

**ETHANOL EXTRACTION, PURIFICATION,
ANTIOXIDANT PROPERTIES, AND IN VIVO
ANTI-AGEING EFFECTS OF WHITE TEA
FLAVONOIDS**

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ANTI-AGEING EFFECTS OF WHITE TEA
FLAVONOIDS**

by

YANG YUHUA

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LIST OF SYMBOLS AND ABBREVIATIONS

SMT	<i>Shou Mei</i>
GMT	<i>Gong Mei</i>
WPT	White Peony
SNT	Silver Needle
EC	Ethanol Concentration
LSR	Liquid-Solid Ratio
UP	Ultrasonic Power
ET	Extraction Time
ABTS	2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)
DPPH	1,1-diphenyl-2-picrylhydrazyl
SOD	Superoxide Dismutase
CAT	Catalase
MDA	Malondialdehyde
MTH	Methuselah
CFWTE	Crude flavonoid white tea extract
PFWTE	Purified flavonoid white tea extract
HPLC	High-performance liquid chromatograph
Vit C	Ascorbic acid
CTL	Control group
LDG	Low dose group
MDG	Medium dose group
HDG	High dose group
H ₂ O ₂	Hydrogen peroxide

**PENGEKSTRAKAN BERASASKAN ETANOL, PENULENAN, SIFAT
ANTIOKSIDAN, DAN KESAN ANTI-PENUAAN SECARA IN VIVO
FLAVONOID TEH PUTIH**

ABSTRAK

Berdasarkan perbezaan dalam kualiti, teh putih boleh dibahagikan kepada empat gred: "*Shou Mei*" (SMT), "*Gong Mei*" (GMT), "*Bai Mu Dan*" ("*White Peony*", WPT), dan "*Bai Hao Yin Zhen*" ("*Silver Needle*", SNT). Teh-teh ini dilaporkan mempunyai pelbagai bioaktiviti yang kuat, seperti antioksidan, anti-radang, anti-mutagenik, dan aktiviti anti-kanser. Kajian mengenai kesan anti-penuaan teh putih masih agak kurang. Fungsi kesihatan ini dapat dikaitkan kembali kepada flavonoid yang secara semulajadi terdapat dalam teh. Kajian ini bertujuan untuk menyiasat kesan anti-penuaan daripada flavonoid tulen daripada teh putih. Untuk mencapai tujuan ini, kajian ini dibahagikan kepada 3 fasa. Untuk Fasa 1, empat gred teh putih telah dikenakan kepada pengekstrakan flavonoid dengan bantuan ultrasonik. Kesan kepekatan etanol (X_1), nisbah cecair-pepejal (X_2), suhu pengekstrakan (X_3), dan kuasa ultrasonik (X_4) terhadap hasil pengekstrakan ditentukan melalui analisis faktor tunggal dan permukaan respons. Dengan menggunakan parameter proses pengekstrakan flavonoid yang optimum, hasil pengekstrakan dalam urutan menaik adalah GMT < SMT < WPT < SNT. Parameter pengekstrakan yang dioptimumkan untuk SMT adalah 60% (X_1), 42:1 (X_2), 130 W (X_3), dan 45 min (X_4). Selepas mempertimbangkan kos dan ketersediaan bahan mentah, ekstrak teh putih flavonoid mentah (CFWTE) daripada SMT dipilih untuk penulenan (Fasa 2). AB-8 dipilih untuk penulenan flavonoid berdasarkan prestasi penyerapan dan penyaherapan yang baik. Proses penulenan yang optimal ditentukan adalah seperti berikut: suhu

penyerapan (25 °C), pH 3, kepekatan etanol (80%), kadar aliran pemuatan sampel dan kadar aliran elusi (1.5 BV/min), jumlah elusi (40 BV). Di bawah parameter penulenan ini, ketulenan flavonoid total dalam ekstrak teh putih flavonoid tulen (PFWTE) meningkat dari 17.69 ± 0.62 menjadi $46.23 \pm 0.82\%$, mencapai peningkatan sebanyak 2.61 kali. Dalam Fasa 3, HPLC mengkuantifikasikan 4 sebatian flavonoid, rutin, myricetin, quercetin, dan kaempferol, dalam CFWTE dan PFWTE. Kedua-dua ekstrak menunjukkan peningkatan kadar penyingkiran radikal bebas DPPH, radikal bebas hidroksil, radikal bebas anion superoksida, dan radikal bebas ABTS seiring dengan peningkatan kepekatan. Fasa terakhir dalam kajian ini meneroka kesan anti-penuaan PFWTE menggunakan lalat buah (*Drosophila melanogaster*) sebagai model haiwan. Keputusan menunjukkan bahawa PFWTE mempunyai kesan antioksidan yang lebih kuat pada lalat buah berbanding dengan kumpulan kawalan (tanpa PFWTE). Pada kepekatan 3 mg/mL PFWTE, hari kelangsungan hidup 50%, jangka hayat purata, dan jangka hayat maksimum lalat buah jantan meningkat sebanyak 15.75, 15.41, dan 21.05%, masing-masing. Secara mengejutkan, pada kepekatan yang sama, hari kelangsungan hidup 50%, jangka hayat purata, dan jangka hayat maksimum lalat buah betina meningkat sebanyak 34.48, 30.39, dan 32.27%, masing-masing. Keupayaan menentang tekanan oksidatif hidroksil pada lalat buah ditingkatkan secara signifikan ($P < 0.01$) dalam kedua-dua kumpulan dos sederhana dan tinggi. Aktiviti enzim antioksidan dalam kumpulan yang dirawat dengan PFWTE meningkat secara signifikan ($P < 0.05$), dan kandungan MDA berkurangan secara signifikan ($P < 0.05$). Ekspresi gen-gen berkaitan antioksidan SOD1, SOD2, dan CAT dinaikkan secara signifikan ($P < 0.05$) pada lalat buah, sementara gen MTH diturunkan secara signifikan ($P < 0.05$). Kesimpulannya,

PFWTE memiliki sifat antioksidan *in vitro* yang baik dan kesan anti-penuaan *in vivo* yang menjanjikan, yang mungkin membantu melambatkan penuaan pada lalat buah.

