Modeling of Patulin Degradation in Apple Based Beverages by Multiple Wavelengths of Ultraviolet-C Irradiation

By

Yan Zhu

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ABSTRACT

Modeling of Patulin Degradation in Apple Based Beverages by Multiple Wavelengths of Ultraviolet-C Irradiation

Yan Zhu
University of Guelph, 2014

Advisor: Professor Keith Warriner

The mycotoxin patulin is a secondary metabolite produced by a range of molds and is commonly associated with apple juice derived from spoiled fruit. The study investigated the application of UV-C radiation (200-300 nm) to degrade patulin in apple juice/cider and inactivate patulin-producing molds. The degradation of patulin irradiated at UV 254 nm followed first-order kinetics the rate of which was influenced by the fluence rate, sample thickness and absorption coefficient of the sample. The UV fluence required at 254 nm to support 90% degradation of patulin was 84.3 mJ·cm\(^{-2}\) which compares to 19.6 and 55.0 mJ·cm\(^{-2}\) when wavelengths of 222 nm and 282 nm were applied respectively. When all three UV-C wavelengths were applied simultaneously the dose required to decrease patulin by 90% was 36.6 mJ·cm\(^{-2}\). The higher degradation rates at 222 nm was by virtue of the higher photon energy compared to the other wavelengths tested. At 222 nm there was a significant change in juice color but this did not impact on the overall sensory characteristics. By using Saccharomyces cerevisiae and Drosophila melanogaster toxicity assays the photoproducts generated by the UV degradation of patulin were found to be less toxic compared to the native mycotoxin. Further work demonstrated that the UV-C fluence applied to support a 3 log reduction of the Penicillium expansum spore levels in apple juice resulted in > 90% patulin degradation. The inactivation of Penicillium by UV-C
followed tri-phasic kinetics with distinct shoulders and tailing effects. In the course of storage at 4 °C, the UV treated apple juice inoculated with *Penicillin* accumulated significantly less patulin compared to non-UV irradiated controls. In conclusion, the study has modelled the degradation kinetics of patulin in apple juice and illustrated that molds surviving UV treatment have a reduced capacity to produce the mycotoxin.
Publications


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# Table of Contents

Chapter One .................................................................................................................................. 1

Literature Review .......................................................................................................................... 1

1.1. Introduction .......................................................................................................................... 2

1.2. Patulin and patulin-producing molds .................................................................................... 2

1.2.1. Patulin .............................................................................................................................. 2

1.2.1.1. Stability ...................................................................................................................... 4

1.2.1.2. Toxicity ...................................................................................................................... 8

1.2.1.3. Detection assays ........................................................................................................ 10

1.2.2. Patulin-producing molds .................................................................................................. 12

1.2.2.1. Species and prevalence on fruits ................................................................................ 12

1.2.2.2. Biosynthesis of patulin .............................................................................................. 13

1.3. Patulin contamination in apple cider and juice .................................................................... 15

1.3.1. Occurrence of patulin contamination ............................................................................. 15

1.3.2. Current regulations .......................................................................................................... 21

1.3.3. Control of patulin contamination in liquid apple products ............................................ 22

1.3.3.1. Sources of patulin contamination in apple juice production ................................... 22

1.3.3.2. Prevention of patulin contamination ......................................................................... 23

1.4. UV technique ....................................................................................................................... 27

1.4.1. Principle of UV light ....................................................................................................... 27

1.4.2. UV sources ...................................................................................................................... 30

1.4.3. Calculation of UV fluence .............................................................................................. 33

1.4.3.1. Technique terms ......................................................................................................... 33
1.4.3.2. Determination of UV fluence in Batch UV reactors ........................................... 37
1.4.4. Effects of UV irradiation on pathogen inactivation and chemical degradation ........ 47
1.5. Research hypothesis and objectives ......................................................................... 51

Chapter Two ....................................................................................................................... 53

Kinetics of Patulin Degradation in Model Solution, Apple Cider and Apple Juice by Ultraviolet Radiation .............................................................................................................................. 53

Abstract ............................................................................................................................... 54

2.1. Introduction ..................................................................................................................... 55

2.2. Materials and Methods .................................................................................................. 57

2.2.1. Reagents ..................................................................................................................... 57

2.2.2. UV exposure ............................................................................................................... 57

2.2.3. HPLC analysis ........................................................................................................... 58

2.2.4. Determination of the absorption coefficient ............................................................... 60

2.2.5. Modeling of patulin degradation kinetics .................................................................. 60

2.2.6. Determination of applied UV fluence and average UV fluence ................................. 62

2.2.7. Statistical analysis ...................................................................................................... 62

2.3. Results ........................................................................................................................ 62

2.3.1. Degradation of patulin with UV exposure ................................................................. 62

2.3.2. Kinetics of patulin degradation ................................................................................. 64

2.4. Discussion ...................................................................................................................... 73

2.5. Conclusions ................................................................................................................... 76

Chapter Three ....................................................................................................................... 77
Reduction of Patulin in Apple Juice Products by Ultraviolet Light of Different Wavelengths in UV-C Range .................................................................................................................. 77

Abstract .......................................................................................................................... 78

3.1. Introduction ................................................................................................................ 79

3.2. Materials and Methods ............................................................................................ 81

3.2.1. Materials .............................................................................................................. 81

3.2.2. UV processing unit ............................................................................................ 81

3.2.3. Determination of UV fluence ............................................................................ 83

3.2.4. Modeling of patulin reduction kinetics ............................................................... 87

3.2.5. UV radiation ....................................................................................................... 88

3.2.6. HPLC analysis for patulin and ascorbic acid concentration ............................... 89

3.2.7. Optical properties, pH and color of treated apple juice ...................................... 90

3.2.8. Sensory evaluation ............................................................................................. 91

3.2.9. Statistical analysis .............................................................................................. 92

3.3. Results ...................................................................................................................... 92

3.3.1. Reduction of patulin with UV exposure at three wavelengths ............................ 92

3.3.2. Efficiencies of Far UVC (222nm), UVC (254nm) and Far UVC plus (282nm) lamps ......................................................................................................................... 94

3.3.3. Quality attributes study ....................................................................................... 95

3.3.4. Temperature monitoring during UV exposure .................................................... 98

3.4. Discussion ................................................................................................................ 99

3.5. Conclusions ............................................................................................................ 103

Chapter Four .................................................................................................................. 104
Decrease of Patulin Toxicities in Apple Juice after Ultraviolet (254 nm) Irradiation Using both Saccharomyces cerevisiae and Drosophila melanogaster as Model Systems

Abstract

4.1. Introduction

4.2. Materials and Methods

4.2.1. Materials

4.2.2. UV irradiation

4.2.3. Growth condition of S. cerevisiae

4.2.4. Sample preparation for S. cerevisiae model system

4.2.5. Preparation of apple juice agar and Sokolowski lab fly food

4.2.6. Sample preparation for D. melanogaster model system

4.2.7. Inoculation of fruit fly eggs

4.2.8. Observation

4.2.9. HPLC analysis

4.2.10. Statistics analysis

4.3. Results

4.3.1. Patulin sensitivity evaluation in S. cerevisiae model system

4.3.2. UV-detoxification evaluation in S. cerevisiae model system

4.3.3. Patulin sensitivity evaluation in D. melanogaster model system

4.3.4. UV-detoxification evaluation in D. melanogaster model system

4.4. Discussion

4.5. Conclusions

Chapter Five

ix
Inactivation and Patulin Accumulating Ability of *Penicillium expansum* Inoculated into Apple Juice and Irradiated with Ultraviolet Light at 254 nm

Abstract .................................................................................................................................................. 128

5.1. Introduction ..................................................................................................................................... 129

5.2. Materials and Methods ................................................................................................................... 130

5.2.1. Inoculum preparation .................................................................................................................. 130

5.2.2. UV irradiation ............................................................................................................................. 131

5.2.3. Calculation of average UV fluence ............................................................................................. 131

5.2.4. UV inactivation of *P. expansum* ............................................................................................. 132

5.2.5. Modeling of *P. expansum* inactivation .................................................................................... 132

5.2.6. Indirect impedance assay .......................................................................................................... 133

5.2.7. Spore germination after UV irradiation ..................................................................................... 134

5.2.8. Challenge testing and patulin accumulation .............................................................................. 134

5.2.9. HPLC analysis of patulin .......................................................................................................... 134

5.2.10. Statistical analysis .................................................................................................................... 135

5.3. Results .............................................................................................................................................. 136

5.3.1. UV inactivation of *P. expansum* ............................................................................................. 136

5.3.2. Indirect impedance assay .......................................................................................................... 138

5.3.3. Spore germination after UV irradiation ..................................................................................... 139

5.3.4. Challenge testing ....................................................................................................................... 141

5.3.5. Patulin accumulation ................................................................................................................. 141

5.4. Discussion ....................................................................................................................................... 144

5.5. Conclusions ..................................................................................................................................... 147
Chapter Six.................................................................................................................. 148

Conclusions and Future Studies.................................................................................. 148

6.1. Conclusions........................................................................................................... 149

6.2. Future studies ....................................................................................................... 151

References.................................................................................................................... 153
## List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The chemical structure of patulin</td>
</tr>
<tr>
<td>1.2</td>
<td>The absorbance spectra of patulin between 200 and 350 nm (10 mg·L(^{-1}), 5 mg·L(^{-1}) and 1 mg·L(^{-1}))</td>
</tr>
<tr>
<td>1.3</td>
<td>Mechanism of metal-catalysed oxidation of ascorbic acid (Source: Drusch et al., 2007)</td>
</tr>
<tr>
<td>1.4</td>
<td>Mechanism of metal chelate-catalysed oxidation of ascorbic acid (Source: Drusch et al., 2007)</td>
</tr>
<tr>
<td>1.5</td>
<td>Hypothetical mechanisms for patulin degradation after 80°C, pH 8 for 40 min. (Source: Collin et al., 2012)</td>
</tr>
<tr>
<td>1.6</td>
<td>Patulin biosynthetic pathway (Source: Moake et al., 2005)</td>
</tr>
<tr>
<td>1.7</td>
<td>Electromagnetic spectral categories from gamma-rays through radio wavelengths (Source: ISO, 2007)</td>
</tr>
<tr>
<td>1.8</td>
<td>Spectrum of UV Lamps and cell deactivation curve (Source: Schalk et al., 2006)</td>
</tr>
<tr>
<td>1.9</td>
<td>Schematic diagrams of batch UV reactors</td>
</tr>
<tr>
<td>2.1</td>
<td>Representative HPLC chromatograms of patulin</td>
</tr>
<tr>
<td>2.2</td>
<td>Degradation of patulin in model solution by UV exposure with uniform sample thickness (l = 0.5) cm and various incident fluence rates</td>
</tr>
<tr>
<td>2.3</td>
<td>Degradation of patulin in model solution by UV exposure with uniform incident fluence rate (E_0 = 3.0) mW·cm(^{-2}) and various sample thicknesses</td>
</tr>
<tr>
<td>2.4</td>
<td>Degradation of patulin in apple juice without ascorbic acid addition by UV exposure with uniform incident fluence rate (E_0 = 3.0) mW·cm(^{-2}) and various sample thicknesses</td>
</tr>
</tbody>
</table>
Figure 2.5. Degradation of patulin in apple cider, apple juice with and without ascorbic acid addition by UV exposure with uniform incident fluence rate \( (E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}) \) and sample thickness \( (l = 0.2 \text{ cm}) \) ..................................................................................................................................................69

Figure 2.6. Comparison of patulin degradation rates in 4 kinds of media. \( (E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}, l = 0.2 \text{ cm}) \) ..................................................................................................................................................70

Figure 2.7. Residual concentration of patulin during UV exposure. Compendium of 50 experimental data comparing fluence-based first-order degradation model predictions..............71

Figure 2.8. Comparison of patulin degradation rates in model solution and apple juice without ascorbic acid addition in both dynamic and static systems. \( (E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}, l = 0.2 \text{ cm}) \) ......72

Figure 3.1. Schematics of triple wavelengths box UV reactor .................................................................82

Figure 3.2. Diagrammatic sketch of mathematic model on the determination of UV fluence rate ..................................................................................................................................................86

Figure 3.3. Reduction of patulin in apple cider by UV exposure with individual 222, 254, 282 nm wavelength UV lamps and the combination of three UV lamps ............................................93

Figure 3.4. Reduction of patulin in apple juice by UV exposure with individual 222, 254, 282 nm wavelength UV lamps and the combination of three UV lamps .................................................................94

Figure 3.5. Color change in apple juice by UV exposure with individual 222, 254, 282 nm wavelength UV lamps and the combination of three UV lamps.................................................................96

Figure 3.6. Relationship between the photon energy and the absorbance spectrums of apple juice (0.02 cm of light pathlength) and 10 mg·L\(^{-1}\)patulin (1 cm of light pathlength) .........................103

Figure 4.1. Growth curves of \( S. \text{cerevisiae} \) in minimal media with various patulin concentrations ..................................................................................................................................................115
Figure 4.2. Growth curves of *S. cerevisiae* in the blank (PAT= 0 mg·L\(^{-1}\) without UV treatment), UV-CK (PAT= 0 mg·L\(^{-1}\) with UV treatment), treated (PAT = 5.85 mg·L\(^{-1}\) with UV treatment), spiked (PAT = 5.85 mg·L\(^{-1}\) without UV treatment) and PAT-CK (PAT = 50 mg·L\(^{-1}\) without UV treatment) samples ................................................................. 116

Figure 4.3. Daily percentage of survived larva in the blank (PAT= 0 mg·L\(^{-1}\) without UV treatment), UV-CK (PAT = 0 mg·L\(^{-1}\) with UV treatment), treated (PAT = 87.9 mg·L\(^{-1}\) with UV treatment), spiked (PAT = 87.9 mg·L\(^{-1}\) without UV treatment) and PAT-CK (PAT = 500 mg·L\(^{-1}\) without UV treatment) samples. ........................................................................................................ 118

Figure 4.4. Daily percentage of pupa in the blank (PAT= 0 mg·L\(^{-1}\) without UV treatment), UV-CK (PAT = 0 mg·L\(^{-1}\) with UV treatment), treated (PAT = 87.9 mg·L\(^{-1}\) with UV treatment), spiked (PAT = 87.9 mg·L\(^{-1}\) without UV treatment) and PAT-CK (PAT = 500 mg·L\(^{-1}\) without UV treatment) samples ........................................................................................................ 119

Figure 4.5. Daily percentage of adult fly in the blank (PAT= 0 mg·L\(^{-1}\) without UV treatment), UV-CK (PAT = 0 mg·L\(^{-1}\) with UV treatment), treated (PAT = 87.9 mg·L\(^{-1}\) with UV treatment), spiked (PAT = 87.9 mg·L\(^{-1}\) without UV treatment) and PAT-CK (PAT = 500 mg·L\(^{-1}\) without UV treatment) samples ........................................................................................................ 120

Figure 4.6. Pictures of larvae at day 6 ........................................................................................................ 121

Figure 4.7. Pictures of pupae at day 10 .................................................................................................... 122

Figure 4.8. Pictures of adult flies at day 15 ............................................................................................ 123

Figure 5.1. Survival data of *P. expansum* spores in apple juices after UV irradiation fitted first-order, di-phasic (log-linear plus tail) and tri-phasic (log-linear plus shoulder and tail) kinetics models ......................................................................................................................... 137
Figure 5.2. Growth curves of *P. expansum* in apple juice at 25 °C presented by M-value using indirect impedance assay .................................................................139

Figure 5.3. Germination of *P. expansum* spores in apple juice .................................................................140

Figure 5.4. Growth of *P. expansum* and specific activity of patulin accumulation in apple juice during the storage at 4 °C ...........................................................................................................143
List of Tables

Table 1.1. Occurrence of patulin in fruits, fruit juices and other fruit products .................................19
Table 1.2. Typical bond energies of important biological moieties and their corresponding wavelengths (Source: Blatchley and Peel, 2001) ..................................................................................................................29
Table 1.3. Matrix of excimers (X_2*, Rg_2*) and exciplexes (RgX*) obtained from halogens and rare gases and their emission maxima (nm) (Source: Oppenländer and Sosnin, 2005). ............33
Table 1.4. Inactivation of microorganisms by UV irradiation in apple cider and juice ......................50
Table 2.1. Degradation of patulin with UV exposure .............................................................................63
Table 2.2. pH and absorption coefficient of apple juice products before and after UV exposure ..........................................................................................................................................................................................72
Table 3.1. Experimental conditions of UV exposure ............................................................................88
Table 3.2. Patulin reduction by triple wavelength box UV reactor.......................................................89
Table 3.3. Lamp efficiency of patulin reduction ......................................................................................95
Table 3.4. Effects of the UV irradiation on pH, total soluble solid, absorption coefficient, color and concentration of ascorbic acid .................................................................................................................97
Table 3.5. Triangle test for determination of color changes in apple juice after UV irradiation with fluence supporting to degrade 90 % of patulin ........................................................................................................98
Table 4.1. Sample preparation for S. cerevisiae model system .............................................................110
Table 4.2. Sample preparation for D. melanogaster model system .......................................................112
Table 4.3. Hatching and development rate at day 6, 10 and 15 in various patulin concentrations .................................................................................................................................................................................117
Table 4.4. Hatching and development rate at Day 6, 10 and 15 in the blank (PAT = 0 mg∙L⁻¹ without UV treatment), UV-CK (PAT= 0 mg∙L⁻¹ with UV treatment), treated (PAT = 87.9 mg∙L⁻¹
with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples...

Table 4.5. Length of larva, pupa and adult fly at Day 6, 10 and 15 (mm) in the blank (PAT = 0 mg·L⁻¹ without UV treatment), UV-CK (PAT = 0 mg·L⁻¹ with UV treatment), treated (PAT = 87.9 mg·L⁻¹ with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples...

Table 5.1. Model parameters and statistical indices for UV-C inactivation kinetics of P.expansum spores in apple juices...
## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Absorption coefficient</td>
<td>cm⁻¹</td>
</tr>
<tr>
<td>α₂₅₄</td>
<td>Absorption coefficient at 254 nm wavelength</td>
<td>cm⁻¹</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength</td>
<td>m</td>
</tr>
<tr>
<td>φ₁</td>
<td>Incident angle</td>
<td></td>
</tr>
<tr>
<td>φ₂</td>
<td>Refracted angle</td>
<td></td>
</tr>
<tr>
<td>a*</td>
<td>Parameter in the CIELAB color scale positioned between green and red</td>
<td></td>
</tr>
<tr>
<td>b*</td>
<td>Parameter in the CIELAB color scale positioned between blue and yellow</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>Speed of light</td>
<td>m·s⁻¹</td>
</tr>
<tr>
<td>d</td>
<td>Distance between the infinite small segment of UV source and the any infinity small volume in the sample</td>
<td>cm</td>
</tr>
<tr>
<td>d'</td>
<td>Part of d in the liquid sample</td>
<td>cm</td>
</tr>
<tr>
<td>D</td>
<td>Vertically distance between the UV lamp and the plane which across the center of petri dish and is perpendicular to the surface of sample</td>
<td>cm</td>
</tr>
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<td>Dᵢ</td>
<td>Average UV fluence results in 90 % reduction of patulin</td>
<td>mJ·cm⁻²</td>
</tr>
<tr>
<td>Dᵣ</td>
<td>UV exposure time results in 90 % reduction of patulin</td>
<td>min</td>
</tr>
<tr>
<td>E₀</td>
<td>Incident UV fluence rate (at the center of petri dish)</td>
<td>mW·cm⁻²</td>
</tr>
<tr>
<td>E₍ᵢ,ᵢ₎</td>
<td>Fluence rate at a point in the sample which emitted from small part of UV lamp at position l</td>
<td>mW·cm⁻²</td>
</tr>
<tr>
<td>Eₐₚₐₖ</td>
<td>Applied UV fluence rate</td>
<td>mW·cm⁻²</td>
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<tr>
<td>Eₐₐvg</td>
<td>Average UV fluence rate</td>
<td>mW·cm⁻²</td>
</tr>
<tr>
<td>Eₑₐₐvg</td>
<td>Fluence rate at the center of petri dish</td>
<td>mW·cm⁻²</td>
</tr>
<tr>
<td>Eₚ</td>
<td>Photon energy per mole</td>
<td>J·mol⁻¹</td>
</tr>
<tr>
<td>ΔE*</td>
<td>Total color difference in the CIELAB color scale</td>
<td></td>
</tr>
<tr>
<td>h</td>
<td>Planck’s constant</td>
<td>J·s</td>
</tr>
<tr>
<td>H</td>
<td>Distance between the UV lamp and the surface of sample</td>
<td>cm</td>
</tr>
<tr>
<td>Hₐₐvg</td>
<td>Average UV fluence</td>
<td>mJ·cm⁻²</td>
</tr>
<tr>
<td>kᵥ</td>
<td>Average-fluence-based first-order reaction rate constant</td>
<td>mW⁻¹·cm²·s⁻¹</td>
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</tr>
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<td>kₘₐₓ</td>
<td>Inactivation rate constant of log-linear part of the survival curve</td>
<td>mW⁻¹·cm²·s⁻¹</td>
</tr>
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<td>kₜ</td>
<td>Time-based first-order reaction rate constant</td>
<td>s⁻¹</td>
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<tr>
<td>l</td>
<td>UV path length of sample (sample thickness)</td>
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</tr>
<tr>
<td>l'</td>
<td>Distance between sample and UV source</td>
<td>cm</td>
</tr>
<tr>
<td>L</td>
<td>Length of UV lamp</td>
<td>cm</td>
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<tr>
<td>n₁</td>
<td>Refraction index of air</td>
<td></td>
</tr>
<tr>
<td>n₂</td>
<td>Refraction index of apple juice</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Patulin concentration (Chapter 2 and 3)</td>
<td>mol·L⁻¹</td>
</tr>
<tr>
<td>P. expansum</td>
<td>spore concentration (Chapter 5)</td>
<td>CFU·mL⁻¹</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
<td>Unit</td>
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<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>N&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Initial patulin concentration (before UV irradiation) (Chapter 2 and 3)</td>
<td>mol·L&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Initial &lt;i&gt;P. expansum&lt;/i&gt; spore concentration (before UV irradiation) (Chapter 5)</td>
<td>CFU·mL&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>N&lt;sub&gt;A&lt;/sub&gt;</td>
<td>Avogadro’s number</td>
<td></td>
</tr>
<tr>
<td>N&lt;sub&gt;res&lt;/sub&gt;</td>
<td>Residual &lt;i&gt;P. expansum&lt;/i&gt; spore concentration</td>
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<td>q&lt;sub&gt;n,p&lt;/sub&gt;</td>
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<td>Re</td>
<td>Reflectance</td>
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<td>SI</td>
<td>Shoulder length</td>
<td>mJ·cm&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
<tr>
<td>t</td>
<td>UV exposure time</td>
<td>s</td>
</tr>
</tbody>
</table>
Chapter One

Literature Review
1.1. Introduction

Patulin is a common mycotoxin produced as a secondary metabolite by a range of *Penicillium*, *Aspergillus* and *Byssochlamys* typically encountered on spoiled apples (Moake *et al*., 2005; Puel *et al*., 2010). Patulin is commonly associated with juice prepared from spoiled apples and can lead to acute, but more commonly, chronic toxicity when consumed at levels greater than 50 ppb (Lindroth and von Wright, 1978; McKinley and Carlton, 1980a; McKinley and Carlton, 1980b; McKinley *et al*., 1982; Becci *et al*., 1981). The toxicity and high occurrence of patulin in apple cider/juice prompted regulatory bodies establish tolerance limits in these products (U.S.FDA, 2001; EC, 2006). Insufficient application of good agricultural practices (GAP) and good manufacturing practices (GMP) during pre- and post-harvest, however, results in the processing of damaged apples with high concentrations of patulin. The limited ability to reduce patulin levels in traditional downstream processing such as clarification and pasteurization encouraged the development of novel techniques to control the contamination in the final product. Ultraviolet (UV) irradiation technique, based on the strengths of high efficiency, low energy consumption and approval of pasteurization method in apple cider and juice, became a potential strategy to reduce patulin contamination before the food products were released to the consumers. The following thesis described studies that modelled patulin degradation by mono- and mutli-wavelength UV, and identified factors affecting photosensitivity of the mycotoxin.

1.2. Patulin and patulin-producing molds

1.2.1. Patulin

Patulin, 4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one, is a mycotoxin produced by various moulds. It has a polyketide lactone structures (Figure 1.1) with a molecular weight of 154.1 (Ciegler,
Patulin, a colorless to white crystalline solid, is soluble in water, aqueous solutions, methanol, ethanol, acetone, and ethyl acetate and less soluble in diethyl ether and benzene. Patulin has a melting point at 110.5 °C and maximally absorbs ultraviolet light at 276 nm (Figure 1.2). Patulin was firstly isolated and identified in 1943 (Birkinshaw et al., 1943). The early findings of broad-spectrum antibiotic properties of patulin led to research interests on the possibility of its use in treatment of the human cold. However, toxic effects which included stomach irritation, nausea and vomiting, ceased the studies of possible medical applications (Ciegler, 1977).

Figure 1.1. The chemical structure of patulin
1.2.1. Stability

Knowledge of patulin stability in aqueous solutions such as fruit juices may be useful when seeking effective chemical, physical or biological control methods, as well as for establishing reasonable extraction and detection assays during the experimental design. The stability of patulin had been shown to be dependent on the pH value, compounds containing sulfhydryl groups, presence of ascorbic acid, and thermal treatment. Brackett and Marth (1979b) proved that patulin was unstable in an alkaline pH range. It was reported that the half-life of patulin at pH = 8 in phosphate buffer at 25 °C was 63 hours compared to the 1308 hours at pH = 6. In the acidic to neutral pH range, Drusch et al. (2007) showed that patulin was reduced to 68 % ~ 71 % of its initial concentration in McIlvaine buffer (pH 2.5 ~ 5.5) after 30 days. When the pH was
increased to 7.0, only 36 % of the initial patulin concentration was observed. Scott and Somers (1968) evaluated the stability of patulin during three weeks storage at 22 °C in various fruit juices. The authors hypothesized that the presence of higher levels of sulfhydryl compounds in canned orange juice (0.02 mmole SH per 100 ml) contributed to a greater decrease in patulin levels (75 %) than grape (35 %) and apple juice (25 %). In addition to sulfhydryl compounds, ascorbic acid as a natural constituent and as a commercial additive was also reported to accelerate patulin degradation (Drusch et al., 2007; Brackett and Marth, 1979a; Koca and Eksi, 2005). Drusch et al. (2007) observed that 29 % to 32 % of patulin (2 mg/L) was reduced in McIlvaine buffer without ascorbic acid compared to the 70 % of patulin degradation in the presence of ascorbic acid (482 mg/L) after storage of 34 days. The authors elucidated that patulin was degraded by free radicals generated by either rapid metal-catalysed oxidation of ascorbic acid in the presence of oxygen or slow metal-chelate-catalysed oxidation of ascorbic acid without oxygen (Figure 1.3 and 1.4). The thermal stability of patulin was investigated by several researchers. Wheeler et al. (1987) reported an 18.5 % decrease in patulin concentration in apple cider after a HTST treatment at 90 °C for 10 second. Kadakal and Nas (2003) observed 19 % and 26 % of patulin reduction after thermal treatment for 20 min at 90 °C and 100 °C, respectively. The longer exposure time during heating was evaluated by Kryger (2001), which showed 33 % degradation of patulin in apple juice treated at 100°C for 177 min. The degradation products of heat-treated patulin (80 °C, pH = 8, 40 min) were identified as 3-keto-5-hydroxypentanal and glyoxylic acid (Figure 1.5) using gas chromatography-mass spectrometry (Collin et al, 2008). The authors explained that 3-keto-5-hydroxypentanal may form from opening of the patulin hemiacetal moiety followed by retroaldolization and lactone hydrolysis.
Figure 1.3. Mechanism of metal-catalysed oxidation of ascorbic acid (Source: Drusch et al., 2007)
Figure 1.4. Mechanism of metal chelate-catalysed oxidation of ascorbic acid (Source: Drusch et al., 2007)

Figure 1.5. Hypothetical mechanisms for patulin degradation after 80°C, pH 8 for 40 min. (Source: Collin et al., 2012)
1.2.1.2. Toxicity

Patulin has a strong affinity for sulphydryl groups, which inhibits the activities of critical enzymes by reacting with their sulphydryl groups (Atkinson and Stanley, 1943; Cavallito and Bailey, 1944; Geiger and Conn, 1945). By feeding patulin adducts (reaction mixture between patulin and cysteine) instead of patulin, less teratogenic effects in chicken embryo and higher oral median lethal doses (LD50) in mice were observed (Ciegler et al., 1976; Lindroth and von Wright, 1978). The toxicological studies of patulin covered both acute and chronic adverse effects. Carcinogenicity, teratogenicity, immunotoxicity and genotoxicity were reported by several in vivo and in vitro studies (Becci et al., 1981; Dailey et al., 1977; Sorenson et al., 1986; Escoula et al., 1988; Thust et al., 1982).

