France). The pork fat was finely chopped and the starter cultures (*P. pentosaceus* and *S. carnosus* mixture) were added to yield 8 and 6 log CFU/g, respectively. The pork and beef were added and chopped to 3mm particles and then the dry ingredients were added. The ingredients included: salt (2.91% w/w; HyGrade, Sifto Canada Corp., Mississauga, ON, Canada), D-glucose (0.06% w/w; Sigma Chemical Co.), Cervelat spice mixture codeC719 (0.44% w/w; Wiberg Corp., Oakville, ON, Canada), pickle cure concentrate (0.31% w/w; Canada Compound Corp., Winnipeg, MB, Canada) with 6.25% (w/w) sodium nitrite/nitrate and sodium erythorbate (0.05%w/w; Canada Compound Corp.). Yellow mustard powder was added at the end of the chopping process. Then, the sausage batter was transferred to a pre-cooled vacuum stuffer (VF 608, Handtmann, Waterloo, ON, Canada) and mechanically stuffed into water softened 55 mm diameter fibrous casings (Kalle GmbH, Wiesbaden, Germany). Each sausage weighed approximately 500g. Sausages were then hung on horizontal aluminum sticks and placed

into a single rack automated smokehouse (ASR 1495 EL/WA, Titan, Maurer AG, Reichenau, Germany) with programmable temperature, relative humidity (RH) and pH controller. Sausages were smoked intermittently during fermentation (2 h total). The initial temperature was set at 26 ℃ and decreased by 2 ℃ every 24 until 20 ℃was reached. Thereafter, 2 ℃ drops occurred every 12 h until 14 ℃ was achieved. The RH was initially set at 88% and dropped to 80% during the first 24 h. Then, the RH was decreased stepwise by 2%/ day until a final RH of 75% was achieved. Sausages were transferred after 3 days to a second single rack smokehouse (AFR-Fishmaster "Roundair"; Rauch und Warmetchnik Gmbh and Corp., Reichenau, Germany) with a temperature and RH controller and dried for the next 25 days at 14 ℃ and 75% RH. The total production time was 28 days.

4.3.3 Deheated yellow mustard powder preparation

The method of deheated yellow mustard powder preparation was from Luciano, Belland & Holley. (2011).The hot ground mustard powder was placed on a metal tray in a 1 cm layer and covered with aluminum foil, and then autoclaved at 115 ℃ for 15 min. The powder was cooled to room temperature and finely mixed. The "deheated" or "deodorized" yellow mustard powder was freshly prepared the day before sausage production, and stored in a closed container at ambient temperature. Thermal treatment deactivated the endogenous myrosinase and successfully stabilized the sinalbin level in the yellow mustard powder (Luciano, Belland & Holley., 2011; Nilson and Holley, 2012).

4.3.4 Microbial and physico-chemical analysis of sausages

Inoculated meat batter was sampled at day 0 and analyzed for microbial and physico-chemical properties. Dry sausages were sampled and tested on days 7, 14, 21 and

28. A 25 g sample was aseptically taken from the core of the sausage and placed into a stomacher bag (Filtra-bag; VWR, Edmonton, AB, Canada). The sample was homogenized with 225 mL 0.1% peptone water (BagMixer 400, Intersciences Inc., Markham, ON, Canada) for 1 min. Serial dilutions from 10^{-2} to 10^{-5} were prepared. Samples were then plated using an Autoplate 4000 Spiral Plater (Spiral Biotech, Bethesda, MD, USA). *P. pentosaceus* was plated on MRS agar (Oxoid) and *S. carnosus* was cultured on mannitol salt agar (MSA; Oxoid). Inoculated plates were incubated at 35℃ for 24-48 h.

The sausage water activity was measured using a Novasina AW-Sprint Machine (Axion AG, Pfaffikon, Switzerland). Twenty gram samples were homogenized with 180 mL sterilized distilled water in stomacher bags, and then sausage pH was measured (Accumet Basic pH meter; Denver Instrument Co., Denver, CO, USA).

4.3.5 Texture evaluation of sausage

The shear value of dry-fermented sausage (from day 7 to day 28 of ripening) was measured using the Zwick/Roell material tester (Kennesaw, GA, USA) with a 1 KN load cell and a Warner-Bratzler straight blade. Salami samples were sliced to 1cm thick and shear forces were quantified using testXpert II software (Zwick/ Roell). Crosshead speed was set at 400 mm/min. Shear values were the maximum force required for a complete cut through the salami samples.

4.3.6The preparation of sensory evaluation samples

The salami was harvested on day 28, vacuum packed in Deli *1 barrier bags (WinPak, Winnipeg, MB, Canada), and stored unsliced at 4℃. The salami was

aseptically sliced in 1mm thick pieces about 2 days before the sensory tests. Approximately 15 salami slices were vacuum-packed per bag (Deli*1) for each treatment and stored at 4℃ in the sensory laboratory prior to the sensory evaluation. The salami was 3 to 4 months old before sensory tests were conducted.

4.3.7 Sensory analysis

Untrained panellists (n=86; age 16~24, n=53; age >24, n=53) were recruited from the staff and students at the University of Manitoba (Winnipeg, MB, Canada) according the procedures approved by the Human Ethics Research Board at the University of Manitoba. The sensory analysis was performed in private booths equipped with Sensory Management System (2006) hardware and computerized sensory software (Sensory Integrated Management System, Morristown, NJ, USA) under incandescent light. Five different samples were randomly coded with 3 digit numbers. One slice of each sample was served to the panellists. Room temperature water was provided to clean the palate between tasting samples. The hedonic test was performed using 9 point scales (1=dislike extremely and 9=like extremely). The following sensory attributes were evaluated: appearance, colour, flavour, texture and overall acceptability. At the end of the test, the panellists were asked to indicate their gender, age and how often they normally consumed salami.

4.3.8 Statistical analyses

In physico-chemical and microbiological analysis, one-way analysis of variance (ANOVA) was performed using SAS (windows 9.0) software (Statistical Analysis System, Cary, NC, USA). Treatments were compared using Tukey's test to find statistical differences ($p<0.05$).

In sensory analysis, a four-factor ANOVA was performed (PROC MIXED) using SAS (windows 9.0) software. Tukey's multiple comparison test was employed to find significant differences ($p<0.05$) between treatments.

4.4 Results and Discussion

4.4.1 Microbiological characteristics

The population of *P. pentosaceus* was virtually stable among the control and treatment groups throughout production (Table 4.1). Also, no differences in *P. pentosaceus* population were found among the control and 4 mustard treatment groups. These results were expected because even in salami produced with 6% autoclaved (deactivated myrosinase), non-deheated or non-deheated/autoclaved yellow mustard powder, the *P. pentosaceus* population remained virtually static during fermentation and drying (Luciano, Belland & Holley., 2011).In addition, the resistance of *P. pentosaceus* to allyl isothiocyanate and *para*-hydroxybenzyl isothiocyanate (p-HBIT) has been shown in previous studies (Luciano and Holley, 2010).

However, numbers of *S. carnosus* cells were reduced by 1 log CFU/g in all treatments (Table 4.2). This reduction of *S. carnosus* is common during meat fermentation since the organism does not tolerate pH values around 5.0 very well. Such a reduction has been previously reported (Luciano, Belland &Holley, 2011). In the present test, the reduction of *S. carnosus* viability during sausage maturation was only slightly related to the use of mustard powder. At higher levels of mustard powder addition slightly greater inhibitory effects of mustard against *S. carnosus* has been noted (1.2 log at 6%, Graumann and Holley, 2009). In contrast, lower resistance of *S. carnosus* to AIT

and p-HBIT than that of *P. pentosaceus* has been reported (Luciano and Holley, 2010).In addition, 6% autoclaved (deactivated myrosinase) yellow mustard powder (w/w) in dry-fermented sausage showed a significant (2-3 log CFU/g) reduction in *S. carnosus* numbers during production (Luciano, Belland & Holley, 2011). The greater reduction with autoclaved mustard may have been due to the generation of inhibitory phenolic compounds during thermal treatment. Deodorized (deactivated myrosinase) yellow mustard powder (4 and 6% w/w) in Westphalian ham also rapidly reduced the *S. carnosus* population (Nilson and Holley, 2012). The difference might have been due to the use of different *S. carnosus* strains in sausage and Westphalian ham.

