Photocatalytic disinfection of patulin using titania in apple juice

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DECLARATION

I, Morelle Merlina Ngandjou Douanla declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology and the North-West University.

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ABSTRACT

The food industry is facing many challenges to provide safe food free from microbial contamination and especially free from mycotoxins, which seems to bypass the pasteurisation treatment frequently used for microbial deactivation in industry. Mycotoxins are secondary metabolites produced by numerous microorganisms. Among mycotoxins, patulin is well known to affect apples and is therefore found in apple juice and apple cider. Patulin is in fact a mycotoxin produced by *Penicillium expansum*. It is toxic; hence, there is a need to remediate the toxin from juices. After the ingestion of patulin, gastrointestinal symptoms such as diarrhoea, vomiting, nausea and may ensue leading to death.

In this study, different method of patulin treatment was elaborated on but the emphasis was on photocatalysis. The purpose of this thesis was to treat patulin in apple juice by the means of photocatalysis. Photocatalysis requires nanoparticles and light. The choice of nanoparticles was (TiO_2) which were synthesized from titanium chloride ($TiCl_4$) in different solubilising matrices using a wet chemical method. The quantification of patulin from apple juice was measured using the LC/MS instrument. As a result, the best TiO_2 nanoparticles derived from $TiCl_4$ dissolved in water. Photocatalysis experiment was done in 2 different conditions under different Ultraviolet (UV) light intensity of 15V and 30 V. The results shown that UV 30 provided the greater percentage degradation as compared to UV 15 demonstrating that, the efficiency of photocatalysis depends on the light intensity. Overall, the patulin level, were reduced to below 10 ng/L within 180 min of treatment, with the juice adhering to the internal quality standard of patulin in apple juices. In conclusion, photocatalysis was determined as an efficient treatment for patulin degradation in apple juice. This is therefore, a cheap and easy method of patulin treatment for small-scale juice producers.

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DEDICATION

To my parents

Mr Simeon Ngandjou and Jeannette Ngoualem Ngandjou

And to

All men and women who believe in ladies' empowerment through education.

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GLOSSARY

Abbreviation	Definition
DLLME	Dispersive liquid-liquid microextraction
DNA	Deoxyribose nucleic acid
EDS	Energy dispersive X-ray spectroscopy
FTIR	Fourier transmittance infrared
GC	Gas chromatography
HMF	5- hydroxymethyl-2-furfural
HPLC	High performance liquid chromatography
LC/MS	Liquid chromatography / massspectroscopy
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
NP	Nanoparticles
QuEchERS	Quick easy cheap effective rugged safe
SPE	Transmission electron microscopy
TEM	Transmission electron microscopy
TLC	Thin layer chromatography
EU	European Union
WHO	World Health Organisation
XRD	X-ray diffraction
CVD	Chemical vapour decomposition

OUTLINE OF THE THESIS

The research presented in this thesis was conducted in the BioERG laboratory based on experimental studies. BioERG is located at the Faculty of Applied Sciences, Cape Peninsula University of Technology, Cape Town, South Africa in Collaboration with the Cape Town Cold drink Company Ltd (Pty), South Africa. This thesis was structured in 6 chapters, as follows:

- CHAPTER 1: Introduction and motivation

A general background on Patulin, and an understanding of its origins and health effect in humans is discussed. It also provides the main objectives achieve in this thesis as well as the questions raised to develop the research.

- CHAPTER 2: Literature review

In this chapter, a literature review on patulin with the different method for its quantification and detection are discussed as well as its treatment methods in apple juice using photocatalysis approach and a synthesized TiO_2as nanomaterial.

- CHAPTER 3: Effect of solubilizing matrices for $TiCl_4$ on the formation of TiO_2 nanoparticles

The synthesis of TiO_2 was conducted through wet chemical method, which is mainly discussed in this chapter. The focus of this chapter is to investigate the best solubilizing matrices between water, methylene chlorine and toluene for TiO_2 synthesis, which was chosen for photocatalytic experimental.

- CHAPTER 4: Photocatalytic application of nanoparticles TiO_2 synthesised from $Ticl_4$ in the degradation of Patulin in water matrices and apple juice

This chapter investigates the effectiveness of photocatalysis in the treatment of patulin from water matrice and apple juice.

- CHAPTER 5: General discussion, conclusions and recommendations

This chapter provides a general discussion, conclusion and recommendations for future studies.

- The references section, provides a list of bibliographical references consulted for the study.

CHAPTER 1

BACKGROUND AND MOTIVATION

CHAPTER 1

INTRODUCTION

1.1 Background and motivation

Patulin is a secondary metabolite produced by moulds such as *Penicillium expansum*. This psychrophilic blue mould is responsible for fruit spoilage on apples and pears including patulin contamination as it is produced as a by-product. Overall, the remediation of patulin is a great challenge for the fruit juice industry. Several researches have conducted studies on patulin detection, quantification and on other properties such as its volatility. About 80% - 90% of patulin in juice is quantified by HPLC combined with UV detection at 276 ηm (Lin et al., 2014).

Several methods around the treatment of patulin have been recently reported. Regal et al. (2017) has reported the efficiency of a photodegradation process using UV/visible light to inhibit microbial growth of patulin producers and to reduce the patulin toxin in the juice for a reaction, which was described by first order kinetics. Overall, the pasteurisation process on its own does not have any significant effect on patulin reduction but a combination of SO_4 and heat can reduce the level of patulin in apple juice. In addition, the heating process does not affect neither the quality nor the physical properties of the juices as well as their sensory properties (Tabatabaei Yazdiet al., 2010), with evaporation having a significant effect in the clarity of apple juice as compared to heat treatment (Kadakal et al., 2003).

Currently, photocatalysis is one of the alternative, innovative and promising techniques for degradation of mycotoxins in food matrices. Photocatalysis is energy dependant (Mayer et al., 2019). It is more efficient in a batch system compared to a continuous system (Rodriguez-Gonzalez et al., 2019); albeit, a continuous system will greatly improve the productivity of the system being used. Photocatalysis involves both UV/visible light and nanoparticles. Nanoparticles are generally used for the treatment of innocuous compounds in wastewater treatment, dye degradation, bacterial deactivation and in the pharmaceutical industry for production of toothpaste, cosmetic products including in the production of paints. Several nanoparticles (Cu_2O, SnO, ZnO) have been intensively studied and applied in wastewater treatment research, but TiO_2 has gained popularity because of its stability, affordability, large band gap, recyclability and its efficiency in photocatalysis (Athanasekouet al. , 2018).

1.2 Research questions

The gaps identified above raised the following questions:

- What is the appropriate method and technique for patulin quantification in apple juice?
- What is the best solubilising material for TiO_2 synthesis from $TiCl_4$?
- What is the impact of photocatalysis in patulin treatment in an apple juice using TiO_2 from $TiCl_4$?

1.3 General objectives

General aim: This study is contributing to human health protection by treating patulin from apple juice.

Objective1: To screen the level of patulin in commercial apple juice produced from the Cape Town cold drink company.

Activity 1: To extract patulin from apple juice using solid phase extraction (SPE) and liquid-liquid microextraction and to develop rapid and valid HPLC MS MS method for patulin detection and quantification in apple juice. The best extraction method was selected and reported herein.

Activity 2: To compare the results obtained with the acceptable national and international level of patulin in juices from the association of analytical chemists (AOAC) and food and drugs Administration (FDA).

Objective 2: To remove patulin from apple juice by UV photocatalysis using TiO_2. This objective was performed in two parts.

Part A: To synthesize TiO_2 using wet chemical method.

Activity 1: To synthesise TiO_2 from different $TiCl_4$ solutions in different matrices such as water, toluene, methylene chloride in order to find the best solvent for the $TiCl_4$ salt.

Activity 2: To characterise TiO_2 nanoparticles and select of the best TiO_2 which is going to be coated on the surface of a solid glass for patulin removal in apple juice.

Part B: To apply TiO_2 photocatalysis using UV light for the degradation of patulin.

Activity 1: To design and built a single flatbed reactor for patulin degradation in apple juice.

Activity 2: To assess the effectiveness of the flat bed reactor under the variation of the energy level of light at a laboratory scale.

CHAPTER 2

LITERATURE REVIEW

PHOTOCATALYSIS: AN APPROACH FOR PATULIN DEGRADATION IN APPLE JUICE FOR SMALL-SCALE JUICE PRODUCERS

CHAPTER 2

LITERATURE REVIEW

Photocatalysis: An approach for patulin degradation in apple juice for small-scale juice producers

General overview of the article

This chapter provides an understanding on Patulin, it origins, toxicology effect in human, the regulations and quantification using different analytical instruments. This chapter also provides a brief discussion on TiO_2 nanoparticles as good a catalyst in photocatalysis. Different methods for patulin treatments are also discussed with the emphasis on photocatalysis which is the main purpose of this study.

Photocatalysis: An approach for patulin degradation in apple juice for small-scale juice producers

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2.1 Abstract

This literature review explores photocatalysis as a promising approach for patulin degradation in apple juice. Mycotoxins are secondary metabolites produced by microbial growth, specifically soil microorganisms, during postharvest under specific and favourable environmental conditions. Patulin is a mycotoxin produced by fungi in a variety of perishable, and semi perishable foods. However, the predominant source of patulin production is found in apple juice and apple cider. There are side effects of patulin in humans and animals, which are related to both gastro intestinal clinical outcomes depending on the level of ingestion. Hence, regulations, proper farming methods and efficient treatments need to be developed. Therefore, this literature review present different methods for patulin degradation with an emphasising on photocatalysis as an efficient treatment method for patulin degradation in apple juice.

Keywords: *Apple Juice, Nanomaterials, Patulin, Photocatalysis,* TiO_2

2.1 Introduction

Food spoilages are generally a results of microbial growth in food. In fruits, vegetable and dairy products, fungi are the main spoilage agents (Garnier *et al.* , 2017,Samuel *et al.*,2015, Udoh *et al.* , 2015). In fact, under appropriated conditions, several fungus species produce some metabolites with the evolution of the specie producing secondary metaboltes under specific ecological conditions (Oroian *et al.* , 2014). There exists a variety of useful secondary metabolites such as: Strobilurin (antifungal), Gibberellins (growth Hormones), Herbicides (control weeds), Insecticides (control insects), Enzymes (proteins), Pigments (dyes), Antibiotics (drugs), Pharmacological drugs, and Mycotoxins which are poisonous (Gallo *et al.* , 2015, Stoev, 2015). However, one of the most harmful mycotoxins produced by fungal species in fruit is called patulin (Li *et al.* , 2017, Diao *et al.* , 2019).

Patulin is a mycotoxin produced by fungus species such as *Penicillium sp.*, *Aspergillus sp.* and *Byssachlamys sp*. These fungal species are responsible for high level of patulin production and are responsible for the high level of losses in term of fruits spoilage (Artigot *et al.* , 2009). Patulin appears in blue molds growing either at the surface or in the middle part of apples (Oroian *et al.* , 2014). It has also been identified in oranges, peaches, mangoes, plumbs and lemons, including figs up to a concentration of 87,6 µg/kg (Ji *et al.* , 2017). After ingestion of patulin, symptoms could include vomiting, diarrhea and nausea. Patulin could also cause tetragenic and carcinogenic diseases by affecting the nucleic material of human and animal cells. These diseases caused by patulin in humans and animals have lead countries and regulatory bodies such as WHO (World Health Organisation) to agree on a 50 µg/kg concentration limit of patulin in juices for adult and 10 µg/kg for infants (Ji *et al.* , 2017). Although patulin has been found in a variety of food, studies on patulin has been more focused on apple products such as apple juice, including apple ciders as the most ingested beverages in the world (Li *et al.* , 2017, Zhong *et al.* , 2018).

Several methods can be applied to remove patulin from juice. These methods include thermal and non-thermal methods. Avsaroglu *et al.* , (2015) reported pulsed-high hydrostatic pressure (Phhp) as a promising method for patulin reduction in clear apple juice. Although, also effective for the degradation of pathogenic microorganisms including patulin producers' and related pathogens, the method is only effective in apple juice containing very low patulin concentrations and the results from this method does not follow a regular pattern. Ozone has been found to be effective for patulin degradation but the treatment is limited by the fact that it decreases the level of nutrients in the juices with a reduction in the malic acid, phenolic acid and ascorbic acid being observed (Diao *et al.* , 2018).

The degradation of patulin in apple juice using UV light is feasible and requires a higher light intensity as compared to the light intensity required to reduce 90 % of pathogen in the humans' body. However, to achieve a successful patulin degradation using UV treatment without affecting the nutritional value of the juice, factors such as clarity of the juice, phenolic compounds and accelerating agents must be known. Accelerating agents could be biological compounds such as fructose (Tikekar *et al.* , 2014) or chemical compounds acting as catalysts. Catalysts are often used to speed up chemical reactions and one of the processes in which catalysts have been widely used in the past decade, is photocatalysis. This process required the presence of light including catalysts known as nanoparticles. In the past decade, TiO_2 is one the most used nanoparticles in the world. It is nontoxic, cheap and can easily be produced, which in turn gives good advantages to the photocatalysis method currently observable as a good, reliable, fast, easy to operate technique. Therefore, this literature review discusses appropriate methods to remove patulin from apple juice focusing on the quantification and detection methods and the concept of photocatalysis TiO_2 nanoparticles, as a promising method of patulin treatment in apple juice.