It was reported the oral LD50 of patulin ranged between 29 and 55 mg/kg b.w. (body weight) of NMRI male mice, Swiss ICR mice, Syrian hamster and male Sprague-Dawley rats (Lindroth and von Wright, 1978; McKinley and Carlton, 1980a; McKinley and Carlton, 1980b; McKinley et al., 1982). Compared to rodents, poultry have a higher tolerance to patulin. The oral LD50 of patulin in white leghorn cockerels was 170 mg/kg b.w. (Lovett, 1972). Toxic symptoms included gastrointestinal hyperaemia, distension, haemorrhage and ulceration (JECFA, 1995). One explanation for acute toxicity of patulin was proposed that the antibiotic activity leads to alternations in the bacteria flora of intestinal tract through inhibiting the Gram positive organisms and providing selective advantage to the pathogenic Gram negative bacteria (McKinley and Carlton, 1980a; McKinley and Carlton, 1980b).
In chronic toxicity studies of feeding lower level of patulin, the weight loss, gastric and intestinal changes and renal malfunction were observed (Puel et al., 2010). A long-term toxicity study of patulin in the rat was conducted with lifetime administration of patulin by gastric intubation three times per week (Becci et al., 1981). The study evaluated the patulin level of 0, 0.1, 0.5 and 1.5 mg/kg b.w. and found a significant increase in mortality rate with a patulin level of 1.5 mg/kg b.w., a significant decrease in body weight of male rats with patulin levels of 0.5 and 1.5 mg/kg b.w., as well as no tumorigenic effect with all tested patulin levels. Combined with the evaluation of acute, long-term and tumorigenic toxicity, a patulin level of 0.1 mg/kg b.w. was considered as no observed adverse effect level (NOAEL), which was adopted by U.S. Food and Drug Administration (FDA) and European Commission (EC) to establish the maximum tolerance of patulin in liquid apple products (U.S.FDA, 2001; JECFA, 1995; EC, 2006).

To investigate the carcinogenicity of patulin, two long-term animal studies were performed by orally feeding 2.5 mg/kg b.w. to Sprague-Dawley rats twice a week for 74 weeks and 1.0 mg/kg b.w. to Swiss mice three times a week for 104 weeks. No carcinogenic effects were noted in these studies (Osswald et al., 1978; Becci et al., 1981). The International Agency for Research on Cancer (IARC) concluded that “there is inadequate evidence for the carcinogenicity of patulin in experimental animals” and “no evaluation could be made of the carcinogenicity of patulin to humans” (IARC, 1986).

The teratogenicity of patulin was not noted at the level up to 1.5 mg/kg b.w. in the rats studies although the increase of frequency of abortion of embryos at higher patulin level indicated the embryotoxicity of the mycotoxin (Dailey et al., 1977; Reddy et al., 1978). In the chicken
embryos, however, the teratogenicity (eg. primarily ankle malrotation and splayed feet) was observed at the paulin levels of 1-2 µg/egg (Ciegler et al., 1976).

The immunosuppressive properties of paulin have been examined by numerous in vitro and in vivo studies. Patulin was found to inhibit several rats and mouse macrophage functions including inhibition of protein synthesis, alternation of membrane functions, as well as decreased production of O2-, phagosome-lysosome fusion, phagocytosis and lysosomal enzyme (Sorenson et al., 1986; Bourdiol et al., 1990). The in vivo mice studies indicated patulin can increase the number of splenic T lymphocytes, depress serum immunoglobulin concentration and depress delayed hypersensitivity responses (Escoula et al., 1988; Paucod et al., 1990).

The genotoxicity studies of patuin showed mainly negative results with bacteria but were mainly positive when using mammalian cells (JECFA, 1995). Patulin has been demonstrated to act as a clastogen in mammalian cells (Thust et al., 1982; Pfeiffer et al., 1998; Alves et al., 2000). Moreover, some studies indicated that patulin may impair DNA synthesis through reacting with sulphydryl groups of enzymes involving with replication of genetic material (JECFA, 1995).

1.2.1.3. Detection assays

Apple cider and juice have been identified as high risk foods with respect to being contaminated with patulin-producing molds thereby acting as vehicles for the mycotoxin. Given the stability of patulin, there is a need for diagnostic tools for detection and quantification of the toxic agent. The first Association of Official Analytical Chemists (AOAC) method on patulin detection used thin-layer chromatography (TLC) for separation and 3-methyl-2-benzothiazolinone hydrazine-
HCl for detection (Scott, 1974). Along with the development of the chromatography techniques, several high performance liquid chromatography (HPLC) methods have been reported (Brause et al. 1996; MacDonald et al., 2000). In 1996, Brause et al. completed a collaborative study of an LC method for determination of patulin in apple juice, and the procedure was adopted as a first action method by AOAC international (995.10). According to the method, patulin samples were extracted with ethyl acetate and cleaned up by extraction with anhydrous sodium sulfate. The HPLC conditions included the use of a C-18 column, a mobile phase of various concentrations of tetrahydrofuran (THF) (up to 5 %) and acetonitrile (ACN) (up to 10 %), a mobile phase flow rate of 0.5 mL/min and a UV detection of the analyte at a wavelength of 276 nm. The method successfully separated patulin from 5-(hydroxymethyl)furfural (HMF), a main interference in LC analyses of apple juice. Compared to the TLC assay, HPLC methods showed lower detection limits, better precision and ease of LC automation. In order to extend the application of LC method to cloudy apple juice and apple puree, a new AOAC method (2000.02), which introduced treatment of pectinase enzyme on cloudy apple juice and apple puree before extraction, has been approved (MacDonald et al., 2000). The extraction and clean-up procedures have also been improved. Compared to the multistep time-consuming liquid-liquid extraction (LLE), solid-phase extraction (SPE) procedures have been introduced due to their attributes of better recoveries, increased sensitivities, quickness and ease of operation (Katerere et al., 2007; Li et al., 2007; Eisele and Gibson, 2003). Besides LC assays, gas chromatography mass spectrometry (GC-MS, Tarter and Scott, 1991; Llovera et al., 1999; Cunha et al., 2009; Kharandi et al., 2013) and liquid chromatography mass spectrometry (LC-MS, Kataoka et al., 2009; Desmarchelier et al., 2011) assays have been proposed to enhance reliability and reduce detection limits.
Chromatography assays provide reliable, sensitive and accurate patulin analysis. However, the drawback of off-line analysis limits their applications in the field and food processing facilities. Nowadays, rapid on-line detection assays have been preliminarily investigated, which include immunochemical methods with selectivity for patulin (McElroy and Weiss, 1993), application of a chemiluminescence sensor based on enhanced luminescence of luminol when reacted with hydrogen peroxide in the presence of patulin (Liu et al., 2008), as well as an assay based on the inhibition of bioluminescence in Vibrio fisheri when exposed to patulin (Sarter and Zakhia, 2004). Although these efforts have shown limited success, the development of rapid patulin detection assays is still a need for the future.

1.2.2. Patulin-producing molds

1.2.2.1. Species and prevalence on fruits

Patulin was originally named as claviformin due to its isolation from Penicillium claviforme (Chain et al., 1942). The name of “patulin” was come from the patulin-producing molds, P. patulum (Birkmshaw et al., 1943). Several papers reviewed the species of patulin-producing molds belonging to the genera, Penicillium, Aspergillus, Byssochlamys and Paecilomyces (IARC, 1986; Moake et al., 2005; Puel et al., 2010). The genus, Penicillium, contributes the most patulin-producing species. Frisvad et al. (2004) identified and reported 13 species from a total of 58 species in Penicillium subgenus Penicillium, which included P. carneum, P. clavigerum, P. concentricum, P. coprobiun, P. dipodomyicola, P. expansum, P. glandicola, P. gladioli, P. griseofulvum, P. marinum, P. paneum, P. sclerotigenum and P. vulpinum. The list also included three Aspergillus species (A. clavatus, A. giganteus and A. longivesica) (Varga et al., 2007). For
the genera of *Byssochlamys* and *Paecylomyces*, only *Byssochlamys nivea* and *Paecylomyces saturatus* have the ability to produce patulin (Samson *et al.*, 2009).

*P. expansum* has been considered as the main source of patulin in pomaceous fruits including apples and pears since it rapidly invades the fruits and causes rot and decay (Puel *et al.*, 2010; McKinley and Carlton, 1991). In other fruits and vegetables, such as peaches, apricots, greengages, bananas, strawberries, honeydew melons, tomatoes, red and green paprika, cucumbers and carrots, *P. expansum*, *P. urticae* and *B. nivea* have also been responsible for patulin production (Frank *et al.*, 1977). Inoculation experiments were performed to investigate the effects of environmental factors on the patulin production by the molds. Hasan (2000) compared the growth and patulin production of *P. expansum* in glucose-Czapek's-apple medium and observed that patulin production increased when growth reached the stationary phase. One proposed explanation was that patulin, as a secondary metabolite of patulin-producing molds, can be biosynthesized when the energy source in the medium is nearly depleted and enough intermediates have accumulated (Hasan, 2000; Grootwassink and Gaucher, 1980). The authors also reported that the patulin accumulation maximum occurred at a lower incubation temperature of 15 °C and a decrease in patulin production due to the degradation by some chemicals as organic acids leaching from the vacuoles and by mycelia of *P. expansum* (Hasan, 2000).

### 1.2.2.2. Biosynthesis of patulin

Although the biosynthesis of patulin appears complicated, it has been fully characterized. The entire pathway includes ten biochemical reactions and is catalyzed by specific enzymes (Figure 1.6; Moake *et al.*, 2005; Puel *et al.*, 2010). The first step in patulin biosynthesis involves the
condensation of one acetyl-CoA and three malonyl-CoA units and the formation of 6-methylsalicylic acid (6-MSA) by the action of the enzyme, 6-MSA synthetase (Birch et al., 1955; Tanenbaum and Bassett, 1959; Scott et al., 1971). Historically, patulin production was demonstrated through oxidative fission of the aromatic ring and rearrangement of $^{14}$C labelled 6-MSA (Bu’Lock and Ryan, 1958; Tannenbaum and Bassett, 1959). By applying the kinetic pulse-labelling study in which radiolabelled acetate and other pertinent secondary metabolites were fed to the culture and the kinetics of the incorporation of radioactivity into subsequent metabolites was examined, acetate, 6-MSA, m-cresol, m-hydroxybenzyl alcohol, m-hydroxybenzaldehyde and gentisaldehyde were verified as the intermediates of patulin production (Forrester and Gaucher, 1972). The following steps include decarboxylation of 6-MSA to m-cresol by 6-MSA decarboxylase, oxidation of m-cresol to form m-hydroxybenzyl alcohol, as well as the formation of gentisaldehyde (Light, 1969; Murphy and Lynen, 1975; Scott and Yalpani, 1967; Scott et al., 1973). Finally, gentisaldehyde is converted to patulin with several intermediates including isoepoxydon, phyllostine, neopatulin and E-ascladiol (Sekiguchi and Gaucher, 1979; Sekiguchi and Gaucher, 1978; Sekiguchi et al., 1979; Sekiguchi et al. 1983).
1.3. Patulin contamination in apple cider and juice

1.3.1. Occurrence of patulin contamination

Worldwide interest in the occurrence of patulin in food has dramatically increased due to the health concerns of its acute and chronic toxicity. Although patulin has been isolated from cheddar cheese and barley malts (Stott and Bullerman, 1976; Lopez-Diaz and Flannigan, 1997), the mycotoxin has been mainly reported as a nature contaminant in fruits such as apples, pears, peaches, cherries, blueberries, strawberries, raspberries, lingon barriers, black mulberries and white mulberries (de Sylos and Rodriguez-Amaya, 1999; Beretta et al., 2000; Martins et al., 2002; Drusch and Ragab, 2003). Consequently, patulin also occurred in the fruit juice and other fruit products including apple juice, apple cider, apple marmalade, apple puree, nectar, pear,
marmalade, pear juice, peach juice, apricot juice, orange juice, grape must, mixed fruit juice and baby food with fruits (Funes and Resnik, 2009; Yuan et al., 2010; Majerus et al., 2008; Spadaro et al., 2008; Leggott and Shephard, 2001; Cho et al., 2010; Harris et al., 2009).

In the last twenty years, a series of surveys on patulin occurrence in fruit products were conducted in different countries including Argentina (Funes and Resnik, 2009), Belgium (Baert et al., 2006), Brazil (de Sylos and Rodriguez-Amaya, 1999; Iha and Sabina, 2008), China (Yuan et al., 2010; Guo et al., 2013), India (Saxena et al., 2008), Iran (Cheraghali et al., 2005), Italy (Beretta et al., 2000; Ritieni, 2003; Spadaro et al., 2007; Spadaro et al., 2008), Japan (Watanabe and Shimizu, 2005), Portugal (Martins et al., 2002; Barreira et al., 2010), South Africa (Leggott and Shephard, 2001), South Korea (Cho et al., 2010), Spain (Marín et al., 2011; Piqué et al., 2013), Tunisia (Zaied et al., 2013), Turkey (Gökmen and Acar, 1998; Yurdun et al., 2001) and U.S.A. (Harris et al., 2009) (Table 1.1).

The surveillance data revealed different frequency and concentrations of patulin contamination in fruit juice especially apple juice. In some regions like India, Iran, Tunisia and Turkey, the mean concentrations of patulin in apple juice (845 µg/L, 48.1 µg/L, 80 µg/L and N/A, respectively) and percentage of samples exceeding 50 µg/L or 50 µg/kg (20 %, 33 %, N/A and 44 %, respectively) were dramatically high (Saxena et al., 2008; Cheraghali et al., 2005; Zaied et al., 2013; Yurdun et al., 2001). One possible reason was proposed that either a Mediterranean or a tropical climate favors growth of patulin-producing fungi and accumulation of the mycotoxin. The more direct reason, however, was attributed to poor agricultural and manufacturing practices where damaged and rotten fruits were not removed prior to processing (Saxena et al., 2008;
Cheraghali et al., 2005; Zaied et al., 2013). The difference of patulin levels also occurred among the variant processing years. A survey of patulin contamination levels of Michigan apple cider has shown mean patulin concentration values of 55.2, 23.9 and 39.7 µg/L in the year of 2002, 2003 and 2004, respectively (Harris et al., 2009). The authors explained higher paulin level in 2002 and 2004 resulted from climatic factors including a late frost in 2002 and a hail storm in 2004. To compensate for reduced apple production and poor apple quality due to adverse climate conditions, processors may do less culling and trimming of damaged or rotten fruits (Harris et al., 2009).

In addition to the contamination level in fruit products, many surveys compared the occurrence of patulin based on agricultural practices (conventional and organic) and clarity (clear and cloudy). Different observations were obtained when comparing the patulin level in apple juice produced from grown conventionally and organically. Ritieni (2003) and Spadaro et al. (2007) reported no significant difference between the two agricultural practices, as both production chains removed decayed and damaged fruits prior to juice processing. In other studies, however, significantly lower patulin concentrations were found in apple juice from conventional compared to organic agriculture (1.01 vs 7.69 µg/kg, Beretta et al., 2000; 2.3 vs 9.1 µg/kg, Piqué et al., 2013; 10.2 vs 43.1 µg/L, Baert et al., 2006). Avoiding application of insecticides and fungicides in organic agricultural practices may favor insect damage and fungal invasion and consequently produce more rotten fruits with a high level of patulin. Spadaro et al. (2007) found no significant difference between clear and cloudy apple juices and presumed that the clarification of apple juice probably did not significantly change the level of patulin. Nevertheless, the data from another survey in Spain showed that the patulin level in cloudy juice
was significantly higher than in clear juice (Piqué et al., 2013). The authors explained the particles of cloudy juice were richer in proteins which may interact with patulin than what is found in the liquid phase.

Finally, the natural occurrence of patulin in spoiled fruits has been reported to be extremely high. The mean patulin concentrations of 220 µg/kg in spoiled apples, 196 µg/kg in spoiled pears and 120 µg/kg in spoiled peaches were found in a survey in Brazil (de Sylos and Rodriguez-Amaya, 1999). Comparing 7 varieties of spoiled apples, Richared showed the highest mean concentration of patulin (80.5 mg/kg). Golden Delicious, Rome Beauty, Red Delicious and Reineta had lower mean values (3.05, 3.06, 4.37, 5.37 mg/kg, respectively, Martins et al., 2002). Beretta et al. (2000) analysed the patulin concentration of rotten areas (0.002 - 113 mg/kg), unaffected areas with peel (ND - 1.17 mg/kg) and unaffected areas without peel (ND – 0.093 mg/kg) of spoiled apples. This study suggests that stricter control should be made prior to juice processing as high levels of patulin were presented not only in the rotten areas but also in unaffected parts of the spoiled fruits.
Table 1.1. Occurrence of patulin in fruits, fruit juices and other fruit products

<table>
<thead>
<tr>
<th>Country</th>
<th>Samples</th>
<th>No. of samples with patulin&gt;50µg/L / no. of samples analysed</th>
<th>Mean concentration of patulin</th>
<th>Maximum concentration of patulin</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina</td>
<td>Apple, marmalade</td>
<td>-/26</td>
<td>6.2 µg/kg</td>
<td>39 µg/kg</td>
<td>Funes and Resnik (2009)</td>
</tr>
<tr>
<td></td>
<td>Apple, puree</td>
<td>-/8</td>
<td>61.5 µg/kg</td>
<td>221 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple, jelly</td>
<td>-/7</td>
<td>0 µg/kg</td>
<td>0 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple, sweet</td>
<td>-/4</td>
<td>0 µg/kg</td>
<td>0 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pear, marmalade</td>
<td>-/6</td>
<td>4.2 µg/kg</td>
<td>25 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Belgium</td>
<td>Apple juice</td>
<td>2/177</td>
<td>5.8 µg/L</td>
<td>328.7 µg/L</td>
<td>Baert et al. (2006)</td>
</tr>
<tr>
<td>Brazil</td>
<td>Apple juice</td>
<td>0/30</td>
<td>-</td>
<td>17 µg/L</td>
<td>de Sylos and Rodriguez-Amaya (1999)</td>
</tr>
<tr>
<td></td>
<td>Other juice (grape, pineapple,</td>
<td>-/8</td>
<td>-</td>
<td>0 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>papaya, guava, banana, mango)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spoiled apples</td>
<td>-/6</td>
<td>220 µg/kg</td>
<td>267 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spoiled pears</td>
<td>-/4</td>
<td>196 µg/kg</td>
<td>245 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spoiled peaches</td>
<td>-/4</td>
<td>120 µg/kg</td>
<td>174 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>Apple-based drink</td>
<td>0/134</td>
<td>-</td>
<td>7 µg/L</td>
<td>Iha and Sabina (2008)</td>
</tr>
<tr>
<td>China (Northeast)</td>
<td>Apple juice</td>
<td>1/15</td>
<td>22.8 µg/kg</td>
<td>90.3 µg/kg</td>
<td>Yuan et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Apple juice, conc.</td>
<td>6/20</td>
<td>28.6 µg/kg</td>
<td>94.7 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baby food</td>
<td>1/30 (~10 µg/kg:11/30)</td>
<td>9.3 µg/kg</td>
<td>67.3 µg/kg</td>
<td>Saxena et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>6/30</td>
<td>24.9 µg/kg</td>
<td>91.8 µg/kg</td>
<td></td>
</tr>
<tr>
<td>China (Shaanxi)</td>
<td>Apple juice, conc.</td>
<td>4/1987</td>
<td>8.44 µg/kg</td>
<td>78 µg/kg</td>
<td>Guo et al. (2013)</td>
</tr>
<tr>
<td>Germany</td>
<td>Grape must</td>
<td>-/96</td>
<td>15.6 µg/L</td>
<td>80 µg/L</td>
<td>Majerus et al. (2008)</td>
</tr>
<tr>
<td>India</td>
<td>Apple juice</td>
<td>3/15</td>
<td>845 µg/L</td>
<td>1839 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple juice, local vendor</td>
<td>5/25</td>
<td>191 µg/L</td>
<td>325 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>0/10</td>
<td>46 µg/L</td>
<td>70 µg/L</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>Apple juice</td>
<td>14/42</td>
<td>48.1 µg/L</td>
<td>285.3 µg/L</td>
<td>Cheraghali et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Apple juice, conc.</td>
<td>13/23</td>
<td>61.7 µg/L</td>
<td>148.8 µg/L</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Apple juice</td>
<td>0/23</td>
<td>-</td>
<td>28.24 µg/kg</td>
<td>Beretta et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>Apple juice with pulp</td>
<td>1/12</td>
<td>-</td>
<td>1150 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baby food</td>
<td>0/23</td>
<td>-</td>
<td>6.39 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spoiled apples (unaffected area)</td>
<td>-/26</td>
<td>-</td>
<td>1170 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Apple products (juice, vinegar,</td>
<td>2/40</td>
<td>26.7 µg/L</td>
<td>74.2 µg/L</td>
<td>Ritiieni (2003)</td>
</tr>
<tr>
<td></td>
<td>puree, baby food)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Country</td>
<td>Samples</td>
<td>No. of samples with patulin &gt; 50 µg/L / no. of samples analysed</td>
<td>Mean concentration of patulin / µg/kg</td>
<td>Maximum concentration of patulin / µg/kg</td>
<td>Reference</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td>----------------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>Italy</td>
<td>Apple juice</td>
<td>0/53</td>
<td>9.32</td>
<td>47.91</td>
<td>Spadaro et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>1/82</td>
<td>4.54</td>
<td>55.41</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Apricot juice</td>
<td>0/27</td>
<td>3.6</td>
<td>32.4</td>
<td>Spadaro et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>Pear juice</td>
<td>0/39</td>
<td>5.1</td>
<td>33.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peach juice</td>
<td>0/30</td>
<td>0.3</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>0/29</td>
<td>4.9</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>Apple juice</td>
<td>0/188</td>
<td>8 µg/L</td>
<td>15 µg/L</td>
<td>Watanabe and Shimizu (2005)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Spoiled apples</td>
<td>-/351</td>
<td>3.05-80.5 mg/kg</td>
<td>-</td>
<td>Martins et al. (2002)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Apple juice</td>
<td>0/68</td>
<td>-</td>
<td>42 µg/kg</td>
<td>Barreira et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Apple puree</td>
<td>0/76</td>
<td>-</td>
<td>5.7 µg/kg</td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>Apple juice</td>
<td>0/13</td>
<td>-</td>
<td>10 µg/L</td>
<td>Leggott and Shephard (2001)</td>
</tr>
<tr>
<td></td>
<td>Apple, carbonated</td>
<td>0/4</td>
<td>-</td>
<td>45 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>0/6</td>
<td>-</td>
<td>5 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple cider, alcoholic</td>
<td>0/8</td>
<td>-</td>
<td>10 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Infant fruit juice and puree</td>
<td>0/17 (&gt;10 µg/kg; 3/17)</td>
<td>-</td>
<td>20 µg/L</td>
<td></td>
</tr>
<tr>
<td>South Korea</td>
<td>Apple juice</td>
<td>0/24</td>
<td>-</td>
<td>8.9 µg/L</td>
<td>Cho et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Orange juice</td>
<td>0/24</td>
<td>-</td>
<td>30.9 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grape juice</td>
<td>0/24</td>
<td>-</td>
<td>14.5 µg/L</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>Apple juice</td>
<td>0/28</td>
<td>-</td>
<td>6 µg/kg</td>
<td>Marin et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Nectar, jam</td>
<td>0/5</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baby food</td>
<td>0/17</td>
<td>-</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple juice, conc.</td>
<td>5/33</td>
<td>-</td>
<td>74.4 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pear juice, conc.</td>
<td>5/10</td>
<td>-</td>
<td>126.9 µg/kg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peach juice, conc.</td>
<td>0/15</td>
<td>-</td>
<td>20.7 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>Apple juice</td>
<td>0/47</td>
<td>5.5 µg/kg</td>
<td>36.5 µg/kg</td>
<td>Piqué et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Apple puree</td>
<td>1/46</td>
<td>3.8 µg/kg</td>
<td>50.3 µg/kg</td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td>Apple juice</td>
<td>-/30</td>
<td>80 µg/L</td>
<td>167 µg/L</td>
<td>Zaied et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>Mixed juice</td>
<td>-/30</td>
<td>55 µg/L</td>
<td>125 µg/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Baby food</td>
<td>-/25</td>
<td>68 µg/L</td>
<td>165 µg/L</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>Apple juice, conc.</td>
<td>98/215</td>
<td>-</td>
<td>376 µg/L</td>
<td>Gökmen and Acar (1998)</td>
</tr>
<tr>
<td>Turkey</td>
<td>Apple juice</td>
<td>20/45</td>
<td>-</td>
<td>732.8 µg/L</td>
<td>Yurdun et al. (2001)</td>
</tr>
<tr>
<td>USA</td>
<td>Apple cider</td>
<td>11/493</td>
<td>36.9 µg/L</td>
<td>467.4 µg/L</td>
<td>Harris et al. (2009)</td>
</tr>
</tbody>
</table>
1.3.2. Current regulations

The health concerns regarding the toxicity of patulin and the frequent occurrence of the mycotoxin in fruit products (especially apple juice, the popular drink to the consumers) motivated government efforts to establish tolerance levels for patulin in fruit products. Scientific options included identification of a no observed adverse effect level (NOAEL) and a provisional tolerable daily intake (PTDI) and patulin exposure assessments (U.S.FDA, 2001). First, the U.S.FDA reviewed toxicological studies of patulin and adopted the results from a long-term (104 weeks) feeding study on rats (Becci et al., 1981). In that study, the NOAEL of patulin in rats was identified as 0.1 mg/kg b.w. three times a week (or 0.043 mg/kg b.w. per day). In order to estimate the acceptable exposure level for patulin in humans, a safety factor (also called uncertain factor) was used to extrapolate the NOAEL from animal experiments (Lehman and Fitzhugh, 1953). The FDA chose a 100-fold safety factor, which included two 10-fold safety factors accounting for the interspecies extrapolation from rats to humans, and the intraspecies variation reflecting typical population subgroups (Dourson and Stara, 1983; Dourson et al., 1996). The PTDI was finally calculated as 0.43 µg/kg b.w. per day by dividing the NOAEL of patulin on rats by a safety factor of 100. Secondly, the FDA applied a "Monte Carlo analysis" to estimate patulin exposure (Rubinstein, 1981) based on the consumption data for apple products and the occurrence level of patulin in these products. The 90th percentile apple patulin exposure were calculated and compared with the PTDI in the following groups: all ages, age 1 to 2, and age under 1. The data showed there was enough margin of protection when juice samples with patulin level exceeding 50 µg/kg were excluded. When all samples were included, however, the 90th percentile apple patulin exposure was almost four times the PTDI in the 1 to 2 age group. The U.S.FDA believed that consumers would be at negligible risk of adverse health effects from
patulin if processors controlled the patulin concentration in apple juice to 50 µg/kg or below (U.S.FDA, 2001).

Based on the study described above, the U.S. FDA set a maximum level (50 µg/L) in single-strength apple juice, reconstituted single-strength apple juice, or single-strength apple juice used as an ingredient in food (U.S.FDA, 2005). In Canada, Health Canada adopted a maximum concentration of patulin (50 µg/kg) in apple juice, including the apple juice portion of any juice blends or drinks, and unfermented apple cider (Health Canada, 2012). The EU conducted a similar study and limited the patulin concentration in fruit juice, spirit drinks and cider derived from apples to 50 µg/kg (EU, 2006). The EU also set lower limitations in solid apple products (25 µg/kg) and apple juice and solid apple products for infants and young children (10 µg/ kg) (EU, 2006). The action level of 50 µg/kg has also been adopted by other countries such as Brazil, China, GCC (Gulf Cooperation Council), India, Japan, Kenya, Nigeria, Russia, Singapore and South Africa (Kubo, 2012; Anonymous, 2014).

1.3.3. Control of patulin contamination in liquid apple products

1.3.3.1. Sources of patulin contamination in apple juice production

It has been widely agreed that the main source of patulin contamination in liquid apple products comes from unhealthy and damaged fruits which may contain high concentration of patulin by the invasion of molds (Drusch and Ragab, 2003; Sant’Ana et al., 2008). The damaged fruits were the result of either adverse environmental conditions such as a late frost and a hail storm (Harris et al., 2009), or application of organic agricultural practices without appropriate pest control (Beretta et al., 2000; Piqué et al., 2013; Baert et al., 2006). Without careful culling, the
contaminated apples can be mixed with sound fruit resulting in transfer of patulin into the final apple juice. Moreover, lower quality apples unsuitable for edible retail purposes are frequently used to process apple juice, which further raises the risk of patulin contamination (Moake et al., 2005). The typical apple juice production flow from farm to table includes pre-harvest growth in orchards, harvest, post-harvest (fruit transport, storage, reception, washing and selection) and juice processing (pressing, clarification, pasteurisation, concentration, formulation, filling, storage and commercialisation) (Sant’Ana et al., 2008). Thus, the strategies for controlling patulin in liquid apple products would be to prevent patulin contamination during the pre-harvest, harvest and post-harvest steps, or by decreasing patulin levels in contaminated juice during processing.

1.3.3.2. Prevention of patulin contamination

Implementation of an integrated management system such as hazard analysis and critical control points (HACCP) with prerequisite good agricultural practices (GAP) and good manufacturing practices (GMP) is the best method to minimize patulin in each phase of juice production (Lopez-Garcia and Park, 1998; Park et al., 1999). Based on the GAP and GMP, the EU issued a code of practices on the prevention and reduction of patulin contamination in apple juice and apple juice ingredients in other beverages (EC, 2003). During the pre-harvest, the code suggests removal of diseased wood and fruits in the dormant season, control of pests and diseases that lead to the rotting or introduce create entry sites for patulin-producing molds, and application of fungicide to inhibit fungal growth. During the harvest, the code recommended gentle handling apples to minimize physical damage, discarding fallen fruit, transporting apples to processing facilities within 3 days after harvest, and ensuring apple collection containers are dry, clean and
free of debris. During the post-harvest, the code advised careful sorting of apples to remove visually moldy fruit, checking core rots in varieties with an open calyx, chilling fruit and maintaining the temperature of < 5 ºC, storing apples under controlled atmosphere conditions (< 1.8 % of oxygen), and washing fruit thoroughly before pressing (EC, 2003).

