EFFECTS OF ULTRASOUND-ASSISTED EXTRACTION AND TANNASE TREATMENT ON PHYSICOCHEMICAL AND FUNCTIONAL PROPERTIES OF GREEN TEA (*Camellia sinensis* var. *assamica*) CATECHINS

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EFFECTS OF ULTRASOUND-ASSISTED EXTRACTION AND TANNASE TREATMENT ON PHYSICOCHEMICAL, FUNCTIONAL PROPERTIES, AND STABILITY OF GREEN TEA (*Camellia sinensis* var. *assamica*) EXTRACT

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Haoyuan Su

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ABSTRACT

This thesis explores the enhancement of catechin extraction from Green Tea (*Camellia sinensis* var. *assamica*) efficiency, extract quality and catechin stability, through various methodologies, including solvent extraction, ultrasound-assisted extraction (UAE), and tannase treatment, as well as the impact of storage conditions on catechin properties. In the first, the study compared solvent extraction and UAE methods for their effects on the physicochemical properties of catechins extract. UAE demonstrated superior efficiency in catechin extraction, achieving higher yields and improved higher total polyphenol content (TPC) and antioxidant capacity of catechin extract compared to conventional solvent extraction. UAE on condition tea : 50% ethanol v/v (1 : 20) at 20min showed the highest extract yield 22.99 ± 0.08%, catechin content 33.75 ± 0.15 (g/100 g db), TPC 64.02 ± 0.16 (g/100 g db) and antioxidant capacity. The second investigated the effects of tannase in modifying the physicochemical and functional properties of catechin extract. Tannase treatment significantly impacted the TPC and antioxidant capacity of catechins. Although tannase

treatment reduced content of catechins, and it provided greater stability during storage by minimizing degradation. This stabilization effect was more pronounced at lower temperatures. In the final, the stability of catechins was assessed under different storage conditions, specifically at freezer (-20°C) and incubator (30°C). Untreated catechin samples showed considerable degradation in TPC, antioxidant activity, and individual catechin content over an 8-week period, with more severe declines at incubator. Tannase-treated samples, however, exhibited better stability, with slower declines in catechin content and antioxidant activity. Overall, the results demonstrated that ultrasound-assisted extraction (UAE) is a highly effective technique for obtaining catechin-rich extracts with superior antioxidant properties compared to traditional solvent extraction methods. Furthermore, tannase treatment proved to be a promising strategy for improving the long-term stability of catechins, despite its initial reduction in catechin content. This stability enhancement is especially critical for applications where maintaining antioxidant activity over extended storage periods is essential. The research also highlighted the significant impact of storage conditions on catechin degradation. Lower temperatures, such as those in freezer storage (-20°C), were shown to significantly mitigate the loss of catechin content, total polyphenols, and antioxidant capacity compared to higher storage temperatures (30°C). These findings provide valuable insights for the development and preservation of catechin-rich products, emphasizing the importance of employing advanced extraction techniques and stabilizing agents to maintain the quality and efficacy of catechins.

Keywords: Catechins, Ultrasound-associated Extract (UAE), Tannase

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CHAPTER 1

INTRODUCTION

1.1 Introduction to the Research Problem and Its Significance

Tea is one of the most widely consumed drinks in the world. There are many similar kinds of tea, which are named according to the processing technology. Among which the famous ones are as green, white, black, yellow, oolong and dark tea. Among all of these, the consumption of green tea has shown the most notable effects on human health. Numerous substances, such as proteins, amino acids, carbohydrates, minerals, and trace elements, are found in tea. The 30% of soluble compounds are found in tea, and these vary depending on the cultivar, environment, production area, and handling and processing techniques. The active constituents of tea are polyphenols, commonly known as tea catechins, which make up more than 75% of the soluble compounds in tea. Epigallocatechin-3-O-gallate (EGCG) is the most important and most abundant catechins, accounting for more than 40% of the total catechins in green tea (Dong et al., 2011). Catechin is the most important component of tea extract, but also the most nutritious part. Catechins are important roles for healthy benefit, including antioxidant (Yan et al., 2020), anti-diabetes (Fu et al., 2017), anti-β-Amyloid (Polito et al., 2018), anti-cancers (Wang et al., 2018, 2019), anti-allergy (Li et al., 2021) and antiviral effects (Wang et al., 2021).

Catechins can be extracted and isolated from tea leaves through several key processes. These include the initial treatment of the tea leaves to prepare them for extraction, followed by the use of specific solvents to extract catechins. After extraction, catechins must be separated from other components present in the extract. The final step often involves drying techniques to produce catechin extracts in powder form, such as freezer dry or spray dry. Throughout this process, factors such as solvent

type, extraction time, and temperature, as well as the purity and yield of catechins, play crucial roles in optimizing the efficiency and quality of the extract. However, the biggest challenge for catechins is their bitter taste and low bioavailability, which leads to low sensory preference and low absorption efficiency in the body. Catechins play a key role in contributing to the astringency and bitterness of green tea infusions. While increasing concentration of catechin increases taste intensity, it also reduces the palatability of the flavor (Zhang et al., 2016). Since catechin has a high molecular size and many hydrogen bonds, its bioavailability is quite low. According to Fan et al. (2016), catechins's steric configuration and molecular structure are linked to their bioactivities and intermolecular interactions, potentially causing varied behaviors in various catechins. Tea polyphenols also possess anti-nutritional properties due to their capacity to bind and precipitate a number of digestive enzymes, such as amylase, pepsin, and lipase. Treatment with tannase is one of the best approaches to solve those issues mentioned above.

Nowadays enzymatic treatment in tannase (tannin acyl hydrolase, EC 3.1.1.20) catalyzes ester and disulfide bond hydrolysis in hydrolysable tannins or gallic acid esters, such as EGCG or ECG, to release glucose or gallic acid. And Inhibiting aggregation of macromolecules and precipitate formation occur during the storage of tea beverages, tannase hydrolytic activity reduces EGCG interaction and EGCG interacts with the protein as the ester bond breaks. According to recent studies, reported that the negative effects of tannins may be eliminated by using tannase in food and beverage industry goods. Additionally, tannase is frequently employed in the production of green tea beverages, green tea concentrate, and instant green tea powder to improve their taste quality. The enzyme helps to reduce the astringency caused by tannins, thereby improving the overall flavor profile. Tannase also contributes to the clarity and stability of the final product, making it more appealing to consumers. Tannase not only enhances the taste by reducing bitterness and astringency, but it also makes it easier to extract other beneficial compounds from the tea, thereby improving its overall quality (Chávez-González et al., 2012). Tannase-treated green tea infusion exhibited an improved mouthfeel compared to the control (Lu et al., 2009). Condensed and hydrolysable tannins exhibit stronger antioxidant activity by quenching peroxyl radicals than simple phenolics (Hagerman et al., 2009).

So, this research to investigate the performance between solvent conventional extraction by ethanol and ultrasound-assisted extraction (UAE) . Moreover, tannase treatment was investigated to reduce catechin bitterness and improve functional properties. Analyses of catechin constituents and their biological component activities in tea extracts were performed to evaluate the quality enhancement of green tea catechins.

1.2 Objectives

1.2.1 To investigate the performance between solvent extraction and ultrasound-assisted extraction (UAE) on physicochemical properties of green tea catechin.

1.2.2 To study the effect of tannase on physicochemical and functional properties of green tea catechin.

1.2.3 To study stability of green tea catechin during storage time.

1.3 Scope of Research

Fresh tea (*Camellia sinensis* var. *assamica*) was used as the raw material for this study. Solvents and Ultrasound-Assisted Extraction (UAE) were performed for catechin extraction methods. Secondly, tannase treatment on the physicochemical and functional properties of catechins, assessing properties was altered. Lastly, the stability of catechins were examined with different temperature at -20°C and 30°C, to explore their properties and preservation during storage time.

1.4 Expected Benefits

1.4.1 Research Outputs

1.4.1.1 It provided a comprehensive performance of extraction method of solvent and ultrasound-assisted extraction.

1.4.1.2 Tannase treatment provided information of physicochemical and functional properties of catechin extract.

1.4.1.3 It provided the influence of temperature and tannase treatment during catechin extract storage.

1.4.2 Research Outcomes

1.4.2.1 The research suggested to performance of extraction method between solvent and ultrasound-assisted extraction to apply in food industry.

1.4.2.2 The research provided more information about catechins extract after tannase treatment, and explore their stability for future study.

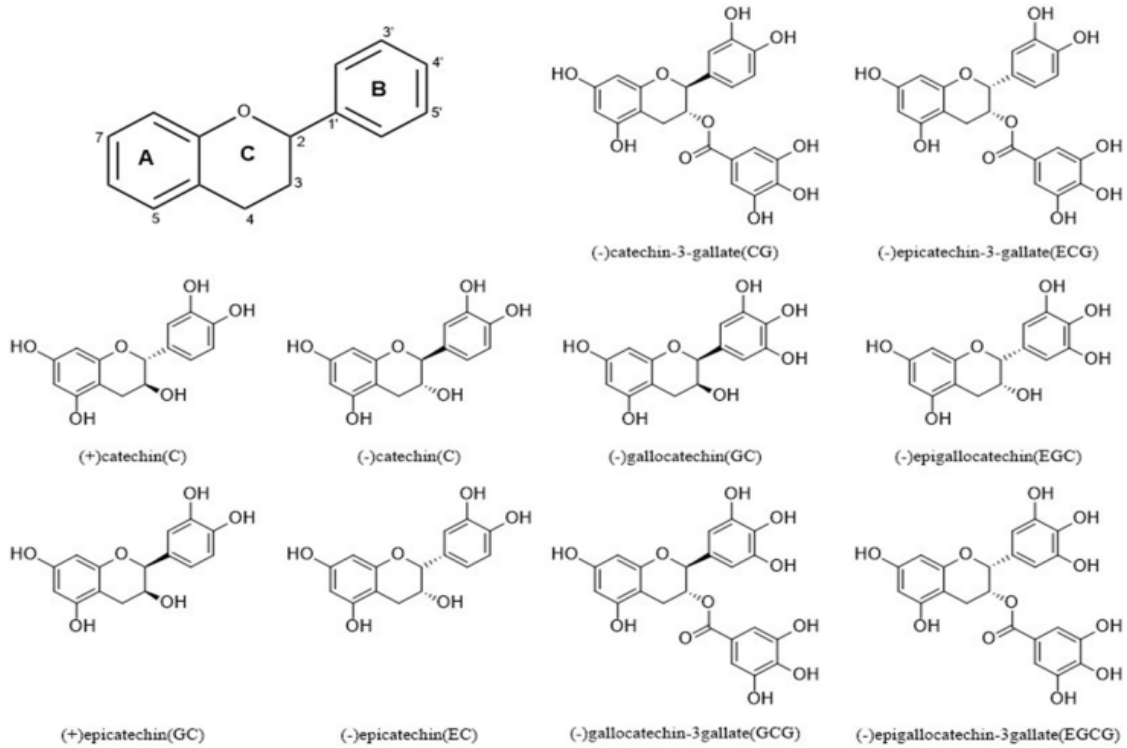
CHAPTER 2

LITERATURE REVIEW

2.1 Green Tea Catechin

Green tea is rich in polyphenolic compounds known as green tea catechins, which are powerful antioxidants. Products derived from green tea, such as liquid extracts and powders, vary significantly in their polyphenol content, ranging from 45% to 90%, and in their caffeine content, which can range from 0.4% to 10%. Numerous studies have investigated the potential therapeutic benefits of green tea catechins, particularly their use as chemotherapeutic agents in the treatment of neoplastic disorders and infectious diseases. These catechins have shown promise not only for their antioxidant properties but also for their role in modulating cellular pathways involved in cancer progression and immune response. A total of twelve catechins have been identified through chromatography, but only eight are present in tea in notable amounts. However, most studies typically concentrate on a small subset of catechins, primarily focusing on the most prevalent ones: epigallocatechin gallate (EGCG) and epigallocatechin (EGC). These two catechins alone constitute over 70 percent of the total catechin content in fresh tea leave. Due to their prevalence, EGCG and EGC are often the primary subjects of research, as they are believed to play significant roles in the health benefits associated with green tea, including its potential anti-cancer and anti-inflammatory effects. Despite this, there is growing interest in exploring the bioactivity and therapeutic potential of other less abundant catechins to gain a more comprehensive understanding of green tea's health benefits. As shown in Figure 2.1, Catechins consist of a phenolic structure with two benzene rings (the C6 parts) that are connected by a three-carbon chain, specifically a 2,3-dihydro-2H-chromene unit. Catechins are composed of a basic 3-carbon unit, and it connected to two aromatic rings, characteristic of the flavonoid structure, which features a C6-C3-C6 skeleton consisting of two phenolic nuclei. These rings contain various hydroxyl groups (Vuong et al., 2011), including those in epigallocatechin-3-gallate (EGCG),

epigallocatechin (EGC), and epicatechin-3-gallate (ECG) (Sano et al., 2001), and those catechin have different substituents, particularly at the 3 and 5 positions of the rings, influencing their solubility and bioactivity. Among the various catechins, EGCG, it is also known as epigallocatechin-3-gallate, stands out due to its potent biological and pharmacological activities. Chemically described as [(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl] and 4,5-trihydroxybenzoate, with the molecular formula C22H18O11, EGCG is an ester formed from epigallocatechin and gallic acid. Catechins contain multiple hydroxyl (-OH) groups, which contribute to their antioxidant properties. The positioning of these hydroxyl groups on the rings is crucial for their biological activities. This compound is known for its powerful antioxidant properties and has been extensively studied for its potential role in preventing and treating various diseases, including antioxidant, antiinflammatory, antiangiogenic, antiproliferative, pro-apoptotic and antimetastatic properties (Singh et al., 2011; Zhang et al., 2013).



Source Rashidinejad (2021)

Figure 2.1 The Chemical Structure of the Major Catechins in Green Tea

2.2 Catechin Extraction

Although there are many studies on the extraction of catechins, however catechin extraction is challenging for reasons: first, catechins can be found in plant tissue bound to proteins, sugars, or both; second, they can form polymerized derivatives with varying degrees of dissolution; and third, the interactions and chemical structures of catechin with other food components remain inadequately comprehended. They are also very vulnerable to oxidation, high temperatures, light, and an alkaline environment, making it challenging to create an effective extraction method. During extraction, solvents infiltrate the solid plant material and dissolve compounds with similar polarities. This process allows the targeted compounds, such as catechins in the case of tea leaves, to be selectively solubilized and separated from the plant matrix. The efficiency of extraction is largely influenced by solvent's polarity, the extraction

temperature, and the duration of the process. These variables are essential for optimizing the yield of the target compounds while reducing the extraction of undesired substances (Remington, 2006; Ncube et al., 2008). Some common extraction methods comprise maceration, infusion, heat reflux solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical carbon dioxide extraction. The effectiveness of extraction is significantly influenced by factors such as pH, temperature, the solvent-to-material ratio, and the duration of each extraction phase. These variables, including the quantity and timing of each step, are key to optimizing the extraction process (Stalikas, 2007). Catechins, being polar compounds, dissolve in polar organic solvents like ethanol, methanol, water, and acetone. However, these solvents also extract additional substances including caffeine and chlorophyll. In green tea, theanine and caffeine, with lower molecular weights than catechins, are more soluble and thus are extracted in earlier fractions. Their melting points are between those of catechins, which facilitates their co-extraction during the solvent extraction process of green tea (Ho et al., 2009). This means that while extracting catechins, it is important to consider the presence of other components that might be co-extracted, potentially influencing the overall composition of the extract. On the other hand, to obtain crude catechins from green tea, it is essential to consider their properties and optimize the extraction method accordingly.

2.2.1 Conventional Solvent Extraction

Solvent extraction is a separation process that involves the distribution of a solute between two immiscible or partially miscible liquids. The principle of conventional solvent extraction involves using a solvent to dissolve and separate target compounds from a solid or liquid mixture. This process is based on the selective solubility of compounds in the chosen solvent, where factors like polarity, temperature, pH, and solvent-to-material ratio are optimized to enhance the extraction of desired substances while minimizing impurities. The solvent interacts with the sample, dissolving the components of interest, which can then be isolated through subsequent steps such as filtration or evaporation. It is one of most convenient method of catechins extraction. Fruits, leafy vegetables, and fresh green tea leaves are all excellent sources of catechins. Since these catechins have a highly polar nature, polar solvents such water,

ethanol, acetone, and methanol can be used to extract them (Turkmen et al., 2007). Moderate heat treatment can enhance the solubility of catechins in polar solvents, leading to an increased yield of extractable solids, including catechins and other soluble components. The choice of solvent significantly affects both the extraction yield and the composition of the extracts, as noted by Nwuha (2000) and Perva-Uzunalić et al. (2006). In their study, Perva-Uzunalić et al. (2006) compared the extraction efficiencies of catechins and caffeine from green tea using various solvents, such as water, pure organic solvents (acetone, ethanol, methanol, and acetonitrile), and their aqueous solutions at boiling temperatures. The study revealed that the lowest yields of caffeine and catechins were obtained when using absolute acetone and acetonitrile as extraction solvents. In contrast, the highest yield of catechins was achieved when absolute ethanol was used for extraction. In addition, Liang et al. (2007) reported that fresh leaves of green tea may be effectively extracted using 75% ethanol and 30–70% aqueous ethanol to extract catechins. Solvent type, temperature, duration, and the ratio of material to solvent are other elements that influence extraction efficiency.