**ETHANOL EXTRACTION, PURIFICATION, ANTIOXIDANT
PROPERTIES, AND IN VIVO ANTI-AGEING EFFECTS OF WHITE TEA
FLAVONOIDS**

ABSTRACT

Based on the differences in qualities, white tea can be divided into four grades: “*Shou Mei*” (SMT), “*Gong Mei*” (GMT), “*Bai Mu Dan*” (White Peony, WPT), and “*Bai Hao Yin Zhen*” (Silver Needle, SNT). These teas were reported to have various potent bioactivities, such as antioxidant, anti-inflammatory, and anti-cancer activities. These health functionalities can be traced back to flavonoids found naturally in tea. This study aims to investigate the anti-ageing effects of purified flavonoids from white tea. To achieve this aim, this study was separated into 3 phases. For Phase 1, four grades of white teas were subjected to ultrasonic-assisted flavonoid extraction. The effects of ethanol concentration (X_1), liquid-solid ratio (X_2), extraction temperature (X_3), and ultrasonic power (X_4) on the extraction yield were determined through single-factor and response surface analyses. Under the optimal flavonoid extraction process parameters, the extraction yield in ascending order is GMT < SMT < WPT < SNT. The optimised extraction parameters for SMT are 60% (X_1), 42:1 (X_2), 130 W (X_3), and 45 min (X_4). After considering the cost and availability of the tea leaves, crude flavonoid white tea extract (CFWTE) from SMT was selected for purification (Phase 2). AB-8 macroporous resin was chosen for flavonoid purification based on its good adsorption and desorption performance. The optimal purification process determined was as follows: adsorption temperature (25°C), pH 3, ethanol concentration (80%), sample loading flowrate and elution flowrate (1.5 BV/min), and elution volume (40 BV). Under this purification

parameter, the purity of the total flavonoids in the purified flavonoid white tea extract (PFWTE) increased from 17.69 ± 0.62 to $46.23 \pm 0.82\%$, achieving a 261% improvement. In Phase 3, HPLC quantified 4 flavonoid compounds, rutin, myricetin, quercetin, and kaempferol, in CFWTE and PFWTE. Both extracts exhibited an increasing scavenging rate towards DPPH free radicals, hydroxyl free radicals, superoxide anion free radicals, and ABTS free radicals as the concentration increased. The last phase of this study explored the anti-ageing effects of PFWTE using fruit flies (*Drosophila melanogaster*) as the animal model. The results showed that PFWTE had a more potent antioxidant effect on the fruit flies when compared with the control group (without PFWTE). At 3 mg/mL of PFWTE, the 50% survival days, mean lifespan, and maximum lifespan of male fruit flies were increased by 15.75, 15.41, and 21.05%, respectively. Surprisingly, at the same concentration, the 50% survival days, mean lifespan, and maximum lifespan of female fruit flies were increased by 34.48, 30.39, and 32.27%, respectively. The activity of antioxidant enzymes in the PFWTE-treated group was significantly increased ($P < 0.05$), and the content of MDA was significantly reduced ($P < 0.05$). The expression of antioxidant-related genes SOD1, SOD2, and CAT were up-regulated significantly ($P < 0.05$) in fruit flies, while MTH genes were significantly down-regulated ($P < 0.05$). In summary, PFWTE has good in vitro antioxidant properties and a promising in vivo anti-ageing effect, which may help delay fruit flies' ageing.

CHAPTER 1

INTRODUCTION

1.1 Research background

Ageing is a gradual decline in various physiological functions of the organism over time, influenced by genetic, lifestyle, and environmental factors. Additionally, ageing often impacts various bodily functions, such as decreased physical and reproductive capabilities, and these processes are associated with free radicals (Ajagun-Ogunleye & Ebuehi, 2020). Currently, the ageing mechanism needs to be clearly understood, and the most broadly recognised hypothesis is the free radical theory. Harman first proposed this theory in 1956, pointing out that the body maintains a dynamic balance through the constant production and removal of free radicals. Oxygen-free radicals accumulate in the body as age advances, leading to an imbalance and disrupting cellular structures. This accumulation results in lipid peroxidation and cross-linking of proteins and nucleic acid molecules (Jo & Imm, 2017).

Additionally, there is a decline in enzymatic activity and issues such as alterations in DNA sequence or irregular duplication. These mechanisms disrupt regular metabolic functions, play a role in the development of illnesses, and hasten the ageing process in humans. Simultaneously, the enzymatic antioxidant defence system in the body includes the production of enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and peroxiredoxins. These enzymatic antioxidant systems work collaboratively and coordinate to protect cells from the damaging effects of oxidative stress (Ma et al., 2019). They maintain a stable level of reactive oxygen species (ROS) within cells, preserving cellular health and slowing ageing.

Consequently, the search for natural and safe antioxidants that effectively prevent and treat diseases has garnered increasing attention.

Tea is the second most widely consumed beverage after water, owing to its health benefits and satisfying sensory experiences. White tea, classified as lightly fermented tea, is one of the six major categories of tea in China. White tea is categorised into Silver Needle, White Peony, “*Shou Mei*”, and “*Gong Mei*” based on different standards for picking fresh leaves. It is mainly produced in Fuding, Zhenghe, Songxi, and Jianyang, Fujian, China. Compared to other types of tea, the processing of white tea is relatively simple, involving only two steps: withering and drying. The straightforward and natural production process of white tea imparts it with a unique aroma, flavour, and distinctive medicinal and health benefits. Currently, research by scientists both domestically and internationally on the chemical composition of white tea indicates that it primarily contains various functional and nutritional components such as amino acids, tea polyphenols, tea pigments, flavonol glycosides, tea polysaccharides, alkaloids, trace elements, vitamins, aromatic substances, and more (Pastoriza et al., 2017). Research on the medicinal value of active components in white tea has indicated various health benefits in recent years. These include antioxidant activity, anti-ageing effects, anti-cancer and anti-tumour properties, mutation resistance, blood pressure regulation, blood sugar control, lipid-lowering effects, free radical scavenging, antibacterial and anti-inflammatory properties, prevention of cardiovascular diseases, protection against UV radiation, invigorating effects, anti-fatigue properties, and enhancement of memory (Hinojosa-Nogueira et al., 2021). Studies on the pharmacological effects and mechanisms of white tea primarily focus on these aspects. In vitro experiments by Zhao et al. (2019) indicated that the infusions of 30 selected teas were prepared

mimicking daily practices, and their antioxidant activities were evaluated using different determination methods. This study investigates the anti-ageing properties of white tea flavonoids on the lifespan of *Drosophila melanogaster*. The research could further elucidate the mechanisms behind the life-extending effects of white tea flavonoid, laying the groundwork for the future development of natural plant resources using animal model systems.

1.2 Research objectives

The primary objective of this study is to obtain the optimal crude flavonoid extraction rate from different varieties of white tea (Silver Needle, White Peony, “*Shou Mei*”, and “*Gong Mei*”). Subsequently, the crude flavonoid white tea extraction was further purified using macroporous adsorption resin. A comparison was made between the in vitro antioxidant activities of the crude flavonoids white tea extraction and purified flavonoids white tea extraction. Additionally, the study aims to investigate the in vivo anti-ageing effects of the purified flavonoid white tea extraction in fruit flies. The specific goals of this research are as follows:

1. To optimise the ultrasonic-assisted extraction parameters to obtain crude extract with the highest extraction yield from 4 grades of white tea.
2. To investigate the most suitable purification conditions based on adsorption and desorption performance.
3. To determine the in vitro antioxidant activities and efficacy of purified tea flavonoids in comparison to crude extract.
4. To investigate the in vivo antioxidant efficacy of the purified tea flavonoids as an anti-ageing model using fruit flies.

