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MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Green extraction and protection of bioactives from green tea waste and their incorporation into a functional kiwifruit juice

A thesis presented in partial fulfilment of the requirements for the degree of

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New Zealand

Weerawich Athirojthanakij

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Abstract

Several tea products are manufactured from *Camellia sinensis*, yet about one-third of tea is wasted globally during harvest and processing. Bioactive compounds (mostly catechins), which possess numerous health-promoting properties in humans, are greatly found in such a huge waste. Thus, the objectives of this study were: 1) to utilise green tea waste by finding an appropriate, efficient, green, and industry-relevant extraction method for its catechins; 2) to protect and deliver the extracted catechins using a suitable encapsulation technology; and, 3) to test the behaviour of the encapsulated extract/catechins in a functional kiwifruit juice.

The optimisation and standardisation of catechin extraction from green tea waste were investigated by considering various extraction techniques (hot water, ultrasound-assisted, and ethanol extractions) and ratios of solvents (1:100, 1:50, and 1:20, green tea waste:solvent w/v) with an extraction temperature of 80 °C for 20 min. Distilled water was used in both hot water and ultrasound-assisted extraction methods, while ethanol was applied for the last extraction technique where ethanol was removed using a vacuum evaporation process. The results showed that hot water extraction achieved the highest extraction efficiency in the case of all ratios, and the highest extraction yield was obtained at the ratio of 1:50 over both ultrasonic-assisted and ethanol extractions, indicating that the hot water extraction was more effective than the other two methods.

Green tea waste extract (GTWE) was encapsulated into Single- and Double-layer liposomes by using soy lecithin and soy lecithin + chitosan, respectively. Single-layer liposomes possessed high encapsulation efficiency (~70 %), encapsulation yield (~75 %),

and loading capacity (~37 %) together with a small particle size (~180 nm) and high values of zeta potential (>-35 mV). However, the Double-layer liposomes showed a large particle size (~430 nm) and the opposite zeta potential values (>25 mV) during the 28 days of storage. These results indicated that both types of liposomes (i.e., Single-layer and Double-layer) had high stability up to the end of the storage period.

Kiwifruit juice was chosen as the suitable delivery vehicle for both Single- and Double-layer liposomes, due to the potential synergistic effect between catechins and vitamin C. The enrichment of kiwifruit juice with of the Single-layer liposomes increased the total phenolic content (TPC), total antioxidant activity (TAA), and total catechin concentration in the kiwifruit juice analysed by Folin-Ciocâlteu assay, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical assay, and high-performance liquid chromatography (HPLC), respectively, without the alteration of its pH during the entire 28 days of storage. Although the addition of the liposomes caused an increase in the particle size and zeta-potential of the kiwifruit juice, the TEM micrographs illustrated the separation of the Single-layer liposomes and kiwifruit fibre molecules, which confirms their stability in the juice matrix.

In terms of the application of Double-layer liposomes in kiwifruit juice, this type of liposomes could not protect the catechins in GTWE as efficiently as the Single-layer liposomes; i.e., both TPC and TAA values were lower, when compared with the Single-layer liposomes. This was possibly due to the leakage of the encapsulated catechins from the sedimented liposomes (caused by the interactions between chitosan and fibre) and their consequent degradation. In addition, TEM images of the kiwifruit juice containing these liposomes showed that the attraction of the chitosan layer and kiwifruit fibre

molecules increased the size of the particles in this beverage. Taken together, the findings of this research confirmed that green tea waste can be utilised for the extraction of its high-value bioactive compounds (i.e., catechins) that could be efficiently extracted using a green method (hot water) and be incorporated into a kiwifruit juice for the enhancement of its functionality. This research will be of high interest for the valorisation of a low-value by-product that is currently wasted and its utilisation of its extract as an added-value ingredient that can be used in the food industry for the creation of functional food products. However, further research is required to investigate the behaviour of such a developed ingredient in various functional food products and the bioaccessibility/bioavailability of its catechins after food consumption.

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Abbreviations

C	(+)-catechin
CG	Catechins gallate
CCL	Chitosan-coated liposomes
CVDs	Cardiovascular diseases
CTE	Catechins equivalent
DPPH	2,2-diphenyl-1-picryl-hydrazyl assay
EC	(-)-epicatechin
ECG	(-)-epicatechin-3-gallate
EGC	(-)-epigallocatechin
EGCG	(-)-epigallocatechin-3-gallate
EL	Empty liposomes
EthE	Ethanol extraction
EY	Extraction yield
FC	Free catechins
FRAP	Ferric reducing antioxidant power
FW	Flesh weight
GAE	Gallic acid equivalent

GC	Gallocatechin
GCG	Gallocatechin-3-gallate
GIT	Gastrointestinal tract
GTE	Green tea extract
GTW	Green tea waste
GTWE	Green tea waste extract
HPLC	High-performance liquid chromatography
HWE	Hot water extraction
KJ	Kiwifruit juice
LDL	Low-density lipoprotein
LL	Loaded liposomes
LUV	Large unilamellar vesicles
MDA	Malondialdehyde
MLV	Multilamellar vesicles
MVV	Multivesicular vesicles
NP	Normal phase
ORAC	Oxygen radical absorbance capacity

PC	Phosphatidylcholine
PME	Pectin methylesterase
PWR	Precipitate weight ratio
RP	Reverse phase
SUV	Small unilamellar vesicles
T_m	Transition temperature
TAA	Total antioxidant activity
TBARS	Thiobarbituric acid reactive substance assay
TE	Trolox equivalent
TEM	Transmission electron microscopy
TPC	Total phenolic content
UE	Ultrasound-assisted extraction
UV	Ultraviolet

Chapter 1

General Introduction

Tea (*Camellia sinensis*) was accidentally discovered around 6,000 years ago in the age of ancient China. Tea leaves were harvested and prepared by immersing in boiling water. Chinese believed that tea could refresh and revitalize their health. Moreover, tea was used as a medicine to cure a variety of illnesses in the country; relieving headaches, dissipating fever, and assisting digestion (Ho et al., 2009; Zhen et al., 2002). Presently, tea has been classified and developed from medicine to a beverage that has a high demand for healthy people. The worldwide consumption of tea is around 3.5 billion kilograms and continues to increase every year (Pastoriza et al., 2017). Therefore, a large scale of tea production has been expanded in order to serve customer needs (Zhen et al., 2002).

Typically, tea products are classified into three major types consisting of green tea, oolong tea, and black tea. A variety of tea types results in different production processes, particularly the duration of the fermentation. Green tea is produced without any fermentation while oolong tea and black tea are manufactured by applying semi- and full fermentation, respectively (Ho et al., 2009). Although to date, there have been numerous tea products manufactured from *Camellia sinensis*, yet about one-third of tea is wasted during harvest and processing (Wang et al., 2016; Wang et al., 2012). To minimize such a huge waste, it is usually transformed into a feed additive, an activated carbon, a fertilizer, or a low-cost absorbent, which might not gain the maximum benefit of such a high-value product. One of the most efficient ways for valorisation of tea waste

is to extract its bioactive compounds, which could be used as food additives and/or supplements in the health-promoting products, such as functional foods or nutraceuticals (Sui et al., 2019).

Bioactive constituents, such as vitamins, fatty acids, and phenolic compounds, can provide a wide range of health benefits via interaction with living tissues (Guaadaoui et al., 2014). Catechins are a part of the phenolic compounds in green tea, accounting for 8-15 percent of dried green tea leaves and are considered as the major phenolic compounds providing the antioxidant activity and bitterness. The main types of catechins in green tea consist of (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG) (Choung et al., 2014; Rashidinejad et al., 2014). Among those catechins, EGCG is the most abundant and potent catechin, preoccupying half of the total catechins in green tea, while it possesses five times higher antioxidant capacity than some renowned antioxidants such as vitamin C and E (Choung et al., 2014; Rashidinejad et al., 2014). Although it is proven that green tea catechins possess several health benefits such as antioxidative, antibacterial, antiviral, and antitumor properties (Kim et al., 1994; Rice-Evans, 1999; Roccaro et al., 2004), it is difficult to maintain their functionalities in food products due to the instability of catechins under both environmental and gastrointestinal tract (GIT) conditions. Therefore, an effective delivery system needs to be applied for the protection of catechins against such conditions and their controlled release in the GIT (Rashidinejad et al., 2014).

Encapsulation technology has been developed as a delivery approach to retain valuable substances (known as core materials) by their entrapment in the coating materials (also known as shell, carrier, or encapsulant) (Madene et al., 2006). There are

several encapsulation methods, such as freeze drying, spray drying, emulsions, nanoprecipitation, and liposomes, which are suitable for different kinds of core materials. In terms of catechins, liposomes have been known as a suitable and efficient method, because they are flexible carriers with amphiphilic, biocompatibility, biodegradability, and non-toxicity properties. To enhance the efficiency of encapsulation, soy lecithin, which is a phospholipid obtained from soybeans, has been successfully applied for catechins sustainability in both pharmaceutical and food industries (Rashidinejad et al., 2014; Tamjidi et al., 2013).

The stability of liposomes is one of the most essential features that need to be considered before applying liposome-encapsulated catechins in foods. This parameter could be maximized by controlling the external factors such as ionic strength, pH, and temperature and the internal factors such as composition and concentration of phospholipid and encapsulated compounds (Taylor et al., 2005). Regarding the external factors, zeta potential is a reliable method to measure the ionic strength of liposomes. The higher zeta potential values gain for liposomes, the more stable they will be; because, the increase of zeta potential promotes the repulsion effect between liposome particles that, in turn, results in greater stability of the colloidal dispersion (Mady et al., 2009; Rashidinejad et al., 2014). Optimum pH for liposomal encapsulation formed by phosphatidylcholine (PC) is 4.15, in order to perform the maximal interfacial tension (Petelska & Figaszewski, 2002). High temperature (>80°C) may disrupt the liposomal structure (Rodriguez-Nogales & Delgadillo, 2005), depending on the heat intenstiy. In terms of the internal factors, phospholipid composition has a substantial impact on the distinct surface charge, ionic strength, and permeability of the membrane (Thompson &

Singh, 2006). Proper phospholipid concentration can create a complete liposomal system by covering almost all of catechin molecules (Ostro, 1987). Theoretically, the ordinary meaning of functional foods is the foods possessing the same appearance as the conventional foods and being consumed regularly, but providing other benefits such as the physiological benefits and the risk reduction of chronic diseases beyond the traditional nutrients (Gul et al., 2016; Kaur & Das, 2011). The efficient concentration of most flavonoids towards the achievement of their health-promoting effects is in the range of 50 to 400 mg per day, which is considered a high concentration and difficult to achieve by consuming merely conventional diets (Ahn-Jarvis et al., 2019). Therefore, the development of functional foods and beverages plays an important role in delivering the required amount/dosage of these bioactive compounds to the human body through the most convenient way (i.e., food).

Kiwifruit juice can be a suitable candidate as a functional beverage for applying the encapsulated catechins, because of its exceptional nutritional value. Kiwifruit juice is an excellent source of vitamins (especially, vitamin C), minerals, and phenolic compounds (e.g., coumaric acid, caffeic acid, procyanidins, as well as trace amounts of catechin (0.19-8.66 mg/100 g flesh weight (FW)), and epicatechin (0.02-0.56 mg/100 g FW)), providing free radical scavenging property to prevent oxidation reaction, cancers, and cardiovascular diseases (Dawes & Keene, 1999; Guo et al., 2017; Ma, Lan, Ju, et al., 2019). Moreover, the physicochemical properties of kiwifruit juice are suitable for the incorporation of the delivery systems since the juice provides an environment with optimum acidity that supports the stability of these systems. For example, the pH of kiwifruit juice is approximately 3.5-6 (depending on kiwifruit species), which is situated

in the effective range of PC liposomes (Petelska & Figaszewski, 2002). Additionally, kiwifruit juice contains a low amount of catechins (approximately 0.19-8.66 mg/100 g FW) (Ma, Lan, Ju, et al., 2019), indicating that the enrichment of the encapsulated green tea catechins can enhance its nutritional value and promote this juice to become a functional drink. Therefore, this thesis aimed to find the most effective and practical method to extract and utilise catechins from green tea waste.

Due to the high sensitivity of catechins, they can be degraded by processing and biological transformation; thus, encapsulation is one of the most suitable delivery approaches that can protect catechins against degradation. In addition, although the health-promoting properties are the main advantages of catechins, a high concentration (approximately 280 mg per day) of these bioactives should be consumed daily in order to provide such benefits. Obviously, the consumption of this amount of catechins through a normal diet (especially, in the Western culture) is not practical. Hence, in this study, the author aimed to investigate the behaviour of the encapsulated catechins from green tea waste after their incorporation into a kiwifruit juice (as a functional beverage), to meet the amount of the daily requirement of catechins via the most convenient way (i.e., a functional beverage).

Chapter 2

Review of the literature

2.1 Background

This review discusses the alternative ways for the utilisation of green tea waste by finding an appropriate extraction method that can obtain the highest yield of bioactive compounds (i.e., catechins). Encapsulation technologies, in particular liposomal encapsulation, as the suitable approach for the protection and delivery of green tea bioactives, are also highlighted. Additionally, the applications of the encapsulated catechins in functional food/beverage products, with a focus on kiwifruit juice as a delivery vehicle for these valuable compounds from tea waste, are explained.

2.2 Green tea production

The global tea production gradually increased from 5.06 to 8.96 million tonnes in 2013 and 2018, respectively. According to these records, the world leader of tea production was China, which produced 2.63 million tonnes, accounting for about 29% of the world's total production, followed by India (1.34 million tonnes), Kenya (0.49 million tonnes), and Sri Lanka (0.30 million tonnes) (Table 2.1) (Chang, 2015). In 2017, the primary producers of black tea were India, Kenya, and Sri Lanka while China and Japan focused more on green tea cultivation (Lernoud et al., 2017).

Green tea production includes the cultivation of the plant *Camellia sinensis* to the harvest of its leaves, for which the right seasons need to be concerned. In China, spring is the only season that green tea leaves can be harvested. The first plucking is in the middle of March until the end of April and there are three to four plucking for four weeks after

the first plucking. After the harvest season, tea could not be plucked until the next spring. On the contrary, Japan has a different way of harvest for green tea leaves, which can be divided into three crops. The first crop starts from late April to the middle of May, followed by the second crop in late June, and finally, the third crop in late July. However, the similarity of the green tea harvest in both countries is that the slower tea plucking is, the lower tea quality and quantity get (Willson & Clifford, 1992).

Table 2.1 World ranking of tea production in 2018.

Rank	Country	Quantity (Tonnes)
1	China	2,625,138
2	India	1,344,827
3	Kenya	492,990
4	Sri Lanka	303,840
5	Turkey	270,000
6	Viet Nam	270,000
7	Indonesia	141,342
8	Iran	109,357
9	Japan	83,052
10	Argentina	81,981

Normal and high qualities of green tea leaves are transferred to the manufacturing processes. The first process is steaming the green tea leaves for 45-60 s, in order to inactivate endogenous enzymes. If the enzymes are not deactivated, they can lead to the fermentation process (the oxidation of catechins) (Ho et al., 2009; Willson & Clifford, 1992). To extend the shelf-life of green tea leaves, the drying process is indispensable to reduce the moisture content. To achieve the desired moisture content (about 6%) for the desirable storage of tea leaves, there are three drying stages (depending on the temperature and time). First, tea leaves are curled and dried in hot air at 90-110°C for 40-50 min, to decrease the moisture content from 76% to 50%. Second, green tea leaves are pressed and dried at 50-60°C for 30-40 min to reduce the moisture to 30%. Last, the goal

of 6% moisture content is accomplished by drying the tea leaves at 80-90°C for 40 min (Willson & Clifford, 1992). The final stage in green tea manufacturing is the refining process and quality screening. It is during this final process that the leaves with poor appearance and disunity (known as crude tea leaves) are separated to remove dust and sold as a low-quality tea product or tea waste; whereas, the purified leaves are packed as a high-quality tea product (Ho et al., 2009; Willson & Clifford, 1992).

2.3 Green tea waste

About 1 million tonnes of tea products, which account for almost 30 percent of the annual global tea production (approximately 3.5 million tonnes), is wasted every year (Sui et al., 2019). Chinese tea factories create almost 1 million tonnes of tea waste while Indian and Malaysian tea factories generate about 0.19 and 0.10 million tonnes of the waste per annum, respectively (Hussain et al., 2018). Regarding the factors that affect the quality of tea leaves and result in the production of low-quality leaves, various constituents relate to tea quality such as tea plant cultivars, seasons, harvesting procedure, fertilisation, and processing technology. Tea species have a repercussion on green tea quality, especially the ratio of amino acids and polyphenols. For example, Longjing 43, Fuding Dabaicha (China species), and Yabukita (Japan species) tend to give a better quality of green tea, compared with other species in the same crop and manufacturing conditions. Zheng et al. (2015) studied the remaining polyphenols and amino acids in green tea waste and found over 60% of those compounds, together with 30% of crude protein, in the waste.

Tea shading is another factor that affects the catechins and nitrogen levels in green tea. It has been reported that the green tea plant, grown under the shade before plucking,

produces a greater number of amino acids in the leaves than the unshaded leaves (Zhen et al., 2002). On the other hand, it has been reported that the shading treatment could harm the concentration of catechins, because shaded Yabukita green tea produced fewer catechins content than unshaded green tea. EGC and EC were decreased by 48% and 41%, respectively, while the galloylated catechins (ECG, EGCG) were decreased by 20-25% (Iwasa, 1976).

Yet, the most influential factor is green tea processing, which focuses on fixing and rolling processes. To begin with the fixing process, there is a fixing principle indicating that heavy fixing is suitable for the tender leaves while light fixing is used for the mature leaves. Theoretically, tender leaves usually contain substantial enzymes, which can lead to the oxidation reaction so that heavy fixing, which needs to be implemented at a high temperature for a long period, can inactivate enzymes and increase the hydrolysis of the proteins. Nevertheless, over-fixing can cause a smoky aroma and damage the leaves, while short fixing generates red leaves because of the oxidation of polyphenols representing the low quality of green tea. Turning to the rolling process, rolling time and pressure levels are the key parameters affecting the quality of green tea. Using a high level of pressure and a long time could promote yellowish and broken leaves. Due to the excessive water evaporation, chlorophyll hydrolysis, and polyphenol oxidation can occur and result in adverse effects on green tea taste (Zhen et al., 2002). For these reasons, green tea waste can be developed and utilized by highly efficient methods to gain the maximum potential for humans. Normally, the waste can only be transformed into a feed additive, an activated carbon, a fertilizer, and a low-cost absorbent. Interestingly, this waste contains a great concentration of polyphenolic

compounds such as catechins, deserving a more efficient way for the extraction of such high-value compounds and their utilisation in the nutraceutical and food industries (Sui et al., 2019).

2.4 Bioactive compounds

Bioactive compounds (e.g., polyphenols, carotenoids, essential oils, vitamins, minerals, peptides, and prebiotics) are defined as substances with the ability to provide an abundance of probable effects by interacting with other components in the living tissues (Guaadaoui et al., 2014; Mahfoudhi et al., 2016). Due to their established health-promoting effects, bioactive compounds can be used in a wide range of applications such as medicines, cosmetics, and functional foods (Guaadaoui et al., 2014). Focusing on the application of functional foods, bioactive substances can be extracted from natural sources such as fruits, cereals, vegetables, and food by-products/wastes (Sibele et al., 2019). The extracted compounds are then incorporated into various food matrices (e.g., liquids, semi-solids, or solids) for enhancing the functional, nutritional, and sensorial properties of such products (Mahfoudhi et al., 2016). Importantly, bioactive compounds in foods are categorised as natural antioxidants and have the radical-scavenging properties that can prevent oxidation reactions.

