UNIVERSITI PUTRA MALAYSIA

SURFACE DECONTAMINATION OF FRESH-CUT PENNYWORT (Centella asiatica L.) LEAVES THROUGH PULSED LIGHT TECHNOLOGY AND ACIDIC ELECTROLYSED WATER

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By

SITI ZAHARAH BINTI ROSLI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

June 2022

DEDICATIONS

I wish to dedicate this thesis to Allah, my creator, my pillar of strength, and my source of inspiration, wisdom, knowledge, and understanding. This work is also dedicated to my husband, Mohd Zamzury, our children, Ariff and the twins Aafiya and Affiq, my father, Rosli, my mother, Zoriyah, and members of my family for their never-ending love, understanding, and support throughout my entire life. I cannot express how thankful I am to have each and every one of you in my life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Minimally processed vegetables are in high demand as it is convenient and have fresh-like quality. However, it remains the main cause of foodborne outbreaks. These outbreaks are allied with the consumption of fresh-cut products such as pennywort (*Centella asiatica*) leaves. Pennywort is an herbaceous vegetable that is usually eaten fresh as 'ulam' or salad. Fresh-cut products are exposed to faster many physiological changes that lead to the biochemical and microbiological deterioration that affect the quality and shelf-life. Thus, this research aimed to extend the shelf life of fresh-cut pennywort by the potential applications of pulsed light (PL) treatment and acidic electrolysed water (AEW) in reducing microbial load, retain physicochemical qualities, physiological changes, nutritional values and acceptance of sensory quality during refrigerated storage. The reduction of inoculated *Escherichia coli* on the pennywort leaves after exposure to PL was examined and viewed using the scanning electron microscopy. The effect of sample weight and leaves arrangement using PL fluence on the microbiological properties of fresh-cut pennywort leaves were evaluated. PL at a fluence of 6.9 J/cm^2 was found to be the optimal fluence for reducing total plate counts while keeping the physical qualities of pennywort leaves and increasing shelf life to approximately 12 days, while control sample about 8 days. Unfortunately, higher PL fluence (9.6 and 12.3 J/cm^2) exposure negatively affected the visual quality of the leaves. The inactivation of *E. coli* population was significantly higher at PL fluence of 6.9 J/cm^2 with the value of reduction about 2.27 log CFU/g. Sample weight, sample arrangement and pre-treatment step were able to reduce microbial loads in pennywort leaves and were found necessary to maximise impact of pulsed light. Exposure to PL was effective when smaller sample weight was used, where 5 g > 10 g > 15 g with the significant highest log reduction were 0.64 log CFU/g and 0.55 log CFU/g for TPC and YM, respectively for 5 g. In term of sanitiser efficacy, acidic electrolysed water (AEW) was shown to be the most effective in preserving the physiological and physicochemical properties of pennywort and was highly accepted by consumers based on sensory quality. The combination of PL treatment and AEW indicated a synergistic effect. Higher microbial reduction was achieved about 1.80 and 1.11 log CFU/g (TPC and YM) of fresh-cut pennywort leaves through combination of PL technology and AEW and preserved the fresh-like sensory quality of pennywort leaves. Meanwhile, the bioactive compounds from triterpene glycosides namely, asiaticoside, madecassoside, asiatic acid, and madecassic acid are preserved. In conclusion, the combination of pulsed light technology and acidic electrolysed water was effective to extend shelf life of fresh-cut pennywort leaves up to 16 days by ensuring its safety while maintaining the physicochemical, nutritional and sensory qualities during storage at 4±1 °C.