4.4.2 Physico-chemical characteristics

The a_w of the control and 4 treatment groups were similar during dry sausage manufacture (Table 4.3). Though the a_w values were significantly different between the control and 2% treatment group at day 28, differences were small and were unrelated to the addition of mustard (from examination of a_w values from other treatments). In contrast, a previous study reported that salami with 6% autoclaved yellow mustard powder had a higher a_w (Luciano, Belland & Holley., 2011). In addition, the previous authors suggested that the mustard deheating procedure using steam and pressure might result in better water-binding capacity of the mustard powder and yield higher a_w (Hampton, Shantz, Gallo & Unger, 1975, Van Eylen,Indrawati, Hendrickx&Van Loey, 2006). Nonetheless, no significant differences in a_w were found between control and deodorized mustard-treated (4% and 6% w/w)Westphalian ham (Nilson and Holley, 2012). The relationship between a_w and texture development during drying has been studied and a_w is considered an important feature which determines the texture of ripened dry-fermented sausage

(Leistner, 1992; Casiraghi, Pompei, Dellaglio, Parolari & Virgili, 1996). Additional deheated mustard powder, even at the higher level used in the present study (4%), did not influence the a_w of sausage during drying. However, this does not mean that the texture development of mustard-containing salami was unaffected.

Changes in pH values during sausage maturation are shown in Table 4.4. Difference appeared to be related to the addition of mustard powder since treatment with higher concentrations (3 and 4%) showed slightly lower pH values at 28 d. In addition, the differences in pH values appeared between the control and sausage containing 2% mustard at 7, 14 and 21 d during fermentation and drying. Similar results were observed in the Luciano, Belland & Holley (2011) study. Also, Graumann and Holley (2008) noted that the pH differences observed were not associated with the starter culture types and were possibly caused by the carbohydrate from added mustard powder. Carbohydrate fermentation in fermented meat products is suggested to be one of the major reactions involved in flavour and aroma development. Lactic and acetic acid resulting from carbohydrate fermentation by LAB are related to the acidic taste of fermented meat products, especially the northern European type sausage. Ramihone, Sirami, Larpent & Girard (1988) indicated that excess acid production might result an undesirable astringent, sour flavour in the sausage. Moreover, the pH decrease is important for colour and texture development in fermented sausage. Additional carbohydrate provided by yellow mustard powder might have contributed to the lower pH, and thus affected the texture and colour of dry-fermented sausage, as well as its acidic taste.

4.4.3 Texture changes in dry sausage containing yellow mustard

The effect of deheatd yellow mustard on dry-fermented sausage texture during fermentation and drying is presented in Table 4.5. Results showed the shear resistance differences between the control and treatment groups were not significant from day 7 to day 28. The results in this study did not match results from previous work (Graumann and Holley, 2008), which showed a higher shear force in control (no mustard) than treatment groups (2%, 4% and 6% non-deheated mustard and 6% deheated mustard). This may have been related to the use of greater amount of mustard in the latter work. In addition, instrumental measurement of texture may not be an accurate reflection of sensory perception of texture.

4.4.4 Sensory analysis

Results from a four-factor ANOVA are shown in Table 4.6 and Table 4.7 for the salami, including the F-values associated with probabilities of the effects and interactions analyzed for all the sensory attributes.

The effect of gender and age

Gender and age had no significant effect on the appearance, colour, flavour, texture and overall acceptability of all 5 treatments (Table 4.6). Further, the cross factors of gender and age had no influence on the sensory attributes reported by the untrained panellists for the 5 treatments. Therefore, the sensory evaluation was reliable and unbiased. In addition, the 9-point hedonic-scale questionnaire was administered on campus where the largest age group was between 16 and 24 and it had a slightly higher

mean value of sausage sensory scores than other age groups. Also, the female gender group had a slightly higher overall mean value than the male group.

The consumption frequency of salami

Answers to the salami consumption frequency question indicated that 91% of participants had eaten salami before, but 9% of participants had never tasted salami before (Table 4.8). Most of the participants (29%) ate salami at least once per month. In addition, nearly half of the participants had eaten salami about 1 to 3 times per year. It is notable that consumption frequency had no effect on any of the sensory attributes (data not shown).

Appearance

There was no difference in salami appearance between the control and 1% mustard salami, whereas a detectable difference was observed between 1 and 2% mustard salami (Table 4.7). Also, there was a further difference in appearance between 2 and3% mustard salami. However, the appearance difference between 3% and 4% mustard salami was not significant. The preference of control, 1 and 2% mustard salami's appearance were both notably different from 3 and 4% mustard salami. The results indicated that sausages with higher concentrations of mustard had less appealing appearance. The appearance of the control and 1% mustard salami were appreciated moderately, whereas 3 and 4% mustard salami were liked slightly. Thus, the influence of higher levels of mustard powder on salami appearance was still acceptable. However, fat particles in the mustard-treated sausage slices were more visible. Normally, deheated ground yellow mustard is used in cooked processed meat products because it is an excellent emulsifier,

and water binding is due to the bran mucilage content (approx 15~25%)(Cui and Eskin, 1998).The fat particles in the mustard-containing sausage slices might have been less tightly bound by the protein gel and became more visible. Further, 4% (w/w) yellow mustard powder is the minimum amount thought to be needed to effectively eliminate *E. coli* O157:H7 from dry-fermented sausage (Graumann and Holley, 2008). Lower levels of yellow mustard powder did not reduce the numbers of *E. coli* O157:H7 by the 5 log CFU/g required during maturation (Graumann and Holley, 2008). At a 6% (w/w)level yellow mustard powder was even more effective and eliminated *E. coli* O157:H7 (>5log CFU/g)from dry-fermented sausage and Westphalian ham in less than 1 month and in 45 days, respectively(Graumann and Holley, 2008; Luciano, Belland & Holley, 2011; Nilson and Holley, 2012). The salami treated with 4% yellow mustard powder was still slightly liked in terms of appearance, which might mean that if used at this level to eliminate the *E. coli* O157:H7 threat, customers would be less likely to buy the product.

Colour

Yellow/white mustard powder has a natural golden colour, while deheated (autoclaved/deodorized) yellow mustard powder had a darker golden colour. However, colour differences were not observed among the control or 1, and 2 or 3% mustard salami (Table 4.7). Two and 3% mustard were similar, but the 4% mustard sausages were different from the other 4 formulations. The results indicated that the highest concentration of mustard powder used negatively affected salami colour quality; yet, 4% mustard salami did not show a substantial colour difference from the 1% salami. Further, the colour of control and 1% mustard salami was appreciated moderately, while 4% mustard salami was liked slightly. The consumer scores of 2% and 3% mustard salami

were between 7 and 6, which corresponded to between “like moderately” and “like slightly”. Results suggest that the salami formulated with comparatively low mustard still had good colour quality; though mustard powder did change the salami colour in an undesirable manner. It should be noted that food poisoning caused by *E. coli* O157:H7 could be prevented by adding ≤ 4% (w/w) yellow mustard powder during production. It is possible that the colour of 4% mustard-treated salami might not be a strongest factor influencing consumer acceptability of these products. Additionally, mustard-treated sausages had a slightly lower pH, especially the 3 and 4% mustard treatments that would add further antimicrobial protection.

Flavour

There was also no significant flavour difference between the control and 1% mustard salami, or between 1% and 2% mustard salami, whereas 2% mustard salami was different from the control (Table 4.7). Four percent mustard salami had a significant flavour difference from the other 4 groups of salami. In contrast, 3% and 4% had lower flavour scores than the other groups and were different from each other. The flavour of 3% mustard salami was still liked slightly (average score=6.0, corresponds to “liked slightly”) and 4% mustard salami had a less desirable flavour. Increased concentrations of mustard reduced the flavour scores of salami. From the perspective of microbial safety, 2% mustard was not sufficient to achieve the required 5 log CFU/g reduction of *E. coli* O157:H7 in 30 days (Graumann and Holley, 2008). Although this requirement has been consistently met at 4% mustard powder, preliminary evidence suggests that 3% mustard may be inadequate for controlling this pathogen in fermented sausage (unpublished, this laboratory).

Deheated mustard flour contains a high level of (30~35%) proteins, which in turn contain a large amount of lysine and sulphur-containing amino acids (Table 2.1) (Cui and Eskin, 1998).Pripis-Nicolau, de Revel, Bertrand & Maujean (2000) indicated that the reaction of carbonyl compounds with amino acids, in particular sulphur-containing amino acids, could release aromatic compounds and contribute to sulphurous nutty, toasty, rotten fruity aromas. However, in a further review of the flavour of peptides, amino acids and proteins it was found that lysine contributed to a plane taste (Solm, 1969). The breakdown of the amino acids leucine, isoleucine, valine, and phenylalanine could produce a large amount of aromatic compounds, which can contribute a strong flavour and aroma to food products (Talon, Ldroy-Setrin & Fadda, 2004). This may explain in part, the reason that salami with higher levels of deheated mustard had a less appreciated flavour. Some panellists noted that 4% mustard salami had a bitter or greasy taste (data not shown). Deheated yellow mustard powder is used in cooked processed meat products at relatively low levels of between 1% and 5%. At these concentrations it has not shown the sensory issues noted here with uncooked sausages. It may be that cooking significantly reduced mustard-related adverse sensory attribute development in meat products. In addition, storage time after manufacture can negatively affect the sensory characteristics (flavour, lipid oxidation and colour) of dry-fermented sausage. However, vacuum packaging improved and maintained sausage sensory stability during long-term (6 to 12 months) storage (Rubio, Martinez, Garcia-Cachan, Rovira & Jaime, 2008; Soyer and Ertas, 2006; Summo, Caponio, Paradiso, Pasqualone & Gomes, 2009). Age following manufacture may have reduced the flavour intensity of sausage (both control and treatment groups)during the 3 month interval before sensory analysis, however under

vacuum and unsliced, these products would have at least a 9 month shelf-life at 4℃. Nonetheless, how the deheated yellow mustard affected salami sensory properties is an unanswered question. Further study is necessary to understand the changes in volatiles between deheated mustard-treated salami and salami without mustard powder to improve salami flavour quality and ensure sausage safety.