2.2.2 Ultrasound-assisted Extraction

Ultrasound-induced cavitation bubbles create hydrophobic surfaces within the extraction liquid, which enhances the medium's hydrophobic characteristics. The principle behind UAE involves the generation by intense frequency waves of sound, which make cavitation bubbles in the solvent (Mason et al., 1996). When these bubbles collapse, they generate intense pressure and localized heating, causing disruption of the plant or material cell and it improves the penetration of the solvent into the cells and enhances the extractions of bioactive compounds. This facilitates the extraction of polar components into hydrophilic aqueous media, reducing the reliance on less desirable hydrophobic or strongly polar solvents. (Vinatoru, 2001). Ultrasound baths is common configurations of ultrasound-assisted extractors. Mass transfer is enhanced by the mechanical effects of ultrasound, which provide a higher penetration of solvent into biological materials (Mason et al., 1996). The sonochemical effects of ultrasound in a liquid are attributed to the phenomenon of acoustic cavitation (Suslick & Price, 1999). During the ultrasound on progressing, acoustic cavitation typically involves the formation, growth, and implosion of bubbles as ultrasound waves propagate through a

liquid medium (Kentish & Ashokkumar, 2010). The molecules within the liquid are bound by attractive forces (Suslick, 1989). When an ultrasound wave passes through an elastic medium, it causes alternating cycles of compression and rarefaction. Hot water and organic solvents are employed to extract the catechins from green tea. Although using hot water for extraction is safe, the heat used degrades the quality of green tea and destroys catechins. Although low temperatures are preferred for extraction, extraction efficiency is poor. recently reported, Albu (2004) studied the effects of different solvents and Ultrasound-Assisted Extraction (UAE) on extracting carnosic acid from rosemary. Compared to conventional extraction methods, ethanol was much less effective than ethyl acetate and butanone. However, the use of UAE enhanced the performance of ethanol, making it more competitive as a solvent. In decade years, reports have been numerous researches on UAE for isolating various phytochemicals. Examples include the extraction of bioactive compounds from herbs (Vinatoru, 2001), ginsenosides from ginseng roots and cultured cells (Wu et al., 2001), polysaccharides from *Salvia officinalis* L. (Hromadkova et al., 1999), rutin from *Sophora japonica* (Paniwnyk et al., 2001), and essential oils from plant materials (Toma et al., 2001).

Ultrasonic-assisted extraction (UAE) can greatly enhance extraction efficiency, reducing both processing time and solvent usage. Additionally, UAE can be performed at lower temperatures, thereby preventing thermal degradation of the extracts and minimizing the loss of volatile compounds that would typically occur during boiling (Wu et al., 2001). The improved solvent extraction in UAE is primarily attributed to the mechanical effects of acoustic cavitation, which facilitates deeper solvent penetration into plant tissues and promotes the release of intracellular compounds by disrupting cell walls (Mason et al., 1996). An effective tool for speeding a number of analytical procedures is ultrasonic radiation. In comparison to traditional extraction methods, ultrasound-assisted leaching is a quick and efficient means to extract analytes from various matrices (Dobiá et al., 2010). Because it enhances solvent penetration into the cellular matrix and improves mass transfer of constituents, it has proven more at extracting bioactive components than standard methods (Mason et al., 1996). Another benefit over SWE is that it uses lower extraction temperatures, which lessens the chance of catechin degradation. There are few researches that use ultrasound-assisted extraction

and effects to promote or extract processing, compared to how frequently industrial processing is studied. As with soxhlet extraction, a range of natural substances may be extracted using ultrasound-assisted extraction with any solvent (Wang & Weller, 2006). This technique involves mixing the sample with an appropriate solvent before adding it to the ultrasonic bath at a certain temperature and time.

An effective tool for speeding a number of analytical procedures is ultrasonic radiation. In comparison to traditional extraction methods, ultrasound-assisted leaching is a quick and efficient means to extract analytes from various matrices (Dobiá et al., 2010). Because it enhances solvent penetration into the cellular matrix and improves mass transfer of constituents, it has proven more effective at extracting bioactive components than standard methods (Mason et al., 1996). Another benefit it uses lower extraction temperatures, which lessens the chance of catechin degradation. There are few researches that use ultrasound-assisted extraction and effects to promote or extract processing, compared to how frequently industrial processing is studied. As with soxhlet extraction, a range of natural substances may be extracted using the ultrasound-assisted extraction with any solvent (Wang & Weller, 2006). This technique involves mixing the sample with an appropriate solvent before adding it to the ultrasonic bath at a certain temperature and time.

In comparison, while traditional solvent extraction is simple and cost-effective, UAE offers advantages in terms of efficiency, speed, and environmental impact, making it a preferable choice for high-quality catechin extraction, especially in research and industrial applications where these factors are critical. Conventional stirred extraction showed ethanol to be significantly less effective than ethyl acetate and butanone. However, ultrasound improved ethanol's performance to levels comparable with butanone and ethyl acetate. Thus, ultrasonication can reduce reliance on certain solvents and enable the use of alternative ones.

2.3 Effects of Catechin on Human Body

Catechins offer a number of benefits. Catechins are important components of tea leaves, known for their potent antioxidant properties and various physiological effects. Such as antioxidant anticancer, antimicrobial, capacity, anti-inflammatory and anti-allergic capacity, these are the most prominent functions of catechins. Due to these beneficial effects, tea catechins are increasingly utilized in medical, pharmaceutical, and cosmetic products and are actively researched through various approaches.

2.3.1 Antioxidant Effects

Numerous studies suggest that the polyphenols in green tea leaves have antitumor effects by inhibiting cell division and inducing phase II antioxidant such as superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase and reductase. Antioxidants are substances that defend cells from reactive oxygen species, including singlet oxygen, superoxide, peroxyl, hydroxyl, and peroxynitrite radicals. A significant natural antioxidant with potent anti-oxidant action is EGCG. Sano et al. (2001) reported the inhibitory effect of green tea leaves on lipid peroxidation induced by tert-butyl hydrogen peroxide and the effect of antioxidant on the kidney after oral administration of EGCG, an important tea polyphenol. The antioxidant capacity of crude catechin powder and individual catechin was studied by active oxygen species method. Crude catechins are more effective than DL-A-tocopherol in reducing peroxide formation. *In vivo* studies have shown that green tea catechins enhance total plasma antioxidant activity (Ananingsih et al., 2001). Consumption of green tea extracts has been found to increase serum superoxide dismutase activity and catalase expression in the aorta, aiding in cellular protection against reactive oxygen species (Skrzydlewska et al., 2002; Negishi et al., 2004). Additionally, these extracts directly impact reactive oxygen species by reducing plasma nitric oxide levels (Negishi et al., 2004). These findings suggest that catechins may exert antioxidant effects either directly or indirectly by boosting the activity or expression of protective enzymes.

Catechins are believed to help protect against diseases by contributing to the overall antioxidant defense system, along with antioxidant vitamins and enzymes. Numerous studies have reported that green tea catechins enhance total plasma

antioxidant activity. These findings imply that catechins may have an impact, either directly or indirectly. Catechins may inhibit the oxidation of other antioxidants, such as vitamin E, because they can function as antioxidants *in vitro*. However, consuming green tea catechins *in vivo* had no effect on the plasma status of vitamins E and C (Skrzydlewska et al., 2002; Tiburg et al., 1997; Alessio et al., 2002).

2.3.2 Anticancer of Catechins

Scientific research indicates that EGCG, the predominant polyphenol in tea, along with other polyphenols, demonstrates significant anticancer potential. EGCG, a polyphenolic flavonoid abundant in tea, has shown promise in influencing human health and combating disease. It has been found to inhibit tumor cell metastasis and angiogenesis, and may also help overcome drug resistance in cancer cells, positioning it as a promising candidate for combination therapies. EGCG can neutralize reactive oxygen species, including hydrogen peroxide, superoxide anions, and hydroxyl radicals, thus mitigating oxidative DNA damage, mutations, tumor progression, and carcinogenesis (Wang et al., 2019; Hidgn et al., 2017; Lambert et al., 2009). Furthermore, EGCG exhibits anticancer effects by modulating Toll-like receptor 4 and Toll-interacting protein via the 67-kDa laminin receptor (67LR). This receptor plays a crucial role in cellular adhesion and cancer cell metastasis, and also functions as a cell-surface receptor for EGCG (Byun et al., 2010; Byun et al., 2014).

2.3.3 Antimicrobial of Catechins

Research on recent years has shown that green tea catechins, particularly (+)-epigallocatechin gallate (EGCG) and (+)-epicatechin gallate (ECG), effectively inhibit the growth of both Gram-positive and Gram-negative bacteria to a moderate degree. The antimicrobial mechanisms of green tea catechins include damage to the bacterial cell membrane, inhibition of bacterial fatty acid synthesis, and suppression of various enzymes such as protein tyrosine kinase, cysteine proteinases, DNA gyrase, and ATP synthase, as well as reduction of efflux pump activity (Reygaert et al., 2014). Ikigai et al. (1993) observed that high concentrations of EGCG could cause permanent damage to the bacterial cytoplasmic membrane, evidenced by leakage of fluorescent dye from phosphatidylcholine (PC) liposomes. However, Incorporating charged lipids into the liposomal bilayer, which more closely resembles the composition of

cytoplasmic membranes in both Gram-positive and Gram-negative bacteria, helped reduce the extent of this damage.

2.3.4 Anti-inflammatory of Catechins

Green tea extract, rich in catechins, has been widely studied for its anti-inflammatory properties, especially in preclinical models. Research highlights its effectiveness in addressing inflammatory responses related to nonalcoholic steatohepatitis (NASH). Specifically, supplementation with green tea extract or its key catechin, epigallocatechin gallate (EGCG), has been shown to reduce liver steatosis and damage while mitigating NFκB-dependent inflammation in both genetic and diet-induced NASH models (Park et al., 2011; Chung et al., 2012; Park et al., 2012). Conversely, EGCG's regulation of the NF-κB pathway reduces NF-κB DNA binding activity and lowers the expression of pro-inflammatory mediators, including interleukin-1β (IL-1β), IL-6, IL-8, and tumor necrosis factor-α (TNF-α). It also decreases the levels of downstream enzymes such as poly [ADP-ribose] polymerase (PARP), COX-2, and iNOS, and reduces the recruitment of inflammatory cells. (Kanlava et al., 2019; Riegseckker et al., 2013; Wang et al., 2015). Anti-inflammatory effects of EGCG are linked to elevated levels of longevity factors like sirtuin-1 and Forkhead box O3 (FOXO3a) (Niu et al., 2013). Additionally, Ludwig et al. (2004) proposed that EGCG might be beneficial for treating atherosclerosis by down regulating vascular cell adhesion molecule-1 (VCAM-1). Nonetheless, further comparative *in vivo* and *in vitro* research is required to fully studied the specific antioxidant capacity and anti-inflammatory mechanisms about EGCG.

2.3.5 Anti-allergic of Catechins

Recent evidence indicates that leaves from specific tea cultivars with high levels of methylated catechins are more effective in alleviating allergic symptoms. The anti-allergic activities of catechins may be linked to their binding to the 67-kDa laminin receptor (67LR) (a cell surface receptor), the 67 kDa laminin receptor (67LR) as a cell-surface receptor that mediates the anti-cancer action of EGCG (Tachibana et al., 2004). Fujimura et al. (2008) found that down regulating the expression of 67LR resulted in decreased activities of galloylated catechins. This suggests that both the galloyl group and the hydroxylation pattern on the B-ring are crucial for the biological activities of

tea catechins and their interaction with 67 LR. Recent evidence indicates that leaves from specific tea cultivars with high levels of methylated catechins are more effective in alleviating allergic symptoms.

2.3.6 Inhibit α-Glucosidase Activity

α-Glucosidase plays a key role in carbohydrate digestion, and its inhibition can reduce excessive glucose absorption in the small intestine, making it an effective strategy for managing blood glucose levels and preventing diabetes and obesity (Azuma et al., 2011). Matsui et al. (2007) demonstrated that the inhibitory activity of catechins against α-glucosidase follows this order: EGCG > ECG > EC > EGC > C. This suggested that catechins with a 2,3-cis structure, such as ECG and EGCG were more effective inhibitors compared to those with a 2,3-trans structure, such as CG and GCG. Additionally, galloylated catechins were more potent inhibitors than nongalloylated ones.

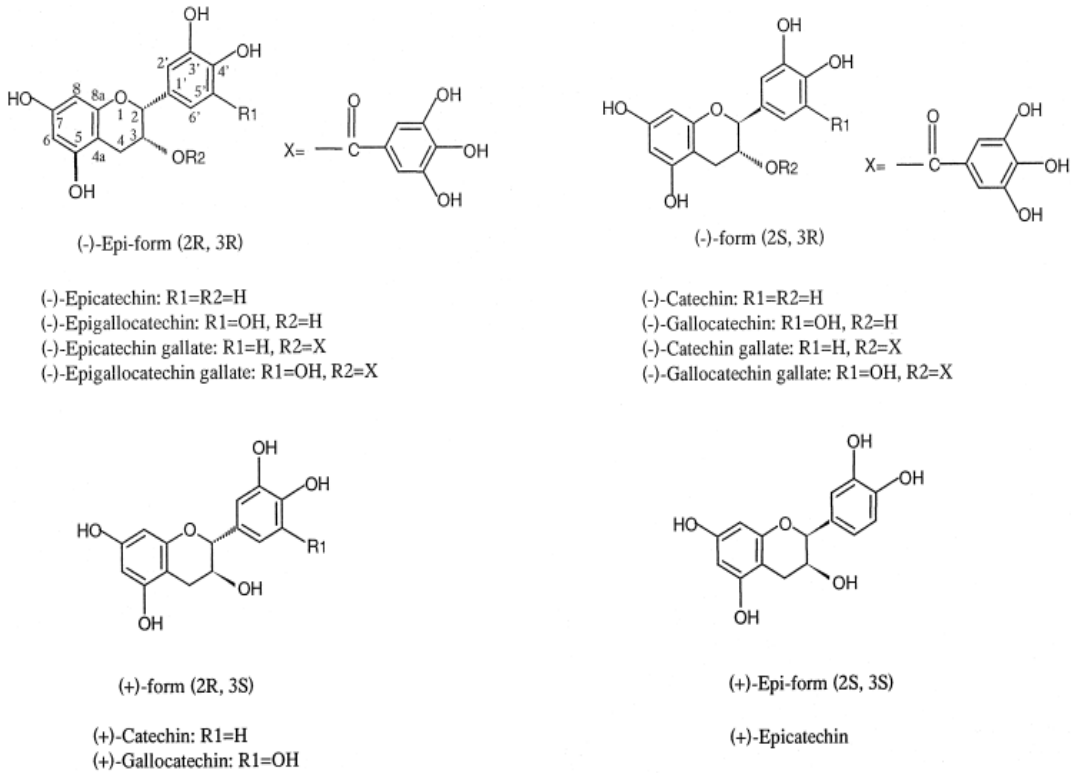
2.3.7 Inhibit α-amylase Activity

α-Amylases (EC 3.2.1.1) catalyze the hydrolysis of α-1,4-glucosidic linkages in starch, glycogen, and various oligosaccharides. Inhibiting their activity in the human digestive tract can help control obesity and diabetes by reducing glucose absorption from starch (Gong et al., 2020). As a result, there has been considerable interest in finding effective and non-toxic α-amylase inhibitors. Recent research has focused on catechins as potential inhibitors. For example, tea polyphenols with a concentration of 0.05 mg/mL, including 5.1% C/EGC, 40.9% EGCG, 30.4% ECG, 10.9% GCG, and 6.3% EC, achieved an α-amylase inhibition ratio of about 61% (He et al., 2007). Tadera et al. (2006) compared the inhibitory effects of C, EC, EGC, and EGCG on human pancreatic α-amylase and found the order to be: EGCG > EC > EGC > C. These findings indicate that galloylated catechins have higher inhibitory activity than non-galloylated catechins, and catechins with a catechol structure (CG and ECG) are twice as effective as those with a pyrogallol structure (GCG and EGCG). Furthermore, catechins with a 2,3-trans structure (CG and GCG) showed ten times higher inhibition compared to those with a 2,3-cis structure (ECG and EGCG) (Hara & Honda, 1990).