Keyword: Pulsed light; electrolysed water; leafy greens; *Centella asiatica*; ‘ulam’; shelf life

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENCEGAHAN KONTAMINASI PADA PERMUKAAN POTONGAN DAUN PEGAGA (*Centella asiatica* L.) SEGAR MELALUI TEKNOLOGI CAHAYA DENYUT DAN AIR ELEKTROLISIS BERASID

Oleh

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Sayur-sayuran yang diproses secara minimum mempunyai permintaan yang tinggi kerana ianya mudah disediakan dan masih dalam keadaan segar. Namun, ia merupakan penyebab utama kepada wabak bawaan makanan. Wabak ini berkait rapat dengan pengambilan produk potongan sayur segar seperti daun pegaga (*Centella asiatica*). Pegaga merupakan sayuran herba yang selalu dimakan segar sebagai ulam atau 'salad'. Produk potongan sayur segar yang terdedah mempercepatkan lebih banyak perubahan fisiologi dan menyebabkan kemerosotan biokimia dan mikrobiologi yang menjejaskan kualiti dan jangka hayat produk. Oleh yang demikian, kajian ini bertujuan untuk memanjangkan jangka hayat potongan daun pegaga segar dengan aplikasi berpotensi iaitu rawatan cahaya denyut (PL) dan air elektrolisis berasid (AEW) bagi mengurangkan bilangan mikroorganisma, mengekalkan kualiti fizikokimia, perubahan fisiologi, nilai nutrien dan penerimaan terhadap kualiti deria semasa penyimpanan pada suhu sejuk. Kesan pengurangan *Escherichia coli* yang diinokulasi keatas daun pegaga selepas didedahkan kepada PL telah diperiksa dan diperhatikan menggunakan mikroskop pengimbasan elektron. Kesan kepada saiz berat sampel dan jenis susunan daun menggunakan teknologi PL terhadap sifat mikrobiologikal potongan daun pegaga segar dinilai. PL pada dos 6.9 J/cm^2 didapati sebagai dos yang optimum untuk mengurangkan jumlah kiraan mikroorganisma sambil mengekalkan kualiti fizikal daun pegaga dan memanjangkan jangka hayat kepada 12 hari, manakala sampel kawalan ialah 8 hari. Malangnya, pendedahan terhadap PL pada dos yang tinggi (9.6 and 12.3 J/cm^2) memberi kesan negatif yang menjejaskan kualiti visual daun. ketidakaktifan populasi *E. coli* jauh lebih tinggi pada PL dengan dos 6.9 J/cm² secara signifikan dengan pengurangan sebanyak 2.27 log CFU/g. Pemilihan berat sampel, jenis susunan daun dan langkah pra-rawatan didapati mampu mengurangkan bilangan mikroorganisma pada daun pegaga dan didapati sesuai untuk memaksimumkan kesan cahaya denyut. Hasil kajian menunjukkan pendedahan kepada PL lebih berkesan untuk berat sampel yang lebih kecil, di mana 5 g > 10 g > 15 g dengan pengurangan log yang signifikan sebanyak 0.64 log CFU/g (TPC) dan 0.55 log CFU/g (YM), masing-masing untuk 5 g. Dari segi keberkesanan pembersihan kuman, air elektrolisis berasid (AEW) telah menunjukkan kesan terbaik dalam mengekalkan sifat fisiologi dan fizikokimia potongan daun pegaga segar dan sangat diterima baik oleh pengguna berdasarkan kualiti deria. Kombinasi rawatan PL dan AEW dan menunjukkan kesan sinergistik. Pengurangan bilangan mikroorganisma pada potongan daun pegaga segar telah dicapai lebih tinggi pada 1.80 dan 1.11 log CFU/g (TPC dan YM) melalui kombinasi PL dan AEW dan mengekalkan kualiti deria daun pegaga seperti sayur segar. Sementara itu, kehadiran komponen bioaktif daripada glikosida triterpen, yang dinamakan asiaticoside, madecassoside, medecassic acid, dan asiatic acid dalam potongan daun pegaga tetap terjaga. Kesimpulannya, kombinasi teknologi cahaya denyut dan air elektrolisis berasid adalah berkesan untuk memanjangkan jangka hayat potongan daun pegaga segar sehingga 16 hari dengan memastikan keselamatannya disamping mengekalkan kualiti fizikokimia, nilai nutrien dan penerimaan kualiti deria semasa penyimpanan pada suhu 4±1 ºC.

