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Title: Effect of Mustard Powder on the Survival of Salmonella ser. Typhimurium and Penicillium chrysogenum in Shredded Mozzarella Cheese

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**Abstract**

Glucosinolates, components of mustard, when hydrolyzed, produce strong antimicrobial agents called isothiocyanates. The objective of this study was to evaluate the effect of mustard powder on the survival of *Salmonella* ser. Typhimurium and *Penicillium chrysogenum* in shredded Mozzarella cheese. Mustard powder at 0, 3, 5, 9, and 17% (w/w) was mixed into shredded cheese and packaged in polyethylene retail bags. The challenge study against *S.* Typhimurium and *P. chrysogenum* was conducted under modified (70% nitrogen, 30% carbon dioxide) and normal atmospheric conditions, respectively. Sample treatments were inoculated with the test organisms and stored at 4°C or 25°C. Increased concentrations of mustard powder resulted in an increased rate of reduction of *S.* Typhimurium. Addition of 17% mustard powder to the cheese reduced *S.* Typhimurium populations by greater than 5-log CFU/gram in 20 days. Treatments had no significant effect in inhibiting the growth of *P. chrysogenum*. Consumer preference of important sensory attributes and acceptability was significantly lowered due to the addition of mustard powder to shredded Mozzarella cheese (p<0.05). The studies provide evidence that addition of mustard powder may not be a viable strategy to enhance safety or quality of shredded cheese products.
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# Table of Contents

Abstract ........................................................................................................................................... 2  

List of Tables .................................................................................................................................. 9  

List of Figures ............................................................................................................................... 10  

Chapter I: Introduction.................................................................................................................. 11  
  Statement of the Problem .................................................................................................. 14  
  Purpose of the Study ......................................................................................................... 15  
  Assumptions of the Study ................................................................................................. 15  
  Definition of Terms........................................................................................................... 15  
  Limitations of the Study.................................................................................................... 16  
  Methodology ..................................................................................................................... 17  

Chapter II: Literature Review ....................................................................................................... 18  
  Mozzarella Cheese ................................................................................................................ 18  
    Background ....................................................................................................................... 18  
    Mozzarella Cheese Composition .................................................................................. 19  
    Cheese Contamination .................................................................................................. 19  
    Routes of Cheese Contamination ................................................................................... 20  
  Microorganisms Relevant to Food Safety and Quality of Cheese Products ................. 21  
    *Salmonella* Species ........................................................................................................ 21  
    Mode of Infection ........................................................................................................... 23  
    Prevalence of *Salmonella* spp. in Food ........................................................................ 23  
    Salmonella Outbreaks in Cheese ............................................................................... 24  
    *Penicillium* Species ........................................................................................................... 24
Spoilage Molds in Cheese.......................................................................................... 25

*Penicillium chrysogenum* ...................................................................................... 25

*Penicillium* Species in Ripened Cheese................................................................ 26

Preservation of Cheese .............................................................................................. 26

Control of Mold in Cheese ...................................................................................... 26

Control of Spoilage and Pathogenic Bacteria in Cheese ......................................... 27

Packaging for the Preservation of Cheese ................................................................ 28

Modified Atmosphere Packaging (MAP) .................................................................. 28

Modified Atmosphere Packaging of Cheese .............................................................. 30

Mustard .......................................................................................................................... 31

Uses ............................................................................................................................... 32

Composition ................................................................................................................ 32

Mustard Types .............................................................................................................. 32

Processing of Mustard ............................................................................................... 33

Products of Mustard .................................................................................................... 34

Mustard Oil .................................................................................................................... 34

Prepared Mustard ....................................................................................................... 34

Mustard Flour .............................................................................................................. 34

Mustard Powder or Ground Mustard ....................................................................... 34

Functional Properties of Mustard Powder ............................................................... 34

Antioxidant .................................................................................................................. 34

Anti-carcinogen .......................................................................................................... 34

Antimicrobial .............................................................................................................. 35
Isothiocyanates in Mustard Powder .................................................................................. 35

Chapter III: Methodology ............................................................................................................. 38

Microbial Challenge Study: Effect of Mustard Powder on Salmonella ser. Typhimurium and Penicillium chrysogenum ..................................................................................... 38

Sample Selection and Description ......................................................................................... 38

Preparation of Mustard Treatment to Cheese ........................................................................ 39

Data Collection Methodology ........................................................................................................ 39

*Salmonella* ser. Typhimurium Strain Preparation ................................................................ 39

*Penicillium chrysogenum* Strain Preparation ......................................................................... 40

*Salmonella* ser. Typhimurium Inoculation ........................................................................... 40

*Penicillium chrysogenum* Inoculation ................................................................................. 40

Enumeration of *Salmonella* ser. Typhimurium ........................................................................ 40

Enrichment of Cheese Samples for the Enumeration of *Salmonella* ser. Typhimurium ........ 41

Effect of Mustard in the Growth of *Penicillium chrysogenum* ............................................ 41

Statistical Analysis ......................................................................................................................... 41

Sensory Study ................................................................................................................................. 42

Sample Selection and Description .............................................................................................. 42

Sample Preparation ....................................................................................................................... 42

Panelist Selection and Description ............................................................................................. 43

Sample Serving .............................................................................................................................. 43

Data Collection Methodology ......................................................................................................... 43

Statistical Analysis ......................................................................................................................... 44
Chapter IV: Results

Effect of Mustard Powder on the Survival of *Salmonella* ser. Typhimurium at 4°C Storage Temperature

Effect of Various Treatments on Decimal Reduction Time (D-value)

Effect of Mustard Powder on the Survival of *Penicillium chrysogenum* in Shredded Mozzarella Cheese Stored at 25°C

Sensory Study

Gender and Age Distribution

Consumption of Cheese

Overall Liking of Appearance

Liking of Cheese Flavor

Overall Liking of Flavor

Overall Liking of Texture

Overall Acceptability

Multiple Comparisons

Limitations

Chapter V: Discussion

Effect of Mustard Powder Treatments on the Survival of *Salmonella* ser. Typhimurium

Effect of Mustard Powder on the Survival of *Penicillium chrysogenum*

Sensory Evaluation of Shredded Mozzarella Cheese Treated with Mustard Powder

Conclusions

Recommendations

References
Appendix A: Consent Form: Sensory Evaluation of Cheese........................................................ 70
Appendix B: Cheese Evaluation Form ......................................................................................... 71
Appendix C: Institutional Review Board (IRB) Approval Form.................................................. 73
List of Tables

Table 1: Reports of Outbreaks in the US. Associated with the Consumption of Cheese made with Pasteurized Milk from 1998-2012 .......................................................... 12

Table 2: General Consumption of Mozzarella Cheese ................................................. 20

Table 3: Recommended Modified Atmosphere Packaging (MAP) Conditions for Various Food Products ........................................................................................................... 30

Table 4: Population Number of Salmonella ser. Typhimurium in Modified Atmosphere Packaged Shredded Mozzarella Cheese with or without Mustard Powder and Stored at 4°C ................. 46

Table 5: Calculated Decimal Reduction Time (D-value) for Salmonella ser. Typhimurium in Mozzarella Cheese with Various Concentrations of Mustard Powder ...................... 48

Table 6: Presence or Absence of Penicillium chrysogenum Sporulation or Mycelial Growth in Mozzarella Cheese Samples with Various Treatments and Stored at 25°C .................. 49

Table 7: Descriptive Statistics (Mean Ratings and Standard Deviations) of the Liking for Appearance, Cheese Flavor, Overall Flavor and Overall Acceptability of the Cheese Samples Treated with or without Mustard Powder ................................................. 56
List of Figures

Figure 1: Processing of Mustard and Mustard Products.................................................................33
Figure 2: Mechanism of Enzymatic Hydrolysis of Glucosinolates in Mustard Powder..............36
Figure 3: Destruction Rate of *Salmonella* ser. Typhimurium in Cheese Samples Treated with or
without Mustard Powder............................................................................................................47
Figure 4. Gender Distribution of Sensory Panelists........................................................................50
Figure 5: Age Distribution of Sensory Panelists...........................................................................51
Figure 6: Cheese Consumption Pattern of Sensory Panelists........................................................51
Figure 7: Mean Scores for Overall Liking of Appearance for Treatment Samples as Evaluated
Along a 7-point Hedonic Scale......................................................................................................52
Figure 8: Mean Scores for Overall Liking of Cheese Flavor for Treatment Samples as Evaluated
Along a 7-point Hedonic Scale......................................................................................................53
Figure 9: Mean Scores for Overall Liking of Flavor for Treatment Samples as Evaluated Along a
7-point Hedonic Scale................................................................................................................54
Figure 10: Mean Scores for Overall Liking of Texture for Treatment Samples as Evaluated
Along a 7-point Hedonic Scale......................................................................................................54
Figure 11: Mean Scores for Overall Acceptability of Texture for Treatment Samples as
Evaluated Along a 7-point Hedonic Scale...................................................................................55
Chapter I: Introduction

Foodborne pathogens are found in a wide variety of foods and are responsible for outbreaks of many illnesses each year. Each year in the United States, 48 million people (about 1 in 6) get sick, 128,000 are hospitalized, and 3000 die of foodborne diseases (Centers for Disease Control and Prevention, 2014b). *Salmonella* is the major contributor of the foodborne illnesses, hospitalization and death in United States. *Salmonella* accounts for 11% of the total foodborne illnesses, 35% of total foodborne illnesses resulting in hospitalization and 28% of the total foodborne illnesses resulting in death (Centers for Disease Control and Prevention, 2014a).

Even though most cases of outbreaks have been associated with undercooked ground beef and raw milk, there have been many cases of outbreaks in other foods including cheese, apple juice, salami, yogurt, and mayonnaise. Milk and milk products are considered as the second most common source of foodborne infection that is responsible for 14% of total illnesses and 10% of total deaths (Painter et al., 2013). There have been several reports of severe food poisoning from pasteurized dairy products around the world, although pasteurized they are considered very safe to consume. Surface post-processing contamination by vegetative pathogens at home or in the food-service environment is considered potential risk factor (Glass, Kaufman, & Johnson, 1998).