In the human body, the physiological conditions are balanced between oxidising agents and antioxidants. If the generated free radicals overcome the antioxidant capacity of the body, they may interact with proteins, lipids, and DNA, destroying enzymes and genetic materials, and the phenomenon known as oxidative stress. Since the oxidation reaction, in turn, can cause various diseases and disorders such as cancer and cardiovascular and neurodegenerative diseases, antioxidants have drawn a lot of attention

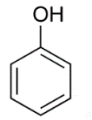
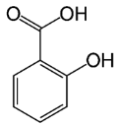
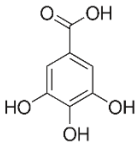
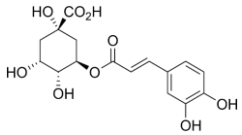
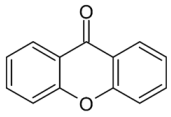
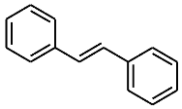
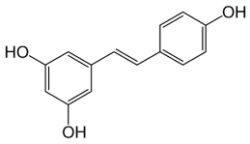
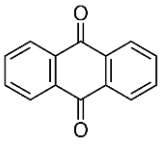
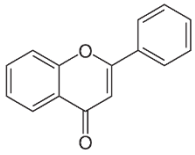
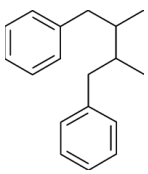
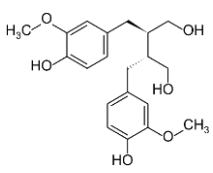
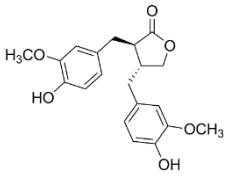
among scientists to study their extraction, isolation, protection, delivery, bioavailability, and bioefficacy (Rojas & Buitrago, 2019).

2.5 Phenolic compounds

Phenolic compounds originating from natural sources are one of the main compounds under the class of bioactive compounds and their chemical structures consist of at least one hydroxyl group attached to an aromatic benzene ring. Correspondingly, polyphenols are aromatic compounds resulting from more than one phenol unit. They can be found in plants in forms of esters or glycosides, which could be counted as secondary metabolites for these sources. Moreover, polyphenols can inhibit microorganisms, become chemical messengers, and contribute to growth and reproduction in plants (Bravo, 1998). Recently, phenolic compounds are widely studied because of their health-promoting properties (Drummond, 2013; Guo et al., 2017; Lorenzo & Munekata, 2016; Trigo et al., 2020; Zokti et al., 2016).

Regarding the classification of phenolic compounds, there is a rampant of these structures (approximately 8,000 forms), which are amalgamated in plant tissues during normal development. Therefore, classification methods of phenolic compounds are necessary for creating groups of the compounds having the same structure. Classification based on the carbon skeletons of phenolics is the most renowned method to differentiate the subclasses of these compounds. The subclasses consist of simple phenols (C6), phenolic acids (C6-C1), hydroxycinnamic acids (C6-C3), xanthones (C6-C1-C6), stilbenes and anthraquinones (C6-C2-C6), and flavonoids (C6-C3-C6) (Table 2.2) (Bravo, 1998; Robards & Antolovich, 1997).

Table 2.2 The basic structures of the phenol, phenolic subclasses, and derivatives.

Basic structure	Main derivative(s)	
Phenol 		
Phenolic acid 	Gallic acid 	Chlorogenic acid 
Xanthone 		
Stilbene 	Resveratrol 	
Anthraquinone 		
Flavonoid 		
Lignan 	Secoisolariciresinol 	Matairesinol 

2.5.1 Polyphenols in foods

Polyphenols are the major antioxidants in foods. The adequate intake of polyphenols is 1 g per day that is quite a high quantity comparing to other antioxidants. For instance, the recommended daily amount for vitamin C and E is 0.1, 0.01 g per day, respectively (Scalbert et al., 2005). The major food sources of polyphenols are fruits, vegetables, cereals, chocolate, legumes, and plant-derived beverages such as tea, coffee, and wine (Cheynier, 2005). Regarding the diversity of polyphenols, they are normally classified into four types: phenolic acids, lignans, stilbenes, and flavonoids (Table 2.2) (Manach et al., 2004). Phenolic acids have two sub-classes including benzoic acid and cinnamic acid derivatives. The hydroxybenzoic acid is found at low concentration in edible plants, except for onions, black radish, red fruits, and tea. Gallic acid is one of the essential hydroxybenzoic acids, which can be extensively found in tea leaves (about 4.5 g/kg of fresh leaves) (Clifford & Scalbert, 2000; Tomas-Barberan & Clifford, 2000). Concerning hydroxycinnamic acids, chlorogenic acid, which is originated from the combination of caffeic and quinic acid, is a distinct substance found in coffee; a single cup of coffee may contain 70-350 mg of chlorogenic acid, having a substantial influence on the taste of coffee drinks (e.g., astringency, sweet, and sour) (Clifford, 1999).

Lignans stem from two phenylpropane units and are situated in the bran layer of cereal grain. The plant containing the highest amount of lignans is linseed that contain secoisolariciresinol (around 3.7 g/kg dry weight) and small amount of matairesinol (Table 2.2) (Adlercreutz & Mazur, 1997; Heinonen et al., 2001). Lignans can be converted into enterolignans, enterodiol, and enterolactone by the intestinal bacteria. Enterolignans have many biological effects, such as antioxidant, anti-inflammatory, and anti-apoptosis

(Yoder et al., 2015). Stilbenes are detected in a very low amount in human food. However, resveratrol, which is one of the most effective forms of stilbenes, has an anticarcinogenic effect and can be found in red wine with small quantities (ranging from 0.1 to 42 mg/L, depending on the origin of red wine) (Frémont, 2000; Manach et al., 2004). Nevertheless, it is nearly impossible to consume only red wine and gain the health-promoting effects of resveratrol, because the recommended daily intake of resveratrol is 12.5 mg/kg of body weight (Weiskirchen & Weiskirchen, 2016).

2.5.2 Flavonoids

Flavonoids, which are the most potent group of polyphenols, can be divided into flavonols, flavanones, flavones, isoflavones, flavanols, and anthocyanidins. Table 2.2 provides some in-depth information about the structure of flavonoids synthesized by two aromatic rings (i.e., A- and B-rings), which are bound together with an oxygenated heterocycle or C-ring formed by three carbon atoms (Mérillon & Ramawat, 2019).

Flavonoids are naturally found in fruits, vegetables, cereals, seeds, tea, wine, and certain spices (e.g., turmeric, saffron, and ginger). A large quantity of flavonoids is discovered in berries, kale, parsley, oregano, dark chocolate, black tea, and green tea. However, the presence of flavonoids in fruits and vegetables may vary, based on the species of plant, crop variety, seasonality, processing, and storage. Variation of flavonoid contents in the plant sources occurs ordinarily, but in some cases, there is a considerable discrepancy of flavonoid contents recorded (e.g., berries and tea) (Mérillon & Ramawat, 2019).

The flavonoid contents of several foods are presented in Table 2.3. Each subclass of flavonoids might be found in different types of foods. For instance, blueberries, blackberries, and strawberries are rich in anthocyanidins while both green tea and black tea are filled with flavanols (e.g., catechins). Different types of flavanols can be discovered in various kinds of foods. For example, fruits are full of (+)-catechin (C) and (-)-epicatechin (EC); whereas, tea, mainly green tea, contains of plenty of (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-gallate (EGCG) (Mérillon & Ramawat, 2019). However, tea source and tea production have significant effects on the ratio of flavanols, meaning that EGCG may not be the most abundant of catechins in all kinds of green tea (Koch et al., 2018).

Table 2.3 The concentration of flavonoid subclasses in various foods. Modified from Mérillon and Ramawat (2019).

Anthocyanins (mg/100g)		**Flavanols (mg/100g)**	
Chokeberry	349.79	Green tea, brewed	116.15
Bilberries	285.21	Black tea, brewed	115.57
Chickpeas	262.49	Dark chocolate	108.60
Flavonones (mg/100g)		**Flavones (mg/100g)**	
Dried Maxican oregano	412.13	Dried parsley	4523.25
Grapefruit	54.5	Dried oregano	1046.46
Lemon	49.81	Fresh parsley	216.15
Flavonols (mg/100g)		**Isoflavones (mg/100g)**	
Fresh capers	493.03	Soy flour	166.66
Dried parsley	331.24	Natto	82.29
Goji berries	31.20	Soybeans, raw	48.95

2.5.3 Green tea catechins

Green tea is one of the most popular beverages consumed worldwide and renowned as a potent source of antioxidants. All of the antioxidant constituents in green tea are mainly polyphenols including phenolic acids and catechins, especially EGCG, which provides the highest potential of antioxidant activity among catechin derivatives

(Du et al., 2012). Catechins in green tea can be classified into two classes; a) the flavan-3-ol unit including (+)-catechin, (+)-epicatechin, (+)-gallocatechin, and (+)-epigallocatechin, and b) the galloylated or esterified catechins including (-)-epicatechin gallate, (-)-epigallocatechin gallate, and (-)-gallocatechin gallate (Figure 2.1) (Du et al., 2012; Hara, 2001). Naturally, fresh green tea leaves contain about 30-40% catechins per dry weight basis (de Mejia et al., 2009). Moreover, green tea has an astringency taste originated from the galloylated catechins (e.g., ECG, EGCG) together with a bitter taste (e.g., ECG), while the flavan-3-ol unit has lower astringency with a bit sweet taste (Hara, 2001).

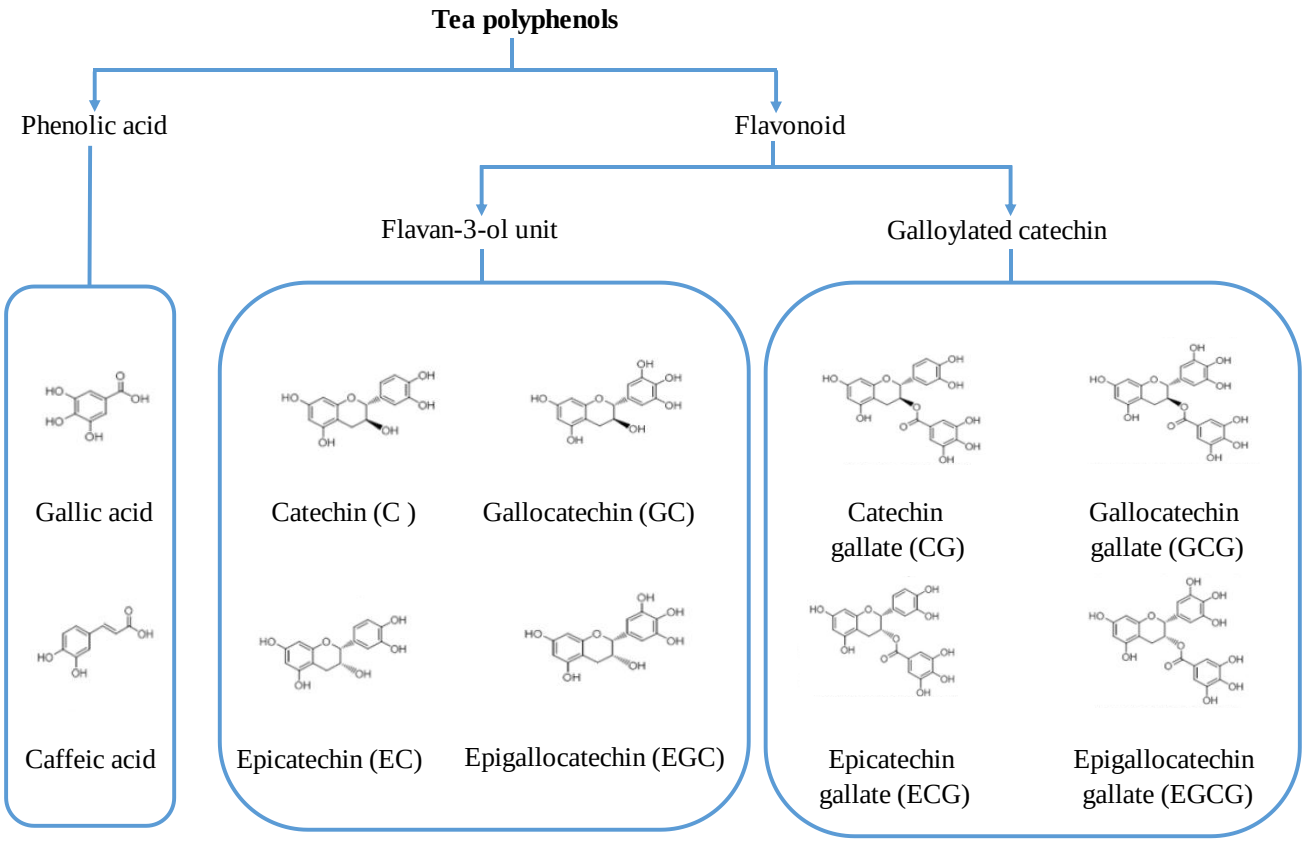


Figure 2.1 The classifications of tea polyphenols with their chemical structures.

2.5.3.1 Stability of green tea catechins

The stability of green tea catechins has been studied in various conditions to understand the degradation of each type of catechin. According to Lun Su et al. (2003), when green tea drinks were heated up to 100°C for 3 h, it was found that EC, ECG, EGC, and EGCG had the same degradation rate. The effect of pH was also tested by comparing the natural pH (pH 4) and the adjusted pH of green tea drinks (pH 7.4). The results showed that all catechins were stable at pH 4, but EGC and EGCG in the green tea drink pH of which was adjusted to 7.4 was degraded during 6 h of incubation, while EC and ECG were degraded only by 35% or less.

Chen et al. (2001) studied the effect of extreme temperatures applied in the canned green tea drinks via autoclaving at 120°C for 20 min, and the results demonstrated that the epimerization of the galloylated catechins occurred via the transformation of EGCG to gallated catechin gallate (GCG); i.e., because GCG was significantly increased while EGCG was drastically decreased after autoclaving. Wang and Zhou (2004) applied green tea extract, which is a rich source of catechins, in the bread-making process, consisting of unfrozen and frozen dough, to study the stability of green tea catechins. Interestingly, all green tea catechins were stable in the frozen dough at -20°C for nine weeks, but after the bread-making process, EGCG and EGC were found less stable than EC and ECG. In the study carried out by Rashidinejad, Birch and Everett (2016), encapsulated green tea catechins were added in a full-fat cheese in order to study the stability of the catechins during storage at 8°C for 0, 30, and 90 days. The results indicated that the supplemented green tea catechins increased the total phenolic content

and antioxidant activity of the full-fat cheese, even when the cheese was kept up to 90 days. In conclusion, the extremely high temperatures (e.g., 120°C) can drastically reduce the amount of EGCG, while the low temperatures (e.g., -20°C) can preserve catechins up to nine weeks. In terms of acidity, it seems that almost all kinds of catechins are stable at pH 4 (Chen et al., 2001; Lun Su et al., 2003; Rashidinejad, Birch, & Everett, 2016).

2.6 Green tea polyphenols and their association with human health

Green tea polyphenols are dominated by catechins and their derivatives, which are EC, ECG, EGC, and EGCG, together with a small concentration of flavonols and phenolic acids (Lorenzo & Munekata, 2016). These polyphenols play an important role in increasing the amounts of antioxidants in the human body, decreasing oxidative stress, and subsequently, reducing the risk of chronic diseases such as cancer, diabetes, cardiovascular, neurodegenerative, and Alzheimer's diseases. These bioactive compounds have been studied for the adverse association between their bioactivity and the risk reduction of medical ailments such as cancer, diabetes, cardiovascular, neurodegenerative, and Alzheimer's diseases (Lorenzo & Munekata, 2016). The clinical studies so far have reported that green tea polyphenols had been linked with antioxidant, anti-cancer, anti-microbial, anti-cardiovascular, anti-hyperglycemic, and anti-obesity properties, which may promote a protective effect on human health (Lorenzo & Munekata, 2016; Xing et al., 2019). However, to gain the corresponding biological effect, tea polyphenols have to meet the requirement of bioavailability and bioaccessibility, which starts from the food matrix accompanied by absorption in the digestive tract (Xing et al., 2019).

Bioavailability could be described as the ratio of the ingested substances involving in the systemic circulation while bioaccessibility accounts for the proportion of substances released from the food matrix and their absorption availability in the intestinal tract (Tagliazucchi et al., 2010). Various *in vitro* reports suggested that the range of bioaccessibility is different depending on the source of organs. In the oral cavity, green tea polyphenols can be absorbed through the saliva at the concentration of about 2.83 to 50.39 mg/g dry weight, while the bioaccessibility in the stomach has been reported to range from 1.65 to 35.62 mg/g. Furthermore, the bioaccessibility of polyphenols in the intestine has been elucidated in the order of EC (0.61 mg/g), ECG (0.35 mg/g), EGC (0.41 mg/g), and EGCG (2.49 mg/g). To sum up, up to 90% of green tea polyphenols can be lost through gastrointestinal digestion (Tenore et al., 2015).

The bioavailability of green tea polyphenols has been reported in the same manner as their bioaccessibility. In one study (Tenore et al., 2015), the simulation of intestinal absorption was set to determine the bioavailability of the polyphenols and found that EGCG possessed the highest bioavailability at 0.32 mg/g, followed by EGC (0.05 mg/g), ECG (0.04 mg/g), and EC (0.04 mg/g), respectively. The reasons for the poor bioavailability of the polyphenols might be linked to their polymerization, including poor intestinal transportation and harsh environment in the intestinal tract (Xing et al., 2019). However, during absorption, green tea catechins were absorbed in the native structure and simultaneously metabolized by phase II enzyme, which is situated in the liver and small intestine. Then, the catechins are glucuronidated, sulfated, and methylated to become catechins conjugates (Stalmach et al., 2010). Glucuronidation and sulfation play a vital role in the assistance of the catechins solubility as well as elimination through

urine (Chow et al., 2005). Thus, upon the colon, green tea catechins can be recovered and found in the form of sulfates and methyl sulfates at a concentration of approximately 37% (Stalmach et al., 2010). To improve the bioavailability of green tea catechins, nanotechnologies may need to be executed for developing their stability and absorption for maximizing the health-promoting properties such as anti-cancer, anti-cardiovascular, and anti-obesity (Lorenzo & Munekata, 2016; Xing et al., 2019).

2.6.1 Anti-cancer property of green tea catechins

Cancer is a leading cause of death worldwide, accounting for an estimated 18.1 million new cases and 9.6 million deaths from cancer in 2018 (Ferlay et al., 2019). The most common organs related to cancer are prostate, breast, colorectal, bronchus, and lung. In the United States and European countries, pancreatic, stomach, and leukemia cancers are the leading causes of death. The risk factors for developing cancers are smoking, overweight, improper diet, and lack of exercise (Lorenzo & Munekata, 2016). A variety of studies revealed that green tea polyphenols had a positive effect on cell proliferation, cell apoptosis, cell migration, and cell cycle in tumour growth related to the reduction of cancer risk (Xing et al., 2019).

The relationship between green tea consumption and the risk of digestive system cancers was studied by Nechuta et al. (2012). In this study, 69,310 Shanghai women were evaluated for 11 years in order to collect the data related to tea consumption and cancer risk. The results showed that regular consumption of green tea contributed to an inverse association with cancer development as a whole digestive system. The risk of digestive cancer development was decreased by 21% for the women who usually consumed two to three cups of green tea per day. Therefore, the increase in the amount and time of green

tea consumption has a positive effect on the reduction of the risk of digestive system cancers. In terms of prostate cancer, Kurahashi et al. (2008) suggested that five cups or more of green tea consumption could reduce the risk of the advanced type of this cancer, but one (or fewer) cup of green tea consumption did not show any positive result. Bettuzzi et al. (2006) conducted a one-year treatment for 60 male suffering from high-grade prostate intraepithelial neoplasia by taking an adequate dose of green tea polyphenols (600 mg/day). The results after one year of the intervention demonstrated that green tea polyphenols enhanced the chemoprevention efficacy up to 90% with the absence of adverse effects on the patients.