1.3 Outline of thesis

CHAPTER ONE provides a general introduction to the research background of this project, including an overview of the development of tea, the current state of research on the functionality of tea, and a summary of the technical approach and critical points of the present study.

CHAPTER TWO is a literature review based on the current study. It discusses the tea classification, the compositional elements of tea, the structure of tea flavonoids, the classification of flavonoids, the functionality of tea, methods for extracting and purifying flavonoids, and an introduction to the in vitro antioxidant activity and in vivo anti-ageing activity.

CHAPTER THREE uses ultrasound-assisted extraction of total flavonoids from four types of Zhenghe white tea (Silver Needle, White Peony, *Shou Mei*, and *Gong Mei*). Factors such as ethanol concentration, liquid-to-solid ratio, extraction temperature, ultrasonic power, and extraction time are chosen as single-factor experimental parameters. After analysing the factors significantly influencing the response value, a four-factor, three-level response surface optimisation experiment is conducted. The optimal extraction process for total flavonoids from the four types of Zhenghe white tea is determined, with a particular emphasis on “*Shou Mei*” as the subsequent research subject.

CHAPTER FOUR builds upon the findings of chapter three and investigates the purification process of crude flavonoid white tea extract (CFWTE) using macroporous adsorption resin. The optimal resin is selected from five types of macroporous adsorption resins based on the adsorption and desorption rates of

CFWTE. Parameters such as sample solution pH 3, adsorption temperature, desorption agent concentration, sample solution flow rate, desorption flow rate, and desorption agent volume are examined for their impact on the adsorption and desorption performance. Subsequently, the purification process is determined based on these factors.

CHAPTER FIVE focuses on the in vitro antioxidant activities of CFWTE and PFWTE, including their abilities to scavenge hydroxyl radicals, superoxide radicals, DPPH radicals, and ABTS radicals. The flavonoid components and contents in CFWTE and PFWTE are analysed using HPLC. The in vivo antioxidant activity of purified flavonoid white tea extract (PFWTE) and its effects on the anti-ageing model using *Drosophila melanogaster*. Different doses of PFWTE are added to the basic culture medium. The evaluation includes measurements of fruit flies gustatory, lifespan, climbing ability, antioxidant stress resistance activity, antioxidant enzyme activity, and mRNA expression levels of anti-ageing related genes. By observing various indicators in *Drosophila*, this chapter aims to enhance the in vivo antioxidant capacity of PFWTE and assess their anti-ageing activity in the fruit fly model.

CHAPTER SIX provides an overall summary and conclusion of the present study and recommendations for future research directions in this field.

CHAPTER 2

LITERATURE REVIEW

2.1 Tea

Tea is a common beverage made from the leaves, buds, and tender stems of the *Camellia sinensis* plant. It is used as a plant with medicinal properties. For several centuries, the commercialisation of tea commenced along Chinese trade routes, experiencing rapid global expansion. It traversed different Asian regions and was transported to Europe and the Americas via commercial sailing vessels. Tea carries unique cultural meaning across diverse backgrounds. Its delightful sensory qualities and wide-ranging health benefits have contributed to its enduring popularity as a beverage. The tea industry is expanding rapidly, with global annual tea production and consumption reaching approximately three billion kilograms. Tea is cultivated in at least 45 countries worldwide, with China taking a substantial share. Significant contributions come from countries such as India, Turkey, Japan, Vietnam, and Indonesia (Pastoriza et al., 2017).

Following the harvest, tea leaves undergo processing into six distinct varieties using different techniques based on the processing method. These six types of tea are categorised according to the fermentation/oxidation process (Figure 2.1). Tea leaves acquire unique taste, aroma, visual characteristics, and specific chemical properties throughout each processing stage.

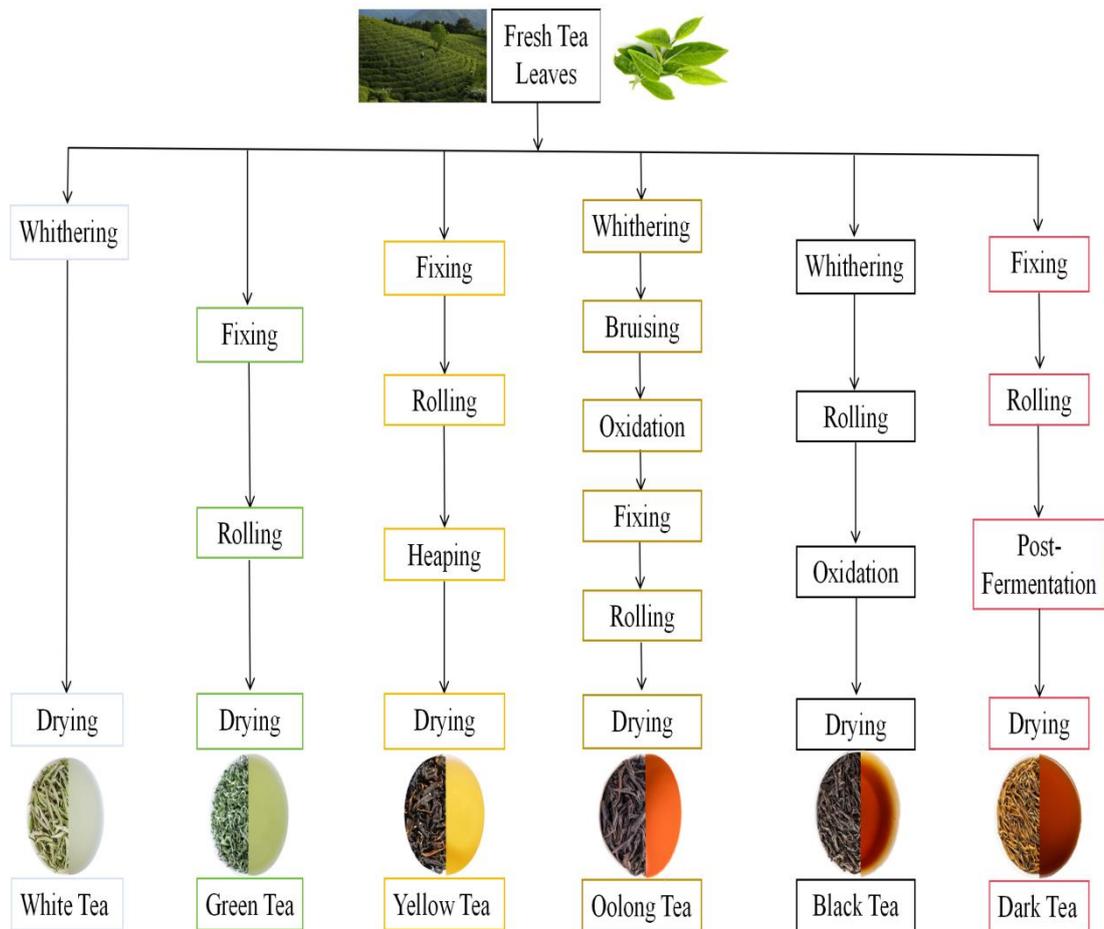


Figure 2.1 Six types of tea processing (Hinojosa-Nogueira et al., 2021)