According to the definition of ‘potentially hazardous food’ defined by the Food and Drug Administration (FDA), cheese is a potentially hazardous food because it falls under the category of foods with pH >4.6 and water activity >0.85 (Glass et al., 1998). There have been a total of 29 outbreaks related to the consumption of pasteurized dairy products among which 19 outbreaks are related to the consumption of cheese made from pasteurized milk from 1998-2012 (Real Raw Milk Facts, 2011).
Table 1

Reports of Outbreaks in the U.S. Associated with the Consumption of Cheese made with Pasteurized Milk from 1998-2012 (Real Raw Milk Facts, 2011)

<table>
<thead>
<tr>
<th>Year</th>
<th>Causative Pathogen</th>
<th>Causative Food</th>
<th>Number of Illnesses</th>
<th>Hospitalized /Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ricotta Salata cheese</td>
<td>22</td>
<td>20/4</td>
</tr>
<tr>
<td>2010</td>
<td><em>Listeria monocytogenes</em></td>
<td>Queso Fresco cheese</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2010</td>
<td><em>Campylobacter jejuni</em></td>
<td>Cheese curds</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2009</td>
<td><em>Listeria monocytogenes</em></td>
<td>Cheese</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2008</td>
<td><em>Listeria monocytogenes</em></td>
<td>Queso Fresco cheese</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2008</td>
<td><em>Salmonella Java</em></td>
<td>Cheddar cheese</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>2007</td>
<td><em>Salmonella Montevideo</em></td>
<td>Shredded cheese</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>2006</td>
<td><em>Norovirus</em></td>
<td>Swiss cheese</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>2006</td>
<td><em>Listeria monocytogenes</em></td>
<td>Cheese</td>
<td>3</td>
<td>2/1</td>
</tr>
<tr>
<td>2004</td>
<td><em>Norovirus</em></td>
<td>Cheese</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>2003</td>
<td><em>Norovirus</em></td>
<td>Cheddar cheese</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>2002</td>
<td><em>Norovirus</em></td>
<td>Cheese</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td><em>Norovirus</em></td>
<td>Swiss cheese</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td><em>Salmonella Newport</em></td>
<td>Cheese</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2001</td>
<td><em>Staphylococcus aureus</em></td>
<td>Cheese</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>2001</td>
<td><em>Norovirus</em></td>
<td>Swiss cheese</td>
<td>73</td>
<td>0</td>
</tr>
<tr>
<td>1999</td>
<td><em>Norovirus</em></td>
<td>Cheese</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>1998</td>
<td><em>Norovirus</em></td>
<td>Cheese</td>
<td>258</td>
<td>4</td>
</tr>
</tbody>
</table>
The outbreaks consisted of a total of 2,824 illnesses and 8 deaths related to the consumption of pasteurized dairy product. The outbreaks include, 575 illnesses and 5 deaths from the consumption of contaminated cheeses made with pasteurized milk (Real Raw Milk Facts, 2013). Table 1 shows reports of outbreaks associated with the consumption of cheese made with pasteurized milk from 1998-2012.

Cheese, specifically Mozzarella is a popular ready-to-eat food product among consumers. Microbiological studies on fresh Mozzarella shows the presence of several microbiological species including *Lactobacillus lactis* subsp. *lactis, L. lactis* subsp. *diacetylactis, L. lactis subsp. cremoris, Streptococcus thermophilus, Enterococcus faecium*, *E. faecalis, Escherichia coli*, yeasts and molds, and various other spoilage microorganisms (Altieri, Scrocco, Sinigaglia, & Nobile, 2005). Presence of these microorganisms makes Mozzarella cheese prone to spoilage and a shorter shelf life. Fungal contamination during processing or packaging and post-processing (unclean equipment, dirty packaging materials, poor packaging material etc.), has been a significant challenge to food industry (Grove, 1998). Contamination during shredding can lead to reduced shelf life of shredded Mozzarella cheeses (Ryser, 2001, p. 476). *Penicillium* and *Aspergillus* species are the major fungal flora found in spoiled cheese products. Among the 371 cheese spoilage fungi identified by Lund, Filtenborg, and Frisvad (1995), *Penicillium* species accounted for 91% of the fungal contaminants. Spoilage of cheese due to these fungal contaminants has a serious economic effect on the cheese industry as well as on the consumers.

Fresh Mozzarella cheese, a ready-to-eat food, is usually eaten without further cooking and can easily be contaminated on the surface by undesirable microorganisms (Lara-Lledó, Olaimat, & Holley, 2012). The treatment with natural or synthetic antimicrobial agents can be a solution but these agents can be easily absorbed into the food or neutralized (Shin, Harte, Ryser,
Since most microbial contaminations occur due to surface contamination, maintaining antimicrobial properties on the product surface is a very important factor to extend food shelf life and to enhance the safety of food. Consumers do not prefer the use of antimicrobial agents like organic acids and their salts, bacteriocins and nitrites. However, natural antimicrobials such as essential oils and spices are popular among consumers because of their perceived lower risk to consumers (Lara-Lledó et al., 2012). Consumer awareness, clean label demands by retailers, and the restrictions imposed on the use of synthetic food preservatives by regulatory agencies, have all led to an interest in natural food preservatives, especially those derived from plants (Delaquis & Mazza, 1995). In this context, glucosinolates, components of many cruciferous vegetables including especially mustard, horseradish and wasabi, have a potential for use as an antimicrobial agent in various food products (Lara-Lledó et al., 2012; Shin et al., 2010).

This study evaluated the antimicrobial property of yellow mustard powder in shredded Mozzarella cheese, under normal and modified atmosphere (MA) packaged conditions. More specifically this study tested the hypothesis that mustard powder can inhibit *Salmonella* ser. Typhimurium and *Penicillium chrysogenum*.

**Statement of the Problem**

There have been several reports around the world of severe food poisoning related to cheese consumption. Cheese spoilage due to fungal contaminants also causes serious financial losses to the food industry and to consumers. Since most contamination occur due to surface contamination, maintaining antimicrobial and antifungal properties on the surface of cheese is very important to enhance the safety and quality of cheese. Treatment with antimicrobial agents can be a solution but these treatments can be easily absorbed into food or neutralized. Consumer
awareness, clean labels demands from retailers and consumers alike, as well as restrictions imposed on the use of synthetic food preservatives by regulatory agencies has led to an interest in natural food preservatives, especially those derived from plants.

**Purpose of the Study**

The purpose of this study was to evaluate the antimicrobial property of yellow mustard powder in shredded Mozzarella cheese packaged under modified atmosphere (MA) packaging stored at 4°C, and under normal atmosphere packaging stored at 25°C. More specifically this study tested the hypothesis that mustard powder can inhibit *Salmonella* ser. Typhimurium and *Penicillium chrysogenum*.

**Assumptions of the Study**

One of the assumptions in the study was that the mustard powder and cheese samples were assumed to not contribute to the microbiology of the product. This assumption may not be true and may have had some effect on the mycelial presence and sporulation during the evaluation of the effect of mustard powder on the survival of *Penicillium chrysogenum* when stored at 25°C.

**Definition of Terms**

**Antimicrobial preservatives.** “Compounds, either present naturally, formed during processing, or legally added as ingredients that can kill microorganisms or control their growth in food” (Ray & Bhunia, 2007, p. 403).

**Bacterial colony.** “Large group of bacteria clustered together visible to naked eye” (Perry & Pawsey, 1973, p. 6).

**Bactericidal.** “Capable of killing bacteria, but not necessarily bacterial spores” (Perry & Pawsey, 1973, p. 32).

Decimal reduction time (D-value). “The time during which the number of a specific microbial population exposed to a specific temperature is reduced by 90% or 1-log” (Ray & Bhunia, 2007, p. 374).


Modified Atmosphere Packaging (MAP). “Type of packaging method to extend the shelf life of product where food is enclosed in high barrier packaging material; air is removed from the package, which is then flushed with a particular gas or combination of gases and the package is hermetically sealed” (Ray & Bhunia, 2007, p. 397).


Limitations of the Study

A first limitation was the low number (n=64) of panelists for sensory study. At least 100 panelists are usually recommended for a valuable sensory analysis. A second limitation was the length of study. Number of samples packaged for the experiment was only enough until day 20 for the study of the survival of Salmonella ser. Typhimurium. Rate of destruction of Salmonella ser. Typhimurium after 20 days would provide a better understanding of days required for complete destruction of bacterial population.
Methodology

The variables for this research model includes 0% mustard powder (control), 3% mustard powder, 5% mustard powder, 9% mustard powder and 17% mustard powder and length of storage (days). Shredded Mozzarella cheese was obtained from Cady Cheese LLC and packaged under modified atmosphere or normal atmosphere conditions. For data collection, shredded Mozzarella cheese was aseptically mixed with mustard powder at the concentration of 0, 3, 5, 9 and 17% (w/w). Packages containing fifty grams of cheese treatments were package sealed with or without a gas flush (70% nitrogen and 30% carbon dioxide). *Salmonella* ser. Typhimurium or *Penicillium chrysogenum* was inoculated at the concentration of 6.2-log CFU/gram or $10^4$ spores/ml, respectively. Cheese samples were sampled periodically during storage for enumeration of *Salmonella* populations (days 0, 1, 2, 3, 5, 8, 11, 15 and 20). A decimal dilution of the sample was plated on Xylose Lysine Deoxycholate (XLD) agar and the plates were incubated at 4°C for 24 hours before colonies were counted. Growth of *Penicillium chrysogenum* was monitored in packages packaged under normal atmospheric condition, through visual observation for mycelial presence and sporulation on days 1, 3, 5, 7, 9, 12 and 15. In another experimental series of consumer acceptance, a total of 64 panelists evaluated consumer liking and acceptability of cheese to which mustard powder was added at the test concentrations. Significant differences between treatment means in microbiology studies were analyzed using Analysis of Variance (ANOVA) or Chi-square analysis using IBM Statistical Package for the Social Sciences (IBM SPSS Statistic 20). Consumer acceptability data was analyzed using CompuSense® software (5.0 version, CompuSense Inc, Guleph, Ontario, Canada).
Chapter II: Literature Review

This study evaluated the antimicrobial property of mustard powder, in shredded Mozzarella cheese packaged under modified atmosphere (MA) packaging stored at 4°C, and under normal atmosphere packaging stored at 25°C. More specifically this study tested the hypothesis that mustard powder can inhibit *Salmonella* ser. Typhimurium and *Penicillium Chrysogenum*.

The following review of literature begins with the discussion of Mozzarella cheese and microorganisms related to the foodborne illness and spoilage of cheese. A discussion of preservation of cheese is followed. Mustard powder and its potential use as a natural antimicrobial in food are also discussed. The literature review also addresses the theory behind the antimicrobial property of Isothiocyanates (ITCs) present in mustard powder and its potential application in food packaging to extend the shelf life and enhance food safety.