2.6.2 Cardiovascular diseases and the association with green tea catechins

Cardiovascular diseases (CVDs) are the world number one cause of death supported by 17.9 million deaths in 2016. Over 85% of the deaths came from a heart attack and stroke (WHO, 2017). The cause of heart attack and stroke is that the blood circulation is blocked due to fatty deposits on the inner walls of the blood vessel resulting in the limitation of blood flow so that the hearts' muscle cells may die from lack of oxygen (Ambrose & Singh, 2015). There are several risk factors enhancing CVDs such as high blood pressure, smoking, diabetes, lack of exercise, obesity, high blood cholesterol, poor diet, depression, family history, and excessive alcohol (Charlson et al., 2013). However, recent studies showed that catechins and their derivatives might exert a cardioprotective effect leading to the reduction of CVDs risk via the interaction between catechins and low-density lipoprotein (LDL) in plasma (Zheng et al., 2011).

To prove the beneficial effect of green tea catechins in the risk reduction of CVDs mortality, Kuriyama et al. (2006) researched 40,530 participants in Northeastern Japan.

The results revealed that women who consumed five cups or more (≥ 500 mL) of green tea per day, presented a higher risk reduction of CVDs at 31% than those who consumed one cup or fewer (≤ 100 mL) per day. Sano et al. (2004) supported the previous study that increased regular consumption of green tea to 5.9 cups per day can diminish the risk of CVDs in 203 patients. Furthermore, the main catechins consisting of EC, EGC, ECG, and EGCG were proven to show a positive effect in obstructing LDL peroxidation by Cai et al. (2002). The authors reported that peroxyl radicals were interacted and trapped by those catechins in the initiating and propagating processes. Suzuki-Sugihara et al. (2016) supported the prior study that galloylated catechins (EGCG and ECG) were present in plasma after regular green tea consumption. Both catechins interacted with LDL particles without the conjugation process and the incorporated LDL were extremely resistant to radical molecule resulting in the prevention of oxidation reaction. In summary, these pieces of evidence demonstrate that green tea catechins and their derivatives promote the inverse relationship with the risk reduction of CVDs.

2.6.3 The effect of green tea catechins on diabetes and obesity

There has been a continuous increase in the number of diabetic patients (from 108 million in 1980 to 422 million in 2014) and in 2016, about 1.6 million deaths were originated from diabetes. The cause of diabetes is insufficient insulin produced from the pancreas in the bloodstream due to a lack of insulin secretion (WHO, 2018). One of the significant health problems leading to an increased risk of diabetes is obesity or being overweight, because the fat cells of an obese person need to process more nutrients, which is over their average ability. The stress of fat cells leads to the production of a protein named cytokines that can block the signal of insulin receptors, so the cells are

gradually resistant to insulin that takes responsibility for the conversion of glucose to energy. When glucose presents in the bloodstream, insulin resists converting the glucose into energy resulting in a high level of glucose in the blood (Golay & Ybarra, 2005; Verma & Hussain, 2017). Moreover, various studies suggested that diabetes has negative effects on physical health in the long term; e.g., retinopathy, nephropathy, and neuropathy. Interestingly, green tea polyphenols have been continuously studied as a choice for anti-diabetic and anti-obesity properties (Rizvi & Zaid, 2001 ; Rizvi et al., 2005).

Green tea polyphenols may interact with glucose in blood via different mechanisms, including the impediment of glucose absorption in the gut and outer tissues (Matsui et al., 2002). Chen et al. (2020) studied 10,825 Chinese diabetes patients to clarify the influence of tea consumption on diabetes. The results showed that regular tea consumption was associated with the risk reduction of diabetes, accounting for 32% in females, 24% in elder (>45 years), and 34% overweight participants. Some studies had the goal to determine the association between green tea consumption and body weight reduction, which is the most essential risk factor leading to diabetes. Chen et al. (2016) revealed that the daily consumption of a high-dose green tea extract (EGCG) of about 800 mg per day had a significant effect on the weight loss of 115 obese women. This anti-obesity mechanism was attributed to the high dose of EGCG and the subsequent ghrelin inhibition that occurred after the intake of this catechin. The inhibition of ghrelin secretion can increase the levels of adiponectin, which is a protein with the advantages of anti-atherogenic and anti-diabetic properties (Chen et al., 2016). In conclusion, the regular drinking of green tea could decrease the diabetes risk in females, elderly, and

overweight people, up to about 30% together with the anti-obesity property of green tea catechins.

2.7 Nanotechnology as a delivery approach for green tea catechins

The National Nanotechnology Initiative presented a definition of 'nanotechnology' as "the understanding and control of matter at the nanoscale, at dimensions between roughly 1 and 100 nm, where unique phenomena enable novel applications", as well as another definition for 'nanomaterial' as "a discrete entity that has one or more dimensions of the order of 100 nm or less" (SCENIHR, 2007). According to Tamjidi et al. (2013), the size range should be adjusted to 1 to 600 nm, because in the human gastrointestinal tract (GIT), the particle sizes that are in the range of 1-100 and 600 nm will behave similarly. Nanotechnology has been fast developed and accepted in a variety of industries such as chemical, cosmetics, pharmaceutical, and food (Madene et al., 2006). There are abundant benefits of nanotechnologies in foods such as packing materials, productive nutrient delivery system, and formations of a new molecule together with bioactivity improvement (Tamjidi et al., 2013). To entrap/encapsulate the bioactive molecules in food for their protection against the environmental and gastrointestinal conditions, a suitable delivery system needs to be concerned, because of the direct effects on the controlled release rate, targeting the encapsulated bioactive molecules, and improving thermal stability. Moreover, the delivery system could generate more surface area, which can result in the enhancement of the solubility and bioavailability of the encapsulated bioactive compounds (Madene et al., 2006; Tamjidi et al., 2013).

Nanodelivery systems can be divided into polymer- and lipid-based systems. The former system has crucial disadvantages on the toxicity in polymers, the lack of suitable biopolymers, the difficulty of implementation in large-scale production, and the requirement of organic solvents (Muller et al., 2000; Tamjidi et al., 2013). Furthermore, almost all bioactive compounds (e.g., carotenoids, flavonoids, polyphenols, tocopherols, fatty acids, and oil-soluble vitamins) are naturally hydrophobic, meaning that they require oil in water type of colloidal dispersion for their encapsulation. For these reasons, the lipid-based system such as nanoemulsions, microemulsions, liposomes, solid lipid nanoparticles, and nanostructured lipid carriers, can gain more attention on the effectiveness of the encapsulation such as flexibility of the carrier(s), droplet stability, and full availability of the bioactive compounds to be encapsulated (Tamjidi et al., 2013).

Nanoencapsulation in the food industry is the technique by which minuscule particles (e.g., acidulants, fats, and flavours) are entrapped with one or more coating materials. The coating material is called shell, carrier, wall, or encapsulant and the coated material is called active or core material (Madene et al., 2006; Tamjidi et al., 2013). Nanoencapsulation has several advantages in the food industry as follows:

- Protecting the core material from the outside environmental factors such as temperature, oxygen, light, and water.
- Masking the undesirable taste of the core material.
- Controlling the release rate of the core material to accomplish a suitable delay.
- Reducing the evaporation rate of the core material during transfer to the outer atmosphere.

- Increasing the precision of the core material dilution when it is used in a little amount and enhancing the dispersion uniformity in the host material.
- Providing better core material management; lumping prevention, core material uniformity, and availability of a liquid to a solid form conversion (Tamjidi et al., 2013).

2.7.1 Liposomes as an efficient delivery system for catechins

Liposomes or lipid vesicles are one of the lipid-based delivery systems that can be applied for a wide range of bioactive compounds. The shape of liposomes is spherical with particle sizes ranging from nanometre to micrometre (Taylor et al., 2005). Single or multi bilayer membranes are the primary advantage of liposomes having an ability to deliver the core material into a target area by imitating the natural lipid fraction of the cell membrane. Liposomes can be divided into three types, based on the number of the bilayer membranes. Small (SUV) and large unilamellar vesicles (LUV) represent a single bilayer membrane that can be measured < 30 and 30-100 nm in size, respectively. Multilamellar vesicles (MLV) illustrate liposomes with multilayer membranes and multivesicular vesicles (MVV) contain several randomly sized vesicles that are situated in the interior of a larger vesicle (Figure 2.2) (New, 1990). Most importantly, liposomal functionalities rely on extrinsic factors such as pH, temperature, ionic strength, as well as intrinsic factors such as concentration and nature of the entrapped compounds, the concentration of phospholipids, and the composition of phospholipid. All these factors affect the chemical and physical stability of liposomes. The chemical instability of liposomes may be a result of hydrolysis and oxidation of polar lipids. In contrast, the physical instability of these vesicles may occur due to the phase transition and aggregation of the bilayer

membranes, which can result in the release of the encapsulated compounds and the rise of particle size distributions (New, 1990; Taylor et al., 2005).

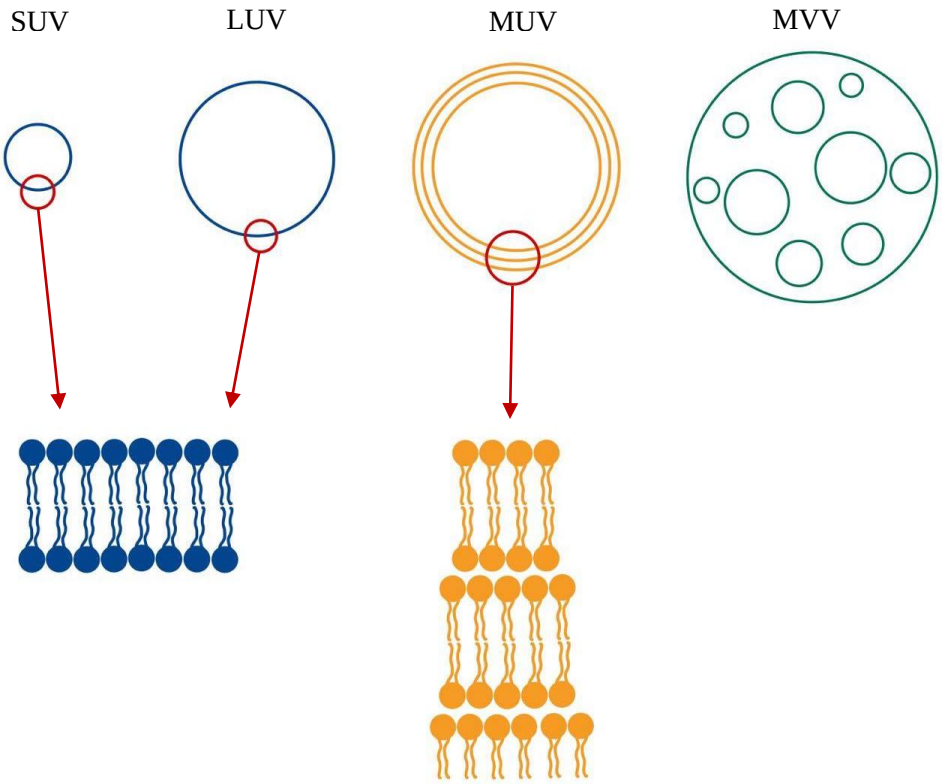


Figure 2.2 Structure illustration of small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV), multilamellar vesicles (MLV), and multivesicular vesicles (MVV)

The chemical structure of liposomes mostly consists of phospholipids, but sometimes other lipids (e.g., galactolipids) may also be involved. Phospholipids are considered as biological membranes that are a part of either phosphodiglycerides or sphingolipids (Barenholz et al., 1977; Lasch et al., 2003). Lecithin or phosphatidylcholine (PC) is the most renowned phospholipid. The PC chemical structure is consisted of two acyl hydrocarbon chain connected with the glycerol in the position of *sn*-1 and *sn*-2 that are connected via ester linkage and the third glycerol carbon (*sn*-3) linked to a choline group. The polarity of a phospholipid is provided by hydrophilic

heads and hydrophobic tails. The polar head group originates from the hydrophilic phosphocholine, while the hydrophobic tail stems from the two acyl hydrocarbon chains (Koynova & Caffrey, 1995). In terms of the phase transition in phospholipids, the transition temperature (T_m) and the composition of phospholipids are critical factors for the phospholipids phase transition. Phospholipids are stable in one or more liquid-crystalline or mesomorphic forms. However, high temperature (over the melting point of hydrocarbon chains) results in losing the phospholipid structure. The longer hydrocarbon chains are involved in a phospholipid structure, the higher T_m that particular phospholipids get (New, 1990). Interestingly, almost all natural phospholipids are fatty acid chains composed of saturated and unsaturated fatty acids, so that the level of unsaturated fatty acids affect the phase transition, because the T_m will be increased due to the rise of saturation (Marčelja & Wolfe, 1979; New, 1990).

Liposomal functionalities and properties rely on both external (e.g., ionic strength, pH, and temperature) and internal factors (e.g., composition and concentration of phospholipid and encapsulated compound), which can affect the physical and chemical instabilities (Marčelja & Wolfe, 1979; New, 1990; Taylor et al., 2005). The phase transition of phospholipids brings about the deformation of liposomes, leading to physical instability. The combination of bilayer structures has negative effects on the leakage of encapsulated substances and also the increase of the liposomal particle size (Taylor et al., 2005). In terms of chemical instability, oxidation and hydrolysis reactions may occur in the position of polar lipids leading to losing the amphiphilic property of the bilayer (Taylor et al., 2005). For these reasons, entrapped components in liposomes need to be

studied thoroughly about the capsule stability under food processing and food application to prevent physical and chemical instability.

Concerning the production of the high stability of liposomes, the types of liposomes along with the production technologies need to be considered. Different kinds of liposomes require different levels of input energy. LUV and SUV need substantial energy to interrupt MLV and MVV structures leading to generate unimodal vesicles (Taylor et al., 2005). Regarding the encapsulation techniques, various encapsulation methods (e.g., emulsions, freeze drying, nanoprecipitation, and yeast cells) have been applied to protect a wide range of polyphenols as core materials. Nonetheless, some drawbacks from those methods have been reported; solvent uses, capsule instability, low encapsulation efficiency, and large particle sizes (Rashidinejad, Birch, & Everett, 2016). Liposomes are an effective encapsulation technique to deliver a variety of polyphenols due to their advantages such as non-toxicity, small particle size, biodegradability, and biocompatibility (de Leeuw et al., 2009). Therefore, liposomal encapsulation is an efficient technique that has been chosen to protect green tea catechins.

2.8 Functional foods

Food is one of the necessities for maintaining primary functions in the human body, such as energy production, nutrient supply, and metabolic activities. In the 20th century, scientists have paid more attention to food promoting better health conditions and to the modified traditional foods to become functional foods (Doyon & Labrecque, 2008). There is no exact definition of functional foods as each ingested food performs different functions in the body. However, the ordinary meaning of the term involves the foods possessing the same appearance as the conventional foods and being consumed

regularly, but providing other benefits such as the physiological benefits and the risk reduction of chronic diseases, beyond the traditional nutrients (Gul et al., 2016; Kaur & Das, 2011).

To clarify the functional food categories, there are three kinds of foods related to the additional bioactive compounds. Firstly, basic foods are food or a food product containing indigenous bioactive compounds such as beta-carotene in carrot, lycopene in tomato, and lutein in kale. Secondly, processed foods enriched with bioactive compounds are a food product that contains some added bioactive compounds during food processing; e.g., pasteurised milk fortified with omega-3 fatty acids and bread enriched with iron. Thirdly, foods with enhanced concentrations of bioactive compounds, which include natural foods modified by breeding or genetic engineering; e.g., the genetic modification of chicken producing eggs with a high-level of omega-3 fatty acids (Gul et al., 2016).

The development of functional foods can solve both personal and public health problems, mainly in nutritional deficiency from the traditional foods and changing lifestyle of the people in different communities (German et al., 1999). Food manufacturers have been trying to develop functional foods that can launch into the market in order to solve the abovementioned problems, as well as meeting the customer requirement (Charalampopoulos et al., 2002; Sanders, 1998). For example, in dairy products, milk is considered as one of the foods rich in calcium, which can diminish the risk of osteoporosis and colon cancer. Calpis Co. (Japan) has developed sour milk enriched with some amino acids such as valine, proline, and isoleucine to promote the additional function of lowering blood pressure (Kaur & Das, 2011).

As well established so far, tea is renowned as the great source of polyphenols, primarily catechins and their derivatives, with well-known health-promoting properties. Taiyo (2020, Japan) introduced a green tea product that was produced from purified polyphenols under 'Sunphenon' brand (Kaur & Das, 2011). Sunphenon was obtained from the solvent extraction of high-quality green tea leaves. Then, the green tea extract was dried to become a powder. The product specification of Sunphenon relied on the compositions and concentrations of polyphenols, catechins, EGCG, and caffeine. For example, Sunphenon 90LB contained >90% polyphenols, >80% catechins, >40% EGCG and <1% caffeine. Moreover, Sunphenon could be applied in several products such as energy drinks, dietary supplements, and hair nourishment (Taiyo, 2020).

Zokti et al. (2016) applied encapsulated catechins from green tea extracts to mango juice in order to enhance the health-promoting properties. The results showed that the encapsulated catechins were stable leading to promote additional nutrition in the juice, since the degradation rate of the encapsulated catechins was determined to be in the range of 16.47-29.72% that was lower than non-encapsulated compounds (46.46%). Additionally, high encapsulation efficiency (71.41-88.04%), total phenolic content (19.32-24.90 g/100g gallic acid equivalent (GAE)), and antioxidant capacity (29.52-38.05%) were achieved. On the other hand, the physicochemical properties (pH, total solid (°Brix), and viscosity) of the juice were not been interrupted by encapsulated catechins. Rashidinejad, Birch, Sun-Waterhouse, et al. (2016) investigated the antioxidant properties of the encapsulated green tea catechins incorporated into a hard low-fat cheese that was digested using an *in vitro* digestive model. C and EGCG were encapsulated in soy lecithin using liposomal encapsulation technology. The cheese samples fortified with

the encapsulated catechins were kept at 8°C for 90 days. The total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) methods were used to determine the antioxidant capacity of cheese samples. The results stated that both C and EGCG at the proper concentration of 250 ppm provided the enhancement of the total phenolic content and antioxidant capacities, without any adverse effects on pH or compositions of the cheese. After 90b days of ripening and six hours of digestion, a substantial amount of catechins was recovered. Rashidinejad, Birch and Everett (2016) also investigated the incorporation of liposomal encapsulated green tea catechins into a full-fat cheese. Various concentrations of encapsulated catechins in the cheese enhanced its antioxidant capacities and the phenolic contents (determined by TPC, ORAC, and FRAP methods), without influencing the pH or compositions of the functional cheese. Additionally, the authors also studied the stability of the encapsulated catechins during 90 days of storage and found that the encapsulated catechins were stable during this period, meaning that the liposomal encapsulation technology limited the degradation of catechins in the cheese matrix over time.

2.8.1 Kiwifruit juice as a functional beverage

Kiwifruit and the juice obtained from this fruit are nutrient-rich products that if consumed on a regular basis, can have numerous positive effects on human health, primarily on the metabolic, immunity, and digestive system (Boeing et al., 2012). Various studies investigated the origin, species, and nutrition of kiwifruit (Richardson et al., 2018). Kiwifruit was an indigenous plant in the southwest of China. In 1904, the first kiwi tree was planted in New Zealand and gained acceptance from the native

agriculturists, because it could ripen and substitute other fruits in the winter (Ward & Courtney, 2013). Therefore, the plantation of kiwifruit has continuously grown in New Zealand until now. Currently, New Zealand is a notable exporter of Kiwifruit due to the rapid geographic expansion (Warrington & Weston, 1990).