Selection or culling apple fruit upstream of juice processing has been shown to effectively reduce patulin levels (Wilson and Nuovo, 1973; Sydenham et al., 1995; Sydenham et al., 1997; Jackson et al., 2003). Wilson and Nuovo (1973) examined 100 samples of fresh apple cider and found that five samples with higher patulin concentrations (up to 45 mg/L) were made from decayed and insect damaged apples. By removing damaged fruits and applying a wash treatment prior to processing, Sydenham et al. (1995) found that the patulin concentration reduced to 55 µg/kg, which was significant lower than a wash treatment alone (190 µg/kg). Jackson et al. (2003) reported that the patulin concentration of apple cider pressed from four cultivars (Golden Delicious, Granny Smith, Red Delicious and Fuji) of ground-harvested apples were in the range between 40.2 and 374 µg/L, and that the mycotoxin was not detected in cider produced from tree-picked apples. The authors also found 0.97 to 64 µg/L of patulin in apple cider made from unculled tree-picked apples after 4 to 6 weeks storage at 0 to 2 ºC, compared to no detectable amounts of patulin in apple cider made by culled tree-picked apples.

The relationship between the production of patulin and the storage conditions (temperature and atmosphere composition) has also been investigated (Paster et al., 1995; Lovett et al., 1975; Sitton and Patterson, 1992; Johnson et al., 1993). Paster et al. (1995) compared patulin production on apples kept at various storage temperatures of 0, 3, 6, 17 and 25 ºC and found the
highest patulin concentration at 17 °C. The authors also observed total inhibition of patulin production when controlled atmosphere (CA) condition (3 % CO₂ / 2 % O₂, 25 °C) were used. The data matched a previous study that found lower patulin concentrations (500 µg/L) in juice made from apples subjected to CA storage (1-3 % CO₂ / 3 % O₂, 0-3.3 °C) compared to normal atmospheric storage (2000 to 3000 µg/L) for 14 weeks (Lovett et al., 1975). The higher level of CO₂ (> 3 % and < 8 %) and lower O₂ level (0.75 %) were also reported to be more effective at controlling rot in stored apples (Sitton and Patterson, 1992; Johnson et al., 1993).

Washing treatment is a final measure for preventing patulin contaminated apples from entering juice processing. By immersing ground-harvested apples in wash solutions (potable water, 100 and 200 ppm chlorine) for 2 min, patulin levels can be reduced by 10 to 100 % depending on the initial concentration and wash solutions (Jackson et al., 2003). Using a high-pressure water spraying, 54 % of patulin was removed from apples (Acar et al., 1998). Sydenham et al. (1995) reported that apples with an initial patulin concentration of 920 ng/g dropped to 190 ng/g after a water wash treatment. Although wash treatment is easily operated and effective at eliminating most of the patulin, it is insufficient for completely removing patulin since it can diffuse into healthy part of tissue (Marín et al., 2006). Another limitation of wash treatment may come from the wash water. The water which contains the patulin and suspended fungal spores from rotten fruits, could cross-contaminate other fruit if the sanitation measures are insufficient (Sydenham et al., 1995; Sant’Ana et al., 2008).
1.3.3.3. Reduction of patulin during juice processing

Once the patulin transfers to juice during processing, measures should be taken to decrease the toxin to a safe level. During juice clarification and filtration, application of activated carbon to remove patulin has been widely studied (Kadakal and Nas, 2002; Mutlu et al., 1997; Huebner et al., 2000; Leggott et al., 2001). Kadakal and Nas (2002) reported that mixing 3 g/L of activated charcoal for 5 min decreased the patulin levels from 62.3 ppb to 30.8 ppb. The efficacy of a composite carbon adsorbent (CCA) with ultrafine activated carbon bonded onto granular quartz was evaluated for patulin reduction in apple juice. The 50% breakthrough capacities for patulin in 1.0, 0.5 and 0.25 g CCA columns were 137.5, 38.5 and 19.9 µg (Huebner et al., 2000). Leggott et al. (2001) compared the adsorptions of patulin in apple juice in two kinds of steam-activated carbons and one chemically-activated carbon. The authors found lower patulin removal when using the chemically-activated carbon (45%) than the steam-activated ones (70% and 80%) at a dosage of 1 g/L. Although activated carbon adsorption was able to reduce patulin levels, negative effects such as changes in color, pH, °Brix, fumaric acid content and taste impeded the commercial application of the technique (Kadakal and Nas, 2002; Huebner et al., 2000). Some conventional techniques were therefore investigated. Acar et al. (1998) reported that application of a rotary vacuum pre-coat filter removed 39% of patulin from apple juice, which was more effective than ultrafiltration (25%). The use of paper filtration, pectinase treatment, fining with bentonite and centrifugation were reported to reduce patulin levels in apple pulp spiked with patulin by 70%, 73%, 77% and 89%, respectively (Bissessur et al., 2001).

Patulin degradation during thermal treatments was investigated by several studies, although these treatments showed limited effectiveness. For examples, Lovett and Peeler (1973) described the
kinetics of thermal degradation of patulin. The D value at 105 °C in a pH 3.5 solution was 1058 min. Kryger (2001) reported 33 % patulin reduction in apple juice after heating at 100 °C for 177 min. Kadakal and Nas (2003) found that patulin concentration in apple juice decreased with increased heating times at 90 °C and 100 °C. After 20 min, 18.81 % and 25.99 % of patulin reductions were observed, respectively. The effect of high temperature short time (HTST) pasteurization on the patulin reduction was also investigated. The mycotoxin levels in apple juice decreased by 19 % at 90 °C for 10 s (Wheeler et al., 1987). Although pasteurization is unlikely to reduce patulin levels sufficiently, it can inactivate the spores of Penicillium expansum and prevent subsequent patulin production in apple juice (Sant’Ana et al., 2008).

Electromagnetic irradiation is an alternate choice instead of thermal treatment due to its ability to inactivate pathogens and degrade mycotoxins. Zegota et al. (1988) reported that an ionizing radiation dose of 0.35 kGy caused a 50 % reduction of patulin in apple juice. Although not studied extensively, it is also possible that patulin could be degraded using ultraviolet (UV) treatments. Recently, it was reported that UV exposures of 14.2 to 99.4 mJ·cm⁻² resulted in a significant decrease in patulin levels of 9.4 to 43.4% while producing no quantifiable changes in the chemical composition (i.e., pH, Brix, and total acids) or organoleptic properties of cider (Dong et al., 2010).

1.4. UV technique

1.4.1. Principle of UV light

As part of the electromagnetic radiation in the range between 100 and 400nm, UV light is divided into the range of vacuum UV (VUV, 100-200nm), UV-C (200-280nm), UV-B (280-
315nm) and UV-A (315-400nm) (Figure 1.7; ISO, 2007). In principle, a discrete amount of energy (photon, E) is generated and released by a transition of electrons of atoms and ions from a higher energy state (E₂) to a lower one (E₁) (Equation 1.1). The spectrum of emitted light is determined by the particular atoms or ions and their interaction with external force fields (Koutchma, 2009).

\[ E = E_2 - E_1 = \frac{hc}{\lambda} \]  

(1.1)

Where \( h \) is Plank’s constant \( (6.23 \times 10^{-34} \text{ J}\cdot\text{s}) \), \( c \) is the speed of light \( (2.998 \times 10^8 \text{ m}\cdot\text{s}^{-1}) \) and \( \lambda \) is the wavelength of radiation (m).

Generated UV light from UV sources will propagate away from the atoms and ions. The intensity of the light attenuates as it interacts with encountered materials through absorption, reflection, refraction and scattering. UV photons may react with a reactant molecule (A) when the photons are absorbed by the molecule and they have sufficient energy to promote a reaction (Koutchma et al., 2009; Hall, 2000) (Equation 1.2).

\[ A + hv \rightarrow A^+_\text{products} \]  

(1.2)

The typical bond energies of important biological moieties and the corresponding wavelengths are summarized in Table 1.2 (Blatchley and Peel, 2001). Evidently, photons in the UV spectrum possess sufficient energy to promote photochemical reactions.
Figure 1.7. Electromagnetic spectral categories from gamma-rays through radio wavelengths
(Source: ISO, 2007)

Table 1.2. Typical bond energies of important biological moieties and their corresponding wavelengths (Source: Blatchley and Peel, 2001)

<table>
<thead>
<tr>
<th>Bond</th>
<th>Typical bond energy (kJ/mol)</th>
<th>Corresponding wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-H</td>
<td>460</td>
<td>260</td>
</tr>
<tr>
<td>C-H</td>
<td>410</td>
<td>290</td>
</tr>
<tr>
<td>N-H</td>
<td>390</td>
<td>310</td>
</tr>
<tr>
<td>C-O</td>
<td>370</td>
<td>320</td>
</tr>
<tr>
<td>C=O</td>
<td>740</td>
<td>160</td>
</tr>
<tr>
<td>C=C</td>
<td>620</td>
<td>190</td>
</tr>
<tr>
<td>C≡C</td>
<td>830</td>
<td>140</td>
</tr>
<tr>
<td>C=N</td>
<td>600</td>
<td>200</td>
</tr>
<tr>
<td>C≡N</td>
<td>850</td>
<td>140</td>
</tr>
</tbody>
</table>
1.4.2. UV sources

As interest in the use of the UV technique for non-thermal processing of liquid food has increased, a number of traditional and advanced UV sources have become available for laboratory studies and commercial applications. They include mercury lamps, amalgam lamps, excimer lamps, pulsed lamps, microwave lamps and UV-light-emitting diodes (Koutchma et al., 2009). Figure 1.8 shows the spectra of different UV lamps and the cell deactivation curve (Schalk et al., 2006).

Mercury vapour UV lamp sources are considered reliable sources for disinfecting liquids such as water and fruit juices due to good performance and low cost. Low pressure mercury (LPM) and medium pressure mercury (MPM) UV sources based on the vapour pressure of mercury are the two typical mercury vapour lamps. LPM lamps are operated at a nominal total gas pressure of $10^2$ to $10^3$ Pa, which corresponds to the vapour pressure of mercury at a temperature of 40 °C. The emission spectrum of LPM is concentrated at the resonance lines of 253.7 and 185 nm (Masschelein, 2002). The 253.7 nm line represents approximately 85 % of the total emitted UV intensity and shows a strong germicidal effect since the photons at this wavelength can be efficiently absorbed by the DNA of microorganisms (Bolton and Linden, 2003; Falguera et al.; 2011b). MPM lamps are polychromatic and operated at a total gas pressure of $10^4$ to $10^6$ Pa (Masschelein, 2002). The emission spectrum of MPM covers wavelengths from about 200 nm to almost 600 nm, which results from a series of emissions in the UV and in the visible ranges. Although the wider spectrogram of MPM lamps is not efficient to target germicidal treatment, they have been useful in waste treatment for series oxidation and photochemistry reactions. The
stronger photon flux in these units results in high penetration depth which may promote photo-degradation in food matrixes with higher UV absorption.

Instead of mercury lamps, an alternative low pressure amalgam (LPA) UV lamp was developed with the characteristics of high power output and long life time. Compared with LPM lamps, LPA lamps reach the optimum mercury vapor press of $10^2$ Pa at a higher wall temperature of approximately 100 °C. Therefore, they consumed more electrical power and generate up to 1000 mW/cm of specific UV-C-flux per unit arc length (Schalk et al., 2006). The higher operational temperatures of LPA lamps reduce the sensitivity to temperature fluctuations and thus result in a constant UV flux. Another advantage of LPA lamps is a long operation life time up to 16000 hours if a long life technique is applied (Schalk et al., 2006).

UV lamps used for water treatment typically contains 5 to 400 mg of mercury content (Koutchma et al., 2009). Health concerns of mercury release due to the breakage of the UV lamp sleeve encouraged the development of mercury-free UV sources. Excilamps, also called excimer lamps or exciplex lamps, have been designed based on the formation of rare gas ($Rg_2^*$), halogen excimers ($X_2^*$) or rare gas halide exciplexes ($RgX^*$), and the efficient fluorescence of these molecules in different types of discharges (Oppenländer and Sosnin, 2005). According to the excimers and exciplexes obtained from halogens and rare gases, the excilamps can emit various quasi monochromatic spectrums in the VUV and UV range (Table 1.3). The available narrow band of emission at a specific wavelength encouraged the research interests on potential applications in photochemistry, photobiology, and photo-medicine fields. Along with being mercury free and the narrow band of emission, excilamps also have the advantage of long life
time, instant on lamps without warming-up, and variable geometries. Their weaknesses include a lower UV-C efficiency and higher investment cost (Koutchma et al., 2009; Oppenländer and Sosnin, 2005; Schalk et al., 2006).

Figure 1.8. Spectrum of UV Lamps and cell deactivation curve (Source: Schalk et al., 2006)


b. MPM lamp, high performance

c. MPM lamp, standard

d. 222 nm Excimer lamp

e. LPM lamp

f. 282 nm Excimer lamp
Table 1.3. Matrix of excimers \((X_2^*, \text{Rg}_2^*)\) and exciplexes \((\text{RgX}^*)\) obtained from halogens and rare gases and their emission maxima (nm) (Source: Oppenländer and Sosnin, 2005).

<table>
<thead>
<tr>
<th>Halogen ((X_2^*))</th>
<th>He</th>
<th>Ne</th>
<th>Ar</th>
<th>Kr</th>
<th>Xe</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>74</td>
<td>84</td>
<td>126</td>
<td>146</td>
<td>172</td>
</tr>
<tr>
<td>Cl</td>
<td>-</td>
<td>108</td>
<td>193</td>
<td>248</td>
<td>354</td>
</tr>
<tr>
<td>Br</td>
<td>-</td>
<td>-</td>
<td>175</td>
<td>222</td>
<td>308</td>
</tr>
<tr>
<td>I</td>
<td>-</td>
<td>-</td>
<td>165</td>
<td>207</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>190</td>
<td>253</td>
</tr>
</tbody>
</table>

1.4.3. Calculation of UV fluence

1.4.3.1. Technique terms

Various terms, symbols and formulas were used to describe the quantity, distribution and behaviour of UV light in a medium. The International Union of Pure and Applied Chemistry (IUPAC) recommended the definition and application of the following terms (Braslavsky et al., 2011). Photon flux \((q_p)\) is the number of photons per time interval with an SI unit of \(s^{-1}\). The photon flux can be also expressed as photon flux, amount basis \((q_{n,p})\) with an SI unit of \(\text{mol} \cdot s^{-1}\). Photon fluence rate \((E_{p,o})\) is the photon flux incident from all directions on a small element of surface containing the point under consideration divided by the area of the element, with an SI unit of \(m^{-2} \cdot s^{-1}\). Photon irradiance \((E_p)\) is used for a beam incident from all upward directions with an SI unit of \(\text{mol} \cdot m^{-2} \cdot s^{-1}\). Radiant power \((P)\) is energy of UV light per time interval with an SI unit of \(W\). Radiant intensity \((I)\) is the radiant power at all wavelengths per solid angle \((\Omega)\) with an SI unit of \(W \cdot \text{sr}^{-1}\). For a point source, the radiant intensity does not decrease with distance in a non-absorbing medium (Equation 1.3).

\[
I = \frac{P}{4\pi}
\]  \hspace{1cm} (1.3)
**Fluence rate** \( (E_o) \) is the radiant power of all wavelengths incident from all directions on a small element of surface containing the point under consideration divided by the area of the element (Equation 1.4). The SI unit of fluence rate is W·m\(^{-2}\). **Irradiance** \( (E) \) is used for a beam incident from all upward directions.

\[
E_o = \frac{dP}{dS}
\]  

(Equation 1.4)

**Fluence** \( (H_o \text{ or } F_o) \) is the radiant energy incident on a small sphere from all direction divided by the cross-sectional area of that sphere. The SI unit of fluence is J·m\(^{-2}\). Fluence can be calculated according to equation 1.5.

\[
H_o = \int E_o \, dt
\]  

(Equation 1.5)

**Dose** is the energy or amount of photons absorbed per surface area or per volume by an irradiated object during a particular exposure time. The SI unit of dose is J·m\(^{-2}\) or J·m\(^{-3}\). The term is used widely in UV disinfection applications, which have the same meaning as fluence.

In a specific research, some derived terms were defined and adopted. **Applied UV fluence** \( (H_{app}, \text{SI unit: J·m}^{-2}) \) is the energy per unit area of sample surface without considering energy loses due to reflection and scattering. It is independent of the nature of the material to be irradiated. Knowledge of the applied fluence is important to select a correct power and type of UV source by taking into account their UV efficiency in order to achieve a targeted degradation or inactivation of material. **Applied UV fluence rate** \( (E_{app}, \text{SI unit: W·m}^{-2}) \) is easily measured with a radiometer or a chemical actinometer. **Average UV fluence** \( (H_{avg}, \text{SI unit: J·m}^{-2}) \) reflects average energy distribution in liquid sample. The concept considers the attenuation of UV energy in the UV reactor vessel and food material due to the reflection, refraction, absorption, and
divergence. The average UV fluence provides accurate information on the actual energy used for the microbial disinfection and chemical degradation. The value is comparable amongst different UV reactors. Unlike applied UV fluence rate, **average UV fluence rate** \( E_{\text{avg}} \) (SI unit: W \cdot m^{-2}) cannot be measured directly. Correction factors combined with a mathematic model could be used to determine its value.

Bolton and Linden (2003) proposed a serial correction factors for determination of UV fluence in collimated beam UV reactor. Some of these factors are also applicable to other UV reactors with necessary modifications.

**Petri factor** (PF) is defined as a ratio of the average value of UV fluence rate on the surface of a sample in a petri dish (or other containers) over the value at the sample’s center. The UV fluence rate is measured by a radiometer at each small grid (eg. 5 mm) on the petri dish. The PF is used to characterize the non-uniformity of UV light on the surface of a particular sample.

**Reflection factor** (RF) is defined as the percentage of UV light entering a medium from another medium with a different refractive index. Based on Fresnel Law (Meyer-Arendt, 1984), the reflectance \( Re \), which is the percentage of reflected light at the interface, can be calculated using equation 1.6:

\[
Re = \frac{1}{2} \left[ \left( \frac{n_2 \cdot \cos \varphi_1 - n_1 \cdot \cos \varphi_2}{n_1 \cdot \cos \varphi_1 + n_2 \cdot \cos \varphi_2} \right)^2 + \left( \frac{n_1 \cdot \cos \varphi_1 - n_2 \cdot \cos \varphi_2}{n_1 \cdot \cos \varphi_1 + n_2 \cdot \cos \varphi_2} \right)^2 \right]^{1/2}
\]

where, \( \varphi_1 \) is the angle of incidence, \( \varphi_2 \) is the angle of refraction, \( n_1 \) and \( n_2 \) are refractive indices of the media on the incidence and refraction sides, respectively. If the medium is exposed to the
UV light from the upward direction, the reflectance can be expressed in a simpler manner (Equation 1.7).

\[ Re = \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2 \]  

(1.7)

Thus, the RF can be calculated using equation 1.8.

\[ RF = 1 - Re \]  

(1.8)

**Refraction factor (RfF)** impacts the UV energy distribution in a liquid sample by altering the light pathway at an interface between two media. There is no particular equation for calculating RfF, but the factor should be considered when developing mathematic models for the fluence determination in complex UV reactors. For systems in which UV light radiates in an upward direction, the refraction factor is not applicable.

**Divergence factor (DF)** characterizes the attenuation of UV fluence due to the divergence of UV light (Equation 1.9).

\[ E_r = \frac{P}{4\pi r^2} \]  

(1.9)

where, \( E_r \) is the fluence rate at the point at a distance \( r \) from the point source. In the case of collimated beam UV reactors, the DF is defined as the ratio of the average UV fluence rate in the sample over the value at the center of the material surface without consideration of the energy loses due to absorption in the medium (Bolton and Linden, 2003) (Equation 1.10):

\[ DF = \frac{l'}{l' + l} \]  

(1.10)

where, \( l' \) is the distance between the sample surface and the UV source, and \( l \) is the sample thickness.
Absorption factor (AF) characterizes the attenuation of UV fluence rate when the UV light is absorbed by the medium (Equation 1.11):

\[ E_x = E_0 \cdot 10^{-\alpha_{10}x} = E_0 \cdot \exp(-\alpha x) \]  

(1.11)

where, \( E_x \) is the fluence rate at point with a light path length \( x \) in the liquid sample. \( E_0 \) is the fluence rate at the cross point between the light path length and the sample surface. \( \alpha \) is the absorption coefficient of the liquid sample. In the case of collimated beam UV reactors, the AF can also be named the Water Factor (WF) which is defined as the ratio of the average UV fluence rate in the sample over the value at the center of material surface without consideration of the energy loses due to divergence (Bolton and Linden, 2003) (Equation 1.12):

\[ AF = \frac{1 - 10^{-\alpha_{10}l}}{\alpha_{10}l \cdot \ln(10)} \]  

(1.12)

Sensor factor (SF) is applied when a radiometer calibrated at 254 nm is used to measure the fluence rate of a polychromatic UV lamp (eg. MPM lamp). It is defined as the ratio of the sensitivity of the radiometer at 254nm over the weighted average sensitivity of the detector in the UV range of interest (eg. 200~300nm) (Bolton and Linden, 2003) (Equation 1.13):

\[ SF = \frac{S_{254}}{\sum_i N_{\lambda i} S_{\lambda i}} \]  

(1.13)

where \( S_{254} \) is the radiometer sensitivity at 254 nm, \( N_{\lambda i} \) and \( S_{\lambda i} \) are the relative lamp emission and the radiometer sensitivity in a narrow wavelength band centered at a wavelength of \( \lambda_i \).

1.4.3.2. Determination of UV fluence in Batch UV reactors

Collimated beam UV reactors emit parallel light as the UV light has been collimated by either a long cylindrical tube or successive apertures (Figure 1.9A). The sample is placed horizontally
under the light output and receives UV radiation in the upward direction. Therefore, the fluence rate is equal to the irradiance. Bolton and Linden (2003) established a standard equation to determine the average fluence rate of collimated UV reactors with monochromatic light (Equation 1.14):

\[ E_{avg} = E_0 \cdot (PF) \cdot (RF) \cdot (AF) \cdot (DF) \]  

(1.14)

Substitute equation 1.7, 1.8 and 1.10 into equation 1.14 gives:

\[ E_{avg} = E_0 \cdot (PF) \cdot \left[ 1 - \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2 \right] \cdot \frac{1 - 10^{-al}}{al \cdot \ln(10)} \cdot \frac{t'}{t'+l} \]  

(1.15)

The applied UV fluence rate can then be:

\[ E_{app} = E_0 \cdot (PF) \]  

(1.16)

In a collimated beam UV reactor, \( E_0 \) is the UV fluence rate at the centre of the petri dish and is easily measured with a radiometer. For polychromatic collimated beam reactors, \( E_0 \) should be adjusted by the sensor factor.

Point light source UV reactors are the simplest reactors as the light source is assumed as a point (Figure 1.9B). One point source UV reactor is a UV light emitting diode (UV-LED) lamp (Bowker et al., 2011). In a point light source UV reactor, a petri dish containing a liquid sample is placed under the UV source. The projection of the point source is on the center of petri dish. The fluence rate at any point on the surface of sample with a distance \( r \) from the center of petri dish can be described as:

\[ E_\lambda(r) = \frac{P_\lambda}{4\pi(L^2 + r^2)} \]  

(1.17)
where $P_{\lambda}$ is the output power of the point source, and $L$ is the distance between the point source and the centre of petri dish. Consequently, the applied UV fluence rate can be calculated by integrating all the points on the surface of liquid sample (Equation 1.18):

$$E_{\lambda,\text{app}} = \frac{P_{\lambda}}{4\pi R^2} \cdot \ln \frac{L^2 + R^2}{L^2}$$

(1.18)

where, $R$ is the radius of petri dish. The calculation of the average UV fluence rate is complex due to energy loses through reflection, refraction, absorption and divergence. Equation 1.19 shows the UV fluence rate at any point with distance $r$ from the center axis of the petri dish and distance $h$ from the surface of sample.

$$E_{\lambda(r,h)} = (1 - Re) \cdot \frac{P_{\lambda}}{4\pi (l_1 + l_2)^2} \cdot \exp(-\alpha_{\lambda} l_2)$$

(1.19)

where $l_1$ and $l_2$ are the light path length between the point source and the surface of sample as well as between the surface of the sample and the point $(r,h)$, respectively. $Re$ is the reflectance, calculated using equation 1.6. The average UV fluence rate can be expressed as:

$$E_{\lambda,\text{avg}} = \int_0^R \int_0^H E_{\lambda(r,h)} dr dh$$

(1.20)

As equation 1.20 is difficult to resolve, in practice, a finite number of points equally spaced from each other in the sample can be used to calculate $E_{\lambda,\text{avg}}$ based on equation 1.19.

In order to calculate $E_{\lambda,\text{app}}$ and $E_{\lambda,\text{avg}}$, the power output $P_{\lambda}$ must be known. It can be calculated using equation 1.21:

$$P_{\lambda} = 4\pi L^2 \cdot E_{\lambda,0}$$

(1.21)

where $E_{\lambda,0}$ is the fluence rate (or irradiance) at the centre of petri dish and can be measured by a radiometer. It should be noted that the discussion above is based on a monochromatic UV source.

In the case of a polychromatic source, the applied and average UV fluence rates are expressed as:
\[ E_{app} = \int_{\lambda} E_{\lambda,app} d\lambda \quad , \quad E_{avg} = \int_{\lambda} E_{\lambda,avg} d\lambda \]  

(1.22)

In practice, the total applied and average UV fluence rates are achieved by the summation of infinite narrow wavelength bands (e.g. 5 nm) in the spectrum of interest (e.g. 200-300nm).

Linear UV lamps are the most popular UV sources in both research and industrial. The lamps are widely used in either annular or plane photo reactors (Figure 1.9C,D,E,F) (Harris and Dranoff, 1965; Jacobm and Dranoff, 1970; Irazoqui et al., 1973; Suidan and Severin, 1986; Blatchley 1997; Esplugas et al., 1983; Bolton, 2000; Falguera et al., 2011a). A series of models were developed to investigate the UV energy distribution in reactors with a single linear lamp. Harris and Dranoff (1965) proposed a radial linear model (also call an infinite-length lamp model or paralleled radiation model) which assumes that the linear lamp is composed of a continual point source with the same power which emits UV photons in a plane that is perpendicular to the lamp (Figure 1.9C). The UV fluence rate at any point in the annular reactor without consideration of reflection and refraction is expressed as:

\[ E_{\lambda,(r,z)} = \frac{E_{\lambda,r0}r_0}{r} \exp[-\alpha_{\lambda}(r-r_0)] \]  

(1.23)

where, \( E_{\lambda,r0} \) is the fluence rate at the inner radius \( r_0 \) on the perpendicular plane to the lamp; \( r \) and \( z \) are the radial and axial coordinates, respectively; \( \alpha_{\lambda} \) is the Napierian coefficient of the liquid sample. The average fluence rate can be expressed as : (Suidan and Severin, 1986).

\[ E_{\lambda,avg} = \frac{2E_{\lambda,r0}r_0}{(R^2 - r_0^2)} \int_{r_0}^{R} \exp[-\alpha_{\lambda} (r-r_0)] dr \]  

(1.24)

where, \( R \) is the outer radius of the reactor.