Kata kunci: Cahaya denyut; air elektrolisis; sayuran berdaun hijau; pegaga; ‘ulam’; jangka hayat

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AEW	Acidic electrolysed water
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASA	Ascorbic acid
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
CFU	Colony Forming Unit
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl 1-picrylhydrazyl
DoA	Department of Agriculture
EW	Electrolysed water
FAMA	Federal Agriculture Marketing Authority
FDA	Food and Drug Association
GC-MS	Gas Chromatography-Mass Spectrometry
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
h^o	Hue angle
HPLC	High Performance Liquid Chromatography
IBS	Institute of Bioscience
IFPA	International Fresh-cut Produce Association
J	Joules
Na_2CO_3	Sodium carbonate
NIST	National Institute of Standards and Technology
MAP	Modified Atmosphere Packaging

O_2	Oxygen
PCA	Plate Count Agar
PDA	Potato Dextrose Agar
POD	Peroxidases
PPO	Polyphenol oxidase
PL	Pulsed light
ppm	parts per million
rpm	Revolutions per minute
SEM	Scanning Electron Microscopy
TC	Total chlorophyll
TEM	Transmission Electron Microscopy
TPC	Total Plate Count
USDA	United State Department of Agriculture
UV	Ultraviolet
UPM	Universiti Putra Malaysia
WHO	World Health organization

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Foodborne pathogens are the main cause of foodborne diseases. Improper control of foodborne pathogens poses a food safety issue and is one of the major public concerns. This is a common issue especially in places with poor hygiene practices, improper food handling and lack of sanitation facilities for food preparation and storage. Study of the sources of contamination and the type of pathogenic etiological agents isolated from fresh fruits and vegetables includes *Bacillus cereus, Salmonella spp., Campylobacter jejuni, Staphylococcus, Clostridium botulinum, E. coli* O157:H7, *Listeria monocytogenes, Shigella,* and *Vibrio cholera* (Balali et al., 2020).

The FDA inspected an outbreak of *Salmonella* Typhimurium contaminations connected with the consumption of bagged salad greens that cause illnesses and hospitalizations in July 2021 (FDA, 2021). There were 550 million cases of foodborne diarrhoeal diseases and 230000 deaths globally each year (WHO, 2016). Public health has been seriously affected by the sharp increase in outbreak cases linked to the consumption of fresh vegetables (Moravkova et al., 2018). Between 2016 to 2018, several outbreaks were reported, involving fresh-cut products such as leafy greens, salad mix, sprouts, cucumbers, papaya, frozen strawberries, prepared fresh and frozen vegetable, pre-cut melons, and dried coconut (Johnson, 2019). These outbreaks are caused by several pathogenic bacteria such as *Salmonella, Escherichia coli*, and *Listeria monocytogenes*, which are linked with fresh-cut products or raw vegetable (Berthold-Pluta et al., 2017). The increase in reported food poisoning cases increase throughout the years reflects the true food safety situation at the same time increase the foodborne disease burden (New et al., 2017).

Meanwhile, minimally processed vegetables have grown in popularity because of their favourable reputation for being both healthful and practical. Consumers choose ready-to-eat goods, such as freshly cut vegetables and fruits due to their busy and complex lifestyles. Vegetables are a great source of vitamins, minerals, and dietary fiber, as well as other important nutrients (Varadaraju, 2019). The WHO recommends a consumption of more than 400 g of fruits and vegetables daily (Jayawardena et al., 2020). Consuming vegetables could help improve gastrointestinal health, lower risk of heart attack, some types of cancer, and chronic illnesses, including diabetes (Ramya & Patel, 2019). In addition, the consumption of raw fruits and vegetables differentially predicted better mental health than the consumption of processed fruits and vegetables even when controlling for demographic, socioeconomic, and health covariates (Brookie et al., 2018).