Texture

Mustard powder negatively affected sausage texture at the highest concentration. Texture differences were not found between the control and 1% mustard salami, the 1% and 2% mustard salami, and 2% and 3% mustard salami, whereas 2% and 3% mustard salami were different from the control(Table 4.7). In contrast, 4% mustard salami was significantly different from the other 4 groups of salami and had a consumer score ranging from “like slightly” to “neither like nor dislike”, and as such, this treatment had the least appreciated texture. Sausage treated with 4% mustard was evaluated as having a texture score similar to that observed for the flavour attribute. The texture of dry-fermented sausage is developed by the denaturation of salt-soluble muscle proteins, which starts when meat pH reaches 5.3. Protein denaturation yields a gel-like texture in the sausage that is further stabilized during drying. Significant differences in pH between the control and 3 and 4% mustard treatments were noted from day 7 of ripening onward, although these differences (≤ 0.2 pH units) were not large. Similar differences were noted in previous studies (Graumann and Holley et al., 2008; Luciano, Belland & Holley., 2011). Mustard contains approximately 12%~ 18%carbohydrates (AAFC, 2009b), some of which could be fermented by LAB in the sausage to produce lactic acid, and thus affect the pH drop. This could contribute to the texture change in the sausage treated with

the larger amount of mustard. Texture difference noted might also have been due to mustard mucilage which accounts for 2% of the dry seed and majorly contributes to emulsification, thickening and water-biding (Cui and Eskin, 1998).

Overall acceptability

The overall acceptability of control and 1% mustard salami, and 1% and 2% mustard salami were not different (Table 4.7). Three and 4% mustard salami were both significantly different from the other treatments and were significantly different from each other. It was found that 4% mustard-containing salami was the least preferred overall. Thus, the use of higher levels of mustard powder in salami resulted in a reduction of sensory quality. In contrast, the insignificant sensory difference between the control and 1% suggested that the lower level mustard powder had a limited effect on salami sensory quality. It would appear that there would be little uncertainty in terms of consumer acceptability if 2% mustard powder were used in commercial products. Lower overall acceptability of the 3% mustard treatment was influenced by both lower appearance and flavour scores, suggesting that at this level of mustard addition, consumers could detect its addition and only slightly liked the product. As consumers neither liked nor disliked salami with 4% mustard, this product would not likely be successful in the marketplace.

4.5 Conclusion

Deheated ground mustard has been used commercially as an emulsifier, binder and thickener in cooked processed meat products for a long time. Its antimicrobial activity has also been demonstrated in uncooked dry-cured fermented meats. Its effect on dry-fermented sausage sensory properties showed that the appearance, colour, flavour

and texture quality of mustard-containing salami were reduced with increasing amounts of yellow mustard. Sausage treated with 4% deheated yellow mustard received the lowest consumer scores over all sensory attributes. However, 4% mustard-containing salami had a “like slightly” appearance and colour, which was scored on average at 6. The flavour of 4% mustard salami was referred to as “neither like nor dislike” and the texture quality was between “like slightly” and “neither like nor dislike”. In overall acceptability the 4% mustard salami was neither liked nor disliked. The sensory evaluation also indicated that the sausage treated with 3% deheated yellow mustard had detectable changes in sensory quality that might affect its acceptance. The 2% mustard salami quality was ranked between “liked moderately” and “liked slightly”. At present, 4% (w/w) ground yellow mustard is the minimum amount required to effectively eliminate detectable (5 log CFU/g) *E. coli* O157:H7 from dry-fermented sausage; however, this value is likely somewhere between 3 to 4%. Food appearance and colour are considered to be the two most important quality issues in relation to consumer satisfaction and customer appreciation. Flavour and texture are the features that determine the real eating quality of food. However, food safety is the most critical issue above all other factors. It is important to find ways to ensure the safety of fermented sausage, without altering its eating quality. In addition, further study of the sensory profiles of the mustard-containing salami in order to characterize the contribution of the volatile compounds present which may influence sensory changes, would be of value in understanding how to improve treated salami eating quality.

Table 4.1 Recovery of *Pediococcus pentosaceus* starter culture during fermentation and drying of fermented sausage for the control and treatments groups containing 1, 2, 3 and 4% (w/w) deheated mustard powder.

	P. pentosaceus (log CFU/g)				
Day	Control	1% deheated mustard	2% deheated mustard	3% deheated mustard	4% deheated mustard
0	7.47 ±0.10a	7.21 ±0.21a	7.19 ±0.20a	7.20 ±0.18a	7.21 ±0.20a
7	8.28 ±0.20a	8.37 ±0.20a	8.37 ±0.30a	8.40 ±0.26a	8.20 ±0.28a
14	8.71 ±0.20a	8.68 ±0.27a	8.68 ±0.20a	8.77 ±0.25a	8.28 ±0.25b
21	8.22 ±0.11a	8.31 ±0.06a	8.34 ±0.13a	8.35 ±0.09a	8.22 ±0.23a
28	8.34 ±0.06a	8.41 ±0.18a	8.39 ±0.22a	8.40 ±0.22a	8.24 ±0.26a

Values are the mean ± standard deviation of two trials replicated four times. Numbers in the same row with different letters are significantly different ($p<0.05$).

Table 4.2 Recovery of *Staphylococcus carnosus* starter culture during fermentation and drying of fermented sausage for the control and treatments groups containing 1, 2, 3 and 4% (w/w) deheated mustard powder.

	S. carnosus (log CFU/g)				
Day	Control	1% deheated mustard	2% deheated mustard	3% deheated mustard	4% deheated mustard
0	6.14 ± 0.08a	5.71 ± 0.26ab	5.61 ± 0.17b	5.59 ± 0.24b	5.61 ± 0.23b
7	5.78 ± 0.13a	5.38 ± 0.23b	5.32 ± 0.25b	5.14 ± 0.14b	5.11 ± 0.25b
14	5.66 ± 0.12a	5.59 ± 0.20a	5.45 ± 0.30a	5.39 ± 0.24a	5.33 ± 0.24a
21	5.72 ± 0.16a	5.40 ± 0.05ab	5.17 ± 0.20b	5.17 ± 0.35b	5.07 ± 0.23b
28	5.67 ± 0.16a	5.41 ± 0.04ab	5.17 ± 0.16b	5.18 ± 0.29b	5.12 ± 0.35b

Values are the mean ± standard deviation of two trials replicated four times. Numbers in the same row with different letters are significantly different ($p<0.05$).

Table 4.3 Changes in water activity (a_w) during fermentation and drying of fermented sausage for the control and treatment groups containing 1, 2, 3 and 4% (w/w) deheated mustard powder.

Day	Control	1%deheated mustard	2% deheated mustard	3% deheated mustard	4% deheated mustard
0	0.947 ±0.01a	0.951 ±0.01a	0.949 ±0.01a	0.950 ±0.01a	0.948 ±0.01a
7	0.931 ±0.01a	0.933 ±0.01a	0.933 ±0.01a	0.931 ±0.01a	0.931 ±0.01a
14	0.903 ±0.01a	0.907 ±0.01a	0.905 ±0.01a	0.907 ±0.01a	0.908 ±0.01a
21	0.884 ±0.01a	0.884 ±0.02a	0.881 ±0.01a	0.888 ±0.01a	0.888 ±0.01a
28	0.874 ±0.02a	0.865 ±0.01ab	0.857 ±0.01b	0.861 ±0.01ab	0.862 ±0.01ab

Values are the mean ± standard deviation of two trials replicated four times. Numbers in the same row with different letters are significantly different ($p<0.05$).

Table 4.4 Changes in pH during fermentation and drying of fermented sausage for the control and treatment groups containing 1, 2, 3 and 4% (w/w) deheated mustard powder.