2.2.Patulin: toxicity, quantification and removal methods in apple juice

Patulin ($C_7H_6O_4$) (Figure 2.1) has a low molecular weight and belongs to the family of ketones (Khan *et al.* , 2019). The chemical structure of patulin presents a lactone group and hemiacetal group. It is a white crystal compound soluble in polar solvents and insoluble in very low pH solutions (González-Osnaya *et al.* , 2007). The short history on patulin stipulate that patulin was firstly described in 1942 by Wisner as an antibiotic to inhibit bacteria growth. However, this pronouncement was discredited when studies proved the detrimental effect of patulin in humans and animals health (Mayer *et al.* , 1969).

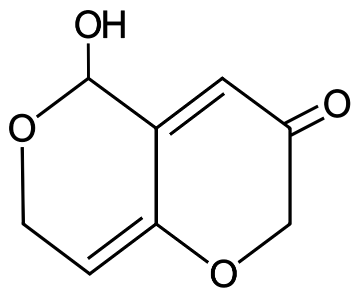


Figure 2-1: Patulin chemical structure (Collin et al., 2008)

Patulin is carcinogenic, tetragenic, mutagenic, genotoxic, with its effects generating dysfunctions of the human gastrointestinal track. The associated ingestion symptoms include (among many others): vomiting, nausea and diarrhoea (Andersen et al., 2004, McCallum et al., 2002).

2.2 Toxicity of patulin

2.2.1 Genotoxic effect of patulin

The genotoxic effect of patulin has tremendous consequences on human's genetic material precisely on the chromosomes by forming micronucleus and nucleoplasmic bridges which disturb DNA structure and formation (Jayashree *et al.* , 2017). The destruction of the genetic material occurs when the level of glutathione decreases within the cell. In fact, glutathione is a major compound used by the cell for defense. The presence of patulin in the cell reduces the level of glutathione; hence, increases the genotoxic consequences. Modifications in the genome may lead to a wrong division of chromosomes during cell division and mitosis (Glaser and Stopper, 2012; Fliege and Metzler, 2000). The mutagenicity of patulin was assessed on V79 cells and HepG2 cells and the evidence that patulin creates deletion and point to mutation in the cell was due to the decrease of glutathione levels (Schumacher *et al*., 2005).

2.2.2 Carcinogenicity

The carcinogenicity of patulin in human and animals was demonstrated by the increase of exponential growth of tumour cells (Ciegleret al., 1971) and by the process of autophagy. In this process, cell degrades their own amino acids and their intracellular components such as mitochondria. Endoplasmic reticulum autophagy induced by patulin plays a tremendous role on skin carcinogenesis (Guo et al., 2013). The genotoxicity and carcinogenicity of patulin in apple juice is of great threat for humans and animals and need to be addressed. Hence, the need of pursuing studies to develop new approaches for patulin prevalence detection and eradication in apple juice.

2.2.3 Patulin prevention and pre-elimination

The primary stage for patulin prevention and accumulation in apple juice is to monitor the pre-harvest condition by the means of proper farming methods and the regulation of the postharvest conditions such as storage, sorting of fruits and washing. For small-scale producer of apple juices, Nachman paster (2008) suggested that trimming away the rotten and the affected part, can have a great significance in reducing the level of patulin in juice. However, this proposition can pose a big challenge for large-scale producers where fruits are processed in batches and when all process stages are automated. Therefore, trimming each affected apple would be time consuming and costly for companies in terms of labour. The other challenge is that patulin can also develop in the centre of the apple (Sulyok *et al.* , 2010). It is also important to understand that one way by which patulin can be handle by small-scale juice producers, is by understanding the manufacturing process as highlighted in Figure 2.2.

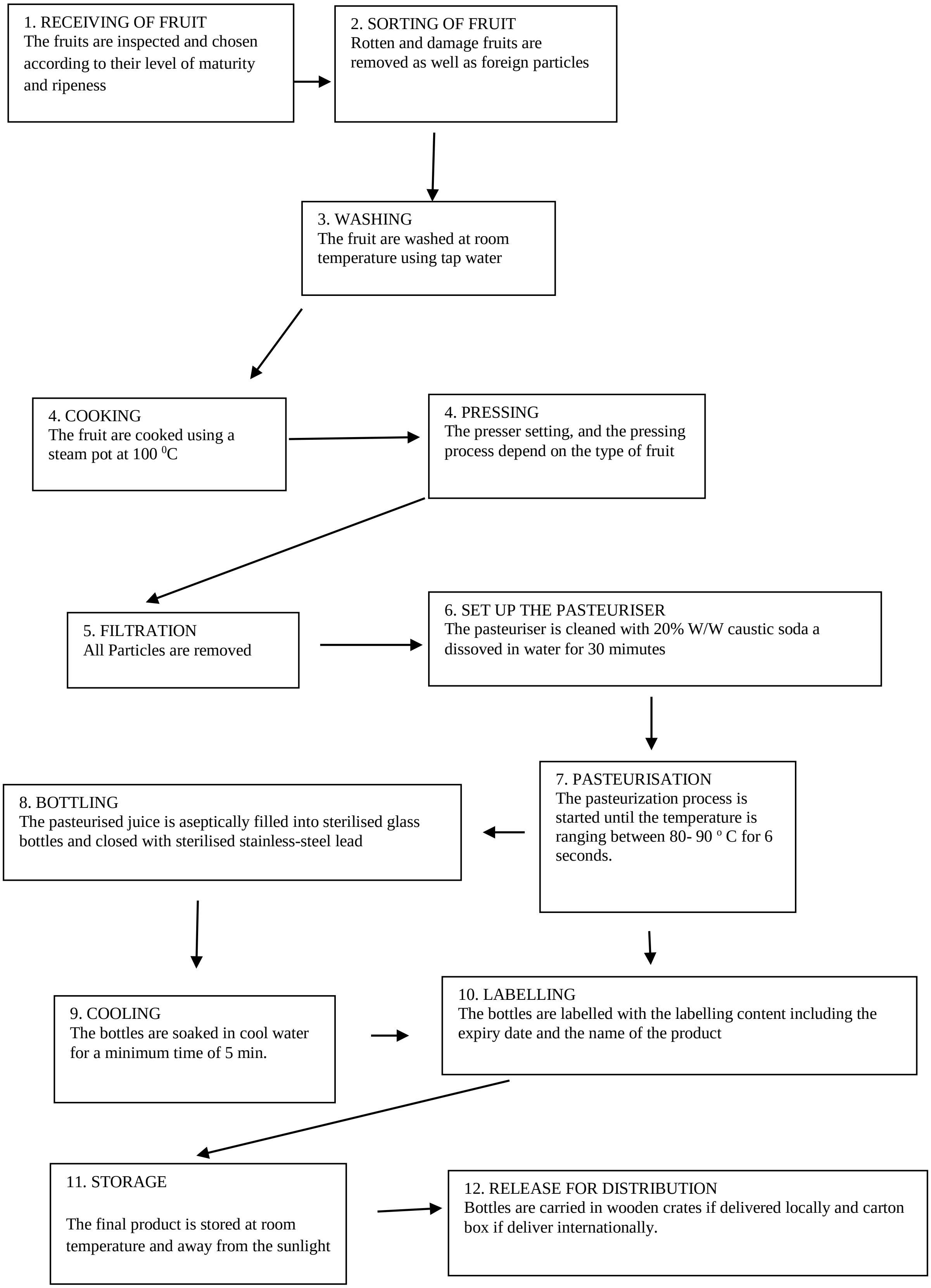


Figure 2-2: Industrial process for apple juice production.

2.3 Methodology of patulin quantification in apple juice

Chromatography is reliable, fast and cost-effective tool to separate and quantify molecules in a mixture. Chromatography is applying in several areas such as the food, pharmaceutical, and wastewater treatment industried. The principle of chromatography relies on the level of adhesion between the components in the mixture and the stationary phase. The stronger the adhesion between the components of separation and the stationary phase, the longer the retention time. The components are usually pushed through the column by the mobile phase. Based on the mode of action, chromatography exists in four main types, i.e. Liquid Chromatography, gas Chromatography, Thin-Layer chromatography and Paper Chromatography.

Several methods for patulin quantification in apple juice have been developed but prior to quantification, patulin must be extracted from the juice. Some of the well-known technique for patulin extraction are; QuEchERS extraction (Kharandi et al., 2013), solid- phase extraction (SPE) (Bobeldijk and van Osenbruggen, 2005) and liquid-liquid extraction (LLE) with ethyl acetate (LLE) been adopted by the Association Of Analytical Chemists (AOAC) as an official method for patulin extraction. Table 2.1 illustrates different extraction methods with more details on the limit of quantification (LOQ) and the limit of detection (LOD). Although, LLE has been adopted by the AOAC as an official method for patulin extraction in apple juice, solid phase extraction presents some advantages such as low solvent consumption, ease to perform, feasibility, less time consumption and low cost involved.

Table 2.1: Patulin quantification methods in apple juice

Methods	LOD/LOQ	Extraction and detection method	Matrix	References
HPLC -MS/MS	LOD:0.25 ng/mL^{-1} LOQ:0.76 ng /mL ^{1}	Liquid extraction Mass spectrometry	Apple puree	(Lee et al., 2014)
(SPE–HPLC–UV)	LOD: 1.2 µg/kg to 42 µg/kg	Solid phase extraction UV detection	Apple puree	(Alvito and Almeida, 2010)
(HPLC–DAD)	LOQ & LOD:25 µg/kg	Solid phase extraction Diode array detection	Apple juice	(Bobeldijk and van Osenbruggen, 2005)
HPLC	None	Reversed-phase isocratic UV detection	Apple juice and apple puree	(Leggott and Shephard, 2001)
SPE prior HPLC	LOD: 20 µg/l	Liquid-liquid extraction UV detection	apple juice	(Permaul and Odhav, 2001)

2.3.1 Chemical reagents used in chromatography for patulin quantification

The chemical approaches of patulin quantification begin with sample preparation step which consists of extracting patulin from the juice matrix. Sample preparation in any test is of crucial importance as most often the quality of the result relies upon this technique. In fact, the sample preparation consists of extracting the analyte from the matrices. After extraction, the analyte is injected in the chromatography apparatus depending on the nature of the analyte.

2.3.1.1 Gas chromatography (GC) - mass spectrometry

GC is often used the component to be analysed is in a gaseous form. The use of GC for patulin quantification has not been the way to follow in many studies because patulin has a low volatility and has a low molecular weight. Also GC requires an extra step that involves chemical reagents like acylation and trimethylsilyl ether which are chemical that increase the volatility of patulin. The loss of the analyte and the contamination of sample easily occur in GC. The technique also lacks a clean-up step and requires derivation step (Li et al., 2017).

2.3.1.2 Liquid chromatography – mass spectrometry (LC – MS)

LC has been proven to be effective the determination of patulin in food matrices. It works at its best when it is coupled with mass spectrometry. LC does not require derivative step. It has a high sensitivity; low selectivity. It is of good repeatability and reproductively. Although the qualities of this technique are to be praised, it also has some shortcoming it is affected by the presence of interfering agents and require high solvent usage (Li et al., 2017).

Patulin studies in South Africa has not been the focus of many studies for the past 18 years. Studies are very few, albeit not well elucidated and some of the results are outdated. Leggott and Shephard (2001) have conducted a survey on patulin concentration in 60 commercial apple juice and puree produce in Cape Town. The results were satisfactory for adult's food as they raged between 5 and 45 µg/L but very unsatisfactory for infant as the range were between 5 and 20 µg/L which is not in line with laws from to the regulatory bodies which stipulate that the concentration of patulin in infant food should not exceed 10 µg/L. Hence the need to develop studies in this area.

2.4 Patulin removal methods

Biological and chemical methods of patulin treatment in apple juice and apple cider have been developed. Biological methods usually are related to fermentation processes while other methods relies on the use of chemical and hydrothermal processes.

2.4.1 Fermentation as a biological process: relationship between patulin and *Saccharomyces cerevisiae*

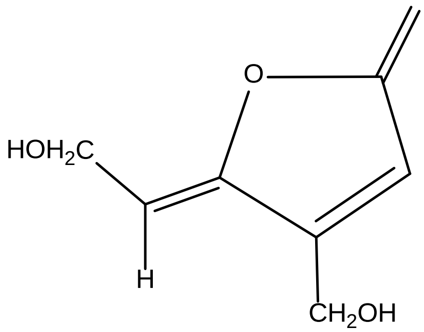
Fermentation is a biological process by which yeasts uses sugar to produce alcohol. Fermentation is either aerobic or anaerobic and, in both cases, the success of the process depends on the quality of the inoculum, the fermentation conditions and operation, the nature of the fermenter and the growth kinetics of the cells used. There are several yeasts capable of degrading patulin such as *Rhodosporidiumkratochvilovae, Gluconobacteroxydans, Saccharomyces cerevisiae.*

Biological degradation of patulin put emphasis on alcohol fermentation as a promising way of patulin degradation and the focus has been put on *Saccharomyces cerevisiae* as the main fermentation agent. There are two hypotheses by which *S. cerevisiae* could degrade patulin, it is either by enzymatic reactions under specific conditions or by the ability of patulin not to disrupt the yeast cell wall (Coelho *et al.* , 2008). Patulin was able to produce forwards mutations in extrachromosomal cell of *S. cerevisiae* by changing the genetic material of the microorganism from wild type to petite. The mutations were confirmed by the ability of the microorganism to utilise glycerol. Patulin can also inhibit microbial growth by increasing the percentage of cell death during exponential phase. This is due to the immature formation of mitochondria cells during exponential growth, thus minimising the respiration process (Mayer *et al.* , 1969).