Kiwifruit was arranged in *Actinidia* genus with many cultivars. Two major cultivars are *A. deliciosa* and *A. chinensis*, which can be easily identified by "green" and "gold" colour of kiwifruit pulp, respectively (Richardson et al., 2018). In terms of the compositions, the fruit contains water (approximately 80 g/100g raw kiwifruits) in combination with carbohydrate, sugar, fibre, protein, fat, and vitamin C (14, 9, 3, 1, 0.5, and 161 mg/100g of raw kiwifruit, respectively) (Drummond, 2013). Interestingly, the vitamin C content in kiwifruit is three times higher than strawberries (58.8 g/100g raw fruit) and oranges (53.8 g/100g raw fruit), but the sugar content of kiwifruit remains almost the same as those fruits (~8 g/100g raw fruit) (Drummond, 2013; Richardson et al., 2018). Nevertheless, the total antioxidant capacity of kiwifruit is less than strawberries and oranges (Beekwilder et al., 2005; Wang et al., 1996).

Some studies have tried to increase the total antioxidant capacity of kiwifruit by focusing on the effect of growing practices and geographical location, but the results of the increase of antioxidant compounds were not consistently significant (Giangrieco et al., 2016; Park et al., 2013). For these reasons, kiwifruit is considered as an eligible delivery vehicle for antioxidants such as green tea catechins, where such an approach can add functional values to the products obtained from this fruit (e.g., kiwifruit juice). In particular, if the antioxidants are extracted by a green technology (i.e., no organic solvents or chemicals involved) and from a waste source (e.g., green tea waste that is

currently used as only a landfill), the manufacture of such a functional food product can be very cost-effective and feasible for the food industry as well.

In regard to the nutritional and biological properties of kiwifruit juice, Ma, Lan, Geng, et al. (2019) determined the total phenolic, total flavonoid, and total flavan-3-ol content of this juice that was obtained from different cultivars (Hayward, Huayou, Hongyang, and Qinmei). The results showed that Huayou contained the highest total phenolic content (109.27 mg GAE/100 g flesh weight (FW)) followed by Hongyang, Qinmei, and Hayward (89.09, 62.09, and 58.91 mg GAE/100 g FW, respectively). On the contrary, Hayward possessed about 112.39 mg catechin equivalent (CTE)/100 g FW that was considered as the highest total flavonoid content among the kiwifruit juices prepared from four kiwifruit species, while Huayou presented the lowest value (62.39 mg CTE/100 g FW). The total flavan-3-ol content of these kiwifruit juices ranged from 3.01 to 7.03 mg CTE/100 g FW, in the order of Hongyang > Huayou > Hayward > Qinmei. Similar to the order of the total flavan-3-ol content, Hongyang contained the highest amount of catechin (~6 mg/100 g FW) and epicatechin (~0.45 mg/100 g FW), followed by Huayou (~3, and ~0.30 mg/100 g FW of catechin, and epicatechin, respectively); whereas, Hayward and Qinmei demonstrated the lowest values, accounting for one-tenth of the content determined in Hongyang.

The biological properties of kiwifruit juice were provided by Ko et al. (2005). The authors suggested that the kiwifruit juice from *A. chinensis* or *A. Deliciosa* could increase the antioxidant capacity in the human bloodstream within 30 min and remain stable up to 90 min. In addition, an antimutagenic property was also attributed to the kiwifruit juice because of the high levels of vitamin C (161 mg/100g of raw kiwifruit). The radical

scavenging activity caused by vitamin C could inhibit nitrosation reactions, leading to the reduction of oxidative stress as well as the risk of cancer (Normington et al., 1986). Nevertheless, the variety of kiwifruit species played a significant role in the total phenolic, total flavonoid, and total flavan-3-ol content related to the biological properties of the kiwifruit juice. The total catechin and epicatechin contents in the kiwifruit juice ranged from 0.6-6 and 0.04-0.45 mg/100 g FW, respectively, because of the difference of kiwifruit cultivars (Normington et al., 1986). Finally, it is worth noting that with all promising aspects of functional food development so far, there appear to be unclear regulations (or in the case of some countries, lack of regulations) around the functional food products containing encapsulated bioactives and the corresponding health claims. Although this cannot be sorted during a scientific experiment as such, it should be taken into attention when the development of functional foods and beverages are considered.

2.9 Chemical interactions between green tea catechins and the components of kiwifruit juice

As explained so far, brewed green tea contains the total catechins (EC, ECG, EGC, and EGCG) at the concentration of 110 mg/100 g (Koch et al., 2018; Mérillon & Ramawat, 2019), while kiwifruit consists of carbohydrate, sugar, fibre, protein, fat, and vitamin C (14, 9, 3, 1, 0.5, and 161 mg/100g of raw kiwifruit, respectively) (Drummond, 2013). This juice also contains a minor amount of catechins such as catechin and epicatechin (6 and 0.45 mg/100 g FW, respectively), which can be varied by differences in kiwifruit cultivars. In fact, the concentration of both green tea catechins and kiwifruit compositions can vary depending on the type of green tea and the cultivar of kiwifruit. According to a systematic search in the Google Scholar and Web of Science (on 10 May

2020), the author was not able to find any relevant publications on the interactions between green tea catechins and kiwifruit components. Thus, due to the limitation of the evidence on the specific interaction between green tea catechins and kiwifruit components, this section illustrates the previous studies about the interactions related to green tea catechins and other food products.

2.9.1 The interactions between green tea catechins and food proteins

Proteins are a vital part of most food systems with several functions such as biocatalysts, metal chelation, transport, protection, and storage. The functional properties of food proteins depend on their structure, which is consisted of primary, secondary, tertiary, and quaternary forms (Damodaran, 2017). Accordingly, the interactions between green tea catechins and food proteins may affect the functionality of green tea catechins; i.e., their bioavailability, bioaccessibility, and antioxidant capacity (Haratifar & Corredig, 2014; Yuksel et al., 2010). In theory, green tea catechins can link with proteins by hydrogen and hydrophobic bonds, resulting in insoluble complexes. The binding affinity of the catechin—protein complexes tend to increase with their particle size (Kanakis et al., 2011); however, several interactions have not been well studied and reported.

There is no evidence supporting the interactions between catechins and actinidin, which is the major type of protein in kiwifruit juice. Nevertheless, the quantity of actinidin in kiwifruit is quite low (1.0 g/100 g FW), so it may not have a significant role in the bioavailability and bioaccessibility of polyphenols when green tea catechins are added to a kiwifruit juice.

Various studies have focused on the interactions between milk proteins and green tea catechins to understand the increase in their bioavailability and bioaccessibility, besides understanding the complex structure of the catechins interacted with proteins (Haratifar & Corredig, 2014; Ye et al., 2013). Influential interactions have been investigated between green tea catechins and milk proteins. Green tea catechins bond tightly with both caseins (e.g., α-, β-, and κ-caseins) and whey proteins (e.g., α-lactoglobulin, β-lactoglobulin, and bovine serum albumin) (Haratifar & Corredig, 2014; Kartsova & Alekseeva, 2008; Stojadinovic et al., 2013). Hofmann et al. (2006) reported that the interactions between β-caseins and green tea catechins resulted in the reduction of astringency. To support this interaction, the relationship between catechins and proteins filled with proline was presented. Proline was rapidly bound with catechins and developed an open structure with high flexibility so that the astringency, provided by catechins, could be reduced because of the interaction (Bandyopadhyay et al., 2012; Charlton et al., 2002; Hofmann et al., 2006). On the contrary, some researchers revealed that the interactions between catechins and proteins might decrease EGCG bioavailability (Jöbstl et al., 2004). Therefore, such interactions are still a controversial issue in the sense of the usefulness of bioaccessibility and bioavailability (Haratifar & Corredig, 2014).

The structures and characteristics of individual green tea catechins interacted with proteins in whole milk were studied by Ye et al. (2013). The interaction between milk proteins (e.g., α- casein, β-casein, and β-lactoglobulin) and isolated catechins (e.g., C, EC, EGC, and EGCG) could develop the complexes of catechin—protein (Kanakis et al., 2011). The results indicated that the strength of the catechin—protein formation relied on the molecular structure of green tea catechins. The *cis*-form and a gallate group in the

catechin structure played an essential role in the strength of the formation, while a pyrogallol group could deliberate the interaction, because EGCG provides higher affinities during the interactions than C, EC, and EGC (Xiao et al., 2011). The irregular structures of caseins (e.g., random coil and large loop) were transformed into the secondary structure (α-helix and β-pleated sheet) by the interactions between catechins and milk proteins (Alaimo et al., 1999). The irregular structure is caused by proline as it interrupts the formation of the secondary structure. Hence, the interactions between catechins and proline contributes to the release segments of proteins leading to the α-helix and β-pleated sheet formation (Ye et al., 2013).

The interactions between EGCG and ovalbumin associated with the reduction of allergy was investigated by Ognjenovic et al. (2014). Ovalbumin (OVA) is one of the major proteins in the egg white, which is the main cause of human allergy when consuming egg and egg-based products, especially in children (Sampson, 2004). The structure of this protein consists of three β-sheets and 8-9 α-helices presented in a three-dimensional form. The addition of EGCG to OVA at the ratio of 1:1 M affected the secondary structure of OVA, which was shown by the reduction of random coils and the increase of β-sheets. This alteration of the OVA secondary structure might relate to the decrease in the egg allergy, since the protein structure plays a substantial role in the sensitising ability of food allergens (Ognjenovic et al., 2014).

2.9.2 The interactions between green tea catechin and sugars

It has been reported that the addition of sugars might have a negative influence on antioxidant activity of some bioactive polyphenols (Samra et al., 2011). Kopjar et al. (2016) investigated the antagonistic effect of polyphenols in the presence of sugars by

comparing catechins with and without the addition of sucrose. DPPH and FRAP assays were used to determine the amount of antioxidant activity. The DPPH results showed that catechins without sugars obtained 191.4 µmol Trolox equivalents (TE)/100 ml of the sample, which was higher than catechins with the addition of sucrose (145.7 µmol TE/100 ml). The same trend of the antioxidant activity results was observed for the FRAP assay. The catechins without sugars gained the antioxidant activity (215.4 µmol TE/100 ml) as twice as the catechins with the addition of sucrose (117.2 µmol TE/100 ml) (Kopjar et al., 2016). It could be summarised that the addition of sugars to catechins can result in the antagonistic effect leading to the reduction of the antioxidant activity. Still, the mechanism of this interaction needs to be investigated further.

The thermal stability of catechin and epicatechin with the addition of disaccharides was studied by Lončarić et al. (2018). 10% of sucrose and trehalose was added to the catechin and epicatechin solutions. After that, the solutions were heated at the temperature of 50, 80, and 100°C for 2, 30, and 60 min. The results (analysed by HPLC) indicated that the addition of both sugars had an influence on the epimerisation of catechin and epicatechin at 80 and 100°C. At 80°C and 30 min, the highest epimerisation of catechin was shown in the catechins with sucrose (20.33%) which was dramatically higher than the catechins with trehalose (4.46%). The utmost epimerisation of epicatechin was discovered at the condition of 100°C and 60 min. The epicatechin with sucrose received 35.78% of epimerisation while the epicatechin with trehalose got only 7.05%. It could be summarised that the addition of trehalose had the potential to protect the epimerisation of both catechin and epicatechin during thermal processing. In contrast, the addition of sucrose diminished the stability of both catechins.

The association between green tea polyphenols and sugars, resulting in sedimentation in tea production, was studied by Xu et al. (2017). They reported that the addition of sugars could decrease the tea sedimentation, due to the effect on the viscosity that stabilised the colloidal system; however, theoretically, such sedimentation stems from the interactions of catechins with caffeine and protein (Chao & Chiang, 1999; Xu et al., 2014). Fructose and glucose have a fructosyl group which plays an important role in the decrease of tea sediment due to the rapid reaction over other kinds of sugars (Yuerong & Bee, 1995). The addition of maltose, glucose, fructose, or sucrose significantly reduced the formation of the tea sedimentation in the range of 31.4-86.4% (compared with the tea without sugar) because, the addition of sugars could increase the viscosity as well as the stability of the colloidal system. Although the addition of sugars affects the reduction of the ratios of catechins and caffeine in the sediment, it still contained some remaining catechins in the residue (Xu et al., 2017).

The total sugar content of kiwifruit juice is about 9 g/100 g FW, including fructose (4.53 g), glucose (4.11 g), maltose (0.19 g), and sucrose (0.15 g/100 g) FW (Drummond, 2013). Thus, as according to the results form Kopjar et al. (2016), Lončarić et al. (2018), and Xu et al. (2017) stating that green tea catechins interact with sugars that leads to the reduction of the total catechins, in the case of the current study, the presence of the sugars in kiwifruit juice may have a negative effect on the antioxidant activity of green tea catechins. However, this problem can be solved by implementing an encapsulation technique that can prevent catechins from interacting with sugars in kiwifruit juice.

2.9.3 The interactions between green tea catechins and dietary fibres

The definition of dietary fibre is the consumable parts of plants or carbohydrates resisting to absorption or digestion in the human digestive system. Dietary fibre comprises of polysaccharides, oligosaccharides, lignin, and associated plant compounds (Phillips & Cui, 2011). The dietary fibre of kiwifruit is originated from the cell walls of the plant, especially polysaccharides. Approximately 2-3 % FW of kiwifruit is non-starch polysaccharides that can be separated into soluble and insoluble fibres, while about 0.5% FW is minor compounds of the dietary fibre, such as lignin and tannin (Ferguson & Ferguson, 2002; Kennedy et al., 1999). The proportion of the soluble and insoluble fibres in kiwifruit is 1:1 to 2:1, which is considered to be the same ratio as apple, tomato, and date (Ellench et al., 2011; Kennedy et al., 1999). Most of the soluble fibre in kiwifruit is comprised of pectin polysaccharides (472-708 mg/100 FW); whereas, cellulose and hemicelluloses dominate the insoluble fibre shown as 339-1029 and 182-574 mg/100 FW, respectively (Sims & Monro, 2013). In this study, to make kiwifruit juice, kiwifruits were peeled and squeezed, meaning that almost all insoluble fibre was separated and the juice contained mainly soluble fibre.

Few studies have been carried out on the interactions between catechins and polysaccharides, especially soluble fibre (Wang et al., 2013). Hayashi et al. (2005) investigated the effect of the interactions between catechins, consisting of EC, ECG, EGC, and EGCG, and pectin on the reduction of green tea astringency (originated from catechins). The taste sensor system was applied to measure the astringency in the mixed solutions between catechins and pectin via the specific probe. The results indicated that the complex of pectin—galloylated catechins (ECG and EGCG) had a higher potential to

reduce the astringency of catechins than the pectin—non-galloylated catechins (EC and EGC), because the galloylated catechins provided stronger affinity towards pectin. Such a stronger affinity resulted from the strength of their hydrophobicity, several hydroxyl groups, and high flexibility of the structure. Thus, referring to these findings, in the case of the current study, the interactions between catechins and soluble fibre may occur. This has an advantage with regards to the decrease in astringency via the formation of the pectin—galloylated catechins complex.

2.10 High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is an analytical technique to identify, quantify, and isolate the constituents in a mixture (Danilo, 2011). HPLC recently has become the primary technique in chemical, pharmaceutical, and food industries because of its spontaneous response, high sensitivity, and high efficiency of substance separation. Moreover, HPLC provides more convenience for users about the ease of mobile phase adjustment, as well as a wide range of stationary phases (Danilo, 2011; Lees, 2003; Leo & Fidel, 2013). In the case of the current experiment, the quantitative analysis of green tea catechins is precisely and rapidly measured by HPLC, because the combination of advanced equipment with suitable sample preparation results in the efficient detection and separation of catechins in the analyte (Valls et al., 2009).

The principle of HPLC is based on the distribution of substances (analyte) between two phases, which are composed of a stationary phase and a mobile phase (Lees, 2003). A column filled with packing material is called the stationary phase, while pure or mixture eluents are represented as the mobile phase. Additionally, HPLC instruments are

generally composed of the pump, the injector, the column, the detector, and data system control, as shown in Figure 2.3 (Lees, 2003; Leo & Fidel, 2013).

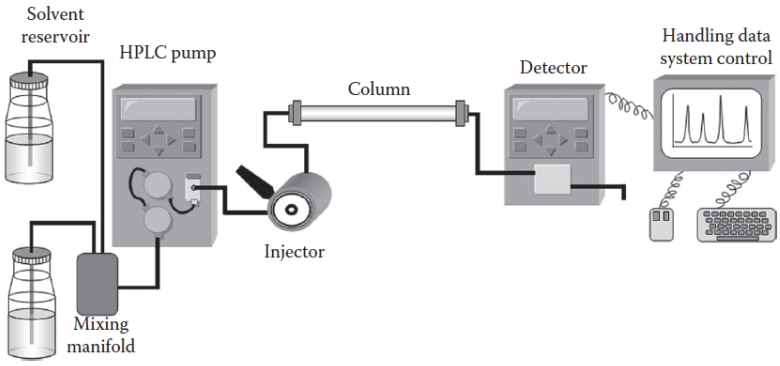


Figure 2.3 Schematic representation of HPLC instruments applied for the separations of green tea catechins (Leo & Fidel, 2013).

The eluent (mobile phase), which is stored in a solvent reservoir, is delivered by the pump. This solvent must be degassed before and during an analysis period, because air bubbles result in the reduction of flow stability and separation ability. A sample is injected by the injector, which is commonly set at a specific injection volume (e.g., 10 µl), in the eluent (Lees, 2003; Leo & Fidel, 2013). The detectors can be divided into optical and luminescent detectors, based on wavelength and sensitivity. The optical detector can separate the compounds present in the mobile phase via the detection of the ultraviolet (UV) intensity determined by a diode array, where the source of UV light is a deuterium lamp providing the range of wavelength from 190 to 400 nm. In terms of the luminescent detector, fluorescence is a light source used to separate compounds in the range of wavelength from 480 to 570 nm and it has an advantage on high sensitivity that is significantly higher than UV and diode array detection. However, it can be applied in a limited number of organic compounds such as monomeric flavanols, dimeric

procyanidins, and trans-resveratrol, although derivatisation with proper reagent could increase the detection rate (Leo & Fidel, 2013).

HPLC techniques could be specified by the type of mobile phase. In the modern chromatographic practice, the normal phase (NP) and reverse phase (RP), which are the major retention mechanisms, are widely used in the food industry (Danilo, 2011; Leo & Fidel, 2013). Theoretically, the retention mechanisms have an important role in the separation of substances in analytes depending on an adsorption mechanism between mobile and stationary phases (Leo & Fidel, 2013). To begin with NP chromatography, the polar of the stationary phase is more than the mobile phase that leads to the positive effects on high stability and homogeneity of the compounds in the mobile phase. However, NP equilibration process is slow, so it is a major hindrance for applying the gradient eluent, which is the next step for the improvement of substance separation. As an advancement, RP is developed from NP to get more efficient compound separation, because the equilibration process of RP is faster than NP. Therefore, RP method can be applied gradient eluent and can be used for both polar and hydrophobic compounds in the stationary phase. In terms of the interaction in the stationary phase, the higher hydrophobic compounds in the mobile phase interact with the stationary phase, the slower those compounds are eluted. In other words, hydrophilic substances would be detected in a shorter period of retention time than hydrophobic substances (Leo & Fidel, 2013).

Regarding HPLC analysis of flavonoids, the chromatographic separation of flavonoids is intricate, because of their six subclasses having a similarity of isomeric oligomers in plant sources (Howard & Beecher, 2000). Several studies have suggested

that RP chromatography with C18 column has been the proper method for the analysis of flavonoids, because this method can separate minuscule oligomers of the same degree of polymerization (DP) (Howard & Beecher, 2000; Valls et al., 2009). Due to the small particle size of catechins from green tea, ultra-performance liquid chromatography with C18 column and UV-vis diode array detector is a suitable method for catechin analysis because of its speed and sensitivity. This method is already applied in the analysis of commercial teas to determine the quantitative of catechins. A regular HPLC chromatogram obtained for catechins present in green tea extract samples is illustrated in Figure 2.4 (Valls et al., 2009).