Day	Control	1% deheated mustard	2% deheated mustard	3% deheated mustard	4% deheated mustard
0	5.87 ±0.05a	5.85 ±0.05a	5.89 ±0.05a	5.88 ±0.05a	5.89 ±0.04a
7	5.09 ±0.04a	5.02 ±0.05b	4.99 ±0.04bc	4.95 ±0.04c	4.83 ±0.04d
14	5.06 ±0.03a	5.00 ±0.05a	4.93 ±0.05b	4.89 ±0.05b	4.81 ±0.05c
21	5.01 ±0.04a	5.00 ±0.05a	4.91 ±0.05b	4.88 ±0.05b	4.80 ±0.05c
28	4.85 ±0.05a	4.87 ±0.05a	4.90 ±0.05ab	4.74 ±0.05c	4.75 ±0.03c

Values are the mean ± standard deviation of two trials replicated four times. Numbers in the same row with different letters are significantly different ($p<0.05$).

Table 4.5 Maximum shear force resistance of dry-fermented sausage during fermentation and drying for control and treatment groups containing 1, 2, 3 and 4% (w/w) deheated mustard powder.

Day	Control(no mustard)	1% deheated mustard	2% deheated mustard	3% deheated mustard	4% deheated mustard
7	39.25 ±4.50a	34.33 ±5.54a	39.73 ±6.54a	37.40 ±4.58a	40.15 ±6.28a
14	45.98 ±5.81a	42.35 ±5.79a	45.43 ±8.40a	39.21 ±5.72a	43.26 ±6.33a
21	65.68 ±7.88a	65.58 ±8.67a	67.04 ±6.57a	65.84 ±7.03a	66.08 ±6.07a
28	75.94 ±6.46a	72.65 ±8.57a	72.94 ±8.50a	76.49 ±6.50a	69.70 ±4.27a

Values are the mean ± standard deviation of two trials replicated eight times. Numbers in the same row with different letters are significantly different ($p<0.05$).

Table 4.6 The sensory analysis results of gender and age from four-way ANOVA and Tukey's test for five salami formulations.

	Source of variance (F-value)					Mean value for Gender		Mean value for age	
Sensory attributes	Sample	Gender	Age	Consumer	Gender* Age	Female n=43	Male n=43	16~24 n=53	>24 n=33
Appearance	9.25***[a]	0.50 NS	3.49NS	3.90***	0.65NS	6.6(0.10)[b]	6.3(0.10)	6.6(0.08)	6.2(0.13)
Colour	18.05***	2.59NS	3.49NS	4.98***	0.00NS	6.8(0.09)	6.4(0.10)	6.8(0.08)	6.3(0.12)
Flavour	32.17***	0.09NS	1.34NS	2.90***	0.49NS	6.3(0.12)	6.2(0.12)	6.4(0.10)	6.1(0.16)
Texture	23.68***	0.60NS	2.54NS	3.47***	0.27NS	6.6(0.10)	6.3(0.12)	6.6(0.09)	6.2(0.15)
Overall Acceptability	36.89***	0.54NS	0.95NS	3.94***	0.69NS	6.5(0.11)	6.3(0.11)	6.5(0.09)	6.2(0.14)

[a]NS: $p>0.05$; ***: $p<0.01$.

[b]Mean (standard error).

Mean with different letter on the same row are significantly different at $p<0.05$.

Table 4.7 The sensory analysis results from four-way ANOVA and Tukey's test for five salami formulations.

Sensory attributes	Mean Intensity value for formulation n=86				
	Control (without mustard)	1% mustard salami	2% mustard salami	3% mustard salami	4% mustard salami
Appearance	6.8(0.14)[a]a	6.8(0.14)a	6.5(0.15)b	6.1(0.16)c	6.0(0.18)c
Colour	7.0(0.11)a	7.1(0.11)a	6.6(0.14)b	6.4(0.15)b	6.0(0.19)c
Flavour	7.1(0.14)a	6.8(0.15)ab	6.6(0.14)b	6.0(0.19)c	4.9(0.25)d
Texture	7.1(0.13)a	6.9(0.13)ab	6.6(0.16)bc	6.3(0.17)c	5.4(0.23)d
Overall Acceptability	7.1(0.12)a	6.9(0.13)ab	6.6(0.14)b	6.2(0.17)c	5.2(0.22)d

[a] Mean (standard error).

Mean with different letter on the same row are significantly different at $p<0.05$.

Table 4.8 Salami consumption frequency among consumer panellists (n=86)

Consumption frequency of salami	Respondents (%)
More than once a month	16
About once a month	29
About 3 times per year	20
Less than 3 times per year	26
Never consumed salami before	9

Chapter 5

General Discussion and Conclusion

5.1 Antimicrobial Influence of Stabilized p-HBIT on *Escherichia coli* O157:H7

p-HBIT, stabilized in maltodextrin, effectively controlled *Escherichia coli* O157:H7 viability (> 4 log CFU/g reduction) in a sausage model system during a 6 day experiment. Starter cultures (*Pediococcus pentosaceus* and *Staphylococcus carnosus*) were also reduced nearly 3 log CFU/g at the end of test. This was believed due to the extended incubation at 26℃. Since p-HBIT is more stable at lower pH, it becomes an excellent natural antimicrobial agent at the ultimate pH of formulated (pH< 5.3) in sausage. The higher concentration of p-HBIT (206 mg, equivalent to 4% (w/w) deheated yellow mustard) was more effectively bactericidal against on *E. coli* O157:H7. The study confirmed that 4% yellow mustard (both hot and cold) has the potential to yield at least a > 5 log CFU/g *E. coli* O157:H7 reduction during fermentation and drying of sausages. Moreover, p-HBIT stabilized in maltodextrin was practical and useful for effectively reducing *E. coli* O157:H7 viability. Though the lower concentration of p-HBIT (103mg, equivalent to 2% (w/w) deheated yellow mustard) significantly reduced *E. coli* O157:H7 numbers, a longer time of ripening would be required to achieve a > 5 log CUF/g reduction of this pathogen. However, purified p-HBIT is not allowed to be added to food directly. In addition, it is difficult to extract and preserve p-HBIT due to its extreme instability. It is degraded to more stable compounds in minutes in the present of moisture at a neutral pH. Its unique pungency limits its application in processed foods. Therefore, yellow mustard powder (both hot and cold) is a better antimicrobial agent, which not only

contains p-HBIT but also has been used in the food industry for a long time. Further, quantification of p-HBIT residues and p-HBIT hydrolysis products would be important to better understand the antimicrobial activity of p-HBIT during fermentation and drying of sausage.

5.2 Sensory effect of ground deheated yellow mustard on dry-fermented sausage

Although higher levels of yellow mustard (both hot and cold) can effectively achieve dry sausage safety, it does not improve sausage eating quality. Sensory defects were noted in fermented sausages formulated with deheated yellow mustard, even though it is used as an excellent source of plant proteins in processed cooked meat products. In these latter applications, it is also considered to be a good water binder, thickener and emulsifier. However, the higher the amounts of deheated yellow mustard that were added to sausage, the worse the eating quality of sausage became. Salami that contained higher levels of mustard (3% and 4%) were least appreciated in terms of appearance, colour, texture and flavour. Dry sausage containing 1% (w/w) yellow mustard did not show significantly sensory scores from the control. Sausage formulated with 2% yellow mustard (w/w) showed slightly less appreciated flavour and texture. Although 1% and 2% mustard salami still had good colour quality, salami which contained 3% and 4% mustard had less desirable colour. Moreover, fat particles in the latter mustard-treated sausage slices were more visible than in the control samples. However, 1% yellow mustard did not suitably reduce *E. coli* O157:H7 viability. Two percent (w/w) mustard was also not sufficient to eliminate *E. coli* O157:H7 and did not achieve a > 5 log CFU/g reduction during sausage manufacture. Nonetheless, sausages formulated with different levels of yellow mustard did not show differences in pH or water activity. Starter cultures (*P.*

pentosaceus and *S. carnosus*) in sausages were not affected by the higher levels of yellow mustard. Mustard powder did not affect the physical properties (instrumental measurement) of sausage. Moreover, higher levels of yellow mustard did not detrimentally affect sausage appearance and colour, which are important first indicator of food quality used by consumers in making purchase decisions. However, sausage texture and flavour changes found suggested that yellow mustard negatively affected these most important eating quality characteristics, which also influence consumers' repeat purchase decision-making. How the deheated yellow mustard influences the characteristic sausage flavour compounds is still an unanswered question. Therefore, targeting a sufficient amount of deheated yellow mustard to satisfy both sausage safety and sensory quality requirements is still an unresolved issue in dry sausage manufacture. It is notable that 3% yellow mustard had a less detrimental effect on sausage flavour and texture than 4% yellow mustard had. Three percent yellow mustard, with higher levels of p-HBIT was more effective against *E. coli* O157:H7 than 2% mustard. Therefore, deheated yellow mustard, at a level of less than 4% (w/w) but higher than 3% may be the optimum for protecting sausage safety with lowered sensory alteration. In addition, further characterization of the contribution of the volatile compounds present in mustard-treated sausage is important for better understanding of how salami eating quality might be improved without negatively affecting sausage safety.