The by-products resulting from patulin degradation are more polar that patulin itself. Table 2.1 shows that E – ascladiol (Figure 2.3) and Z – ascladiol (Figure 2.4) are the main by-products from the degradation of [^{14}C] labelled patulin during alcoholic fermentation. These compounds could be further degraded during fermentation to more polar compounds (Moss *et al.* , 2002, Ricelli *et al.* , 2007). The effect of E- ascladiol was demonstrated to have no toxic effect on humans and animals. Besides ascladiol compound, desoxypatulinic acid was also found as a byproduct of patulin degradation (Tannous *et al.* , 2017, Maidana *et al.* , 2016, Ricelli *et al.* , 2007). Patulin is also heat resistant and cannot be destroyed by pasteurisation process and according to Collin et al. (2008), hydroxypentanal and the glyoxilic acid could be the products from the heat degradation of patulin at 80 ^{o}C for a period of 40 minutes (Figure 2.5).

The chemical structure of patulin presents a lactone group and hemiacetal group. However, the by-products listed lack the presence of lactone and hemiacetal group, and could this could be the reason of less toxicity of E- ascladiol as compare to patulin. A hypothesis was proposed by

(Tannous *et al.* , 2017) stating that it the blocking to the conversion of ascladiol to patulinor by encouraging the conversion of patulin to ascladiol can ensue, which can be a good strategy to eliminate patulin contamination risks.



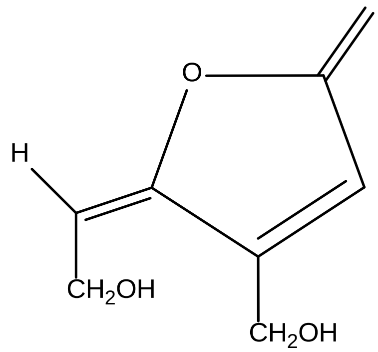


Figure 2-3: E-Ascladiol

Figure 2-4: Z-Ascladiol

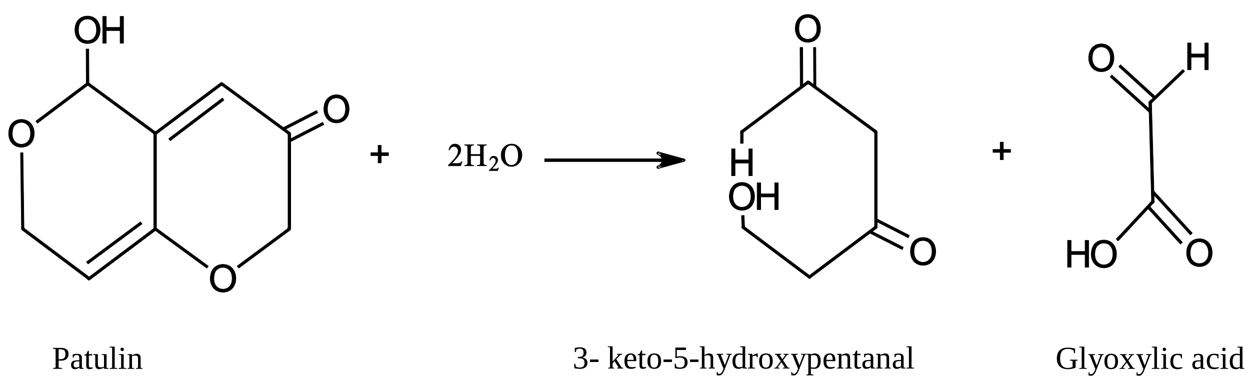


Figure 2-5: Hypothetically degradation by product of Patulin (Collin et al., 2008)

Table 2-1: Microorganisms able to degrade patulin and their by-product.

Bacteria names	**Degradation products**	**References**
Ascomyceta	E- escladiol	(Tannous et al. , 2017)
Basidiomycetaphila	E- escladiol	(Ianiri et al., 2016)
Pucciniomycotinasporobolomyces	Deoxypatulinic acid and Z-escladiol	(Ianiri *et al.* , 2013)
Gluconobacteroxydans	E- escladiol and Z-escladiol	(Ricelli *et al.* , 2007)
Rhodosporidiumkratochvilavae	Deoxypatulinic acid	(Castoria *et al.* , 2011)
Saccharomyces cerevisiae	E- escladiol and Z-escladiol	(Moss *et al.* , 2002)

2.4.2 Chemical treatment of patulin

2.4.2.1 Ozone treatment

Ozone is a powerful chemical oxidant used for the degradation of both biological and chemical contaminants in food. Ozone treatment is a promising industrial technique for the degradation of microorganisms and their toxins. Ozone present advantageous parameters over chlorides in term of food decontamination because it can be effective over a broad spectrum of microorganisms and it has be proven to be 50% more powerful than that chlorides. Ozone can be generated by chemical reaction (conversion of Oxygen to ozone) or phytochemical reaction (conversion of a gas mixture containing oxygen and expose to a power source). However, ozone cannot be produced readily and therefore it is produce when needed. This method is not well developed and understood and it can be very costly for industries (Diao *et al.* , 2018, Diao *et al.* , 2019).

2.4.2.2 UV treatment

Ultraviolet (UV) are electromagnetic radiation ranging from 10nm – 400 nm, is usually produced by the sun and contributes about 10% of sunlight UV. It is known to damage the eyes and skin and causes cancer in cells. Although UV treatments have sides' effects, it is fast, cost effective and very easy to operate method. It is used industrially for bacterial deactivation, heat treatment, sterilisation and others. Additionally, UV have been reported effective to degrade patulin in apple juice without affecting the quality of the juice. In fact, patulin can be significantly decreased when exposed to UV radiation for a period of 15sec without changing the chemical parameters of the juice such as pH, brix and acid content (Dong *et al.* , 2010) . Another study has demonstrated the efficacy of UV to degrade patulin in both apple juice and apple cider, with the degradation being determined to follow first order kinetic reaction. Patulin was significantly degraded from both matrices and the kinetic reaction constants was 5.5 % greater in apple juice compare to apple cider (Assatarakul *et al.* , 2012). The factors affecting patulin degradation using UV treatment are apple juice constituents, suspended compounds and polyphenols. Patulin degradation using UV treatment is more effective in clarified apple juice than that in non-filter apple juice because the radiation is blocked by suspended particulate matter in the apple juice preventing the rays to reach the patulin (Tikekar *et al.* , 2014).

Biological and chemical methods are both effective in patulin reduction and do not totally remediate the toxin from the apple juice. There is a need to develop and explore other methods.

2.5 Patulin photocatalysis using TiO_2 nanoparticles: A promising method of patulin treatment in apples juice

Photocatalysis is a process in which catalysis or a semiconductor activates light, to generate a chemical reaction. The light emits a photon, which excite the band gap of the semiconductor to generate a hole and electron pair production at the surface of the semiconductor (Zhang et al., 1998). It is important that both electrons and holes production occur apart from each other to prevent the process of recombination which nullifies the photocatalytic reaction (Fujishima et al., 2000, Linsebigler et al., 1995).

2.5.1 Mechanism of photocatalysis

Photocatalysis is a combination of two words: photo which means "light" and catalysis also called "semiconductor. The process of photocatalysis result in production of holes positively charged and electron negatively charged due to the absorption of an energy greater than the one of it band gap. After excitation of the band gap by photon, which the energy is greater than the one of the band gap. Electrons and holes are produced. Their behaviour can represent two scenarios; both electron and holes can recombine to produce thermal energy or the can move apart from each other to participate in redox reactions (Figure 2.6).

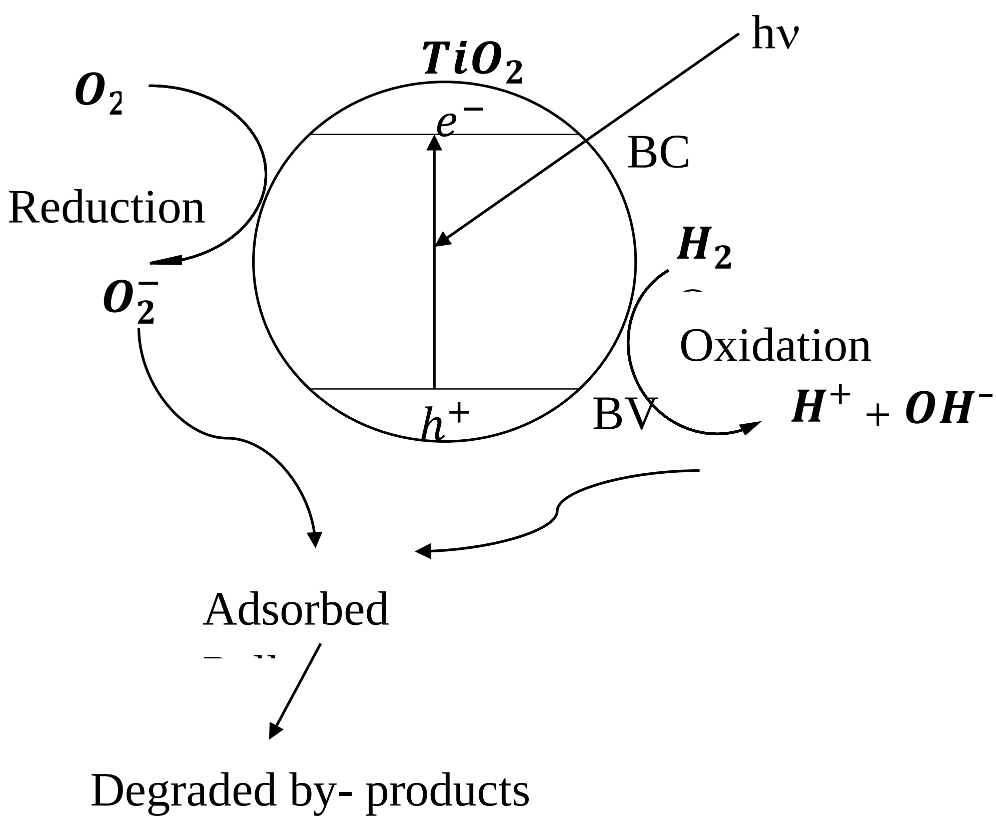


Figure 2-6: Reaction mechanism of photocatalysis adapted from (Li *et al*., 2016)

However, the reaction can take place at the surface or within the catalyst and depending on the location in which they occur two types of reaction are distinguished; homogenous and heterogeneous photocatalysis. Heterogeneous photocatalysis is an exothermic process in which the charge transfer is continuous. The semiconductor or catalyst remains inert after the reaction. It has been highly used in environmental clean-up for the removal of organic molecules in contaminated air and wastewater. Heterogenous reactions has two types of photoreactions depending on the origin of the excitation, i.e. catalyzed photoreaction (interaction between the adsorbate molecule and the catalyst substrate), and sensitized photoreaction (Liesbigher et al, 1995; Wong et al; 2006).

2.5.2 Application of photocatalysis

Photocatalysis has it genesis in the years 1970s (kavitakabra et al, 2004). It has been widely applied in water and air treatment. It is well applied in other disciplines of life such as pharmaceutical, paint, cosmetic and less in the food industries. The first application of photocatalysis was in the splitting of water by Fujishima and Honda (Linsebigler et al; 1994). Thereafter, researches have been intensified to understand and increase the effectiveness of photocatalysis process.

Photocatalysis is a promising technology widely used in the past decade for the treatment of wastewater and the remediation of hazardous compounds in the environment. Unlike other treatment methods that uses energy- intensive methods, photocatalysis uses solar energy to deteriorate life threatening compounds in aqueous samples. In addition, the by-product of photocatalysis are often innocuous product compared to other treatments, which transfer hazardous product from one area to the other. Therefore, photocatalysis can be described as an effective for aqueous and gaseous phase treatments. The reaction conditions are often moderate and the outcome waste are minimal. The application of photocatalysis has been approved for self-cleaning glasses (Puricha et al; 2008), dye removal in wastewater treatment (Li et al., 2003; Augustina et al., 2003; Mc cullagh et al., 2007), environmental purification (Liquang et al., 2003) and removal of organic pollutant such as cyanide (Chatterjel and Dasgupta, 2005; Kabra et al., 2004).