The six types of tea are black tea, green tea, Oolong tea, yellow tea, dark tea, and white tea. The processing of black tea mainly involves oxidation fermentation facilitated by 80-95% polyphenol oxidase, endowing it with a smoother taste, a prolonged fragrance, and a rich, mellow flavour (Tanaka & Matsuo, 2020). Simply put, green tea is produced by subjecting fresh tea leaves to three main steps: fixation, rolling, and drying. This process deactivates enzyme activity, allowing green tea to exhibit its characteristic qualities of a fresh and robust flavour profile unaffected by enzymatic influences (Suzuki et al., 2016). Oolong and yellow tea are both types of tea that undergo partial oxidation. Oolong tea typically has an oxidation level of 10-

20%, resulting in a rich, sweet, and fresh taste profile with a lingering sweetness. Yellow tea, with an oxidation level of 30–60%, employs high-temperature fixation to deactivate enzymes and produce coloured compounds, resulting in a yellow-coloured infusion and leaves (Fang et al., 2019). Dark tea is crafted through an external microbial post-fermentation process, giving it a wealth of substances such as vitamins, minerals, proteins, amino acids, and sugars. This unique method also contributes to dark tea's distinct aged and fragrant flavour characteristics (Zhu et al., 2020). White tea is crafted from tender buds and young leaves adorned with downy white hairs, lending the tea its name. Subject to minimal processing, white tea retains elevated levels of antioxidant properties. It imparts a unique, gentle, and sweet taste with hints of floral and fruity aromas (Hao et al., 2023). The last stage in the production of the six types of tea mentioned involves drying. The varying types of tea and the differences in drying temperatures lead to changes in the composition of the tea leaves, particularly breaking down heat-resistant molecules. As a result, different types of tea exhibit distinct characteristics.

White tea is mainly produced in Fuding, Zhenghe, Jianyang, and Songxi in Fujian province, China. Based on the freshness and variety of the leaves, they can be categorised into “*Bai Hao Yin Zhen*” (White Silver Needle), “*Bai Mu Dan*” (White Peony), “*Gong Mei*”, and “*Shou Mei*”. The presence of buds characterises fresh-leaf tea and leaves from the same stem, displaying a deep green colour with a silver-white lustre. It possesses internal qualities that are fresh and sweet, accompanied by a noticeable and gentle fragrance. White Silver Needle is a finished tea made from whole and robust tea buds, named for its silver-white colour and needle-like appearance. White Peony is a finished tea crafted using one bud and one or two leaves as raw materials. “*Gong Mei*” is a finished tea made from plucking one bud

and leaves from a specific tea variety. The raw material for “*Shou Mei*” consists of the leaves remaining after removing the buds from the tender shoots picked during the production of White Silver Needle.

Compared to other types of tea, the processing of white tea is relatively simple, involving only two main steps: withering and drying. The straightforward and natural production process of white tea imparts unique aroma and flavour qualities and distinct medicinal and health benefits. It contains various beneficial components and functions for the body. At present, the chemical composition of white tea predominantly comprises a variety of functional components, including flavonoids, amino acids, tea polyphenols, tea pigments, alkaloids, tea polysaccharides, trace elements, vitamins, aromatic substances, and more (Hao et al., 2023; Dai et al., 2017).

2.2 Composition of white tea

White tea components include flavonoids, polyphenols, amino acids, alkaloids, vitamins and minerals, tea pigments, and water.

2.2.1 Flavonoid

Flavonoid compounds, also known as bioflavonoids, typically exhibit a pale yellow or yellow colour. Flavonoid compounds are a structurally simple natural plant product widely distributed throughout the plant kingdom. They belong to a group of polyphenolic compounds exceeding 4000 varieties, consisting of 15 carbon atoms arranged in a C₆-C₃-C₆ configuration. The diverse functional groups and their relative positions in the molecular structure determine the biological effects of flavonoids (Jiang et al., 2017). These structural characteristics contribute to their antioxidant properties and role in inhibiting the carcinogenic process, making them beneficial for

human health. It is reported by Wang et al. (2024) comprehensively studied the metabolic mechanism of flavonoid glycosides and their contribution to flavour evolution during white tea processing using quantitative descriptive analysis, metabolomics, dose-over-threshold factors, and pseudo-first-order kinetics. A total of 223 flavonoids were identified. The results showed that water loss stress induced the degradation of key flavour flavonoid glycosides during the wilting process, which contributed to the formation of a unique flavour of white tea. Wang et al. (2024) studied the conversion mechanism of flavonoid glycosides in white tea and its effect on taste during drying at 60, 80 and 100 °C. There were 45 different flavonoid glycosides in white tea at 3 drying temperatures. Compared with the wilted samples for 48 h, the contents of total flavonoid glycosides decreased at three drying temperatures, and quercetin 3-O-galactoside and kaempferol 3-o-glucoside showed the greatest degradation degree. These results were confirmed by a simulated drying reaction using the flavonoid glycosides standard. Zhang et al. (2024) studied 46 kinds of flavonoids in white tea of different storage years, indicating that quercetin, rutin, and hesperidin are important sources of ageing charm and aroma of “*Shoumei*” white tea, and the cooperation with bacteria and fungi may lead to changes in the composition of flavonoids and amino acids during the storage of white tea.

2.2.1(a) Flavanol

Flavanol, or flavanol-3-ol, is a flavanone derivative with a 2-phenyl-3,4-dihydro-2H-chromen-3-ol backbone. It includes catechins and galocatechin (Figure 2.2). Tea flavanols have been extensively studied for their potential health benefits and anti-ageing properties. The primary flavanols in tea, such as catechins, are known for their potent antioxidant and anti-inflammatory effects, which contribute to

their ability to protect against age-related diseases and promote longevity. A study on catechins in green tea can prolong lifespan and attenuate A β by inducing *Caenorhabditis elegans* through anti-oxidation and peptide dis-aggregation. The result found that tea catechins prolonged the lifespan and healthspan of paralytic *C. elegans*, improving stress resistance, reducing ROS levels, MDA contents, and A β deposition, enhancing GSH-Px and SOD activities, reinstating benzaldehyde chemotaxis, reducing 5-HT sensitivity, and alleviating glutamate-induced neurotoxicity (Yang et al., 2024).