**Mozzarella Cheese**

**Background.** Mozzarella cheese is a soft, unripened cheese, which originated in the Battipaglia region of Italy (Jana & Mandal, 2011). Originally, Mozzarella cheese was made from buffalo milk. However today it is mostly made from cow milk (Ghosh, Singh, & Kanawjia, 1990). Fresh Mozzarella cheese is white in color, has a glossy texture and has an excellent stretch property making it suitable for pizza topping (Jana & Mandal, 2011). Mozzarella cheese is one of the most popular cheeses in the world. It is widely used in the preparation of many dishes, especially lasagna and pizza. According to the U.S. Department of Agriculture, Dairy Products Annual Summary, in 2012 approximately one third of the total cheeses produced in the United States were Mozzarella cheese (Wisconsin Cheese, 2013). Mozzarella cheese, along with Cheddar cheese is the most popular cheese in the United States with an average annual
consumption of 10.6 and 10 pounds per person, respectively (Geisler, 2012). Mozzarella is also the cheese of choice for pizza because it becomes very stringy when cooked and has an excellent stretch property (Ridgway, 1999). The major characteristics of Mozzarella cheese are the stretchability and melting characteristics which is influenced by the composition and product pH (Fox & Guinee, 1987). The authors in their study suggested that among the 11 varieties of cheese examined, Mozzarella had the highest value of elasticity. The shredded form of Mozzarella cheeses is popular because it is ready to use and the convenience is highly preferred by consumers.

**Mozzarella cheese composition.** Several factors influencing the composition of Mozzarella cheese includes the type of milk used, procedure of cheese manufacturing, as well as the class of Mozzarella cheese being manufactured (Jana & Mandal, 2011). Based on moisture and fat composition, the USDA classifies Mozzarella cheese into five groups, regular, part-skim, low-moisture, low-moisture part skim, and Lite. Mozzarella cheeses can be found in various forms like shredded, diced, loaf, sliced etc. The majority of the composition of Mozzarella cheese includes moisture, fat and protein. Jana and Mandal (2011) reported the general composition of Mozzarella cheese, which is shown Table 2.

**Cheese contamination.** Generally, cheeses are regarded as one of the safest food products. However, there have been several reports of severe food poisoning linked to the consumption of cheese and cheese products (Kousta, Mataragas, Skandamis, & Drosinos, 2010). Ready-to-eat cheeses like Mozzarella are usually eaten without further cooking. Cheese can also be contaminated by undesirable microorganisms at the post-processing level (Lara-Lledó et al., 2012)
Table 2

*General Composition of Mozzarella Cheese*

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>48–51</td>
</tr>
<tr>
<td>Fat</td>
<td>25–28</td>
</tr>
<tr>
<td>Protein</td>
<td>20–21</td>
</tr>
<tr>
<td>Lactose</td>
<td>1–2</td>
</tr>
<tr>
<td>Ash</td>
<td>2–4</td>
</tr>
<tr>
<td>Salt</td>
<td>1–2</td>
</tr>
<tr>
<td>Acidity (lactic acid)</td>
<td>0.6–0.8</td>
</tr>
</tbody>
</table>

**Routes of cheese contamination.** The contamination of cheese can have various routes. Several routes of microbial contamination of cheese have been reported including milk, starter culture, brine, floor, packaging material, cheese vat, cheesecloth, knife, storage coolers, production room, production workers and production environment (Kousta et al., 2010).

Studies have reported the contamination of cheese by pathogenic bacteria that survived outside the product, on equipment and storage facilities. A study conducted in Ireland at 5 different processing plants found the prevalence of *Listeria monocytogenes* on food-contact surfaces (2.9% of sampled surfaces) and non-food-contact surfaces (4.6% of sampled surfaces) (Cox et al., 1989 as cited in Kousta et al., 2010). A similar study conducted at two soft cheese processing plants found 3.3% prevalence of *Listeria monocytogenes* from non-food contact surfaces but did not find any on food-contact surface (Silva et al., 1989 as cited in Kousta et al., 2010). Food handlers also have been identified to be one of the major sources of contamination of cheese products. A 2.5 years study of dairy processing plant by Tondo, Guimarães,
Henriques, and Ayub (2000), found food handlers to be the route of *S. aureus* contamination of final product coming from raw milk. Literatures suggest that raw milk, food handlers, processing equipment, storage facilities and the exterior of cheese products itself can be routes of contamination of cheese.

Secondary contamination of cheese by pathogenic and spoilage bacteria as well as mold at consumer home is regarded as one of the major issue of ready-to-eat products like cheese. Cheese products can be easily cross contaminated at consumer homes or at retail locations. At consumer homes ready-to-eat products like cheese are highly prone to be cross contamination with raw meat products due to unsafe handling practices (Kennedy et al., 2011). In 2008, the largest listeriosis outbreak in Quebec was associated with the consumption of cross-contaminated cheese. A total of 38 confirmed cases were linked with the cross contaminated cheese shipped to more than 300 retailers (Gaulin, Ramsay, & Bekal, 2012).

**Microorganisms Relevant to Food Safety and Quality of Cheese Products**

*Salmonella* species. *Salmonella* is a gram-negative facultative anaerobic bacilli bacterium, which consists of more than 2600 serovars (Portillo, 1999, p. 3). The genus *Salmonella* has been divided into two species, *Salmonella enterica* (*S. enterica*) and *Salmonella bongori*. The species *S. enterica* is further subdivided into six subspecies including enterica, which consist of serovars commonly isolated from human and food products and are potentially pathogenic to human beings (D’Aoust, 2001, p. 164). The subspecies group enterica includes various serovars such as *S. enterica ser. Typhi, Paratyphi, Sendai, Typhimurium, Enteritidis, Choleraesuis, Dublin, Gallinarum/Pullarom, and Abortusovis*. The infections caused by *Salmonella enterica* are host and serovar specific. Some serovars cause disease only to human, some only in specific animals, whereas some cause infections in both human and animals.
Serovars Typhimurium and Enteritidis have caused several incidence of disease in human, cattle, poultry, swine, horses and rodents (Portillo, 1999, p. 4). In the present study, *Salmonella enterica enterica* ser. *Typhimurium*, commonly referred as *Salmonella* ser. Typhimurium, was used in the assessment of the safety of shredded Mozzarella cheese.

*Salmonella* species are widely found in the environment including soil, water, and food products. Gastrointestinal tracts of animals, birds, and insects as well as human are the habitats of *Salmonella* and act as a carrier of disease, Salmonellosis, which is characterized by diarrhea and abdominal cramps (Ray & Bhunia, 2008, p. 286). The survival of *Salmonella* for long period in hostile environment has been a major concern for human health (D’Aoust, 2001, p. 169). Generally a higher dose, as high as $10^5$ cells of *Salmonella* is required to cause the infection, but some strains of *Salmonella* that are acid-resistant can cause disease with significantly less number of cells. The *Salmonella* dose to cause disease is lowered when consumed with food, especially milk and cheese that neutralizes the stomach acidity (Ray & Bhunia, 2008, p. 286).

According to Centers for Disease Control and Prevention (CDC) estimates, each year in the United States, *Salmonella* causes about 1.2 million illness, 23,000 hospitalization and 450 deaths (CDC, 2014). *Salmonella* accounts for 11% of the total foodborne illnesses, 35% of total foodborne illnesses resulting in hospitalization and 28% of the total foodborne illnesses resulting in death (CDC, 2014). Every year in United States, several outbreaks related to foodborne pathogens like *Escherichia coli* O157:H7, *Salmonella* ser. Typhimurium, and *Listeria monocytogenes* are reported and *Salmonella* ser. Typhimurium accounts for about one-fourth of all cases of *salmonella* outbreaks (Rhee, Lee, Dougherty, & Kang, 2003). It causes typhoid-like disease in mice. In humans, it does not cause too severe symptoms and is not normally fatal. It
is characterized by diarrhea, abdominal cramps, vomiting and nausea and generally lasts up to 7 days. Most infections with *Salmonella* are traced back to dairy, poultry, meat, and fresh fruit and vegetable products including green-leafy vegetables.

**Mode of infection.** Ray and Bhunia (2008) outline the detailed steps involved in the pathogenesis of *Salmonella*. Pathogens begin to colonize in the large and small intestine after consuming contaminated food. They then attach themselves to the mucosal membrane in the intestines and attack the mucosal cells. This process is followed by the pathogen multiplying in the epithelial cells. The pathogen can also start to extend to other parts of the body but does not usually leave the GI tract. The targeted areas of the body depend on the strain of *Salmonella* and the status of the host body’s defense system. The multiplication of the pathogen in the epithelial cells ultimately leads to the lysis of the cells, causing severe inflammation at the infected area. The wound surrounding the affected area generates the release of prostaglandins and thus increasing the cyclic adenosine monophosphate (cAMP) levels in the mucosal cells. This interferes with the electrolyte balance of the mucosal cells. Increasing levels of cAMP prohibits the uptake of sodium (Na\(^+\)) ions and releases chloride (Cl\(^-\)) ions into the intestinal tract, instigating fluid loss in the intestinal tract, causing diarrhea.

**Prevalence of *Salmonella* spp. in food.** *Salmonella* species are widely found in the environment including soil, water, and food products. Foodborne *Salmonella* species responsible for illnesses have been isolated from various food sources. The prevalence of *Salmonella* species is very high in meat products including chicken, turkey, duck, pork, lamb, and beef. Consumption of fresh cantaloupes, watermelons, alfalfa and other vegetable sprouts, tomatoes and orange juice has widely been reported be a source of human Salmonellosis. Fish and shellfish have also been responsible as a source of *Salmonella*. Milk and milk products are
considered as one of the major food vehicle for *Salmonella* due to the occurrence of *Salmonella* in more than 9% of raw milk (D’Aoust, 2001, p. 171).

**Salmonella outbreaks in cheese.** Food products originating especially from animals including beef, chicken, turkey, pork, eggs, milk, and cheese have been responsible for many illnesses and outbreaks related to *Salmonella* (Ray & Bhunia, 2008, p. 287). Dairy related outbreaks of *Salmonella* are mostly linked with the consumption or contamination of raw milk however pasteurized milk and cheese has been responsible for foodborne outbreaks. There have been several reports of *Salmonella* outbreaks specifically linked with the consumption of cheese (Ryser, 2001, p. 473). In 1984, the largest cheeseborne salmonellosis outbreak occurred in Ontario and the four Maritime Provinces causing more than 2000 culture confirmed cases linked to Cheddar cheese made from pasteurized milk. Two distinct strains of *Salmonella* Typhimurium were identified in this incident. In 1989, 164 cases of salmonellosis related to Mozzarella cheese were detected in Minnesota, Wisconsin, Michigan, and New York. The two identified modes of infection were *Salmonella* Javiana and *Salmonella* Oranienburg. The cause of this outbreak was suspected to be inadequate factory sanitation practices and contamination of the cheese by infected production workers. A total of 339 cases of *Salmonella* Heidelberg gastroenteritis were identified in Colorado during 1976 and was linked to cheddar cheese manufactured in Kansas using pasteurized milk (Ryser, 2001, p. 473).