However, the radial linear model is not exact in its description of UV light radiation (Esplugas et al., 1983); it can predict a higher average fluence rate than the value from a spherical linear
model (Suidan and Severin, 1986). The spherical linear model (also called a finite line source model or line source integration model) assumes that the lamp is a line forming from consecutive point sources which emit photons spherically (Jacobm and Dranoff, 1970; Suidan and Severin, 1986; Blatchley 1997; Esplugas et al., 1983; Bolton, 2000) (Figure 1.9D). Without consideration of reflection, refraction and absorption of the quartz jacket, the UV fluence rate at any point \((r, z)\) can be expressed as:

\[
E_{\lambda}(r,z) = \frac{S_{\lambda,L}}{4\pi} \int_x^{x+L} \frac{\exp(-\alpha_{\lambda}B)}{r^2 + (z - x')^2} \, dx'
\]  

(1.25)

\[
S_{\lambda,L} = \frac{P_{\lambda}}{L}
\]  

(1.26)

\[
B = \left(1 - \frac{r_0}{r}\right) \sqrt{r^2 + (z - x')^2}
\]  

(1.27)

where, \(S_{\lambda,L}\) is the output power of the UV lamp per unit length; \(r\) and \(z\) are the radial and axial coordinates, respectively; \(x\) is the distance between the bottom of the reactor and the bottom of the lamp; \(x'\) is the distance between the bottom of the reactor and the point \((r,z)\); \(\alpha_{\lambda}\) is the Napierian coefficient of liquid sample. \(r_0\) is the inner radius of the reactor; \(B\) is the light path length in the liquid sample. If the absorption of the quartz jacket is accounted for, equation 1.25 should be rewritten as:

\[
E_{\lambda}(r,z) = \frac{S_{\lambda,L}}{4\pi} \int_x^{x+L} \frac{\exp\left[-(\alpha_{\lambda,q}B' + \alpha_{\lambda}B)\right]}{r^2 + (z - x')^2} \, dx'
\]  

(1.28)

\[
B' = \frac{t_q}{r} \sqrt{r^2 + (z - x')^2}
\]  

(1.29)

where, \(t_q\) is the thickness of the quartz jacket; \(B'\) is the path length of UV light in the quartz jacket; and \(\alpha_{\lambda,q}\) is the Napierian coefficient of quartz at wavelength \(\lambda\).
Equations 1.25 and 1.28 cannot be integrated analytically unless there is no UV absorption in the reactor or absorption can be neglected. In such situations, the solution of equation 1.25 can be expressed as:

\[ E_{\lambda(r,z)} = \frac{S_{\lambda,L}}{4\pi r} \left[ \arctan \left( \frac{z - x}{r} \right) - \arctan \left( \frac{z - x - L}{r} \right) \right] \] (1.30)

For a more accurate determination of average fluence rate in an annular reactor, Bolton (2000) modified the spherical linear model by considering the significance of reflection and refraction (Figure 1.9E). In a general annular UV reactor, the reflection is taking place at least two interfaces (the air/quartz and quartz/sample). The number of interfaces could be increased if other functional appliances (eg. cooling water jacket) are applied. The reflection factor can then be expressed as:

\[ RF = (1 - Re_1)(1 - Re_2) \cdots (1 - Re_n) \] (1.31)

where, Re_n is the reflectance at the interface in question. Refraction alters the light pathway and influences the energy distribution in the reactor vessel. If the refraction factor is considered, the refraction of light should be determined by Snell’s Law and the given geometry of the reactor. Equation 1.32 was proposed by Bolton et al. (2000) to describe the fluence rate in an annular reactor considering both reflection and refraction.

\[ E_{\lambda(r,\theta,z)} = (RF) \frac{P \cdot \sin \theta \cdot \Delta \theta}{2n \cdot \Delta S} \exp(-\alpha_{\lambda}l) \] (1.32)

where, \( E_{\lambda(r,\theta,z)} \) is the fluence rate at position \( (r,z) \) with light emitted from a specific position on the linear lamp with an azimuthal angle of \( \theta \); \( P \) is the output power of the UV lamp, which is divided by \( n \) parts of equally-spaced point sources; \( r \) and \( z \) are the radial and axial coordinates, respectively; \( \Delta S \) is the circular radiation area with distance \( r \) to the lamp axis generated by a small change of the azimuthal angle \( (\theta) \); \( \alpha_{\lambda} \) is the Napierian coefficient of medium in the reactor;
and \( l \) is the light path length in the liquid sample. \( \Delta S \) and \( l \) can be calculated based on the reactor geometry (Figure 1.9C). The average UV fluence rate can be obtained based on a range of \( r \) and \( z \) values.

Due to the assumption of UV light emitting from the axis of the lamp, the spherical linear model should be restricted to applications in which the radius of UV lamp can be neglected compared to the radius of the reactor. A more sophisticated spherical cylindrical model (also called extense source model) which assumed that the light emitted from the elementary volume of the UV source cylinder was proposed by Irazoqui (1973). Although the model is not widely used due to its complexity, it is the best theoretical approximation for predicting the radiation behaviour from a cylindrical source (Irazoqui et al, 1973).

Except for an annular reactor, the spherical linear model can also be used for a plane reactor. Falguera (2011a) established a model which assumes that a sample in a plane reactor under a linear UV lamp and receives light radiation emitted from the lamp spherically (Figure 1.9F). The UV fluence rate at any point \((x,y,z)\) in the plane reactor can be expressed as:

\[
E_{\lambda(x,y,z)} = \frac{P_{\lambda}}{4\pi L} \int_{y_0}^{y_0+L} \frac{\exp\left(-\frac{\alpha_{\lambda}z}{\sin\phi}\right)}{(x_0-x)^2 + (l-y)^2 + (z_0+z)^2} \, dl
\]  

(1.33)

\[
\sin\phi = \frac{z_0 + z}{\sqrt{(x_0-x)^2 + (l-y)^2 + (z_0-z)^2}}
\]  

(1.34)

where, \( P_{\lambda} \) is the output power of UV lamp at wavelength \( \lambda \); \( x, y \) and \( z \) are the coordinates shown in the Figure 1.9F ; \( \alpha_{\lambda} \) is the Napierian coefficient of the liquid sample; \( L \) is the length of UV lamp; and \( \phi \) is the angle between the light pathway and the surface of the reactor.
In summary, determination of applied or average UV fluence rate in a given reactor should follow several steps. First, an applicable model should be chosen and necessary modifications should be made if required based on the geometric profile of the UV source and reactor. Secondly, the reactor should be evaluated and determination should be made if the reflection, refraction and absorption of the quartz sleeve are accounted for in the model. Thirdly, the UV fluence rate at any point in the reactor vessel should be calculated. The applied and average UV fluence rates should be determined by either summation of the weighted fluence rate of points along the coordinate axis or use an analytical equation if it is available (eg. the situation that absorption of liquid medium can be neglect). Finally, if a polychromatic UV source is adopted, the fluence rate should be obtained by integrating the value of the specific wavelength over the whole range of interest (eg. 200-300nm).
Figure 1.9. Schematic diagrams of batch UV reactors.

A. Collimated reactor (Source: Bolton and Linden, 2003 with modification)

B. Point source reactor

C. Annular reactor with radial linear model (Source: Harris and Dranoff, 1965 with modification)

D. Annular reactor with spherical linear model (Source: Jacobm and Dranoff, 1970 with modification)

E. Annular reactor with considering reflection and refraction (Source: Bolton, 2000 with modification)

F. Plane reactor (Source: Falguera et al., 2011a with modification)
1.4.4. Effects of UV irradiation on pathogen inactivation and chemical degradation

UV irradiation for liquid food products has been used for the last 20 years for several purposes including disinfection of spoilage and pathogenic microorganisms without significant loss of nutritional and sensorial qualities, absence of known toxic effects, and lower processing costs than thermal processing due to low energy consumption (Gayán et al., 2013). Successful application of UV pasteurization for apple cider and apple juice has been developed due to outbreaks of pathogens, especially Escherichia coli O157:H7 in apple cider (Vojdani et al., 2008) and the consumer preference of fresher, more natural and healthier food without thermal treatment (Caminiti et al., 2012). The inactivation of microorganisms is caused by the crosslinking of pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand caused by absorption of UV energy. This mutation blocks DNA transcription and replication, and finally leads to cell death (Bolton and Linden, 2003; Jin et al., 2006; Sastry et al., 2000). UV light with a wavelength in the UV-C range (200-280 nm), especially between 250 and 270 nm, is lethal to most microorganisms (Bintsis et al., 2000; Kuo et al., 2003; Gayán et al., 2013).

Although almost all types of microorganisms, including viruses, bacteria, yeasts, molds and algae have been reported to be inactivated with UV light (Guerrero-Beltrán and Barbosa-Cánovas, 2004), the most research interest has focused on the use of UV to inactivate food borne pathogens and spoilage microbes in apple cider and juice (Table 1.4). The target pathogens in these studies were Escherichia coli O157:H7 (Wright et al., 2000; Ngadi et al., 2003; Basaran et al., 2004; Gabriel, 2012) and its surrogate strains such as non-pathogenic E. coli and E. coli K12 (Duffy et al., 2000; Ukuku and Geveke, 2000; Quintero-Ramos et al., 2004; Geveke, 2005; Guerrero-Beltrán and Barbosa-Cánovas, 2005; Keyser et al., 2008; Gachovska et al., 2008;
Franz et al., 2009; Caminiti et al., 2012; Gayán et al., 2013), as well as non-pathogenic *Listeria innocua* (Guerrero-Beltrán and Barbosa-Cánovas, 2005; Geveke, 2005; Caminiti et al., 2012). The effects of UV irradiation on spoilage microorganisms in apple cider and juice such as *Lactobacillus brevis*, *Alicyclobacillus acidoterrestris* spores and *Saccharomyces cerevisiae* were also reported (Guerrero-Beltrán and Barbosa-Cánovas, 2005; Franz et al., 2009; Gabriel, 2012; Baysal et al., 2013). Wright et al. (2000) reported a 5.4 log reduction of a five-strain mixture of *E.coli* O157:H7 in apple cider after UV treatment with a thin film UV disinfection unit at a fluence of 61 mJ·cm². The authors believed that the background yeast and molds in cider influenced the disinfection effectiveness due to a low transmittance of UV light. Treatment by a CiderSure 3500 unit with a UV fluence of 14 mJ·cm², 5.93-6.63 log reductions of three *E.coli* O157:H7 strains were observed in apple cider prepared from eight apple cultivars (Basaran et al., 2004). In another study, a higher UV fluence of 390 mJ·cm² released from a collimated UV lamp inactivated more than 5 log of *E.coli* O157:H7 in apple juice without color change during four weeks of storage at 25 °C (Ngadi et al., 2003). Compared to pathogenic bacteria, spoilage yeast exhibited higher resistance to UV treatments. Guerrero-Beltrán and Barbosa-Cánovas (2005) treated apple juice with a UV fluence of 450 kJ·m² and achieved a 5.10 and 4.29 log reduction of *E.coli* and *Listeria innocua*, respectively. However, the same UV fluence only reduced *S. cerevisiae* by 1.34 log. Similarly, a 1-2 log reduction of *S. cerevisiae* compared to 4-5 log reduction of *E.coli* was achieved by passing cloudy apple juice through a LPM UV source with helically wound tubing at a flow rate of 4-8 L/h (Franz et al., 2009).

The kinetics of inactivation of microorganisms has been evaluated by various mathematic models. The simplest and most traditional is a first-order kinetics model which gives a linear
relationship between log of viable cells and UV fluence (Guerrero-Beltrán and Barbosa-Cánovas, 2005; Geveke, 2005; Hijnen et al., 2006). However, a number of studies observed non-log-linear survival curves when applying UV irradiation (Ngadi et al., 2003; Quintero-Ramos et al., 2004; Baysal et al., 2013; Gayán et al., 2013). These survival curves exhibited a sigmoidal shape with either a shoulder (an initial plateau) and/or a tail. Shoulders are caused by an injury phase of microorganisms in response to UV exposure. Tailings take place due to either a generation of UV resistance in microorganisms or aggregation of microorganisms (Sastry et al., 2000; Hijnen et al., 2006; Izquier and Gómez-López, 2011). In order to assess UV treatment and predict the inactivation of microorganisms, more complicated but accurate models were developed and applied. These models included a log-linear plus tail model (Baysal et al., 2013), a two-phase kinetics model (Ngadi et al., 2003) and a series-event inactivation model (Ye et al., 2007).

Unlike inactivation of pathogenic and spoilage microorganisms, degradation of chemicals in fruit juice by UV irradiation has both positive and negative effects. Several studies reported a reduction of ascorbic acid and loss in the activity of the enzyme, polyphenol oxidase. Orłowska et al. (2013) reported 1.30 % and 5.45 % reductions of ascorbic acid exposed by LPM and MPM UV lamps at a fluence of 10 mJ·cm⁻². In another study, the reduction of ascorbic acid in apple juice was observed from 170 mg·L⁻¹ to 60 mg·L⁻¹ after 30 min of UV irradiation (Tikekar et al., 2011). Guerrero-Beltrán and Barbosa-Cánovas (2006) found UV irradiation at 450 kJ·m⁻² can decrease the activity of polyphenol oxidase in mango nectar by 73-81 %. Similarly, an approximately 80 % of polyphenol oxidase activity was reduced after 100 min of UV treatment at an irradiance of 21.9 W·m⁻² (Manzocco et al., 2009). One positive effect of UV treatment is photo degradation of mycotoxins present in fruit juice. Dong et al. (2010) reported the validation
of patulin reduction in apple cider by UV irradiation. However, there are no studies that quantified patulin degradation in various apple products with UV exposure using kinetic models.

Table 1.4. Inactivation of microorganisms by UV irradiation in apple cider and juice

<table>
<thead>
<tr>
<th>Product</th>
<th>Microorganism</th>
<th>UV reactor / UV lamp</th>
<th>UV fluence (mJ·cm⁻²)</th>
<th>Inactivation (log)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple juice</td>
<td>E. coli O157:H7</td>
<td>Collimated beam Apparatus / LPM</td>
<td>390</td>
<td>&gt; 5</td>
<td>Ngadi et al. (2003)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Alicyclobacillus acidoterrestris</td>
<td>Collimated beam Apparatus / LPM</td>
<td>539</td>
<td>2.1</td>
<td>Baysal et al. (2013)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>E. coli</td>
<td>Double tube UV disinfection system</td>
<td>45000</td>
<td>5.10</td>
<td>Guerrero-Beltrán and Barbosa-Cánovas, 2005</td>
</tr>
<tr>
<td>Apple juice</td>
<td>L. innocua S. cerevisiae</td>
<td>Continuous flow UV treatment chamber</td>
<td>-</td>
<td>3.46</td>
<td>Gachovska et al. (2008)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>E. coli K12</td>
<td>Tubular UV unit / LPM (30 W)</td>
<td>-</td>
<td>4.0</td>
<td>Ukuku and Geveke (2000)</td>
</tr>
<tr>
<td>Apple juice</td>
<td>E. coli K12</td>
<td>UV rising film reactor / LPM (30W)</td>
<td>2660</td>
<td>4.59</td>
<td>Caminiti et al. (2012)</td>
</tr>
<tr>
<td>Apple cider</td>
<td>E. coli O157:H7</td>
<td>Thin film UV disinfection unit /10 LPM</td>
<td>61</td>
<td>5.4</td>
<td>Wright et al. (2000)</td>
</tr>
<tr>
<td>Apple cider</td>
<td>E. coli</td>
<td>CiderSure 3500</td>
<td>-</td>
<td>5</td>
<td>Duffy et al. (2000)</td>
</tr>
<tr>
<td>Apple cider</td>
<td>E. coli</td>
<td>CiderSure 3500</td>
<td>6.5</td>
<td>&gt; 5</td>
<td>Quintero-Ramos et al. (2004)</td>
</tr>
<tr>
<td>Apple cider</td>
<td>E. coli O157:H7</td>
<td>CiderSure 3500</td>
<td>14</td>
<td>5.93-6.63</td>
<td>Basaran et al. (2004)</td>
</tr>
<tr>
<td>Apple cider</td>
<td>E. coli L. innocua</td>
<td>LPM surrounded by a coil of tubing</td>
<td>-</td>
<td>3.4</td>
<td>Geveke (2005)</td>
</tr>
</tbody>
</table>
1.5. Research hypothesis and objectives

Because of the health risk of patulin contamination in apple based beverages and insufficient controls during the harvest and processing to prevent patulin contamination of juice, novel techniques has been sought to reduce the mycotoxin to a safe level. UV irradiation is a good candidate as patulin has a strong absorbance in the UV-C range with a maximum absorbance at 276 nm. Moreover, the approval of UV light as an alternative treatment to thermal pasteurization of fresh juice by U.S. FDA and Health Canada (U.S.FDA, 2000; Health Canada, 2004) makes it possible to adopt the technique in commercial applications.

The research hypothesis of this study is that UV irradiation (200-300nm) is an effective approach for reducing the patulin and patulin-producing mold levels in apple cider and juice. It is also hypothesized that degradation of patulin follows first-order reaction kinetics, that the degradation rate and quality attributes are wavelength-dependant. And that the toxicological effects of apple juice spiked with patulin decreases after UV irradiation.

The specific objectives are listed below:

**Objective 1:**

a) Model the UV (254 nm) degradation kinetics of patulin in apple cider, apple juice and a model solution.

b) Compare the patulin degradation rate as affected by various *in-situ* (eg. chemical compounds and absorption coefficient of media) and configuration (eg. UV fluence rate and sample thickness) factors.
Objective 2:

a) Compare the efficiency of patulin degradation by various UV wavelengths.

b) Evaluate the quality attributes (pH, color, soluble solid content, ascorbic acid and absorbance) of apple juice after UV irradiation.

Objective 3:

a) Evaluate the toxicological effects of apple juice spiked with patulin after UV (254 nm) irradiation using Drosophila melanogaster (fruit fly) and Saccharomyces cerevisiae (yeast) as model systems.

Objective 4:

a) Determine the kinetics of photo-inactivation of Penicillium expansum treated with a UV source (254 nm).

b) Compare the growth and patulin accumulating ability of Penicillium expansum in apple juice before and after irradiation with UV light at 254 nm.
Chapter Two

Kinetics of Patulin Degradation in Model Solution, Apple Cider and Apple Juice by Ultraviolet Radiation
Abstract

Patulin is a mycotoxin produced by a wide range of molds implicated in fruit spoilage and remains a significant food safety concern. The current study evaluated the feasibility of monochromatic ultraviolet (UV) radiation at 254 nm as an intervention to degrade patulin in fresh apple cider and juice. By using a UV reactor with a 2 mm of sample thickness and an incident fluence rate of 3.00 mW·cm⁻² for 40 min, it was demonstrated that patulin levels could be reduced from an initial level of 1 mg·L⁻¹ by 56.5 % (model solution), 87.5 % (apple cider), 94.8 % (apple juice without ascorbic acid) and 98.6 % (apple juice with ascorbic acid). The degradation of patulin followed a first-order reaction model with mixing enhancing the patulin degradation rate. The reaction rate constant of patulin degradation in apple juice was significantly higher (P < 0.05) than in buffered solutions suggesting interaction of the mycotoxin with sample constituents. In conclusion, it was demonstrated that UV radiation is an effective method for decreasing patulin levels in apple cider and juice.
2.1. Introduction

Patulin [4-hydroxy-4H-furo (3, 2-c)-pyran-2-(6H)-one] is a mycotoxin produced by certain species of *Aspergillus*, *Penicillium* and *Byssochlamys* (Lai *et al.*, 2000) with *P. expansum* being the most significant (Andersen *et al.*, 2004). As with the majority of mycotoxins, patulin is stable and can persist in juice over extended time periods. Patulin can cause acute, but more frequently, chronic intoxications leading to nervousness, convulsions, lung congestion, oedema, hyperaemia, immunotoxic, immunosuppressive and teratogenic effects (Roll *et al.*, 1990). Because of the prevalence and toxicity of patulin, the Codex Alimentarius (Codex, 2003), in addition to the U.S. FDA (2005), have set limits of 0.05 mg·L\(^{-1}\) (50 ppb) for apple based products. The European Commission (EC, 2006) has gone further and imposed a maximum limit of 0.01 mg·L\(^{-1}\) (10 ppb) in baby foods. Given the stability of patulin the main initiatives to minimize contamination is to ensure juice producers do not use spoiled fruit in juice production and to undertake extensive sampling to ensure contaminated end products do not reach the market.

Although GMPs and screening are useful, both are relatively ineffective in ensuring products are free from patulin. In this respect there is an identified need for intervention methods that can degrade patulin. Patulin is relatively stable under acid conditions (pH < 5.5) but becomes unstable at neutral to alkali pH values (Heatley and Philpot, 1947; Lovett and Peeler, 1973; McCallum *et al.*, 2002). There are conflicting reports on the thermal stability of patulin in apple juice. For example, Doyle *et al.* (1982) reported that approximately 90 % degradation of patulin can be achieved by heat treatment at 105 °C for 29 s. However, Wheeler *et al.* (1987) reported only an 18.5 % reduction at 90 °C for 10 s. Similarly, Kadakal *et al.* (2003) observed a 9.4 % - 14.6 % reduction in patulin levels at 70 and 80 °C for 20 min during the evaporation process in
apple concentrate production. The basis for thermal stabilities of patulin is unclear, although it is likely related to the reaction of the mycotoxin with apple juice components, most notably ascorbic acid (Kabak, 2008). However, in the absence of oxygen (such as in cartons or bottles) or the addition of metal chelating agents, patulin is stable in the presence of ascorbic acid thereby limiting the practical application of the approach (Drusch et al., 2007).

UV based decontamination methods are widely used in the apple juice industry to address the food safety risk associated with Escherichia coli O157:H7 (Donahue et al., 2004; Koutchma et al., 2004). UV-C (254 nm) disrupts the DNA leading to lethal mutations that inactivate microbes (Donahue et al., 2004). UV light has also been found to degrade the mycotoxin aflatoxin M1 via a free radical mechanism (Yousef and Marth, 1985; Moreno et al., 1987). Although not studied extensively, it is also possible that patulin could be degraded in addition to inactivating using UV based treatments. Recently, it was reported that UV exposure of 14.2 to 99.4 mJ·cm⁻² (The CiderSure 3500, FPE, Inc., Macedon, NY) resulted in a significant, and nearly linear decrease in patulin levels while producing no quantifiable changes in the chemical composition (i.e., pH, Brix, and total acids) or organoleptic properties of the cider (Dong et al., 2010). However, there were no subsequent studies that quantified patulin degradation in various apple products with UV exposure using kinetic models. The current study was directed towards investigating efficiency of patulin degradation by UV light and determining the degradation kinetics of patulin in model solution, in addition to apple cider and juice, by taking into consideration effects of pH and ascorbic acid content.
2.2. Materials and Methods

2.2.1. Reagents

Patulin [4-hydroxy-4H-furo (3,2-c)-pyran-2-(6H)-one] was obtained from the Sigma Chemical Company (St. Louis., U.S.A.) and used directly without further purification. The model solution was prepared using 50 mM citrate buffer pH 3.4 (Gomori, 1955). Apple cider (pH = 3.47), apple juice with ascorbic acid addition (pH = 3.57) and apple juice without ascorbic acid addition (pH = 3.38) were purchased from a local supermarket. The concentration of ascorbic acid in apple cider, apple juice without ascorbic acid addition and apple juice with ascorbic acid addition was determined as 7.3, 9.9 and 125 mg·L⁻¹, respectively, according to the AOAC official method 985.33 (OMA, 2005).

2.2.2. UV treatments

A low-pressure mercury (LPM) UV light (254 nm) reactor (R-52G MINERALIGHT® UV Lamp UVP Inc, CA, USA) was used in the study. The incident fluence rate was varied by adjusting the distance between the sample surface and the UV lamp. The incident fluence rate was measured using a digital ILT1700 radiometer (International Light Technologies, MA, USA). Samples were spiked with patulin to a final concentration of 1.0 mg·L⁻¹ then dispensed into 5.2 cm diameter petri dishes. The petri dish was placed under the UV lamp and irradiated at different incident fluence rates (1.0, 3.0 and 5.0 mW·cm⁻²) with the sample thickness varying between 0.2 – 0.8 cm for 40 min. Treatments were delivered with no mixing or under dynamic conditions. For the latter, mixing was achieved using a magnetic stirrer and a micro stirring bar (2×7 mm) rotating at approximately 200 rpm. The temperature was measured before and following UV treatment with a Thermo Fisher probe thermometer.
2.2.3. HPLC analysis

Patulin samples were extracted using the solid phase extraction as described by Eisele and Gibson (2003). Oasis HLB extraction cartridges (3 mL/60 mg, Waters, MA) were conditioned by passing 2 mL of water, 2 mL of methanol and 2 mL of water at a flow rate of approximately 0.1 mL·s⁻¹. The sample (1 mL) was applied to the column followed by 2 mL of a 1.0 % (w/v) sodium bicarbonate solution then 2 mL of 1.0 % (v/v) acetic acid. The patulin was then eluted using 1.0 mL of ethyl acetate and evaporated to dryness under a stream of nitrogen. The residue was then dissolved in 0.5 mL 0.1 % (v/v) acetic acid prior to analysis. The standard patulin solutions of 0.01, 0.05, 0.1, 0.5, and 1 mg·L⁻¹ were prepared and extracted using the same method described above to form the linear working curve (0.01 to 1 mg·L⁻¹). Samples and standards were analyzed using a HPLC system (Agilent Technology 1200 Series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, and a diode array detector (DAD) set at 276 nm. A Phenomenex Luna® 3 µ C18 column (250 x 2.0 mm) with a C18 guard column (Torrance, CA) was used for the separation. Patulin was eluted isocratically using 0.8 % tetrahydrofuran in water at a flow rate of 0.2 mL·min⁻¹ with run time of 25 minutes. The retention time of patulin was around 20 min. Figure 2.1 showed chromatograms of standard patulin solution (1 mg·L⁻¹), and apple juice spiked with patulin (1 mg·L⁻¹) before and after UV treatment. Control samples were measured to conform that samples before UV treatment were patulin-free. All samples and standards were analyzed in triplicate.
Figure 2.1. Representative HPLC chromatograms of patulin.

A: Standard patulin solution (1 mg·L⁻¹)

B: Apple juice spiked with patulin (1 mg·L⁻¹) before UV treatment

C: Apple juice spiked with patulin (1 mg·L⁻¹) after UV treatment
2.2.4. Determination of the absorption coefficient

Absorbance of the samples was measured using a UV/visible spectrometer (Ultrospec 3100 Pro) (Biochrom Ltd., Cambridge, England). Each sample was tested in triplicate before and after UV treatments using demountable fused-quartz cuvettes (NSG Precision Cell. Inc., Farmingdale, NY). The model solution samples with patulin exhibited high UV transmission and hence be tested using quartz cuvettes with a 1 cm path length. For the juice samples, the UV transmission was measured at 0.01, 0.02, 0.05, 0.075 and 0.1 cm path lengths then plotted to calculate the absorption coefficient.

2.2.5. Modeling of patulin degradation kinetics

The time-based first-order degradation model was used in this study to describe the kinetics of patulin degradation by UV light. This model assumes that the degradation rate is associated with the patulin concentration (Equation 2.1). The reaction rate constant (k_t) can be obtained from linear regression between ln (N/N_0) and time (t). Although the time-based first-order degradation model can be used to predict the patulin degradation, the rate constant varied along with the change in experimental conditions even in the same material. An alternative fluence-based first-order degradation model was developed by Severin et al. (1983) and Ye et al. (2007) for inactivation of microorganisms. For the model, the k_f is fluence-based first-order reaction rate constant with unit of mW^{-1}·cm^2·s^{-1}. It is obtained from linear regression between ln(N/N_0) and average UV fluences (E_{avg}·t) (Equation 2.2a, 2.2b). In the current study, k_f was proposed to predict patulin degradation when incident UV fluence rate, sample thickness and media were known.

\[
\frac{N}{N_0} = e^{-k_f t} \quad \text{or} \quad \ln \frac{N}{N_0} = -k_f t
\]  

(2.1)
\[
\ln \frac{N}{N_0} = -k_f E_{avg} t
\]  
\hspace{1cm} (2.2a)

where,
\[
E_{avg} = E_0 \cdot (PF) \cdot (1 - Re) \cdot \frac{1 - 10^{-\alpha l}}{\alpha l \ln(10)} \cdot \frac{l'}{l' + l}
\]  
\hspace{1cm} (2.2b)

\(E_{avg}\) is the average UV fluence rate throughout the sample and is calculated from the incident UV fluence rate at the center of the surface of sample (\(E_0\)) and corrective factors (Bolton and Linden, 2003). The petri factor (PF) was defined as a ratio of the average UV fluence rate on the surface of sample to the incident fluence rate at the center, which modified non-uniformity of UV fluence rate on the surface of sample. Re is reflectance, and a value of 0.025 was determined using the average refractive indexes of air (1.000) and water (1.372) at wavelengths between 200 and 300 nm (Bolton and Linden, 2003). \(\alpha\) is the absorption coefficient of sample which was measured with a Ultrospec 3100 Pro UV/visible spectrometer (Biochrom Ltd., Cambridge, England). \(l'\) is the distance between the sample surface and the UV source. \(l\) is the thickness of the sample. \(t\) is the UV exposure time. Comparing equation 2.1 and 2.2a, the relationship between \(k_i\) and \(k_f\) was obtained as follows:
\[
k_i = k_f E_{avg}
\]  
\hspace{1cm} (2.3)

According to the definition of decimal reduction time (\(D_t\)-value), which is the time required for 90 \% reduction of patulin, equation 2.4 was obtained.
\[
D_t = \frac{-\ln(0.1)}{k_f \cdot E_{avg} \cdot 60}
\]  
\hspace{1cm} (2.4)

where, \(D_t\) is decimal reduction time with a unit of minutes, 60 is a unit conversion factor.
2.2.6. Determination of applied UV fluence and average UV fluence

An applied UV fluence is energy generated by an incident UV fluence rate modified by a petri factor (PF) on the surface of sample in a certain exposure time (Equation 2.5):

\[ H_{app} = E_0 \cdot (PF) \cdot t \]  \hspace{1cm} (2.5)

An average UV fluence is average energy distribution in the whole sample considering the attenuation of UV energy due to reflection, refraction, absorption, and divergence (Equation 2.6):

\[ H_{avg} = E_0 \cdot (PF) \cdot (1 - Re) \cdot \frac{1 - 10^{-al}}{al\ln(10)} \cdot \frac{l'}{l' + l} \cdot t \]  \hspace{1cm} (2.6)

2.2.7. Statistical analysis

All UV processing conditions were performed in triplicate with a completely independent and randomized design. The statistical analyses of experimental data were carried out by SPSS version 20 (IBM, Armonk, NY). The pair data before and after UV exposures, as well as the data between statistic and dynamic UV treatment were performed to test significance (paired t-test). All patulin degradation rate constants were calculated from regression plots based on the series data of patulin concentration at sampling points during the UV exposure. The coefficients of determination R² were calculated.