2.5.2.1 Introduction to nanoparticles

Nanoparticles also called semiconductors, are substances used to speed up chemical reactions without taking part in the reaction themselves. The choice of catalysis is based on the factors such as the ability to increases the rate of the reaction, selectivity and stability. Catalysis have three ways of actions: (i) they can be involved in a reaction by interrelating with other reactants or products, (ii) They can also change the speed of a reaction and (iii) reappear to it in an authentic form. The advantages associates with the use of a catalyst are, minimal activation energy to form the products, with no transitional form being required while requiring less suitable conditions to operate (Fujishima and Zhang, 2006; Zhang et al., 1998).

2.5.2.2 Toxicology parameters and health effects of TiO_2

Several research fields address the issue of hazardous nanomaterials but, nanotechnology is one of the well understood, promising and innovative technologies which can be applied for the degradation of biological and chemical pathogens in environmental sciences and in food. Nanotechnology relies on the application of nanoparticles with most not naturally occurring in the environment. They are often synthesised via biological (plants, agro-waste) or chemical (chemical salts) techniques. Up to date, chemical methods are specifically applied for TiO_2 and a large number of other nanoparticle have been developed using methods such as chemical precipitation (Collazzo *et al.* , 2011, Jaggessar *et al.* , 2018, Syuhada *et al.* , 2018); the sol gel method (Mutuma *et al.* , 2015, Pinjari *et al.* , 2015, Vetrivel *et al.* , 2015), hydrothermal (Cano-Casanova *et al.* , 2018, Gao *et al.* , 2015, Li *et al.* , 2016, Wu *et al.* , 2015), solvothermal processes (Xie *et al.* , 2010, Yang *et al.* , 2009), combustion method (Ma *et al.* , 2015, Umale *et al.* , 2018), chemical vapour deposition (CVD) (Chimupala *et al.* , 2016), electrochemical synthesis (Döşlü *et al.* , 2018, Nur *et al.* , 2016) and fungus-mediated synthesis (Rajakumar *et al.* , 2015, Santhoshkumar *et al.* , 2014). Amongst this, wet chemical method is known to be effective as chemical precipitation is applied with low chemical consumption, in a cost efficient many with quality product outcomes.

TiO_2 appears in three forms in nature, i.e. as anatase, rutile and brookite. Anatase is predominantly used in solar cells and its amorphous phase ease of conductivity allows for electrons to move freely. Anatase can also be easily doped with certain chemicals to increase its conductivity with both anatase and rutile being known to have a band gap of 3.0 and 3.2 eV, respectively (Kordouli *et al.* , 2015) which is suitable for photocatalytic degradation of pollutants. TiO_2 has a low absorption coefficient, a high refractive index, high surface area and a great photocatalytic activity with a high ion – exchange capacity where holes and electrons are produced for redox reactions to oxidise organic pollutant to non-toxic constituents such as CO_2 and water in wastewater treatment plants and to sanitise air. Therefore, TiO_2 could be used in various applications such as: photocatalysis for self-cleaning glasses (Calia *et al.* , 2017, Naufal *et al.* , 2017), photocatalysis for the remediation of naturally occurring organic matter (Sousa, 2017), wastewater treatment (Bhanvase *et al.* , 2017, Borges *et al.* , 2016, Chong *et al.* , 2015), environmental purification (Liqiang *et al.* , 2003), interfacial charge carrier transfer, and the removal of organic pollutant such as cyanide (Aguado *et al.* , 2002).

The wet chemical method has been intensively adopted and perform to synthesise nanoparticles but with the growth of hazardous compound in nature, researchers are more concern on finding a green and environmental benign approach to TiO_2 synthesis. Wet chemical method relies on the use of chemical reagents and it is advantageous in terms of low solvent consumption, ease of performance and cost effectiveness. All the reagents involved are used in a liquid form, thus ease of handling. More often, ammonium hydroxide is mixed with an amorphous $TiCl_4$ or $TiOCl_2$ in an aqueous solution (Yin *et al.* , 2001). Many of the previous study used ammonium hydroxide and ethanol (Gupta *et al.* , 2010) as solvents for $TiCl_4$ salt. In this study, $Ticl_4$ was purchased in a liquid state dissolved in water, methylene chloride and toluene and the choice solubilising matrices were intentionally selected based on polarity. According to our knowledge, no study has presented the effect of solubilising agent for $TiCl_4$ on TiO_2 nanoparticles synthesis. Therefore, this study reports the influence of solubilising matrices on TiO_2 synthesis.

TiO_2 has worked best when it is coupled with UV light. Most of the time, TiO_2 is usually coated on the surface of glass, plastic, paper and cloth. It has been proven by (Maneerat *et al.* , 2006) that TiO_2 coated in plastic and combined with UV light can reduce the level of fruit spoilage at the postharvest stage. The study reported the number of colonies observe in plates that TiO_2 combined with UV treatment has more efficiency in *Penicillium* sp compared to when TiO_2 was used alone which is in line with the finding of (Hur et al., 2005). The availability of data regarding the toxic effect of TiO_2 is still not reported. Skocaj et al (2011) have reported some attributes for which TiO_2 is known for, i.e.:

- TiO_2 is inert and non-toxic,

- TiO_2 is not regarded as a carcinogenic material, and
- It is safely used in foods and pharmaceuticals industries, paints and beauty products.

2.5.2.3 Doping

Doping is a process of modifying the chemical properties of a semiconductor or catalysts. The utilisation of Fe^{3+} Mo^{5+}, Ru^{3+}, Os^{3+}, Re^{5+}, V^{4+} and Rh^{3+} between 0.1-0.5 % was reported to increase the redox potential of catalysts during photocatalysis (Choi et al., 1994). The different methods used to dope catalysts are: sol-gel method in acidic media, homogeneous precipitation or hydrothermal method and through hydrolysis.

The advantages of doping a semiconductor is as follows: 1) increase the photocatalytic activity by reducing the hole and electrons recombination. A comparative study conducted by Cong et al. (2007) on pure TiO_2 and TiO_2 co-doped with nitrogen and 0.5% iron (III) concluded that the photocatalytic reaction with co-doped particles was increased by 75% under visible and 5% under UV light compared to pure TiO_2, 2) a shift in the photocatalytic region from UV to visible light occurs by narrowing the band gap of the catalyst and by increasing the degradation process of targeted pollutant and/or contaminants (Cong et al., 2007). The degradation of methyl orange using TiO_2 dope Fe^{3+} orange under UV and visible light showed a great efficiency under visible light compare to UV irradiation due to the reduction of the band gap through the process of doping (Wang et al., 2006). Another study conducted by Ananpattarachai et al. (2009) on TiO_2 using a variety of dopants such as diethonolamine, trimethylamine and urea, reported that effect of doping increased adsorption in visible light region and also increase the rate of degradation process.

2.5.2.4 Application of photocatalysis for microbial disinfection

Researches done by Wong et al. (2006) has proven that nitrogen – doped TiO_2 as a disinfectant for environmental and medical purposes was infact more effective. It was demonstrated that TiO_2 doped Ni is more effective and have some great bactericidal activities on a broad number of pathogenic microorganisms such as *Shigella flexneri*, and *Listeria monocytogenes*.

TiO_2 has a broad biocidal spectrum that prevent and act against infections. TiO_2 has the same microbial effect as H_2O_2. In fact, hydroxyl group, oxygen and water molecules produced from the irradiation of TiO_2 disrupt the phospholipid of the cell membranes and therefore attack the DNA at a specific point. A variety of studies demonstrated that TiO_2 is effective for microbial disinfection under UV light and result in a more productive result under visible light when the particles are doped with metals (Wong et al., 2006; Mc cullagh, 2007). In fact, the optimum concentration of TiO_2 required for antimicrobial activity was shown (Sousa, 2017). Photocatalysis has also been approved effective for the remediation of naturally occurring organic matter (Li et al., 2003; Sousa, 2017). It is beneficial to use photocatalysis facilitated by TiO_2 for mycotoxin removal in a liquefied form to obtain a satisfactory result after the photocalytic process.

Photocatalysis has been highly used as a green technology for various industrial processes such as potable water and wastewater treatment including the concentration of spoilage organisms in fresh juices, hydrogen generation and CO_2 conversion (Zhang et al., 1998). However, the application and the development of photocatalytic materials for perishable agricultural produce industries (juicing) still pose some challenges; therefore, an effort should be done to explore this area (Robert et al., 2017), for the benefit of small scale juice producers.

2.6 Conclusion

In summary, patulin is a mycotoxin produce by blue mould on rotten apple. It was proven to be carcinogenic for both humans and animals; hence, the need to degrade the toxin from food. Patulin can be quantify using LC/MS with the most predominant extraction from apple juice being liquid-liquid extraction and solid phase extraction. Biological and chemical methods of patulin removal in apple juice and apple cider have been discussed. The biological methods are related to fermentation processes while other methods rely on the use of chemical and hydrothermal processes. Most of these methods affect the apple juice quality and resulting in it becoming unsuitable for consumption. However, UV light have been reported to be effective to degrade patulin in apple juice without affecting the quality of the juice. Ozone treatment is another promising industrial technique for the degradation of patulin in food. Nevertheless, ozone cannot be conserved and should be produced when needed which could be costly for industry; however, photocatalysis have been widely used in pathogen microorganism disinfection namely *Eschericia* *coli, Basilussubtilus, Staphylococcus aureus* and it is proposed that patulin can also be treated using this technique for juicing operations in combination with TiO_2.

2.7 References

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CHAPTER 3

IMPACT OF SOLUBILIZING MATRICES FOR $TICL_4$ ON THE FORMATION OF TIO_2 NANOPARTICLES

CHAPTER 3

RESULTS

IMPACT OF SOLUBILISING MATRICES FOR $TICL_4$ ON THE FORMATION OF TIO_2 NANOPARTICLES

General overview of the article

This paper aimed to synthesised TiO_2 from $TiCl_4$. To achieve this, a wet chemical method was adopted. The main objective was to find the best solubilizing matrice for $TiCl_4$. Thus, $TiCl_4$ was dissolved in three solubilizing matrice such as water, methylene chloride and toluene.

As a result, TiO_2 was successfully synthesis from the three solvent and after annealing, the samples were subjected to SEM, TEM, FTIR. The results showed that the samples have good optical properties. A comparison of particles showed that, the nanoparticles produce by $TiCl_4$ dissolve in water provided the best particles size and the good characteristics.

Impact of solubilising matrices for $TiCl_4$ on the formation of TiO_2 nanoparticles

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4.1 Abstract

Nanoparticles are generally used for the treatment of innocuous compounds in wastewater treatment, dye degradation, bacterial deactivation and in the pharmaceutical industry for the production of toothpaste, cosmetic products including in the production of paints. Several nanoparticles (Cu_2O, SnO, ZnO) have been intensively studied and applied in wastewater treatment research, but TiO2 has gained popularity because of its stability, affordability, large band gap, recyclability and its efficiency in photocatalysis. This study reports on the influence of solubilising matrices on TiO_2 synthesis. A wet Chemical method was used to synthesis TiO_2 nanoparticles by solubilising $TiCl_4$ in three types of solvent: water, toluene and methylene chloride. Physical, chemical and optical properties of TiO_2 nanoparticles obtained from these various solvents were characterised by XRD, UV-Vis, FTIR and SEM. Results are compared for each solvent with TiO_2 nanoparticles solubilised in water having the best properties.

Index Terms --- Titanium Chloride, Titanium Oxide, Nanomaterials, Wet Chemical method.

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4.2 Introduction

Several research fields address the issue of hazardous compounds in wastewater treatment but, nanotechnology is one of the well understood, promising and innovative technologies which can be applied for the degradation of biological and chemical pathogens in environmental sciences. Nanotechnology relies on the application of nanoparticles with most not naturally occurring in the environment. They are often synthesised via biological (plants, agro-waste) or chemical (chemical salts) techniques. Up to date, chemical methods are specifically applied for TiO_2.A large number of other nanoparticle have been developed using methods, such as: chemical precipitation (Syuhada and Yuliarta, 2018 ; Coleazzo et al., 2011), the sol gel method (Mutuma et al., 2015 ; Vetrivel et al., 2015), hydrothermal (Umale et al., 2018 ; Ma et al., 2015), solvothermal processes (Xie et al., 2010 ;Yang et al., 2009), combustion method (Umale et al., 2018 ; Ma et al., 2015), chemical vapour deposition (CVD) (Chimupala et al., 2016), electrochemical synthesis (Döşlü et al., 2018 ; Nur et al., 2016) and fungus-mediated synthesis (Rajakumar et al., 2015 ; Santhoshkumar et al., 2014). Amongst, these the wet chemical method is known to be effective as chemical precipitation is applied with low chemical consumption, in cost efficiency and quality product outcomes.