**Penicillium species.** *Penicillium* belongs to the kingdom *Fungi* and commonly referred as mold. They belong to the Hyphomycetes class and Eumycota division, class of fungi of significant importance to food and beverage industries. *Penicillium* species produce branch like structures known as mycelia. Conidia (spores) are produced directly on the tip of mycelia, usually as an individual unit and branch at the top and form chain-like structure and can be either
transparent or brightly colored (Pitt, 1979 as cited in Grove, 1998). *Penicillium* species are found almost everywhere mostly on moist, damp areas and soils. They cause spoilage in fruits, vegetables, grains, meat, breads and cheese (Grove, 1998; Ray & Bhunia, 2008).

Some of the *Penicillium* spp. includes *P. camemberti, P. caseicolum, P. roqueforti, P. commune,* and *P. chrysogenum.* *P. camemberti* and *P. roqueforti* are widely used in the cheese manufacturing process but also have been isolated from spoiled cheese (Grove, 1998). Methyl ketones and free fatty acids produced by *Penicillium* species give a distinct flavor and aroma to the cheeses (Hassan & Frank, 2001, p. 160).

**Spoilage molds in cheese.** Cheese is predominantly spoiled by *Aspergillus* and *Penicillium* spp. A study conducted on fungal flora in cheese spoilage indicated that around 91% of contamination was linked to *Penicillium* species (Lund et al., 1995) Another study conducted on semi-hard cheeses indicated *Aspergillus* and *Penicillium* species to be dominant in the cheese samples (Hoekstra et al. 1998 as cited in Grove, 1998). Among 144 spoilage fungi isolated from 14 varieties of cheeses, *Penicillium* species accounted for 69% of the spoilage (Marth and Yousef 1999 as cited in Grove, 1998). *Penicillium roqueforti* and *Penicillium camemberti* are widely used in mold-ripened cheese production and are associated with the cheese spoilage. Some other *Penicillium* spp. such as *P. bevicompactum, P. chrysogenum, P. commune,* and *P. coryliphilum* were also found responsible for the spoilage of cheese (Hoekstra et al., 1998 as cited in Grove, 1998).

*Penicillium chrysogenum.* *P. chrysogenum* was derived from the word *Penicillium griseoroseum* (Cruickshank & Pitt, 1987; Frisvad & Filtenborg, 1989 as cited in Pitt & Hocking, 2009, p. 235). It is a mesophilic species, grows at a minimum temperature of 4°C, optimum at 23°C and maximum at 37°. *P. chrysogenum* germinates at a water activity rate of 0.78-0.81
P. chrysogenum is widely spread and usually causes tainting and produces undesirable flavors in foods. P. chrysogenum has been isolated from various spoiled food products including, cheese and dairy products, bakery, margarine, cantaloupes and grapes (Pitt & Hocking, 2009, p. 237)

Penicillium species in ripened cheese. Spores of Penicillium species, especially P. camemberti can usually be added directly to the milk during some cheese preparation. Once the cheese is manufactured, it is ripened for about two weeks where the molds eventually grow all over the surface of cheese. As the mold grows, the cheese becomes soft with a creamy texture and appears yellowish in color. With further ripening, the cheese becomes softer just like the surface (Johnson, 2001, p. 361). P. camemberti is highly tolerable to salt content in cheese. Hence it is easy to grow on the cheese surface. The mold breaks down the milk fat and converts free fatty acids to methyl ketones. It also degrades milk protein casein resulting in an increased pH. At this point of stage, ammonia is released resulting in the aroma formation covering the interior and exterior portions of cheese (Jespersen & Josephsen, 2006, p. 177.13).

Preservation of Cheese

Control of mold in cheese. In general, mold growth is seen on the surface of cheese when there is adequate oxygen available i.e. exposed to air for a longer period of time (Johnson, 2001, p. 374). Controlling the amount of residual oxygen in the cheese packaging will control the mold growth, which can be achieved by modified atmosphere or vacuum packaging. Addition of antimicrobials like potassium sorbate and natamycin to cheese has been shown to
inhibit mold growth. The use of 0.3% of sorbic acid in cheese, which is the maximum permitted limit, can inhibit *Aspergillus spp.* but not enough for all strains of *Penicillium* (Liewen & Marth 1984, as cited in Johnson, 2001, p. 374). A polymer coating incorporated with natamycin or sorbates may be applied to prevent mold in cheeses, however, sorbates may cause off flavors due to diffusion to cheese (de Ruig & van den Berg, as cited in Johnson, 2001, p. 374). Use of ozone in cheese ripening rooms has been demonstrated and is widely used by cheese industries to reduce mold (Serra, Abrunhosa, Kozakiewicz, Venâncio, & Lima, 2003).

To prevent undesirable mold growth in cheese, following important steps are to be considered (Byrne & Bishop, 2001):

1. Maintaining the cheese plant hygiene by following HACCP plan.
2. Prevent any cross contamination by following good manufacturing practices (GMP) and proper sanitation techniques (SSOPs).
3. Maintain the environment where mold growth can be limited (optimum temperature, salt concentration, pH, moisture content, fermentation, storage temperatures etc.)

**Control of spoilage and pathogenic bacteria in cheese.** Unpasteurized milk and poor hygienic conditions are the main factors for the microbial spoilage in cheese (Johnson et al., 1990 as cited in Johnson, 2001). Some of the microbial genera that survive pasteurization include *Mycobacterium, Clostridium* spores, *Micrococcus, Bacillus* spores, *Strepococcus, Enterococcus, Coryneform, and Lactobacillus* and may be responsible for the abnormal and undesirable colors and flavors in cheese (Hull et al., 1992 as cited in Johnson, 2001 p. 253). Some pathogenic bacteria like *Salmonella* species, *Listeria monocytogenes* and *E. coli* may be exposed to cheese by cross contamination. To control the growth of microbes, several procedures can be employed. This starts with cleaning and sanitizing the pasteurizer from time
to time. Sanitation techniques will reduce the growth of pathogenic and spoilage bacteria.

Some European countries use sodium nitrate to control the growth of *Clostridium tyrobutyricum* spores (Johnson, 2001).

Since many cheese products may naturally contain bacterial species, identification of microorganisms is very crucial to maintain the quality and safety of cheese products. Molecular techniques such as polymerase chain reaction (PCR) have been applied to identify the presence of specific bacterial strains in cheese (Atlas & Bej, 1994 as cited in Johnson, 2001).

Several methods and techniques can be utilized to enhance the safety and quality of cheese. Methods that have the potential to prevent spoilage and pathogenic bacteria in cheese are outlined below (Champagne & Laing, 1994).

1. Low temperature- Slows down the growth of bacteria.
2. High temperature- Denaturation of cell components
3. Hydrogen peroxide- Activates lactoperoxidase system.
4. Chlorine- Kills bacteria
5. Carbon dioxide injection- Influences membrane permeability
6. Sorbate- Inhibits enzyme system
7. Lysozyme- Attacks cell wall

**Packaging for the Preservation of Cheese**

**Modified atmosphere packaging (MAP).** It is the type of packaging method of food using high barrier packaging material in which air is removed from the package and then replaced with particular gas or combination of gases and the package (Ray & Bhunia, 2007, p. 397). The objectives of the MAP system is to control microbial growth, prevent any undesirable changes in the nutritive and sensory quality of food, and hence extend the shelf life of the food
product (Floros & Matsos, 2005, p. 160). Major gases used in MAP are nitrogen, oxygen and carbon dioxide and play an important role in the preservation of food. Nitrogen is an inert and tasteless gas, and its role is to displace oxygen and prevent collapse of the package. Oxygen is an important gas in the MAP system and is usually avoided because it promotes lipid oxidation, rapid ripening of fruits and vegetables and spoilage due to microbial growth. Carbon dioxide inhibits the growth of microorganism and also slows down the respiration of food product (Floros & Matsos, 2005, p. 161). Carbon dioxide in MAP has been demonstrated to inhibit most food spoilage microorganisms, especially psychrotrophic species such as *Pseudomonas*, which can grow in wide range of refrigeration temperatures. Growth of several microorganisms has been shown to be inhibited in MAP foods including, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Plesiomonas spp.*, *Staphylococcus aureus*, *Vibrio cholera*, and *Vibrio parahaemolyticus* (Robertson, 2013, p. 438). The composition of gas used in the MAP system varies depending on the type of product. Table 3 below displays the recommended MAP conditions to maximize the shelf life of various food products (Floros & Matsos, 2005, p. 165).

MAP has several advantages and has been demonstrated to potentially extend the shelf life by 50%. MAP has also shown to prevent spoilage and reduce economic loss, enhance quality, and maintain the integrity of the product (Farber, 1991).
Table 3

*Recommended Modified Atmosphere Packaging (MAP) Conditions for Various Food Products*

<table>
<thead>
<tr>
<th>Food</th>
<th>Temperature (°C)</th>
<th>Oxygen (%)</th>
<th>Carbon dioxide (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard cheese</td>
<td>1–4</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Soft cheese</td>
<td>1–4</td>
<td>0</td>
<td>20–40</td>
</tr>
<tr>
<td>Beef</td>
<td>-1–2</td>
<td>60–80</td>
<td>20–40</td>
</tr>
<tr>
<td>Pork</td>
<td>-1–2</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Poultry</td>
<td>-1–2</td>
<td>0</td>
<td>25–35</td>
</tr>
<tr>
<td>Bread (sliced)</td>
<td>Ambient</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

**Modified atmosphere packaging of cheese.** Packaging of cheese depends on the type of the cheese and their composition. Both MAP and vacuum packaging is used to preserve and extend the shelf life of cheese. Vacuum packaging is used by manufacturers to package cheese but with the disadvantage of possible damage of product and inconvenience associated with opening vacuum packaged products. Because of the disadvantage, cheeses especially soft are packaged in modified atmosphere. A study of the stability of shredded Mozzarella cheese under modified atmospheres demonstrated 75% carbon dioxide level to be very effective in maintaining microbial quality and safety of cheese by inhibiting yeast and mold (Eliot, Vuillelmerd, & Emond, 2006). However, the effect of higher concentration of carbon dioxide on sensory attributes of cheese was not mentioned in the study.

Hard cheeses are usually packaged with 100% carbon dioxide while soft cheeses are usually packaged with the mixture of 20-40% carbon dioxide and 60-80% nitrogen (Floros &
Matsos, 2005, p. 167). For the current study, MAP condition of 70/30% mixture of nitrogen/carbon dioxide was used for shredded Mozzarella cheese packaging.