Malaysia's planted herbaceous area expanded from 2312.42 to 3010.53 hectares with the production increased from 11648.72 to 12389.43 metric tonnes and total of planted area for pennywort in between 27.46 to 33.39 Ha and the production values about 230.75 to 200.09 Mt in 2016-2018 (DoA, 2022b). Herbaceous plant such as pennywort is a popular vegetable in Malaysia. Producing vegetables is a crucial part of farm diversification methods and offers a promising economic prospect for alleviating rural poverty and unemployment in emerging nations (Schreinemachers et al., 2018). Concerns about the sustainability and sufficiency of food production, as well as the consequences for economic development, food security, and nutrition, have guided the development of food and agriculture in Malaysia, as well as related policy interventions throughout many decades (Sundaram et al., 2019). While domestic food production surely contributes to the availability of food in a country, imported items may be more affordable, hence enhancing economic accessibility.

Centella asiatica, commonly referred to as pennywort, is a herbaceous vegetable usually eaten fresh as 'ulam' or salad (Siti Zaharah et al., 2020). In the herbal trade, it is known as Gotu Kola, Asiatic Pennywort or Brahmi and it is a significant element in over 100 health care products on the market (Prasad et al., 2019). Pennywort contains 37 kcal per 100 g, with high potassium (391 mg) and calcium (171 mg), low protein (2%), fat (0.2%), and carbohydrate (6.7%) (Hashim, 2011). Proteins, essential minerals, and other phytonutrients such as tannins, flavonoids, volatile oils, and polyphenol are all present (Chandrika & Prasad Kumara, 2015). Also, its comprises a bioactive constituents including triterpenes, alkaloids, fatty and volatile oils (Mushtaq & Qayyum, 2018).

Additionally, pennywort contains bioactive phytochemicals that promote health, including triterpene glycosides that is asiatic acid, asiaticoside, madecassic acid and madecassoside (Prasad et al., 2019). Pennywort has various therapeutic effects as commonly used to treat several nootropic disorders, amnesia, platelets aggregation, insomnia, psoriasis, hypertonic marks, burn wounds, and skin cell regeneration and curing (Prasad et al., 2019; Ruszymah et al., 2012). These health promoting advantages of leafy greens such as pennywort has been associated with an increased in fresh fruits and vegetables intake. Similar consumption trend was observed for products in the fresh-cut produce as they can provide better nutrition for consumers (Yildiz et al., 2019; Oliveira, 2012).

Unfortunately, processing procedures can hasten degradation of the product due to cutting, peeling, cleaning and packing processes; causing biochemical, sensory and microbiological changes on plant tissue surfaces (Botondi et al., 2021). Freshly cut fruits and vegetables are one example of a little processed product that is susceptible to quality loss due to its perishable nature. The processing operations usually reduce the shelf life of fresh-cut fruits and vegetables through tissue softening, cut surface browning, decreased nutritional value, presence of off flavour and microbiological spoilage during storage (Finnegan & O'Beirne, 2015; Kim et al., 2014; Curutchet et al., 2014). Biological contamination can be primarily hazardous, occurring when the infectious organisms directly contaminate raw materials through cross-contamination during food preparation (Botondi et al., 2021). This study is important to assess the ability of non-thermal technology such as pulsed light (PL) to produce convenient and have fresh-like quality, reduce the microbial activity and retain the physicochemical quality of fresh-cut pennywort leaves which can provide basic knowledge for food preservation.