2.3. Results

2.3.1. Degradation of patulin with UV exposure

Significant reductions (P < 0.05) of patulin were found in a model solution, apple cider, apple juice with or without ascorbic acid when irradiated with UV light. From a starting level of 1.0 mg·L⁻¹ of patulin, an applied UV fluence of 7.06 J·cm⁻² resulted in a 56.5 % (model solution),
87.5 % (apple cider), 94.8 % (apple juice without ascorbic acid addition) and 98.6 % (apple juice with ascorbic acid addition) reduction in levels of the mycotoxin in the different matrices. The actual UV delivered to the different liquid matrices varied as a function of the absorption coefficient and the sample thickness. The average UV fluences were 6.59 J·cm⁻² for model solution, 1.70 J·cm⁻² for apple cider, 2.13 J·cm⁻² for apple juice without ascorbic acid addition and 0.63 J·cm⁻² for apple juice with ascorbic acid addition. From the gradients of the patulin UV degradation curves the D₅₀ values were calculated as 112.6 min for model solution, 44.2 min for apple cider, 32.6 min for apple juice without ascorbic acid addition and 19.4 min for apple juice with ascorbic acid addition (Table 2.1).

Table 2.1. Degradation of patulin with UV exposure

<table>
<thead>
<tr>
<th>Media</th>
<th>Degradation of patulin (%)ᵃ</th>
<th>Applied UV fluence (J·cm⁻²)</th>
<th>Average UV fluence (J·cm⁻²)</th>
<th>D₅₀-value (min)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model solution</td>
<td>56.5 ± 0.6ᶜ</td>
<td>7.06</td>
<td>6.59 ± 0.0009</td>
<td>112.6 ± 1.6</td>
</tr>
<tr>
<td>Apple cider</td>
<td>87.5 ± 2.7</td>
<td>7.06</td>
<td>1.70 ± 0.003</td>
<td>44.2 ± 2.1</td>
</tr>
<tr>
<td>Apple juice without ascorbic acid addition</td>
<td>94.8 ± 1.1</td>
<td>7.06</td>
<td>2.13 ± 0.0002</td>
<td>32.6 ± 1.0</td>
</tr>
<tr>
<td>Apple juice with ascorbic acid addition</td>
<td>98.6 ± 0.2</td>
<td>7.06</td>
<td>0.63 ± 0.0006</td>
<td>19.4 ± 0.4</td>
</tr>
</tbody>
</table>

ᵃ Samples were exposed by UV for 40 minutes with 3.0 mW·cm⁻² of incident fluence rate and 0.2 cm of sample thickness. The initial concentration of patulin was 1 mg·L⁻¹.

ᵇ The doses were calculated based on 1 mg·L⁻¹ of initial patulin concentration.

c Values are averages ± standard deviations (n = 3) for all analysis.
2.3.2. Kinetics of patulin degradation

Effect of different media, UV fluence rate and sample thickness. The experimental data showed that patulin degradation followed a first-order reaction model due to the linear decrease of \( \ln(\frac{N}{N_0}) \) with the exposure time \( (R^2 > 0.95) \) (Figure 2.2, 2.3, 2.4, 2.5). Relationships between patulin degradation rate and incident UV fluence rate, and patulin degradation rate and sample thickness in model solution and apple juice without ascorbic acid addition were observed. Higher incident UV radiance and thinner sample thickness increased the rate of patulin degradation when other parameters were fixed (Figure 2.2, 2.3, 2.4). Figure 2.5 indicated that ascorbic acid would enhance the patulin degradation. The time-based reaction rate constants \( (k_t) \) were affected by the nature of the sample and experimental settings including incident UV fluence rate and absorbance. The fluence-based reaction rate constants \( (k_f) \) were kept constant in the same medium. Figure 2.6 presented summarized calculated \( k_t \) and \( k_f \) in each test media which can be used to predict patulin degradation. Figure 2.7 showed high correlation \( (R^2 > 0.99) \) between predicted reduction of patulin concentrations and experimentally measured values using the fluence-based first-order degradation model. A total of 50 pairs of data evaluated factors such as incident fluence rates \( (1.0, 3.0 \text{ and } 5.0 \text{ mW} \cdot \text{cm}^{-2}) \), sample thicknesses \( (0.2, 0.5 \text{ and } 0.8 \text{ cm}) \), UV exposure time \( (5, 10, 20, 30 \text{ and } 40 \text{ min.}) \) and media including the model solution, apple cider, apple juice with or without ascorbic acid addition. The predicted data were calculated with the determined fluence-based reaction rate constants (Equation 2.2a, 2.2b) whereas the experimental data were directly obtained from HPLC analysis.

Effect of dynamic vs. static treatment regime. The kinetics study revealed that the time-based reaction rate constants of all samples treated in the dynamic regime (model solution: \( 2.95 \times 10^{-4} \text{ s}^{-1} \),
juice: 4.31E-4 s⁻¹) were significantly (P < 0.05) higher than samples treated in static regime (model solution: 2.79E-4 s⁻¹, juice: 3.49E-4 s⁻¹) when incident UV fluence rate and sample thickness were constant (E₀ = 3.0 mJ·cm⁻², l = 0.2 cm; Figure 2.8). Comparing the four test media, the reaction rate constants in apple juice and apple cider were significantly higher than that in the model solution (P < 0.05). The addition of ascorbic acid to apple juice resulted in a dramatic increase in the reaction rate constant as shown in Figure 2.6.

Effect of UV light exposure on pH and absorption coefficient of apple cider and apple juice.

The pH and absorption coefficients of apple cider and apple juice with and without ascorbic acid addition during UV exposure were measured and shown in Table 2.2. Significant decreases in the absorption coefficient (P < 0.05) were observed after UV exposure. There was no significant changes in pH values (P > 0.05) after the UV exposure.

Temperature monitoring during UV exposure. A temperature increase of 0.8 °C was observed when treating apple juice samples (2 mm of thickness) with UV light at an incident UV fluence rate of 5 mW·cm⁻² for 40 min. These parameters resulted in the highest UV energy in this study. The temperature change within 1 °C indicates no significant heating effect resulted from the UV source used in this study.
Figure 2.2. Degradation of patulin in model solution by UV exposure with uniform sample thickness \((l = 0.5 \text{ cm})\) and various incident fluence rates

- ♦: \(E_0 = 1.0 \text{ mW/cm}^2\)
- ■: \(E_0 = 3.0 \text{ mW/cm}^2\)
- ▲: \(E_0 = 5.0 \text{ mW/cm}^2\)
Figure 2.3. Degradation of patulin in model solution by UV exposure with uniform incident fluence rate ($E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}$) and various sample thicknesses

♦: $l = 0.2 \text{ cm}$

■: $l = 0.5 \text{ cm}$

▲: $l = 0.8 \text{ cm}$

$k_t = 0.000279 \text{ s}^{-1}$
$k_t = 0.000315 \text{ s}^{-1}$
$k_t = 0.000341 \text{ s}^{-1}$

$R^2 = 0.9995$
$R^2 = 0.9998$
$R^2 = 0.9991$
Figure 2.4. Degradation of patulin in apple juice without ascorbic acid addition by UV exposure with uniform incident fluence rate ($E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}$) and various sample thickness

♦: $l = 0.2 \text{ cm}$

■: $l = 0.5 \text{ cm}$

▲: $l = 0.8 \text{ cm}$
Figure 2.5. Degradation of patulin in apple cider, apple juice with and without ascorbic acid addition by UV exposure with uniform incident fluence rate ($E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}$) and sample thickness ($l = 0.2 \text{ cm}$)

■: Apple cider

♦: Apple juice without ascorbic acid addition

▲: Apple juice with ascorbic acid addition
Figure 2.6. Comparison of patulin degradation rates in 4 kinds of media. \((E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}, l = 0.2 \text{ cm})\)

A, B, C, D: A different letter indicates significant differences \((P < 0.05)\) in mean values of time-based reaction rate constant \((k_t)\) were observed between different media.

a, b, c: A different letter indicates significant differences \((P < 0.05)\) in mean values of fluence-based reaction rate constant \((k_f)\) were observed between different media.
Figure 2.7. Residual concentration of patulin during UV exposure. Compendium of 50 experimental data comparing fluence-based first-order degradation model predictions. The data covered various experimental conditions of various media (model solution, apple cider, apple juice with and without ascorbic acid addition), incident UV fluence rates (1.0, 3.0 and 5.0 mW·cm⁻²) and sample thickness (0.2, 0.5 and 0.8 cm)
Figure 2.8. Comparison of patulin degradation rates in model solution and apple juice without ascorbic acid addition in both dynamic and static systems. ($E_0 = 3.0 \text{ mW} \cdot \text{cm}^{-2}, l = 0.2 \text{ cm}$)

*: Significant difference ($P < 0.05$) between samples in dynamic and static system.

Table 2.2. pH and absorption coefficient of apple juice products before and after UV exposure

<table>
<thead>
<tr>
<th>Media</th>
<th>pH</th>
<th>P value</th>
<th>Absorption coefficient (cm$^{-1}$)</th>
<th>P value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>40 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple cider</td>
<td>3.47</td>
<td>3.47</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Apple juice without Vc addition</td>
<td>3.38</td>
<td>3.38</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Apple juice with Vc addition</td>
<td>3.57</td>
<td>3.57</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Model solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple juice (without Vc addition)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ P value between 0 min and 40 min.
2.4. Discussion

Results obtained in the course of this study confirmed that UV-C light (254 nm) supported the degradation of patulin in apple based beverages such as apple cider and apple juice. Patulin reductions of 90% were achieved during the time range from 19.4 to 44.2 min in various apple products using the examined experimental conditions described in this study. Considering that most normal post-harvest contamination concentrations of patulin were reported to be < 0.5 mg·L\(^{-1}\) (Gökmen and Acar, 1998; Lai et al., 2000; Leggott et al. 2000), this study provides evidence that application of UV light to apple cider and apple juice can reduce patulin levels below action levels of 0.05 mg·L\(^{-1}\) set by regulatory bodies in U.S., Canada and Europe (Codex, 2003; U.S. FDA, 2005; Health Canada, 2012; EU, 2006).

In general, the applied UV fluence, average UV fluence and degradation kinetics have to be known for scale-up and design of a commercial process. Knowledge of the applied fluence is important to select a correct power and type of UV source by taking into the account their UV efficiency in order to achieve a targeted degradation of material. Ideally, the peak emission wavelength of the UV source should be close to the peak absorption of the target material. In this study, patulin has peak absorption at 276 nm and the emission wavelength of the low pressure UV reactor is 254 nm. Although there are differences between these values, the UV source can still be applied since a related high absorption at 254 nm was observed based on a spectral curve of patulin absorption. The applied fluence characterizes the energy emission from the UV source on the surface of test material and it is independent of the material to be irradiated. Because of reflection, refraction, divergence and absorption by media, only part of applied energy can be used to drive a degradation reaction. Therefore, knowledge of average UV fluence is necessary
to compare UV fluences in different settings of UV exposure (eg. fluence rate and sample thickness) given that it reflects the characteristic of media and is independent of UV exposure parameters.

A few of mathematical models were established to investigate UV degradation for chemicals, bacteriophages and pathogenic bacteria (Tikekar et al., 2011; Rontó et al., 1992; Amos et al., 2001; Severin et al., 1983). In the current study, a time-based first-order model and fluence-based first-order model were adopted for the patulin degradation. It was shown that both models can be used to predict patulin degradation. The fluence-based model can be more beneficial given that the identical degradation rate constant ($k_f$) in the same media can be obtained from the specific experiment, but consequently adopted for a further prediction with different UV fluence rates and sample thicknesses.

Previous studies reported the first-order inactivation constants of *Escherichia coli*, the pathogen of concern in apple based beverages, to be between 0.325 and 0.893 mW$^{-1}$·cm$^2$·s$^{-1}$ in various media (Severin et al., 1984; Taghipour, 2004; Ye et al., 2007). For example, Ye et al. (2007) determined that the first-order reaction rate constant for inactivation of *Escherichia coli* K12 was 0.325 mW$^{-1}$·cm$^2$·s$^{-1}$ in model caramel solution, which is 46 to 263 times lower than the rate constant that required for patulin degradation in apple juice and apple cider. However, the data are not completely comparable since experimental settings and the media used were not the same. Consequently, the UV process designed to inactivate pathogens such as *E coli* O157 cannot be directly applied for the degradation of patulin. High UV fluence can potentially lead to discoloration, generation of photoproducts that may negatively affect sensory quality and cause
loss of nutrient (e.g. vitamin content) although this does not appear to be a problem in previous published works (Dong et al., 2010).

It was noteworthy that the degradation rate of patulin in apple juice was higher compared to the model solution. Moreover, the rate in apple juice with ascorbic acid addition was significantly higher than apple cider and apple juice without ascorbic acid addition. This would suggest that constituents within apple juice enhanced degradation of patulin. Fan and Geveke (2007) reported the major component of apple juice including fructose, malic acid and ascorbic acid, absorbed UV at 254 nm. Of these constitutes, the ascorbic acid may play an important role in decreasing the stability of patulin during UV exposure. Several other studies (Brackett et al., 1979a; Steiner et al., 1999) demonstrated the increased degradation of patulin in the presence of ascorbic acid. The mechanism by which ascorbic acid degrades patulin remains unclear although studies performed to date indicate that the reaction occurs via a free radical mechanism (Brackett et al., 1979a; Drusch et al., 2007). Here, ascorbic acid is oxidized to dehydroascorbic acid in the presence of oxygen catalyzed with metal ions such as Fe$^{2+}$ (Drusch et al., 2007). Free radicals that are generated as intermediate products of the reaction may react with patulin by attacking the conjugated double bond. However the precise breakdown products of the mycotoxin have yet to be elucidated (Morgavi et al., 2003).

The current study also showed that the reaction rate constant of samples in dynamic system were significantly higher than those in the static system. Although the patulin solution is homogeneous, the whole reaction system can be considered heterogeneous because the UV photons are not uniformly absorbed. According to the Beer–Lambert Law, the UV fluence rate
decreases exponentially in liquid samples. The stirring applied in the dynamic system increases the probability of exposure of patulin molecules with UV photons and consequently increases the destruction reaction rate.

Evaluation of the absorption coefficients of three kinds of apple products at 254 nm indicated that the absorbance coefficient of juice with ascorbic acid addition is greater than the original apple juice and cider, which is due to the larger amount of ascorbic acid (Ye et al., 2007).

The products of the patulin degradation by UV photons are still unknown. Based on the previous work involving long-term high-heat incubations, it was revealed that patulin degrades primarily into glyoxylic acid and 3-keto-5hydroxypentanal (Collin et al., 2008). A recently study indicated that patulin degrades into a far less toxic compound ascladiol as determined in an isolated strain of *Gluconobacter oxydans* (Ricetti et al., 2007). These results indicated that different patulin reduction strategies can produce different end products. A better understanding of the UV-generated chemical by-products of patulin degradation is needed to develop a commercial method for the reduction of patulin in liquid apple products in the future.

### 2.5. Conclusions

The current study demonstrated that monochromic UV radiation at 254 nm is a means of reducing the patulin levels in apple based beverage. The fluence-based first-order constant ($k_f$) was determined and accurately prediction of patulin degradation.
Chapter Three

Reduction of Patulin in Apple Juice Products by Ultraviolet Light of Different Wavelengths in UV-C Range
Abstract

This study evaluated three UV-C wavelengths (222 nm, 254 nm and 282 nm) on degradation of patulin added to apple juice or apple cider. The average UV fluences of 19.6, 84.3, 55.0 and 36.6 mJ·cm\(^{-2}\) resulted in the 90 % reduction of patulin in apple juice through the exposure of UV lamps at 222, 254, 282 nm wavelength and the combination of wavelengths, respectively. The efficiency of three wavelength lamps was: Far UV-C 222 nm > Far UV-C plus 282 nm > UV-C 254 nm. In terms of color, treatment of apple juice with 222 nm resulted in an increase in L* value but a decrease in a* and b* values although the changes were not significantly different (P > 0.05) from non-treated controls based on a sensory evaluation. The ascorbic acid loss in juice treated at 222 nm to support 90 % reduction of patulin was 36.5 %. This compares to 45.3 % and 36.1 % ascorbic acid loss with samples treated at 254 nm and 282 nm. The current work demonstrated that the 222 nm wavelength possessed the highest efficiency on patulin reduction in apple juice compared with the 254 nm and 282 nm with no benefit gained from using a combination of wavelengths.
3.1. Introduction

The presence of patulin mycotoxin \([4\text{-hydroxy-4H-furo}\ (3,\ 2\text{-c})\text{-pyran-2-(6H)}\text{-one}]\) in apple juice continues to represent a significant food safety issue (Sant’Ana et al., 2008). Patulin associated with apple juice is commonly linked to the use of fruit contaminated with \textit{Aspergillus}, \textit{Penicillium} and/or \textit{Byssochlamys} and stored under warm and humid environments (Andersen et al., 2004; Jackson et al., 2003; Lai et al., 2000; Sydenham et al., 1995). Resulting from use of poor quality fruits and insufficient control measures, the levels of patulin associated with intact apple fruit or apple juice varies significantly although is typically in the order of \(1 \text{ mg}\cdot\text{kg}^{-1}\) and \(0.5 \text{ mg}\cdot\text{L}^{-1}\), respectively (Beretta et al., 2000; Gökmen and Acar, 1998; Hasan, 2000; Lai et al., 2000; Leggott et al., 2000). Long term exposure to patulin can result in nervousness, convolution, lung congestion, oedema, hyperaemia, immunotoxic, immune-suppressive and teratogenic effect (Roll et al., 1990). Because of the prevalence of patulin in apple products and its toxicity, the U.S. Food and Drug Administration set a maximum level of \(0.05 \text{ mg}\cdot\text{L}^{-1}\) in single-strength apple juice, reconstituted single-strength apple juice or the single-strength apple juice used as an ingredient in foods (U.S. FDA, 2005). The European Union established a \(0.05 \text{ mg}\cdot\text{L}^{-1}\) limit for patulin concentration in fruit juice, spirit drinks and cider derived from apples (EC, 2006). The EU also set a lower limit for solid apple products \((0.025 \text{ mg}\cdot\text{L}^{-1})\), and apple juice and solid apple products for infants and young children \((0.01 \text{ mg}\cdot\text{L}^{-1})\) (EC, 2006).

Ultraviolet (UV) has been approved as a non-thermal method for pasteurization of fresh juice products by U.S. FDA (2000). Health Canada (2004) also approved the application of the CiderSure 3500 UV reactor as an intervention against \textit{Escherichia coli} O157:H7 in apple juice, the pathogen that is the primary target for pasteurization treatment. Although UV treatment was
primarily designed to reduce the risks associated with *E. coli* O157:H7 contamination of apple juice products, it is also possible to reduce mycotoxin levels through photolytic degradation (Assatarakul *et al.*, 2012; Dong *et al.*, 2010; Moreno *et al.*, 1987; Yousef and Marth, 1985; Zhu *et al.*, 2013).

As part of the electromagnetic radiation in the range between 100 and 400 nm, UV light is divided into vacuum UV (100-200 nm), UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm). The available UV sources include mercury lamps, amalgam lamps, excimer lamps, pulsed lamps, microwave lamps and UV-light-emitting diodes (Koutchma, 2009). Low pressure mercury (LPM) lamps were widely applied in the disinfection of pathogens due to the emission wavelength at 254 nm that targets DNA (Koutchma *et al.*, 2009). However, patulin has a peak absorption wavelength at 276 nm (Cole and Cox; 1981) suggesting that alternative UV wavelengths could enhance the degradation process. Excimer lamps were considered as alternative UV lamps as they feature narrow emission bands depending on the choice of rare gas and/or halogen (e.g. *KrCl* *, λ* = 222 nm; *XeBr*, *λ* = 282nm) (Koutchma, 2009; Sosnin *et al.*, 2006). In this study, Far UVC plus novel lamps, monochromatic sources with specific wavelength (222 nm and 282 nm) in the germicidal range, were specially designed for emitting single wavelength light based on the excimer discharge. These UV sources were used to enhance the reduction of patulin based on the potential mechanism of either providing photons with higher energy or matching the peak absorption of patulin (276 nm).

UV based methods have received significant attention as a low cost, reliable, non-thermal pasteurization technique although the approach does have disadvantages such as causing
reductions in levels of nutrients such as ascorbic acid (Ibarz et al., 2005; Tikekar et al., 2011). The generation of photoproducts that alters the sensory properties of juices such as apple can also potentially occur (Caminiti et al., 2012; Ibarz et al., 2005; Orlowska et al., 2013; Tikekar et al., 2011). Consequently, a balance needs to be made with respect to degrading patulin without having adverse effects on the product. The extent of byproduct formation depends on the applied UV wavelength although this aspect has not been studied to any great extent.

The objective of the following study was to investigate patulin degradation kinetics at different UV wavelengths and study the generation of photoproducts that may negatively affect the sensory quality of apple juice.

3.2. Materials and Methods

3.2.1. Materials

Patulin [4-hydroxy-4H-furo (3,2-c)-pyran-2-(6H)-one], L-ascorbic acid, acetic acid, sodium bicarbonate, formic acid, LC grade tetrahydrofuran (THF), acetonitrile and ethyl acetate were obtained from the Sigma Chemical Company (St. Louis., U.S.A.). Pasteurized apple cider (pH 3.45) and apple juice enriched with ascorbic acid addition (pH 3.17) were purchased from a local supermarket and used directly.

3.2.2. UV processing unit.

A triple wavelength box equipped with three monochromatic wavelength UV lamps (Far UVC 222 (222 nm), UVC (254 nm) and Far UVC plus 282 (282 nm)) from HEI (Dover, NH) was used to treat samples (Figure 3.1). Excimer lamps emitting at 282 or 222 nm, along with low pressure
mercury lamp (254 nm) were used as UV sources and placed 5.5 cm (Far UVC 222 and Far UVC plus 282) or 6.5 cm (UVC) above the sample. The apple juice sample was placed in a petri dish with a sample depth of 0.5 cm and diameter of 8.4 cm.

Figure 3.1. Schematics of triple wavelengths box UV reactor. A: Exposure under single UV lamp. B: Exposure under three UV lamps
3.2.3. Determination of UV fluence

Patulin photo-degradation rates in samples treated with UV illumination were determined using treatment wavelength, applied UV fluence and average UV fluence as experimental variables. Applied UV fluence is the energy generated by incident UV irradiance on the surface of the sample at a certain exposure time. Average UV fluence reflects the average energy in the whole liquid sample. Calculation of UV fluences was based on the geometric structure of the UV reactor (Figure 3.2).

At any infinitely small volume in the liquid sample located at coordinate (x,y,z), which receives the UV irradiation from the point on the lamp with a distance (l) to the center of the lamp, the UV fluence rate can be expressed as equation 3.1. It is deduced from the equation proposed by Jacob and Dranoff (1970) with consideration of the reflection factor.

\[
dE(x, y, z, l) = \frac{P \cdot dl}{4\pi d^2 L} \cdot (1 - Re) \cdot 10^{-\alpha d'}
\]  

(3.1)

where, P is the output power of the UV lamp; L is the length of UV lamp; \(\alpha\) is the absorption coefficient of liquid sample; d is the distance between the infinite small segment of UV source and any infinitely small volume (x,y,z), which can be calculated by equation 3.2a for the lamp vertically above the sample, or by equation 3.2b for the lamp diagonally above the sample; \(d'\) is the part of d in the liquid sample can be calculated by equation 3.3; Re is the reflectance which can be calculated by equation 3.4. The refraction of UV light in liquid sample was neglected to simplify the model.

\[
d = \sqrt{(H + z)^2 + (l - x)^2 + y^2}
\]  

(3.2a)

\[
d = \sqrt{(H + z)^2 + (l - x)^2 + (D - y)^2}
\]  

(3.2b)
\[ d' = \frac{zd}{H + z} \]  \hspace{1cm} (3.3)

Where, \( H \) is the distance between the UV lamp and the surface of liquid sample; \( D \) is the vertically distance between the UV lamp and the plane across the center of petri dish and is perpendicular to the ample surface.

\[ Re = \frac{1}{2} \left[ \left( \frac{n_2 \cdot \cos \varphi_1 - n_1 \cdot \cos \varphi_2}{n_1 \cdot \cos \varphi_2 + n_2 \cdot \cos \varphi_1} \right)^2 + \left( \frac{n_1 \cdot \cos \varphi_1 - n_2 \cdot \cos \varphi_2}{n_1 \cdot \cos \varphi_1 + n_2 \cdot \cos \varphi_2} \right)^2 \right] \]  \hspace{1cm} (3.4)

Where, \( \varphi_1 \) and \( \varphi_2 \) are incident and refracted angles at the interface between two media with refraction indices of \( n_1 \) and \( n_2 \).

As the equation 3.1 cannot be integrated analytically, the equation 3.1 can be rewritten in a “finite” form:

\[ E(x, y, z, l) = \frac{P}{4\pi d^2 n} \cdot (1 - Re) \cdot 10^{-ad'} \]  \hspace{1cm} (3.5)

In the equation 3.5, \( n \) is the number of lamp segments with the equal space. The total lamp output power \( P \) can be calculated based on the fluence rate at the center point of the sample surface measured by a radiometer (Equation 3.6, 3.7).

\[ dE_c(l) = \frac{P \cdot dl}{4\pi(l^2 + H^2)L} \]  \hspace{1cm} (3.6)

Equation 8 can be integrated to give equation 3.7.

\[ E_c = \frac{P}{4\pi HL} \left( \arctan \frac{L_1}{H} + \arctan \frac{L_2}{H} \right) \]  \hspace{1cm} (3.7)

where, \( L_1 \) and \( L_2 \) are the parts of lamp length from each edge to the point with the project on the centre of petri dish; \( E_c \) is the fluence rate at the center of petri dish which can be measured by a radiometer.
When the output power $P$ is obtained, fluence rate of any point in the sample with a coordinate of $(x,y,z)$ which received the UV irradiation from all the parts of lamp can be calculated (Equation 3.5). The applied and average UV fluence rate can be calculated from a series of point fluence rates with equal increments along the axis of $x$ (0.2cm), $y$ (0.2cm) and $z$ (0.005cm). The applied and average UV fluence can be calculated by the equation 3.8 and 3.9.

\[ H_{app} = E_{app} \cdot t \]  \hspace{1cm} (3.8)

\[ H_{avg} = E_{avg} \cdot t \]  \hspace{1cm} (3.9)
Figure 3.2. Diagrammatic sketch of mathematic model on the determination of UV fluence rate.
A: Exposure under single UV lamp. B: Exposure under three UV lamps (showed the lamp diagonally above the sample)
3.2.4. Modeling of patulin reduction kinetics

An average fluence-based first-order reduction model was mentioned by Severin et al. (1983) and Ye et al. (2007) for inactivation of microorganisms. Assatarakul et al. (2012) and Zhu et al. (2013) reported that the model was applied to patulin photo-degradation in apple cider and juice. The kinetics model was mathematically expressed as equation 3.10a.

\[
ln \frac{N}{N_0} = -k_f E_{avg} t
\]  

(3.10a)

The \( k_f \) is average-fluence-based first-order reaction rate constant with unit of mW\(^{-1}\)·cm\(^2\)·s\(^{-1}\).

Alternately, the applied-fluence-based first-order reduction model was developed by replacing average UV fluence with applied UV fluence (Equation 3.10b).

\[
ln \frac{N}{N_0} = -k_{f0} E_{app} t
\]  

(3.10b)

The \( k_{f0} \) is applied-fluence-based first-order reaction rate constant with unit of mW\(^{-1}\)·cm\(^2\)·s\(^{-1}\).

In this study, two fluence-based first-order reduction models were adopted. The \( k_f \) was proposed to predict patulin reduction when applied UV fluence rate, sample thickness, mixing condition and media were known and \( k_{f0} \) was used to compare efficiencies of UV lamps with same UV reactor, sample thickness mixing condition and media. The \( D_t \) is the exposure time, with a unit of min, required to reduce 90 % of patulin in apple juice by UV irradiation. It can be obtained from equation 3.11.

\[
D_t = \frac{-\ln(0.1)}{k_f \cdot E_{avg} \cdot 60}
\]  

(3.11)

The average UV fluence required for 90 % reduction of patulin (\( D_f \)) can be calculated from equation 3.12.

\[
D_f = \frac{-\ln(0.1)}{k_f}
\]  

(3.12)
To compare the efficiencies of UV lamps with different wavelengths, the lamp efficiency index (LEI) was defined as the ratio of applied-fluence-based first-order reaction rate constant ($k_{f0}$) of a UV lamp to that of the traditional UV lamp with 254 nm wavelength.

\[
LEI = \frac{k_{f0}}{k_{f0(254nm)}} 
\]  

(3.13)

### 3.2.5. UV radiation

In the patulin reduction study, samples spiked with 1.0 mg·L\(^{-1}\) of patulin were exposed to UV treatments for varying amounts of time. The UV fluence rates and exposure time were given in Table 3.1. In the quality study, the apple juice samples were exposed for the time ($D_t$) which resulted in the equivalent UV fluence for patulin reduction (Table 3.2). The temperatures in samples before and after UV exposure were monitored with a IKA® ETS-D4 digital temperature probe (IKA works, Inc., USA).