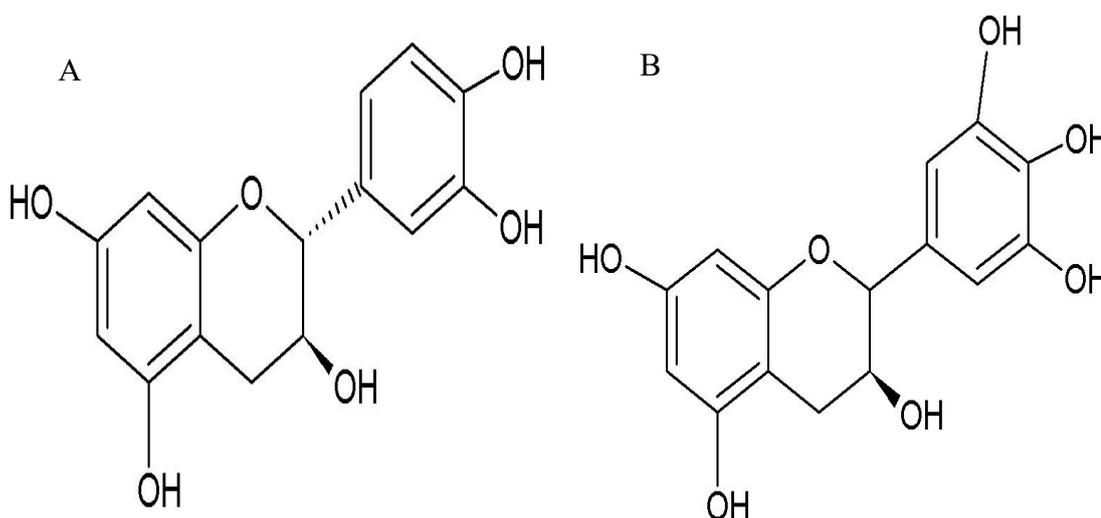


Figure 2.2 Structural of flavanols. A represents catechins, and B represents gallocatechin.

2.2.1(b) Flavonol

Flavonol is a class of compounds with a 3-hydroxyflavone backbone. Its distinctive feature is the presence of a double bond between C2 and C3 atoms, with a hydroxyl group at C3, commonly referred to as 3-hydroxyflavonols. Derivatives with hydroxylation at C3, C5, and C7 are termed 3,5,7-trihydroxy derivatives. There are

three major types of flavonols in tea leaves, such as kaempferol (4'-OH), quercetin (3',4'-diOH), myricetin (3',4',5'-triOH), and rutin with different numbers of hydroxyl groups on the ring B (Figure 2.3).

Flavonols are mainly present in the form of glycosides in tea leaves, and the glycosylation level of flavonol could be the indicator for the discrimination of the processing suitability of tea varieties. Due to diverse physiological health effects, quercetin was developed to regulate multiple dysfunctions (Chen et al., 2019). Regulating iron metabolism genes, quercetin could inhibit duodenal non-haem iron absorption in systemic iron metabolism. This could lead to developing new effective ways of preventing and treating iron-deficiency anaemia. Kaempferol might display an anti-obesity effect by inhibiting adipogenesis and increasing lipolysis (Mahobiya et al., 2018).

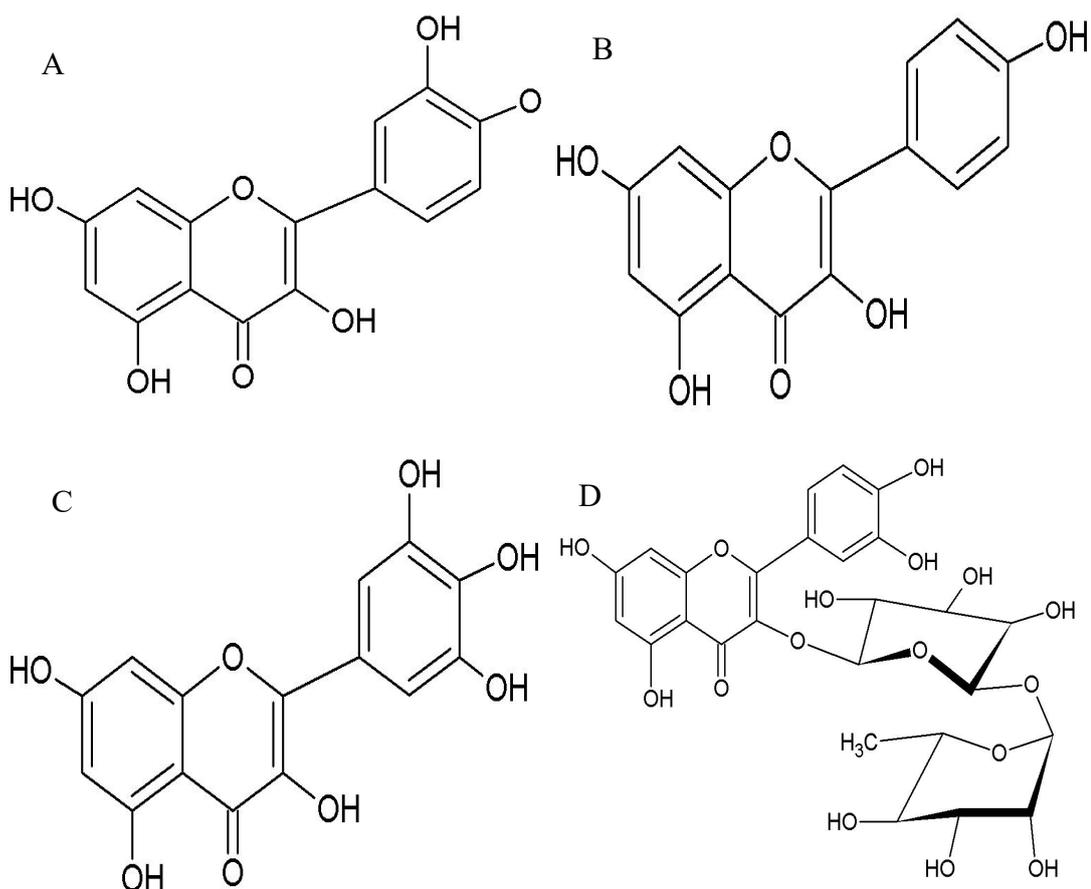


Figure 2.3 Structural of flavonols. A represents quercetin, B represents kaempferol, C represents myricetin, and D represents rutin.

2.2.1(c) Theaflavins

As a bipolymer of catechin, theaflavins consist of two chromanes and a benzotropolone skeleton, which oxidised from the B ring of catechins. Theaflavins exert comparable or even better pharmacological effects as catechins in terms of antioxidation, cancer adjuvant therapy, anti-obesity effect, etc. (He. 2017). In addition to these routine benefits, theaflavins have also shown significant activity in anti-mutagenesis and lowering blood lipids. The signalling pathway of transcription

factors is blocked by inhibiting the automatic phosphorylation of proteins, and theaflavins play a defensive role in preventing DNA damage (Ko et al., 2015).

Besides, for the prevention and regulation of mental disorders, such as anxiety disorder and depression, consumption of theaflavins is associated with the increase of dopamine turnover in the frontal cortex. Theaflavins could induce anxiolytic effects via activation of the dopaminergic system (Figure 2.4) (Kita et al., 2019).