1.2 Problem statements

In both industrialised and developing nations around the world, foodborne disease outbreaks are a major public health concern. Recent years have seen an increase in the number of foodborne illness outbreaks linked to fresh and fresh-cut produce (Yu et al., 2018). Fresh-cut fruits and vegetables are in high demand due to their convenience and fresh-like quality. Nonetheless, processing activities reduce the storage life of fresh-cut fruits and vegetables (Finnegan & O'Beirne, 2015; Kim et al., 2014). New approaches to fruits and vegetables sanitation that uses advanced technologies such as cold plasma, ozone, high hydrostatic pressure and ultrasound have improved efficacy in removing microorganisms and pesticides (Bhilwadikar et al., 2019). Non-thermal technique such as pulsed light technology may be used to inactivate various spoilage and pathogenic microorganisms in various foods have minimal impact on the quality attributes (John & Ramaswamy, 2018). This method relies on intense short-duration broad-spectrum light (Avalos-Llano et al., 2018).

The application of PL treatments to extend freshness of products through inactivation of pathogens, elimination of spoilage microorganisms and retention sensorial or nutritional properties were reported (Koh et al., 2017; Valdivia-Nájar et al., 2017; Agüero et al., 2016). Multiple ultraviolet C (UV-C) treatments can be an effective alternative to maintain the quality and to enhance the shelf life of postharvest leaf vegetable (Liao et al., 2016). Additionally, PL technology has been shown to reduce microbial contamination and extend the shelf life of certain fruits and vegetables, such as apple, blueberries, lettuce, spinach, and tomato (Agüero et al., 2016; Avalos Llano et al., 2016; Cao et al., 2017; Tao et al., 2019; Leng et al., 2020). Several research have reported the possible application of PL treatments to inactivate pathogens with minimum impact on sensory or nutritional characteristics in order to improve shelf life (Koh et al., 2017; Valdivia-Nájar et al., 2017; Agüero et al., 2016; Chen et al., 2015). Since the benefits will allow for enough time for retailing of the products and hence reduce waste, the prospective application of PL treatment for fresh-cut produce shelf life extension should be investigated. Therefore, both manufacturers and consumers will profit from this application.

On the other hand, postharvest decontamination steps such as water washing are essential for reducing dirt, and microbial load from the fresh vegetable (Warriner & Namvar, 2014). Washing fresh vegetables and fruit with electrolysed water has shown promise as a way to kill germs and could be used as an effective sanitiser (Chen et al., 2019). EW is one of the prospective alternatives to sodium hypochlorite usage with an environmentally friendly broad-spectrum microbial disinfection in the food industry furthermore, there is no need to use hazardous chemicals (Sapers, 2014). EW washing decreases cleaning times, is ready to use, and is safer and healthier (Turantaş et al., 2018). Pennywort has never been investigated as a minimally processed food treated with PL and electrolysed water, despite its popularity as a raw salad ingredient. The objective of this study is to determine the influence of PL at varying fluences in combination with acidic electrolysed water on the microbiological, physicochemical, and sensory quality of fresh-cut pennywort (*Centella asiatica*) leaves during storage.

1.3 Objectives

The overall objective of this research is to assess the possible benefits of pulsed light treatment and acidic electrolysed water in extending the shelf life of fresh-cut pennywort leaves as ‘ulam’. Consequently, the following are the specific objectives of this study:

1. To examine the efficacy of pulsed light treatment in lowering microbial load and preserving the quality of fresh-cut pennywort leaves during refrigerated storage.

2. To determine the effect of sample weight and layering on microbial reduction in pulsed light treated fresh-cut pennywort leaves.

3. To examine the efficacy of sanitiser solutions on the microbiological, physicochemical, and sensory aspects of fresh-cut pennywort leaves.

4. To investigate the combined effect of pulsed light technology and acidic electrolysed water on the storage stability of fresh-cut pennywort leaves.

5. To determine the effect of pulsed light technology and acidic electrolysed water on the sensory, morphology and bioactive compound quality of fresh-cut pennywort leaves.

Figure 1.1 shows an overview of the research project. The goals of chapters 4 and 5 are to find ways to solve the problems found in chapter 3. After figuring out how to solve the problems, chapter 6 was about how to use the combinations technique to improve the findings and maintain the freshness of fresh-cut pennywort leaves. The bioactive compound and other quality were investigated in chapter 7.