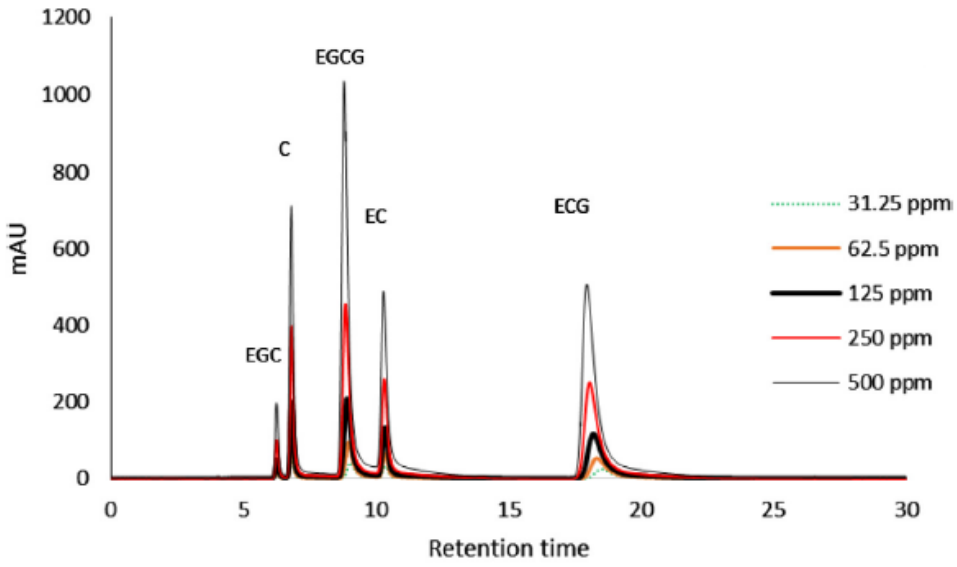


Figure 2.4 Ion chromatogram of green tea analysed via high-performance liquid chromatography (HPLC). The peaks are for epigallocatechin (EGC), catechin (C), epigallocatechin gallate (EGCG), epicatechin (EC), and epicatechin gallate (ECG) (Rashidinejad, Birch, & Everett, 2016).

Chapter 3

Materials and methods

3.1 Materials

Green tea waste was obtained from Guizhou Eight Grams Tea and Agricultural Development Ltd. (Qiannan, China), and kiwifruit was purchased from a local supermarket (PAK'nSAVE, Palmerston North, New Zealand). The high purity (HPLC grade) catechins including catechin (C), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) standards were purchased from Sigma-Aldrich, Inc. (Darmstadt, Germany).

Low viscosity light liquid soy lecithin (Beakin® LV1 Lecithin) was bought from Archer Daniels Midland Co. (Illinois, USA). 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) reagent was procured from Sigma-Aldrich Co., Inc. (Darmstadt, Germany). Folin-Ciocalteu's reagent was purchased from Merck Co., Inc. (New Jersey, USA). All the chemicals and reagents used in this study were of analytical grade.

3.2 Methods

The concentration of the green tea waste that was used for the extraction of catechins as a green tea waste extract (GTWE), as well as the developed experimental parameters, are presented in Table 3.1. Kiwifruit juice was prepared freshly by squeezing the kiwifruit. The overall design of the current study can also be seen in Figure 3.3. This study comprised of four parts: Part I aimed to find out the most suitable extraction method for green tea catechins by applying different solvents including distilled water and ethanol, extraction techniques which were composed of conventional and ultrasound-

assisted, and concentrations consisting of 1, 2, and 5% of the ground green tea waste (GTW, w/v). Part II of the study dealt with the protection and stabilisation of the catechins extracted from green tea waste using liposomal encapsulation technology. In Part III, the encapsulated catechins were incorporated into the kiwifruit juice; whereas, the effect of pasteurisation was also studied. And finally, the purpose of Part IV was to study the behaviour of the encapsulated catechins in the beverage (i.e., kiwifruit juice) and the effect on its properties.

Table 3.1 The experimental parameters for different extraction conditions used for green tea waste.

Parameters	Details
Solvents	Distilled water Ethanol
Extraction techniques	Conventional extraction Ultrasound-assisted extraction Vacuum extraction
Concentration of green tea waste (%, w/v)	1 2 5

3.2.1 Extraction of catechins from GTW

The extraction method of GTW was based on the methods developed by Choung et al. (2014) and Perva-Uzunalić et al. (2006), with modifications. The laboratory-scale of the extraction process is presented in Figure 3.1. The dried leaves of green tea waste were ground using a grinder (Breville Group Ltd., BCG200, Australia), sieved with a 0.425 mm screen, and stored in the sealed plastic bags at 4°C before used. Distilled water was used in both the conventional and ultrasound-assisted extraction methods while ethanol was applied in the vacuum evaporation process. The concentration of the ground green tea waste varied for all extraction methods and it was at 1, 2, and 5% (w/v) with an

extraction temperature of 80°C for 20 min. The extracted green tea waste samples were stored at -20°C overnight, before being freeze-dried in a Cuddon FD18 Freeze Drier (Cuddon Freeze Dry (Manufacturer), Blenheim, New Zealand) set at the following conditions; pressure, temperature, and time of 1 mbar, 20°C, and 72 h, respectively.

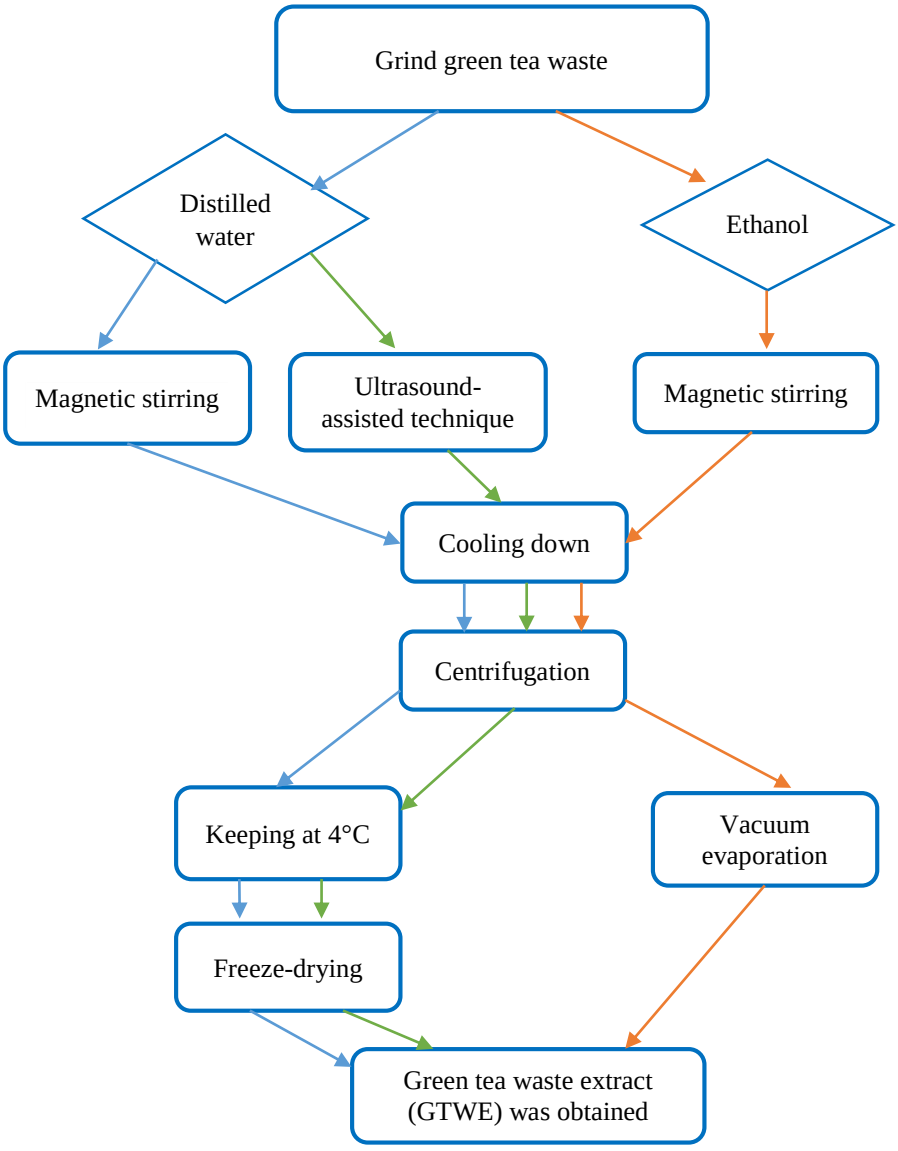


Figure 3.1 The extraction processes applied in the current study: (—) Conventional extraction, (—) Ultrasound-assisted extraction, and (—) Vacuum extraction.

3.2.2 Encapsulation

The preparation of Single-layer liposomes was adapted from the method developed by Rashidinejad et al. (2014) with slight modifications. Soy lecithin was dispersed in the solution of GTWE and/or the acetate buffer at 1% concentration (w/v) and was stirred with a magnetic stirrer for 30 min, followed by high-shear mixing using a high speed mixer (D500 series, Labserv, Germany) at 24,000 rpm (1 min bursts for 5 rounds). The Double-layer liposomes were prepared by following the method of Laye et al. (2008) with some modifications. The preparation of chitosan solution started by dissolving 0.1% (w/v) chitosan in the acetate buffer (pH 3.8), followed by overnight stirring. To create chitosan-coated liposomes, the Single-layer liposomes, prepared with the method described by Rashidinejad et al. (2014), at 0.5% concentration (w/v), were added to the chitosan solution (temperature, time, and speed of stirrer were 20°C, 2 min, and 700 rpm, respectively). The prepared liposomes were stored at 4°C for 24 h before the further investigation and use.

Two controls and one blank were prepared for comparison. The first control was the GTWE solution without lecithin and the second control contained 1% w/v of soy lecithin dispersed in the same acetate buffer without the addition of GTWE. The blank consisted of only acetate buffer (no GTWE or soy lecithin). Controls and blank were processed the same way as the treated samples (i.e., same magnetic stirring and homogenisation processes).

3.2.2.1 Encapsulation efficiency

Liposomes containing GTWE were assessed for encapsulation efficiency, based on the method adapted from that of Rashidinejad et al. (2014). The prepared liposomes

were filtered by Sephadex G100 gel filtration. When the prepared liposomes passed through the gel filtration, non-encapsulated catechins were entrapped in the gel, while the encapsulated catechins passed through the column. The encapsulation efficiency was calculated using the equation below:

$$F_i\ (\%) = \frac{C_{total} - C_{out}}{C_{total}} \times 100 \quad (1)$$

where, F_i = the encapsulation efficiency depended on the concentration of catechins in the liposome structure, C_{total} = the total concentration of both encapsulated and non-encapsulated catechins, and C_{out} = the concentration of non-encapsulated catechins.

3.2.2.2 Encapsulation yield

The encapsulation yield was the ratio of the dry weight of nanoliposomes after the encapsulation process to the dry weight of both GTWE and soy lecithin before the encapsulation process (Rashidinejad et al., 2014). The encapsulation yield was calculated using the equation below:

$$EY\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

where, EY = the encapsulation yield, W_1 = the combination of antioxidant and soy lecithin weight, and W_2 = the nanocapsules weight.

3.2.2.3 Loading capacity

The loading capacity, which represents the maximum catechins that can be encapsulated in liposomes (Rashidinejad et al., 2019), was calculated using the equation below:

$$Loading\ capacity\ (\%) = \frac{C_{total} - C_{out}}{weight\ of\ capsules} \quad (3)$$

where, C_{total} = the total concentration of both encapsulated and non-encapsulated catechins, and C_{out} = the concentration of non-encapsulated catechins.

3.2.3 Kiwifruit juice preparation and the incorporation of the encapsulated catechins

The preparation of kiwifruit juice was adapted from the method reported by Guo et al. (2017) with slight modifications. The flow chart for the preparation of kiwifruit juice in this study is presented in Figure 3.2.

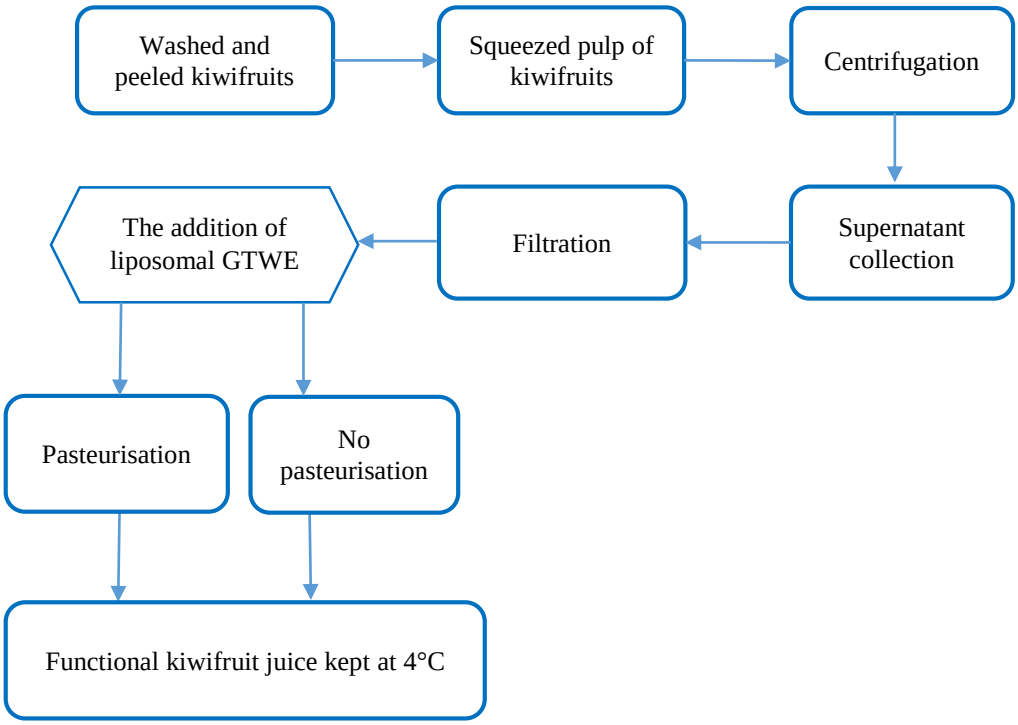


Figure 3.2 A flow chart showing the preparation of kiwifruit juice, incorporation of liposomes, and pasteurisation.

Kiwifruits were peeled and their pulp was squeezed by a juicer (Breville Group Ltd., Juice Fountain JE95, Australia). The juice was centrifuged at 4,000 rpm for 10 min at 20°C to create the phase separation. The supernatant was collected, then passed through a 100 μm filter, to get rid of the remaining pulp. After that, the encapsulated GTWE, including the control and blank samples, were added to the screened kiwifruit juice. The enriched kiwifruit juice was separated into two portions. The first portion went under the pasteurisation process (80°C for 30 s), while the second portion was not pasteurised. Pasteurised and unpasteurised samples were divided into four parts to determine the characteristics of the encapsulated catechins during a certain storage period of 0, 7, 14, and 28 days at 4°C.

3.2.4 The behaviour of the encapsulated GTWE in kiwifruit juice and the effect on its properties

3.2.4.1 Particle size and zeta potential

The particle size of the liposomes was determined using a Zetasizer (Malvern Instruments Ltd., nano ZS, UK) while in the case of the kiwifruit juice fortified with the liposomes the particle size was measured by a Mastersizer (Malvern Instruments Ltd., Hydro 2000MU, UK). The zeta potential of both liposomes and the kiwifruit juice containing liposomes were investigated using a Zetasizer (Malvern Instruments Ltd., nano ZS, UK). The samples, which were required to be analysed by a Zetasizer (Malvern Instruments Ltd., nano ZS, UK), were diluted (1:16) in 0.25 M acetate buffer (pH 3.8), while in the case of Mastersizer, no dilution was required (Rashidinejad et al., 2014).

3.2.4.2 Morphology

Transmission electron microscopy (TEM) was used to examine the morphology of the manufactured liposomes. The sample preparation under the negative staining technique was slightly modified from the method developed by Ruozi et al. (2011). Liposome samples were diluted 20 times before placing a small droplet (~80 µl) on the parafilm. A 200-mesh formvar copper grid (Agar Scientific Ltd., Essex, UK) was placed into the liposome droplet for 4 min. To stain the grid, a drop of 2% uranyl acetate was prepared on the parafilm and the grid (coated with liposomes) was required to incubate in the solution for 4 min. The stained copper grid needed approximately 2 min for drying at room temperature. Finally, the microstructure of the liposomes was seen under TEM (FEI Tecnai G2 Spirit BioTWIN, Czech Republic) at 100 kV. A Veleta CCD Camera (Olympus Soft Imaging Solutions, Germany) was used to capture images.

3.2.4.3 HPLC analysis

Catechins were analysed based on the method reported by Wang et al. (2003) with slight modifications. An Agilent 1200 series high-performance liquid chromatography (HPLC) machine equipped with a C18 reversed phase Synergi 4 µm (150 × 4.6 mm) was used. The mobile phases were composed of eluent A (containing 0.1% orthophosphoric acid in water, v/v) and eluent B (containing 0.1% orthophosphoric acid in methanol, v/v). The gradient was set as 0–5 min, 20% B; 5–7 min, linear gradient from 20 to 24% B; 7–10 min, 24% B; 10–20 min, linear gradient from 24 to 40% B; 20–25 min, linear gradient from 40 to 50% B. Post-run time was 1 min, the flow rate of the eluents was controlled at 0.8 ml/min, and the injection volume was 5 µl. The column temperature was kept at 30°C. In terms of detection, a diode array detector was recorded at 210 and 280 nm. The

identification of catechins was based on the retention times of their peaks and UV-vis spectra compared with the calibration curve made from standards (2-50 ppm).

3.2.4.4 Total phenolic content

Total phenolic content (TPC) was investigated by applying the method developed by Du et al. (2009) with some modifications. The Folin-Ciocâlteu stock solution was prepared by mixing the Folin-Ciocâlteu reagent with distilled water in a ratio of 1:1. Liposome samples were diluted at the ratio of 1:10 (v/v) with methanol, while kiwifruit juice samples containing the encapsulated GTWE were ready for the analysis without any dilution. 0.5 ml of the Folin-Ciocâlteu reagent was mixed with 7.9 ml of distilled water, and 0.1 ml of prepared liposomes or kiwifruit juice samples. After 1 min, 1.5 ml of sodium carbonate (1:5, w/v) was added, vortexed, and kept in dark at room temperature for two hours. The absorbance was measured by spectrophotometer (GENESYS 10 Series, UV-vis, USA) at 765 nm and TPC was compared with the calibration curve made using (+)-catechin as a the standard (0.20-25 ppm).

3.2.4.5 Total antioxidant activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical method was applied due to the method of Kara and Erçelebi (2013) with some modifications. The DPPH stock solution was prepared by dissolving DPPH 1.2 mg with methanol 50 ml. Liposome samples were diluted at the ratio of 1:10 (v/v) with methanol, while kiwifruit juice samples containing the encapsulated GTWE were ready for the analysis without any dilution. 0.1 ml of the prepared samples were mixed with 3.9 ml of DPPH solution. The mixtures were kept in dark at room temperature for 30 min. After that, the absorbance

was determined by spectrophotometer (GENESYS 10 Series, UV-vis, USA) at 515 nm. The %DPPH reduction was calculated using the formula below:

$$\%DPPH\ reduction = \left(\frac{A_C - A_S}{A_C}\right) \times 100 \quad (4)$$

where, A_C = absorbance of a control (t = 0 min), A_S = the absorbance of a tested sample at the end of the reaction (t = 30 min).