MAP extends the shelf life of cheese product by inhibiting spoilage bacteria, including *Pseudomonas, Acinetobacter* and *Moraxella*. However, foodborne pathogens including certain Salmonella strains, *Listeria monocytogenes, Staphylococcus aureus* and *Yersinia enterocolitica* can grow in MAP products, and cause safety issues. MAP alone may not be a viable control technique to enhance the microbial safety and should be combined with other technologies like refrigeration, irradiation, hurdle technology, use of antimicrobial agents including, edible coatings, natural antimicrobials etc. (Floros & Matsos, 2005, p. 168). Many substances derived especially from plants like garlic, olive leaf, cinnamon, clove, onion, tea, thyme, mustard etc. have been used as natural antimicrobial agents to enhance safety and quality of food. Cruciferous vegetables especially mustard, horseradish and wasabi, have a potential for use as an antimicrobial agent in various food products (Lara-Lledó et al., 2012; Shin et al., 2010).

**Mustard**

Mustard has been used for thousands of years and the earliest evidence of use was seen in Sanskrit and Sumerian texts dating back about 3000 years (Cui & Eskin, 1998). The name ‘mustard’, originated from the Latin term “mustum ardens,” which means “burning must”. During the ancient time, mustard was primarily used to mask the taste and flavor of spoiled food, due to its strong flavor. However, later the use of mustard as a masking agent declined due to the advancement in food science and technology (Cui & Eskin, 1998). More than 320 million pounds of mustard is used worldwide per year and it is considered the largest volume spice in the international trade market (Cui & Eskin, 1998) and is primarily grown for spice trade as a source of condiment (Abdul-Fadl, EL-Badry, & Ammar, 2011).
**Uses.** Mustard has been widely used since ancient time as antibacterial and antifungal properties. Mustard powder has been shown to facilitate the secretion of gastric juice and hence aids in digestion (Ammar, 2012). In many communities, mustard is traditionally used as a laxative, medicine and antiseptic agent for various gastrointestinal, respiratory and skin diseases as well as to stimulate appetite (Abdul-Fadl et al., 2011). Mustard protein consists of many essential and non-essential amino acids and is favorable with the requirement for human nutrition (Hendrix et al., 2012). Mustard is a functional food with many beneficial effects on human being. It is considered as a source of many beneficial components like isothiocyanates, phenolics, dithiolthiones and dietary fiber (Hendrix et al., 2012).

Since mustard powder is very rich in lysine with adequate amounts of sulfur-containing amino acids, which is the limiting amino acid in most wheat flour based products, it has the potential to be used to highly enhance nutritive value of products (Ammar, 2012). Food Technology listed protein fourth, on its top ten functional food trends list and suggested it to have a great potential for new product development (Sloan, 2014). There has been an increasing amount of consumer interest in high protein food leading to the interest of protein fortification by manufacturers and mustard being high on protein is of special interest (Abdul-Fadl et al., 2011).

**Composition.** Mustard seeds are typically composed of 23-30% protein, 29-36% of oil and 12-18% of carbohydrate as well as minor components including 4% minerals, (phosphorus, calcium, potassium, and magnesium), 0.8-2.3% isothiocyanates, 2-3% phytin as well as some phenolic compounds (Cui & Eskin, 1998).

**Mustard types.** Mustard belongs to the family of cruciferae family of plants. Two main species of mustard are commonly used in North America, which include *Sinapis alba*, referred as “white” or “yellow” mustard, and *Brasica juncea*, referred as “brown” or “oriental” mustard (Li,
Aliani, & Holley, 2013). Brown or oriental mustard is highly regarded in Indian subcontinent because of its high oil content. Yellow or white mustard contains less oil but is higher in mucilage (Cui & Eskin, 1998). Besides oil, mustard consist of isothiocyanates, euric acid, phenolics, phytin, dithiolthiones and dietary fiber (Cui & Eskin, 1998). In the presence of moisture, brown and oriental mustard have been shown to release abundant amounts of compound allyl isothiocanate (AITC) whereas yellow mustard has shown to release compound 4-hydroxybenxyl isothiocyanate in abundant quantity (Cui & Eskin, 1998).

**Processing of mustard.** After harvesting the mustard seeds for processing, the seeds are dried at the temperature maximum of 32°C to minimize any mold growth as well as to prevent the destruction of enzyme myrosinase. Processing of mustard varies depends on the interest of the final product, which may be mustard oil, mustard flour, ground mustard (mustard powder), or deheated ground mustard. Figure 1 shows the outline of commercial processing of mustard powder into various products (Cui & Eskin, 1998).

![Figure 1. Processing of mustard and mustard products (Cui & Eskin, 1998).](image-url)
**Products of Mustard**

**Mustard oil.** Mustard oil is the second widely used edible oil in India after soybean oil and also is used for body massaging, as hair oil, illuminant and lubricant (Cui & Eskin, 1998). Mustard oil is used as an ingredient in mayonnaise in Sweden; and in some countries is the by-product of condiment industries (Cui & Eskin, 1998).

**Prepared mustard.** Prepared mustard is a condiment product widely used especially in North America and Europe and generally composed of ground mustard powder and/or mustard flour with salt, vinegar, sugar, dextrose, spices and other condiments (Cui & Eskin, 1998).

**Mustard flour.** Mustard flour is obtained from the endosperm of fine ground mustard seed. It is usually used as ingredient in salad dressing, mayonnaise, barbecue sauces, pickles, and processed meats (Cui & Eskin, 1998).

**Mustard powder or ground mustard.** Mustard powder is obtained by grinding whole mustard seeds and is mainly used in the meat industry as an emulsifier, water/fat binder, bulking agent, and seasonings for frankfurters, bologna, salami, and lunch meat (Cui & Eskin, 1998).

**Functional Properties of Mustard Powder**

**Antioxidant.** Mustard powder is an excellent source of antioxidants because it is rich in phenolic compounds (Saleemi, Janitha, Wanasundara, & Shahidi, 1993). The authors demonstrated the addition of 1.5% mustard powder to comminuted pork to have similar property as of 200ppm of butylated hydroxytoluene (BHT) or 30 ppm of tertiary-butyl hydroquinone (TBHQ). The authors also showed that the addition of mustard powder enhanced cooking yield without any unfavorable effect on color of the product.

**Anti-carcinogen.** Isothiocyanates, abundantly present in mustard powder has the potential to prevent bladder cancer (Bhattacharya et al., 2010). The authors demonstrated
mustard powder to inhibit bladder cancer development and progression in a rat bladder cancer model.

**Antimicrobial.** Several authors have reported the antibacterial activity of mustard against bacterial and fungal pathogens, nematodes, insects and weeds (Ammar, 2012; Lara-Lledó et al., 2012). Glucosinolates, grouped in the cells of mustard, are known precursors of isothiocyanates (ITCs) which play a significant role in the antimicrobial property of mustard (Lara-Lledó et al., 2012).

**Isothiocyanates in Mustard Powder**

Mustard consist of an abundant amount of glucosinolates, glycosides stored within cell vacuole (Delaquis & Mazza, 1995). When mustard seed is injured or crushed, glucosinolates are hydrolyzed by enzyme myrosynase to various ITCs (figure 2), components responsible for hot spiciness and pungent aroma of mustard (Cui & Eskin, 1998). As shown in figure 2, brown and oriental mustard release compound allyl isothiocanate (AITC) whereas yellow mustard releases 4-hydroxybenzyl isothiocyanate.
Figure 2. Mechanism of enzymatic hydrolysis of glucosinolates in mustard powder (Cui & Eskin, 1998).

ITCs have been utilized as food preservatives and has demonstrated to be very effective against a wide range of microorganisms (Shin et al., 2010). ITCs are considered to be highly fungitoxic and can be a potent antifungal agent against mycotixin-producing molds such as *Aspergillus flavus*, *Penicillium citrinum* and *Fusarium graminearum* (Cui & Eskin, 1998). The authors also suggested that ITCs have been demonstrated to inhibit the growth of several yeasts including *Neurospora* yeast. Delaquis & Sholberg (1997) demonstrated that ITC slowed the development of bacterial colonies leading to inhibition of bacterial cells and fungal conidia. This suggests the antimicrobial property with bacteriostatic, bactericidal, fungistatic and fungicidal activity. The authors also showed ITCs to be more effective against fungi and gram-negative aerobic bacteria compared to anaerobic foodborne pathogens. Because of the excellent antifungal properties of ITCs in the destruction of spores at low concentration, the authors recommended further research on the use of ITCs in packaged food products. Several studies
have been conducted to determine the minimum inhibitory concentration (MIC) of ITCs required in inhibiting various pathogens. Study conducted to determine the MIC of AITC on *E. coli* B34, *Salmonella* ser. Typhimurium and *L. monocytogenes* found the MIC to be 50, 100 and 200 μg/ml respectively (Lin, Preston, & Wei, 2000). This study also supports the results by previous authors that AITC was more effective on gram-negative than gram-positive bacteria. Lin, Preston, and Wei (2000) further demonstrated AITC to be a strong antimicrobial agent against bacteria at both stationary and exponential phases, suggesting it to be very suitable for application as a food preservative. Winther and Neilsen (2006) utilized the active packaging of semi-hard cheese with AITC labels and demonstrated that AITC to be an effective antimicrobial agent in cheese. They showed that the shelf life of cheese stored in atmospheric air without an AITC label was four and half weeks. The shelf life of cheese was extended to 13 weeks using one AITC label and two AITC label further extended the shelf life to 28 weeks. The shelf life of cheese stored in MAP without AITC labels was 16 weeks. This showed that increasing the AITC concentration extended the shelf life from 18 to 28 weeks (Winther & Nielsen, 2006).
Chapter III: Methodology

The objective of this study was to evaluate the antimicrobial property of yellow mustard powder in shredded Mozzarella cheese, under normal and modified atmosphere (MA) packaged conditions. Specifically this study tested the hypothesis that mustard powder can inhibit Salmonella ser. Typhimurium and Penicillium chrysogenum. This chapter deals with the materials and methodology of the microbial challenge study and consumer sensory evaluation. This chapter discusses sample description, experimental design, preparation, packaging, data collection, and data analysis methodology for the experiments conducted as part of the study.

Microbial Challenge Study: Effect of Mustard Powder on Salmonella ser. Typhimurium and Penicillium chrysogenum

Sample selection and description. Commercially packaged shredded Mozzarella cheese (no added preservative) was purchased from a local manufacturer, Cady Cheese LLC (Wilson, Wisconsin) in five-pound retail polyethylene packages and stored at 4°C until further use. Ingredients used in the manufacturing of the cheese included:

- Pasteurized part-skim milk
- Dairy product sourced cheese culture
- Salt
- Vegetable sourced enzymes

According to the nutrition label, 31 g of Mozzarella cheese contains: eight grams of protein, six grams of total fat, 20 milligrams of cholesterol, 220 milligrams of sodium, and less than 1 gram of carbohydrates.