<table>
<thead>
<tr>
<th>Media</th>
<th>UV lamp</th>
<th>Applied UV fluence rate (mW·cm(^{-2}))</th>
<th>Average UV fluence rate (mW·cm(^{-2}))</th>
<th>Exposure time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple cider</td>
<td>222 nm</td>
<td>4.63</td>
<td>0.10</td>
<td>0, 180, 360, 540, 720, 900</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>8.27</td>
<td>0.31</td>
<td>0, 180, 360, 540, 720, 900</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>7.85</td>
<td>0.31</td>
<td>0, 180, 360, 540, 720, 900</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>13.9</td>
<td>0.44</td>
<td>0, 60, 120, 180, 240, 300</td>
</tr>
<tr>
<td>Apple juice</td>
<td>222 nm</td>
<td>4.95</td>
<td>0.11</td>
<td>0, 60, 120, 180, 240, 300</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>8.18</td>
<td>0.30</td>
<td>0, 60, 120, 180, 240, 300</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>7.76</td>
<td>0.36</td>
<td>0, 60, 120, 180, 240, 300</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>13.9</td>
<td>0.41</td>
<td>0, 20, 40, 60, 80, 100</td>
</tr>
</tbody>
</table>
Table 3.2. Patulin reduction by triple wavelength box UV reactor

<table>
<thead>
<tr>
<th>Media</th>
<th>UV lamp</th>
<th>Average-fluence-based reaction rate constant (mW⁻¹·cm²·s⁻¹)ᵃ</th>
<th>Dᵢ (min)ᵇ</th>
<th>Dₓ (mJ·cm⁻²)ᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple cider</td>
<td>222 nm</td>
<td>1.30E-2± 4.55E-4 𝒜</td>
<td>30.0± 1.0𝒜</td>
<td>178± 6.1 𝒜</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>2.63E-3± 9.69E-5 𝒜</td>
<td>46.9± 1.7Ｂ</td>
<td>877± 31.8 Ｂ</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>3.09E-3± 2.45E-5 𝒜</td>
<td>39.8± 0.3Ｃ</td>
<td>745± 5.9 Ｃ</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>4.22E-3± 1.46E-4 𝒜</td>
<td>20.5± 0.7Ｄ</td>
<td>546± 19.2 Ｄ</td>
</tr>
<tr>
<td>Apple juice</td>
<td>222 nm</td>
<td>1.17E-1± 2.15E-3 𝒜</td>
<td>2.9± 0.1 𝒜</td>
<td>19.6± 0.4 𝒜</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>2.73E-2± 6.01E-4 𝒜</td>
<td>4.8± 0.1 Ｂ</td>
<td>84.2± 1.8 Ｂ</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>4.20E-2± 2.17E-3 𝒜</td>
<td>2.5± 0.1 Ｃ</td>
<td>55.0± 2.8 Ｃ</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>6.30E-2± 2.68E-3 𝒜</td>
<td>1.5± 0.1 Ｄ</td>
<td>36.6± 1.6 Ｄ</td>
</tr>
</tbody>
</table>

ᵃ Values are averages ± standard deviations (n = 3) for all analysis. A different letter (A, B, C, D) indicates that significant differences (P < 0.05) in mean values were observed.

3.2.6. HPLC analysis for patulin and ascorbic acid concentration

Samples were extracted using solid phase extraction as described by Eisele and Gibson (2003). Oasis HLB extraction cartridges (3 mL/60 mg, Waters, MA) were conditioned by passing 2 mL of water, 2 mL of methanol and 2 mL of water at a flow rate of approximately 0.1 mL·s⁻¹. The sample (1 mL) was applied to the column followed by 2 mL of a 1.0 % (w/v) sodium bicarbonate solution then 2 mL of 1.0 % (v/v) acetic acid. The patulin was then eluted using 1.0 mL of ethyl acetate and evaporated to dryness under a stream of nitrogen. The residue was then dissolved in 0.5 mL 0.1 % (v/v) acetic acid prior to analysis. Standard patulin solutions of 0.01, 0.05, 0.1, 0.5, and 1 mg·L⁻¹ were prepared and extracted using the same method described above to form the linear calibration curve (0.01 to 1 mg·L⁻¹). Samples and standards were filtered using syringe filters with 0.45 µm pore size, and then analyzed using a HPLC system (Agilent Technology
1200 Series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, and a diode array detector (DAD) set at 276 nm. A Phenomenex Luna® 3 μ C18 column (250 x 2.0 mm) with a C18 guard column (Torrance, CA) was used for separations. 50 μL of samples and standards were eluted isocratically using 0.8 % (v/v) tetrahydrofuran in water at a flow rate of 0.2 mL·min⁻¹ with run time of 25 min. The retention time of patulin was around 20 min. The 5-hydroxymethylfurfural (HMF), a compound that mostly interfere patulin detection, eluted at approximately 17.5 min, which ensured baseline separation of these two compounds. The patulin detection limit for this method was approximately 0.01 mg·L⁻¹. Control samples were analyzed to ensure that samples before UV treatment were patulin-free. All samples and standards were analyzed in triplicate.

The concentration of ascorbic acid in samples was determined by HPLC. Apple juice samples were diluted 10 times with water. Standard ascorbic acid solutions of 5, 10, 20, 30, and 40 mg·L⁻¹ were prepared to form the linear calibration curve (5 to 40 mg·L⁻¹). Samples and standards were analyzed using a HPLC system (Agilent Technology 1200 Series, Palo Alto, CA) with a Phenomenex Luna® 3 μ C18 column (250 x 2.0 mm) (Torrance, CA) and a diode array detector (DAD) set at 245 nm. The mobile phase was water/acetonitrile/formic acid (95 %) (95:5:0.095 v/v/v, pH=1.8 adjusted by HCl), the flow rate was 0.5 mL·min⁻¹, and the injection volume was 20 µL. The retention time for ascorbic acid was approximately 4 min.

3.2.7. Optical properties, pH and color of treated apple juice
A UV/visible spectrometer (Ultrospec 3100 Pro) (Biochrom Ltd., Cambridge, England) was used to quantify the absorbance values for the samples. Each sample was tested in triplicate before
and after UV treatment using demountable fused-quartz cuvettes (NSG Precision Cell. Inc., Farmingdale, NY). Absorbance values of the apple cider and juice samples were tested in a series of cuvettes (0.01, 0.02, 0.05, 0.075 and 0.1cm). The absorption coefficients were determined by the slope of the linear plot of absorbance verses path length. The pH values were measured with a pH meter (Hack, Germany). Soluble solids content were determined with a Leica Mark II ABBE Refractometer (Leica, Germany). Color was measured in the CIELAB scale with the Lab Scan XE Spectrocolorimeter (HunterLab, USA). The L*(lightness), a* (redness), b* (yellowness) were measured and the total color difference (ΔE*) was calculated using equation 3.14.

\[
\Delta E^* = \sqrt{(L_{control} - L^*)^2 + (a^*_{control} - a^*)^2 + (b^*_{control} - b^*)^2}
\]

A ΔE* value of around 2.3 corresponded to a Just Noticeable Difference (JND) (Mahy et al., 1994) and it was used to evaluate the change of color after UV treatment.

3.2.8. Sensory evaluation

A triangle test was performed to determine if a significant color change occurred in apple juice during UV treatment. Twenty experienced panelists were identified from the employees and students in the Department of Food Science at University of Guelph. The apple juice samples without patulin spiking were treated by UV radiation for the time (D_t) which resulted in the equivalent UV fluence of patulin reduction (Table 3.2). For each treatment, two control samples without UV radiation and one UV treated sample were given a random 3-digit-code and samples were placed in random order. The 20 panelists were asked to identify the odd one from each set of samples. The results were evaluated with the chi-square distribution and the statistically significant differences were determined by comparison between the calculated chi-square value (χ^2) and the reference value of 3.84 (df = 1, α = 0.05) (Lawless and Heymann, 2010). If the
calculated value was lower than the reference value, we concluded there were no significant differences between the control and UV treated samples.

3.2.9. Statistical analysis

All UV processing conditions were performed in triplicate with a completely independent and randomized design. The statistical analysis of experimental data was carried out by SPSS version 20 (IBM, Armonk, NY). Statistical significance between control and UV treatment were evaluated through a paired t-test. The data of applied-fluence-based first-order reaction rate constants, the relative changes of pH, total soluble solids, absorption coefficients at 254 nm, color parameters and ascorbic acid concentration after UV treatments at different wavelengths were evaluated through one-way ANOVA with post-hoc Fisher’s Least Significance Difference significance (LSD) test. All patulin reduction rate constants were calculated from regression plots based on series data of patulin concentration at sampling points during the UV exposure. The coefficients of determination $R^2$ were calculated.

3.3. Results

3.3.1. Reduction of patulin with UV exposure at three wavelengths

UV treatment of apple juice at all three test wavelengths (222, 254 and 282 nm) resulted in a significant reduction ($P < 0.05$) in patulin compared to non-treated controls. UV inactivation kinetics followed first-order inactivation kinetics with the degradation rate being dependent on the applied wavelength (Figure 3.3, 3.4; Table 3.2). Based on the first-order reaction model and equation 2.12, exposure of samples to UV lamps with 222, 254, 282 nm wavelength and the combination of three wavelengths with average UV fluences of 19.6, 84.3, 55.0 and 36.6
mJ·cm$^{-2}$, respectively, resulted in 90% reduction of patulin in apple juice. The average UV fluences which resulted in the 90% reduction of patulin in apple cider were 177.9, 877.2, 744.8 and 545.5 mJ·cm$^{-2}$, respectively.

Figure 3.3. Reduction of patulin in apple cider by UV exposure with individual 222, 254, 282 nm wavelength UV lamps and the combination of three UV lamps
3.3.2. Efficiencies of Far UVC (222nm), UVC (254nm) and Far UVC plus (282nm) lamps

Patulin reduction efficiencies of UV lamps were determined by comparing the applied-fluence-based first-order reaction rate constants ($k_f$). The lamp efficiency indices (LEI) defined by Equation 13 were 2.80, 1.00, 2.00, 1.87 for apple juice and 2.69, 1.00, 1.24, 1.36 for apple cider exposed by the Far UVC (222 nm), UVC (254 nm), Far UVC plus (282 nm) and the combination of three lamps (Table 3.3). The efficiency order of three wavelength lamps was determined as: 222 nm > 282 nm > 254 nm.
Table 3.3. Lamp efficiency of patulin reduction.

<table>
<thead>
<tr>
<th>Media</th>
<th>UV lamp</th>
<th>Applied-fluence-based reaction rate constant (mW$^{-1}$·cm$^2$·s$^{-1}$)$^a$</th>
<th>Lamp efficiency index (LEI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple cider</td>
<td>222 nm</td>
<td>2.77E-4 ± 9.72E-6$^A$</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>9.91E-5 ± 3.66E-6$^B$</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>1.24E-4 ± 9.73E-7$^C$</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>1.34E-4 ± 4.64E-6$^D$</td>
<td>1.36</td>
</tr>
<tr>
<td>Apple juice</td>
<td>222 nm</td>
<td>2.65E-3 ± 4.87E-5$^A$</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td>254 nm</td>
<td>9.86E-4 ± 2.17E-5$^B$</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>282 nm</td>
<td>1.97E-3 ± 1.02E-4$^C$</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Triple lamps</td>
<td>1.84E-3 ± 7.86E-5$^C$</td>
<td>1.87</td>
</tr>
</tbody>
</table>

$^a$ Values are averages ± standard deviations (n = 3) for all analysis. A different letter (A, B, C, D) indicates significant differences (P < 0.05) in mean values were observed.

3.3.3. Quality attributes study

A separate study evaluated the effects of UV treatment on quality factors such as pH, total soluble solids, color, ascorbic acid concentration and absorption coefficients at 254 nm. Apple juice samples were treated at the different UV wavelengths with an equivalent UV fluence to support a 90% reduction of patulin (Table 3.2). Table 3.4 showed that these treatments did not significantly affect (P > 0.05) the pH values or the total soluble solid content (Brix°) of apple juice. The absorption coefficients at 254 nm were compared and all the four treatments showed significant decreases (P < 0.05) after UV exposure (Table 3.4). The greatest decrease in absorption coefficient (23.9%) was observed when juice was treated with a 254 nm UV lamp, which suggests that components in apple juice with peak absorption near 254 nm underwent photodegradation. There was a significant decrease (P < 0.05) in ascorbic acid concentration of apple juice at all the UV wavelengths tested (Table 3.4). The highest loss in ascorbic acid was
observed at 254 nm (45.3 %).

The total color differences (ΔE*) of apple juice after exposure (D_t) under 222 nm, 254 nm, 282 nm and combination of three lamps were 2.97, 0.61, 1.24 and 1.71 respectively (Figure 3.5). Since a ΔE* value of around 2.3 corresponds to a Just Noticeable Difference (JND) (Mahy et al., 1994), the color changes caused by 254 nm, 282 nm and combination UV lamps were not noticeable, whereas the color changes resulting from treatment at 222 nm were slightly noticeable. However, the triangle sensory test showed no significant color changes between samples before and after UV irradiation (Table 3.5).

Figure 3.5. Color change in apple juice by UV exposure with individual 222, 254, 282 nm wavelength UV lamps and the combination of three UV lamps.
A,B,C,D: Average UV fluences of 222, 254, 282 nm and combination of three lamps, respectively, corresponding to time for 90 % patulin reduction (D_t).
Table 3.4. Effects of the UV irradiation on pH, total soluble solid, absorption coefficient, color and concentration of ascorbic acid.

<table>
<thead>
<tr>
<th>Quality attributes</th>
<th>Far UVC lamp (222nm)</th>
<th>UVC lamp (254nm)</th>
<th>Far UVC plus lamp (282nm)</th>
<th>Triple lamps</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>C 3.18±0.01</td>
<td>3.19±0.01</td>
<td>3.16±0.01</td>
<td>3.17±0.01</td>
</tr>
<tr>
<td></td>
<td>T 3.19±0.01</td>
<td>3.18±0.01</td>
<td>3.18±0.01</td>
<td>3.18±0.01</td>
</tr>
<tr>
<td>RC (%)</td>
<td>0.31±0.32&lt;sub&gt;AB&lt;/sub&gt;</td>
<td>-0.10±0.18&lt;sub&gt;A&lt;/sub&gt;</td>
<td>0.42±0.18&lt;sub&gt;B&lt;/sub&gt;</td>
<td>0.32±0.32&lt;sub&gt;AB&lt;/sub&gt;</td>
</tr>
<tr>
<td>Soluble solid (Brix°)</td>
<td>C 11.2±0.06</td>
<td>11.1±0.06</td>
<td>11.2±0.06</td>
<td>11.1±0.00</td>
</tr>
<tr>
<td></td>
<td>T 11.1±0.06</td>
<td>11.2±0.06</td>
<td>11.1±0.06</td>
<td>11.1±0.10</td>
</tr>
<tr>
<td>RC (%)</td>
<td>-0.30±0.51&lt;sub&gt;A&lt;/sub&gt;</td>
<td>0.30±1.03&lt;sub&gt;A&lt;/sub&gt;</td>
<td>-0.30±0.51&lt;sub&gt;A&lt;/sub&gt;</td>
<td>0.00±0.90&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
<tr>
<td>α&lt;sub&gt;254&lt;/sub&gt;</td>
<td>C 24.7±0.02</td>
<td>24.8±0.03</td>
<td>24.7±0.03</td>
<td>24.8±0.01</td>
</tr>
<tr>
<td></td>
<td>T 20.6±0.04</td>
<td>18.9±0.07</td>
<td>20.0±0.07</td>
<td>20.5±0.07</td>
</tr>
<tr>
<td>RC (%)</td>
<td>-16.4±0.11&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>-23.9±0.22&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;B&lt;/sub&gt;</td>
<td>-19.0±0.25&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;C&lt;/sub&gt;</td>
<td>-17.2±0.26&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;D&lt;/sub&gt;</td>
</tr>
<tr>
<td>L*</td>
<td>C 49.3±0.02</td>
<td>49.4±0.07</td>
<td>49.2±0.02</td>
<td>49.3±0.06</td>
</tr>
<tr>
<td></td>
<td>T 50.2±0.07</td>
<td>49.7±0.04</td>
<td>49.7±0.05</td>
<td>49.8±0.06</td>
</tr>
<tr>
<td>RC (%)</td>
<td>1.88±0.13&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>0.72±0.12&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;B&lt;/sub&gt;</td>
<td>1.01±0.11&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;C&lt;/sub&gt;</td>
<td>1.12±0.05&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;C&lt;/sub&gt;</td>
</tr>
<tr>
<td>a*</td>
<td>C -1.67±0.01</td>
<td>-1.67±0.01</td>
<td>-1.64±0.01</td>
<td>-1.63±0.01</td>
</tr>
<tr>
<td></td>
<td>T -2.45±0.01</td>
<td>-1.88±0.01</td>
<td>-2.06±0.01</td>
<td>-2.14±0.01</td>
</tr>
<tr>
<td>RC (%)</td>
<td>-46.4±0.79&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>-12.6±0.60&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;B&lt;/sub&gt;</td>
<td>-26.1±0.41&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;C&lt;/sub&gt;</td>
<td>-31.6±0.47&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;D&lt;/sub&gt;</td>
</tr>
<tr>
<td>b*</td>
<td>C 25.0±0.05</td>
<td>25.0±0.03</td>
<td>25.0±0.02</td>
<td>25.0±0.03</td>
</tr>
<tr>
<td></td>
<td>T 22.3±0.03</td>
<td>24.5±0.03</td>
<td>24.0±0.06</td>
<td>23.4±0.07</td>
</tr>
<tr>
<td>RC (%)</td>
<td>-10.8±0.20&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>-1.81±0.23&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;B&lt;/sub&gt;</td>
<td>-4.19±0.19&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;C&lt;/sub&gt;</td>
<td>-6.13±0.15&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;D&lt;/sub&gt;</td>
</tr>
<tr>
<td>Ascorbic acid (mg L&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>C 278±0.37</td>
<td>267±5.07</td>
<td>272±3.92</td>
<td>271±1.43</td>
</tr>
<tr>
<td></td>
<td>T 176±4.67</td>
<td>146±4.26</td>
<td>174±5.11</td>
<td>173±4.76</td>
</tr>
<tr>
<td>RC (%)</td>
<td>36.5±1.62&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>45.3±0.56&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;B&lt;/sub&gt;</td>
<td>36.1±2.75&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
<td>36.2±1.47&lt;sup&gt;*&lt;/sup&gt;&lt;sub&gt;A&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

C: Control sample without UV treatment. T: Sample treated by UV light for a period of D<sub>c</sub>. RC: Relative change (%). *: Significant difference (P < 0.05) between control and UV treated sample. A, B, C, D: A different letter indicates significant differences (P < 0.05) in mean values were observed between the UV treatments.
Table 3.5. Triangle test for determination of color changes in apple juice after UV irradiation with fluence supporting to degrade 90% of patulin

<table>
<thead>
<tr>
<th>UV lamp</th>
<th>$E_c^a$</th>
<th>$E_i^b$</th>
<th>$O_c^c$</th>
<th>$O_i^d$</th>
<th>$\chi^2_e$</th>
<th>$\chi^2_{(1,0.05)}^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 nm</td>
<td>6.7</td>
<td>13.3</td>
<td>9</td>
<td>11</td>
<td>1.22</td>
<td>3.84</td>
</tr>
<tr>
<td>254 nm</td>
<td>6.7</td>
<td>13.3</td>
<td>8</td>
<td>12</td>
<td>0.40</td>
<td>3.84</td>
</tr>
<tr>
<td>282 nm</td>
<td>6.7</td>
<td>13.3</td>
<td>9</td>
<td>11</td>
<td>1.22</td>
<td>3.84</td>
</tr>
<tr>
<td>Triple lamps</td>
<td>6.7</td>
<td>13.3</td>
<td>9</td>
<td>11</td>
<td>1.22</td>
<td>3.84</td>
</tr>
</tbody>
</table>

$^a$ Expected number of correct responses (panelist = 20)

$^b$ Expected number of incorrect responses (panelist = 20)

$^c$ Observed number of correct responses (panelist = 20)

$^d$ Observed number of incorrect responses (panelist = 20)

$^e$ Calculated $\chi^2$

$^f$ Reference $\chi^2$ (df = 1, $\alpha = 0.05$)

3.3.4. Temperature monitoring during UV exposure

A maximum of 1.9 ºC of temperature increase was observed by measuring apple cider and apple juice samples with various applied UV fluence rates and exposure times listed in Table 3.1. The change of temperature of < 2 ºC indicates minimal heating effect resulted from UV exposures used in this study.
3.4. Discussion

Occurrences of patulin contamination in apple cider and juice were reported by several studies and surveillances (Gökmen and Acar, 1998; Lai et al., 2000; Leggott et al., 2000) and most of the contamination levels were < 0.5 mg·L⁻¹. The UV fluence that results in a 90 % reduction of patulin became an essential parameter for the design of potential commercial UV reactors to reduce levels of the mycotoxin in apple cider and juice to < 0.05 mg·L⁻¹ according to national regulations (U.S.FDA, 2005; EC, 2006). The average-fluence-based first-order reaction rate constants obtained by experimental results can be used to predict required UV fluence leading to a greater reduction of patulin (e.g. 99 %). Assatarakul et al. (2012) investigated the kinetic models of patulin degradation at 254 nm and provided the first-order rate constant of 0.0294 mW⁻¹·cm²·s⁻¹ in apple juice and 0.0053 mW⁻¹·cm²·s⁻¹ in apple cider. The corresponding data determined in the current study (0.0273 mW⁻¹·cm²·s⁻¹ in apple juice and 0.0026 mW⁻¹·cm²·s⁻¹ in apple cider) confirmed the results of the previous study although there were no reported data on the treatment of juice with 222 and 282 nm UV lamps.

As processing of liquid apple products with UV light is designed mainly for inactivation of pathogens, it is worth comparing the UV fluences resulting in 90 % of patulin reduction to those necessary for reduction of microbial pathogens. Many studies identified the germicidal performance of UV reactors at 254 nm on the Escherichia coli O157:H7 and its surrogates (Forney et al., 2004; Koutchma and Parisi, 2004; Wright et al., 2000). Wright et al. (2000) reported that a 5.4 log reduction of Escherichia coli O157:H7 in apple cider was achieved by treatment in a thin film UV disinfection unit with a UV fluence of 61 mJ·cm⁻². Forney et al. (2004) used a Taylor-Couette flow UV reactor and a UV fluence of 21.7 mJ·cm⁻² and determined
a 3 to 5 log reduction of Escherichia coli 15597 in apple juice. In addition, Koutchma and Parisi (2004) determined decimal reduction UV fluences of 4.60 mJ·cm\(^{-2}\) and 6.46 mJ·cm\(^{-2}\) on Escherichia coli K12 in apple juice and apple cider respectively. The average UV fluence resulting in 90 % of patulin reduction in apple juice (84.2 mJ·cm\(^{-2}\)) with treatment with 254 nm UV light indicated that it was sufficient to reduce Escherichia coli O157:H7 levels by 5 logs although the UV reactors and experimental settings were different. Therefore, future work will determine the required UV fluences for the 5 log reduction of pathogens in apple juice using 222, 254 and 282 nm UV light to confirm that the fluence resulting in 90 % of patulin reduction could also reduce 5 log of pathogens simultaneously.

Although the UV irradiation with 254 nm wavelength is widely recognized as a successful non-thermal technique for pathogen inactivation, use of other wavelengths need to be studied. Using same applied UV fluences, the efficiencies of patulin photo-degradation of UV lamps at different wavelengths were influenced by the photon energy, absorption spectrum of patulin and absorption spectrum of apple juice. Among them, the photon energy is inversely proportional to the wavelength (Equation 3.15).

\[
E_p = \frac{hcN_A}{\lambda} \tag{3.15}
\]

Most of bond energies are coincident with the photon energies in UV range (Blatchley and Peel, 2001). In general, the photon with a lower wavelength has sufficient energy to break the bonds and consequently promotes a photo chemical reaction of the desired component. The wavelength of light by which patulin is exposed affects the reduction rate. Based on the Grotthuss–Draper law of photochemistry (Hall, 2000), the light must be absorbed by a chemical substance in order for a photochemical reaction to take place. The photons with a wavelength closer to patulin’s
absorption maximum (276 nm) can be easy absorbed by patulin, and result in photo-degradation of the mycotoxin. A higher absorption coefficient impedes the penetration of UV light and reduces the average UV fluence in the whole sample. The relationships among the three factors are shown in Figure 3.6. The experimental data assessed the overall effects of these three factors on patulin reduction among UV lamps with three wavelengths. The fact that reduction rate caused by 222 nm was greater than 282 nm and 254 nm indicated the priority of choosing a UV lamp with a specific wavelength was the photon energy. Meanwhile, the potential quality change of apple juice should be evaluated.

Liquid apple products are complex matrices containing sugars, organic acids, polyphenolic groups and added ascorbic acid (Buettner and Jurkiewicz, 1996) although the natural concentration of ascorbic acid in apple juice is as low as 9.0 mg·L⁻¹ (USDA, 2013a). Normally, additional ascorbic acid is added to enhance the nutrient value of apple juice to a level of 385 mg·L⁻¹ (USDA, 2013b). The loss of ascorbic acid by all UV treatments was significant and could be viewed as a limitation of the non-thermal pasteurization method. Several researches have reported loss of ascorbic acid in fruit juices when exposed to UV light (Falguera et al., 2011; Koutchma, 2008; Tikekar et al., 2011; Tran and Farid, 2004; Ye et al., 2007). However, by using 222 nm light, not only is a higher level of patulin degradation achieved, but the ascorbic acid losses are minimized. Due to the UV-instability of ascorbic acid, it would be beneficial to replenish the nutrient after UV radiation.

As with other UV treatments, there was a change in color following treatment due to the generation of photo-byproducts. Typically, UV treatment results in an increase in L* and
decrease of a* (from red to green) and b* (from yellow to blue) (Ibarz et al., 2005). The same observation was made in the current study although the color change using 222, 254 and 282 nm did not result in any significant change in the apple juice. Flavor evaluation is another important sensory test to discriminate the quality change of apple juice after UV treatment. The current study did not perform the test due to the non-food grade UV reactor and processing facility. This evaluation will be included in future work.

The patulin degradation products resulting from UV radiation is still unknown. Identifying and quantifying the photo-products of patulin and evaluating the toxicological effects of byproducts of patulin and/or UV treated apple cider and juices which contain patulin should be the subject of future work.
Figure 3.6. Relationship between the photon energy and the absorbance spectrums of apple juice (0.02 cm of light pathlength) and 10 mg·L\(^{-1}\) patulin (1 cm of light pathlength)

### 3.5. Conclusions

The rate of patulin photo-degradation is wavelength dependent. Among the UV lamps with wavelength of 222 nm, 254 nm and 282 nm, the Far UVC lamp (222 nm) was the most effective UV source due to the best performance of the photon energy at this wavelength. Meanwhile, the advantages of no significant changes in pH, total soluble solid and color changes in apple juice after UV exposure at 222 nm for a time to degrade 90 % of patulin \((D_t)\) demonstrate potential of further development of this novel UV source for the commercial applications.
Chapter Four

Decrease of Patulin Toxicities in Apple Juice after Ultraviolet (254 nm) Irradiation Using both Saccharomyces cerevisiae and Drosophila melanogaster as Model Systems
Abstract

The decrease in patulin toxicities in apple juice after UV (254 nm) irradiation using both a single eukaryotic cell (*Saccharomyces cerevisiae*) and a metamorphosis insect (*Drosophila melanogaster*) model systems were evaluated in this study. The UV fluences were 2582 and 1890 mJ·cm⁻², respectively. In the mycotoxin sensitivity evaluations, 50 mg·L⁻¹ of patulin caused a growth inhibition of *S. cerevisiae* as well as 500 mg·L⁻¹ resulted in a larvicidal effect on *D. melanogaster*. In the UV-detoxification evaluation on *S. cerevisiae*, the yeast populations in UV treated samples were significantly higher (P < 0.05) than untreated patulin control samples at an observation point of 66 h. By using *D. melanogaster* model system, hatching rates, development rates of pupae, development rates of adult flies and length of larvae from UV treated samples were significantly higher (P < 0.05) than the untreated patulin control samples (68.5 % versus 21.1 %, 69.2 % versus 0 %, 69.0 % versus 0 %, 3.4 versus 1.3 mm, respectively) and had no significant differences (P > 0.05) with the blank (juice without patulin + no UV treatment), UV control (juice without patulin + UV treatment) and spiked (juice spiked with patulin + no UV treatment) samples. The result suggested the reduction of patulin toxicities after UV irradiation and preliminarily confirmed the UV-C (254 nm) irradiation can be safely applied in the photodegradation of patulin in liquid apple products.
4.1. Introduction

Properties of patulin were initially studied as a potential broad-spectrum antibiotic for medical treatment of the common cold. However, the toxicity of patulin which included stomach irritation, nausea and vomiting ceased studies of possible medical applications of the mycotoxin (Ciegler, 1977). Patulin was reclassified as a mycotoxin during the 1960s and became an important contaminant in food especially liquid apple products (Bennett and Klich, 2003).

The toxicological studies of patulin involve assessments of acute and chronic effects. The oral LD50 values of patulin in mice, hamsters, rats and poultry were reported (Lindroth and Wright, 1978; McKinley and Carlton, 1980a; McKinley and Carlton, 1980b; McKinley et al., 1982; Lovett, 1972). One explanation for the acute toxicity of patulin that has been proposed is that patulin leads to alternations in the bacteria flora of intestinal tract by inhibiting Gram-positive organisms and providing selective advantage to pathogenic Gram-negative bacteria (McKinley and Carlton, 1980a; McKinley and Carlton, 1980b). Becci et al. (1981) conducted a long-term toxicity study of patulin in rats. Combined with the evaluation of acute, long-term and tumorigenic toxicities, a patulin level of 0.1 mg/kg b.w. was found to be the value of no observed adverse effect level (NOAEL). This NOAEL was used by the U.S. Food and Drug Administration (FDA) and European Commission (EC) to establish the maximum tolerances for patuin in liquid apple products (U.S.FDA, 2001; JECFA, 1995; EC, 2006).