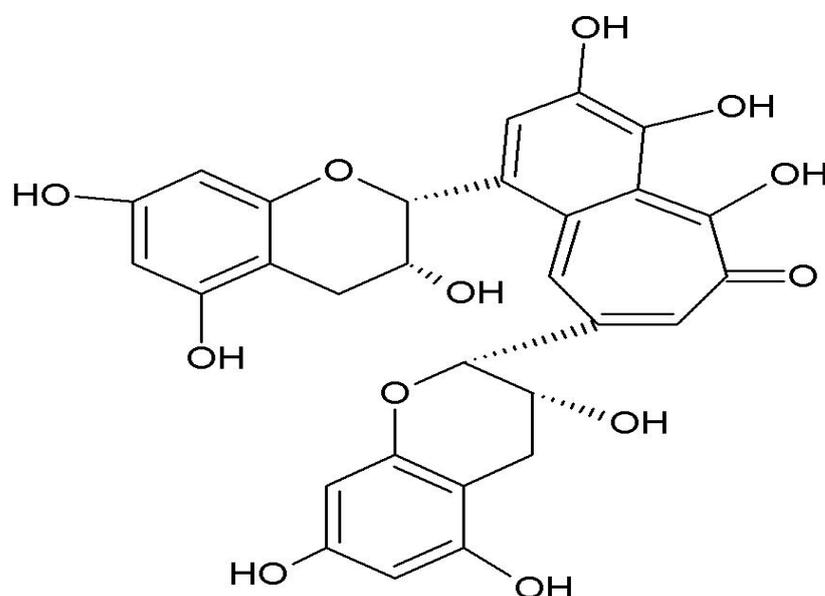


Figure 2.4 Structural of theaflavins

2.2.1(d) Anthocyanidins

Anthocyanidins are a group of water-soluble natural pigments widely present in plants. In tea, cyanidin, pelargonidin, and delphinidin were familiar (Figure 2.5). The difference was embodied in the number of hydroxyl groups in the B ring. Taking tea as a substrate, anthocyanin could be produced by *Lactococcus lacti* in cell factories to afford the bio-utilization. Due to anthocyanin's presence, pH-sensitive and antioxidant packaging films could be prepared for food packaging. It was

reported by Zhang et al. (2019) that modified Eagle's medium, stability of ten of the most common anthocyanins was evaluated. The results showed that both glycosylation and methylation could improve the stability of anthocyanins.

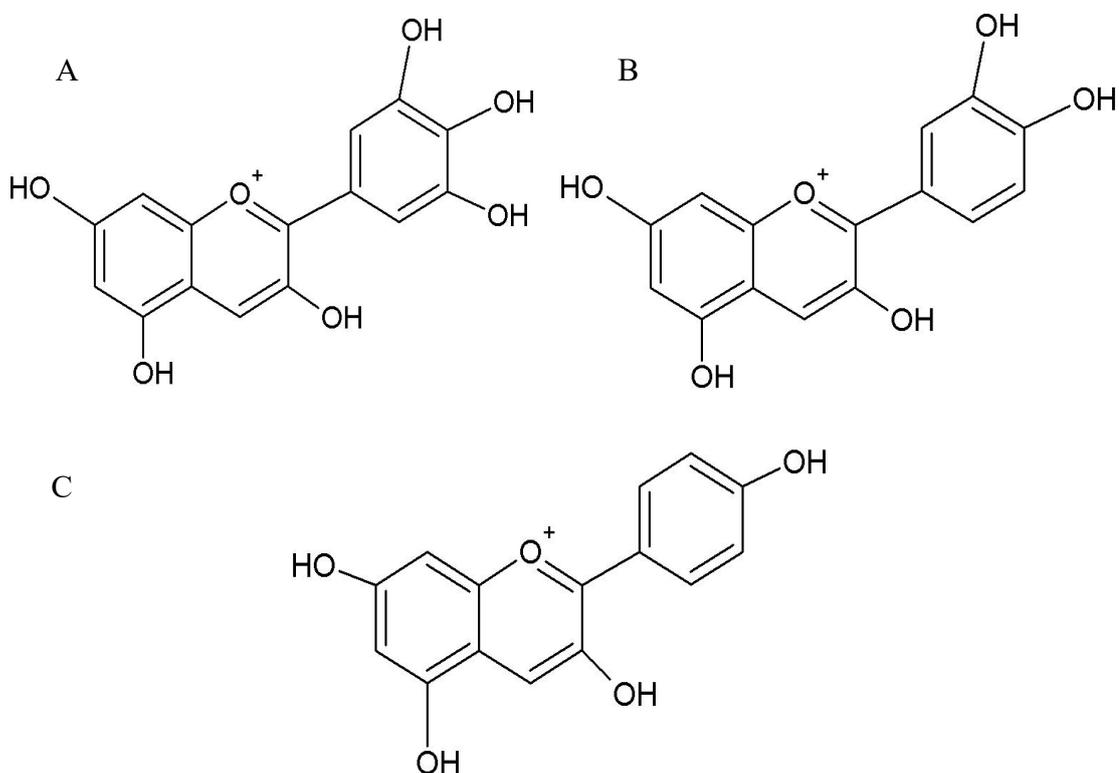


Figure 2.5 Structural of the anthocyanins. A represents delphinidin, B represents cyanidin, and C represents pelargonidin.

2.2.1(e) Catechins

Catechins are the most commonly mentioned and most representative members of the flavan-3-ol family. Typically, being a member of the flavan-3-ols family, 3-hydroxyl substituted chroman (ring A and C) and aryl at the 2-position (ring B) existed in the skeleton of catechins. Catechins are mainly categorised into four types: epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Figure 2.6). Compared with other catechins, the

EGCG skeleton contains up to 8 hydroxyl groups, which has the most outstanding antioxidant and free radical scavenging potential.

Catechins are the primary phenolic compound in tea infusion and are one of the main contributors to the astringent and bitter taste. In addition to directly affecting the sensory quality of tea, catechins, especially EGCG, combine with other molecules in tea, such as *L*-theanine, caffeine, etc., and show good activity for human health. Long-term intake of catechins displayed a therapeutic effect for chronic disease. For instance, in vitro and animal trials verified that EGC possessed blood anticoagulation and antiplatelet activity (Chen et al., 2013).