Retail packaged pure yellow mustard powder was purchased from Zenobia LLC (Bronx New York) and stored at 4°C in a 2 pound bag until further use.
Preparation of mustard treatment to cheese. Shredded Mozzarella cheese was aseptically mixed with yellow mustard powder at the concentration of 0, 3, 5, 9 and 17 % (w/w) and placed into sterile polyethylene packages. Polyethylene pouches (17x15.5 cm) were used for packaging of the samples (Flair Flexible Corp., Appleton, Wisconsin). Packages were sealed (14-TT-VAC-1/4, Mansfield, Texas) with or without gas flush (70% nitrogen and 30% carbon dioxide). The experimental treatments were:

1) Shredded Mozzarella cheese (Control)
2) Shredded Mozzarella cheese with 3% (w/w) mustard powder
3) Shredded Mozzarella cheese with 5% (w/w) mustard powder
4) Shredded Mozzarella cheese with 9% (w/w) mustard powder
5) Shredded Mozzarella cheese with 17% (w/w) mustard powder

Data Collection Methodology

Salmonella ser. Typhimurium strain preparation. Three Salmonella ser. Typhimurium strains (ATCC 14028, 25241, and 13311) were acquired from the American Type Culture Collection (ATCC). Culture strains were stored in Tryptic Soy broth (BD Difco) with 0.85% yeast extract, TSBYE, (BD Difco) at 4°C. To prepare fresh cultures, the three Salmonella ser. Typhimurium strains were streak plated on Luria-bertani, LB, (BD Difco) agar plate and incubated for 24 hours at 37°C. An isolated colony from each plate was individually selected and transferred to 10ml of TSBYE in sterilized centrifuge tubes, vortexed for 5 seconds, and incubated for 24 hours at 37°C. A second subculture was prepared by transferring 100μL- aliquot of the sample to 10 ml of sterilized TSBYE in sterilized centrifuge tubes. Samples were vortexed for 5 seconds and incubated for 24 hours at 37°C. After incubation, the bacterial suspension was centrifuged at 3000 X g for 15 minutes and the cell pellet was resuspended in
10ml of 0.85% sodium chloride. The three suspension cultures were combined to obtain a 3-strain cocktails containing approximately 6.2-log CFU/mL of *Salmonella* ser. Typhimurium.

**Penicillium chrysogenum strain preparation.** *Penicillium chrysogenum* strain was acquired from the American Type Culture Collection (ATCC). The strain was spot inoculated onto malt extract agar (BD Difco) and incubated at 25°C for spore production. After 7 days of storage, the spores were harvested by flooding 5ml of 0.01% (v/v) Tween-80 to the plate. Spores in the suspension were counted using a hemocytometer, which was present at a spore load of $10^5$ spores/ml. The suspension was then diluted to obtain $10^4$ spores/ml.

**Salmonella ser. Typhimurium inoculation.** Packaged cheese sample treatments were inoculated with 0.5 ml of the prepared 3-strain cocktail using a syringe and a septum (PPL-193456, Illinois Instrument, Johnsonburgs, Illinois) and mixed by shaking by hand for 10 seconds for even distribution before the enumeration (day 0). Samples were stored in the refrigerator at 4°C until the experimental period.

**Penicillium chrysogenum inoculation.** Packaged cheese sample treatments were inoculated with 0.1 ml of the prepared *Penicillium chrysogenum* spore suspension using syringe and septum (PPL-193456, Illinois Instrument, Johnsonburgs, Illinois) and mixed by shaking by hand for 10 seconds for even distribution. Samples were incubated at 25°C until the experimental period.

**Enumeration of Salmonella ser. Typhimurium.** Inoculated and stored cheese samples were sampled periodically during storage for enumeration of *Salmonella* populations on days 0, 1, 2, 3, 5, 8, 11, 15 and 20. The samples were stomached (Seward Stomacher 400, UK) in a stomacher bag (BA6041/57R Seward, UK) with 100 mL of sterile 0.1% buffer peptone-water (BPW) for 30 seconds. The samples were then serially diluted in BPW and decimal dilutions of
the stomached sample were plated on xylose lysine deoxycholate (XLD) agar. The plates were incubated for 24 hours at 37°C before the large, black colonies of *Salmonella* ser. Typhimurium were counted.

**Enrichment of cheese samples for the enumeration of *Salmonella* ser. Typhimurium.** The enrichment of the cheese samples was conducted according to the guidelines outlined by Food and Drug Administration’s Bacterial Analytical Manual (BAM) for *Salmonella* (Andrews, Jacobson, and Hammack, 2014). The stomached cheese sample in BPW for enumeration was incubated for 24 hours at 37°C. After the incubation, 0.1 mL of stomached sample was transferred into 10 of Rappaport-Vassiliadis (RV) 10 mL of Tetrathionate (TT) broth kept in sterile tubes. The samples were mixed and incubated for 24 hours at 42°C and 37°C, respectively. After the incubation, each sample was streak plated on XLD agar plate, incubated at 24 hours at 37°C, and examined for the presence of colonies for the qualitative detection of *Salmonella* ser. Typhimurium.

**Effect of mustard in the growth of *Penicillium chrysogenum*.** Growth of *Penicillium chrysogenum* was monitored through visual observation for mycelial presence and sporulation. Number of samples positive and negative for the presence of spores or mycelia was counted on days 1, 3, 5, 7, 9, 12 and 15 and total number of positive and negative samples was determined.

**Statistical Analysis**

All experimental tests were conducted in triplicate. IBM Statistical Package for the Social Sciences (IBM SPSS Statistic 20) was utilized for data analysis. Data from the study of *Salmonella* ser. Typhimurium was analyzed using analysis of variance (ANOVA) followed by Tukey’s to test the significant differences (p< 0.05) between the treatment means. Data from the
Penicillium chrysogenum study was analyzed using Pearson’s chi-square test for the significant difference between the treatments (p< 0.05).

**Sensory Study**

**Sample selection and description.** Shredded Mozzarella cheese samples were purchased from a local manufacturer, Cady Cheese LLC (Wilson, Wisconsin). Cheese samples were stored at 4°C until further use. Retail packaged pure yellow mustard powder was purchased from Zenobia LLC (Bronx, New York) and stored at 4°C until further use.

**Sample preparation.** Cheese samples with and without mustard powder were prepared in the sensory evaluation laboratory at the University of Wisconsin-Stout. Samples with four different mustard powder variations were prepared and stored in refrigerator at 4°C two hours prior to the sensory evaluation. Samples were prescreened before the sensory study to evaluate taste, appearance, flavor, texture and other sensory characteristics in order to eliminate any bias. The screening team decided not to include sample with 17% mustard powder in the sensory study because of its intense aroma and flavor.

The experimental treatments were:

1) Shredded Mozzarella Cheese (control)
2) Shredded Mozzarella cheese with 3% (w/w) mustard powder
3) Shredded Mozzarella cheese with 5% (w/w) mustard powder
4) Shredded Mozzarella cheese with 9% (w/w) mustard powder

Random three-digit codes were assigned to the sample variations. Two-ounce size sample cups were coded according to the assigned codes for presenting the samples to the panelists.
Panelist selection and description. The sensory study was conducted after receiving approval from the Institutional Review Board (IRB). Panelists were recruited from the University of Wisconsin-Stout via emails and fliers. Panelists consisted of university staff, faculty, and students. Most participants in the study ranged from 18-21 years of age.

Sample serving. Samples were served in the sensory evaluation lab at the University of Wisconsin-Stout. Seven panelists were allowed to take the test at once and each individual had a separate booth. Participants were asked to sign the Informed Consent form (Appendix A) before the start of the sensory test. Except for the information on allergens, no other information about the product was provided to the panelists. The panelists received a tray of four samples with a cup of water. Panelists were asked to evaluate the samples based on a series of questions on appearance, flavor, texture and overall acceptability followed by demographic questions on gender, age and frequency of cheese consumption. All the participants received the coded sample cups in randomized manner in order to maintain balanced results without any bias.

Data collection methodology. A score sheet was created and entered into the CompuSense® software (5.0 version, CompuSense Inc, Guleph, Ontario, Canada). Questionnaire was based on the appearance, flavor, texture and overall acceptability. A 7-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely) was used to measure the panelists’ perspective of each attribute for all the samples. Demographic questions included gender, age (18-21, 22-25, 26-29, 29+) and frequency of consumption of cheese (daily, weekly, monthly, yearly, never).
Statistical Analysis

Sensory data was collected using CompuSense® software (5.0 version, CompuSense Inc, Guleph, Ontario, Canada) and analyzed using ANOVA followed by Tukey’s to test the significant differences (p< 0.05) between the treatments.
Chapter IV: Results

The purpose of this study was to evaluate the antimicrobial property of yellow mustard powder in shredded Mozzarella cheese under normal and modified atmosphere (MA) packaging conditions. More specifically this study tested the hypothesis that mustard powder can inhibit *Salmonella* ser. Typhimurium and *Penicillium chrysogenum*. The research design was a quantitative experimental study with mustard concentrations as the variable (0%, 3%, 5%, 9% and 17%). Time of storage was another variable in the study.

**Effect of Mustard Powder on the Survival of *Salmonella* ser. Typhimurium at 4 °C Storage Temperature**

The effect of mustard powder treatments on the survival of *Salmonella* ser. Typhimurium in cheese packaged under modified atmosphere packaging was studied. *Salmonella* population in cheese samples with different mustard treatments was analyzed and compared. Population of *Salmonella* declined during the storage for all treatments (0%, 3% 5%, 9% and 17%) during the storage period. Table 4 shown below, displays the declining rate of *Salmonella* in cheese samples treated with mustard powder.

Increasing the concentration of mustard powder resulted in a higher rate of destruction of populations of *Salmonella* ser. Typhimurium. All of the mustard treated samples were effective in significantly reducing *Salmonella* ser. Typhimurium population compared to the control (p<0.05). Addition of 17% mustard powder to cheese reduced *Salmonella* population by > 5-log CFU/g in 20 days, which was a significant reduction (p<0.05) compared to the control. No significant differences were found between the cheese samples containing 3% and 5% mustard powder (p>0.05), wherein a 2- log reduction was observed in both samples in 20 days. Cheese samples with 17% mustard powder reduced the *Salmonella* population below detection level.
(<10 CFU/g) in 20 days when plated on XLD agar. However, samples enriched in Rappaport Vassiliadis (RV) medium and Tetrathionate (TT) broth on day 20 for the qualitative detection of *Salmonella* showed positive growth on Xylose Lysine Deoxycholate (XLD) agar indicating that a population of the bacterial cells had undergone injury but were not dead.