TiO_2 appears in three forms in nature, anatase, rutile and brookite. Anatase is predominantly used in solar cells and its amorphous phase ease of conductivity allows electrons to move freely. Anatase can also be easily doped with certain chemicals to increase its conductivity with both anatase and rutile being known to have a band gap of 3.0 and 3.2 eV, respectively (Kordouli et al., 2015) which is suitable for photocatalytic degradation of pollutants. TiO_2 has a low absorption coefficient, a high refractive index, high surface area and a great photocatalytic activity with a high ion – exchange capacity where holes and electrons are produced for redox reactions to oxidise organic pollutant to non-toxic constituents such as CO_2 and water in wastewater treatment plants and to sanitise air. Therefore, TiO_2 could be used in various applications such as: photocatalysis for self- cleaning glasses (Calia et al., 2017 ; Naufal et al., 2017), photocatalysis for the remediation of naturally occurring organic matter (Sousa, 2017), wastewater treatment (Bhanvase et al., 2017; Chong et al., 2015), environmental purification (Liqiang et al., 2003), interfacial charge carrier transfer and removal of organic pollutant such as cyanide (Aguado et al., 2002).

Wet Chemical method has been intensively adopted and perform to synthesise nanoparticles but with the growth of hazardous compound in nature, researchers are more concern on finding a green and environmental benign approach to TiO_2 synthesis. Wet chemical method relies to the used to chemical reagents and it is advantageous in terms of low solvent consumption ease of performance and cost effective. All the reagents involved are used in liquid form. More often Ammonium hydroxide is mixed with an amorphous, $TiCl_4$or $TiOCl_2$ aqueous solution (Yin et al., 2001). Many of the previous study used ammonium hydroxide and ethanol (Gupta et al., 2010) as solvent for $Ticl_4$ salt. According to our knowledge, no study has presented the effect of solubilising agent for $TiCl_4$ on TiO_2 nanoparticles synthesis. Therefore, this paper reports the influence of solubilising matrices on TiO_2 synthesis.

The objective of this study was (I) to determine the total PTE content, i.e. Al, Cu, Fe, Mg and Mn, and compare their binding forms in representative soil samples, and, (II) to evaluate metal distribution in the sediment samples using sequential chemical extraction (BCR), including (III) to characterize PTEs constituents in the soil using XRF and FTIR.

1.3 Materials and Methods

4.3.1 TiO_2 synthesis

All the reagents used in the study were of analytical grade purchase from Sigma Aldrich except for acetone (99,5%). All the TiO_2 nanoparticles derived from the use of $TiCl_4$in different solvent were synthesized under the same conditions using the wet chemical method. $TiCl_4$ solution (1M) in toluene, in water, in methylene were purchase from sigma Aldrich. Ammonium hydroxide 32% was used to precipitate the nanoparticles in the solutions.

$TiCl_4$ (2 mL) was added drop- wise in a 100 ml Beaker containing ammonium hydroxide solution under vigorously stirring for 10 min until an amorphous white precipitate was obtained. The synthesis was done at 60 °C in a water bath. The samples were dried in an oven at 80°C to transform the amorphous phase to a solid phase. The dry particles were transferred in tubes and washed several time with warm distilled water to remove the excess of chloride. All the samples were collected by centrifugation. Acetone was used to dry the sample before they were transferred in crucibles for annealing at 350 ^{o}C for 6 h. The annealed powders were characterised.

4.3.2 TiO_2 characterisations

Different properties of the annealed powders were studied. X-Ray Diffraction (XRD) analysis and Scanning Electron Microscopy (SEM) were used to investigate the TiO_2 physical properties, i.e. the TiO_2 nanoparticles crystallisation and its surface topographic, respectively. The average size (D) of the annealed TiO_2 nanoparticles was estimated by using the Debye-Schereer's Equation:

$$D = 0.9\lambda /(ß \times \cos\Theta) \quad (1)$$

Where:

λ: is the wavelength of the copper anode radiation that used during the XRD analysis, with a value 1.5406 Å,

ß: is the full width half maximum (FWHM) of the peak, in radian, and

ϴ: is the Bragg's angle; in degree.

Chemical properties, such as elemental composition and chemical bonding of the annealed TiO2 nanoparticles were identified by Energy Dispersive X-ray Spectroscopy (EDS) and Fourier transform-infrared (FTIR) analysis, respectively. Optical properties were studied by running an UV-VIS-NIR within a spectra range of 200 to 800nm.

4.4 Results and Discussion

All $TiCl_4$ solubilised in different solvents have shown their effectiveness as suitable solvents for the synthesis of TiO_2. The UV-VIS analysed have shown the presence of TiO_2 nanoparticles as shown in Figure 3.1. The TiO_2 nanoparticles UV-VIS absorption spectra was determined to fit within the invisible UV range of sunlight between 100- 400 nm (Behar-Cohen et al., 2014). The TiO_2 nanoparticles could absorb the UV of sunlight for various applications including dermal (Luan et al., 2016).

TiO_2 nanoparticle were adsorbed at 280nm in the UV–VIS spectroscopy. The same adsorption wavelength observed herein was reported by various researchers (Dobrucka et al., 2017 ; Roopan et al., 2012) . However, the adsorption peak was higher for TiO_2 synthesised by solubilised $TiCl_4$ in water than when using methylene chloride and toluene.

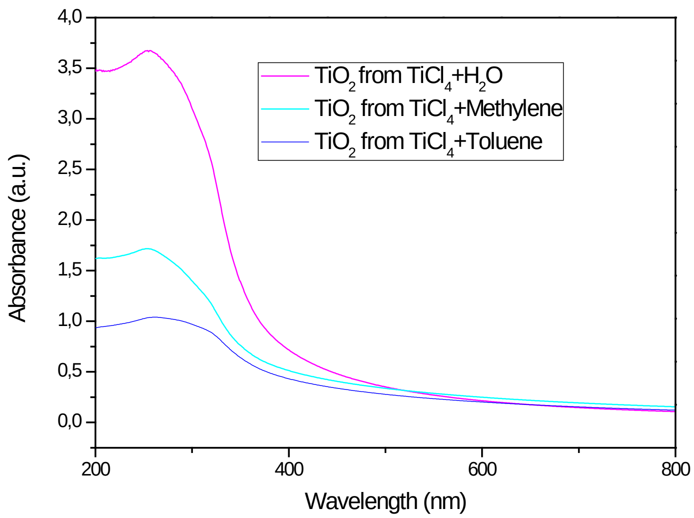

Figure 3-1: UV-VIS absorbance of TiO_2 nanoparticles from various $TiCl_4$ solubilisations

Figure 3.2 shows the XRD patterns of each TiO_2 nanoparticle produced from various $TiCl_4$ solubilisations. The Muller's indices (*hkl*) have detected at 2Θ (degree) = 25.281; 37.801; 48.050; 53.891; 55.062; 62.690 and 75.032 for *hkl* = 101; 004; 200; 105; 211, 204 and 215, respectively. Similar peaks were obtained for all synthesised TiO_2. Based on the *hkl* indices, the atom position of each TiO_2 has a body-centered tetragonal. The average size (D) has a value of 12 nm; 7nm and 10nm for $TiCl_4$ solubilised in water, methylene chloride and toluene, respectively. SEM images presented in Figure 3.5 shows that the TiO_2 nanoparticles were quite polydisperse in methylene chloride and toluene than in water, and their size range was 124 nm, 120 nm and 100 nm size, respectively. The chemical elemental composition of TiO_2 nanoparticles obtained by EDS techniques is shown in Figure 3.3. The area delimited by the rectangular polygon represents the sampling points in which EDS measurement were made. Three elements were detected, such as C, O and Ti: C and O chemical elements which particles the oxidation reactions with which the $TiCl_4$ salt were in derived (Qi et al., 2017). FTIR spectroscopy analyses is shown in Figure 3.4 illustrated chemical bands at 3327.65 cm^{-1}, 1635.33 cm^{-1} and 605.53 cm^{-1} for $TiCl_4$ solubilised in methylene chloride and toluene and 3207.61 cm^{-1}, 2350.40 cm^{-1}, 2030.61 cm^{-1}, 1622.16 cm^{-1} and 659.63 cm^{-1} for $TiCl_4$ solubilised into water. Characteristic bands indicated at 1635.33 cm^{-1} and 1622.16 cm^{-1} represented the saturated hydrocarbons, i.e. the C=C link. Bands 3327.65 cm^{-1} and 3207.61 cm^{-1}

indicated the O-H, at the peaks 2350.40 cm^{-1}, 2030.61 cm^{-1} correspond to the C-O stretching alcohols from methylene chloride and toluene. All bands were generated by the chemical and elemental interaction forms of water, methylene chloride and toluene. The presence of TiO_2 nanoparticles was indicated by the peak 605.53 cm^{-1} and 659.63 cm^{-1} for $TiCl_4$ in water and for $TiCl_4$ methylene chloride and toluene, respectively.

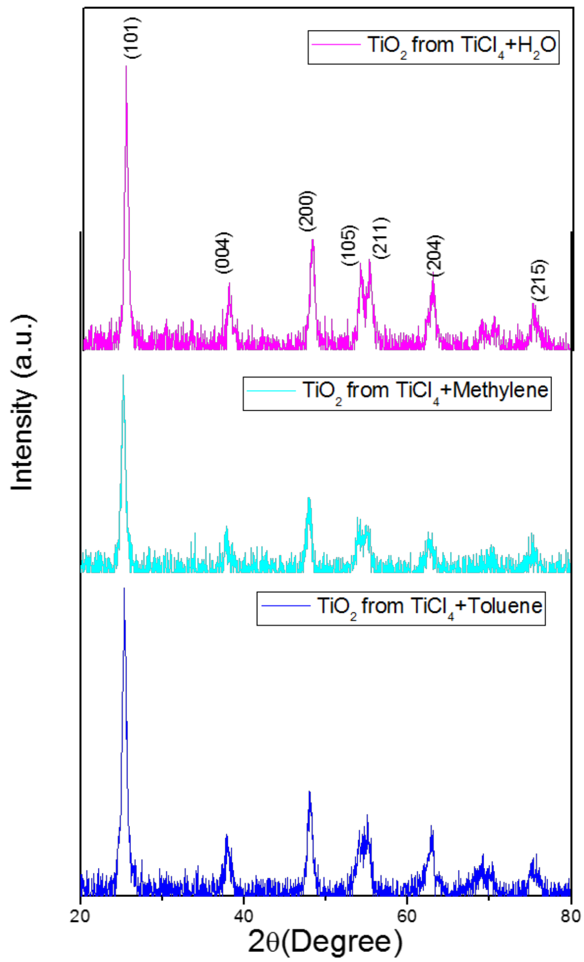

Figure 3-2: XRD patterns of TiO_2 nanoparticles from various $TiCl_4$ solubilisations

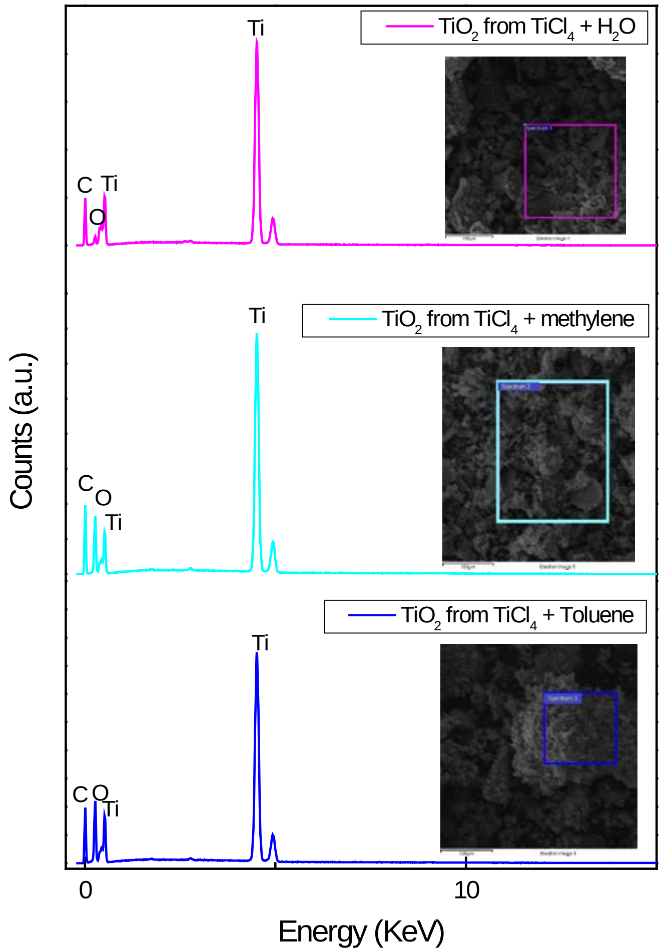

Figure 3-3 :EDS graphs of TiO_2 nanoparticles from various $TiCl_4$ solubilisations

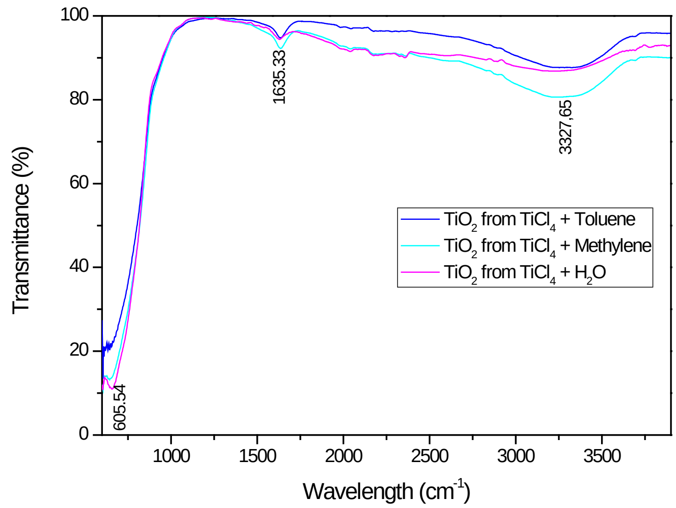

Figure 3-4: FT-IR Spectra of TiO_2 nanoparticles from various $TiCl_4$ solubilisations