In conclusion, dry-fermented sausage formulated with the higher levels of yellow mustard tested successfully eliminated the risks associated with *E. coli* O157:H7, but caused undesirable alteration of eating quality. However, there is still an opportunity to use between 3% and 4% yellow mustard to control *E. coli* O157:H7 viability by

increasing the concentration (by 100-fold) of the *S. carnosus* starter culture which is known to possess elevated levels of myrosinase to more efficiently convert low levels of glucosinolates to the antimicrobial isothiocyanates.

Chapter 6

Future Work

In order to reduce the negative sensory impact of mustard powder on fermented sausage sensory quality at higher concentrations (> 2%), fractionation of the powder should be considered. Yellow mustard seeds contain about 2% mucilage, which accounts for 20~25% of mustard bran. Yellow mustard polysaccharides have unique rheological properties which contribute to stabilization, emulsification and water-binding in many food products. However, its viscosity is affected by pH, temperature, salt and sugar content (Cui, Eskin & Biliaderis, 1993). Yellow mustard mucilage, at pH values higher or lower than 7, at lower temperature and higher salt and sugar content, has higher viscosity. The production of dry-fermented sausage achieves low pH, involves decreased temperature and sugar levels, but higher salt levels (about 3.5%). These properties may increase mucilage viscosity, and contribute to texture changes in sausage during fermentation and drying. The mustard powder used in these tests was made from whole ground seeds, and since much of the mucilage can be found in the mustard seed coat, de-hulling may be a useful step. Removal of the mucilage from yellow mustard seeds prior to the deheating process may be a solution to produce deheated yellow mustard powder which may be more ideally suited for use in dry-fermented sausage manufacture.

Another approach to improve the sensory quality of dry sausage formulated with ground deheated yellow mustard may be to increase the *Staphylococcus carnosus* starter culture concentration. While *S. carnosus* is less resistant to p-HBIT than *Pediococcus pentosaceus*, it is more resistant to p-HBIT than *Escherichia coli* O157:H7. In addition, *S.*

carnosus is able to generate larger amounts of myrosinase-like activity than *P. pentocsaceus* which results in greater hydrolysis of glucosinolates and facilitates p-HBIT formation. This may yield greater lethality of *E. coli* O157:H7 at lower mustard concentrations.

References

Abdel-Aziz, S. A., Esmail, S. A., Hussein, L., and Janssen, F. (1997). Chemical composition and levels of nonmeat proteins in meat brands extended with soy protein concentrate, Food Chem., 60, 389–395.

Agriculture and Agri-Food Canada (AAFC).(2009a). Agri-Food Trade Service: Canada's Agriculture, Food and Beverage Industry, Canada's Mustard Seed Industry. Retrieved from http://www.ats.agr.gc.ca/supply/3311_e.htm.

Agriculture and Agri-Food Canada (AAFC).(2009b). Mustard Seed.Retrieved from http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1175116081724⟨=eng.

Agriculture and Agri-Food Canada (AAFC). 2011. The case of Canadian mustard. Retrieved fromhttp://www.ats.agr.gc.ca/pro/4512-eng.pdf.

Ahmad, S., Rizawi, J.A., and Srivastava, P.K. (2009).Effect of soy protein isolate incorporation on quality characteristics and shelf-life of buffalo meat emulsion sausage. J. Food Sci. Technol., 47(3), 290-294.

Al-Nabulsi, A.A. and Holley, R.A. (2007). Effects on *Escherichia coli* O157:H7 and meat starter cultures of bovine lactoferrin in broth and microencapsulated lactoferrin in dry sausage batters. Int.J.Food Microbiol., 113,84-91.

Andres, A. I., Cava, R. and Ruiz, J. (2002). Monitoring volatile compounds during dry cured ham ripening by solid-phase microextraction coupled to a new direct extraction device. J. Chromatog. A., 963, 83 – 88.

Ansorena, D., Gimeno, O., Astiasarán, I. and Bello, J. (2001). Analysis of volatile compounds by GC-MS of a dry-fermented sausage: Chorizo de Pamplona. Food Res. Int., 34, 67-75.

Ansorena, D. and Astiasaran, I. (2004).Effect of storage and packaging on fatty acid composition and oxidation in dry-fermented sausage made with added olive oil and antioxidants. Meat Sci., 76,237-244.

Barai, B. K., Nayak, R. R., Singhal, R. S., and Kulkarni, P. R.(1992).Approaches to the detection of meat adulteration.Trends Food Sci. Technol.,3, 69–72.

Barbieri, G., Bolzoni, L., Parolari, G., Virgili, R., Butinni, R, Careri, M. and Mangia, A. (1992).Flavour compounds of dry-cured ham. J. Agr. Food Chem., 40, 2389–2394.

Beekhuis, H.A. (1975). Technology and industrial applications.In Newman, A.(Eds).Chemistry and Biochemistry of Thiocyanic Acid and its Derivatives.(pp. 222-225).New York:Academic Press.

Bell, J.M. (1989). Nutritional characteristics and protein uses of oilseed meals.In Downey, R.K., Ashri, A (Eds). Oil Crops of the World.(pp, 192-195). New York:McGraw Hill Publishing Company.

Berdague, J.L., Monteil, P., Montel, M.C.and Talon, R.(1993).Effects of starter cultures on the formation of flavour compounds in dry sausage. Meat Sci., 35, 275-287.

Bloukas, J.G., Paneras, E.D. and Fournitzis, G.C. (1997).Effect of replacing pork backfat with olive oil on processing and quality characteristics of fermented sausages. Meat Sci., 45, 133-144.

Calvo, M.M., Garcia, M.L.and Selgas, M.D. (2008).Dry-fermented sausages enriched with lycopene from tomato peel. Meat Sci, 80,167-172.

Campodel, G., Gallego, B., Berregi, I., and Casado, A. (1998). Creatinine, creatine and protein in cooked meat products. Food Chem.,63, 187–190.1

Canadian Food Inspection Agency (CFIA). (1999). Meat Hygiene Manual of Procedures. Chapter 4.10.15, pp. 48-72.

Casiraghi, E, Pompei, C, Dellaglio, S, Parolari, G. and Virgili, R. (1996).Quality attributes of Milano salami, an Italian dry-cured sausage. J. Agr. Food Chem., 44,1248-1252.

Chacon P.A., Buffo R.A. and Holley R.A. (2006). Inhibitory effects of microencapsulated allyl isothiocyanate (AIT) against *Escherichia coli* O157:H7 in refrigerated, nitrogen packed, finely chopped beef. Int. J. Food Microbiol., 107, 231–237.

Choubdar, N., Li, S. and Holley, R.A. (2010). Supercritical fluid chromatography of myrosinase reaction products in ground yellow mustard seed oil. J. Food Sci., 75(4), C341-C345.

Commission of the European Communities (CEC). (2007). Commission directive 2007/68/ec.Official Journal of the European Union.28.11.2007. L 310/14. Retrieved from http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:310:0011:0014:EN:PDF.

Cordoba, J. J., Antequera Rojas, T., Garcia Gonzales, C., Ventanas Barroso, J., Lopez Bote, C. and Asensio, M. A. (1994). Evolution of free amino acids and amines during ripening of Iberia cured ham. J.Agr. Food Chem., 42, 2296–2301.

Cui, W. and Eskin,N.A.M. (1998).Process and properties of mustard products and components. In: Mazza, G., Shi, J., Le Maguer, M. (Eds.). (pp. 235-245).

Functional foods: Boca Raton, FL:biochemical and processing aspects. CRC Press.

Cui, W., Eskin, N.A.M., and Biliaderis, C.G. (1993). Chemical and physical properties of yellow mustard (Sinapis alba) mucilage. Food Chem., 46, 169-176.

Dainty, R. and Blom, H. (1995). Flavour chemistry of fermented sausages. In:Campbell-Platt, G. and Cook, P.E. (Ed),Fermented Meats. (pp. 176–193). London:Blackie Academic and Professional.

Delaquis, P.J.and Sholberg, P.L. (1997).Antimicrobial activity of gaseous allyl isothiocyanate.J. Food Prot.,60,943-947.

Demeyer, D. and Stahnke, L. (2002).Quality control of fermented meat products.Meat processing.Boca Raton, FL:CRC Press LLC.

Diaz, O., Fernandez, M., Garcia de Fernando, G.D., de la Hoz, L. and Ordonez, J.A. (1997). Proteolysis in dry-fermented sausages: the effect of selected exogenous proteases. Meat Sci. 46, 115–128.

Dierickx, T. (1991).Vetmetabolisme en aromavorming in droge gefermenteerde worst. Afstudeerwerk, Gent Universiteit, Faculteit Landbouwwetenschappen, Gent, Belgium.

Dijkstra, D.S., Loinnemann,A.R. and van Boekel,T.A.J.S. (2003). Towards sustainable production of protein-rich foods: appraisal of eight crops for Western Europe. Part II: analysis of the technological aspects of the production chain.Crit. Rev. Food Sci. Nutr., 4,481–506.