All measurements were carried out at room temperature (25°C). The %DPPH of the samples was compared with the calibration curve made using (+)-catechin as the standard (0.20-25 ppm) (Chen et al., 2013).

3.2.4.6 The pH measurements

The pH of kiwifruit juice was measured by the direct insertion of the pH probe into the juice samples (Rashidinejad, Birch, & Everett, 2016). All evaluation factors were applied to determine the characteristics of the liposomes and the kiwifruit juice containing liposomes, which were stored at 4°C for 0, 7, 14, and 28 days.

3.2.4.7 Sedimentation analysis

The precipitation of kiwifruit juice was determined by applying the method developed by Oke et al. (2010) with a slight modification. 3 g of the kiwifruit juice was precisely weighted into a 5 ml Eppendorf tube. The samples were centrifuged under the condition of 12,800 g, for 30 min at 4°C. The supernatant was discarded after the centrifugation and the precipitate with the tube was reweighted. The precipitate weight ratio (PWR) was calculated using the formula below:

$$PWR = \frac{(Precipitate + tube\ weight) - tube\ weight}{(Initial\ sample + tube\ weight) - tube\ weight} \times 100 \quad (5)$$

3.2.3 Statistic analysis

The reported data are means of at least three measurements. All measurements were performed in triplicates. Minitab (version 17.3.1) Statistical Software (Minitab Inc., State College, PA) was used for carrying out the corresponding statistical analysis. The data were subjected to the analysis of variance (ANOVA) for the mean comparison for any significant differences ($p<0.05$). All graphical presentations were generated by Microsoft Excel 2016.

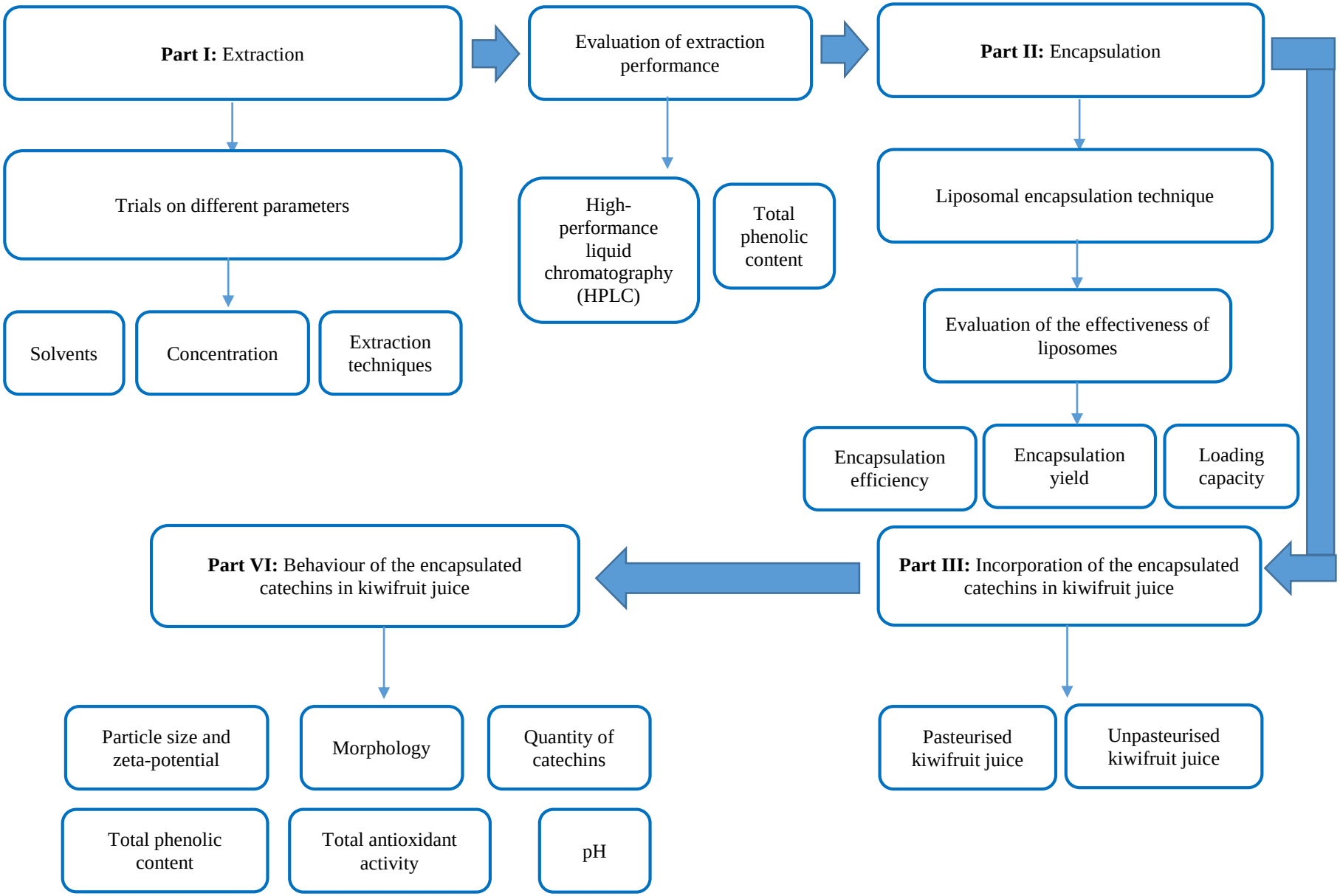


Figure 3.3 An overall overview of the experimental design.

Chapter 4

Results and discussion

4.1 Extraction of catechins from GTW

In this study, the first aim was to optimise and assure the extraction conditions that were reported for green tea catechins so far, by varying solvents (including distilled water and 80% ethanol), the ratio of the green tea waste (GTW) to solvents (1:100, 1:50, and 1:20), and extraction methods (high temperature, ultrasound-assisted, and vacuum extraction). The extraction temperature was fixed at 80°C for hot water extraction (HWE) and the boiling temperature for ethanol extraction (EthE). The extraction time was set at 20 min for all extraction methods. Generally speaking, the catechin extraction efficiency (CEE) depends on several various factors such as the type of the solvent, concentration of each solvent in the solvent mixtures, concentration of the GTW in the solvent, extraction time, and extraction temperature. During the following sections, the effects of various extraction techniques on the extraction efficiency of catechins in GTW and encapsulation yield (EY) are presented and discussed.

4.1.1 The effect of extraction techniques on catechin extraction efficiency (CEE)

The concentration of the extracted green tea catechins relates to the extraction methods, as the first step for the separation of these bioactive compounds from GTW (Choung et al., 2014; Perva-Uzunalić et al., 2006; Tiwari, 2015). In this study, five types of catechins, consisting of C, EGC, EGCG, EC, and ECG, were analysed via HPLC and

DPPH methods, to find the most efficient extraction technique and extraction condition for the separation of catechins from GTW.

The HPLC chromatograms of the standard compounds and the green tea waste extract samples are provided in Figures 4.1 and 4.2, respectively. Peak separation and integrations were implemented based on the guidelines provided by Lee and Ong (2000). The concentration results of five catechins are also presented in Table 4.1. Based on these findings, the ratio of 1:100 demonstrated the highest total catechins at the range of 66.37-70.36 ppm, followed by 1:50 and 1:20 at 62.57-67.83 ppm and 56.57-61.41 ppm, respectively. Total catechins from HWE possessed the highest catechin concentration with statistical significance ($p<0.05$) in the case of every ratio (i.e., 1:100, 1:50, and 1:20), followed by UE and EthE. In terms of the values for individual catechins, EC was found to be the highest concentration detected, ranging from 30.58 to 37.95 ppm in all extraction techniques and ratios of solvents, followed by EGCG (9.71-20.99 ppm), EGC+C (7.95-12.58 ppm), and ECG (1.85-6.05 ppm). In summary, the arranged order of the individual catechins in GTW was EC > EGCG > EGC+C > ECG. It is notable that the reason for reporting EGC together with C (i.e., EGC+C) in this study is that the concentration of C was considered as low and the peak of C was not completely separated from EGC (Figure 4.2); nevertheless, such a combination of peaks/data did not affect the data evaluation.

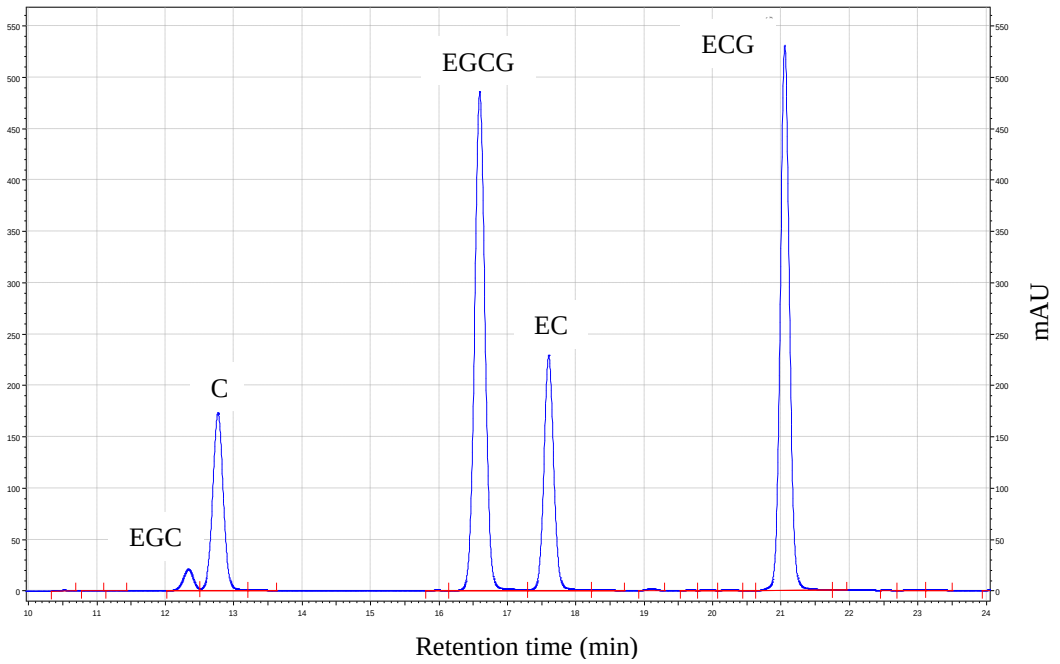


Figure 4.1 The chromatogram of the standard catechin compounds at the concentration of 500 ppm, analysed by high-performance liquid chromatography (HPLC). C: (+)-catechin; EGC: (–)-epigallocatechin; ECG: (–)-epicatechin gallate; EC: (–)-epicatechin; EGCG: (–)-epigallocatechin gallate.

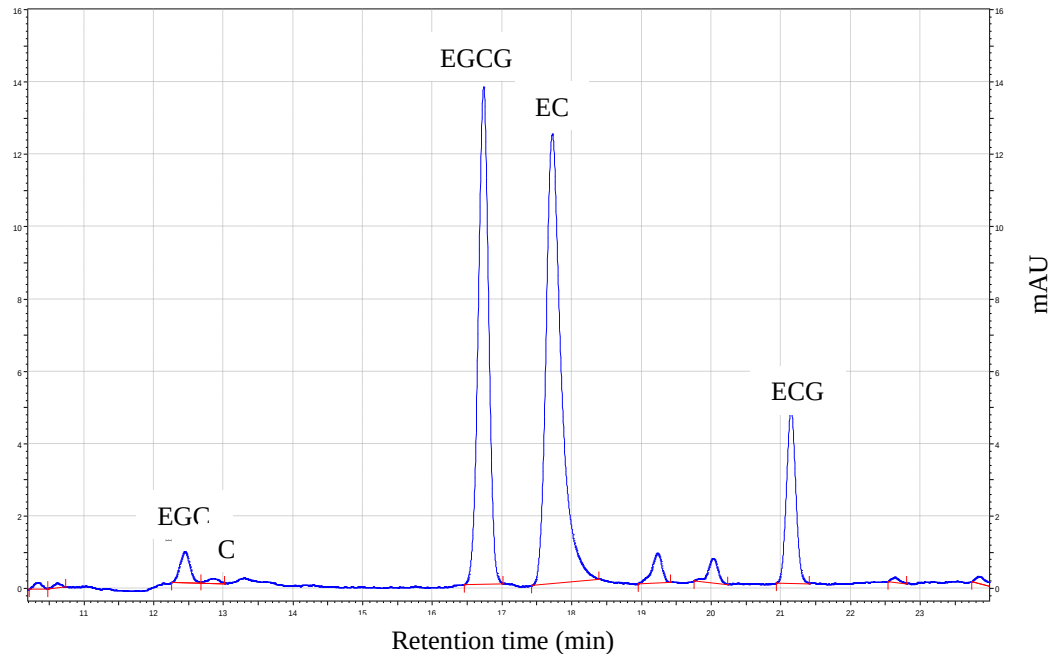


Figure 4.2 The chromatogram of the green tea waste extract (GTWE) analysed by high-performance liquid chromatography (HPLC). C: (+)-catechin; EGC: (–)-epigallocatechin; ECG: (–)-epicatechin gallate; EC: (–)-epicatechin; EGCG: (–)-epigallocatechin gallate. Note: two of the peaks (unmarked) are not known.

Table 4.1 The effect of various extraction techniques and the ratio of the green tea waste to solvents (w/v) on the concentration (ppm) of catechins in green tea waste, analysed by high-performance liquid chromatography (HPLC).

Extraction techniques	Concentration (ppm)*				
	EGC●+C●	EGCG●	EC●	ECG●	Total catechins
1:100▲					
Hot water (HWE)■	*12.58±0.56^{a}*	20.99±1.11^{a}	30.74±0.60^{b}	6.05±0.06^{a}	70.36±1.47^{a}
Ultrasound-assisted (UE)■	*10.93±1.19^{ab}*	16.75±1.41^{b}	35.38±2.39^{a}	5.36±0.42^{ab}	68.42±0.55^{ab}
Ethanol (EthE)Δ	*10.00±0.38^{b}*	13.19±0.48^{c}	37.95±0.84^{a}	5.23±0.29^{b}	66.37±1.27^{b}
1:50▲					
Hot water (HWE)■	12.01±0.93^{a}	19.73±0.50^{a}	31.49±1.09^{b}	4.62±0.49^{ab}	67.83±0.83^{a}
Ultrasound-assisted (UE)■	11.73±1.23^{a}	17.55±0.90^{b}	30.58±1.17^{b}	3.70±0.43^{b}	63.56±1.08^{b}
Ethanol (EthE)Δ	8.65±0.47^{b}	13.11±0.55^{c}	35.81±0.44^{a}	5.00±0.18^{a}	62.57±0.65^{b}
1:20▲					
Hot water (HWE)■	*10.34±0.51^{a}*	14.60±0.88^{a}	32.65±0.76^{b}	3.83±0.54^{a}	61.41±1.00^{a}
Ultrasound-assisted (UE)■	*9.31±0.69^{ab}*	9.71±1.40^{b}	35.71±0.37^{a}	1.85±0.71^{b}	56.57±1.90^{b}
Ethanol (EthE)Δ	*7.95±0.66^{b}*	10.78±0.66^{b}	34.14±1.07^{ab}	4.11±0.55^{a}	56.97±0.49^{b}

a-c: values with different superscripted letters within the same column are significantly different ($p<0.05$).
*Values are mean of three replications (n=3 ± SD)
▲ The ground green tea waste to solvents ratio (w/v)
■ The extraction condition of HWE and UE was 80°C for 20 min, and for EthE boiling temperature for 20 min was applied.
Δ The extraction condition of this extraction method was boiling temperature for 20 min.
● C: (+)-catechin; EGC: (–)-epigallocatechin; ECG: (–)-epicatechin gallate; EC: (–)-epicatechin; EGCG: (–)-epigallocatechin gallate.

Koch et al. (2018) studied the effect of green tea origin on the quantity of individual catechins and suggested that the catechin concentrations of green tea originating from Sri Lanka, Japan, and South Korea could be placed in the following order: EGC > EGCG > ECG > EC > C, while green tea coming from China, India, and Nepal presented a different order; i.e., EGCG > EGC > ECG > EC > C. Moreover, EGC and EGCG were the most dominant catechins in all sources of green tea, accounting for 65-80%. In another study, Henning et al. (2003) reported the content of catechins in 18 commercial tea products. Five catechins from these commercial products were presented from the highest to the lowest quantity. For instance, 'Lipton' green tea contained EGCG

> EGC > ECG > EC > C at the concentrations of 83.9, 76.4, 13.7, 11.9, and 5.8 mg/100 ml, respectively. 'Bigelow' green tea consisted of EGCG > EGC > EC > ECG > C at the concentrations of 42.5, 30.9, 6.5, 3.6, and 0 mg/100 ml, respectively. For these reasons, it could be concluded that the different sources and brands of green tea played a crucial role in the quantity and the proportion of catechins. In the case of the current experiment, the results of HPLC analysis in this study corresponded well with the finding of Koch et al. (2018) and Henning et al. (2003), where HWE was considered as the most efficient extraction technique compared with UE and EthE, because HWE samples contained the highest amount of catechins in every ratio of the solvents.

DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging method was applied to determine the antioxidant capacity of GTW samples and to confirm the efficacy of various extraction methods. Table 4.2 shows the antioxidant capacities of GTW treated with different extraction techniques and the various ratios of solvents. The highest total catechins and %DPPH reduction were found in the case of HWE, while the antioxidant performance of UE was not significantly different than that of EthE. However, at the ratio of 1:100, there was no significant difference (p>0.05) among all methods in terms of total catechins and %DPPH reduction. This might be attributed to the chemical stability of catechins in green tea waste extract (GTWE) under different conditions. Recently, Koch et al. (2020) studied the effect of extraction solvents (ethanol and water) and extraction techniques (HWE, UE, and EthE) on the antioxidant capacity of the green tea samples (from Sri Lanka) using the DPPH method. All extraction methods were kept constant in terms of temperature and time (80°C and 10 min) and the frequency of UE was set at 45 kHz. The results demonstrated that UE gained the lowest

antioxidant activity (59.89%) compared with both HWE and EthE (66.64% and 75.08%, respectively). The reason for the lowest antioxidant activity of UE was speculated to be associated with the applied ultrasonic wave that could have negatively affected the chemical stability of catechins, leading to lower values for DPPH antioxidant activity.

Table 4.2 The effect of various extraction techniques and the ratio of the ground green tea waste to solvents (w/v) on catechin equivalent (ppm) and %DPPH reduction of green tea waste catechins analysed by DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging method.

Extraction techniques	**Catechin equivalent (ppm)***			**%DPPH reduction***		
	1:100▲	1:50▲	1:20▲	1:100▲	1:50▲	1:20▲
Hot water (HWE)■	*215.14±7.59 a*	209.20±16.48[a]	185.11±15.76[a]	35.88±0.66[a]	35.36±1.44[a]	33.26±1.38[a]
Ultrasound-assisted (UE)■	*193.74±18.6 a*	170.04±16.40[b]	149.34±5.95[b]	34.01±1.61[a]	31.94±1.44[b]	30.12±0.52[b]
Ethanol (EthE)Δ	*186.37±17.1 2[a]*	172.83±7.96[b]	149.21±8.23[b]	33.37±1.50[a]	32.18±0.70[b]	30.11±0.72[b]

a-b, values with different superscripted letters within the same column are significantly different ($p<0.05$).
*Values are mean of three replications (n=3 ± SD)
▲ The ground green tea waste to solvents ratio (w/v)
■ The extraction condition of HWE and UE was 80°C for 20 min, and for EthE boiling temperature for 20 min was applied.
Δ The extraction condition of EthE was boiling temperature for 20 min.