2.4 Stability of Catechin

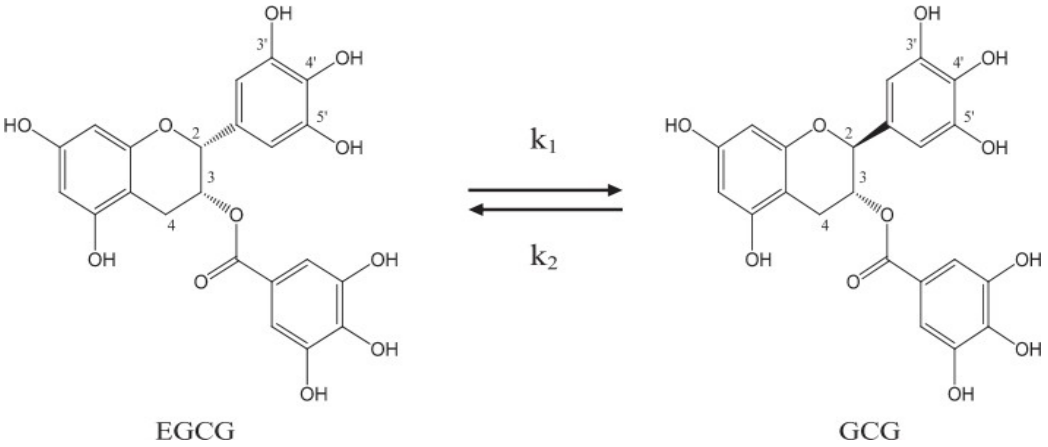
The stability report of green tea catechins to ascertain their chemical alterations during food processing has garnered more attention. Tea leaves are the source of the phytochemicals known as catechins. The lack of fermentation process involved in many productions of green tea, the amount of tea catechins it contains is higher than that of black tea and oolong tea (Toschi et al., 2000). The catchins structures of the main catechins and their epimers are illustrated in Figure 2.2. During manufacturing and brewing, tea catechins undergo various chemical transformations. Research has shown that tea catechins extract be converted to their corresponding epimers during traditional brewing, production, and storage of tea infusions and tea drinks (Chen et al., 2001; Zhu et al., 1997). Epimerization of catechins is influenced by temperature and tends to be sensitive at high temperatures (Wang & Helliwell, 2000). Research has shown catechin was specifically undergo epimerization at the C-2 position when exposed to hot aqueous solutions (Kiatgrajai et al., 1982). It can convert epi-structured catechins to non-epi-structured catechins and vice versa. According to Wang et al. (2008), increasing the temperature leads to a decrease in catechin concentrations while their isomers increase. This indicates catechin degradation, as total catechin levels decline with higher temperatures. During brewing, catechins experience chemical reactions, it including antioxidation and epimerization. The epimerization of catechins is regarded as one of the most important reactions in the production of green tea (Wang & Helliwell, 2000). Research has focused on the oxidative conversion of catechins to theaflavins and thearubigins during black tea production (Davis et al., 1995). During the fermentation of tea, polyphenol oxidase in the tea leaves catalyzes the oxidation of most catechins into theaflavins, thereby reducing the overall catechin content (Friedman et al., 2009). Epimerization, which involves the conversion of tea catechins to their respective isomers, has been observed during the production of tea and tea beverages (Komatsu et al., 1993; Suematsu et al., 1992; Yoshida et al., 1999). Tea catechins are converted to their matching isomers by a process called epimerization. Green tea contains the recognized epicatechins EGCG, EGC, ECG, and EC, which are all trans-structured. These compounds can convert into their non-epicatechin epimers, specifically

GCG, GC, CG, and C (Chen & Chan, 1996; Wang et al., 2008). The chemical conversion between catechin pairs is reversible. The part of chemical structures on epicatechins and non-epicatechins differ in the 2R, 3R (2, 3-cis, epi-form) and 2S, 3R (2, 3-trans, non-epi form) configurations. Figure 2.3 demonstrates the reversible conversion between EGCG and GCG (Ananingsih et al., 2001).



Source Wang H., (2000)

Figure 2.2 Chemical Structures of Tea Catechins and Their Epimers



Source Sharma (2013)

Figure 2.3 Epimerization of Epistructured Catechin

2.4.1 Effect of PH and Temperature

The stability of green tea catechins is influenced by pH and temperature. In acidic solutions (pH < 4), catechins remain exceptionally stable, whereas they become unstable in solutions with pH > 6 (Komatsu et al., 1993; Chen et al., 2001; Su et al., 2006). Additionally, storage temperature significantly affects catechin stability, even at ambient conditions. In alkaline solutions (pH > 8), increasing pH accelerates catechin degradation (Chen et al., 2001). Zhu et al. (1997) showed that (-)-epigallocatechin gallate and (-)-epigallocatechin are less stable than (-)-epicatechin and (-)-epicatechin gallate in basic solutions. This instability could account for the partial absorption of green tea catechins in mouse red blood cells, as the instability of (-)-epigallocatechin gallate and (-)-epigallocatechin in the intestine, where the pH is neutral or alkaline, might be a contributing factor. Research into catechin stability in green tea infusions has shown that epimerization can occur at temperatures as low as 40 °C over time, indicating that both temperature and duration of heating influence catechin epimerization (Wang & Helliwell, 2000). Komatsu et al. (1993) observed that tea catechins could undergo simultaneous degradation and epimerization during thermal processes. Studies by Wang et al. (2006), as well as Wang et al. (2008a) showed that both epimerization

and degradation adhere to pseudo-first-order kinetics. with temperature-dependent kinetic constants described by the Arrhenius equation. At elevated temperatures, green tea catechins become less stable, with heating potentially leading to the conversion of catechins into their isomers through epimerization. For example, epigallocatechin gallate may transform into gallocatechin gallate under high-temperature conditions. Heat treatments can reduce the antioxidant activity of green tea catechins through processes like antioxidation, polymerization, epimerization, and thermal degradation (Ananingsih et al., 2013).

2.4.2 Effects of Oxygen Concentration and Metal Ions

Catechin stability is influenced by factors such as oxygen concentration, free radicals, and metal ions. Sang et al. (2005) found that varying levels of antioxidants can enhance catechin oxidation. Under low oxygen conditions (using nitrogen flushing) at 37 °C and pH 7.4, EGCG showed significant stability with only 5% degradation after 6 hours. This minimal degradation was attributed to the epimerization of EGCG to GCG, as dimers, which are oxidation products of EGCG, were not observed. Conversely, under normal atmospheric conditions, EGCG in aqueous solution at 37 °C and pH 7.4 degraded by 90% within 2 hours, with no EGCG remaining after 6 hours. Additionally, the study highlighted that the presence of ethylenediaminetetraacetic acid (EDTA) improved stability on catechin, likely due to EDTA ability to chelate metal ions that catalyze the oxidation of EGCG.

Metal ions can influence the oxidative activity of catechins by binding to them and forming metal complexes. This interaction can affect the catechins' ability to neutralize free radicals and maintain their antioxidant properties. The formation of these metal complexes can alter the catechins' stability and reactivity, potentially reducing their effectiveness as antioxidants. The study showed by Kumoto et al. (2001) demonstrated that different metal ions in this report have different antioxidant activities of catechins to enhance or reduce this antioxidant activity. The antioxidant activity of EGCG was enhanced by the presence of Cu2+ and Mn2+, whereas Fe2+ diminished that activity. The compounds formed by metal ions and EGCG reduce or enhance the oxidation potential of EGCG. The formation of phenoxy radicals readily occurs in ECG and EGCG because of their gallate group. The presence of Cu2+ and Mn2+ increased

the antioxidant activity of EGCG and promoted the antioxidant activity of EGCG to some extent, hence the use of catechins with Cu2+ is favorable as an antioxidant. The binding of metal ions to EGCG and the change of oxidation potential rationalized the experimental results. In ECG and EGCG, phenoxy radicals are easily formed, which contain gallic acid groups, Cu2+ and Mn2+ to assist the reaction and increase the antioxidant activity of catechins. Therefore, the use of catechins and Cu2+ as antioxidants is advantageous. In addition, the use of metal ions, especially Cu2+ and Fe2+, to measure the antioxidant activity of catechins is not suitable for the correct evaluation of the antioxidant activity of catechins, because metal ions greatly affect the antioxidant activity of catechins.

2.5 Catechin Activity

Tea catechin extract has demonstrated inhibitory effects against tumorigenesis and various other diseases in recent years, highlighting its potential as a natural therapeutic agent. However, the therapeutic efficacy of tea catechins is limited by their low oral bioavailability, which is due to poor stability, rapid metabolism, and inefficient intestinal absorption. Additionally, the strong bitter taste of tea, particularly from compounds like EGCG, further challenges its palatability and consumer acceptance. These factors collectively hinder the full exploitation of tea health benefits in clinical applications. To overcome these limitations, strategies such as encapsulation, formulation with bioenhancers, and structural modification of catechins are being explored to improve their stability and absorption.

2.5.1 The Bitterness

Epigallocatechin gallate (EGCG) is associated with various health benefits; however, its bitter taste can affect the preference for products containing high concentrations of this compound. Gallated catechins, such as EGCG and ECG, exhibit stronger bitterness and astringency compared to non-gallated catechins (Narukawa et al., 2010; Xu et al., 2018). Given the strong bitter taste of catechins, research has focused on bitter taste receptors as potential receptors for catechins among taste receptors (Chandrashekar et al., 2006). Thus, taste receptor cells (TRCs) act as

sensory detectors for bitter compounds (Behrens et al., 2006). TRCs are grouped into taste buds, which are located in the epithelium of the gustatory papillae on the tongue and palate, with each taste bud containing 50 to 100 TRCs (Montmayeur & Matsunami, 2002). These cells express members of the TASTE Receptor type 2 (TAS2R) gene family, responsible for encoding bitter taste receptors (Chandrashekar et al., 2000). The TAS2R family, comprising 25 members in humans, detects bitter compounds. Catechins, known for their strong bitterness, activate taste bud cells in mouse circumvallate papillae when ECG is applied (Narukawa et al., 2010). These findings indicate that catechins are recognized as tastants and suggest the presence of a bitter taste receptor specifically responsive to catechins in taste cells. However, research on ways to alleviate the bitter taste of catechins has become an important topic. Many attempts have been made to reduce the bitter and astringent catechin, in order to improve the sensory quality of catechin extraction. To address the issue of unpleasant flavor, methods for reducing bitterness need to be explored. A cell-based assay using hTAS2R39 can evaluate bitterness in a manner similar to human taste perception. This approach will allow for the objective measurement of bitterness in catechins, such as ECG and EGCG, providing a reliable system for assessing and potentially mitigating the unpleasant taste associated with these compounds.

2.5.2 Low Bioavailability and Digestibility

Consuming foods high in catechins is linked to a lower risk of developing chronic diseases. Although encouraging, it is thought that the low oral bioavailability of catechins from food reduces their potential effectiveness. It is thought that a variety of variables, such as digestive instability, low intestinal transit, and quick metabolism and clearance, all contribute to the low bioavailability of catechins. Catechin bioavailability is limited due to its large molecular size and numerous hydrogen bonds. Fan et al. (2016) noted that the molecular structure and steric configuration of catechins influence their bioactivities and intermolecular interactions, potentially leading to varied behaviors among different catechins. Additionally, hot and clear tea infusions can form tea cream, which are visible, precipitates that appear upon cooling during the production of ready-to-drink tea.

2.6 Tannin

Tannins are secondary metabolites for plant and the second most rich naturally occurring phenols and they were found diffusely in plants (Arbenz & Averous, 2015). These polyphenolic compounds are classified as water-soluble phenolics with a molar mass from 300 to 3000, and are capable of precipitating alkaloids, gelatins, and other proteins. Tannins interact with various biological macromolecules, including polysaccharides, proteins, cellulose, starch, and gelatin, forming complexes whose molecular weight can vary based on the macromolecule involved (Lekha & Lonsane, 1997; Aguilar & Gutierrez-Sanchez, 2001). They exhibit widely much of biological activities, including antioxidant effects, metal ion chelation, and protein precipitation (Hagerman, 2002). Tannins contribute to blood coagulation, support wound healing, and help lower blood pressure, while also displaying antimutagenic, anticancer, and antioxidant properties. Additionally, they serve as immunomodulators and antimicrobial agents (Chung et al., 1998; Chokotho & Van Hasselt, 2005). However, tannins can have undesirable effects. They impart bitterness and astringency to fruits and plants (Aguilar et al., 2007). Their ability to bind proteins can negatively affect the nutritional quality of food by inhibiting digestive enzymes and removing essential proteins, leading to potential digestive issues and making them an anti-nutritional factor (Kumar & Singh, 1984; Lekha & Lonsane, 1997). Improved handling of tannin-rich foods is necessary to enhance their nutritional value while maintaining their essential dietary components.

2.7 Tannase Application

Tannin acyl hydrolase, commonly known as tannase, is an industrially significant enzyme with a broad range of applications, making it a candidate for ongoing development and improvement. Previous research has demonstrated that Tannase treatment can enhance the taste quality and mouthfeel of green tea beverages, concentrates, and instant powders (Chávez-González et al., 2012). Tannase (EC 3.1.1.20) is commonly employed to catalyze the hydrolysis of gallated catechins, converting them into non-gallated

catechins and gallic acid (GA). This process helps reduce tea cream formation and enhances the color of the final product (Lu et al., 2009). Therefore, EGCG can be transformed into EGC and GA using tannase. Previously reported that hydrolyzing EGCG and ECG with tannase increased the green tea infusions' general acceptance and pleasant aftertaste (Zhang et al., 2016). Tannase-mediated transformation of EGCG notably improves the clarity of green tea infusion and enhances its inhibitory antioxidant capacity, as well as its hydroxyl radical scavenging activity (Ni et al., 2015). Another study revealed that enzymatic products like EGC and GA, formed from the degalloylation of EGCG in green tea, displayed significant scavenging and antioxidant properties (Battestin et al., 2008). Furthermore, research by Lu and Chen (2008) showed that tannase-catalyzed hydrolysis of gallated catechins, such as EGCG and ECG, enhanced the radical-scavenging activity of tea extracts against superoxide anions, hydrogen peroxide, and DPPH. The relationship between ferric reducing power and total phenolic content was established by Ercan and Ekrem (2011). The overall amount of catechins did not vary considerably, but tannase treatment increased the tea extract's ability to reduce ferric iron, which may be due to the presence of EGC and gallic acid. McDougall et al. (2009) noted that the number of galloyl groups impacts the protein-binding ability of tannins. Specifically, the protein-binding capacity of EGCG diminishes due to the cleavage of ester bonds by tannase's hydrolytic action. This reduction results in tea extracts from treated leaves having a lower lipase inhibition effect. However, enzymatic extraction must be performed at lower temperatures (30-40°C) for extended durations (over 2 hours) due to the constraints of optimal enzymatic reaction temperatures (Hong et al., 2012). Prolonged treatment can lead to quality degradation during extraction (Murugesh et al., 2014) and poses challenges for industrial-scale production. Prolonged tannase treatment may degrade too much catechin, making it impossible to control the hydrolysis reaction and the production efficiency of secondary products.

Therefore, according to the above reports, it can be known that tannase can bring a lot of benefits in the conclusion of catechin treatment. Tannase treatment of catechins enhances their bioavailability by degrading tannins that bind to them, improves their solubility for better absorption, and may increase antioxidant activity through structural modifications. This enzymatic treatment also reduces astringency, making products more palatable, and can lead to the formation of novel compounds with unique health

benefits, while potentially enhancing the overall antioxidant capacity through synergistic interactions with other phenolic compounds. Thus, it is worthy of further study on the application of tannase in catechin.

The two distinct processes that tannase catalyze. Esterase activity and depsides activity. it catalyzed the degradation of tannin, methyl gallate (MG), epigallocatechin-3-gallate (EGCG) and epicatechin-3-gallate (ECG). As mentioned, the single galloyl binding site of tannase is crucial for both esterase and depside activities (Ren et al., 2013). Esterase activity involves the hydrolysis of ester bonds in compounds such as galloyl glucose and methyl gallate. In contrast, depside activity pertains to the cleavage of depside bonds found in substances like digallic acid, gall tannins, ellagitannins, and complex tannins (Haslam & Stangroom, 1966; Saxena & Saxena, 2004; Sharma et al., 2000). However, tannases do not target C–C bonds and are thus unable to hydrolyze condensed tannins (Haslam & Stangroom, 1966).

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Raw Materials

Dried tea leaves used in this experiment were *Camellia sinensis var. assamica* tea collected from Wawee Tea plantation, Mae Suai District, Chiang Rai, Thailand. Dried fresh tea leaves were plucked and dried at 150 ℃ for 60 minutes. The moisture content was required lower 5% (dry basis). The dried tea leaves were packed with plastic bag and stored in a dry environment at room temperature.

3.1.2 Chemicals and Equipment

All chemical and instruments that used this research are listed in Table 3.1

Table 3.1 Chemical List

Parameters	Chemicals	Company
Extraction	Distill water, 50% ethanol	The Liquor Distillery Organization Thailand
Total polyphenol content	Folin Ciocalteu's phenol, Gallic acid, Na2CO3	Elite Scientific & Meditech Co.
DPPH	DPPH solution, Methanol	Sigma Corporation Japan, Merck & Co Germany
Catechin and Caffeine content	Trifluoroacetic acid, Acetonitrile	Merck & Co Germany
Tannin content	Folin-Ciocalteu reagent, Na2CO3	Elite Scientific & Meditech Co.
Tannase treatment	Tannase	Kikkoman Japan

Table 3.1 (continued)

Parameters	Chemicals	Company
Stomach digestion	Pepsin HCL	Sigma Corporation Japan
Intestine digestion	Pancreatin NaOH	CP Lab Safety U.S.
Glucose consumption	DNS (3, 5-dinitrosalicylic acid reagent)	Merck & Co Germany
α-amylase	DNS (3, 5-dinitrosalicylic acid reagent)	Merck & Co Germany

Table 3.2 Instrument List

Instrument	Model/Brand	Country
Shaker	SK2-PO Wellab	United States
Ultrasonic Cleaner	08895-11 Cole-Parmer	United States
Rotary evaporator	N-1110V-WD Eyela	Japan
Freeze dyer	Delta 2-24 LSC Christ	Germany
High Performance Liquid Chromatography (HPLC)	DSU-405 Bara scientific	Japan
UV-Vis spectroscopy	840-309300 Thermo scientific	United States

3.2 Methodology

This research was divided into 3 experiments. Firstly, the effect of UEA method on physicochemical properties of catechin extract was studied. The suitable condition from UAE is selected for next experiments. Then, the effect of tannase on physicochemical properties of catechin extract was investigated. Lastly, the stability of catechin extract during storage time with different temperature (-20 ℃, 30 ℃) was explored. Overall methodology was shown in Figure 3.1.