Diosady, L.L., Xu L.and Chen, B.-K.(2005). Production of high-quality protein isolates from defatted meals of *Brassica* seed, U.S. Patent 6,905,713.

Dransfield, E. (2008). The taste of fat. Meat Sci., 80, 37–42.

Encinas,J.P., Lopez-Diaz, T.M., Garcia-Lopez, M.L., Otero, A. and Moreno, B. (2000).Yeast populations on Spanish fermented sausages. Meat Sci., 54, 203-208.

Ekanayake, A., Kester,J.J., Li, J.J., Zehentbauer, G.N., Bunke, P.R. and Zent,J.B. (2006). Isogard^{TM}: a natural anti-microbial agent derived from white mustard seed. Acta Hort., 709, 101-108.

Ekanayake, A., Zoutendam, P.H., Strife, R.J., Fu, X., and Jayatilake, G.S. (2012). Development of white mustard (*Sinapis alba L.*) essential oil, a food preservative. Food Chem., 133, 767-774.

Erkkila, S., Venalainen, M., Hielm, S., Petaja, E., Puolanne, E. andMattila-Sandholm, T. (2002). Survival of *Escherichia coli* O157:H7 in dry sausage fermented by probiotic lactic acid bacteria. J. Sci. Food Agr., 80, 2101-2104.

Garcia, M. C., Torre, M., Marina, M. L. and Laborda, F.(1997).Composition and characterization of soybean and related products.Crit. Rev. Food Sci. Nutr., 37,361–391.

Graumann, G.H. and Holley,R.A. (2008). Inhibition of *Escherichia coli*O157:H7 in ripening dry-fermented sausage by ground yellow mustard. J. Food Protect., 71, 486-493.

Hampton, R.J., Shantz, J.T., Gallo, T. and Unger, P. (1975).Process for making free flowing flour. The Ogilvie Flour Mills Company, Limited (Montreal, QC, Canada).United States Patent: 3869558.

Health Canada.(2002). Assessment report of the Canadian Food Inspection Agency activities related to domestic ready-to-eat meat products Retrieved

fromhttp://www.hc-sc.gc.ca/fn-an/securit/raport-rapports/report_cfia-rapport_acia-eng.php.

Health Canada.(2009). Mustard: a priority food allergen in Canada-a systematic review. Retrieved from http://www.hc-sc.ca/fn-an/pubs/label-etiquet/mustard-moutarde/index-eng.php.

Henriksen, A.P. and Stahnke, L.H. (1997).Sensory and chromatographic evaluation of water soluble fractions from dried sausages.J. Agr. Food Chem., 45, 2679–2684.

Herranz, B., de la Hoz, L., Hierro, E., Fernandez, M. and Ordonez, J.A. (2005). Improvement of the sensory properties of dry-fermented sausages by the addition of free amino acids. Food Chem., 91, 673-682.

Hinrichsen, L.L. and Andersen, H.J. (1994). Volatile compounds and chemical changes in cured pork: role of three halotolerant bacteria. J. Agr. Food Chem., 42, 1537–1542.

Hinrichsen, L.L. and Pedersen, S.B. (1995).Relationship among flavour, volatile compounds, chemical change and microflora in Italian type dry cured ham during processing.J. Agr. Food Chem., 43, 2932–2940.

Ho, K. L. G., Wilson, L. A. and Sebranek, J. G.(1997).Dried soy tofu powder effects on frankfurters and pork sausage patties, J. Food Sci., 62, 434–437.

Ildiko, S-G., Klara, K.A., Marianna, T-M., Agnes, B., Zsuzsanna, M-B.and Balint, C. (2006). The effect of radio frequency heat treatment on nutria and colloid-chemical properties of different white mustard (*Sinapis alba*L.) varieties.Innov.Food Sci. Emerg.Technol., 7, 74-79.

International Union of Immunological Societies (IUIS).(2009). Allergen nomenclature sub-committee.Retrieved from http://www. allergen.org/Allergen.aspx.

Isshiki, K., Tokuoka, K., Mori, R.and Chiba, S. (1992). Preliminary examination of allyl isothiocyanate vapour for food preservation. Biosci.Biotech.Biochem., 56,1476-1477.

Jessen, B. (1995). Starter cultures for meat fermentation. In Camp- bell-Platt, G. and Cook, P.E. (Ed.).Fermented Meats. (pp. 130-159). London:Blackie Academic and Professional.

Kinsella, J. E.(1979). Functional properties of soy proteins, J. Am. Oil Chem. Soc. 56: 242 ̈C258.

Kumar, D. and Tanwar, V.K. (2010). Effects of incorporation of ground mustard on quality attributes of chicken nuggets. J. Food Sci. Technol., 48(6), 759-762.

Kyung, K.H., and Fleming, H.P. (1996). Antimicrobial activity of sulfur compounds derived from cabbage. J. Food Prot.,60,67-71.

Larrouture, C., Ardaillon, V., Pepin, M. and Montel, M.C. (2000). Ability of meat starter cultures to catabolise leucine and evaluation of the degradation products by using an HPLC method. Food Microbiol., 17, 563–570.

Lawless, H.T. and Heymann, H. (1998).Sensory Evaluation of Food Principles and Practices.New York, NY:Chapman and Hall.

Leistner, F.(1992).The essentials of producing stable and safe raw fermented sausages. In: Smulders, F.J.M.,Toldra ´,F.,Flores, J and Prieto, M (Eds). New technologies for meat and meat products.(pp 1 – 19). Nijmegen, The Netherlands: Audet.

Lin, C.M., Jeongmok, K., Du., W.X. and Wei, C.I. (2000).Bactericidal activity of isothiocyanate against pathogens on fresh produce. J.Food Prot, 63, 25-30.

Liu, T.T. and Yang, T.S. (2010).Stability and antimicrobial activity of allyl isothiocyanate during long-term storage in an oil-in-water emulsion.J. Food Sci., 75, c445-c451.

Luciano, F.B. and Holley, R.A. (2010). Bacterial degradation of glucosinolates and its influence on the growth of *E. coli* O157:H7 in a dry-fermented sausage model-Part 1. Fleischwirts. Int., 25(6), 67-70.

Luciano, F.B. and Holley, R.A. (2011). Bacterial degradation of glucosinolates and its influence on the growth of *E. coli* O157:H7 in a dry-fermented sausage model-Part 2. Fleischwirts. Int., 26(1), 78-81.

Luciano, F.B., Belland, J. and Holley, R.A. (2011). Microbial and chemical origins of the bactericidal activity of thermally treated yellow mustard powder toward *Escherichia coli*O157:H7 during dry sausage ripening. Int. J. Food Microbiol., 145, 69-76.

Lusas, E. W. and Riaz, M. N. (1995).Soy protein products: processing and use, J. Nutr.,125, 573S-580S.

MacDonald, D.M., Fyfe, M., Paccagnella, A., Trinidad, A., Louie, K. and Patrick, D. (2004).*Escherichia coli* O157:H7 outbreak linked to salami, British Columbia, Canada, 1999. Epidemiol.Infect., 132,283-289.

Marco, A., Navarro, J. L. and Flores, M. (2007). Quantification of selected odor-active constituents in dry-fermented sausages prepared with different curing salts. J. Agr. Food Chem., 55, 3058–3065.

Marnoch, R. and Diosady, L.L. (2006). Production of mustard protein isolates from oriental mustard seed (*Brassica juncea* L.). J. Am.Oil.Chem.Soc., 83, 65-69.

Martuscelli, M., Pittia, P., Casamassima, L.M., Manetta, A.C., Lupieri, L. and Neri, L. (2009).Effect of intensity of smoking treatment on the free amino acids and biogenic amines occurrence in dry cured ham. Food Chem., 116, 955-962.

Masson, F., Hinrichsen, L., Talon, R., and Montel, M.C. (1999).Factors influencing leucine catabolism by a strain of *Staphylococcus carnosus*. Int. J. Food Microbiol., 49,173-178.

Mayton, H.S. (1996). Correlation of fungicidal activity of *Brassica* species with allyl isothiocyanate production in macerated leaf tissue.Phytopathology,86,267-271.

Mendoza, E., Garcia, M.L., Casas, C., and Selgas, M.D. (2001).Inulin as fat substitute in low fat, dry-fermented sausages. Meat Sci., 57, 387-393.

Menendez-Arias, L., Moneo, I., Dominguez, J., and Rodriguez, R. (1988). Primary structure of the major allergen of yellow mustard (*Sinapis alba* L.) seed, Sin a I. Eur. J. Biochem., 177, 159-166.

Montel, M.C., Reitz, J.,Talon, R., Berdague, J.L., and Rousset, A.S. (1996). Biochemical activities of *Micrococcaceae* and their effects on the aromatic profiles and odours of dry sausage model. Food Microbiol., 13, 489-499.