Table 4

**Population Number of Salmonella ser. Typhimurium in Modified Atmosphere Packaged Shredded Mozzarella Cheese with or without Mustard Powder and Stored at 4°C**

<table>
<thead>
<tr>
<th>Day</th>
<th>Mustard Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>0</td>
<td>6.20±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>6.01±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>6.03±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>5.85±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>5.67±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>5.35±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>5.34±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>5.17±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>5.09±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ND</sup><sup>+</sup>, not detectable (<10 CFU/g) after enumeration and plating on XLD, but positive for qualitative counts after enrichment on RV and TT broths. Values with different letters in same row are significantly different (p<0.05).
Effect of Various Treatments on Decimal Reduction Time (D-value)

D-values were calculated for all the treatments by plotting the log of survival counts of *Salmonella* compared to the storage time for each treatment. In other words it was calculated as negative reciprocal of the slope of the line constructed in figure 3. The formula used for calculating D-values is shown below:

\[ D_T = \frac{t}{\log_{10} x - \log_{10} y} = \frac{-1}{\text{slope}} \]

![Figure 3. Destruction rate of *Salmonella* ser. Typhimurium in cheese samples treated with or without mustard powder.](image)

At 4°C, control samples had a significantly higher D-value (p<0.05) compared to the samples with 9% and 17% mustard treatment. Samples with 3% and 5% mustard treatments
were not statistically different from each other (p>0.05). Samples with 17% mustard powder had the lowest D-value suggesting it to be the most effective against *Salmonella*. Increasing the concentration of mustard powder in cheese resulted in a decreased D-value for *Salmonella*. Increasing the concentration of mustard powder in cheese resulted in faster death rate of *Salmonella*. This study suggests that a higher concentration of mustard is needed to achieve faster rate of bacterial death. Table 5 below depicts the D-values for all the experimental treatments.

Table 5

*Calculated Decimal Reduction Time (D-value) for Salmonella ser. Typhimurium in Mozzarella Cheese with Various Concentrations of Mustard Powder*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>D-value (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% Mustard powder</td>
<td>18.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3% Mustard powder</td>
<td>13.23&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% Mustard powder</td>
<td>11.76&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>9% Mustard powder</td>
<td>7.06&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>17% Mustard powder</td>
<td>3.80&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values with different letters are significantly different (p<0.05).

**Effect of Mustard Powder on the Survival of Penicillium chrysogenum in Shredded Mozzarella Cheese Stored at 25°C**

Samples inoculated with *Penicillium chrysogenum* were evaluated for presence of sporulation or mycelial growth on days 1, 3, 5, 7, 9, 12 and 15. Samples with and without mustard stored at 25°C did not show any indication of growth of *Penicillium chrysogenum* until day 3. *Penicillium chrysogenum* sporulation and mycelia was observed during days 5, 7 and 9
for all the treatments. Samples with 9% and 17% mustard were found to have higher percentages of *Penicillium* sporulation. All samples with or without mustard treatments were observed to have mycelial presence or sporulation by day 15. The results of the sporulation or mycelial presence are shown below (Table 6).

Table 6

*Presence or Absence of Penicillium chrysogenum Sporulation or Mycelial Growth in Mozzarella Cheese Samples with Various Concentrations of Mustard and Stored at 25°C*

<table>
<thead>
<tr>
<th></th>
<th>Mustard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
</tr>
</tbody>
</table>

Results of 3 packages for each treatments: + denotes presence of sporulation or mycelial growth and – denotes absence of sporulation or mycelial growth

There was no significant effect of mustard powder in cheese samples observed on the inhibition of *Penicillium chrysogenum* compared to the control (p>0.05).

**Sensory Study**

The sensory study was conducted in order to evaluate the consumer liking and acceptability of shredded Mozzarella cheese incorporated with various levels of mustard powder. A 7-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 =
neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely) was used to determine if the consumers liked various attributes of the Mozzarella cheese samples treated with various levels of mustard powder.

**Gender and age distribution.** A total of 64 panelists participated in the sensory study, of which 69 percent were females and 31 percent were males. A majority of the participants were 18-20 years of age. All the panelists were recruited from University of Wisconsin-Stout, Menomonie, Wisconsin. Figures 3 and 4 shown below displays the gender distribution and the age range of the participants.

![Figure 4. Gender distribution of sensory panelists.](image-url)
**Consumption of cheese.** The frequency distribution rate for the consumption of cheese was based on daily, weekly, monthly, yearly and never scale. Majority of the panelists (48%) consume cheese weekly, 44% consume daily, 6% consume monthly followed by 2% yearly consumption. Figure 5 below shows the consumption frequencies of cheese.

**Overall liking of appearance.** The panelists indicated a significantly greater liking for the appearance for control samples than the samples treated with mustard powder (p<0.05).
Figure 6 below shows the mean distribution of overall appearance for the treatment samples. The liking of the appearance of all cheese samples with mustard treatments was significantly lower as compared to the control (p<0.05). There was no significant difference between samples with 3% (M=4.19) and 5% (M=3.86) mustard treatment (p>0.05). Overall, 9% mustard samples’ appearance was liked the least (M=2.67) and control samples had the most likable (M=5.86) appearance. Addition of mustard powder to cheese resulted in the reduction of mean liking score for appearance.

![Figure 6](image)

**Figure 6.** Mean scores for overall liking of appearance for treatment samples as evaluated along a 7-point hedonic scale. Mean bars having different letters are significantly different (p<0.05). (Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely).

**Liking of cheese flavor.** Figure 7 below shows the mean distribution of overall liking of cheese flavor between the cheese samples treated with various levels of mustard powder. The findings show that the liking of the cheese flavor of all cheese samples with mustard treatments were significantly lowered as compared to the control (p<0.05). Cheese samples with 9% mustard were liked the least (M=2.72); whereas the control samples were liked the most (M=5.89) in terms of cheese flavor. Cheese samples with 3% and 5% mustard samples were liked moderately (M=4.03, M=3.75) by the panelists and there was no significant difference
between these two samples (p>0.05). This study shows that the consumer liking of the cheese flavor was significantly lowered by the addition of mustard powder. This might negatively affect the consumer intention of purchasing the cheese with mustard powder.

**Figure 8.** Mean scores for overall liking of cheese flavor for treatment samples as evaluated along a 7-point hedonic scale. Mean bars having different letters are significantly different (p<0.05).

(Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely).

**Overall liking of flavor.** Figure 8 below shows the mean distribution of liking of overall flavor. The results show that the liking of the overall flavor of all cheese samples with mustard treatments was significantly lowered as compared to the control (p<0.05). In terms of the overall flavor, the control samples were liked the most (M=5.83) and the 9% mustard samples were liked the least (M=2.59). No significant differences were found between the 3% and 5% mustard treated samples. This study shows that the consumer liking of the overall flavor was significantly lowered by the addition of mustard powder and may negatively impact the consumer perception towards the cheese.
Overall liking of texture. Control samples were significantly liked for overall liking of texture compared to the mustard treated samples (p<0.05). Samples with 3% and 5% mustard treatment were moderately liked by the panelists and were not significantly different (p>0.05).

Figure 6 shows the mean distribution of overall liking of texture. This shows that the consumer liking of the cheese flavor was significantly lowered by the addition of mustard powder.

Figure 9. Mean scores for overall liking of flavor for treatment samples as evaluated along a 7-point hedonic scale. Mean bars having different letters are significantly different (p<0.05).
(Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely).

Figure 10. Mean scores for overall liking of texture for treatment samples as evaluated along a 7-point hedonic scale. Mean bars having different letters are significantly different (p<0.05).
(Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely).
Overall acceptability. A one way- ANOVA revealed that the control samples were liked slightly to liked moderately (M=5.53) where as the 2% and 5% mustard treated samples were neither liked nor disliked (M=4.08, M=3.69). There was no significant difference in the overall acceptability of the both samples. 9% mustard samples were disliked moderately (M=2.56) by the panelists. Figure 10 below shows the mean distribution overall acceptability of the samples. This study shows that the consumer liking of the acceptability of cheese with mustard powder was significantly lowered by the addition of mustard powder.

![Bar chart showing overall acceptability](chart.png)

*Figure 11.* Mean scores for overall acceptability of texture for treatment samples as evaluated along a 7-point hedonic scale. Mean bars having different letters are significantly different (p<0.05). (Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely).

Multiple comparisons. Table 7 shows mean rating of the liking for appearance, cheese flavor, overall flavor and overall acceptability of the cheese samples treated with varying levels of mustard powder.

This study shows that the consumer liking of all the cheese attributes tested including appearance, cheese flavor, overall flavor and acceptability were significantly lowered by the addition of 3%, 5% and 9% mustard powder (p<0.05). The findings suggest that there might be
a negative effect on the consumer acceptance of the cheese product formulated with mustard powder.

Table 7

*Descriptive Statistics (Mean Ratings and Standard Deviations) of the Liking for Appearance, Cheese Flavor, Overall Flavor and Overall Acceptability of Cheese Samples Treated with or without Mustard Powder*

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Descriptive Statistics</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Appearance</td>
<td>Mean</td>
<td>5.86</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.26</td>
</tr>
<tr>
<td>Cheese Flavor</td>
<td>Mean</td>
<td>5.89</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.39</td>
</tr>
<tr>
<td>Overall Flavor</td>
<td>Mean</td>
<td>5.83</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.25</td>
</tr>
<tr>
<td>Texture</td>
<td>Mean</td>
<td>5.66</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.30</td>
</tr>
<tr>
<td>Overall Acceptability</td>
<td>Mean</td>
<td>5.53</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.67</td>
</tr>
</tbody>
</table>

*SD means Standard Deviation

(Scale for liking 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, 7 = like extremely)
Limitations

A limitation of the study was the low number (n=64) of panelists for sensory study. At least, 100 panelists are usually recommended for a valuable sensory analysis. A second limitation was the length of study. Number of samples packaged for the experiment was only enough until day 20 for the study of the survival of *Salmonella* ser. Typhimurium. Rate of destruction of *Salmonella* ser. Typhimurium after 20 days would provide a better understanding of days required for complete destruction of bacterial population.
Chapter V: Discussion

Effect of Mustard Powder Treatments on the Survival of *Salmonella* ser. Typhimurium