Using another antioxidant activity assay (oxygen radical absorption capacity assay (ORAC)), Sun et al. (2015) reported that the sonication could enhance the degradation of polyphenols in fresh apple juice. In contrast, several other studies presented that the ultrasonic wave would be beneficial for the extraction of polyphenols because of the endorsement of the heat-sensitive compound extraction and other advantages such as clean energy, green energy, organic solvent rejection, enhancement of EY, and the development of extraction performance (Choung et al., 2014; Tiwari, 2015). For examples, according to Muzaffar et al. (2016), when an ultrasonic wave of 33 kHz with different treatment time (0, 10, 20, 30, 40, and 60 min) was applied to extract the

polyphenols from cherries, %DPPH reduction analysis showed that the longer extraction time resulted in higher antioxidant activity. However, several various factors could negatively affect the extraction efficiency of polyphenols; pH, temperature, time, and characteristics of the food (Koch et al., 2020).

Regarding the implementation of ultrasound-assisted extraction in the pilot plant scale, Saklar et al. (2018) reported that UE had a lower potential to extract all kinds of catechins from green tea leaves than HWE. The extraction condition of HWE was fixed at 85°C for 30 min while the extraction time and temperature of UE were performed at 70 and 80°C for 30 and 60 min. HPLC results of all catechins indicated that HWE was better than UE. For example, the concentration of EGCG extracted from HWE was 3.81 g/100 g dry weight, which was significantly higher than every extraction condition of UE (1.74-2.46 g/100 g dry weight). Therefore, HWE gains a higher possibility to scale up at the pilot plant scale, but more investigation is to be carried out in the case of UE, in terms of ultrasonic probes, extraction tank, and agitation system.

4.1.2 The effect of extraction techniques on the extraction yield (EY)

The EY reflects the CEE, because a higher EY leads to a lower production cost (Pasrija & Anandharamakrishnan, 2015). The application of extraction methods has a direct consequence on EY; thus, as mentioned before, three different techniques, consisting of HWE, UE, and EthE, were implemented in the case of this study in order to demonstrate the most efficient extraction technique for the extraction of green tea catechins from GTW.

As seen in Table 4.3, the extraction techniques and GTW:solvent ratio (w/v) affected the EY of the individual samples differently. Among the three extraction techniques, UE showed the highest yield at the ratio of 1:100 (about 29.93%) and 1:20 (about 20.64%), while HWE provided the highest results at the ratio of 1:50 (about 25.98%). The lowest EY belonged to EthE in any applied ratio. The differences among these obtained values can be attributed to the difference in the extraction techniques and the frequency of an ultrasonic wave. Perva-Uzunalić et al. (2006), who studied the influence of different extraction techniques (HWE and EthE) on the extraction efficiency, also reported that HWE (80°C, 20 min) gained slightly higher EY than EthE (boiling temperature, 20 min) at about 36.5% and 34.5%, respectively.

Table 4.3 The effect of various extraction techniques and the ratio of the green tea waste to solvents (w/v) on the extraction yield (%) of catechins in green tea waste.

Extraction techniques	**Extraction yield (%)***		
	1:100▲	1:50▲	1:20▲
Hot water (HWE)■	*29.30±1.21^{ab}*	25.98±0.75^{a}	18.92±1.45^{a}
Ultrasound-assisted (UE)■	*29.93±0.59^{a}*	24.16±0.95^{b}	20.64±0.51^{a}
Ethanol (EthE)Δ	*27.32±0.34^{b}*	22.59±0.26^{b}	18.46±0.27^{a}

a-b: values with different superscripted letters within the same column are significantly different (p<0.05).
*Values are mean of three replications (n=3 ± SD)
▲ The ground green tea waste to solvents ratio (w/v)
■ The extraction condition of HWE and UE was 80°C for 20 min, and in the case of EthE, boiling temperature for 20 min was applied.
Δ The extraction condition of this extraction technique was boiling temperature for 20 min.

Regarding the effect of ultrasound frequency, Afroz Bakht et al. (2019) reported similar results for the difference in extraction techniques, consisting of EthE and UE, and ultrasonic frequency of branded tea samples (e.g., Lipton, Rabea, Alkbous, Green Gold, and Haritham) that affected the EY. In the current experiment, the EY of EthE was about 12.35%, which was significantly lower than UE applied a frequency of 26 kHz (about 18.61%). The variation in the ultrasonic frequency also played an essential role in the EY

of the samples in this study. The highest EY belonged to 40 kHz (18.61%) followed by 50 kHz (14.89%) and 30 kHz (10.78%). Due to the frequency limitation of the sonicator probe, the highest frequency of UE that was implemented in this experiment was 20 kHz, which is lower than the minimum frequency (30 kHz) applied in the study of Afroz Bakht et al. (2019). This could have negatively affected the EY of UE samples, because the yield of UE was not significantly different ($p<0.05$) from HWE at the ratio of either 1:100 or 1:20, while HWE gained significantly higher EY (25.98%) at the ratio of 1:50. In summary, HWE provided the highest EY over both UE and EthE at the ratio of 1:50, but there was no significant difference in the case of 1:100 and 1:20 ratios. Therefore, UE with low frequency (20 kHz) could not provide a higher EY than other extraction techniques. Further, it can be concluded that the increase in the ratio of GTW to solvents had a positive effect on the EY.

4.2 Liposomal encapsulation of GTWE

Liposomes were the ideal candidate for encapsulation of GTWE, owing to their several advantages including high encapsulation efficiency, high physical stability, small particle size, non-toxicity, biodegradability, and biocompatibility (Rashidinejad, Birch, & Everett, 2016; Rashidinejad et al., 2014). The liposomal encapsulation method used in this study is a fast and simple method where liposomes can be manufactured using a single and simple process (i.e., high-sheer mixing) and using one single coating material (i.e., soy lecithin) and in a short period of time (i.e., 5 min). The characteristics of the liposomes containing GTWE before their incorporation into the fruit juice are presented in the following sections.

4.2.1 Encapsulation efficiency and encapsulation yield

CEE and EY were about 67.85% and 74.59%, respectively. These values correspond with the results of Rashidinejad et al. (2014), who reported the liposomes of green tea catechins incorporated into low-fat hard cheese. However, these results were lower than the corresponding values reported by Zou et al. (2014) and higher than those reported by Ersus and Yurdagel (2007). A possible explanation for the discrepancy between these values may relate to the differences in the encapsulation technique and the type of encapsulant.

With regards to the enhancement of CEE and EY, there are two factors to be considered. Firstly, the encapsulation techniques directly affect the performance of encapsulation. Because, in theory, the encapsulation systems are based on the interference of a disperse phase, composed of bioactive compound(s) and coating material(s), into small particles or nanoparticles using processes such as drying, rearrangement of surface layers, or phase transition (Dons et al., 2019). For example, spray drying is one of the simplest methods of encapsulation with high effectiveness, since the mixture of bioactive compound(s) and coating material(s) is rapidly evaporated after exposure to hot air, where they are converted to small micrometric particles (Fuchs et al., 2006; Gharsallaoui et al., 2007). Zhang et al. (2007) succeeded in producing the microencapsulation of procyanidins using the spray drying method and gained about 88% encapsulation efficiency. Microfluidisation is another example of producing systems such as liposomes with considerably high encapsulation efficiency (Ré et al., 2019). The principle of microfluidisation is applying high pressure to create a direct flow passing through microchannels and the interaction chamber. Due to the implementation of high

pressure, shear forces are produced in both microchannels and the interaction chamber, which leads to the decrease of liposomal droplet size (Jafari et al., 2006). Zou et al. (2014) reported a high encapsulation efficiency (94%) and small particle size (about 70 nm) of EGCG liposomes using high-pressure microfluidisation.

Secondly, coating materials are responsible for protecting bioactive compounds from environmental stimuli such as light and oxygen so that the proper selection of coating material(s) for the entrapment of bioactive compound(s) is also important for the development of encapsulation efficiency (Gadkari & Balaraman, 2015). Zou et al. (2014) strengthened the lipid bilayer of EGCG liposomes by modifying the types and ratios of coating materials. The selected coating materials consisted of phospholipids, cholesterol, and TWEEN 80 and were mixed in the ratio of 16:2.4:4, respectively, to gain the potent EGCG liposomes with 92% of encapsulation efficiency. The improvement of EGCG liposomes using the combination of encapsulants were also studied by Rocha et al. (2011). The researchers chose the mixture of coating materials, specifically in carbohydrate sources to entrap EGCG, which resulted in the achievement of high encapsulation efficiency (85%).

4.2.2 Loading capacity (LC)

The LC represents the maximum catechins capsulated in the liposomes. In this study, the LC of the encapsulated catechins was about 37.12%. This value is similar to the results reported by Ahmad et al. (2016) and lower than the findings of the study carried out by Rashidinejad et al. (2019). The difference in the LC might be linked to the differences in the encapsulation method and the coating material. Moreover, the LC value is related to the CEE, which can be assumed that the greater the CEE, the higher the LC.

4.2.3 Particle size of liposomes

The average particle size is one of the most necessary features for the investigation of colloidal dispersions such as liposomes. This parameter can help with understanding the characteristics of liposomes including solubility, release rate, and stability (i.e., physical, chemical, and biological) (Tamjidi et al., 2013). The particle size results of both empty liposomes and GTWE-loaded liposomes are presented in Figure 4.3. On Day 0 and just after manufacture, the particle size of empty and GTWE-loaded liposomes was about 156.54 and 186.09 nm, respectively, because GTW catechins could possibly adsorb around the surface of lipid bilayers due to the affinity of the catechins, increasing the size of loaded liposomes (Rashidinejad, Birch, & Everett, 2016). It is notable that while the particle size of the empty liposomes slightly increased over time, the loaded liposomes remained at the same size. This can also be confirmed from the visual data (the physical appearance) presented in Table 4.4, where no sedimentation or phase separation can be seen throughout the storage of the loaded liposomes.

The increase in the particle size of the empty liposomes during storage might be attributed to the degradation of liposomes by the oxidative degradation of unsaturated fatty acids (Gibis et al., 2013), which could have been prohibited in the case of the catechin-loaded liposomes due to the antioxidant activity of catechins (Ozkan et al., 2019).

The particle size of the loaded liposomes obtained in the current study is in agreement with the data reported by Guldiken et al. (2018), who manufactured soy lecithin liposomes filled with anthocyanin from black carrot and investigated the stability of liposomes during 21 days of storage. These researchers found that the particle diameter

of the liposomes on Day 0 was almost similar to that of Day 21 (43.9 nm vs 44.5 nm, respectively). Therefore, it can be summarised that the soy lecithin liposomes manufactured in the current study could maintain their original particle size during the 28 days of storage.

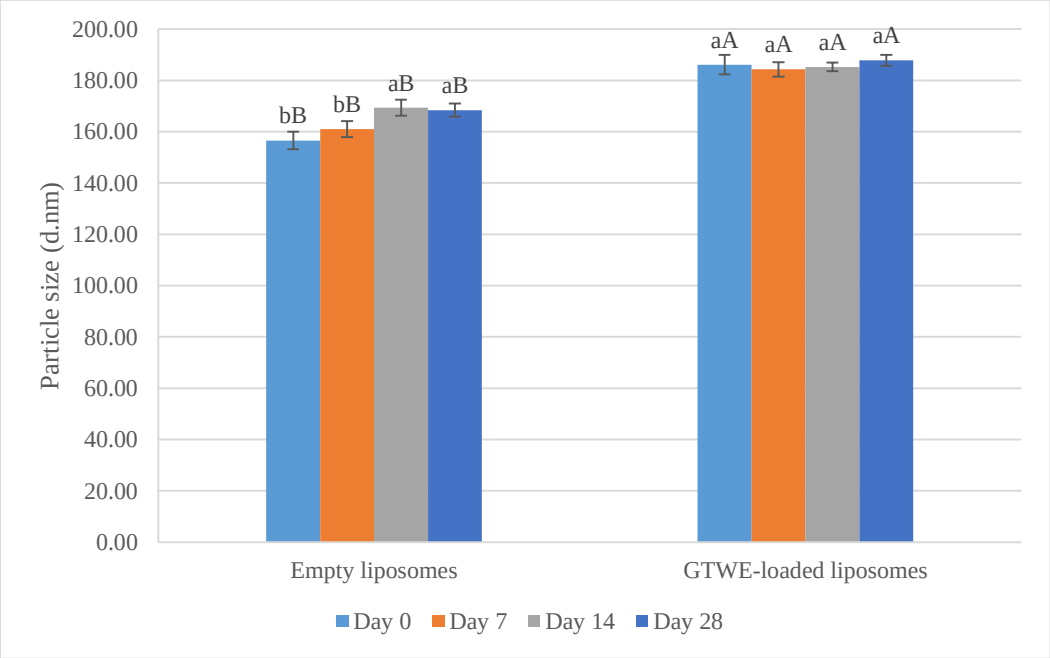


Figure 4.3 The particle size of empty and green tea waste extract (GTWE)-loaded liposomes during 28 days of storage at 4°C. Different lowercase letters indicate statistically significant (p<0.05) differences among the same type of liposomes for different storage times. Different uppercase letters state statistically significant (p<0.05) differences among different types of liposomes at the same storage time.

4.2.4 Zeta potential of liposomes

Zeta potential is defined as the electrical potential at the boundary of the shear plane that covers a charged particle. The counter-ions are strongly bound with the particle resulting in the separation between the particle and enclosing liquid (Lin et al., 2003; Tamjidi et al., 2013). This parameter is an indirect method used for the determination of the stability of colloidally-stable particles and it is well associated with the release

kinetics of the encapsulated material (Tamjidi et al., 2013). In this study, in the case of control liposomes, there was a significant degree of alteration observed in their zeta potential overtime; however, in the case of the GTWE-loaded liposomes, such alteration did not continue after Day 0 (Figure 4.4). This difference between control and loaded liposomes in terms of zeta potential has also been reported in the previous studies. For example, Dag and Oztop (2017) investigated the characteristic of the liposomes loaded with green tea extract and found a significant decrease in the zeta potential of both control and loaded liposomes, which may be associated with the denaturation of structural integration in liposomes during 14 days of storage. Gibis et al. (2012) presented that the primary liposomes were brittle particles with a tendency to lose encapsulation properties for a period of time, due to the kinetic instability. Primary liposomes may be situated in the stage of the slightest free energy when the curve of the surface membrane is about to disrupt. Therefore, the integration of primary liposomes that is likely to occur can result in the denaturation of liposomes.

In the case of the current study, the initial change in the surface charge of GTWE-loaded liposomes might be related to the influence of the interactions between unencapsulated catechins and lipid bilayers of the liposome structure. The possible explanation is that the unencapsulated catechins could attach to the phosphatidylcholine (PC) in the liposomal membrane, which in turn could result in the reduction of zeta potential of the loaded liposomes (Li et al., 2020). Hashimoto et al. (1999) evaluated the affinity of each green tea catechin to PC, which could reflect the hydrophobicity, by the evaluation of the partition coefficient in *n*-octanol/water. The results indicated that ECG and EGCG gained higher lipophilicity (23.9 and 16.0, respectively) than EC and EGC

(1.4 and 0.5, respectively). Therefore, unencapsulated ECG and EGCG had a higher possibility to locate around the surface of phospholipids after liposomal production, due to their lipophilic property. Consequently, in the case of the current experiment, although there was a high encapsulation efficiency obtained, yet the small amount of the unencapsulated catechins were responsible for the alteration of zeta potential during Day 0. Nevertheless, the liposomal system was stable after this point, meaning that this initial alteration in surface charge of the liposomal particles may not be a concern.

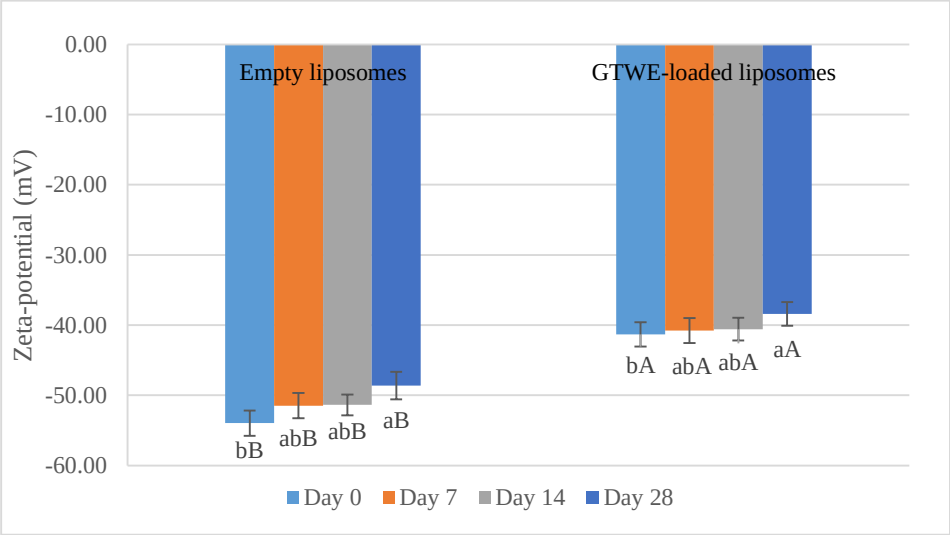
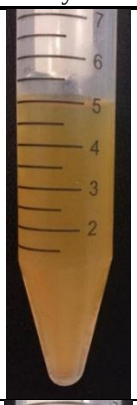
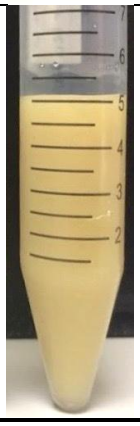


Figure 4.4 The zeta potential of empty and green tea waste extract (GTWE)-loaded liposomes during 28 days of storage at 4°C. Different lowercase letters indicate significant (p<0.05) differences among the same type of liposomes for different storage time. Different uppercase letters state statistically significant (p<0.05) differences among different types of liposomes at the same storage time.

The initial zeta potential values of both liposomes were close to the results of Rashidinejad et al. (2014); -45.1 to -42.4 mV for empty and loaded liposomes, respectively, where 0.5% EGCG was encapsulated in soy lecithin liposomes. As reported by Tamjidi et al. (2013), to attain highly stabile liposomes, the zeta potential should be in the range of ±30 mV or stronger, to stabilise steric and electrostatic repulsions and

prevent the possible liposomes aggregation. Hence, it could be assured that the GTWE-loaded liposomes produced in this study had high stability because of high zeta potential values. Moreover, the physical appearance of the liposomes seen in Table 4.4 supports the high stability of the manufactured liposomes in this study.

Table 4.4 The physical stability (appearance) of free green tea waste extract, empty liposomes, and green tea waste extract (GTWE)-loaded liposomes during 28 days of storage at 4°C.

Sample name	**Storage time**			
	Day 0	Day 7	Day 14	Day 28
Free GTWE				
Empty liposomes				
GTWE-loaded liposomes				

4.2.4 Morphology

Transmission electron microscopy (TEM) has been applied to observe the appearance, size, and distribution of liposomes. This technique can also help with presenting the laminar structure of liposomes, including inner (core material) and outer (coating material) layers, with high-resolution images (Feng et al., 2019; Rashidinejad et al., 2014). As explained in the methods (Section 3.2.4.2), the sample preparation for TEM analysis, implemented in this study, was a negative staining method using uranyl acetate as a dye. The uranyl acetate could enhance the contrast of TEM images; however, it might negatively affect the structure of liposomes (Rashidinejad et al., 2014). The TEM micrographs of both empty liposomes and liposomes containing GTWE are shown in Figure 4.5.