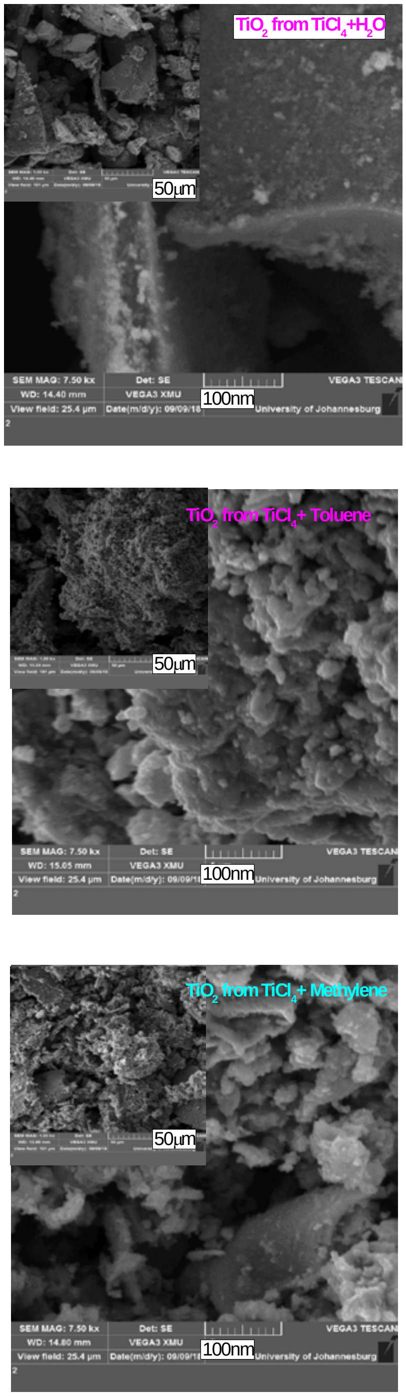

Figure 3-5: SEM images of TiO_2 nanoparticles from various $TiCl_4$ solubilisations

4.5 Conclusion

In summary, TiO_2 was successfully synthesised by solubilising $TiCl_4$ in various solvents, such as water, methylene chlorine and toluene. All TiO_2 nanoparticles synthesised have a single phase anatase structure. However, $TiCl_4$ solubilised in water have shown to have had the best crystallisation. Therefore, water as a solvent is highly recommended to solubilise the matrix $TiCl_4$ to synthesis TiO_2 for photocalytic operation.

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CHAPTER 4

PHOTOCATALYTIC APPLICATION OF TIO_2NANOPARTICLES SYNTHESISED FROM $TICL_4$ IN THE DEGRADATION OF PATULIN IN WATER MATRICES AND APPLE JUICE

CHAPTER 4

RESULTS

PHOTOCATALYTIC APPLICATION OF TIO2NANOPARTICLES SYNTHESISED FROM TICL4 IN THE DEGRADATION OF PATULIN IN WATER MATRICES AND APPLE JUICE

General overview of the article

This chapter provides results from the photocatalysis of patulin in water and apple juice. Patulin in water and apple juice was exposed to conditions at different UV light intensity for a period of 3 h such as UV 15 and UV 30.

Results showed the best degradation level under UV 30 which was recommended as good intensity for patulin treatment in apple juice

Photocatalytic application of TiO_2 nanoparticles synthesised from $TiCl_4$ in the degradation of patulin in water matrices and apple juice

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4.1 Abstract

Patulin is a mycotoxin produce by fungal species in food and especially fruits and fruit juices. Patulin is both chronic and toxic in humans and animal and have fatale destruction of cells, which could lead to death. This paper reports the application of nanoparticles Titanium dioxide (TiO_2) in the degradation of patulin by the means of photocatalysis. TiO_2 was chemically synthesised from the mixture of Titanium Chloride ($TiCl_4$) and ammonium hydroxide. The optical properties of the particle under UV- Vis spectrometry was analysed and the UV-vis absorption spectra fold within the UV visible range of sunlight. The nanoparticles also shown a Polydisperse structures with 120 nm size under the Scanned electron microscopy (SEM).C, O and Ti were elements visualised under the Energy Dispersive Spectroscopy (EDS). The particles also shown, a single-phase anatase indicated at the peak 605.53 cm^{-1} with the average sized range of 100 nm. After exposition of patulin solution containing TiO_2 under UV light, 73.03% of patulin in water was degraded in 160 min. Therefore, photocatalysis is an innovative approach for the degradation of patulin in solution.

Keywords: *Apple Juice, LC-MS/MS, Patulin, TiO_2, Photocatalysis*

4.2 INTRODUCTION

Spoilage in food is usually caused by microbial growth. Microbial growth can produce mycotoxins and others chemical compound which give a spoilage smell to food (Al-Kharousietal, 2016; Pinu et al., 2016). There exist several mycotoxins such as fumonisin, aflatoxin, ochrotoxin, deoxynivalenol, Zeoralenone, and ergotamine (Karlovskyet al., 2016). However, in apple and apple juice, the most studied mycotoxin is patulin. Though it was also found in figs, and mangoes, patulin was showed to predominantly in citrus types fruits and has been determined to be a toxin that can affect humans when ingested from apple juice; hence, international bodies decided on 50 µg/L as the daily intake limit for adults and 10 µg/L for infants (Ji *et al.* , 2017). Methods for mycotoxins avoidance and treatments when producing apple juice involve manual sorting, milling, steeping, and extrusion of the fruits, which can significantly reduce the level of the mycotoxin in the final product (Pinu et al, 2016). However, several methods have been reported for patulin treatment in apples juice. These treatments are of chemical and biological in nature. For example, fermentation, UV treatment, ozone treatment, hydrothermal treatment has been used for patulin degradation. These treatments are usually time consuming, costly and require additional treatments. In addition, the treatment usually reduces the mycotoxin to less toxic by-products. Overall, photocatalysis has showed great efficacy in wastewater treatment for the degradation of innocuous pollutant and dyes, while in the food industry, it has been demonstrated to be effective against microbial deactivation of microorganisms causing food spoilage. Photocatalysis use both nanoparticles and light, in particular UV light. Several nanoparticles exist such as that of Cu, Zn etc. However, TiO_2 has been chosen for these studies because it is inert, non-toxic, cost effective, easy to synthesis and highly reactive. These attributes make photocatalysis using TiO_2 a promising technique for industrial use for the treatment of mycotoxins in apple juice. The aim of this part of the study was to evaluate the efficacy of photocatalysis using TiO_2 in the degradation of patulin in solutions of water and apple juice.

4.3 Material and Methods

4.3.1 Patulin removal

A 5mg patulin standard was purchase from Sigma Aldrich, and was subsequently diluted in 1 L of distilled water, 100 mL of the solution was transferred in a 100 mL volumetric flask and the rest of the solution was stored in the fridge for further use. A mass (0.22 g) of the TiO_2 was added in the flask and keep in the dark and after 30 minutes, the flask was expose to 15 V UV light and sampling was done every 10 minutes of exposure. Patulin degradation efficiency was calculated as in Equation 2:

$$\%\boldsymbol{D} = \frac{\boldsymbol{A_{in}} - \boldsymbol{A_{out}}}{\boldsymbol{A_{in}}}\ x\ 100 \qquad (2)$$

Where A_{in} is the initial concentration of patulin and A_{out} is the concentration of patulin at a given time of exposure.

After the successful reaction of the degradation of patulin in water by the means of photocatalysis, the treatment was proceeded into apple juice, which was the main objective of this part of the study. Apple juice is a complex matrice as compared to water. Therefore, the following was done:

- Designed a reactor suitable for photocatalysis using two (15 V/30 V) UV source,
- Prepared a TiO_2 paste to coat on glass surface to be used in the reactor,
- Etched the glass in acid to mobilise the TiO_2 nanoparticles,
- Clarified the apple juice using bentonite followed by,
- Photocatalysis treatment using a fed batch mode,
- Extracted the patulin from apple juice by solid phase extraction, and prepared it for analysis, and
- Performed LC/MS/MS analysis.

4.3.2 Flatbed reactor design and conception

At the first point, a flatbed reactor was built of polycarbonate material in which two florescent lights was placed across the reactor (Figure 4.1). The long florescent lamps were strategically placed to allow fair distribution of the light within the system. Using as a lock- key system, the head of the light compartment could fit on top of the reactor. Since the reactor was made of a transparent polycarbonate material, a reflective coat (foil paper) outside the reactor was placed to prevent the emission of light out of the system. Consequentially, the light emitted from the light source can reflect into the system to allow for maximum photocatalytic reaction.

Titanium dioxide (TiO_2) particles photocatalytically reacted with the patulin in the juice via photocatalysis. To coat the nanoparticles onto the glass, TiO_2 paste was prepared and paste unto the surface of the glass and characterised by SEM.

Figure 4-1: Flatbed reactor

Table 4-1: flatbed reactor individual components

Components	Names
	Flatbed reactor
	UV light
	Reactor cover
	Glass coated with TiO_2

4.3.3 TiO_2 paste preparation and coating

A mass of 14.4g of TiO_2 was mixed with 12 mL of distilled water, 12 mL of ethanol and 2 mL of acetyl acetone in a mortar and grinded for 20 minutes, after grinding, 2 mL of acetic acid (Merck) and 6 ml of triton X- 100 purchased from Sigma Aldrich was added and the mixture became viscous. The paste was subjected to a Rheological test to measure the shear stress and the viscosity which are the factors affecting the fluidity of a liquid and its attachment and/or fixation to the glass used in the flat-bed reactor. Thus the paste was ready to be coated onto the surface of glass by the doctor blade technique.

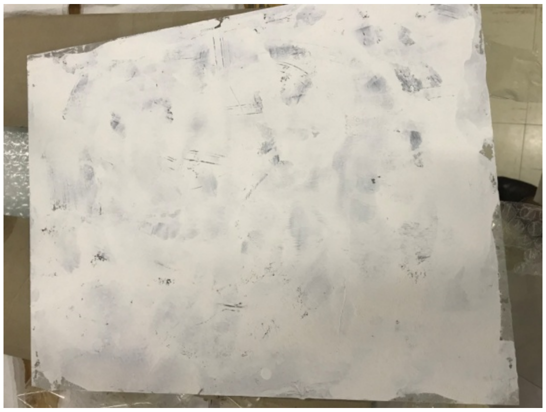

Figure 4-2: Glass coated with TiO_2 paste

4.3.4 Glass etching for reactor

The purpose of using the glasses was to immobilise the TiO_2 to achieve maximum treatment of patulin in apple juice. The glasses were obtained from the builder's warehouse, were smooth and could not bind the NP's without preparing the paste; therefore, the paste was needed post-etching to immobilize the NP's. Thus the glass was place in floric acid which was prepared by adding in 1000 mL of water, with 250 ml of 99.9% H_2SO_4 and 50g of NaF. After two days, the glass was removed and placed in a neutralising solution prepared by dissolving 22.23g of sodium hydroxide pellets and 23.79 g of sodium bicarbonate in 1000mL of water. The pieces of glasses were then coated with a carbon paste and visualised under a SEM device. The results are shown in Figure 4.3.