Yeast, especially the species of Saccharomyces cerevisiae, was used as eukaryotic cell model to investigate gene expression levels after exposure to toxins and to determine the mechanism of their toxicity (Iwahashi et al., 2006). Patulin can bind the sulfhydryl groups of membrane
proteins and reduce the activity of glutathione. Glutathione maintains intracellular condition in order to avoid damage of cellular compounds including nucleotides by oxygen radical species (Suzuki and Iwahashi, 2011; Burghardt et al., 1992). The inhibition of yeast growth induced by patulin has been reported in several studies (Sumbu et al., 1983; Moss and Long, 2002; Iwahashi et al., 2006; Horváth et al., 2010; Suzuki and Iwahashi, 2011; Shao et al., 2012).

*Drosophila melanogaster* serves as a traditional and successful model system in the field of biological studies based on its advantages of: easy observation and manipulation at each developmental stage of metamorphosis; fast growth without complicated feeding conditions; large numbers of offspring; high resistance to the plagues and pathogens; and plenty of developed genetic tools (Stocker and Gallant, 2008). Except for the wide application in genetic studies, the *D. melanogaster* model was also adopted for use in research on the larvicidel, insecticidal and genotoxic activities of mycotoxins including aflatoxin B1, citrinin, stachybotryotoxin, rubratoxin B, diacetoxy-scirpenol and patulin (Cole and Rolinson, 1972; Reiss, 1975; Belitsky et al., 1985; Paterson et al., 1987).

The kinetics of photo-degradation of patulin fits a first-order reaction model and more than 90 % of patulin was removed by UV-C (254 nm) light with fluence of 85.2 mJ·cm⁻² (Assatarakul et al., 2012). Compared to the 254 nm UV light emitted by traditional LPM UV sources, UV light at 222 nm showed higher efficiencies for patulin reduction in apple juice without significant changes in pH, total soluble solids, and color (Zhu et al., 2014). All the previous studies, however, did not examine the change in toxicity of patulin after UV irradiation, although the decrease in mycotoxin levels was determined. In this study, the cytotoxicity and larvicidal effects
of patulin in apple juice before and after UV treatment at 254 nm with fluence resulting in a > 80 % reduction of patulin were evaluated using *S. cerevisiae* and *D. melanogaster* model systems.

**4.2. Materials and Methods**

**4.2.1. Materials**

Standard patulin [4-hydroxy-4H-furo (3,2-\(c\))-pyran-2-(6H)-one] was obtained from the Sigma Chemical Company (St. Louis., U.S.A.). Apple juice (pH = 2.92) was purchased from a local supermarket. *D. melanogaster* were obtained from Bloomington Drosophila stock center, Indiana University (Bloomington, Indiana, U.S.A.). The *S. cerevisiae* strain was obtained from ATCC (ATCC No. 204508). Other chemicals including sucrose, dipotassium phosphate, monopotassium phosphate, diammonium phosphate, magnesium sulfate heptahydrate, iron(II) sulfate, manganese(II) sulfate, 4-methyl-hydroxybenzoate, potassium phosphate, potassium sodium tartrate, sodium chloride, calcium chloride, magnesium chloride, iron (III) sulphate, propionic acid and agar were obtained from Sigma Chemical Company (St. Louis., U.S.A.).

**4.2.2. UV irradiation**

A low-pressure mercury (LPM) UV light reactor (R-52G MINERALIGHT® UV Lamp UVP Inc, CA, USA) was used to deliver a specific dose of UV light at 254 nm to the samples. The samples were treated in a plastic Petri-dish with a diameter of 3.6 cm placed under the lamp. The sample thickness was 1 mm. The incident irradiance was varied by adjusting the distance between samples and UV lamp. Incident irradiance was measured using a digital ILT1700 radiometer (International Light Technologies, MA, USA).
4.2.3. Growth conditions of *S. cerevisiae*

The *S. cerevisiae* strain was re-activated on a Potato Dextrose Agar (PDA, Merck, Kirkland, Canada) plate before being used as inoculum. It was grown aerobically in 5 mL of minimal medium (1 L medium contains 10.0 g sucrose, 2.5 g dipotassium phosphate, 2.5 g monopotassium phosphate, 1.0 g diammonium phosphate, 0.2 g magnesium sulfate heptahydrate, 0.01 g iron(II) sulfate and 0.007 g manganese(II) sulfate) with patulin sample in 50 mL centrifuge tubes at 28 °C. Cell number was monitored using a haemocytomter (Hausser Scientific, Horsham, PA, U.S.A.) under a microscope (SY-LAB, Neupurkersdorf, Austria).

4.2.4. Sample preparation for *S. cerevisiae* model system

In the patulin sensitivity evaluation experiments, acidified water solutions (pH = 4, adjusted with 1 % acetic acid) with various concentrations of patulin (0, 10, 20, 50, 100, 200 and 500 mg·L⁻¹) were prepared and mixed with the minimal medium containing approximate 1×10⁶ cells/mL of *S. cerevisiae* at a ratio of 1:9. Thus the final concentrations of patulin in the minimal medium were 0 (as a blank), 1, 2, 5, 10, 20 and 50 mg·L⁻¹. In the UV-detoxification experiment, five sets of samples were used (Table 4.1). Blank samples were patulin-free apple juice. UV control (UV-CK) samples were patulin-free apple juice treated with an equivalent UV fluence as UV treated sample. Patulin control samples (PAT-CK) were apple juice spiked with an appropriate concentration of patulin which were determined in the sensitivity evaluation. UV treated samples were apple juice spiked with the same patulin concentration as patulin control samples, and then they were treated by UV and resulted in a > 80 % degradation of patulin in samples. The exact concentration of residual patulin was determined by HPLC analysis. Spiked samples were apple juice with the same patulin concentration of samples after UV treatment (treated samples). Thus
spiked samples contained around 20% of original patulin but no UV photoproducts. As mentioned in the patulin sensitivity evaluation, samples were mixed with minimal medium containing approximate $1 \times 10^6$ cells/mL of *S. cerevisiae* at a ratio of 1:9.

Table 4.1. Sample preparation for *S. cerevisiae* model system

<table>
<thead>
<tr>
<th>Samples</th>
<th>Spiked patulin conc. in apple juice (mg·L$^{-1}$)</th>
<th>UV irradiation</th>
<th>Final patulin conc. in apple juice (mg·L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>UV-CK</td>
<td>0</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Treated</td>
<td>500</td>
<td>Yes</td>
<td>58.5</td>
</tr>
<tr>
<td>Spiked</td>
<td>58.5</td>
<td>No</td>
<td>58.5</td>
</tr>
<tr>
<td>PAT-CK</td>
<td>500</td>
<td>No</td>
<td>500</td>
</tr>
</tbody>
</table>

4.2.5. Preparation of apple juice agar and Sokolowski lab fly food

Apple juice agar was used for the collection of fly eggs. It was prepared by weighing out 5 g sucrose, 4.5 g agar and 0.3 g 4-methyl-hydroxybenzoate into 150 mL of distilled water (Sokolowski lab, University of Toronto, Canada). The mixture was heated to boiling and 125 mL of low acidity apple juice was added. The mixture was heated to boiling, then cooled to 70 °C and dispensed into petri dishes (100 mm in diameter).

Sokolowski lab fly food (Sokolowski lab, University of Toronto, Canada) was used in growth experiments. Fly food base (1L medium contains 50 g sucrose, 14.5 g agar, 0.5 g potassium phosphate, 4 g potassium sodium tartrate, 0.25 g sodium chloride, 0.25 g calcium chloride, 0.25 g magnesium chloride and 0.25 g iron (III) sulphate) and yeast medium (500 mL medium contains 25 g dry yeast) were prepared and autoclaved (121 °C, 30 min) separately. After
autoclaving, the yeast medium was transferred into the fly food base. Once thoroughly mixed, 2.5 mL of propionic acid was added.

4.2.6. Sample preparation for D. melanogaster model system
In the patulin sensitivity evaluation, acidified water solutions (pH = 4, adjusted with 1 % acetic acid) with various concentrations of patulin were prepared and mixed with the Sokolowski lab fly food (50 °C) to obtain a ratio of 1: 9 (v/v) of patulin sample: fly food. The final concentrations of patulin in fly food were 0 (as a blank), 0.5, 1, 20, 50, 100, 200 and 500 mg·L\(^{-1}\). The mixtures (1 mL) were immediately distributed into vials. The mixtures were ready for the fruit fly growth experiment after drying overnight at 20 °C. In the UV-detoxification experiment, five sets of samples were used (Table 4.2). Blank samples were patulin-free apple juice. UV control (UV-CK) samples were patulin-free apple juice with equivalent UV fluence of UV treated samples. Patulin control (PAT-CK) samples were apple juice spiked with an appropriate concentration of patulin which was determined by the sensitivity evaluation. UV treated samples were apple juice spiked with the same patulin concentration as patulin control samples, and then they were treated by UV and resulted in a > 80 % degradation of patulin in samples. The exact concentration of residual patulin was determined by HPLC analysis. Spiked samples were apple juice with the same patulin concentration of samples after UV treatment (treated samples). Thus spiked samples contained around 20 % of original patulin but no UV photoproducts. These samples were mixed with the Sokolowski lab fly food at 50 °C at a ratio of 1: 9. 1 mL of the samples was immediately transferred to a vial allowed to dry overnight.
Table 4.2. Sample preparation for *D. melanogaster* model system

<table>
<thead>
<tr>
<th>Samples</th>
<th>Spiked patulin conc. in apple juice (mg·L⁻¹)</th>
<th>UV irradiation</th>
<th>Final patulin conc. in apple juice (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>UV-CK</td>
<td>0</td>
<td>Yes</td>
<td>0</td>
</tr>
<tr>
<td>Treated</td>
<td>5000</td>
<td>Yes</td>
<td>879</td>
</tr>
<tr>
<td>Spiked</td>
<td>879</td>
<td>No</td>
<td>879</td>
</tr>
<tr>
<td>PAT-CK</td>
<td>5000</td>
<td>No</td>
<td>5000</td>
</tr>
</tbody>
</table>

4.2.7. Inoculation of fruit fly eggs

Adult *D. melanogaster* flies were placed into a container with apple juice agar plates and yeast food (prepared by mixing sterile water and dry yeast). The eggs were ready to collect after 16 hours. The first step of collection included removing eggs from apple juice plates and transferring them into a petri dish with a layer of gauze at the bottom. Then, the eggs were decontaminated with 10 % bleach for 2 min followed by washing with sterile water (10 times). Clean eggs were transferred into a 50 mL centrifuge tube and allowed to stand for 10 min. Approximately 30 eggs (27-34) were inoculated into each vial by using a stereo microscope (Carl Zeiss Microscopy GmbH, Jena, German). The vials were incubated at room temperature (approximately 21 ºC) and under a relative humidity around 32 %.

4.2.8. Observation

The number of inoculated fly eggs at day 0 was recorded. The observation was performed every day from day 5. The observation included the number and morphology of larvae, pupae and adult flies. The hatching rate, development rate of pupae and adult flies were calculated by following equations (equation 4.1, 4.2 and 4.3).
\[
Hatching \ rate = \frac{\text{number of survival larvae}}{\text{number of inoculated fly eggs}} \times 100\% \quad (4.1)
\]

\[
Development \ rate \ of \ pupae = \frac{\text{number of survival pupae}}{\text{number of inoculated fly eggs}} \times 100\% \quad (4.2)
\]

\[
Development \ rate \ of \ adult \ flies = \frac{\text{number of survival adult flies}}{\text{number of inoculated fly eggs}} \times 100\% \quad (4.3)
\]

The length of larvae was measured from images taken from a stereo microscope (Carl Zeiss Microscopy GmbH, Jena, German) by comparing the image with one of a ruler taken under the same conditions.

4.2.9. HPLC analysis

In order to measure residual patulin after UV radiation, samples and standards (patulin solutions of 10, 50, 100, 200, 500, and 1000 mg·L\(^{-1}\)) were filtered with syringe filters of 0.45 \(\mu\)m pore size, and then were analyzed using a HPLC system (Agilent Technology 1200 Series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, and a diode array detector (DAD) set at 276 nm. A Phenomenex Luna® 3 \(\mu\) C18 column (250 × 2.0 mm) with a C18 guard column (Torrance, CA) was used for the separations. Samples and standards (10 \(\mu\)L injection volume) were eluted isocratically using 0.8 % (v/v) tetrahydrofuran in water at a flow rate of 0.2 mL·min\(^{-1}\) with run time of 25 min. The retention time of patulin was around 20 min. The 5-hydroxymethylfurfural (HMF), a compound that commonly interferes the patulin detection, eluted at around 17.5 min, which ensured baseline separation of these two compounds.

4.2.10. Statistics analysis

For the sensitivity evaluation experiments, all levels of aqueous patulin solutions were evaluated three times (\(n = 3\)) for the \textit{S. cerevisiae} model system, and six times (\(n = 6\)) for the \textit{D.}
*melanogaster* model system. In the UV-detoxification experiments, all UV-processing conditions (blank, UV-CK, treated, spiked and PAT-CK samples) were performed in triplicate using a completely independent and randomized design. For each replication, six replicate samples were used (n =18) for the *D. melanogaster* model system. The statistical analyses of experimental data were carried out with SPSS version 20 (IBM, Armonk, NY). The data obtained for hatching rate, development rate of pupa and adult fly, length of larva, as well as cell concentration of *S. cerevisiae* model system were evaluated by one-way analysis of variance (ANOVA) with the post hoc Tukey’s honestly significance difference significance test.

4.3. Results

4.3.1. Patulin sensitivity evaluation in *S. cerevisiae* model system

The growth curves for *S. cerevisiae* responding to various concentration of patulin are presented in Figure 4.1. The growth rates decreased with the increasing patulin concentrations. At 58 h, it was observed that yeast in blank samples reached maximum growth, yeast concentrations (2.2 × 10^7 cells/mL) in the 50 mg·L⁻¹ of patulin solution was significantly lower (P < 0.05) than in the blank sample (5.7 × 10^7 cells/mL). The patulin concentration of 50 mg·L⁻¹ was used as the control sample in UV-detoxification experiments.
**Figure 4.1.** Growth curves of *S. cerevisiae* in minimal media with various patulin concentrations

### 4.3.2. UV-detoxification evaluation in *S. cerevisiae* model system

The UV-treated samples contained $5.85 \pm 0.19$ mg·L$^{-1}$ of patulin after UV radiation with average fluence of 2582 mJ·cm$^{-2}$. The growth curves of *S. cerevisiae* of blank, UV-CK, treated, spiked and PAT-CK samples are shown in Figure 4.2. At 66 h, the point at which the yeast in blank sample had a maximum growth, the cell concentration in the treated sample ($1.37 \times 10^8$ cells/mL) was significantly higher ($P < 0.05$) than in the PAT-CK sample ($1.00 \times 10^8$ cells/mL).
Figure 4.2. Growth curves of *S. cerevisiae* in the blank (PAT= 0 mg·L\(^{-1}\) without UV treatment), UV-CK (PAT= 0 mg·L\(^{-1}\) with UV treatment), treated (PAT = 5.85 mg·L\(^{-1}\) with UV treatment), spiked (PAT = 5.85 mg·L\(^{-1}\) without UV treatment) and PAT-CK (PAT = 50 mg·L\(^{-1}\) without UV treatment) samples.

### 4.3.3. Patulin sensitivity evaluation in *D. melanogaster* model system

The life cycle of *D. melanogaster* was observed as larvae (day 1~7), pupae (day 7~13) and adult flies (day 13~16). The hatching rates, development rates of pupae and adult flies were listed in the Table 4.3. There were no significant differences (P > 0.05) in these variables for all of the patulin concentrations excluding the concentration of 500 mg·L\(^{-1}\). The fly food with 500 mg·L\(^{-1}\) of patulin contributed a significant lower (P < 0.05) hatching rate (28.1 %) compared to blank...
and the lower patulin concentrations from 0.5 to 200 mg·L⁻¹ (57.2 % to 64.4 %, respectively). Moreover, the survived larvae stopped developing and died after day 11. The results indicated the threshold concentration of patulin which led to a larvicidal effect on *D. melanogaster* was between 200 and 500 mg·L⁻¹. Thus a concentration of 500 mg·L⁻¹ was used in the control samples in UV-detoxification experiments.

Table 4.3. Hatching and development rate at day 6, 10 and 15 in various patulin concentrations

<table>
<thead>
<tr>
<th>Concentration of patulin (mg·L⁻¹)</th>
<th>Hatching rate at Day 6 (%)ᵃ</th>
<th>Development rate of pupa at Day 10 (%)ᵃ</th>
<th>Development rate of adult at Day 15 (%)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>61.8 ± 12.5 A</td>
<td>63.4 ± 15.7 A</td>
<td>60.5 ± 12.7 A</td>
</tr>
<tr>
<td>0.5</td>
<td>62.3 ± 13.0 A</td>
<td>62.3 ± 13.0 A</td>
<td>57.4 ± 12.0 A</td>
</tr>
<tr>
<td>1</td>
<td>64.4 ± 12.6 A</td>
<td>66.1 ± 15.2 A</td>
<td>60.7 ± 16.3 A</td>
</tr>
<tr>
<td>20</td>
<td>62.5 ± 12.6 A</td>
<td>63.4 ± 13.1 A</td>
<td>60.8 ± 14.1 A</td>
</tr>
<tr>
<td>50</td>
<td>62.7 ± 10.9 A</td>
<td>65.4 ± 11.4 A</td>
<td>61.4 ± 8.1 A</td>
</tr>
<tr>
<td>100</td>
<td>57.2 ± 4.7 A</td>
<td>57.2 ± 4.7 A</td>
<td>56.5 ± 4.5 A</td>
</tr>
<tr>
<td>200</td>
<td>62.2 ± 7.5 A</td>
<td>62.8 ± 6.6 A</td>
<td>59.3 ± 8.2 A</td>
</tr>
<tr>
<td>500</td>
<td>28.1 ± 8.6 B</td>
<td>0 ± 0 B</td>
<td>0 ± 0 B</td>
</tr>
</tbody>
</table>

ᵃ Values are averages ± standard deviations (n = 6). A different letter (A, B) indicates significant differences (P < 0.05) in mean values were observed.

4.3.4. UV-detoxification evaluation in *D. melanogaster* model system

The UV-treated samples which initially contained 500 mg·L⁻¹, contained 87.9 mg·L⁻¹ of patulin after UV treatment of 1890 mJ·cm⁻². The survival curves of larvae, pupae and adult flies among the blank, UV-CK, treated, spiked and PAT-CK samples are shown in Figure 4.3, 4.4 and 4.5. The results showed that survival rate at different development stage among the blank, UV-CK, treated and spiked samples were similar, whereas only larval stage in the PAT-CK samples. The
hatching rates, development rates of pupae and adult flies from UV treated samples were significantly higher (P < 0.05) than the PAT-CK samples (68.5 % versus 21.1 %, 69.2 % versus 0 %, 69.0 % versus 0 %, respectively) and had no significant differences (P > 0.05) with the blank, UV-CK and spiked samples (Table 4.4). The images of larvae, pupae and adult flies at day 6, 10 and 15 are shown in Figure 4.6, 4.7 and 4.8. The length of larvae in treated samples (3.4 mm) were significantly longer (P < 0.05) than those in PAT-CK samples (1.3 mm) but were not significantly different (P > 0.05) from those in UV-CK and spiked samples (Table 4.5).

![Figure 4.3. Daily percentage of survived larva in the blank (PAT= 0 mg·L⁻¹ without UV treatment), UV-CK (PAT = 0 mg·L⁻¹ with UV treatment), treated (PAT = 87.9 mg·L⁻¹ with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples.](image-url)
Figure 4.4. Daily percentage of pupa in the blank (PAT = 0 mg∙L^{-1} without UV treatment), UV-CK (PAT = 0 mg∙L^{-1} with UV treatment), treated (PAT = 87.9 mg∙L^{-1} with UV treatment), spiked (PAT = 87.9 mg∙L^{-1} without UV treatment) and PAT-CK (PAT = 500 mg∙L^{-1} without UV treatment) samples.
Figure 4.5. Daily percentage of adult fly in the blank (PAT = 0 mg·L⁻¹ without UV treatment), UV-CK (PAT = 0 mg·L⁻¹ with UV treatment), treated (PAT = 87.9 mg·L⁻¹ with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples.
Figure 4.6. Pictures of larvae at day 6
Figure 4.7. Pictures of pupae at day 10

A: Blank (PAT = 0 mg·L⁻¹ without UV treatment)
B: UV-Ck (PAT = 0 mg·L⁻¹ with UV treatment)
C: Treated (PAT = 87.9 mg·L⁻¹ with UV treatment)
D: Spiked (PAT = 87.9 mg·L⁻¹ without UV treatment)
E: PAT-Ck (PAT = 500 mg·L⁻¹ without UV treatment)
Figure 4.8. Pictures of adult flies at day 15

A: Blank (PAT = 0 mg·L⁻¹ without UV treatment)
B: UV-CK (PAT = 0 mg·L⁻¹ with UV treatment)
C: Treated (PAT = 87.9 mg·L⁻¹ with UV treatment)
D: Spiked (PAT = 87.9 mg·L⁻¹ without UV treatment)
E: PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment)
Table 4.4. Hatching and development rate at Day 6, 10 and 15 in the blank (PAT = 0 mg·L⁻¹ without UV treatment), UV-CK (PAT= 0 mg·L⁻¹ with UV treatment), treated (PAT = 87.9 mg·L⁻¹ with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Hatching rate at Day 6 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Development rate of pupa at Day 10 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Development rate of adult at Day 15 (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>71.8 ± 13.7&lt;sup&gt; A &lt;/sup&gt;</td>
<td>72.7 ± 14.6&lt;sup&gt; A &lt;/sup&gt;</td>
<td>72.7 ± 14.6&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>UV-CK</td>
<td>67.3 ± 12.4&lt;sup&gt; A &lt;/sup&gt;</td>
<td>68.5 ± 10.9&lt;sup&gt; A &lt;/sup&gt;</td>
<td>68.5 ± 10.9&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>Treated</td>
<td>68.5 ± 14.3&lt;sup&gt; A &lt;/sup&gt;</td>
<td>69.2 ± 14.5&lt;sup&gt; A &lt;/sup&gt;</td>
<td>69.0 ± 14.5&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>Spiked</td>
<td>71.1 ± 15.2&lt;sup&gt; A &lt;/sup&gt;</td>
<td>71.5 ± 15.3&lt;sup&gt; A &lt;/sup&gt;</td>
<td>71.2 ± 15.5&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>PAT-CK</td>
<td>21.1 ± 6.8&lt;sup&gt; B &lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt; B &lt;/sup&gt;</td>
<td>0 ± 0&lt;sup&gt; B &lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are averages ± standard deviations (n = 18). A different letter (A, B) indicates significant differences (P < 0.05) in mean values were observed.

Table 4.5. Length of larva, pupa and adult fly at Day 6, 10 and 15 (mm) in the blank (PAT = 0 mg·L⁻¹ without UV treatment), UV-CK (PAT= 0 mg·L⁻¹ with UV treatment), treated (PAT = 87.9 mg·L⁻¹ with UV treatment), spiked (PAT = 87.9 mg·L⁻¹ without UV treatment) and PAT-CK (PAT = 500 mg·L⁻¹ without UV treatment) samples.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 6 (mm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 10 (mm)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Day 15 (mm)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>3.5 ± 0.2&lt;sup&gt; A &lt;/sup&gt;</td>
<td>3.2 ± 0.2&lt;sup&gt; A &lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>UV-CK</td>
<td>3.4 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
<td>3.3 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
<td>2.5 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>Treated</td>
<td>3.4 ± 0.2&lt;sup&gt; A &lt;/sup&gt;</td>
<td>3.2 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>Spiked</td>
<td>3.4 ± 0.2&lt;sup&gt; A &lt;/sup&gt;</td>
<td>3.2 ± 0.0&lt;sup&gt; A &lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt; A &lt;/sup&gt;</td>
</tr>
<tr>
<td>PAT-CK</td>
<td>1.3 ± 0.1&lt;sup&gt; B &lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are averages ± standard deviations (n = 3). A different letter (A, B) indicates significant differences (P < 0.05) in mean values were observed.
4.4. Discussion

The response of *S. cerevisiae* to exposure to patulin resulted in the growth curve with two stages of inhibition and resumption (Iwahashi *et al.*, 2006; Shao *et al.*, 2012). The duration of the inhibition stage varied depending on the patulin concentration and experimental conditions. Sumbu *et al.* (1983) observed that the synthesis of rRNA, tRNA and probably mRNA were blocked by the action of patulin, which resulted in the inhibition of protein synthesis and cessation in growth of the yeast. Using DNA microarrays technique, Iwahashi *et al.* (2006) explained that patulin inhibits the growth of yeast by activating protein degradation and inducing the repression of protein synthesis. In the current study, the UV treated apple juice which contained 11.7 % of original patulin showed less growth inhibition of the yeast than the patulin control (PAT-CK) sample. The experimental data also indicated no increased toxicities to *S. cerevisiae* resulted from the photoproduts of patulin and other possible UV sensitive compounds in the apple juice.

Most early studies showed none or little reversed effects of patulin in the *D. melanogaster* model system under the given experimental conditions. Reiss (1975) reported there was no larvicidal activity of patulin with the concentrations < 100 mg·L⁻¹ although insecticidal action on *D. melanogaster* was found at these patulin concentrations. Belitsky *et al.* (1985) observed that patulin led to slight but significant increases in the mutation rate at a concentration of $3.2 \times 10^{-3}$ mol·L⁻¹. Paterson *et al.* (1972) reported no significant feeding inhibition of *D. melanogaster* larvae at a patulin concentration of $6.5 \times 10^{-5}$ mol·L⁻¹. In the current study, higher patulin concentrations ( > 100 mg·L⁻¹) were evaluated. 500 mg·L⁻¹ was identified as a patulin level which can influence the survival and metamorphosis of *D. melanogaster*. 
It was difficult to identify and quantify UV photoproducts of patulin. Thus, the toxicity of UV treated apple juice containing patulin rather than only patulin photoproducts was evaluated in this study. Since it was impossible to photo-degrade patulin completely, the treated sample still contained 17.6 % of initially spiked patulin (5000 mg·L⁻¹) under the experimental conditions. Compared to the PAT-CK sample, the higher hatching rate and greater larva length for the UV treated sample strongly indicates that UV irradiation decreases toxicity of patulin contaminated apple juice. Compared to the blank, spiked and UV-CK samples, the similar (insignificant, P > 0.05) effects on hatching and development rate as well as the length of larvae, pupae and flies indicate that no increased toxicities were contributed by the photoproducts of patulin, and possibly other UV sensitive compounds in apple juice.

4.5. Conclusions

In summary, by using both single eukaryotic cell (S. cerevisiae) and metamorphosis insect (D. melanogaster) model systems, the results of current study suggested a reduction of patulin toxicity after UV irradiation with fluences of 2582 and 1890 mJ·cm⁻², respectively. Separation and identification of potential single or multiple UV photoproducts of patulin will be performed in the future work in order to directly evaluate the toxicity of the individual products.
Chapter Five

Inactivation and Patulin Accumulating Ability of *Penicillium expansum* Inoculated into Apple Juice and Irradiated with Ultraviolet Light at 254 nm
Abstract

The present study investigated the inactivation kinetics of *Penicillium expansum* spores in apple juice after exposure to UV-C light at 254 nm and the patulin-producing ability of sub-lethally injured *Penicillium* during post-treatment storage. A 3.1 log reduction in spore numbers was recorded when inoculated into apple juice and irradiated with UV-C at a fluence of 349 mJ·cm$^{-2}$. The inactivation data fitted di-phasic ($R^2 = 0.9782$) and tri-phasic ($R^2 = 0.9885$) inactivation kinetics models. During storage at 4 °C, both UV treated and control samples showed a lag growth phase (10 days) of *P. expansum* and no patulin accumulation. Patulin accumulation was observed after day 17. Compared to the control sample, the UV treated sample presented significantly lower ($P < 0.05$) patulin accumulation (0.12 versus 5.78 mg·L$^{-1}$) and specific activity of patulin accumulation ($4.93 \times 10^{-5}$ versus $1.51 \times 10^{-3}$ ng/CFU). The results suggest that UV-C treatment has advantages of both inactivation of *P. expansum* spores and reduction of patulin accumulation in liquid apple products.
5.1. Introduction

Patulin, a mycotoxin contaminant mainly on the fruits and in the fruit juice, is produced by the genera *Penicillium*, *Aspergillus*, *Byssochlamys* and *Paecilomyces* (IARC, 1986; Moake *et al.*, 2005; Puel *et al.*, 2010). *Penicillium expansum* is well-known as a typical and predominant plant pathogen responsible for the decay of pomaceous fruits and patulin production (Puel *et al.*, 2010; McKinley and Carlton, 1991). As a second metabolite of patulin-producing fungi, patulin levels increase in the stationary phase of mycelial growth because of the depletion of energy sources and the accumulation of sufficient intermediates (Drusch and Ragab, 2003). The biosynthesis of patulin is clearly and well understood with ten steps identified within the biosynthetic pathway (Moake *et al.*, 2005; Puel *et al.*, 2010).

Patulin is commonly encountered in juice prepared from mold spoiled fruit that has been damaged or subjected to high humidity during storage (Drusch and Ragab, 2003; Sant’Ana *et al.*, 2008). The initial damage of fruits can be caused by climatic conditions such as late frost and hail storms (Harris *et al.*, 2009), insect infestation and poor post-harvest handling (Beretta *et al.*, 2000; Piquè *et al.*, 2013; Baert *et al.*, 2006). Once damaged, the fruit becomes susceptible to infection from *P. expansum* that can grow under warm and humid conditions and then subsequently produce patulin (McCallum *et al.*, 2002; Baert *et al.*, 2007; Reddy *et al.*, 2010; Moake *et al.*, 2005).