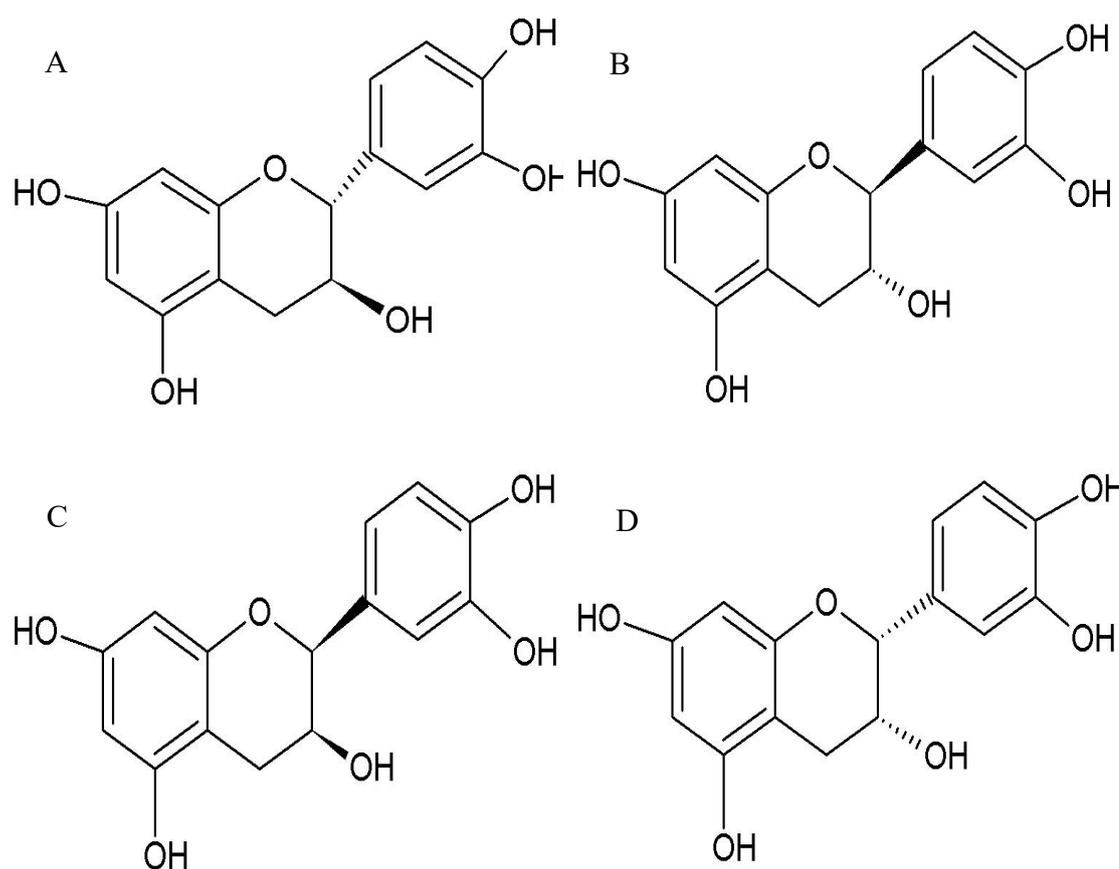


Figure 2.6 Structural of the catechins. A represents (+)-catechin (2R,3S), B represents (-)-catechin (2S,3R), C represents (-)-epicatechin (2R,3R), D represents (+)-epicatechin (2S,3S).

2.2.2 Polyphenols

Tea polyphenols are a collective term for polyphenolic compounds in tea leaves, accounting for about 20-30% of the dry weight of fresh tea leaves. Tea polyphenols are polyphenolic compounds with two or more hydroxy groups in adjacent positions, and they belong to a class of condensed aromatic hydrocarbons with a core structure of 2-phenylbenzopyran and exhibit characteristics typical of phenolic compounds. Tea polyphenols have a molecular structure that can donate protons, making them effective natural antioxidants by neutralising free radicals (Liu et al., 2023). In vivo experiments have shown that tea polyphenols can increase levels of rat serum catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD), and can reduce the production of malondialdehyde (MDA). These findings reveal that tea polyphenols regulate the oxidoreductase system, improve the body's anti-oxidation ability (Ahmed et al., 2017), and prevent oxidative stress caused by bacterial infections and intestinal damage (Zhang et al., 2019). In addition, tea polyphenols can restore the levels of serum total protein, tumour necrosis factor- α (TNF- α), and caspase-3 in the liver of rats with hepatotoxicity induced by azathioprine (El-Beshbishy et al., 2011). This indicates that tea polyphenols protect against liver injury in rats through antioxidant, anti-inflammatory, and antiapoptotic mechanisms.

2.2.3 Amino acid

Amino acids are organic compounds with both an amino group and a carboxyl group, and they are one of the main chemical components of tea leaves. Tea

leaves have 26 different amino acids, making up around 1% to 4% of the weight of fresh leaves. Amino acids, particularly theanine, play a key role in contributing to the fresh taste of white and green tea, with theanine comprising approximately 30-50% of the total amino acids in tea leaves. Theanine in tea has some health benefits. Among the six major tea categories, white tea has the highest content of free amino acids, measuring 617.85 µg/mL, with theanine accounting for 148.5 µg/mL (Pan et al., 2018). It reported that theanine exerted its anticancer function by inhibiting relative pathways, activating the intrinsic apoptosis pathway, and inducing programmed cell death independent of caspase (Shojaei-Zarghani et al., 2021). Nguyen demonstrated that theanine attenuates memory impairment in a genetic ageing model by up-regulation of relative signalling, thereby improving the lifespan of mice (Nguyen et al., 2019).

2.2.4 Alkaloids

Alkaloids in tea pertain to a group of nitrogen-containing organic compounds, making up 2-4% of the dry weight of tea leaves. The primary alkaloids include caffeine, theobromine, and theophylline. Alkaloids are readily soluble in hot water, imparting a bitter taste and serving as important flavour substances in tea infusion. Hydrogen-bonded complexes between caffeine and catechins create a refreshing and pleasant taste, serving as a key factor in the formation of tea quality. Tan et al. (2018) observed that the caffeine content in white tea ranged from 2.23% to 4.94%, with Silver Needle at 4.71%, White Peony at 4.14%, and “*Shou Mei*” at 3.18%, and the theophylline content ranged from 0.04% to 0.15%. Among the six main types of tea processed from the same fresh tea leaves, white tea has the highest caffeine content

relative to other tea types. This difference may be attributed to the relatively extended withering process that white tea undergoes in comparison to other types of tea (Ning et al., 2016).

2.2.5 Tea pigments

Tea pigments are a collective term for a series of natural pigments with biological activity, composed of water-soluble and fat-soluble pigments. Tea pigments include theaflavins, theaflavins, and the brownies, formed by polymerising polyphenols catalysed by enzymes such as polyphenol oxidase and peroxidase, primarily in fermented teas. A recent study by Tan et al. (2017) reported that in different grades of white tea, the content of theaflavins increases as the grade decreases. Specifically, the arrangement of theaflavin content is “*Shou Mei*” > White Peony > Silver Needle. Zhu et al. (2021) revealed that at a concentration of 80 μ g/mL, the origins demonstrated a notable antioxidant capacity with a high hydroxyl radical clearance rate of 79.7%. This suggests a strong antioxidative property of the origins at this specific concentration. Research by Yue et al. (2019) indicated that the brownie has significant potential in reducing the risk of cardiovascular diseases such as diabetes and obesity.