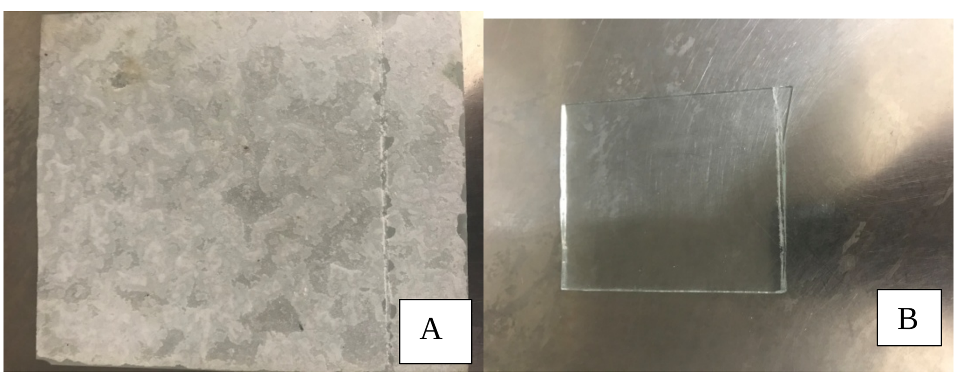

Figure 4-3: Etched piece of glass (A) and plain piece of glass (B)

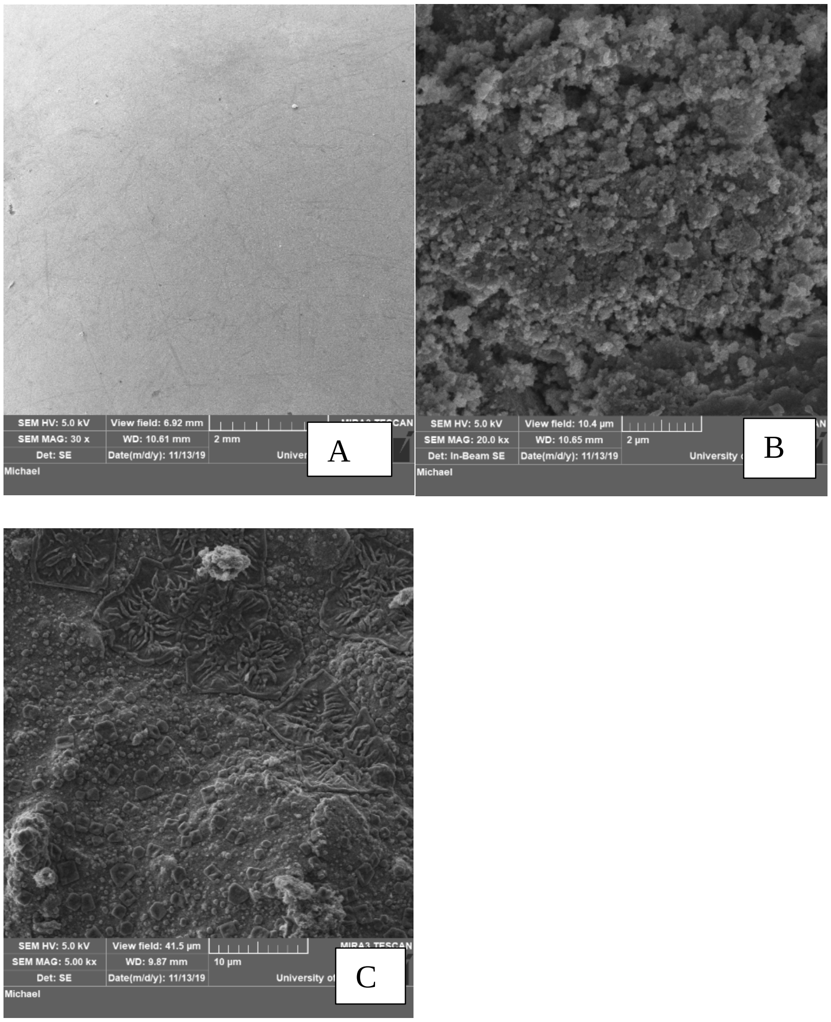

Figure 4-4: SEM analysis of plan piece of glass (A), etched glass (B) and coated glass with TiO_2(C)

4.3.5 Apple juice clarification

The single strength apple juice obtained from the Cape Town cold drink company was a mixture of two types of apples which are branded granny and golden delicious. The cloudy apple juice obtained was clarified to remove all particles which could interfere with the treatment by occupying the active sites of the nanoparticles. Thus, 10 g of bentonite was used for every 1000 mL of apple juice and a clear apple juice was obtained after settling for an hour.

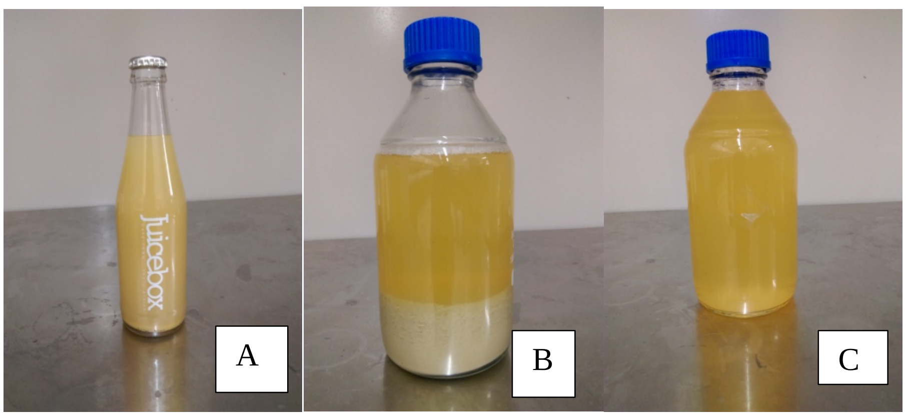

Figure 4-5:Apple juice clarification process: A: sample received, B: apple juice mixed with bentonite after 1hr settlement, C: clear apple juice

4.3.6 Photocatalysis Experimental

4.3.6.1 Chemicals and Reagents

Patulin standard in powder was purchased from Sigma Aldrich. Methanol, ethyl acetate, acetic acid, acetone were also purchased from sigma Aldrich. Ethyl acetate was from Macron fine chemical HPLC grade and hexane was purchased from Honeywell. The study was performed at the Tshwane university of technology (TUT) and the reagents were generously offered by the department of Environmental, Water and Earth Sciences. Polypropylene (PP) membrane filters (0.22 µm, Cameo syringe filters) and syringes, acetonitrile, ammonium acetate, Supelco-Select HLB SPE cartridges (500 mg), were purchased from Sigma-Aldrich (Aston Manor, South Africa).

4.3.6.2 Sample Collection

The apple juice (n= 24x 330 mL) used throughout the study was provided by the juicebox renamed Cape Town cold drink company. The samples were, pasteurised, bottled and ready to be marketed. The samples were kept in room temperature away from sunlight for further usage.

4.3.6.3 Solid phase extraction

Supelco-Select HLB C18- SPE cartridges (500 mg solid phase, 12 mL tubes) (Figure 4.6 A) was prewashed with 10 mL of 99.9 % methanol from Sigma Aldrich and 3 mL of 10% methanol and 10 mL Milli-Q water before utilised. The Supelco-Select HLB C18- SPE cartridges were not allowing to run dry and 4 mL of apple juice with 0.5 mL of acetic acid buffer solution were added onto the Supelco-Select HLB C18- SPE cartridges and allowed to percolate at 2-3 mL / min under gentle suction. The transfer was completed when the solvents was drained to the top of the packing and the Supelco-Select HLB C18- SPE cartridges walls was washed with 5 mL hexane. To avoid cross- contamination, well labelled receiver flasks were placed under the corresponding Supelco-Select HLB C18- SPE cartridges and the Supelco-Select HLB C18- SPE cartridges were eluted with 3x5 mL grade elution solvents (hexane, ethyl acetate, acetone 1:5:4, 1:4:5, 1:3:6). The flow of each solvent was stopped for about a minute to allow the solvent sufficient contact with the Supelco-Select HLB C18- SPE cartridges packing. After elution, 0.5 mL of acetic acid was added and evaporated to dryness under stream of nitrogen (Figure 4.6 B). The residues were reconstituted with 1 mL of acetic acid buffer solution and vortex for 3 minute and transferred in the vials for the injection in the LC/MS/MS for analysis (Lucci et al, 2017; Li et al, 2007).

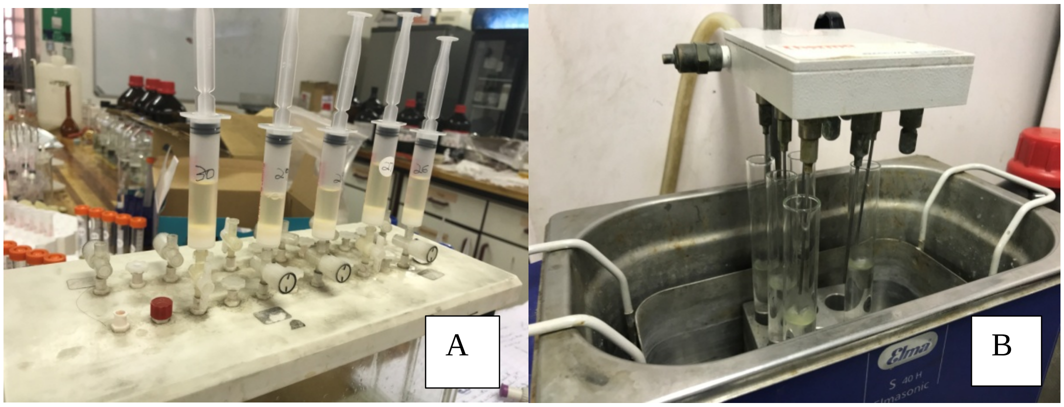

Figure 4-6: SPE extraction device (A) and blow down process (B)

4.4 Results and Discussion

4.4.1 Paste preparation

Figure 4.7 shows that the paste prepared was not a Newtonian fluid. It shows that as the viscosity decreases, the shear stress increases, therefore the paste prepared was a shear thinning material which is good for industrial purposes. The graph shows as well that the paste is smoother and sticky which is suitable for spreading. The paste of TiO_2 was prepared to allow for immobilization onto the glass. The concept of paste preparation started with the construction of the solar cell used for dye degradation for the sole purpose of immobilising nanoparticles onto surface for photocatalysis. This part of the study provided the rheology and the shear stress behaviour of the paste. Ideally, the flux curve for a Newtonian fluid is a straight line through the origin and it's slope represents the viscosity value.

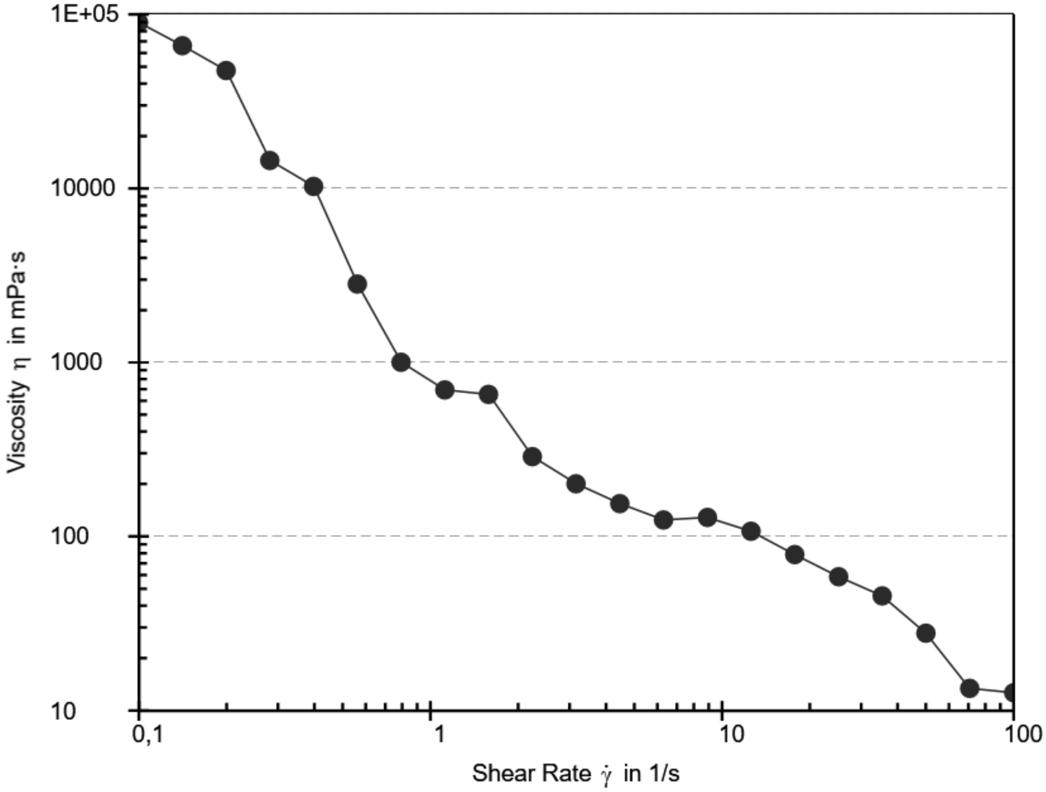

Figure 4-7: Flow curve of Viscosity versus shear rate

4.4.2 LC/MS/MS analysis for patulin determination

4.4.2.1 Sample preparation and irradiation

After connecting all the components of the reactors together, the experiments were performed in a dark room the coated piece of glass was place at the bottom of the reactor as (Figure 4.2). To obtain apple juice with patulin concentration of 1.20 mg/L, 750mL of apple was spiked with 1.8 mL of patulin stock solution of concentration of 500 mg/L. The juice was poured in the reactor and kept in the dark. Then two florescent lights (Philips, the England) were also placed within the flat-bed reactor and after 30 min, the lights were switched on and about 4 ml of sample was collected at the corresponding time interval. The treatment duration was 3 hours, i.e. 180 minutes. The samples collected at different time were centrifuged to remove any NPs that could have enter the juice during sampling subsequent to filtration with 0.2 µm filters. Patulin was extracted using SPE from the juice before being analyse with LC/MS/MS. No stirring process was allowed because it decrease the effectiveness of photocatalysis. Patulin is a carcinogenic compound; therefore, 20% hypochloric acid was used to disinfect all surface area exposed to the patulin.