Cheese samples with varying levels of mustard powder were effective in significantly reducing populations of *Salmonella* ser. Typhimurium indicating the strong bactericidal effect of mustard powder at concentrations as low as 3% (w/w). Increasing the concentration of mustard powder in cheese resulted in a faster death rate of *Salmonella*. This study suggests that a higher concentration of mustard is needed to achieve faster rate of bacterial death. Literature on the study of mustard powder on cheese is very rare but the use of mustard powder on meat products to enhance the safety of meat products have been demonstrated by many authors. The results of this study are in agreement with that of the study conducted by Nadarajah, Han, and Holley (2005). The study on the use of mustard powder to inactivate *Escherichia coli* O157:H7 in ground beef under modified atmosphere packaging showed that 5%, 10% and 20% mustard reduced *Escherichia coli* O157:H7 population by 0.5, 3 and 5.4-log CFU/g respectively after 21 days. Another study conducted on the use of mustard powder on dry fermented sausage on the inhibition of *Escherichia coli* O157:H7 showed significant result (Graumann & Holley, 2008). The authors demonstrated the reduction of *Escherichia coli* O157:H7 by 3.4, 4.4, and 6.9 log-CFU/g, within 30 days, using 2, 4 and 6% mustard powder, respectively, in dry fermented sausage. Both studies concluded that the use of mustard powder had a significantly greater effect on the destruction of pathogens compared to the control. These observations are consistent with the research done in this study. Since coliforms are the indicators for both *Salmonella* and *E. coli*, bacteria of enteric origin, studies on these two pathogens can be comparable.
Effect of Mustard Powder on the Survival of *Penicillium chrysogenum*

This study did not show any significant effect of mustard powder on *Penicillium chrysogenum*. Shredded Mozzarella cheese samples with mustard powder treatment as high 17% (w/w) did not show any significant antifungal property. There is no published literature on the study of use of mustard powder on cheese to inhibit *Penicillium chrysogenum* however, Isothiocyanates (ITCs), antimicrobial compounds released from mustard, have been demonstrated to have strong antifungal activity. Delaquis & Sholberg (1997) demonstrated the inhibition of growth and germination of *Penicillium expansum*, *Aspergillus flavus*, and *Botrytis cinerea* in 100 μg ITC per liter. A study on the effect of natural ITCs on the growth of fruit pathogens showed inhibition of conidial germination and mycelial growth, suggesting potent antifungal activity (Mari et al., 1993 as cited in Delaquis & Mazza, 1995). These observations contradict the research done on this study. One possible reason could be the contribution of mustard powder or cheese to the microbiology of the product, which may have contributed to the presence of sporulation and mycelial growth. Further study on the antifungal property of mustard powder in cheese is recommended. This study suggests that the use of mustard powder may not be a viable option to control fungal spoilage in shredded Mozzarella cheese.

Sensory Evaluation of Shredded Mozzarella Cheese Treated with Mustard Powder

The findings from the sensory study showed that consumer liking of all cheese attributes tested including appearance, cheese flavor, overall flavor and acceptability was significantly lowered by the addition of mustard powder. Cheese samples with 9% mustard powder had the lowest mean liking scores in terms of all the attributes tested, suggesting the product would be least successful in the market. Although, the liking of cheese samples with 3% and 5% mustard powder was reduced, the mean liking score was in a range of ‘like slightly’ and ‘neither like nor
dislike’. The findings suggest that there might be a negative effect on the consumer acceptance of the cheese product formulated with higher concentration of mustard powder and may face a challenge to compete with similar cheese products.

Conclusions

According to the research study presented, there is clear evidence that mustard powder, formulated with cheese, has a strong antimicrobial effect against *Salmonella* ser. Typhimurium. This study suggests that Isothiocyanates (ITCs) present in mustard powder play a vital role in the antimicrobial property of mustard powder as indicated by previous work (Winther & Neilsen, 2006). Even though mustard powder showed strong antimicrobial effect against *Salmonella*, a higher concentration of mustard powder (20%) was required to inhibit *Salmonella* population below detection limit.

This study showed that mustard powder did not inhibit the growth of *Penicillium chrysogenum* in packaged shredded cheese, which contradicts the literatures discussed previously that suggest mustard powder to be a strong antifungal agent. Since there has not been any published research on the antifungal effect of mustard powder in cheese, further research is recommended.

Result from the sensory panel (n=64) suggested that the liking of various attributes (appearance, cheese flavor, overall flavor, texture and overall acceptability) was significantly lowered due to the addition of mustard powder, even at the concentration as low as 3% (w/w). This suggests that the cheese product with mustard added as an ingredient might not be successful in the market.

Even though the addition of mustard powder in shredded Mozzarella cheese showed strong antimicrobial property against, *Salmonella* ser. Typhimurium, it did not show any effect in
the inhibition of mold, *Penicillium chrysogenum*. Mustard powder may be used to control the growth of *Salmonella* ser. Typhimurium in products but may negatively affect the consumer perception. Since the liking of the various attributes was lowered due to the addition of mustard powder, it might not be a viable control strategy to enhance the safety and quality of cheese.

**Recommendations**

Based on the research conducted in this study, following for future research is recommended:

1. A study on the effect of deactivated mustard powder on the survival of *Salmonella* ser. Typhimurium in cheese is recommended. Since the liking of cheese attributes were significantly lowered by the addition of regular mustard powder, enzyme deactivated mustard powder may be more acceptable to the consumers.

2. Sensory evaluation of shredded Mozzarella cheese treated with deactivated mustard powder is recommended.

3. A study on the effect of mustard powder on nutritional content of cheese is recommended. An increase in fiber, protein, and mono and poly unsaturated fatty acid is expected, which may be a good marketing strategy to commercialize the product.

4. The effect of mustard powder on the inhibition of *Penicillium chrysogenum* was studied only through the visual presence of sporulation and mycelia. A quantitative study of the effect of mustard powder in the survival of *Penicillium chrysogenum* through enumeration of fungal spores is recommended.

5. Quantification of Isothiocyanates (ITCs) in cheese packaged with mustard powder was not addressed in this study. A detection and quantification of ITCS in packaged cheese
samples would provide better understanding of antimicrobial property of mustard powder.

6. A focus group study on the acceptance of pizza or similar cooked products using shredded Mozzarella cheese treated with mustard powder as topping is recommended. Since shredded Mozzarella cheese is primarily used in pizza, focus group study would provide better understanding of the acceptance of cheese product in cooked product like pizza. The acceptability score of cheese with mustard incorporated in it may be improved if cheese is used as an ingredient in other food products such as pizza or lasagna.
References


Appendix A: Consent Form: Sensory Evaluation of Cheese

Investigators: Dipak Pokhrel. Advisor: Dr. Cynthia Rohrer, x-2088, room 368 Heritage Hall.

Description: You will be taking part in a sensory evaluation of samples of Mozzarella cheese. If you have any dietary restrictions that would make you unable to eat these items, then you should not take part in the evaluation.

Risks and Benefits: Care has been taken so that all risks associated with food products have been reduced. Samples of cheese have remained refrigerated and freshly served just prior to evaluation. Successful completion of this research project will help minimize the risk of foodborne outbreaks in near future using a natural antimicrobial agent.

Confidentiality: Your name and identity will not be included on any final documents. We do not believe that you can be identified from any of this information. This informed consent will not be kept with any of the other documents completed with this project.

Right to Withdraw: Your participation in this study is entirely voluntary. You may choose not to participate without any adverse consequences to you. Should you choose to participate and later wish to withdraw from the study, you may discontinue your participation at this time without incurring adverse consequences.

IRB Approval: This study is reviewed and approved by the UW-Stout Institutional Review Board. If you have any questions, please contact the investigator below. If you have questions or concerns regarding this study please contact the Investigator or Advisor. If you have any questions, concerns, or reports regarding your rights as a research subject, please contact the IRB Administrator.

Investigator: Dipak Pokhrel
507-210-7247
pokhreld1323@my.uwstout.edu

IRB Administrator:
Sue Foxwell, Director, Research Services
152 Vocational Rehabilitation Building
UW-Stout
Menomonie, WI 54751
715-232-2477
foxwells@uwstout.edu

Statement of Consent:
By signing this consent form you agree to participate in the project entitled, “Sensory Evaluation of Cheese.”

________________________________________  _______________________
Signature                                      Date
# Appendix B: Cheese Evaluation Form

You will be given 4 samples of cheese to taste. Please taste the samples from left to right, drinking water in between and then answer the corresponding questions for each sample.

1. **How do you like the appearance?**

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither like nor dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. **How do you like the cheese flavor?**

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither like nor dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. **How do you like the overall flavor?**

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither like nor dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. **How do you like the overall texture?**

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither like nor dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. **Considering ALL characteristics (appearance, flavor and texture) please indicate overall acceptability of the product.**

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither like nor dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. **How often do you consume cheese?**

<table>
<thead>
<tr>
<th>Daily</th>
<th>Weekly</th>
<th>Monthly</th>
<th>Yearly</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
7. Please indicate what you *LIKED/DISLIKED* about this product.

8. Gender

<table>
<thead>
<tr>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
</table>

9. Age

<table>
<thead>
<tr>
<th>18-21</th>
<th>22-25</th>
<th>26-29</th>
<th>29+</th>
</tr>
</thead>
</table>
Appendix C: Institutional Review Board (IRB) Approval Form

October 29, 2013

Dipak Pokhrel
Food and Nutritional Sciences
UW-Stout

RE: Sensory Evaluation of Cheese treated with Mustard Powder

Dear Dipak,

The IRB has determined your project, "Sensory Evaluation of Cheese treated with Mustard Powder" is **Exempt** from review by the Institutional Review Board for the Protection of Human Subjects. The project is exempt under **Category #6** of the Federal Exempt Guidelines and holds for 5 years. Your project is approved from **October 29, 2013**, through **October 28, 2018**. Should you need to make modifications to your protocol or informed consent forms that do not fall within the exemption categories, you will need to reapply to the IRB for review of your modified study.

If your project involved administration of a survey, please copy and paste the following message to the top of your survey form before dissemination:

"This research has been reviewed by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46."

If you are conducting an **online** survey/interview, please copy and paste the following message to the top of the form:

"This research has been reviewed by the UW-Stout IRB as required by the Code of Federal Regulations Title 45 Part 46."

**Informed Consent:** All UW-Stout faculty, staff, and students conducting human subjects research under an approved “exempt” category are still ethically bound to follow the basic ethical principles of the Belmont Report: 1) respect for persons; 2) beneficence; and 3) justice. These three principles are best reflected in the practice of obtaining informed consent from participants.

If you have questions, please contact Research Services at 715-232-1126, or foxwells@uwstout.edu, and your question will be directed to the appropriate person. I wish you well in completing your study.

Sincerely,

Susan Foxwell
Research Administrator and Human Protections Administrator,
UW-Stout Institutional Review Board for the Protection of Human Subjects in Research (IRB)