Montel, M.C., Masson, F. and Talon, R.(1998). Bacterial role in flavour development.Meat Sci., 49, S111-S123.

Muthukumarasamy, P. and Holley, R.A. (2006).Microbiological and sensory quality of dry-fermented sausages containing alginate-microencapsulated *Lactobacillus reuteri*. Int. J. Food Microbiol., 111, 164-169.

Muthukumarasamy, P. and Holley, R.A. (2007). Survival of *Escherichia coli* O157:H7 in dry-fermented sausages containing micro-encapsulated probiotic lactic acid bacteria. Food Microbiol., 24, 82-88.

Naczk, M., Wanasundara,P.K.J.P.D., and Shahidi, F. (1992).Facile spectrophotometric quantification method of sinapic acid in hexane-extracted and methanol-ammonia-water-treated mustard and rapeseed meals.J. Agr. Food Chem., 40,444–448.

Nadarajah, D., Han. andJ.H. Holley, R.A. (2005). Use of mustard flour to inactivate *Escherichia coli* O157:H7 in ground beef under nitrogen flushed packaging. Int. J. Food Microbiol., 99,257-267.

Naes, H., Holck, A.L., Axelsson, L., Andersen, H.J. and Blom, H. (1995).Accelerated ripening of dry-fermented sausage by addition of a *Lactobacillus* proteinase.Int. J. Food Sci. Technol.,29, 651–659.

Nilson, A.M. and Holley, R.A. (2012). Use of deodorized yellow mustard powder to control *Escherichia coli* O157:H7 in dry cured Westphalian ham. Food Microbiol., 30, 400-407.

Niinimaki, A., Bjorksten, F., Puukka, M., Tolonen, K., and Hannuksela, M. (1995). Spice allergy: results of skin prick tests and RAST with spice extracts. Allergy, 44, 60-65.

Ohta, Y., Takatani, K.and Kawakishi, S. (1995). Decomposition rate of allyl isothiocyanate in aqueous solution.Biosci.Biotechnol.Biochem., 59(1):102–3.

Olesen, P.T., Meyer, A.S., and Stahnke, L.H., (2004). Generation of flavour compounds in fermented sausages-the influence of curing ingredients, *Staphylococcus* starter culture and ripening time. Meat Sci., 66, 675-687.

Olesen, P. and Stahnke, L.H. (2000). The influence of *Debaryomyces hansenii* and *Candida utilis* on the aroma formation garlic spiced fermented sausage and model minces. Meat Sci., 56,357-368.

Olivare, A., Navarro, J.L., and Flores, M. (2009).Distribution of volatile compounds in lean and subcutaneous fat tissues during processing of dry-fermented sausages. Food Res. Int., 42, 1303-1308.

Olivare, A., Navarro, J.L., and Flores, M. (2011).Effect of fat content on aroma generation during processing of dry-fermented sausages.Meat Sci., 87, 264-273.

Ordonez, J. A., Hierro, E. V., Bruna, J. M., and de la Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening.Criti. Rev. FoodSci. and Nutr., 39(4), 329–367.

Papadima, S. N., and Bloukas, J. G. (1999).Effect of level and storage condition on quality characteristics of traditional Greek sausages.Meat Sci., 51, 103 – 113.

Park, C.M. Taormina, P.J. and Beuchat, L.R. (2000). Efficacy of allyl isothiocyanate in killing enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. Int. J. Food Microbiol., 56, 13-20.

Pripis-Nicolau, L., de Revel, G., Bertrand, A., and Maujean, A. (2000). Formation of flavour compounds by the reaction of amino acid and carbonyl compounds in mild conditions. J. Agr.Food Chem., 48(9), 3761-3766.

Ramihone, M., Sirami, J., Larpent, J.P. and Girard, J.P. (1988).Gout acide des saucissons secs. Viande Produits Carnes, 9(6), 291 – 298.

Reed, C. (1995). Challenge study- *Escherichia coli* O157:H7 in fermented sausages (Letter to plant managers, 28 April 1995). USDA, FSIS, Washington, D.C.

Rhee, M.S., Dougherty, R.H., and Kang, D.H. (2003). Combined effects of mustard flour, acetic acid, and salt against *Escherichia coli* O157:H7 stored at 5 and 22 ℃. J.Food Protect., 65, 1632-1636.

Rubio, B., Martinez, B., Garcia-Cachan, M. D., Rovira, J., and Jaime, I. (2008). Effect of the packaging method and storage time on lipid oxidation and colour stability on dry-fermented sausage salchichon manufactured with raw material with high level of mono and polyunsaturated fatty acids. Meat Sci., 80, 1182 – 1187.

Saleemi, Z.O., Janitha, P.K., Wanasundara, P.D., and Shahidi, F. (1993).Effect of low-pungency ground mustard seed on oxidative stability, cooking yield, and colour characteristics of comminuted pork. J. Agr. Food Chem., 41, 641-643.

Schmidt, S. and Berger, R.G. (1998).Microbially formed aroma compounds during the maturation of dry-fermented sausage. Adv. Food Sci., 20, 144–152.

Shahidi, F., Wanasundara, U.N., Amarowicz, R. (1994).Natural antioxidants from low-pungency mustard flour. Food Res. Int., 27, 489-493.

Shofran , B.G., Purrington, S.T., Breidt, F., and Fleming, H.P. (1998). Antimicrobial properties of sinigrin and its hydrolysis products. J.Food Sci., 63, 621-624.

Solm, J. (1969). The taste of amino acids, peptides, and proteins. J. Agr. Food Chem., 17(4), 686-688.

Sondergaard, A, K. and Stahnke, L.H. (2002).Growth and aroma production by *Staphylococcus xylosus*, *Staphylococcus carnosus* and *Staphylococcus equorum*- a comparative study in model systems. Int. J. Food Microbiol., 75(1-2), 99-109.

Soy Protein Council. (1987). Soy Protein Products: Characteristics, Nutritional Aspects and Utilization, (pp. 4-8). Washington, D.C.

Soyer, A. and Ertas, A.H. (2006). Effects of fat level and storage time on lipid and color stability of naturally fermented Turkish sausage (sucuk). J. Muscle Foods, 18, 317-340.

Stahnke, L.H. (1995). Dried sausage fermented with *Staphylococcus xylosus* at chemical and bacteriological data. Meat Sci., 41,179-223.

Stahnke, L.H. (2000). 2-acetyl-1-pyrroline-key aroma compound in Mediterranean dried sausage. Frontiers of Flavour Science. Grarching, Deutsche Forschungsanstalt Fur Lebensmittelchemie, 361-365.

Stahnke, L.H., Sunesen, L.O. and De Smedt, A. (1999). Sensory characteristics of European dried fermented sausages and the correlation to volatile profile In Univ. Gent (Ed). Thirteenth Forum for Applied Biotechnology, Med. Fac. Landbouw. 64/5b, pp. 559–566.

Stone, H. and Sidel, J.L. (1993). Sensory Evaluation Practices, (2nded). Academic Press, San Diego.pp. 1-10.

Stone, H. and Sidel, J.L. (2004). Sensory Evaluation Practices, (3rd ed). Elsevier Academic Press, San Diego,pp. 1-16.

Summo, G., Caponio, F., Pasqualone, A., and Gomes, T. (2010). Vacuum-packed ripened sausages: evolution of volatile compounds during storage. J. Sci. Food Agr., 91, 950-955.

Summo, G., Caponio, F., Paradiso, V.M., Pasqualone, A., and Gomes, T. (2009). Vacuum-packed ripened sausages: Evolution of oxidative and hydrolytic degradation of lipid fraction during long-term storage and influence on the sensory properties. Meat Sci., 84, 147-151.

Sunesen, L.and Stahnke, L.H. (2003).Mold starter cultures for dry sausage-selection, application and effects. Meat Sci.,65, 935-948.

Tainter, D.R. and Grenis, A.T. (1993). Spices and Seasonings: A Food Technology Handbook. (pp. 95-98). New York, N.Y: VCH Publishers, Inc.

Talon, R., Walter, D., and Montel, M.C. (2000).Growth and effect of staphylococci and lactic acid bacteria on unsaturated free fatty acids. Meat Sci., 58,93-97.

Talon, R., Ldroy-Setrin, S., and Fadda, S. (2004).Dry-fermented sausages. Handbook of food and beverage fermentation technology. Marcel Dekker, Inc. New York, N.Y.pp. 457-479.

Teasdale, J.R. and Taylorson, R.B. (1986). Weed seed response to methyl isothiocyanate and metham. Weed Sci. 34,520-524.

Tilden Jr.J., Yong, W., McNamara, A.M., Custer, C., Boesel., B., Lambert-Fair, M.A., Majkowski, J., Vugia, D., Werner, S.B., Hollingsworth, J., and Morris Jr., J.G. (1996). A new route of transmission for *Escherichia coli*: infection from dry fermented salami. Am.J.Public Health, 86, 1142-1145.