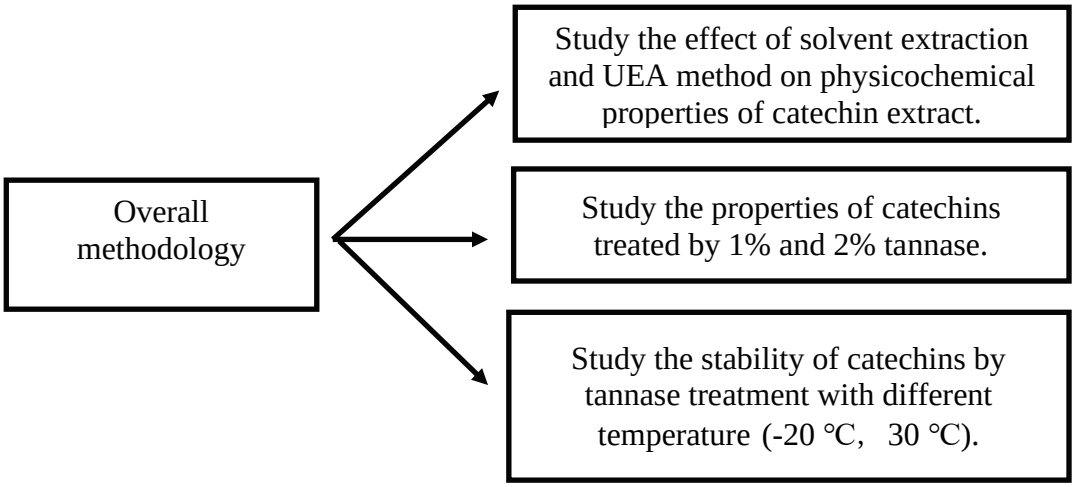


Figure 3.1 Overall Methodology

3.2.1 Experiment I: Study the Effect of Different Extraction Method on Physical and Chemical Properties of Catechin Extract

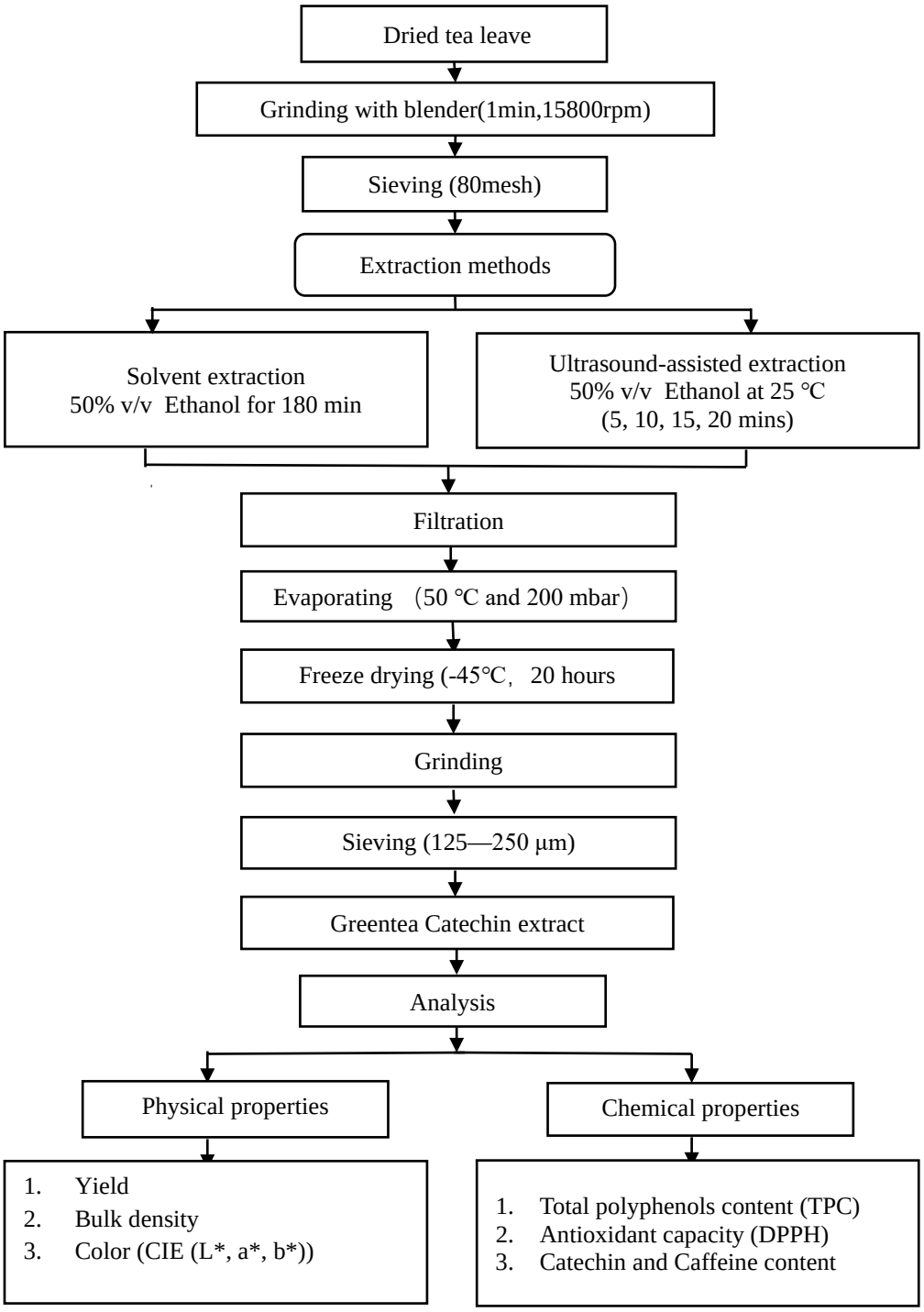


Figure 3.2 Research Framework

3.2.1.1 Dried tea leave preparation

Dried tea where were grinded with a blender (WARING COMMERCIAL, USA) and prepared for extraction .The grinding machine was used to reduce particle size and used sieving machine to obtain the tea leave powder 100-180 μm (80 mesh). Tea leave powder were at room temperature (approximately 25 ℃). The grinding machine were at 15800 rpm for 1minute. Then dried tea leaves were extracted by 50 %v/v ethanol with solvent and ultrasound-assisted extraction, after the processing respectively followed by filtration, evaporation and freeze drying to obtained catechins extract.

3.2.1.2 Green tea catechin preparation

1. Extraction with Solvents Extraction

Tea extracts are prepared from green tea following 3 main steps including: extracting soluble substances from dried tea leaves into tea infusions, concentrating the tea solution, and finally dry it to obtain the tea extracts in powder form. This experiment was modified Kumar and Rajapaksha (2015) developed a previous process to extract crude tea extract from tea leaves involved first grinding the dried tea leaves and then extracted the tea with water ethanol (50% v/v) in a solvent to ground tea ratio of 1:20. Then it was shaken in a shaker at room temperature (about 25 °C) for 90 minutes, and then the tea was brewed and filtered and concentrated in a rotary evaporator (water bath temperature at 50 °C) and freeze-dried to obtain a powdered tea catechin extract. Finally, 150μm catechin extract was screened with a screening machine.

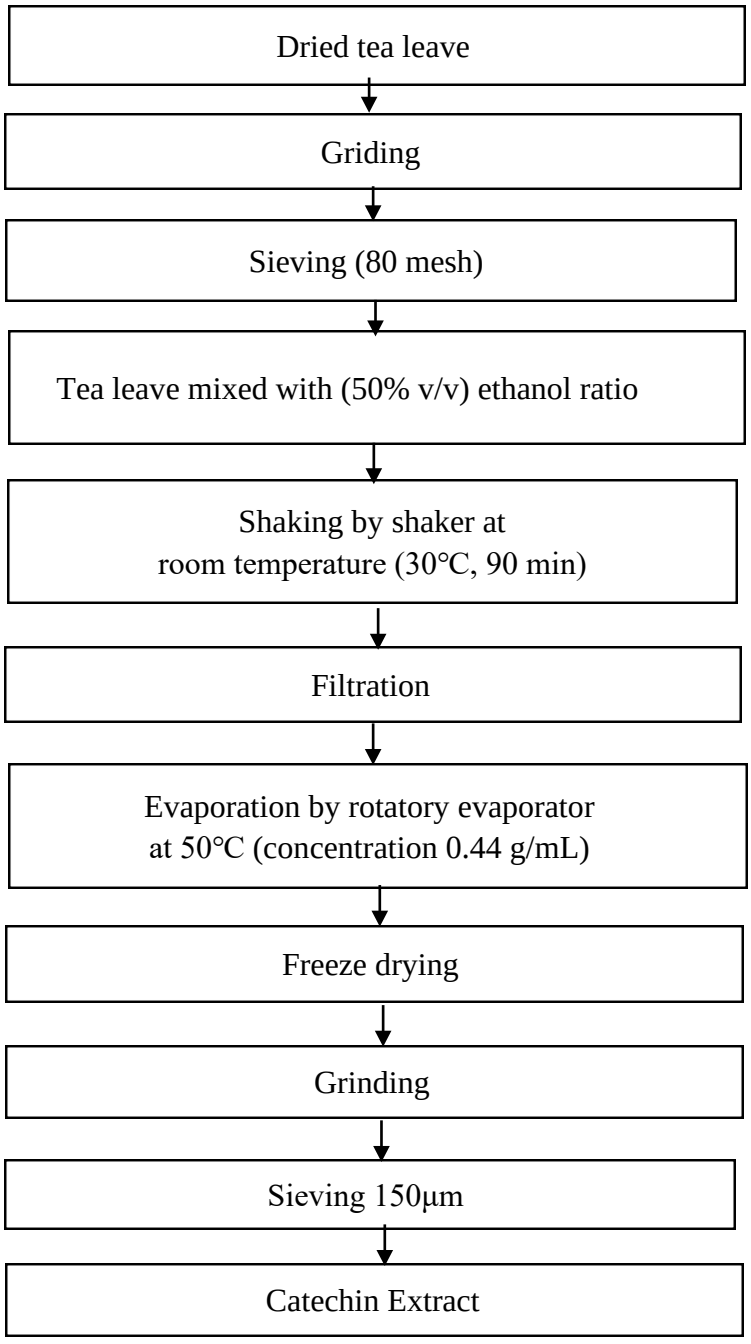


Figure 3.3 Solvent Extraction by 50% Ethanol

2. Ultrasound-Assisted Extraction

This extraction in main followed three steps from solvents extraction: The process involves extracting soluble substances from tea leaves into tea infusions, then concentrating the tea solution, and finally drying it to obtain the final product in powder form. But the grided green tea leaves add ethanol (50% v/v) to mix the ratio between solvent and grinder tea ratio 1:10 and 1:20, then use Ultrasonic machine (Cole-Parmer brand at 25 ^{o}C, every 5 minutes (5 to 20 min), then took the tea infusion to filtration and concentrated on a rotatory evaporator (the temperature of water bath at 50^{o}C) and finally freeze-dried to obtain a powdered catechin extract. Finally used sieving machine to screen green tea catechins and kept in plastic bag and wrap bag with aluminum foil in the freezer at -20 ℃.

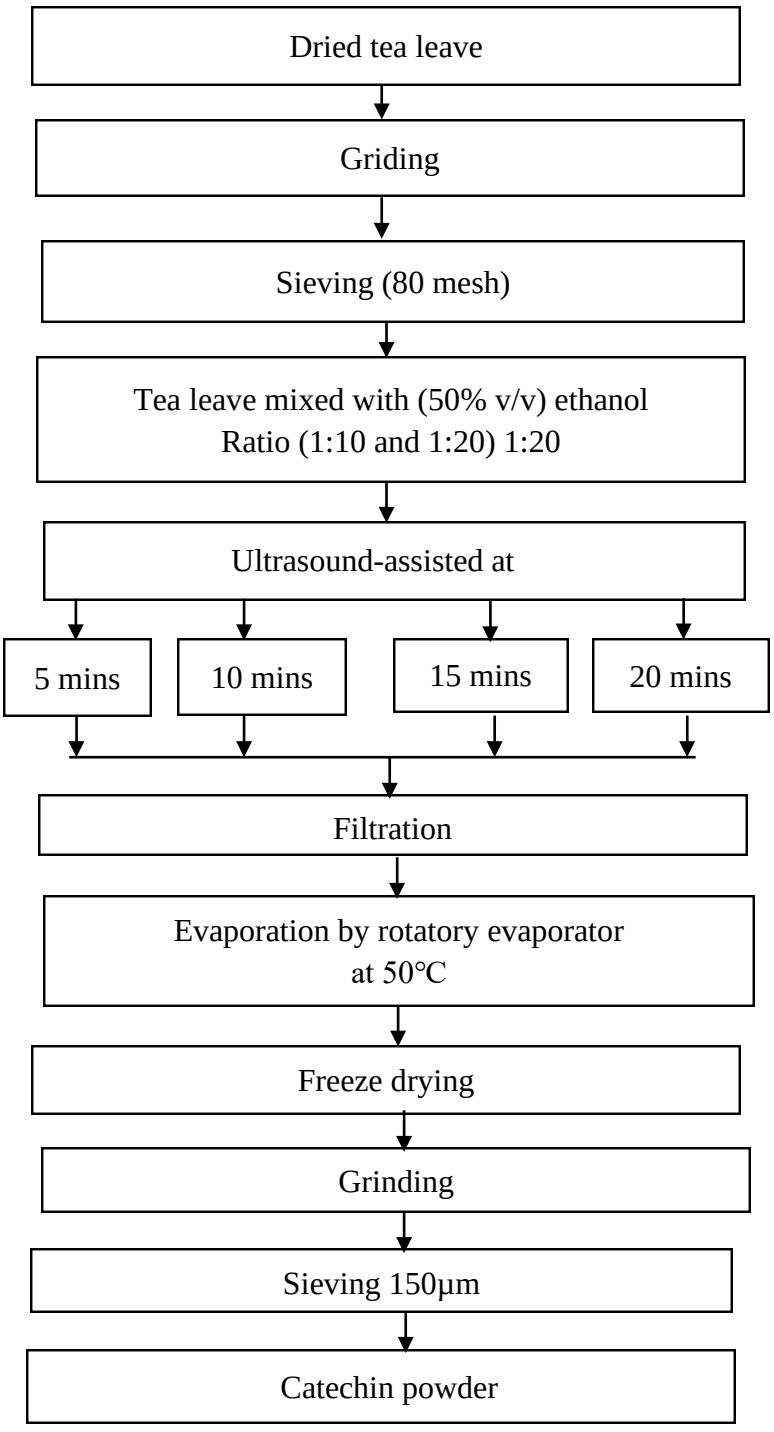


Figure 3.4 Ultrasound-Assisted Extraction

3.2.1.3 Calculation of Yield Extract

The extract yield was calculated using Equation:

$$\text{Extract Yield (\%).} = Wx/Wxy\ 100\%$$

Where, Wx is mass of dry extract in gramme and Wxy is mass of sample in gramme. Based on the calculation of the extract, the extract yielded from using Extraction with conventional solvents and Ultrasound-assisted extraction was compared.

3.2.1.4 Water solubility

Water solubility was evaluated using a modified approach based on the method by Eastman and Moore (1984). To begin, 0.3 g of the inclusion complex (on a dry basis) was placed into a centrifuge tube, and 30 mL of distilled water was added. The mixture was then vortexed at 15,000 rpm for 5 minutes. Following vortexing, the sample underwent centrifugation at 3,000 × g for 2 minutes. Subsequently, 25 mL of the supernatant was transferred to a pre-weighed petri dish and dried in an oven at 105°C for 3 hours. The water solubility (%) was determined using the equation outlined by Cano-Chauca et al. (2005).

$$\text{Water solubility} = W1/W0 \times 100\%$$

Where W1 represents the weight of the recovered sample and W0 is the original weight of the sample added. The solubility value was further validated by determining the concentration of catechin in the aliquot using HPLC.

3.2.1.5 Ture density

The true density of the inclusion complex was measured using a method adapted from Kha et al. (2010). Approximately 1 gram of the inclusion complex was precisely weighed and placed into a 5 mL measuring cylinder, followed by vortexing for 1 minute. The true density was then calculated by dividing the mass of the powder by the volume it occupied in the cylinder.

$$\text{Ture density } (\rho) = \text{Powder mass/ volume of the powder}$$

3.2.1.6 Measurement of color

The color of the catechin extract was assessed using the CIE (L*, a*, b*) system with a Spectrophotometer (CM-600D; Konica Minolta, Osaka, Japan). The L* value measures lightness, with 0 representing black and 100 representing white. The a* value reflects redness (positive values) and greenness (negative values), while the b* value indicates yellowness (positive values) and blueness (negative values).