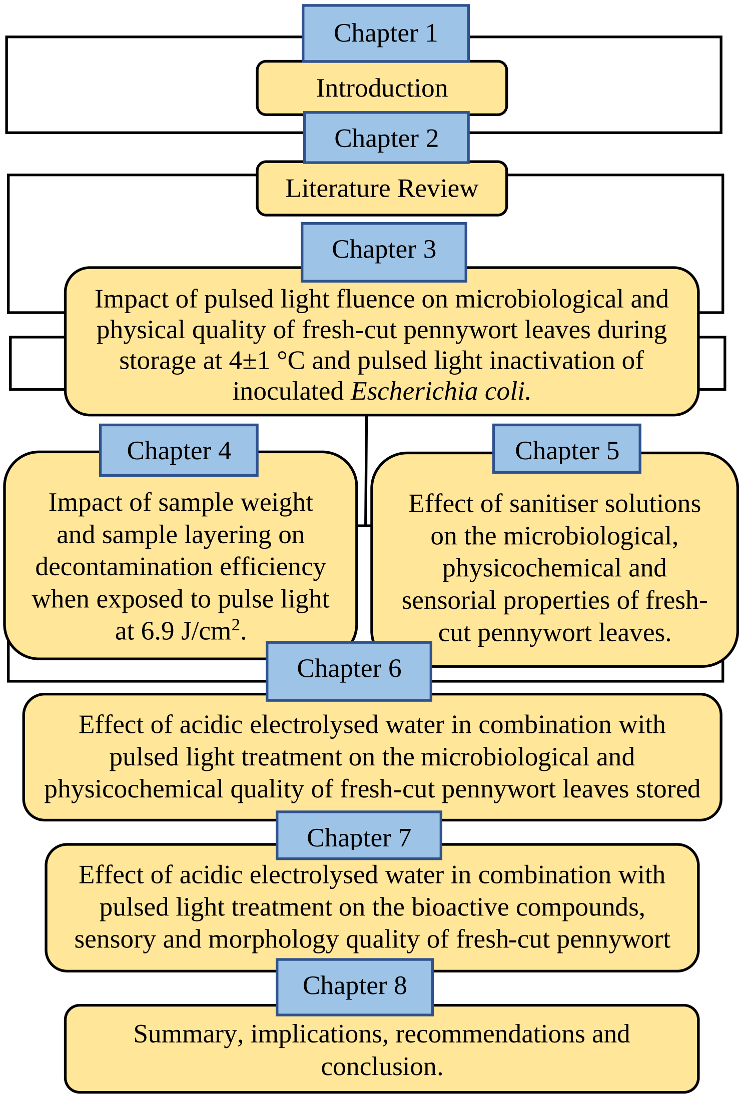

Figure 1.1: Research project overview

1.4 Scope of study

Figure 1.2 is a flowchart illustrating the research activities necessary to identify the quality of fresh-cut pennywort. The first objective was to investigate the effect of PL fluencies on the microbiological and physical quality of fresh-cut pennywort leaves stored at 4±1 °C, as well as the effectiveness of PL to inactivate the inoculated *Escherichia coli* in the samples. Following that, the effect of sample weight and sample layering on decontamination efficiency when exposed to pulsed light fluence of 6.9 J/cm² were examined. The third purpose was to assess the efficacy of sanitiser solutions on microbial reduction when washing pennywort leaves, as washing is a vital stage in the processing of fresh-cut leaves. After washing, the physicochemical and sensory qualities were also assessed.

Based on the results of the third objective, the impact of pulsed light treatment combined with acidic electrolysed water was assessed, with a focus on the microbiological and physicochemical quality of fresh-cut pennywort leaves kept at 4±1 °C. The last goal was to look into how the combined treatment affected the morphological quality, sensory qualities, and bioactive components of freshly cut pennywort leaves.