Tea is a complex beverage with various chemical constituents contributing to its flavour, aroma, and health benefits. The composition of tea can vary depending on the type, the processing method, and the growing conditions. Table 2.1 shows the main ingredients of tea.

Table 2.1 The main constituent and composition of the tea

Constituent	Composition	Reference
Polyphenols	20-30% of dry weight	Liao et al. (2022)
Flavonoids	3-10% of dry weight	Liao et al. (2022)
Catechins	60-80% of total polyphenols	Xu et al. (2023)
Amino Acids	1-4% of dry weight	Liao et al. (2022)
Alkaloids	2-4% of dry weight	Tan et al. (2018)
Caffeine	2-5% of dry weight	Ashihara & Suzuki, (2004)
Vitamins	Trace amounts (various)	Karak et al. (2017)
Minerals	Trace amounts (K, Ca, Mg, Fe, Mn, Zn)	Karak et al. (2017)
Volatile Compounds	Trace amounts (contribute to aroma)	Lv et al. (2014)

2.3 Health benefits of tea

Tea is globally recognised as a natural health drink, and the relationship between tea and health has always been a hot topic in international tea science research. Growing and compelling evidence supports the preventive and therapeutic effects of tea consumption on various diseases. Additionally, it is previously reported that tea has various bioactive functions that contribute to health protection, including antioxidant capacity, anti-inflammatory activity, potential for reducing obesity, lowering the risk of hypertension and cardiovascular diseases, anti-diabetic effects, and anti-cancer properties (Xu et al., 2019; Sanlier et al., 2018) . An increasing

number of studies indicated that tea has significant health benefits for humans and promising preventive and therapeutic effects on diseases. Tea possesses numerous health advantages, such as activities related to antioxidation, tumour inhibition, cardiovascular disease prevention, anti-inflammatory, and ageing resistance (Table 2.2).

People love drinking tea but often extract only a small amount of its bioactive components when consuming it. The valuable substances in tea leaves need to be fully utilised, leading to waste. Hence, to enhance the utilisation of tea components and reduce pollution, this study focuses on extracting the bioactive component, flavonoids, from tea leaves and converting them into powder form. Based on my local advantage, my hometown is in the Fujian Province, China. It is white tea primarily produced in Fujian Province. Therefore, white tea was chosen as a raw ingredient in this project.

Table 2.2 Health properties and effects of the different varieties of teas

Health Properties	Varieties of Teas	Effects	Reference
Anti-ageing Activities	Green tea	Inhibiting reproductive potential	Lopez et al. (2014)
Antioxidant Activities	White tea	Inhibition of free radicals Inhibition on α -Amylase and α -Glucosidase	Xu et al. (2019)
Anti-tumour Activities	Dark brick tea	Reduction of tumour growth and cancer cell proliferation	Liu et al. (2022)
Anti-cardiovascular Diseases	Oolong tea Black tea	Relaxing and anxiety-reducing effect Improvement of mental and sleep health	Zhang et al. (2019)
Anti-inflammatory Activities	Green tea	Reduction of inflammatory markers such as C-reactive protein Increase of anti-inflammatory cytokines such as IL-10	Deo et al. (2019)

2.3.1 Anti-ageing properties

Ageing is a gradual decline in various physiological functions of an organism over time, constituting a complex physiological process. It is accompanied by oxidative damage to tissues, organs, and other components, with the accumulation of reactive oxygen species being one of the primary factors contributing to ageing. The most common reactive oxygen species (ROS) include superoxide radicals (O_2^-) and hydroxyl radicals (OH^-), which stimulate and cause oxidative damage to cells. However, the body possesses various antioxidants, such as superoxide dismutase

(SOD) and catalase (CAT), which neutralise excessive ROS and counteract the damage induced by oxidative stress. Plant natural bioactive ingredients have become a research hotspot in anti-ageing studies due to their efficiency, safety, and cost-effectiveness. The anti-ageing efficacy of these components has been preliminarily confirmed in various in vitro and in vivo experiments. Fei et al. (2017) observed that aqueous extracts from puer tea, black tea, and green tea all extended the life expectancy of worms and delayed the progression of A β -induced paralysis in transgenic worms with Alzheimer's disease. Lopez et al. (2014) observed that water extracts of pure tea, black tea, and green tea could extend the lifespan of worms, demonstrating anti-ageing, anti-Alzheimer's disease, and antioxidant effects. Research has found that green tea polyphenols extend the lifespan of male fruit flies by inhibiting reproductive potential, possibly through restricting iron intake. Ruiz et al. (2018) demonstrated that long-term ingestion of white tea could significantly mitigate oxidative damage associated with ageing and acute oxidative stress in rats. The study highlights the potential of white tea as a dietary intervention to enhance antioxidant defence mechanisms, thus contributing to improved health and longevity. While the study was conducted on rats, the findings suggest the potential benefits of white tea for humans in reducing oxidative stress and possibly prolonging lifespan. Zheng et al. (2017) indicated that chlorogenic acid likely prolonged the lifespan of *Caenorhabditis elegans* mainly by DAF-16 in the insulin/IGF-1 signalling pathway. Li et al. (2020) confirmed that quercetin's protective and lifespan-extending effects in *Caenorhabditis elegans* are attributed to its potent antioxidant capacity and modulation of signalling pathways.

2.3.2 Antioxidant activities

Antioxidant capability is one of the primary functions of tea, and as a natural antioxidant, it has attracted considerable research from researchers. Many diseases are associated with oxidative damage caused by reactive oxygen species (ROS) (Chen et al., 2017). Compounds in tea, especially flavonoids, exhibit strong antioxidant abilities, which are closely related to the structure of flavonoids. Due to the phenolic hydroxyl groups of flavonoids, they can directly provide protons to participate in eliminating free radicals. Their chemical structure allows polyphenols to react with ROS, neutralising them and avoiding their accumulation in the organism. Therefore, the ability of flavonoids to remove free radicals is related to the number of phenolic hydroxyl groups, and the more there are, the stronger the hydrogen-supplying capability, leading to a more potent ability to eliminate free radicals. The studies conducted by Niu et al. (2022) have demonstrated that flavonoids possess antioxidant activity. They can effectively capture free radicals, thereby reducing the harm caused by excessive free radicals to the body. Additionally, the hydroxyl group on the B ring exhibits the strongest proton-donating capability. It serves as the primary site on the flavone molecule for scavenging free radicals and exerting antioxidant activity. Among these, the B ring 3',4'-dihydroxy structure demonstrates the highest activity (Seeram & Nair, 2002).

Antioxidant activity primarily contains two aspects: *in vitro* antioxidation and *in vivo* antioxidation. Standard methods for evaluating *in vitro* antioxidation include the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, superoxide anion radical assay, hydroxyl radical assay, and ABTS radical assay (Xu et al., 2018). *In vitro* antioxidant experiments are characterised by low cost, simplicity, and timeliness, making them widely employed in the initial screening stages of antioxidant activity. However,