3.2.1.7 Determination of total polyphenol content

The content of tea polyphenols in the extract was quantified using a colorimetric assay, employing Folin-Ciocalteu's phenol reagent and gallic acid as the standard, in accordance with the International Standard (ISO/CD 14502-1, 2005). Polyphenols in the extract were calculated as the ratio of total polyphenols to the solid content in the extract. The analysis used a 1000-fold dilution of concentrated green tea. To perform the assay, 5 mL of 10% Folin-Ciocalteu reagent was added to 1 mL of the extract sample (or gallic acid standard solution or pure water). After thorough mixing, the mixture was left to stand for 5 minutes. Subsequently, 4 mL of a 7.5% Na_2CO_3 solution was added. A blank was prepared simultaneously, containing 1 mL of distilled water, 5 mL of 10% Folin-Ciocalteu's reagent, and 4 mL of the 7.5% Na_2CO_3 solution. The samples were then incubated at room temperature for 1 hour. Absorbance was measured at 765 nm using a UV spectrophotometer. Each sample was analyzed in triplicate, and the average absorbance was calculated. A calibration curve was generated from the gallic acid standard solution, and the phenolic concentration (mg/mL) was determined from this curve. The phenolic content in the extracts was expressed as gallic acid equivalents (mg of GA/g of extract).

$$\text{Total polyphenol content} = \frac{(\text{As} - \text{b}) \text{ x } 100}{\text{m x Ws x } 1000 \text{ x DM}}$$

As = Absorbance (765 nm)

B = Y-intercept

Vs = 250 ml

DF = dilution factor

m = Y-ax

Ws = Weight of sample

DM = Dry matter

3.2.1.8 Determination of catechins and caffeine (HPLC analysis)

High-Performance Liquid Chromatography (HPLC) was utilized to analyze concentrated green tea extract samples for caffeine and total catechin content, in accordance with ISO 14502-2:2005 standards. An injection volume of 10 µL was employed for all samples. The mobile phase gradient consisted of solution A (0.05% trifluoroacetic acid in water) and solution B (99.9% acetonitrile). Solution A was filtered through a 0.45 µm cellulose acetate filter, while solution B was filtered using a 0.45 µm nylon membrane filter. Isocratic conditions were established at 87% solution A and 13% solution B. Separation and quantification were carried out using a Platinum C18-EPS 3 µm column (53 mm × 7 mm) from GRACE, with the column temperature maintained at 30 °C. Elution was performed at a flow rate of 2.0 mL/min over a 10-minute period. Detection was conducted at 210 nm with a Waters® 2996 Photodiode Array (PDA) Detector. Standard curves were prepared using catechin and caffeine standards with concentrations ranging from 0.20 to 100.00 µg/mL, each concentration analyzed in duplicate. Catechins and caffeine were identified by comparing their retention times and spectral data with those of authentic standards, and quantification was achieved using the external standard method. The concentrations of catechins and caffeine in the tea extracts were reported as grams per 100 grams of dried sample.

$$\text{Amount (g /100 g dry basis)} = \frac{(\text{As} - \text{b})\text{x FTsd x DF x 100}}{\text{Scf x Ws x 1000}}$$

As = Peak area of sample
b = Y intersection of standard curve caffeine
Scf = Slop of standard curve caffeine
Ftsd = RFi/ RFcf
Vs = Vollum of sample extract
DF = Dilution factor
Ws = Sample weight
DM =% dry matter

3.2.1.9 2-Diphenyl-l-picryl-hydrazyl (DPPH) Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay was performed on Molyneux (2004) with minor modifications. To each 50 μL of diluted sample extract, 1950 μL of 60 μM DPPH solution was added. The mixture was allowed to react in the dark environment and room temperature for 30 minutes. Absorbance was measured at 517 nm, with methanol as the blank. The radical scavenging activity was reported as micromoles of Trolox equivalent per gram of dried sample.

$$\text{DPPH} = \frac{\text{C x Vs x DF x 100 x 100}}{\text{Ws x 10000 x DM}}$$

C= Concentration of the sample

Vs= Vollum of sample extract

DF = Dilution factor

Ws= Sample weight

DM=% dry matter

3.2.2 Experiment II : Study the Effect of Tannase Treatment on Physicochemical of Catechin Extract

3.2.2.1 Sample preparation of catechin

The catechin without enzyme treatment was used for comparing the effect of hydrolysis approaches with tannase. The catechin extracts after extraction was added tannase and the catechin was hydrolyzed using enzyme (tannase) (activity = 500 U/g; optimum pH value 5.0 - 5.5, at temperature 40 °C) used in present experiments was supplied from Kikkoman Co., Japan. the mixture at 40 °C for 180 minutes. The resulting solution was quickly heated to 90°C within 5 minutes to terminate the enzymatic reaction. Samples were cooled down to room temperature and obtained catechin extract from freeze-drying for physicochemical properties analysis. After that, analyze its physical and chemical properties by determined quality every week.

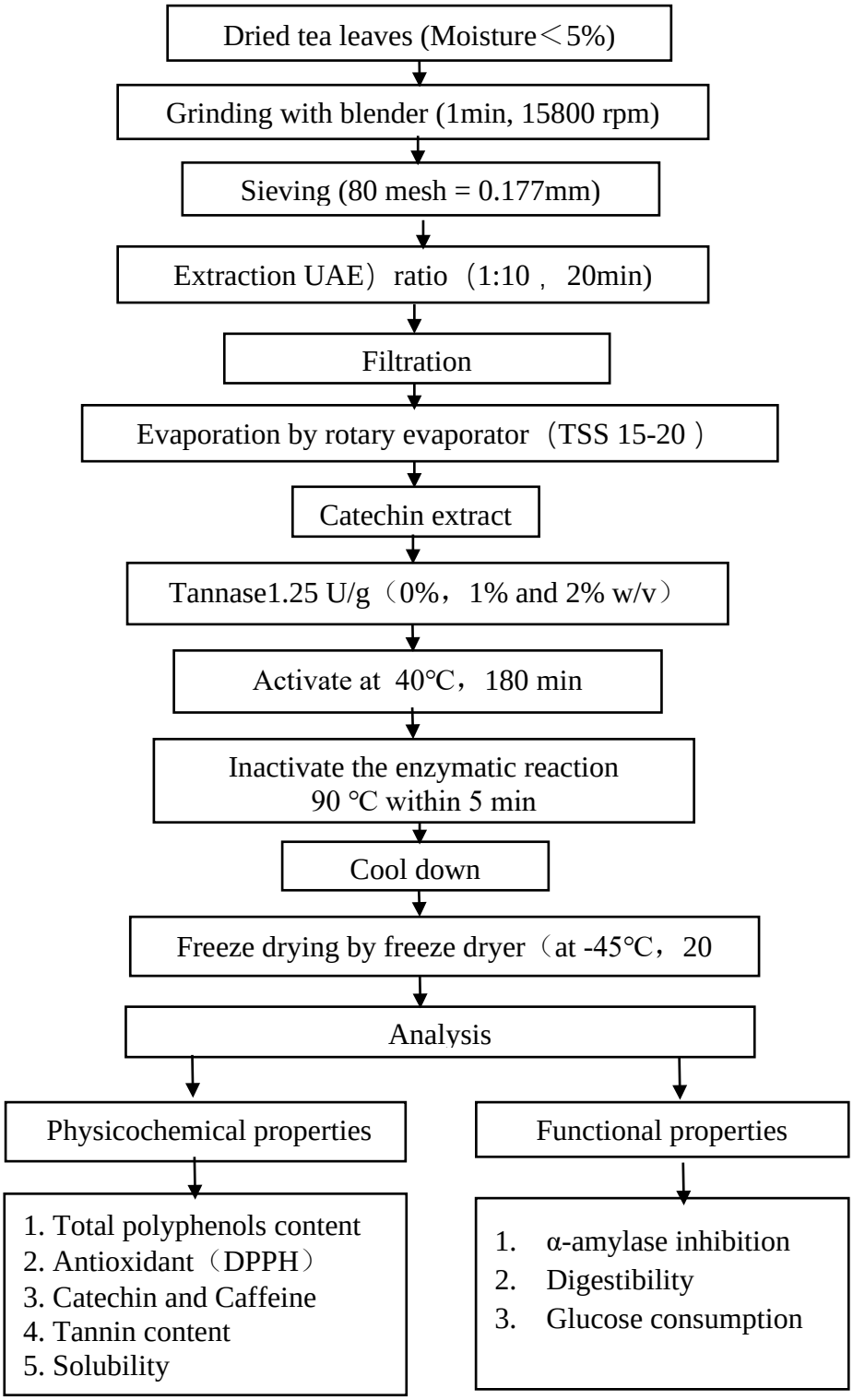


Figure 3.5 The Methodology of Tannase Treatment on Catechin

For the tannase enzymatic treatment, catechins extract, extracted using ultrasound-assisted extraction (UAE), the catechin extract were placed in centrifuge tubes at equal volumes of 40 mL. These samples were then supplemented with a 1.25 U/ml enzyme 1% and 2% w/v. The enzyme-treated samples were promptly incubated in a water bath at 40°C. (the enzyme's optimal operational temperature) for 180 minutes. Following incubation, the samples were heated in a water bath at 90°C for 5 minutes to inactivate the enzyme. After tannase treatment, catechin extraction were used freeze-drying machine to obtaine catechin extract. The analysis of physicochemical properties and functional properties carried out after the samples were cooled at room temperature**.**

3.2.2.2 Determination of total polyphenol content

This method is detailed in section 3.2.1.7. The tea polyphenol content in the extract was determined using a colorimetric assay with Folin-Ciocalteu's phenol reagent, with gallic acid as the standard, following the International Standard (Methods: ISO/CD 14502-1, 2005).

3.2.2.3 2,2-Diphenyl-l-picryl-hydrazyl (DPPH) Assay

This method is described in section 3.2.1.9. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay was performed following Molyneux's (2004) method with slight modifications.

3.2.2.4 Determination of catechins (HPLC analysis)

This method is followed by 3.2.1.8. High Performance Liquid Chromatography (HPLC) was used to analyze concentrated green tea extract samples for catechin content in accordance with ISO 14502-2:2005.

3.2.2.5 Determination of tannin content

Total tannin content of samples was determined by the method described by Son et al. (2013) with modification. Briefly, 400 µL of catechin extract were mixed with 2000 uL of 0.2 N Folin-Ciocalteu reagent and 1600 uL of 7% w/v sodium carbonate solution. After vortexing, the mixture was incubated for 2 h in a dark place and the absorbance was read at 725 nm with a spectrophotometer. Tannic acid was used as standard. The total tannin content was expressed as tannic acid equivalents (TAE), i.e. TAE mg/100 g dry weight (DW).

3.2.2.6 Determination of solubility

Water solubility was assessed following the procedure described by Cano-Chauca et al. (2005), with some modifications. Duplicate samples (1 g each) were added to 100 mL of distilled water at room temperature and stirred with a magnetic stirrer at 600 rpm for 5 minutes. The mixture was then centrifuged at 3000 × g for 5 minutes. An aliquot of 20 mL of the supernatant was transferred to pre-weighed petri dishes and dried in an oven at 70°C for 24 hours until a constant weight was achieved. The percent solubility was determined based on the weight difference and expressed on a dry basis, taking into account the moisture content of each sample.

3.2.2.7 Determination of α-amylase inhibition

The α-amylase assay was conducted according to by Kusano et al. (2010) with minor adjustments. To prepare the starch substrate, 200 mg of starch was dissolved in 25 mL of 0.4 M sodium hydroxide and heated at 100 °C for 5 minutes. After cooling, the pH was adjusted to 7.0, and the solution was made up to 100 mL with distilled water. Acarbose was used as a positive control. For the assay, 40 µL of the starch substrate solution was pre-incubated at 37 °C for 3 minutes with 20 µL of either acarbose or the test material at various concentrations. Following this, 20 µL of 3 U/mL α-amylase (dissolved in 20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9) was added, and the mixture was incubated at 37 °C for 15 minutes. The reaction was terminated by adding 80 µL of 0.1 M HCl, followed by 100 µL of iodine reagent (2.5 mM). Absorbance was then measured at 630 nm. Additionally, DNS (dinitrosalicylic acid) could be used to quantify reducing sugars produced during the reaction, providing further insight into α-amylase activity.

3.2.2.8 Determination of digestibility of catechins

The static *in vitro* gastrointestinal digestion model was adapted from Tamura et al. (2016) with minor modifications. For the pepsin solution, 0.24 g of pepsin from porcine gastric mucosa (800–2500 U/mg protein; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 50 mL of gastric fluid buffer (pH 1.20) using a magnetic stirrer for 10 minutes. The intestinal enzyme solution was prepared by mixing 0.2 g of pancreatin from porcine pancreas (Sigma Aldrich) and 4 mL of amyloglucosidase (Megazume, Co. Wicklow, Ireland) into 25 mL of intestinal fluid buffer (pH 6.8) and stirring for 10 minutes. A 170 mL infusion of catechin extract (catechin : water content,

ratio 3.4g :170 mL) was transferred to a glass reactor connected to a temperature-controlled water bath. The reactor was continuously stirred with a magnetic stirrer (Color Squid White, IKA Works, Wilmington, NC, USA) and maintained at 37°C throughout the experiment. The pH was initially adjusted to 2.00 with 3 M HCl. The gastric phase commenced with the addition of 19 mL of the pepsin solution, and the pH was adjusted to 1.20 ±0.01 using 0.5 M HCl. Samples (0.5 mL) were withdrawn at 0 and 30 minutes and mixed with 4.5 mL of 70% (v/v) methanol to halt the enzyme activity. After 30 minutes, the pH was adjusted to 6.00 with 3 N NaOH to inactivate the pepsin. The small intestinal phase began with the addition of 23 mL of the intestinal enzyme solution, and the pH was adjusted to 6.80 ± 0.01 using 0.5 M NaOH. The sample was maintained under intestinal conditions for 3 hours. At 0, 30, 60, 90, and 120 minutes, 0.5 mL samples were collected and mixed with 4.5 mL of 70% (v/v) methanol.

3.2.2.9 Determination of glucose consumption

The rate of glucose uptake was measured using Brush Border Membrane Vesicles (BBMVs) under Na+-gradient conditions. Prepare a series of dilutions from your tea extract stock solution. Common concentrations to test include a broad range from low to high and add the tea extract before mixing the BBMV suspension with the reaction mixture, The BBMVs were incubated in a 10 mM Hepes/Tris buffer (pH 7.5) with 0.1 µM D-[3H] glucose and 150 mM NaCl. For assessing diffusional glucose uptake, NaCl in the reaction mixture was replaced with an equimolar amount of KCl. The uptake assay was initiated by mixing 40 µL of the BBMV suspension with 160 µL of the reaction mixture. After incubation for the specified time, the reaction was stopped by adding 3 mL of ice-cold stop buffer and then filtering the mixture under vacuum. The stop buffer used in the glucose uptake assay consisted of 10 mM Hepes/Tris (pH 7.5) with 150 mM KCl. After filtering the mixture, the filter was washed three times with 5 mL of this stop buffer to remove any unbound glucose. The washed filter was then placed in a counting vial, and the radioactivity was quantified using an LSC 5100 liquid scintillation counter (Aloka, Tokyo).

3.2.3 Experiment III: Study the Stability of Catechins with Different Temperature

In this experiment, we studied the stability of catechins during storage, we selected catechins without tannase be a control sample and stored with 1% tannase-treated catechins, and compared their physical and chemical properties. Includes Total polyphenols content (TPC), antioxidant (DPPH) and catechin content, the control sample and 1% tannase-treated catechins were stored at incubator 30 ℃ and Freezer -20℃ for 8 weeks, and their physicochemical properties were measured per one week.

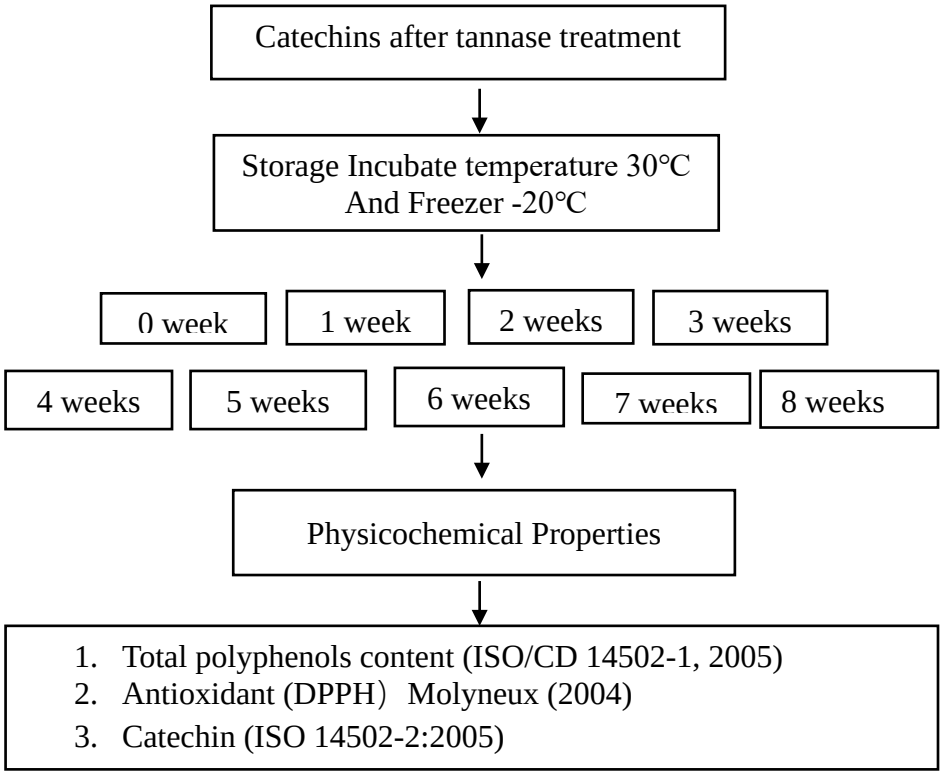


Figure 3.6 The Analysis Physicochemical Properties of Tannase Treatment on Catechin After Different Storage Time

3.2.3.1 Determination of total polyphenol content

This method adheres to section 3.2.1.7. The tea polyphenol content in the extract was evaluated using a colorimetric assay with Folin-Ciocalteu's phenol reagent, with gallic acid serving as the standard, in accordance with the International Standard (ISO/CD 14502-1, 2005).

3.2.3.2 2,2-Diphenyl-l-picryl-hydrazyl (DPPH) Assay

This method adheres to section 3.2.1.9. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging assay was performed following Molyneux's method (2004) with minor adjustments.

3.2.3.3 Determination of catechins (HPLC analysis)

This method is followed by 3.2.1.8. High Performance Liquid Chromatography (HPLC)was used to analyze concentrated green tea extract samples for catechin content in accordance with ISO 14502-2:2005.

3.3 Statistical Analysis

Statistical analysis was carried out using Statistical Packages for the Social Sciences (SPSS version 17), with all analyses performed in triplicate. The data are presented as means ± standard deviation (SD). To assess the significance of differences between samples, Analysis of Variance (ANOVA) was utilized, and Duncan's multiple range tests were employed for further comparisons. Differences were determined significant at $P < 0.05$.