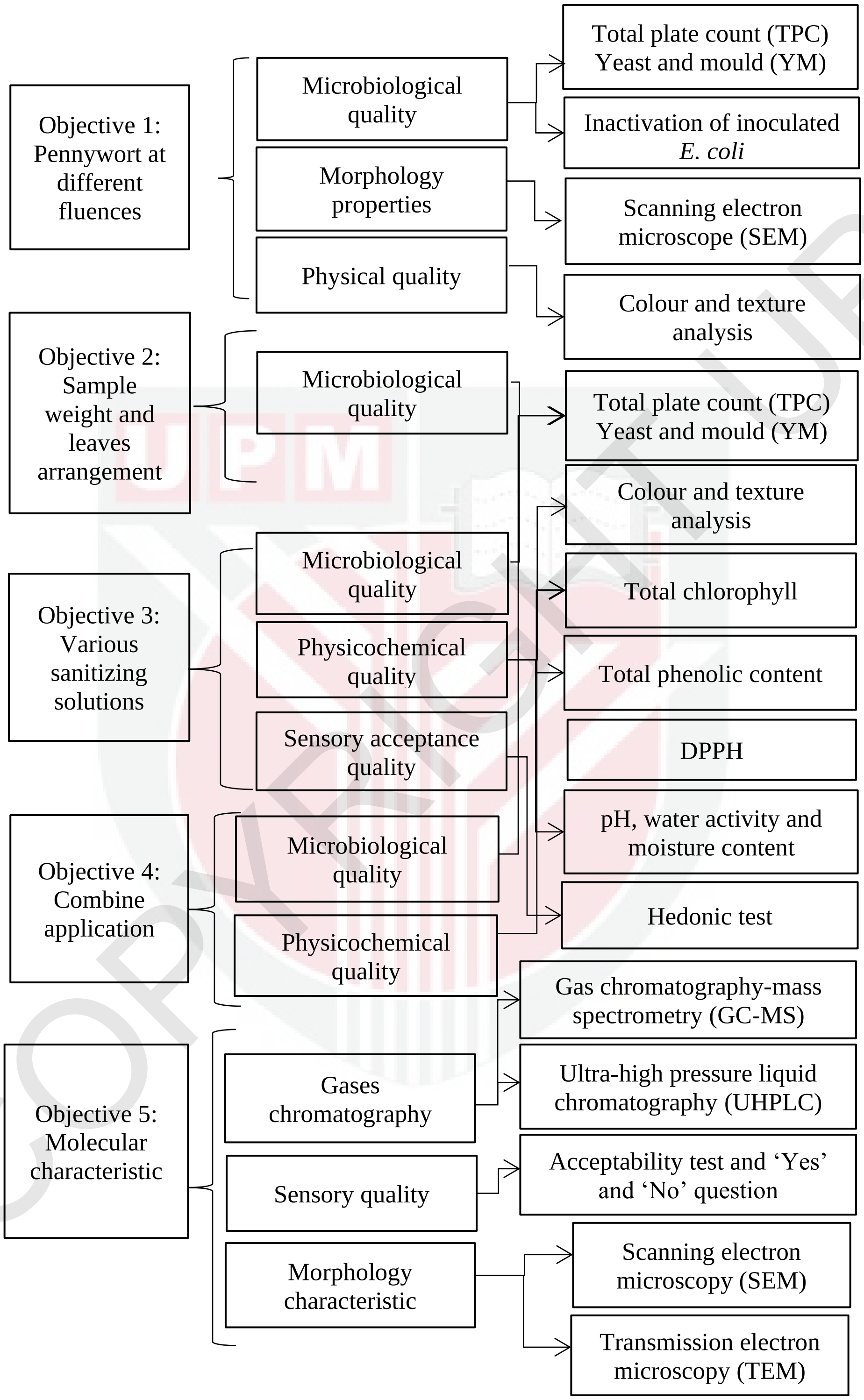

Figure 1.2: Flow chart activities in the study

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