4.4.2.2 LC/MS-8030 configuration for patulin quantification

Liquid chromatography (LC) was performed on a LC/ MS – 8030 Shimadzu system, USA. The LC was coupled with triple quadrupole linear ion trap tandem mass spectrometrer. The column used for separation was a Luna® Omega polar C18 column (2.1 × 100 mm, 3.0 µm, Phenomenex, Aschaffenburg, Germany). The column temperature was maintained at 40 ^{o} C and the autosampler at 4^{o} C. The mobile phase used were 5 mM of NH_4AC (LC/ MS grade from sigma Aldrich) in Milli-Q water and 99.9% acetonitrile (Sigma Aldrich). The flow rate was 0.3 mL/min on an isocratic mode. The total running time was 4 min with the retention time for patulin being 1 min as shown in (Figure 4.8). The injection volume was 10 µl and the sample were run on a negative mode on an electrospray ionization (ESI) source and the injection were duplicate. 5 mg of patulin powder was dissolved in methanol for a final volume of 10 mL. This gave a concentration of 500ppm or 500 mg/L. From this volume, 10 ppm, 1 ppm and 10 ppb were respectively prepared for the calibration curve (Figure 4.9).

4.4.2.3 Validation of Method

The precision and accuracy of the patulin quantification using the LC/MS/MS was done by preparing some blanks made of pure methanol. These blanks were analysed and run at an interval of five samples to evaluate the level of contamination and errors. The samples were run in a duplicate manner and the limit of detection and quantification were calculated based on signal to noise ratio. In this case the LOD, the corresponding concentration of a value equal or closer to 3 was used to calculate the LOD and the corresponding concentration closer from the signal of noise ratio closer to 10 was used to calculate the LOQ. The LOD in this case was determined to be 0.009 µg/mL and LOQ is 0.017 µg/mL.

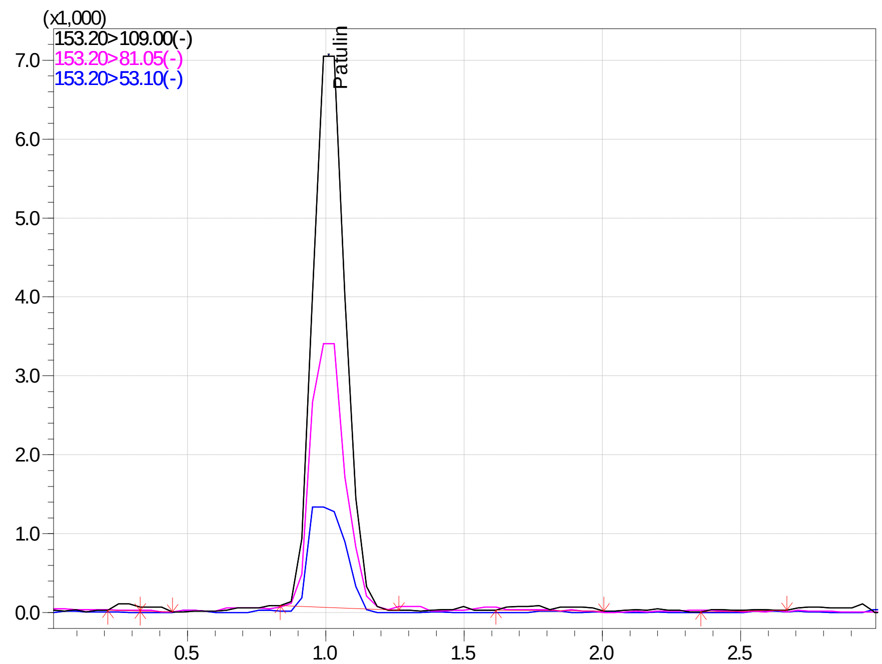

Figure 4-8: Patulin chromatogram and retention time

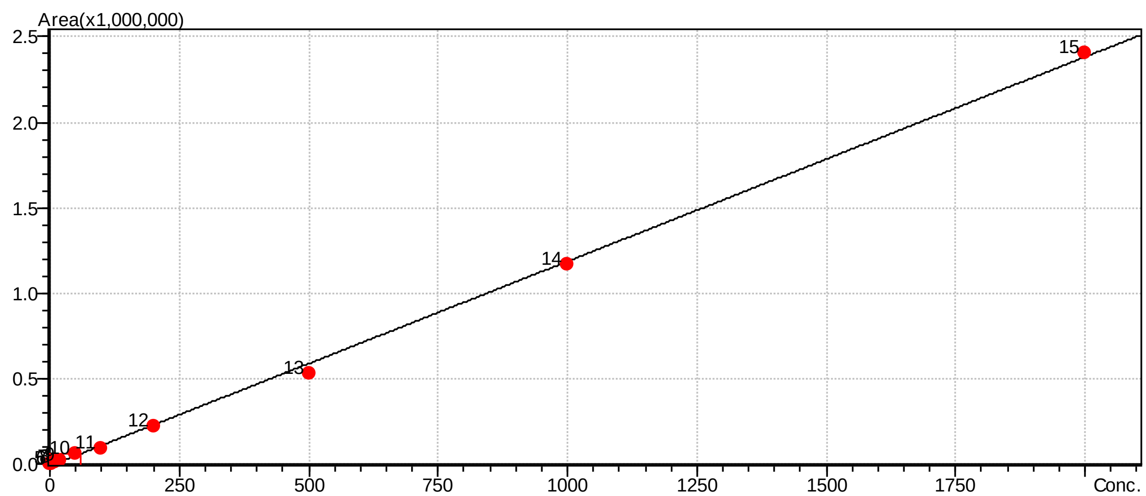

Figure 4-9: Standard preparation and calibration curve

Table 4-2: % degradation of patulin under UV15 and UV 30 in apple juice

Time (Min)	Patulin conc. (ng/L)	% degradation UV 15	Patulin conc. (ng/L)	% degradation UV 30
0	72.576	0	72.576	0
10	62.445	13.959	59.232	18.386
20	53.296	26.565	28.933	60.134
30	52.873	27.151	27.218	62.497
40	44.127	39.191	23.684	67.366
50	43.114	40.594		72.999
			19.596	
60	41.856	42.320	15.912	78.075
90	14.711	79.730		84.082
			11.552	
120	13.019	82.061	10.754	85.182
180	12.362	82.966	8.945	87.674

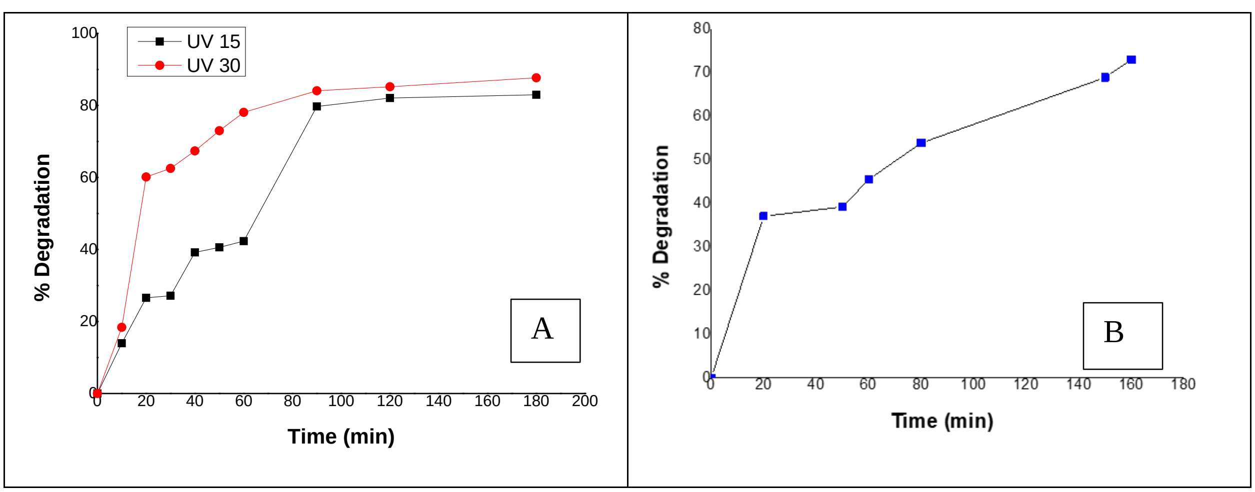

Figure 4-10: A) Patulin degradation curve from UV 15 and UV 30 in apple juice and B) in water (UV 30)

Patulin and nanoparticles were kept in the dark for a period of 30 minutes to allow a great excitation to holes and electrons within the particles in the contact of light. The degradation of patulin was initiated and significant degradation was observed after 20 minutes, and maximum degradation was observed in 160 minutes. The percentage degradation was calculating with Equation 2. Photocatalysis is a promising technique for degradation of toxins even in water as demonstrated. The efficacy of photocatalysis depends upon the catalysts, pH and light intensity. The wavelength between 255 and 355 nm is absorbed by patulin. However, germs and sporulating microorganisms are irradiated under 255 nm (Ibarz et al, 2014). Figure 4.10 shows that percentage degradation of patulin has an exponential increase between 10 to 20 minutes, this could be due to a high excitation of nanoparticles in contact of light after storage in the dark for a period 30 minutes.

Unlike water which is a simple matrice for solutes to dissolve in, apple juice presented some challenges such as cloudiness, to overcome these challenges the juice needed to be clarify in order to prevent interaction of particles with patulin onto the surface of nanoparticles. Another challenge is to extract patulin from the apple juice after treatment in order to quantify. SPE is the most used and adopted method because of the simplicity and the less steps required to perform. Figure 4.10 shows significant decrease in UV 30 V as compared to UV 15V which means that the 30 V light was more effective than the UV 15 V. It was therefore recommended that UV 30 V light be used for the treatment of patulin in apple juice by small scale juicers in South Africa.

4.5 Conclusion

In summary, TiO_2 was synthesised from $TiCl_4$ as a precursor and the particles showed good and great optical properties as catalysts. UV/TiO_2 was demonstrated to have a good symbiotic relationship for patulin degradation, in a suitably designed flat-bed reactor. Additionally, the immobilised TiO_2 can be reused in numerous cycles due to it non-degradability which can give an industrial advantage to small scale juicers. It was shown that photocalysis is effective for patulin treatment in apple juice. This study therefore provides prospective solution for mycotoxin treatment from beverages and especially patulin from apple juice for large and small-scale juice producers.

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The contribution of this work to scientific knowledge was:

The development and assessment of nano semiconductors (nano-materials) TiO_2 for application in UV and/or visible light for rapid photo-oxidation of Patulin. The performance was assessed in the presence of possible interference matrices, ascorbic acid, reducible sugars (glucose, sucrose fructose, etc.) in apple juice without dilution.

The designing of a portable laboratory scale patulin treatment system which uses UV/visible light in a miniaturized mobile patulin treatment unit, which can be used at the source point of the agricultural produce, i.e. to attach it to a juicing process. Overall, this portable unit is able to function using low energy input and be up-scaled to function on-site where the juicing takes place. This creates a platform to stimulate local small scale farmers/juicers, agro-processors to participate in the Bioeconomy. Similarly, the designed system can be used in a variety of industries, especially those that are interested in photo oxidation operations to reduce contaminants.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

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5.1 General Discussion and Conclusion

In summary, patulin is a volatile mycotoxin which is soluble in water. It is produced by fungi and mostly by blue molds in the surface or in the center of apples. These blue molds, produce patulin which is toxic to humans and animals, thus the need to be regulated and treated in apple juice. In this study, it was shown that patulin could be treated via photocatalysis using UV light. The nanoparticles TiO_2 were synthesised from three solubilising matrices and water was concluded to be the best solvent by reason of the quality of the nanoparticles produced. Patulin quantification, detection and method of treatment were developed. In regards to the treatment, the reactor designed – i.e. a flatbed reactor, did not provide any inconvenience for the treatment of patulin with suitable patulin degradation efficiency; however, the apple juice provided was a single strength cloudy apple juice which could not be a representation of different small scale juicers. Prior to juice clarification, the suspended particles in the juice interfered with the treatment thus minimising or slowing the down the degradation process for patulin. Thus, there was a need for clarification, and this was performed by using bentonite to clarify the apple juice. Bentonite is mostly used in wine industry for wine clarification. The other challenge was to immobilize the nanoparticles onto a suitable surface to allow uniform distribution of nanoparticles within the flatbed system and to create a good recovery of nanoparticles after treatment. To troubleshoot this issue, TiO_2 paste was prepared and coated on a surface of glasses by the doctor blade technique. To extract patulin from the matrices, SPE was perform and the patulin extracted was quantify using LC/MS/MS under an isocratic mode. As a result, a significant degradation of patulin was observed and the maximum degradation was observed after 3 hours of treatment. Therefore, photocatalysis is recommended as cheap, fast and simple method for the treatment of patulin in clarified apple juice and ciders for apple juice producers, wine industry and many others.

5.2 General Recommendations

The authors would like to recommend that:

- A nutritional analysis be performed on the juice prior to the photocatalysis treatment and after the treatment to study the variation of the juice quality prior and after treatment.
- The detection and the quantification of by-products of patulin degradation be performed using LC/MS/MS.
- Optimisation studies be performed to determine the quantity of TiO_2 needed for patulin treatment in the flatbed reactor designed.
- Kinetic degradation rates of patulin be performed over time.

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