CHAPTER 4

RESULTS AND DISSCUSION

4.1 Study the Effects of Solvent Extraction and Ultrasound-Assisted Extraction (UAE) on Physicochemical Properties

4.1.1 Physical Properties of Catechin Extract

The results regarding to the physical properties of catechin extract by solvent extraction and ultrasound-assisted extraction (UAE) shown in Table 4.1.1. The extract yield of solvent extraction was 20.18% Higher than 1:10 (tea: 50%ethanol) by UAE (18.22-18.41%) lower than 1:20 (tea: 50%ethanol) by UAE (22.62-22.99%) The extract yield of 1:20 (tea: 50%ethanol) by UAE in 20 min was the highest (22.99%) and was significantly ($P < 0.05$) different from another sample. UAE at a 1:20 ratio (tea: 50% ethanol) is more effective than solvent extraction, as it results in a higher yield of catechin extract. UAE increased yield of extracted components and increased rate of extraction, particularly early in the extraction cycle enabling major reduction in extraction time and higher processing throughput (Caili et al., 2006).

Ture density is one of food powder properties. The true density of catechins extract is in the range of 1.96-1.98g/ml. True density due to an increase in particle size and a greater tendency for the particles to be hollow. Adamapoulos (2008) reported that the residual moisture content of the powder affects the powder properties such as true density. During freeze drying, depending on the sublimation of the ice moisture content of remaining solid decreases. The former can be caused by particle inffation and “ballooning or puffing” (Walton, 2000). The characteristics when choosing the dehydration method to be utilized. Rehydration is an important step in the utilization of dried powder, which interconnects closely with porosity (Hawlader et al., 2006).

Color is one of the important sensory attributes of food and a major quality parameter in dried food product. For color, the highest L-value and a-value which means the brightest color of catechin extract, obtained from the condition of solvent extraction and UAE with different extract time. In contrast, the highest L-value and a-value were found in solvent extraction and the condition of UAE with different extract time the highest L-value and a-value were increase by longer extraction time and higher L-value and a-value were showed in ratio 1:20 (tea: 50%ethanol),when extract time with 20min, L-value was highest (59.39) and a-value was highest (7.52).for the b-value of UAE ratio 1:10 (tea: 50%ethanol) with 5-20 min in range about 13.49-15.06,it lower than UAE ratio 1:20 (tea: 50%ethanol) with 5-20 min about 15.46-16.76. Increasing drying temperature resulted in increasing L, a and b value. Both solvent extraction and UAE can produce high-quality catechin extracts with desirable color attributes. However, UAE, particularly at the 1:20 ratio and with longer extraction times, can achieve higher brightness and vividness, making it a highly effective method.

The moisture content in extract of green tea is a critical aspect in catechins from and thus lower moisture content implies a longer and more effective functional properties. The moisture content of catechins extract powder ranged from 2.12 % to 3.32 %.

Table 4.1 The Physical Properties of Catechin Extracts from the Different Extraction Methods

Treatments	Extract condition	Extract Yield (%)	Density (ns) (g/ml)	Color		
				L*	a*	b*
Solvent extraction		20.18±0.12^{b}	1.98±0.02	56.27±0.43^{e}	8.20±0.13^{a}	13.31±0.20^{d}
1:10 (tea：50% ethanol) UAE	5mins	18.22±0.10^{c}	1.98±0.01	56.69±0.45^{de}	7.61±0.14^{ab}	13.49±0.42^{d}
	10mins	18.27±0.15^{c}	1.96±0.00	58.11±0.45^{bc}	6.88±0.28^{cd}	13.39±0.28^{d}
	15mins	18.01±0.18^{c}	1.96±0.02	58.39±0.33^{abc}	6.64±0.10^{d}	13.07±0.28^{d}
	20mins	18.41±0.19^{c}	1.96±0.01	58.44±0.30^{adc}	7.87±0.02^{ab}	15.06±0.22^{c}
1:20 (tea50% ethanol) UAE	5mins	22.71±0.14^{a}	1.96±0.01	57.50±0.32^{cd}	6.36±0.09^{d}	15.46±0.21^{bc}
	10mins	22.62±0.09^{a}	1.98±0.02	58.71±0.32^{ab}	7.29±0.10^{bc}	14.86±0.06^{c}
	15mins	22.64±0.10^{a}	1.98±0.01	59.09±0.55^{ab}	7.44±0.51^{bc}	16.03±0.56^{ab}
	20mins	22.99±0.08^{a}	1.97±0.00	59.39±0.42^{a}	7.52±0.35^{b}	16.76±0.50^{a}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$). ns was non-significant($p \geq 0.05$).

4.1.2 Chemical Properties with Catechin Extract of Different Extraction Method

Moisture content is an important factor in influencing food product shelf life. Moisture absorption was affected by the nature of the product (hygroscopic or hydrophobic), the permeability of the packing material, and storage ambient conditions such as temperature variations and relative humidity (Kamble et al., 2020). The water activity and moisture content of all the samples are shown in Table 4.2. The result showed that the moisture content of the catechin extracts varied depending on the extraction condition used. The highest moisture content was observed in the 20-minute extraction with 1:20 (tea:50% ethanol) condition by UAE, with a value of 2.82±0.0%. Meanwhile, the lowest moisture content was observed in the 5-minute extraction with 1:10 (tea: 50% ethanol) condition, both with a value of 2.12±0.12%. The solvent extraction and the 20-minute extraction with 1:20 (tea:50% ethanol) condition had moisture content values of 2.24±0.1% and 2.82±0.01%, respectively. Overall, the moisture content of the catechin extracts obtained by different extraction conditions can

vary significantly, and this may have implications for the stability and shelf life of the extract.

Phenolic compounds associated with various health benefits, the total phenol content is an important quality parameter. The total polyphenol content (TPC) of the catechin extracts ranged from 56.28±0.40 g/100 g db to 64.02±0.16 g/100 g db and the similar results suggest that the sample is a good source of polyphenols, with TPC levels similar to those previously reported in Van Cuong et al. (2018). The data showed that the longer extraction time and a higher ratio may result in higher TPC values. As the extraction time increased from 10 minutes to 20 minutes, there was an increase in TPC values, with the highest TPC value of 64.02±0.16 g/100 g db obtained at the 20-minute mark on the 1:20 ratio. When using a 1:20 ratio of tea to 50% v/v ethanol solvent, the TPC values ranged from 59.49 g/100 g db to 64.02 g/100 g db. However, it is important to note that other factors, such as the type of tea and the quality of the solvent, may also play a role in determining the TPC values. Additionally, the study showed that the choice of solvent (ethanol or methanol) used to extract the phenolic compounds from the tea also affected the TPC values obtained. Saeed et al. (2012) investigated the impact of solvent quality on TPC values in plant extracts. The results showed that using solvents of varying polarities (ethanol, methanol, and water) to extract phenolic compounds from the plant material resulted in significantly different TPC values, with ethanol producing the highest TPC values.

Catechins are a type of natural phenol and antioxidant belonging to the flavonoid group, their potent antioxidant properties, which contribute to numerous health benefits. Table 4.2 shows the Trolox equivalent antioxidant capacity (mmol/100 g db) of the catechin extracts obtained under different extraction conditions. The results indicated that the UAE with longer extraction time generally leads to higher antioxidant activity, as indicated by the higher Trolox equivalent values. The highest antioxidant capacity was obtained with ultrasound-assisted extraction using a tea: 50% v/v ethanol ratio of 1:20 and an extraction time of 20 minutes, with a Trolox equivalent value of 496.21±4.48 mmol/100 g db. These results suggested that the extraction time can have a significant impact on the antioxidant activity of the catechin extracts, and longer extraction times may be beneficial for obtaining extracts with higher antioxidant capacity. In previous studies have shown that Trolox has equivalent antioxidant

capacity values of EGCG and other catechins ranging from 474 to 1307 μmol TE/g DW (Adhami et al., 2005).

Caffeine is a natural stimulant present in varying amounts in tea leaves. Caffeine contents in tea extracts not only influences its stimulating effects but also impacts its taste, aroma, and overall quality. The study found that the solvent extraction treatment at a 90-minute 1:20 ratio of tea: 50% v/v ethanol yielded a caffeine content of 8.29 g/100 g db. For the 1:10 tea: 50% v/v ethanol extraction treatment, caffeine values ranged from 8.05 to 8.61 g/100 g db, depending on the extraction duration. Similarly, the 1:20 tea: 50% ethanol extraction treatment produced caffeine values ranging from 7.77 to 8.48 g/100 g db, depending on the extraction time. All UAE extractions were conducted for 20 minutes, and it was observed that longer extraction times generally resulted in higher caffeine content. This can be attributed to caffeine's polarity and high solubility in ethanol, making it easily extractable. However, prolonged exposure to the extraction solvent can lead to the extraction of unwanted, bitter-tasting compounds, emphasizing the need for careful optimization of extraction times. And the study highlights the potential of UAE as a rapid and efficient method for the extraction of caffeine from tea.

Catechins are a type of flavonoid with strong antioxidant properties, predominantly found in tea leaves. These compounds significantly influence the quality, health benefits, and sensory attributes of tea. Table 4.3 shown that the highest total catechin content was obtained in the 1:20 extraction condition at 20 minutes, with a value of 33.75±0.15 g/100 g db. This was significantly higher than the total catechin content obtained from the solvent extraction, which had a value of 31.43±0.17 g/100 g db. There was a significant difference ($p \leq 0.05$) between UAE and solve extraction conditions on total catechin content and their derivatives. As for the effect of extraction time, this study revealed that the total catechin content generally increased with longer extraction times. The highest total catechin content was observed at 20 minutes for all extraction conditions. The ethanol concentration and prolonging the extraction time can result in higher total catechin content in the extract. Moreover, the increase in total catechin content with longer extraction times implies that prolonged extraction time can be beneficial in extracting catechins from tea leaves, except the 1:10 (tea:50% ethanol) extraction condition at 5 minutes. This is due to be since shorter extraction times are

insufficient to fully extract catechins from the tea leaves under this specific condition. When optimized for ethanol concentration and extraction time, UAE offers a more efficient and effective method for extracting catechins from tea leaves compared to traditional solvent extraction. This method not only yields higher catechin content but also suggests potential improvements in the sensory attributes and health benefits of the resulting tea products. Vinatoru (2001) published an overview of the UAE of bioactive principles from herbs. The improvement in extractive value by UAE compared with classic methods in water and ethanol for different plants, the content of each extracts was increased.

Table 4.2 Chemical Properties of Catechin Extract of Different Extraction Methods

Treatments	Extract condition (mins)	Moisture Content (%)	Total Polyphenol Content (g/100 g db)	DPPH Trolox (mmol/100 g db)	Caffeine (g/100 g db)
Solvent extraction	180mins	2.24±0.10^{b}	56.59±1.79^{c}	390.33±7.72^{d}	8.29±0.15^{abc}
1:10	5mins	2.12±0.12^{c}	56.28±0.40^{c}	413.09±7.18^{d}	8.13±0.20^{abc}
(tea：50%	10mins	2.21±0.10^{bc}	57.70±0.28^{bc}	424.47±4.39^{d}	8.05±0.00^{abc}
ethanol)	15mins	2.17±0.16^{c}	62.17±0.16^{a}	466.09±4.42^{bc}	8.24±0.07^{abc}
UAE	20mins	2.50±0.26^{a}	63.98±0.43^{a}	475.61±0.00^{b}	8.61±0.00^{a}
1:20	5mins	2.44±0.28^{c}	59.49±1.20^{b}	449.49±4.33^{c}	7.96±0.00^{bc}
(tea：50%	10mins	2.32±0.52^{d}	59.70±0.90^{b}	464.31±11.65^{bc}	7.87±0.00^{bc}
Ethanol）	15mins	2.52±0.33^{bc}	62.32±0.28^{a}	477.79±4.46^{b}	7.77±0.00^{c}
UAE	20mins	2.82±0.00^{a}	64.02±0.16^{a}	496.21±4.48^{a}	8.48±0.04^{ab}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$)

Table 4.3 showed that the solvent extraction method had the lowest content of all catechins except for CG. Tea: ethanol ratio (1:10 and 1:20). Tea leaves with ethanol extract had similar levels of catechins, with slightly higher levels in the 1:10 extract. In general, increasing the extraction time led to higher levels of catechins, although this effect was not consistent across all catechins. The EGCG catechin increased in the UAE extract with increasing extraction time. From the results, it can be seen that the significantly highest EGCG content (10.18±0.08 g/100 g db) was obtained at 1: 20 tea-

ethanol for 20 minutes. The EGCG content increased consistently with increasing extraction time, as was observed for the total catechin content.

The highest EGCG content was obtained at 20 minutes in both the 1:10 and 1:20 extraction conditions by UAE. The observed highest EGCG content at 20 minutes, suggests that prolonged extraction time can improve the extraction of catechins from tea leaves. The content of GC and C catechins were in the range 0.52±0.01 to 0.56±0.00 g/100 g db across all treatments, while the levels of EC, EGC, GCG, and CG varied more. This variability may be attributed to the influence of extraction conditions on the interactions between catechins and other components in tea leaves. Factors such as temperature, solvent to material ratio, and the time of extraction and individual extraction steps play an important role in the extraction process (Stalikas, 2007). It has been shown that extraction time and extraction temperature are the key factors for extracting more amount of catechins (Qu et al., 2010). The significantly highest catechin content was obtained at 1: 20 tea- ethanol for 20minutes by UAE. UAE highlights that the extraction time and tea-to-ethanol ratio significantly impact the levels of catechins, particularly EGCG. The highest EGCG content (10.18 ± 0.08 g/100 g db) was observed at a 1:20 tea-ethanol ratio with a 20-minute extraction time, demonstrating that prolonged extraction increases catechin yields.

Catechins such as GC and C showed relatively stable concentrations across all treatments (0.52 ± 0.01 to 0.56 ± 0.00 g/100 g db), while other catechins (EC, EGC, GCG, and CG) exhibited more variability due to the extraction conditions. The findings emphasize that key factors such as temperature, solvent-to-material ratio, and extraction time influence catechin extraction efficiency, with UAE showing particular promise in maximizing catechin yields.

Table 4.3 Total Catechin Content and Their Derivatives

Treatments	Extract condition	Total Catechin	GC	EGC$^{(ns)}$	C$^{(ns)}$	EC	EGCG	GCG$^{(ns)}$	ECG	CG
Solvent extraction		31.43±0.17^{b}	0.52±0.01^{c}	3.76±0.00	2.53±0.14	3.93±0.9.03^{b}	9.47±0.00^{bc}	0.01±0.00	11.13±0.00^{abc}	0.08±0.00^{d}
1:10 (tea : 50%ethanol) UAE	5 mins	29.98±0.23^{cd}	0.53±0.03^{c}	3.68±0.00	2.66±0.20	3.94±0.00^{c}	8.86±0.00^{c}	0.01±0.00	10.23±0.00^{cd}	0.08±0.00^{d}
	10 mins	31.08±0.17^{bc}	0.55±0.01^{b}	3.85±0.05	2.87±0.11	4.55±0.00^{a}	9.02±0.00^{c}	0.01±0.00	10.14±0.00^{cd}	0.09±0.00^{c}
	15 mins	31.69±0.19^{bc}	0.55±0.00^{b}	3.86±0.00	2.89±0.00	4.34±0.00^{ab}	9.25±0.00^{cd}	0.01±0.00	10.71±0.00^{bc}	0.09±0.00^{c}
	20 mins	32.76±0.33^{ab}	0.53±0.00^{c}	3.79±0.12	2.80±0.08	4.29±0.10^{ab}	9.85±0.00^{ab}	0.01±0.00	11.38±0.00^{ab}	0.11±0.01^{a}
1:20 (tea : 50%ethanol) UAE	5 mins	29.11±0.28^{cd}	0.53±0.00^{c}	3.76±0.13	2.83±0.12	4.23±0.21^{ab}	8.31±0.00^{d}	0.01±0.00	9.37±0.00^{d}	0.07±0.03^{e}
	10 mins	30.75±0.71^{bcd}	0.53±0.00^{c}	3.79±0.00	2.70±0.01	4.14±0.02^{ab}	8.85±0.57^{c}	0.01±0.01	10.64±0.10^{bc}	0.10±0.00^{b}
	15 mins	31.73±0.37^{bc}	0.57±0.01^{a}	3.76±0.02	2.85±0.01	4.24±0.25^{ab}	9.02±0.00^{c}	0.01±0.01	11.19±0.28^{abc}	0.09±0.01^{c}
	20 mins	33.75±0.15^{a}	0.56±0.00^{a}	3.82±0.05	2.81±0.01	4.15±0.01^{ab}	10.18±0.08^{a}	0.01±0.00	12.13±0.00^{a}	0.10±0.00^{b}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different (P≤0.05). ns was non-significant. GC: Gallic acid; EGC: Epigallocatechin; C: Catechin; EC: Epicatechin; EGCG: Epigallocatechin gallate; GCG: Gallocatechin gallate; ECG: Epicatechin gallate; CG: Catechin gallate. (Report in g/100g db)

4.2 Study the Effect of Tannase on Physicochemical and Functional Properties of Catechin

The water solubility (WS) of catechins was across all samples, with the control at 78.88%, 1% tannase-treated sample at 78.98%, and 2% tannase-treated sample at 78.95%. This uniformity indicates that tannase treatment did not significantly impact the solubility of catechins in water. The tannase treatment was not affect water solubility, it suggests that the structural components or bonds targeted by the enzyme were not significantly involved in determining the solubility of the material in water. They were significant in applications where maintaining or altering solubility is important, as it indicates that enzyme treatment alone may not be an effective method for modifying water solubility.

The total polyphenol content decreased with increasing tannase concentration. The decrease was statistically significant ($p < 0.05$) with tannase treatment. The control sample, which had the highest polyphenol content, showed a statistically significant reduction when treated with tannase. The polyphenol content draped to 61.28% with 1% tannase and further decreased to 58.86% with 2% tannase. This reduction aligns with the role of tannase in hydrolyzing polyphenolic compounds, particularly tannins. As polyphenols contribute significantly to the antioxidant properties of tea, their reduction could impact the tea's overall health benefits.