Tjener, K., Stahnke, L.H., Andersen, L., and Martinussen, J. (2004). Growth and production of volatiles by *Staphylococcus carnosus* in dry sausages: Influence of inoculation level and ripening time. Meat Sci., 67, 447-452.

Tuorila, H. and Pangborn, R.M. (1988).Prediction of reported consumption of selected fat-containing foods. Appetite, 11(2), 81-95.

Tyopponen, S., Petaja, E., and Mattila-Sandholm, T. (2003). Bioprotectives and probiotics for dry sausages. Int.J.Food Microbiol., 83,233-244.

Valencia, I., Ansorena, D., and Astiasarán, I. (2006). Stability of linseed oil and antioxidants containing dry-fermented sausages: A study of the lipid fraction during different storage conditions. Meat Sci., 73, 269 – 277.

Van Eylen, D., Indrawati, Hendrickx, M., and Van Loey, A., (2006). Temperature and pressure stability of mustard seed (*Sinapis alba* L.) myrosinase. Food Chem.,97, 263–271.

Van Opstaele, F. and Dirinck, P. (1999).Volatile composition of fermented products (dry-cured hams and dry sausages). Proceeding part II in Univ. Gent (ed). Thirteenth Forum for Applied Biotechnology, Med. Fac. Landbouw, pp. 551–558.

Van't Hooft, B.J. (1999).Development of binding and structure in semi-dry-fermented sausages.A multifactorial approach. (Doctoral Thesis).University of Utrecht, Utrecht.

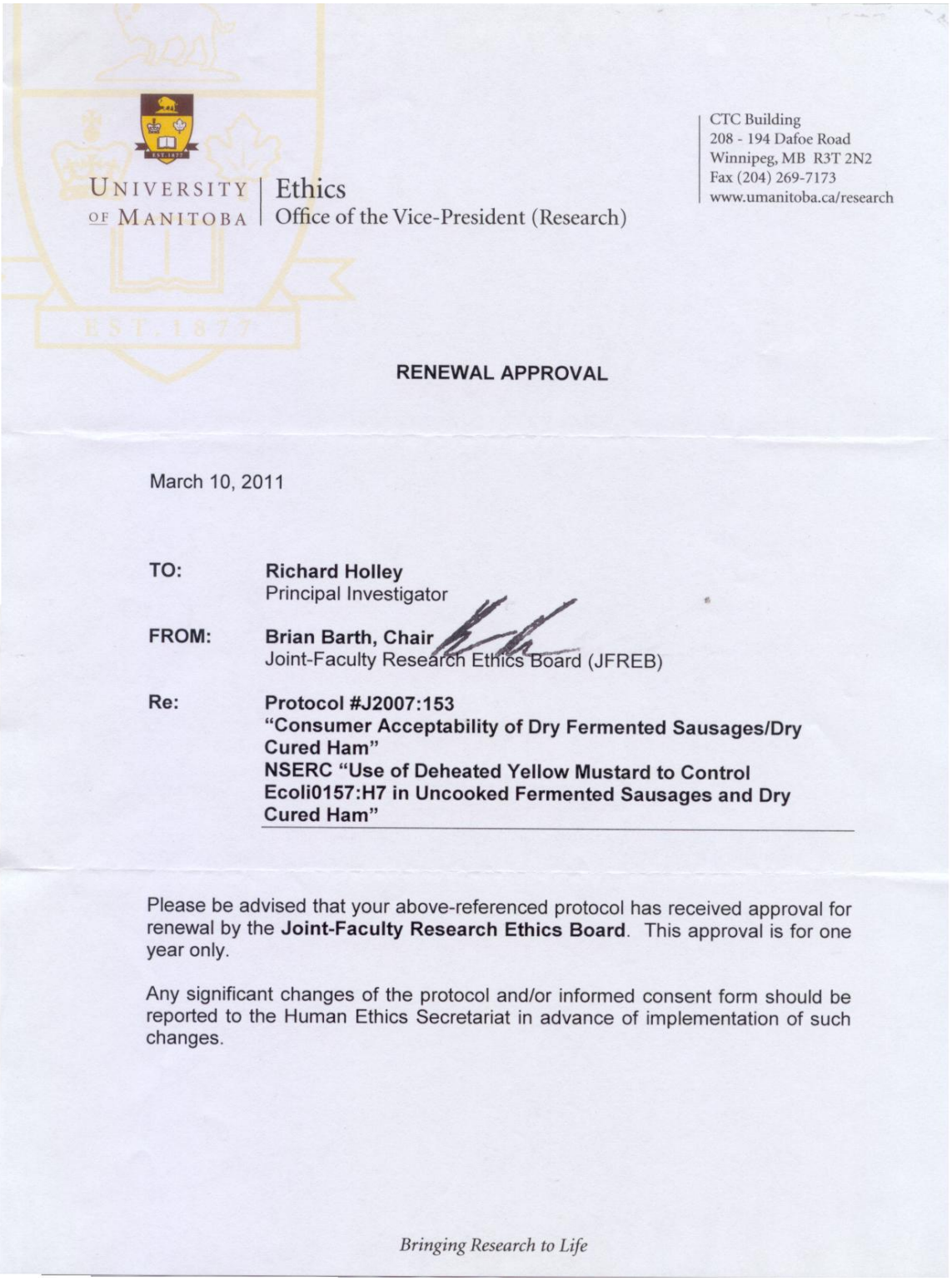
Verplaetse, A., Van Hoye, S., and Demeyer, D. (1990). The effect of chopping condition on dry sausage metabolism.36th ICoMST. Havana, Cuba, III, 920-927.

Williams, R.C., Isaacs, S., Decou, M.L., Richardson, E.A., Buffett, M.C., Slinger, R.W., Brodsky, M.H., Ciebin, B.W., Ellis, A., Hockin, J., and the *E. coli* O157:H7

Working Group. (2000). Illness outbreak associated with *Escherichia coli* O157:H7 in Genoa Salami. Can. Med. Assoc. J., 162, 1409-1413.

Appendices

Appendix A: Ethic approval form for sensory evaluation test



UNIVERSITY OF MANITOBA | Ethics
Office of the Vice-President (Research)

CTC Building
208 - 194 Dafoe Road
Winnipeg, MB R3T 2N2
Fax (204) 269-7173
www.umanitoba.ca/research

RENEWAL APPROVAL

March 10, 2011

TO: **Richard Holley**
Principal Investigator

FROM: **Brian Barth, Chair**
Joint-Faculty Research Ethics Board (JFREB)

Re: **Protocol #J2007:153**
"Consumer Acceptability of Dry Fermented Sausages/Dry Cured Ham"
NSERC "Use of Deheated Yellow Mustard to Control Ecoli0157:H7 in Uncooked Fermented Sausages and Dry Cured Ham"

Please be advised that your above-referenced protocol has received approval for renewal by the **Joint-Faculty Research Ethics Board**. This approval is for one year only.

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Bringing Research to Life

Appendix B: Consent form of sensory evaluation test

Consumer Acceptability of Dry Fermented Sausages

Researcher: Dr. R. Holley,
This consent form is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study is being done to evaluate the acceptability of dry fermented sausages made from pork and beef. Criteria for volunteers are that you like dry fermented sausages and have eaten it at least four times per year. You will be asked to taste no more than 5 samples in total and check the descriptor on a nine point scale how much you like the product overall as well as the color, flavour and texture of it. The single session you would attend would be about 30 minutes. You would receive a snack such as a bag of chips, chocolate bar, granola bar or juice box for participating. Information regarding the project will be sent to participants within one month from completion of data collection.

A concern is a possible allergy to the ingredients of the meat products. The questionnaire (attached to this consent form) regarding allergies to be completed by participants will be used to screen panellists with a potential health risk.

All data related to personal information will be kept in locked cabinet and destroyed one feedback about the study has been provided. Data collected will be summarized as group means and frequencies and kept in a locked cabinet for five years or until data are published whichever comes first. Access to information will be limited strictly to the researcher named above. All data will be shredded after the time has expired.

Your signature on this form indicates that you have understood to your satisfaction regarding participation in the research project and agree to serve as a subject. In no way does this waive your legal rights nor release the researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and/or refrain from answering any questions you prefer to omit, without prejudice or consequence. You continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. This study is being conducted by Dr. Holley, Professor, Department of Food Science 474-9601 rick_holley@umanitoba.ca.
Any questions you have can be directed to Shuliu Li at umli293@cc.umanitoba.ca.

This research has been approved by the Joint-Faculty Research Board of Ethical Review at the University of Manitoba. If you have any concerns or complaints about this project you may contact any of the above-named persons or the Human Ethics Secretariat at 474-7122. A copy of this consent form will be given to you to keep for your records and references.

_____

Participant's Signature Date

Telephone Number_____ Email Address_____

_____

Research and/or Delegate's Signature Date