UV irradiation became an alternative non-thermal control in the processing of beverages due to such advantages as inactivation of spoilage and pathogenic microorganisms without significant losses in nutritional and sensory qualities (Gayán *et al.*, 2013). A successful application of UV
pasteurization of apple cider and apple juice was developed after the outbreaks of illness due to consumption of cider contaminated with pathogens, such as *Escherichia coli* O157:H7 (Vojdani *et al.*, 2008). In addition there is a preference of customers for non-thermally treated juice (Caminiti *et al.*, 2012). In order to thoroughly evaluate the UV treatment, a model used for description of survival of microorganisms is essential. The first-order kinetics model is the simplest log-linear model (Guerrero-Beltrán and Barbosa-Cánovas, 2005; Geveke, 2005; Hijnen *et al.*, 2006). Considering the phenomenon of a shoulder (an initial plateau) and/or a tail that can be observed for UV inactivation, more complicated but accurate models were developed and applied. The models included the log-linear plus tail model (Baysal *et al.*, 2013), the two-phase kinetics model (Ngadi *et al.*, 2003), and the series-event inactivation model (Ye *et al.*, 2007).

Recently, validation and kinetics studies on the patulin degradation during UV irradiation (Dong *et al.*, 2010; Assatarakul *et al.*, 2012; Zhu *et al.*, 2013) provided an opportunity to incorporate patulin reduction and inactivation of patulin-producing mold using the same UV treatment. The objectives of this study were to determine the UV-C inactivation kinetics of *P. expansum* spores in the apple juice, as well as the patulin-production capability of *P. expansum* spores that survive UV treatment.

### 5.2. Materials and Methods

#### 5.2.1. Inoculum preparation

A strain of *P. expansum* was obtained from Agriculture and Agri-food Canada (*P. expansum*-P18-15B), and sub-cultured on plates of potato dextrose agar (PDA, Merck, Kirkland, Canada). The plates were incubated at 25 °C for 2 weeks, and the spores were harvested using sterile
0.1 % w/v of Tween 80 (Sigma, St. Louis., U.S.A) in water. The spore suspension was filtered by sterile cheesecloth and the spore concentration in filtrate was counted by a haemocytometer (Hausser Scientific, Horsham, PA, U.S.A.) and adjusted to a concentration of $5 \times 10^8$ spores/mL with 0.1 % w/v of Tween 80.

5.2.2. UV irradiation

A low-pressure mercury (LPM) UV light reactor (R-52G MINERALIGHT® UV Lamp UVP Inc, CA, USA) was used to deliver a specific dose of UV light at 254 nm to the samples. The samples were treated in a plastic Petri-dish with a diameter of 5.2 cm placed under the lamp. The thickness of juice sample was 2 mm. The incident irradiance was varied by adjusting the distance between samples and the UV lamp. Incident irradiance was measured by a digital ILT1700 radiometer (International Light Technologies, MA, U.S.A.).

5.2.3. Calculation of average UV fluence

The average UV fluence rate ($E_{avg}$) was determined by equation 5.1 (Bolton and Linden, 2003).

$$E_{avg} = E_0 \cdot (PF) \cdot (1 - Re) \cdot \frac{1-10^{-a{l}}}{{a{l}\cdot\ln(10)}} \cdot \frac{l'}{l'+l}$$  (5.1)

where, $E_0$ is the incident irradiance measured by a radiometer. PF, the petri factor is defined as a ratio of the average value of UV fluence rate on the surface of sample in petri dish (or other containers) over to the value at the center. Re is the reflectance, and the value of 0.025 was determined by the average refractive indexes of air (1.000) and water (1.372) in the wavelength between 200 and 300 nm (Bolton and Linden, 2003). $a$ is the absorption coefficient of sample which was measured with a Ultrospec 3100 Pro UV/visible spectrometer (Biochrom Ltd., Cambridge, England). $l'$ is the distance between sample surface and the UV source, $l$ is the
thickness of sample. The average UV fluence is calculated by equation 5.2.

\[ H_{avg} = E_{avg} \cdot t \]  

(5.2)

5.2.4. UV inactivation of *P. expansum*

*P. expansum* spores were spiked into pasteurized apple juice purchased from a local market. The final concentration of spores in apple juice was approximately \(5 \times 10^5\) spores/mL. The samples were irradiated with UV light (254 nm) at an incident irradiance of 5 mW·cm\(^{-2}\) for 0, 2, 4, 6, 8 and 10 min. The treated samples were serially diluted with 0.85 % v/w of saline, and plated on the Rose-Bengal chloramphenicol agar (Oxoid, Nepean, Canada) using the spread plate technique. The colony forming units (CFU) were counted after incubation at 25 °C for 3-4 days.

5.2.5. Modeling of *P. expansum* inactivation

Survival data of *P. expansum* were plotted by plotting the logarithm of the ratio of survival spore concentration (N) to initial spore concentration (\(N_0\)) at sampling points of 0, 2, 4, 6, 8 and 10 min versus the UV fluence (\(H_{avg}\)). The survival curves were obtained by fitting the data to first-order, di-phasic (log-linear plus tail) and tri-phasic (log-linear plus shoulder and tail) kinetics models through a freeware tool of GInaFit (Geeraerd et al., 2005).

In detail, the first-order kinetics model was expressed with equation 5.3.

\[ N = N_0 \cdot e^{-k_{max} \cdot H_{avg}} \]  

(5.3)

Where, \(N\) is the spore concentration during UV irradiation (CFU·mL\(^{-1}\)) and \(N_0\) is the initial spore concentration (CFU·mL\(^{-1}\)). \(k_{max}\) is the inactivation rate constant (mW\(^{-1}\)·cm\(^2\)·s\(^{-1}\)). \(H_{avg}\) is the UV fluence (mJ·cm\(^{-2}\)).
The di-phasic kinetics model was expressed with equation 5.4.

\[ N = (N_0 - N_{res}) \cdot e^{-k_{max} \cdot H_{avg}} + N_{res} \]  \hspace{1cm} (5.4)  

Where, \( N_{res} \) is the residual spore concentration (CFU·mL\(^{-1}\)). \( k_{max} \) is the inactivation rate constant of the log-linear part of the curve (mW\(^{-1}\)·cm\(^2\)·s\(^{-1}\)).

The tri-phasic kinetics model was expressed with equation 5.5.

\[ N = (N_0 - N_{res}) \cdot e^{-k_{max} \cdot H_{avg}} \cdot \frac{e^{-k_{max} \cdot S_I}}{1 + (e^{-k_{max} \cdot S_I - 1}) e^{-k_{max} \cdot H_{avg}}} + N_{res} \]  \hspace{1cm} (5.5)  

Where, \( S_I \) is the shoulder length (mJ·cm\(^{-2}\)) estimated by the GInaFit.

### 5.2.6. Indirect impedance assay

The growth of \textit{P. expansum} was monitored with a BacTrac 4300 microbiological analyzer (SY-LAB, Neupurkersdorf, Austria) based on the change in impedance in the solution. The growth and proliferation of aerobic microorganisms form carbon dioxide as the final product in cell metabolism. The carbon dioxide is absorbed by a potassium hydroxide solution and forms potassium carbonate which increases the impedance of the solution. In this study, the apple juice samples were filtered with a 0.22 µm filter to remove bacteria and fungi before UV treatment, and then spiked with the pore suspension to form a final concentration of \( 5 \times 10^6 \) spores/mL. The samples were treated with UV light for 10 min (335.4 mJ·cm\(^{-2}\)). 4 mL of apple juice samples before and after UV irradiation were added to sterile sample vessels which were placed to measuring cells containing 1 mL of 0.2 % potassium hydroxide solution. The samples were incubated in BacTrac 4300 microbiological analyzer at 25 °C for 72 h. The M-value (media impedance) was monitored and recorded every 20 min.
5.2.7. Spore germination after UV irradiation

The apple juice samples were spiked with *P. expansum* spores to reach a final concentration of $5 \times 10^6$ spores/mL. The samples were treated with UV light for 10 min (335 mJ·cm$^{-2}$). 20 µL of samples before and after UV irradiation were spread on small disks of water agar (20g agar in 1L distilled water) which were cut from water agar plates with a sterile cork borer of 11 mm diameter. Samples were incubated at room temperature. Spores and germ tubes were observed with a microscope (Carl Zeiss Microscopy, Thornwood, NY, U.S.A.) at 0, 6 and 18 h. The 200-fold magnification was obtained by choosing a 10 × eyepiece and a 20 × objective. Photographs were taken with a camera system of GCCD 1350 EX Model B (Qimaging, Surrey, BC, Canada).

5.2.8. Challenge testing and patulin accumulation

The apple juice samples spiked with *P. expansum* spores (final concentration of $5 \times 10^5$ spores/mL) were treated with UV light for 10 min (349 mJ·cm$^{-2}$). The samples before and after UV treatment were stored at 4 ºC aerobically for day 0, 3, 6, 10, 17 and 30 days. The treated samples were made serially diluted with 0.85 % v/w of saline and plated on the Rose-Bengal chloramphenicol agar (Oxoid, Nepean, Canada) using the spread plate technique. The colony forming units (CFU) were counted after incubation at 25 ºC for 3-4 days. Simultaneously, the samples were tested for patulin concentration by HPLC.

5.2.9. HPLC analysis of patulin

Samples were extracted using solid phase extraction as described by Eisele and Gibson (2003). Oasis HLB extraction cartridges (3 mL/60 mg, Waters, MA) were conditioned by passing 2 mL of water, 2mL of methanol and 2 mL of water at a flow rate of approximately 0.1 ml·s$^{-1}$. The 0.5
mL sample was diluted to 2.5 ml with 0.1 % (v/v) acetic acid. The diluted sample (2.5 mL) was applied to the column followed by 2 mL of a 1.0 % (w/v) sodium bicarbonate solution then 2 mL of 1.0 % (v/v) acetic acid. The patulin was then eluted using 1.0 mL of ethyl acetate, and the solution was evaporated to dryness under a stream of nitrogen. The residue was then dissolved in 0.5 mL 0.1 % (v/v) acetic acid prior to analysis. The standard patulin solutions of 0.01, 0.05, 0.1, 0.2, 0.5, and 1 mg·L⁻¹ were prepared and extracted using the same method described above to form the linear working curve (0.01 to 1 mg·L⁻¹). Samples and standards were filtered with syringe filters of 0.45 µm pore size and then were analyzed using a HPLC system (Agilent Technology 1200 Series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, and a diode array detector (DAD) set at 276 nm. A Phenomenex Luna® 3 µ C18 column (250 x 2.0 mm) with a C18 guard column (Torrance, CA) was used for the separation. 10 µL of samples and standards were eluted isocratically using 0.8 % (v/v) tetrahydrofuran in water at a flow rate of 0.2 mL·min⁻¹ with run time of 25 min. The retention time of patulin was approximately 20 min. 5-hydroxymethylfurfural (HMF), a compound that interferes with patulin detection, eluted from the column at approximately 17.5 min, which ensured baseline separation of these two compounds. The patulin detection limit in this system was approximate 0.01 mg·L⁻¹. Control samples were analyzed to conform that samples before UV treatment were patulin-free. All samples and standards were analyzed in triplicate.

5.2.10. Statistical analysis

All UV processing experiments were performed in triplicate with a completely independent and randomized design. The statistical analyses of experimental data were carried out by SPSS version 20 (IBM, Armonk, NY). Statistical significance of concentrations of P. expansum spores
and patulin levels in control and UV treated samples, was evaluated through a paired T-test. All 
P. expansum inactivation rate constants were calculated from regression plots based on the tool of GInaFit (Geeraerd et al., 2005). The coefficients of determination R² were calculated.

5.3. Results

5.3.1. UV inactivation of P. expansum

UV irradiation of apple juice at a wavelength of 254 nm resulted in a significant reduction (P < 0.05) in P. expansum spores compared to non-treated controls. The reduction of population of P. expansum spores depended on the increase of UV fluence. A UV fluence of 349 mJ cm⁻² led to a 3.1 log reduction in the spore concentration in spiked apple juice. The experimental data were fitted to first-order, di-phasic and tri-phasic kinetics models (Geeraerd et al., 2005) based on equations 5.3, 5.4 and 5.5, respectively. Survival curves were regressed by GInaFit program and shown in Figure 5.1. Three models were compared statistically based on the experimental data points (Table 5.1). The first-order kinetics model was not suitable for the experimental data due to a lower coefficient of determination (R² = 0.9008). Both di-phasic and tri-phasic kinetics models had a high degree of fit with the survival data for P. expansum spores although the latter showed a better degree of fit (R² = 0.9782 and 0.9885, respectively). The inactivation rate constants of log-linear part of the curves (k_max) were 0.0334 and 0.0514 mW⁻¹ cm² s⁻¹ in di-phasic and tri-phasic kinetics models, respectively.
Figure 5.1. Survival data of *P. expansum* spores in apple juices after UV irradiation fitted first-order, di-phasic (log-linear plus tail) and tri-phasic (log-linear plus shoulder and tail) kinetics models.
Table 5.1. Model parameters and statistical indices for UV-C inactivation kinetics of *P. expansum* spores in apple juices

<table>
<thead>
<tr>
<th>Model</th>
<th>$k_{\text{max}}$ $^a$</th>
<th>log(N$_{\text{res}}$) $^b$</th>
<th>SI $^c$</th>
<th>RMSE $^d$</th>
<th>$R^2$ $^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>First-order</td>
<td>$0.021 \pm 0.004,^f$</td>
<td>N/A</td>
<td>N/A</td>
<td>0.4489</td>
<td>0.9008</td>
</tr>
<tr>
<td>Di-phasic</td>
<td>$0.033 \pm 0.005$</td>
<td>$2.65 \pm 0.19$</td>
<td>N/A</td>
<td>0.2428</td>
<td>0.9782</td>
</tr>
<tr>
<td>Tri-phasic</td>
<td>$0.051 \pm 0.013$</td>
<td>$2.76 \pm 0.14$</td>
<td>$45.7 \pm 22.3$</td>
<td>0.2165</td>
<td>0.9885</td>
</tr>
</tbody>
</table>

$^a$ Inactivation rate constant of log-linear part of the curve (mW$^{-1}\cdot$cm$^2\cdot$s$^{-1}$)

$^b$ Logarithm of residual spore concentration (log CFU$\cdot$mL$^{-1}$).

$^c$ Shoulder length (mJ$\cdot$cm$^2$)

$^d$ Root mean sum of squared error

$^e$ R-square

$^f$ Values are mean $\pm$ standard error

### 5.3.2. Indirect impedance assay

In the indirect impedance assay, there was a negative correlation between the M-value and growth of *P. expansum*. The decrease of M-value indicates the growth of tested microorganisms.

In the current study, the curves plotted for the M-value versus incubation time of apple juice samples before and after UV treatment (10 min, 335.4 mJ$\cdot$cm$^2$) were compared (Figure 5.2). The times that were needed to reach the threshold M-value (25 %) were $19.7 \pm 1.9$ h in control samples without UV irradiation, and $46.2 \pm 3.5$ h in samples after UV treatment, which revealed a significant longer (P < 0.05) lag phase of *P. expansum* in UV treated samples due to the recovery of spores caused by the UV treatment.
Figure 5.2. Growth curves of *P. expansum* in apple juice at 25 °C presented by M-value using indirect impedance assay

- Growth curve of control sample without UV treatment
- Growth curve of UV treated sample (UV fluence: 335.4 mJ·cm\(^{-2}\))

5.3.3. Spore germination after UV irradiation

The morphology of spore and germ tubes during the germination is presented in Figure 5.3. At 4 h of incubation, the spores from control samples germinated with mycelia length in the range of 3 to 5 µm (Figure 5.3C). No mycelia were found in the microscopic field in the samples after UV irradiation (Figure 5.3D) because of the low density of residual spores. The mycelia of control samples grew rapidly and most of them reached 20 ~ 40 µm length in 18 h, whereas only occasional germinations were found in the samples after UV irradiation (Figure 5.3E, F).
Figure 5.3. Germination of *P. expansum* spores in apple juice

A. Sample: before UV treatment; Germination time: 0 h

B. Sample: after UV treatment (335.4 mJ·cm⁻²); Germination time: 0 h

C. Sample: before UV treatment; Germination time: 6 h

D. Sample: after UV treatment (335.4 mJ·cm⁻²); Germination time: 6 h

E. Sample: before UV treatment; Germination time: 18 h

F. Sample: after UV treatment (335.4 mJ·cm⁻²); Germination time: 18 h

5.3.4. Challenge testing

The growth curves for *P. expansum* in UV treated (10 min, 348.7 mJ·cm⁻²) apple juice sample at 4 °C were compared to the control sample without UV irradiation (Figure 5.4). Both UV treated and control samples presented either a significant decrease (P < 0.05) or no significant change (P > 0.05) in spore concentration in the first 10 days of the lag period. There was a significant increase (2.36 log CFU·mL⁻¹, P < 0.05) in *P. expansum* levels in UV treated samples between days 10 and 17. Then the populations increased to 6.40 log CFU·mL⁻¹ at day 30 (Figure 5.4). The growth of *P. expansum* spores in the UV treated sample at 4 °C was suppressed during the first 10 days, then increased thereafter.

5.3.5. Patulin accumulation

Patulin was not detected (< 0.01 mg·L⁻¹) in un-spiked apple juice used for these studies. During incubation at 4 °C, no patulin was produced during the first 10 days in UV treated and control samples spiked initially with spores. The production and accumulation of patulin were observed
from day 17 onward. The UV treated sample showed a significant lower (P < 0.05) patulin concentration (0.12 mg·L⁻¹) than the control sample (5.78 mg·L⁻¹) at day 30. The UV treated sample took 30 days to reach 0.05 mg·L⁻¹, a widely recognized patulin tolerance in apple cider and juice, whereas 17 days for the control sample without UV irradiation. The specific activity of patulin accumulation is defined as a ratio of the patulin concentration to the \( P. \) expansum spore concentration. The value is used to evaluate the patulin accumulation ability per unit of \( P. \) expansum (Figure 5.4). It clearly indicates a significantly lower (P < 0.05) specific activity of \( P. \) expansum in UV treated samples (4.93 \( \times \) 10⁻⁵ ng/CFU) than the control samples (1.51 \( \times \) 10⁻³ ng/CFU) at the day 30 of storage.
Figure 5.4. Growth of *P. expansum* and specific activity of patulin accumulation in apple juice during the storage at 4 °C.

- : Growth curve of *P. expansum* without UV treatment (control sample)
- : Growth curve of UV treated *P. expansum* (UV fluence: 349 mJ·cm⁻²)
- : Specific activity of patulin accumulation of *P. expansum* without UV treatment (control sample)
- : Specific activity of patulin accumulation of UV treated *P. expansum* (UV fluence: 348.7 mJ·cm⁻²)
5.4. Discussion

UV treatments can serve multiple functions such as reduction of patulin and inactivation of foodborne pathogens, spoilage microorganisms and patulin-producing fungi. These functions would be beneficial for improving safety level of liquid apple products. The UV fluence is an essential parameter to evaluate the efficiency of UV irradiation for the multiple objectives. The current study identified that a UV fluence of 349 mJ·cm⁻² can result in a 3.1 log reduction in the concentration of *P. expansum* spores spiked into apple juice. This UV fluence is sufficient to inactivate 5-log of *E.coli* O157:H7, a main pathogen in apple cider and juice, according to reported studies. For example, Wright et al. (2000) reported a 5.4 log reduction of mixture of five *E.coli* O157:H7 strains in apple cider after UV treatment by thin film UV disinfection unit at fluence of 61 mJ·cm⁻². Treatment with the CiderSure 3500 with a UV fluence of 14 mJ·cm⁻², 5.93-6.65 log reductions of three *E.coli* O157:H7 strains were observed in apple cider prepared from eight apple cultivars (Basaran et al., 2004). In another study, a higher UV fluence of 6.5 mW·min·cm⁻² (or 390 mJ·cm⁻²) obtained with a collimated UV lamp inactivated more than five logs of *E.coli* O157:H7 in apple juice (Ngadi et al., 2003). For patulin photo-degradation, Assatarakul et al. (2012) reported 94.9 % of patulin in apple juice was eliminated by UV exposure with fluence of 99.4 mJ·cm⁻². A similar UV fluence of 84.2 mJ·cm⁻² caused 90 % of patulin degradation in apple juice (Zhu et al., 2014). A higher UV fluence should be applied for inactivation of fungal spores than bacteria as they are more resistant to the UV exposure (Kowalski, 2009). Thus, the fluence leading to a 3.1 log reduction of *P. expansum* spore is sufficient to reduce five logs of *E.coli* O157:H7 and 90 % of patulin in apple juice in a single treatment.
The major adverse effect of UV-C radiation on microorganisms is direct damage DNA through crosslinking of neighbouring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand. The mutation blocks DNA transcription and replication and finally leads to cell death (Bolton and Linden, 2003; Jin et al., 2006; Sastry et al., 2000). The relative high UV resistance of P. expansum spores in this study can be explained by the presence of the pigment related to 1,4-naphthaquinone, which may play a role in protecting the fungal DNA from UV damage (Lennox and Tuveson, 1967; Asthana and Tuveson, 1992).

Modeling the inactivation of P. expansum spores in apple juice helps in our understanding of its responses to effects of UV irradiation. The experimental data fit non-log-linear models (di-phasic and tri-phsic) with sigmoidal survival curves. The data matched some earlier findings of sigmoidal survival curves which were obtained from the UV-C treatment of fungal spores such as Rhizopus suinus, Mucor disperses, Glomerella spp, Aspergillus nigar (Zahl et al., 1939; Dimond and Duggar, 1941; Markert, 1953). The short initial plateau (shoulder) of 45.7 mJ·cm⁻² was observed due to an injury phase of the microorganisms in response to UV irradiation (Sastry et al., 2000). Because of the lower UV fluence in this phase, the probability of accumulation of lethal hits to an individual cell is low. Along with the increase of UV fluence, more and more cells received sufficient hits and were inactivated, which showed a steeper decrease in the survival curve (Ye et al., 2007). Tailings were found in both non-log-linear models. The phase with a long plateau represented a small part of UV resistant spores among the sensitive population (Markert, 1953; Sastry et al., 2000; Izquier and Gómez-López, 2011). The tail can be described by the logarithm of the residual spore concentration (logN_{res}). In the di-phasic and tri-phasic models, logN_{res} is a constant which indicates no further inactivation of spores although the
UV fluence is increased. Thus, the models could help to determine maximum UV treatment needed to inactivate spores.

The indirect impedance assay was adopted to evaluate the proliferation of *P. expansum* spores. Unlike the traditional plate technique, the impedance assay is a dynamic process which monitors not only the number of proliferation-capable cells but also their metabolic activities. Moreover, directly using the liquid food matrix as the media in this analysis provides a real growth situation of tested microorganisms in juice. In this study, the longer detection time of UV treated samples than control samples reflected the longer lag phase due to the lower initial spore concentration caused by UV irradiation. In the germination testing, most spores in UV treated samples lost the ability to germinate due to the lethal damage by UV and kept the original size when the spores in control samples germinated.

Due to the phenomenon of tailing in the survival curve of UV treated *P. expansum* spores, it was not possible to completely inactivate the patulin-producing mold if the initial contamination level is high or the UV fluence is not sufficient. The current study used UV treated samples with spores of a concentration of 2.58 log CFU·mL⁻¹ to evaluate the growth and patulin-producing ability during storage at 4 °C. A correlation between *P. expansum* growth and patulin accumulation was found that the concentration of patulin reached the highest level in samples with a high population of *P. expansum* and stationary growth phase. It can be explained that patulin, as a secondary metabolite of patulin-producing molds, can be biosynthesized when the energy source in the medium is nearly depleted and when enough intermediates are accumulated (Hasan, 2000; Grootwassink and Gaucher, 1980). The lower specific activity of patulin
accumulation of *P. expansum* after UV treatment indicated that the spores that survived may contain mutants induced by UV irradiation and the mutations may alter the metabolic pathway of patulin production. Besides, the higher initial concentration of *P. expansum* spores in control the culture entered the stationary phase sooner compared to that derived from UV irradiated samples. The finding of significant lower patulin accumulation in the apple juice after UV irradiation, suggests that UV treatment has advantages of both inactivation of *P. expansum* spores and reduction of patulin production held under appropriate storage condition.

5.5. Conclusions

In conclusion, UV-C (254 nm) irradiation (349 mJ·cm⁻²) supported a 3 log cfu reduction of *P. expansum* spores in apple juice. The UV inactivation followed di-phasic and tri-phasic kinetics models. Those spores that survived UV treatment and subsequently germinated during the storage appeared to have a reduced capacity to accumulate patulin.
Chapter Six

Conclusions and Future Studies
6.1. Conclusions

The study was set out to explore the kinetics modeling of patulin degradation in apple cider and apple juice treated with UV (254 nm) irradiation, the relationships between patulin degradation rate and interior (absorption coefficient of media) and exterior (UV fluence rate and sample thickness) factors, the efficacy of patulin degradation and the effects on sensory quality of apple juice treated with different wavelengths (222 nm and 282 nm) in the UV-C range, and the reduction of patulin toxicities after UV exposure using D. melanogaster and S. cerevisiae model systems. The study has also sought to investigate the inactivation model of patulin-producing fungi (P. expansum) under UV exposure and the growth and patulin-producing ability of survived organisms.

The current study modeled the kinetics of patulin photo-degradation by UV light with various wavelengths (222 nm, 254 nm and 282 nm) in apple cider and apple juice. The fluence-based first-order reaction rate constants (FFRCs) calculated by the experimental data and mathematical models were used to evaluate the efficacy of UV sources, which may provide strong supports for the development of novel UV systems. In addition to determining the decrease of patulin concentration caused by treatment UV, the reduction in patulin toxicity was also confirmed using model systems of D. melanogaster and S. cerevisiae. Moreover, the research evaluated the behaviors of patulin-producing mold (P. expansum) when exposed to UV light, providing a potential strategy to simultaneously incorporate pathogen inactivation, patulin reduction and patulin-producing mold inactivation using UV treatment.
The main findings of this study are summarized below.

- Monochromic UV radiation at 254 nm was an effective physical method for reducing patulin levels in liquid apple products.
- The kinetics of patulin degradation followed a first-order reaction model. The fluence-based first-order constant \( k_f \) was determined for prediction of patulin degradation.
- The time-based reaction rate constants of samples treated in a dynamic regime were significantly higher than samples treated in a static regime when applied incident UV fluence rate and sample thickness were kept constant.
- The patulin degradation reaction rate constants in apple juice and apple cider were significantly higher than that in a model solution. The addition of ascorbic acid in juice resulted in a dramatic increase in the reaction rate constant.
- The rate of patulin photodegradation is wavelength dependent. Among UV lamps with wavelengths of 222, 254, and 282 nm, the order of efficiency was as follows: far UVC (222 nm) > far UVC plus (282 nm) > UVC (254 nm).
- The far UVC lamp (222 nm) was the most effective UV source due to the highest performance of the photon energy at this wavelength.
- UV exposure at 222 nm with fluence sufficient to degrade 90% of patulin caused no significant changes in pH, total soluble solids, and did not impact on the overall sensory characteristics in apple juice.
- In the mycotoxin sensitivity evaluations, 500 mg·L⁻¹ of patulin caused a larvicidal effect on *D. melanogaster* as well as 50 mg·L⁻¹ of patulin resulted in a growth inhibition of *S. cerevisiae*.
- The reduction of patulin toxicities in apple juice after UV irradiation at 254 nm (UV fluence
of 2582 mJ·cm$^{-2}$ in *S. cerevisiae* model system and 1890 mJ·cm$^{-2}$ in *D. melanogaster* model system) were determined by comparing cell concentration during the growth in *S. cerevisiae* model system and by comparing hatching rates, development rates of pupae, development rates of adult flies and size of larvae in *D. melanogaster* model system.

- UV-C (254 nm) irradiation was proved to effectively inactivate the *P. expansum* spores in apple juice. The inactivation followed the di-phasic (log-linear plus tail) and tri-phasic (log-linear plus shoulder and tail) kinetics models.

- The *P. expansum* spores that survived UV treatment and subsequently germinated during the storage appeared to have a reduced capacity to accumulate patulin.

### 6.2. Future studies

In order to investigate the validation and kinetics of patulin photo degradation and *P. expansum* inactivation, lab-scale UV reactors were used in this study. However, the commercial application of UV irradiation requires more complicated UV reactors with continuous working mode (eg. CiderSure 3500). The findings in this study will be examined in the plant-scale UV system. Moreover, the combination of UV and other technique such as ultrasound may also be studied to improve the efficiency of patulin reduction.

It was difficult to extract and identify the UV photoproduct(s) of patulin using HPLC. The effort can be made continually by using analysis methods with GC-MS and LC-MS. The first step will focus on the simple solution of patulin in water. If the photoproduct(s) was identified, the media of model solution and apple juice will be studied.
Toxicological study of patulin after UV treatment can be performed deeply using human cell lines and experimental animals such as rats or pigs. In addition to the lethal effects, non-lethal effects such as weight loss, gastric and intestinal changes and renal malfunction will be observed to reflect the possible reverse effects of patulin photoproduce(s) at lower concentration. If the photoproduce(s) is identified, the toxic level ($LD_{50}$) compared to patulin will be examined.
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