A similar trend was observed in the total catechin content. The control sample has the highest catechin concentration, which significantly decreased to 20.08 g/100 g with 1% tannase and to 18.57 g/100 g with 2% tannase. This decline is expected due to the hydrolytic action of tannase, which can break down catechins, particularly the galloylated forms. The reduction in catechins is also significant ($p < 0.05$), reflecting the enzyme's efficiency in altering catechin levels. This reduction impacts the antioxidant potential and possibly the flavor profile of the tea, as catechins contribute to both.

The caffeine content decreases as the concentration of tannase increases, with the control sample showing the highest caffeine level. Specifically, the caffeine content drops to 8.82 g/100 g with 1% tannase and further to 8.00 g/100 g with 2% tannase. Although these reductions are relatively small, they are statistically significant. This decrease in caffeine may be due to tannase interacting with other components in the tea matrix, possibly affecting the solubility or extraction of caffeine. The reduction may have only a minimal impact on the tea's stimulant properties.

The tannin content decreases significantly ($p < 0.05$) with tannase treatment. The control sample shows the highest tannin content, which decreased to 59.35% with 1% tannase and further to 52.40% with 2% tannase. This reduction was expected due to tannase's specific activity, which hydrolyzes tannins. The tannins demonstrated the enzyme's effectiveness in reducing astringency and enhancing the sensory quality of the tea. Additionally, this reduction in tannin levels may impact the flavor profile by decreasing bitterness and astringency.

Antioxidant activity, measured in micromoles of Trolox equivalents per 100 grams of dry base (μmol/100 g db), exhibited a noticeable decrease with increasing tannase concentration. The control sample (catechin without tannase treatment) displayed the highest antioxidant activity at 528.61 ± 2.78 μmol/100 g db. In contrast, the 1% tannase-treated sample showed a reduced antioxidant activity of 504.30 ± 2.68 μmol/100 g db, and the 2% tannase-treated sample demonstrated the lowest antioxidant activity at 497.98 ± 2.69 μmol/100 g db. The observed reduction in antioxidant activity with tannase treatment can be attributed to the enzyme's hydrolytic action on catechins, particularly the galloylated forms like epigallocatechin gallate (EGCG), which are known to contribute significantly to the antioxidant capacity of tea.

Table 4.4 Physicochemical Properties of Tea Extract by Tannase Treatment

Sample Catechins	Water Solubility (%) ns	Total Polyphenol (%w/w db)	Total Catechins (g /100g db)	Caffeine content (g /100g db)	Tannin (%w/w db)	DPPH Trolox (µmol /100 g db)
Control	78.88	63.28±0.54[a]	25.70±1.05[a]	10.23±0.40[a]	66.11±0.42[a]	528.61±2.78[a]
Tannase 1%	78.98	61.28±0.30[b]	20.08±0.61[b]	8.82±0.03[b]	59.35±0.41[b]	504.30±2.68[b]
Tannase 2%	78.95	58.86±0.30[c]	18.57±0.36[c]	8.00±0.26[c]	52.40±0.41[c]	497.98±2.69[c]

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$). ns was non-significant ($p \geq 0.05$).

The study analyzed the impact of tannase treatment on the levels of various catechins in tea. The catechins measured include Gallic Catechin (GC), Epigallocatechin (EGC), Catechin (C), Epicatechin (EC), Epigallocatechin Gallate (EGCG), Gallocatechin Gallate (GCG), Epicatechin Gallate (ECG), and Catechin Gallate (CG) was shown in Table 4.5, GC content increased significantly ($p < 0.05$) with tannase treatment. The enzyme hydrolyzes galloylated catechins, releasing more GC in the process. This increase suggested that tannase effectively breaks down complex catechins into simpler forms. A substantial rise in EGC content was observed with tannase treatment. This increase was likely due to the hydrolysis of galloylated catechins, leading to the release of free EGC. Catechin content decreased with increasing tannase concentration. This reduction indicates the conversion of catechin into other simpler compounds or its further degradation. A slight increase in EC content was noted with tannase treatment, likely due to the breakdown of galloylated forms, resulting in free EC. This increase might contribute to improved taste and reduced bitterness. EGCG content decreased significantly ($p < 0.05$) with tannase treatment. Tannase hydrolyzes the ester bond in EGCG, resulting in its reduction. This decrease

may impact the tea's antioxidant properties, as EGCG was a major contributor to the antioxidant activity. GCG content dropped sharply and became undetectable in the 2% tannase-treated sample. This indicates that GCG was highly susceptible to tannase hydrolysis, resulting in its complete breakdown. ECG content decreased drastically with tannase treatment, similar to EGCG and GCG. The reduction of galloylated catechins like ECG confirms tannase's efficiency in hydrolyzing these compounds. CG content became undetectable in tannase-treated samples, suggesting complete hydrolysis by tannase. This outcome supports the enzyme's strong activity against galloylated catechins. The total catechin content decreased significantly with tannase treatment. This reduction reflects the breakdown of complex catechins into simpler forms and other degradation products. Despite this reduction, the increased presence of simpler catechins like GC and EGC could potentially improve flavor and reduce bitterness. Tannase catalyzes ester and disulfide bond hydrolysis in hydrolysable tannins or gallic acid esters, such as EGCG or ECG, to release glucose or gallic acid (Ni et al., 2014). Many papers have reported the application of tannase in instant tea to reduce tea cream formation and improve color appearance. The hydrolytic action of tannase decreases EGCG binding with protein due to ester bond cleavage (Xiao et al., 2015). The esterified catechins contribute astringency with a bitter taste and free catechins was less astringency with slight sweet in taste (Hara, 2001). The catechins possessing ester binding such as EGCG and ECG had a greater ability to form precipitates with enzymes than EC and EGC which lead to the cream formation (Sekiya et al., 1984). This reduction, although minor, could affect the stimulant

Table 4.5 Total and Individual Catechins of Tea Extract by Tannase Treatment

Sample	GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total Catechins	Caffeine content
Control	1.34± 0.29^{c}	2.71± 0.01^{c}	5.42± 0.39^{a}	0.69± 0.01^{c}	7.39± 0.26^{a}	0.66± 0.03	7.74± 0.02^{a}	0.02± 0.05	25.70± 1.05^{a}	10.23± 0.40^{a}
Tannase 1%	1.99± 0.63^{b}	6.62± 0.01^{b}	5.24± 0.28^{b}	0.83± 0.00^{b}	5.43± 0.18^{b}	0.04± 0.04	0.05± 0.00^{b}	ND	20.08± 0.61^{b}	8.82± 0.03^{b}
Tannase 2%	2.09± 0.00^{a}	7.01± 0.26^{a}	4.23± 0.00^{c}	0.88± 0.03^{a}	4.29± 0.03^{c}	ND	0.08± 0.00^{b}	ND	18.57± 0.36^{c}	8.00± 0.26^{c}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$). ns was non-significant($p \geq 0.05$). ND was non-detect. (Report in g/100g db)

Catechin exhibit numerous biological activities, including the inhibition of α-amylase and the regulation of glucose metabolism. This section will be discussed the effects of catechins on α-amylase inhibitory activity and glucose consumption, along with how tannase treatment modifies these activities were showed in table.4.6. The impact of tannase treatment on the α-amylase inhibitory activity and glucose consumption of tea catechins. The samples analyzed include a control sample, 1% tannase-treated catechins.

A lower IC50 value signifies higher inhibitory activity. The control sample exhibited the highest α-amylase inhibitory activity with the lowest IC50 value (0.638 mg/ml). Tannase treatment resulted in a decrease in α-amylase inhibitory activity, with the IC50 value increasing to 0.715 mg/ml for the 1% tannase-treated sample and further to 0.953 mg/ml for the 2% tannase-treated sample. This trend suggested that tannase treatment reduces the effectiveness of catechins in inhibiting α-amylase activity. The reduction in inhibitory activity could be due to the hydrolysis of galloylated catechins, which were known to possess strong α-amylase inhibitory properties. The reduction in

α-amylase inhibitory activity following tannase treatment could diminish these effects, potentially altering the tea's efficacy in glycemic control. However, it's also worth considering that the reduction in α-amylase inhibition might not entirely negate the health benefits of the tea. The decrease in tannin content, which is also a result of tannase treatment, could improve the tea's palatability by reducing bitterness and astringency. Tannin also acts as an anti-nutritional factor by forming complex reactions. (Chung et al., 1998). This balance between improved sensory qualities and modified health-related properties should be carefully considered when applying tannase treatment in tea processing.

Glucose consumption is measured as the IC50 value, representing the concentration required to achieve 50% glucose consumption. A lower IC50 value indicates higher glucose consumption activity. The control sample showed the highest IC50 value (204.97 mg/ml), indicating the lowest glucose consumption activity. The 1% tannase-treated sample demonstrated the most significant glucose consumption activity with the lowest IC50 value (145.45 mg/ml). However, the 2% tannase-treated sample exhibited a reduction in glucose consumption activity compared to the 1% tannase-treated sample, with an IC50 value of 197.24 mg/ml, although it was still higher than the control sample. The enhanced glucose consumption activity in the 1% tannase-treated sample suggested that moderate tannase treatment may improve the glucose metabolism potential of tea catechins. This trend highlights the importance of optimizing tannase treatment to balance the enhancement of beneficial properties with the preservation of bioactivity. The 1% tannase treatment appeared to be the most effective in improving glucose metabolism, likely due to a favorable balance between tannin reduction and catechin preservation. In contrast, the 2% treatment may lead to diminishing returns, where further tannin reduction is outweighed by the loss of catechin activity. The findings suggest that while moderate tannase treatment can enhance the glucose metabolism potential of tea catechins, care must be taken not to over-treat, as this can lead to a reduction in the desired metabolic effects. Future research could focus on identifying the optimal concentration of tannase for maximizing health benefits while minimizing any adverse effects on catechin activity.

Additionally, understanding the specific structural changed in catechins resulting from varying tannase treatments could provide deeper insights into the mechanisms underlying these observed effects. However, excessive tannase treatment (2%) may not provide additional benefits and could slightly reduce the activity. The tannase-mediated biotransformation of tea extracts caused predictable changes the transformed extracts retained or improved their ability to inhibit the α-glucosidase (Roberto et al., 2016).

Table 4.6 α-Amylase Inhibitory Activity and Glucose Consumption of Catechins by Tannase Treatment

Sample	α-amylase inhibitory activity（IC_{50}mg/ml）	Glucose consumption （IC_{50}， mg/ml）
Control sample	0.638 ± 0.019^{c}	204.97 ± 2.31^{a}
1% tannase treatment on catechin	0.715 ± 0.032^{b}	145.45 ± 2.16^{c}
2%tannase treatment on catechin	0.953 ± 0.001^{a}	197.24 ± 2.97^{b}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p\leq0.05$). ns was non-significant ($p\geq0.05$).

This study examined the changes in total catechin content during simulated digestion, comparing a control sample with samples treated with 1% and 2% tannase. The digestion process was simulated in both the stomach and intestinal phases over various time intervals.

The total catechin content was measured in the stomach phase at 0 minutes and after 30 minutes of digestion. The initial measurement showed that the control sample had the highest total catechin content, while the tannase-treated samples had lower catechin contents. This reduction in catechin content in tannase-treated samples was expected, as tannase hydrolyzes ester bonds in catechins, leading to a decrease in their overall concentration. After 30 minutes of stomach digestion, the total catechin content

decreased for all samples. The control sample retain the highest catechin content, followed by the tannase-treated samples, which show a significant reduction. The decrease was more significant in tannase-treated samples, suggesting that the hydrolyzed catechins are more prone to degradation in the stomach.

The intestinal phase further simulates digestion over time, with measurements taken at 0, 30, 60, 90, and 120 minutes. At the beginning of the intestinal phase, the catechin content continues to be highest in the control sample. The tannase-treated samples exhibited a further reduction in catechin content compared to the stomach phase, highlighting the impact of both tannase treatment and the digestion process on catechin stability. The control sample consistently showed higher catechin content throughout the intestinal phase compared to tannase-treated samples. After 90 and 120 minutes of intestinal digestion, catechins in the tannase-treated samples become non-detectable (ND), indicating that they are either completely degraded . In contrast, the control sample still retains a measurable amount of catechins, though significantly reduced from the initial content.

Table 4.7 *In Vitro* Digestion of Catechin on Tannase Treatment

Digestion time (mins)	Total catechin (g /100g db)		
	Control sample	**Tannase 1%**	**Tannase 2%**
Stomach 0	9.50±0.39	7.51±0.09	7.40±0.15
Stomach 30	9.06±0.44	4.85±0.12	4.58±0.04
Intestinal 0	9.03±0.13	4.57±0.14	4.40±0.05
Intestinal 30	7.75±0.15	2.80±0.08	2.88±0.15
Intestinal 60	6.27±0.15	1.01±0.06	1.11±0.04
Intestinal 90	4.68±0.15	ND	ND
Intestinal 120	3.36±0.23	ND	ND

Note ND was non-detected.

4.3 Study of the Stability of Catechin Storage Time

The total catechin content is one of the most important property of catechins storage, it was also found that tea catechins were sensitive to vulnerable to isomerization during storage (Kim et al., 2007; Ito et al., 2003). Consequently, catechins were not expected to be stable over more than few days (Wang & Helliwell, 2000) or weeks (Ito et al., 2003) according to the conditions of production and temperature of storage. The changes in total polyphenol content (TPC) over an 8-week storage period under different conditions: untreated (0% tannase) and treated with 1% tannase, both stored at freezer temperatures (-20°C) and at 30°C. The changed of TPC value are shown in Table 4.9. The initial TPC values indicate that untreated samples have higher polyphenol content compared to those treated with 1% tannase. This is consistent across both storage conditions, highlighting the impact of tannase treatment on reducing polyphenol content. Tannase treatment (1%) results in a lower initial TPC comparedto the untreated control. This reduction is evident across both storage conditions (-20°C and 30°C). Tannase-treated samples maintain a more stable TPC over

time compared to the untreated samples. This tannase treatment may help preserve polyphenol content during storage, especially at lower temperatures. Samples stored at freezer temperatures exhibit a slower decline in TPC over time compared to those stored at 30°C. This trend was consistent for both untreated and tannase-treated samples, indicating that lower temperatures help preserve polyphenol content. Samples stored at 30°C show TPC value was decreased rapidly. The untreated samples, in particular, experience a significant reduction in polyphenol content, indicating that higher storage temperatures accelerate the degradation of polyphenols. The TPC of untreated samples stored at freezer temperatures decreased from 68.46% to 51.90% over 8 weeks, while those stored at 30°C decreased to 49.67%. This indicates that even under freezer storage, polyphenol content declines significantly over time. The TPC of tannase-treated samples stored at freezer temperatures decreased from 63.84% to 55.38% over 8 weeks, while those stored at 30°C decreased to 50.29%. The reduction was less pronounced in tannase-treated samples, suggesting better stability. Tannase treatment enhances the stability of polyphenol content during storage, especially at lower temperatures. Freezer storage is more effective in preserving TPC compared to incubator storage, which is crucial for maintaining the quality of catechin-rich products. Incorporating tannase treatment in product formulation can help preserve polyphenol content during storage, ensuring that the health benefits associated with polyphenols are retained over a longer period. Proper storage conditions, such as maintaining lower temperatures, are essential to maximize the shelf life and efficacy of polyphenol-rich products.

Table 4.8 The Change of Total Catechin Content on Tannase Treatment During Storage

Sample Storage Time	Total Polyphenol (%w/w db)								
	0 week	**1 week**	**2 weeks**	**3 weeks**	**4 weeks**	**5 weeks**	**6 weeks**	**7 weeks**	**8 weeks**
Control at -20˚C	68.46±1.31^{a}	67.62±0.72^{a}	65.47±0.12^{a}	63.51±0.21^{a}	61.14±0.13^{a}	58.18±0.13^{b}	56.53±0.22^{b}	54.85±0.13^{b}	51.90±0.27^{b}
Control at 30˚C	68.46±1.31^{a}	65.76±0.41^{b}	63.21±0.25^{b}	61.11±0.26^{b}	59.09±0.13^{c}	56.78±0.24^{c}	54.70±0.13^{c}	52.70±0.14^{c}	49.67±0.16^{d}
1% tannase at -20˚C	63.84±1.50^{b}	63.77±0.20^{c}	62.32±0.00^{c}	61.03±0.00^{b}	60.24±0.13^{b}	59.08±0.24^{a}	58.26±0.14^{a}	57.45±0.14^{a}	55.38±0.16^{a}
1% tannase at 30 ˚C	63.84±1.50^{b}	61.43±0.21^{d}	59.27±0.13^{d}	57.40±0.13^{c}	56.81±0.00^{d}	55.43±0.00^{d}	54.18±0.14^{d}	52.74±0.14^{c}	50.29±0.29^{c}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different (p≤0.05). ns was non-significant

Table 4.9 Percent Degradation of Total Catechin Content During Storage

Storage time	Total catechin content（%）Degradation			
	Control -20 ˚C	Control 30 ˚C	1%tannase -20 ˚C	1%tannase 30 ˚C
0-1 Week	1.23	3.94	0.11	3.78
0-2 Week	4.37	7.67	2.38	7.16
0-3 Week	7.23	10.74	4.40	10.09
0-4 Week	10.70	13.69	5.64	11.01
0-5 Week	15.02	17.06	7.46	13.17
0-6 Week	17.43	20.10	8.74	19.83
0-7 Week	19.89	23.021	10.01	17.39
0-8 Week	24.19	27.45	13.25	21.22

The antioxidant activity of catechins by DPPH, measured by Trolox equivalent (umol/100 g dry basis), during an 8-week storage period under different conditions: untreated (0% tannase) and treated with 1% tannase, both stored at freezer temperatures and at 30°C. The initial antioxidant activity values indicate that untreated samples had higher antioxidant activity compared to those treated with 1% tannase. This is consistent across both storage conditions, highlighting the impact of tannase treatment on reducing initial antioxidant activity. Tannase treatment (1%) resulted in a lower initial antioxidant activity compared to the untreated control. This reduction was evident across both storage conditions (freezer and 30°C). Tannase-treated samples maintain a more stable antioxidant activity over time compared to the untreated samples. This suggested that tannase treatment may help preserve antioxidant activity during storage, especially at lower temperatures. Samples stored at freezer temperatures exhibit a slower decline in antioxidant activity over time compared to those stored at 30°C. This trend was consistent for both untreated and tannase-treated samples, indicating that lower temperatures help preserve antioxidant activity. Samples stored at 30°C show a more rapid decline in antioxidant activity. The untreated samples, in particular, experience a significant reduction in antioxidant activity, indicating that higher storage temperatures accelerate the degradation of antioxidants. The antioxidant activity of untreated samples stored at freezer temperatures decreased from 513.72 umol/100 g db to 396.21 umol/100 g db over 8 weeks, while those stored at 30°C

decreased to 347.49 umol/100 g db. This indicated that even under freezer storage, antioxidant activity declines significantly over time. The antioxidant activity of tannase-treated samples stored at freezer temperatures decreased from 482.28 umol/100 g db to 404.69 umol/100 g db over 8 weeks, while those stored at 30°C decreased to 365.70 umol/100 g db. The reduction is less pronounced in tannase-treated samples, suggesting better stability. Control Samples at -20°C, degradation was slower (22.87% after 8 weeks) compared to 30°C (32.36%), showing that low temperatures help preserve antioxidant activity.Tannase treatment significantly reduced degradation at both temperatures, with the lowest loss at -20°C (16.09%) and a moderate loss at 30°C (24.17%). Freezer storage is more effective in preserving antioxidant capacity compared to incubator storage. Incorporating tannase treatment in product formulation can help preserve polyphenol content during storage, ensuring that the health benefits associated with antioxidant capacity are retained over a longer period. Proper storage conditions, such as maintaining lower temperatures, are essential to maximize the shelf life and efficacy of antioxidant food products.

Table 4.10 The Changed of Antioxidant Capacity on Tannase Treatment Turing Storage

Sample / Storage Time	DPPH Trolox (umol/100 g db)								
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Control at-20˚	513.72±4.98^{a}	502.80±2.70^{a}	492.25±2.68^{a}	475.27±0.00^{a}	456.50±2.66^{a}	442.81±1.22^{b}	427.45±0.23^{b}	412.49±0.00^{b}	396.21±2.59^{b}
Control at 30 ˚C	513.72±4.98^{a}	490.63±2.62^{b}	475.96±4.78^{d}	452.32±00^{c}	436.70±0.00^{b}	417.34±0.73^{c}	399.71±2.70^{c}	371.62±2.57^{d}	347.49±26.2^{d}
1% tannase at -20˚C	482.28±2.94^{b}	478.87±2.81^{c}	474.45±4.87^{d}	468.13±4.14^{b}	454.56±2.71^{a}	445.06±0.74^{a}	434.91±0.00^{a}	421.76±0.00^{a}	404.69±2.61^{a}
1% tannase at 30 ˚C	482.28±2.94^{b}	469.22±2.72^{d}	459.34±2.74^{c}	448.22±0.00^{d}	437.62±2.74^{b}	415.85±0.76^{c}	399.03±2.76^{c}	382.90±2.63^{c}	365.70±2.60^{c}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$). ns was non-significant($p \geq 0.05$).

Table 4.11 Percent Degradation of Antioxidant Capacity on Tannase Treatment

Storage time (Week)	**Antioxidant capacity (%)Degradation**			
	Control -20 ˚C	**Control 30 ˚C**	**1% tannase -20 ˚C**	**1% tannase 30 ˚C**
0-1	2.13	4.494	0.71	2.71
0-2	4.18	7.35	1.62	4.76
0-3	7.48	11.95	2.93	7.06
0-4	11.14	14.99	5.75	9.26
0-5	13.80	18.76	7.72	13.77
0-6	16.79	22.19	9.82	17.26
0-7	19.71	27.66	12.55	20.61
0-8	22.87	32.36	16.09	24.17

Epigallocatechin gallate (EGCG) is regarded as the most important of the tea catechins (Ju et al., 2007; Yang & Landau, 2000), due to its high content in tea and its protective bioactivity(Yu et al., 2004). Table 4.12 showed the changes in EGCG content, measured in grams per 100 grams dry basis, an 8-week storage period for samples treated with control sample and 1% tannase, stored at both freezer temperatures (-20°C) and at 30°C. Tannase treatment (1%) resulted in a lower initial EGCG content compared to the untreated control. This reduction was consistent across both storage conditions (-20°C and 30°C), indicating that tannase treatment initially reduces the EGCG content. Tannase-treated samples exhibited a more gradual decline in EGCG content over time compared to untreated samples. This suggested that tannase treatment may help in better preserving the EGCG content during storage, particularly at lower temperatures. Samples stored at freezer temperatures exhibit a slower decline in EGCG content over time compared to those stored at 30°C. This trend was consistent for both untreated and tannase-treated samples, indicating that lower temperatures help preserve EGCG content. Samples stored at 30°C show a more rapid decline in EGCG content. Untreated samples, in particular, experience a significant reduction in EGCG content, indicating that higher storage temperatures accelerate the degradation of EGCG.

The EGCG content of untreated samples stored at freezer temperatures decreased from 8.64 g/100g db to 5.44 g/100g db over 8 weeks, while those stored at 30°C decreased to 4.03 g/100g db. This indicated that even under freezer storage, EGCG content declines significantly over time. The EGCG content of tannase-treated samples stored at freezer temperatures decreased from 6.70 g/100g db to 4.67 g/100g db over 8 weeks, while those stored at 30°C decreased to 3.56 g/100g db. The reduction is less pronounced in tannase-treated samples, suggesting better stability

Table 4.12 The Change of EGCG on Tannase Treatment

Storage time (Week)	**EGCG (g /100g dry basis)**			
	Control （-20 ˚C）	**Control （30 ˚C）**	**1% tannase （-20 ˚C）**	**1% tannase （30 ˚C）**
0-1	8.64±0.05^{a}	8.64±0.05^{a}	6.70±0.01^{a}	6.70±0.01^{a}
0-2	8.56±0.09^{a}	8.11±0.00^{b}	6.67±0.07^{ab}	6.39±0.10^{b}
0-3	7.94±0.00^{b}	7.55±0.17^{c}	6.45±0.02^{b}	6.06±0.02^{c}
0-4	7.61±0.02^{c}	7.04±0.14^{d}	6.16±0.01^{c}	5.59±0.01^{d}
0-5	7.22±0.05^{d}	6.22±0.19^{e}	5.92±0.04^{d}	5.14±0.06^{e}
0-6	6.87±0.16^{e}	5.35±0.33^{f}	5.67±0.06^{e}	4.79±0.03^{f}
0-7	6.43±0.02^{f}	4.82±0.14^{g}	5.33±0.04^{f}	4.36±0.06^{g}
0-8	6.09±0.18^{g}	4.40±0.26^{h}	5.01±0.01^{c}	3.89±0.01^{h}
0-1	5.44±0.04^{e}	4.03±0.02^{i}	4.67±0.02^{d}	3.56±0.10^{i}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$). ns was non-significant($p \geq 0.05$)

Table 4.13 Percent Degradation of EGCG Value During Storage

Storage time (Week)	Storage condition EGCG (%) Degradation			
	Control -20 ˚C	Control 30 ˚C	1%tannase -20 ˚C	1%tannase 30 ˚C
0-1	0.93	6.13	0.45	4.63
0-2	8.10	12.61	3.73	9.55
0-3	11.92	18.52	8.06	16.57
0-4	16.43	28.01	11.64	23.28
0-5	20.49	38.08	15.37	28.51
0-6	25.58	44.21	20.45	34.93
0-7	29.51	49.07	25.22	41.94
0-8	37.04	53.36	30.30	46.87

Table 4.14 showed this epigallocatechin (EGC) content, measured in grams per 100 grams dry basis, over an 8-week storage period for samples treated with 0% and 1% tannase, stored at both freezer temperatures (-20°C) and at 30°C. The initial EGC values indicate that untreated samples have higher EGC content compared to those treated with 1% tannase. This trend is consistent across both storage conditions, suggesting that tannase treatment initially reduces the EGC content. Tannase treatment (1%) results in a lower initial EGC content compared to the untreated control. This reduction is consistent across both storage conditions (-20°C and 30°C), indicating that tannase treatment initially reduces the EGC content. Tannase-treated samples exhibit a more gradual decline in EGC content over time compared to untreated samples. This suggests that tannase treatment may help in better preserving the EGC content during storage, particularly at lower temperatures. Samples stored at freezer temperatures exhibit a slower decline in EGC content over time compared to those stored at 30°C. This trend is consistent for both untreated and tannase-treated samples, indicating that lower temperatures help preserve EGC content. Samples stored at 30°C show a more rapid decline in EGC content. Untreated samples, in particular, experience a significant

reduction in EGC content, indicating that higher storage temperatures accelerate the degradation of EGC. The EGC content of untreated samples stored at freezer temperatures decreased from 2.97 g/100g db to 1.13 g/100g db over 8 weeks, while those stored at 30°C decreased to 1.07 g/100g db. This indicates that even under freezer storage, EGC content declines significantly over time. The EGC content of tannase-treated samples stored at freezer temperatures decreased from 1.86 g/100g db to 1.29 g/100g db over 8 weeks, while those stored at 30°C decreased to 1.10 g/100g db. The reduction is less pronounced in tannase-treated samples, suggesting better stability.

Table 4.14 The Change of EGC on Tannase Treatment

Storage time (Week)	**Storage condition EGC (g/100g dry basis)**			
	Control (-20 ˚C)	**Control (30 ˚C)**	**Control (-20 ˚C)**	**1% tannase (30 ˚C)**
0-1	2.97±0.03^{a}	2.97±0.03^{a}	1.86±0.01^{a}	1.86±0.01^{a}
0-2	2.91±0.12^{a}	1.34±0.08^{b}	1.82±0.01^{a}	1.82±0.02^{a}
0-3	2.64±0.05^{b}	1.32±0.20^{b}	1.79±0.02^{ab}	1.83±0.01^{ab}
0-4	1.41±0.07^{c}	1.24±0.01^{c}	1.79±0.01^{bc}	1.76±0.03^{ab}
0-5	1.17±0.03^{d}	1.11±0.05^{d}	1.76±0.03^{c}	1.76±0.01^{b}
0-6	1.15±0.00^{e}	1.09±0.10^{e}	1.76±0.10^{c}	1.70±0.00^{c}
0-7	1.13±0.04^{e}	1.07±0.00^{f}	1.57±0.16^{d}	1.16±0.10^{d}
0-8	1.12±0.00^{e}	1.07±0.01^{f}	1.34±0.04^{e}	1.15±0.02^{e}
0-1	1.13±0.04^{e}	1.07±0.01^{f}	1.29±0.04^{e}	1.10±0.01^{f}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p \leq 0.05$).

Table 4.15 Percent Degradation of EGC Value During Storage

Storage time (Week)	Storage condition EGC (%) Degradation			
	Control -20 ˚C	Control 30 ˚C	1%tannase -20 ˚C	1%tannase 30 ˚C
0-1	2.02	54.88	2.15	2.15
0-2	11.11	55.56	3.76	1.61
0-3	52.53	58.25	3.76	5.38
0-4	60.61	62.63	5.38	5.38
0-5	61.28	63.30	5.38	8.60
0-6	61.95	63.97	15.60	37.63
0-7	62.29	63.97	27.96	38.17
0-8	61.95	63.97	30.65	40.86

This epicatechin (EC) content, measured in grams per 100 grams dry basis, over an 8-week storage period for samples treated with 0% and 1% tannase, stored at both freezer temperatures -20°C and at 30°C. The initial EC values show that the samples treated with 1% tannase have higher EC content compared to those without tannase treatment. This trend is consistent across both storage conditions, indicating that tannase treatment initially increases the EC content. Tannase treatment (1%) results in a higher initial EC content compared to the untreated control. This increase is consistent across both storage conditions, indicating that tannase treatment initially boosts the EC content. Tannase-treated samples show a gradual decline in EC content over time, but the rate of decline is slower compared to untreated samples, particularly at -20°C. Samples stored at freezer temperatures exhibit a slower decline in EC content over time compared to those stored at 30°C. This trend is consistent for both untreated and tannase-treated samples, indicating that lower temperatures help preserve EC content. Samples stored at 30°C show a more rapid decline in EC content. Untreated samples experience a significant reduction in EC content, indicating that higher storage temperatures accelerate the degradation of EC. The EC content of untreated samples

stored at freezer temperatures decreased from 0.55 g/100g db to 0.46 g/100g db over 8 weeks, while those stored at 30°C decreased to 0.37 g/100g db. This indicates that even under freezer storage, EC content declines significantly over time. The EC content of tannase-treated samples stored at freezer temperatures decreased from 0.70 g/100g db to 0.50 g/100g db over 8 weeks, while those stored at 30°C decreased to 0.46 g/100g db. The reduction is less pronounced in tannase-treated samples, suggesting better stability.

Table 4.16 The Changed of EC on Tannase Treatment

Storage time (Week)	**Storage condition EC (g /100g dry basis)**			
	Control （-20 ˚C）	**Control （30 ˚C）**	**1% tannase （-20 ˚C）**	**1% tannase （30 ˚C）**
0-1	0.55 ± 0.00^{a}	0.55 ± 0.02^{a}	0.70 ± 00.0^{a}	0.70 ± 00.0^{a}
0-2	0.55 ± 0.01^{a}	0.48 ± 0.00^{b}	0.68 ± 0.01^{b}	0.56 ± 0.01^{a}
0-3	0.55 ± 0.00^{a}	0.47 ± 0.00^{c}	0.60 ± 0.01^{c}	0.55 ± 0.00^{a}
0-4	0.55 ± 0.00^{a}	0.45 ± 0.00^{d}	0.57 ± 0.06^{c}	0.53 ± 0.01^{b}
0-5	0.55 ± 0.00^{a}	0.43 ± 0.01^{e}	0.52 ± 0.05^{d}	0.53 ± 0.01^{b}
0-6	0.54 ± 0.04^{ab}	0.42 ± 0.01^{ef}	0.52 ± 0.08^{d}	0.51 ± 0.00^{c}
0-7	0.51 ± 0.02^{bc}	0.42 ± 0.01^{ef}	0.52 ± 0.00^{c}	0.51 ± 0.01^{c}
0-8	0.50 ± 0.00^{c}	0.39 ± 0.02^{ef}	0.50 ± 0.01^{d}	0.48 ± 0.01^{d}
0-1	0.46 ± 0.01^{d}	0.37 ± 0.01^{f}	0.50 ± 0.01^{d}	0.46 ± 0.02^{d}

Note Data were presented as mean ± standard deviation. Means with different superscript letters in the same column were significantly different ($p\leq0.05$).

Table 4.17 Percent Degradation of EC Value During Storage

Storage time (Week)	Storage condition EC (%) Degradation			
	Control -20 ˚C	**Control 30 ˚C**	**1%tannase -20 ˚C**	**1%tannase 30 ˚C**
0-1	1.79	14.29	2.86	20.00
0-2	1.79	16.07	14.29	21.43
0-3	1.79	19.64	18.57	24.29
0-4	1.79	23.21	25.71	24.29
0-5	3.57	23.21	25.71	27.14
0-6	8.93	25.00	25.71	27.14
0-7	10.71	25.00	28.57	31.43
0-8	17.86	30.36	28.57	34.29

CHAPTER 5

CONCLUSIONS

The study aimed to assess potential utilization of the extraction of catechin, tannase treatment on catechin and the quality of catechin extract, according to their putative properties. However, as catechin extracts are widely used as functional substances at present, they are explored and analyzed. The catechins from dried tea leaves was extracted by solvent extraction and Ultrasound -assisted extraction (UAE) and then dried to extract powder. UAE provides a higher extract yield compared to solvent extraction. The extract yield was higher at ratio 1:20 (tea: 50%ethanol). UAE offerd higher total polyphenol content (TPC), antioxidant capacity, and catechin content.

The change of catechin properties after tannase treatment was studied. Compared with the control sample, the increase of tannase concentration decreased TPC, antioxidant capacity, total catechin content, caffeine content and tannin content, and the content of EGCG and ECG significantly decreased, while the content of GC, EGC and EC increased. Tannase treatment reduced the effectiveness of catechins in inhibiting α-amylase activity. Due to the hydrolysis of gallate catechins by tannase, the treatment reduced the α-amylase inhibitory activity of catechin extracts. Meanwhile, glucose consumption initially increased with 1% tannase treatment but excessive tannase treatment (2%) may not provide additional benefits and could slightly reduce the activity. But provided better degradation of catechin in digestion, and the percentage loss of tannase treat-catechins is higher than the control sample, the digestibility be better with higher tannase concentration in the same digestion time.

Storage 8 week for explore the stability of catechins, the TPC of catechins, antioxidant capacity, major catechins (EGCG, EGC and EC) showed the impact of storage conditions was also evident, as lower temperatures (-20°C) significantly slowed catechin degradation compared to higher temperatures (30°C). For instance, tannase-

treated catechins stored at -20°C exhibited the least degradation in TPC, antioxidant capacity and total catechin content, a decreasing trend with the increase of storage time. The decreasing trend of catechins treated with 1% tannase was slower than that of control sample, and the decreasing trend was related to temperature. The control sample and 1% tannase treated catechins, the attenuation trend of catechins storage at -20^{o}C is lower than that at 30^{o}C. The TPC, antioxidant capacity and major catechin content of catechin were stable under tannase treatment and frozen storage.

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