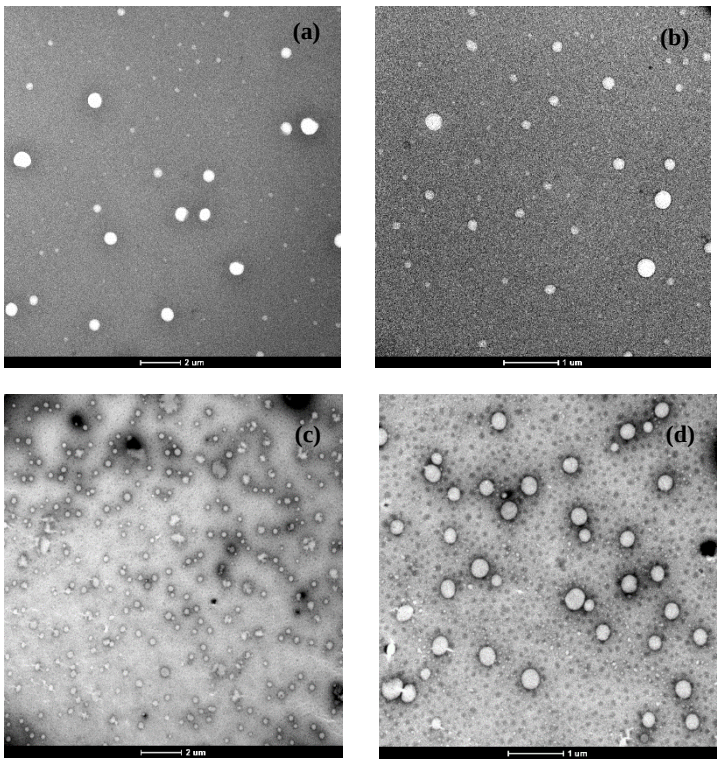


Figure 4.5 Transmission electron micrographs of nanoliposomes encapsulating; (a) and (b): no catechins, (c) and (d): green tea waste catechins. The scale bars can be found at the bottom of each micrograph. Magnifications: 6000× for (a), (c), and 16500× for (b), (d).

As can be seen in Figure 4.5, the particle size of the empty liposomes (Figure 4.5, a and b) was smaller than the nanoliposomes encapsulating GTW catechins, confirming the data obtained for particle size (Figure 4.3). In terms of the morphology, the empty liposomes (Figure 4.5, a and b) were seen to be lighter than the nanoliposomes encapsulating GTW catechins (Figure 4.5, c and d), particularly in the wall of the liposomes, because of the interactions between catechins and phospholipids (Rashidinejad, Birch, & Everett, 2016).

Different catechins in green tea have different affinities for binding to phospholipids, depending on the number of hydroxyl groups on the B ring and the existence of galloyl group; i.e., the galloylated catechins containing higher affinity than the non-galloyled catechins, gain a higher possibility to bind with the phospholipid membranes, leading to the high density of electrons (Du et al., 2012; Hara, 2001; Rashidinejad, Birch, & Everett, 2016). Therefore, the TEM images of the liposomes containing GTW catechins (Figure 4.5, c and d) showed the darker particles than the empty liposomes (Figure 4.5a and b), due to the difference of the electrons around the phospholipid membranes, which agrees with the results published in the previous studies (Rashidinejad, Birch, & Everett, 2016; Tunick et al., 2002)

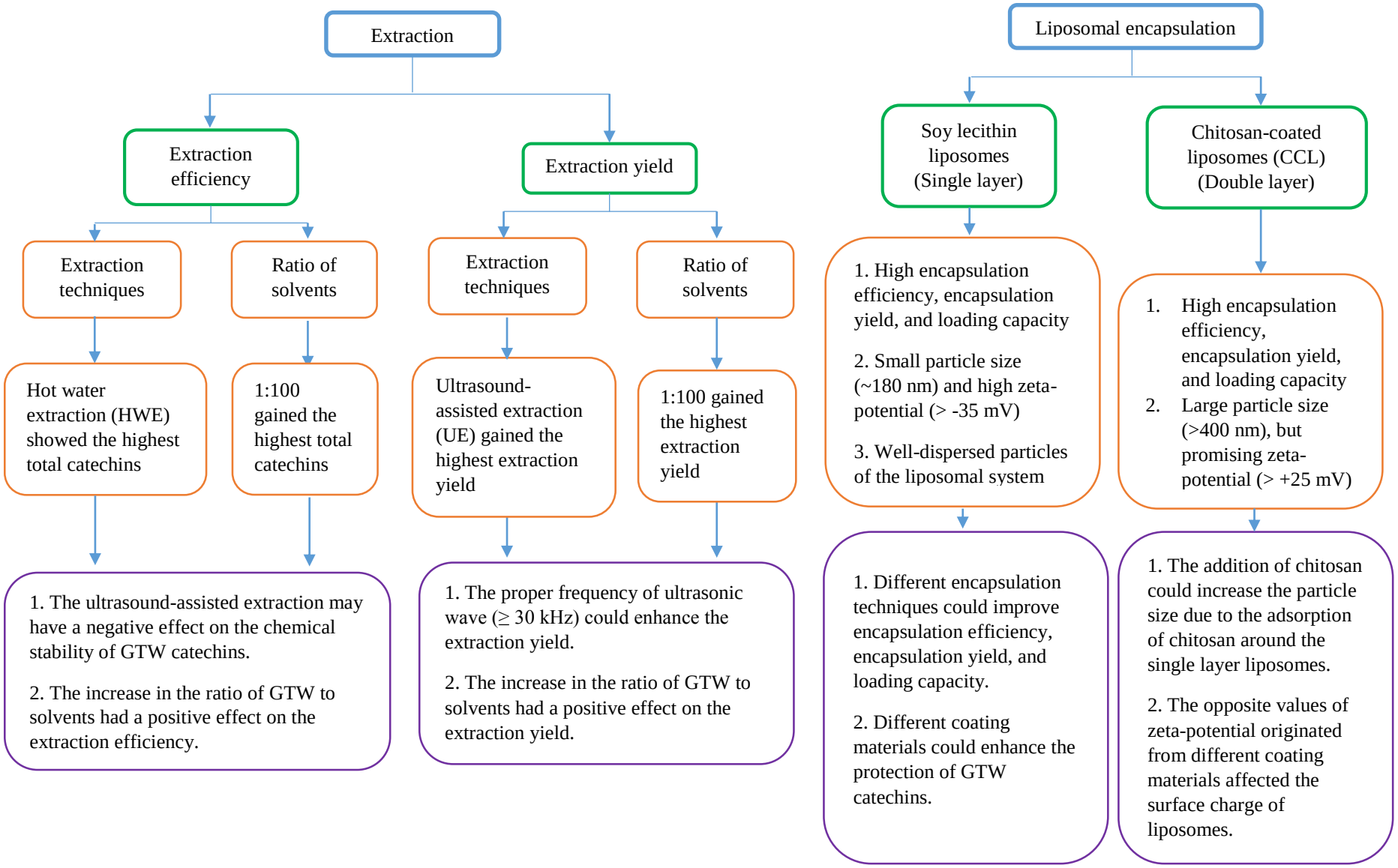


Figure 4.6 A summary flowchart for the results and discussion of extraction and encapsulation sections. : Section name, : Evaluation factors, : Results, and : Discussions.

4.3 The behaviour of the encapsulated GTWE in the kiwifruit juice and the effect on its properties

Encapsulated GTWE was incorporated into the kiwifruit juice to enrich the nutritional value and assess the possibility of the manufacture of a functional kiwifruit drink with added antioxidant activity. Although kiwifruit juice is known as a source of antioxidants such as vitamin C, the incorporation of catechins from green tea waste can result in boosting the antioxidant activity of not only kiwifruit juice but also green tea catechins themselves. This is due to the synergistic mechanisms known for various bioactive compounds (Drummond, 2013; Hidalgo et al., 2010; Salminen & Russotti, 2017). Saucier and Waterhouse (1999) investigated the synergistic effect between vitamin C and (+)-catechin. The results showed that the combination of these two compounds gained significantly higher antioxidant capacity than that of each alone. Therefore, the synergistic effect of such compounds could enhance the obtained antioxidant capacity. Nakagawa et al. (2000) examined the influence of catechin supplementation on the development of the ascorbate metabolism in red sea bream (a type of fish). The concentration of vitamin C in serum, liver, and muscle of the fish was determined and found that the level of ascorbic acid in the fish supplemented with catechin was two times higher than the control fish.

In the following sections, the results obtained for the characterisations of the encapsulated GTWE in the kiwifruit juice are presented and discussed.

4.3.1 Particle size

The particle size (D[4, 3]-Volume weighted mean) of kiwifruit juice fortified with GTWE-loaded liposomes was determined to investigate the stability of the liposomes suspended in the kiwifruit juice during 28 days of storage at 4°C. According to Figure 4.7, the particle size of control kiwifruit juice and kiwifruit juice containing free GTWE significantly decreased (p<0.05) after 14 days of storage. The reduction in particle size of both samples may link to the activity of pectin methylesterase (PME) that is considered as a cause of cloud loss and gelation problems in fruit juice manufacturers (Igual et al., 2013). PME is a catalyst related to the hydrolysis of homogalacturonan, which is a part of pectin polysaccharide. Due to the hydrolysis process, the methoxy and carboxylic groups are released bringing about the production of free radicals that can decrease the particle size as well as molecular weights (Igual et al., 2013; Pelloux et al., 2007). The kiwifruit juice containing empty liposomes had a consistent particle size until the end of storage.

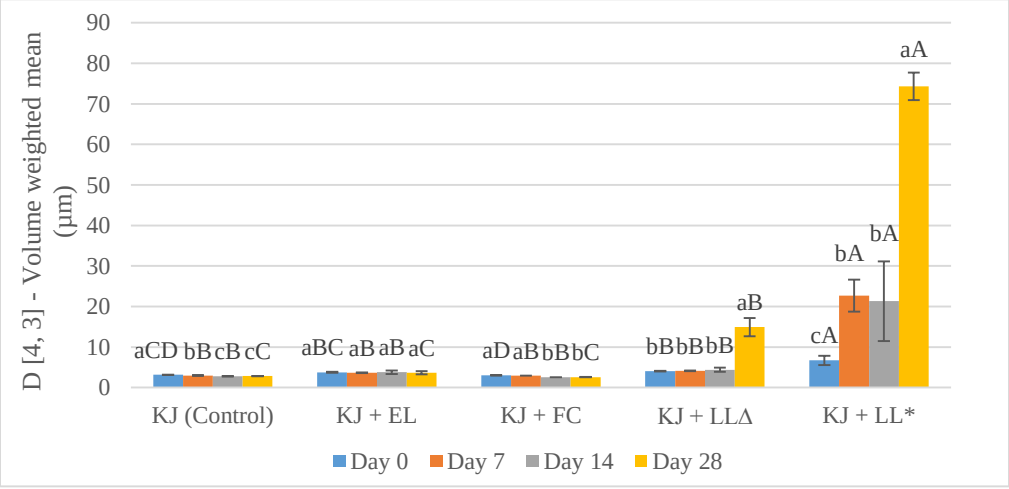


Figure 4.7 The particle size (D [4, 3]-Volume weighted mean (µm)) of kiwifruit juice containing empty and green tea waste extract (GTWE)-loaded liposomes during the storage at 4°C. KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free GTW catechins; KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice enriched with GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes. Different lowercase letters indicate significant (p<0.05) differences among the same kiwifruit juice samples for different storage time; Different capital letters stand for statistically significant (p<0.05) differences among different kiwifruit juice samples on the same storage day.

In terms of the kiwifruit juice enriched with GTWE-loaded liposomes, the pasteurisation process was carried out in the experiment to investigate the stability of the liposomes by comparing the results of pasteurised and unpasteurised kiwifruit juice samples. The particle size of the unpasteurised kiwifruit juice was not significantly affected ($p>0.05$) from Day 0 to Day 14 and significantly increased ($p<0.05$) on Day 28. However, the particle size of the pasteurised kiwifruit juice significantly shifted ($p<0.05$) to the larger particles since Day 7, which was bigger than the size of unpasteurised kiwifruit juice on Day 28, and then soared up to the biggest particles among all kiwifruit juice samples on Day 28 (about 74 μm). This might be related to the effect of pasteurisation, the presence of calcium ion (Ca^{2+}), and the interaction between fibre and polymer chains. However, in this study, the effect of only one methods of pasteurisation was experimented, indicating that the effect of other pasteurisation methods/heat treatment is unknown. Typically, raw green kiwifruit contains calcium (approximately 34 mg/100 g FW), which may be present in the kiwifruit juice after squeezing (Drummond, 2013). The presence of Ca^{2+} can play a crucial role in the interactions between particles that results in the aggregation of liposomes, because the dehydration of the bilayers could happen due to the induction of Ca^{2+}. This reaction could decrease the number of water binding sites on the bilayers, which in turn, could result in the decrease of repulsive hydration forces, and then the particles could easily aggregate (because of the lower repulsion force). Nevertheless, the thermal treatments such as pasteurisation, may enhance such a phenomenon (Marsanasco et al., 2011).

As reported by Sun-Waterhouse et al. (2013), the increase in particle size of fibre may be related to the interactions between fibre and polymer chains. Smoothie beverages that were treated with high-temperature short-time pasteurisation (85°C, 15 s) and were fortified with apple fibre and carboxymethylcellulose (CMC) (stabilizer), to boost the nutritional value of the beverages. The CMC polymer chains could interact with the fibre particles in two different ways. First, the CMC chains were loosened and coated the particles of apple fibre on the external surface resulting in the increase in particle size and decrease in viscosity. Second, the CMC chains were intertwined and suspended in the solution, resulting in the elevation of viscosity. In this experiment, the former case was the reason for the increase in particle size of both the pasteurised and unpasteurised kiwifruit juice samples during 28 days of storage. Because, some GTWE-loaded liposomes might be degraded over time and destroyed during the pasteurisation process, leading to the disentanglement of the phospholipids (becoming polymer chains) with a possibility to react with fibre in the kiwifruit juice.

4.3.2 Zeta potential

In this study, the zeta potential of all kiwifruit juice samples was not significantly (p>0.05) different during the 28 days of storage, except for the pasteurised kiwifruit juice fortified with GTWE-loaded liposomes (Figure 4.8). First of all, the kiwifruit juice samples with no liposomes, control kiwifruit juice, and kiwifruit juice fortified with free GTWE were stable within a certain period of storage. This was due to the consistency of zeta potential values (-8.28 to -7.89 and -8.31 to -7.97 mV, respectively) throughout the storage period, in combination with the smallest particle sizes reported in the previous section (Section 4.3.1). To the best of author's knowledge, there was no supporting

evidence, found in the Google Scholar and Web of Science (on 1 September 2020), about the zeta potential of kiwifruit juice. Therefore, the findings of this experiment are the first piece of evidence to reveal that the zeta potential of control kiwifruit juice was about -8 mV and the addition of free GTWE in kiwifruit juice did not change the zeta potential.

Secondly, the kiwifruit juice containing empty liposomes also showed the compatible zeta potential values, in the range of -8.26 to -8.08 mV, together with the small particle size presented pefore (Section 4.3.1). This value was similar to the results of the kiwifruit juice samples with the absence of liposomes which could be assumed that the addition of empty liposomes did not affect the zeta potential value of the kiwifruit juice.

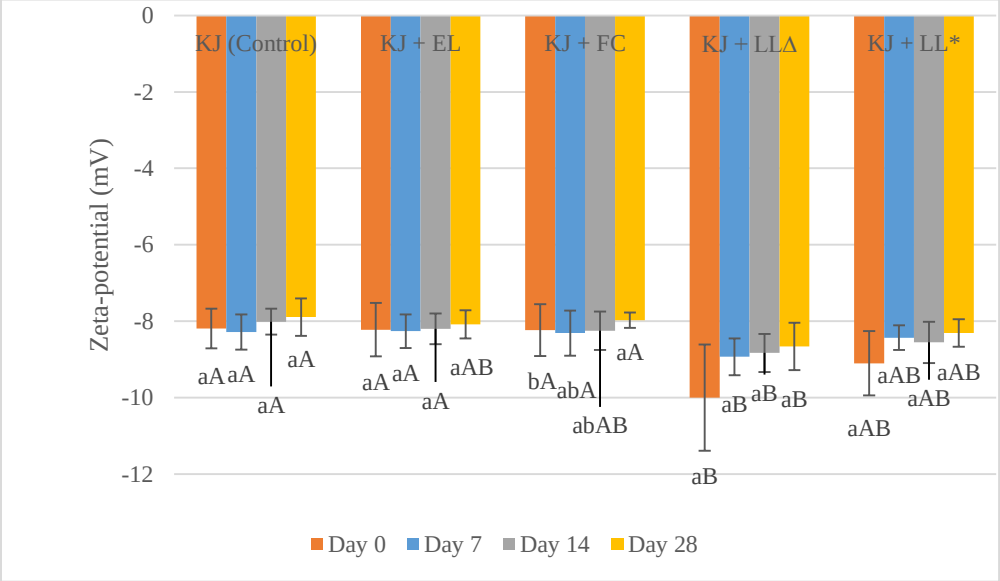


Figure 4.8 The zeta potential of kiwifruit juice fortified with empty and loaded liposomes during storage at 4°C. KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free green tea waste extract (GTWE); KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice enriched with GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes. Different lowercase letters indicate significant (p<0.05) differences among the same kiwifruit juice samples for different storage time; Different capital letters stand for statistically significant (p<0.05) differences among the different kiwifruit juice samples in the same storage time.

Last, both pasteurised and unpasteurised kiwifruit juice samples containing GTWE-loaded liposomes presented higher zeta potential values (-9.10 to -8.31 and -10.00 to -8.66, respectively) than other juices, because of the effect of the liposomal enrichment (Zhu et al., 2020). The addition of GTWE-loaded liposomes elevated the population of particles in the solution, which reduced the distance between particles, while it also increased the inter-particle interactions linked to the zeta potential. The lower the size of suspended particles appears in the solution, the greater the zeta potential is achieved, which results in the colloidal stability of the system. Moreover, these results were partially in agreement with the finding of Rashidinejad, Birch and Everett (2016) who determined the zeta potential of catechins (EGCG) and green tea extract (GTE), applied in a dispersion of milk fat globules, with different concentrations (125, 250, 500, and 1000 ppm). The higher concentration of EGCG and GTE applied in the milk fat, the higher the zeta potential was recorded, because of the interactions between the milk fat globules membrane and green tea catechins that resulted in the alteration of phospholipid affinity. Additionally, the increase in particle size of both samples during storage (explained in Section 4.3.1), slightly decreased the zeta potential values. In summary, the addition of free GTWE and empty liposomes in the kiwifruit juice did not change the zeta potential value compared with the control kiwifruit juice. In contrast, the enrichment of GTWE-loaded liposomes could develop the stability of the dispersed particles in the kiwifruit juice by increasing the zeta potential.

4.3.3 Morphology

As can be seen from Figure 4.9, the soluble dietary fibres in the kiwifruit juice, which mostly comprised of pectin (472-708 mg/100 FW) (Sims & Monro, 2013), were appeared in the a-g micrographs that is supported by the findings of Sorrivas et al. (2006) who reported almost the same microstructure for apple fibres. The pectin structure is an open network with single or multiple-strands formed as loose or bundle structures (Sorrivas et al., 2006). It is also notable that when free GTWE was added to the kiwifruit juice, the microstructure was different than the juice without any catechins (Figure 4.9, a vs b). The morphological data found for liposomes added to kiwifruit juice in this study are in agreement with those reported by Rashidinejad et al. (2014), who incorporated green tea catechin liposomes into a low-fat hard cheese. The empty liposomes (c) gained slightly smaller particle size than the GTWE-loaded liposomes (Figure 4.9, c vs d). Most importantly, pasteurisation affected the size of liposomes in a way that the liposomes in the pasteurised juice appeared bigger. The reasons for this phenomenon were explained in Section 4.3.1 and Figure 4.7, where the effect of pasteurisation on the size of liposomes in the kiwifruit juice was discussed. In brief, the presence of Ca^{2+} resulted in the aggregation of liposomes, because of the dehydration of the bilayers that could happen due to the induction of Ca2+, which could be accelerated by the thermal treatment (i.e., pasteurisation). Additionally, the interactions between fibre and phospholipid polymer chains were involved in the increase of the particle size, because GTWE-loaded liposomes might have been degraded and destroyed during the pasteurisation process, leading to the disentanglement of their phospholipids. This, in

turn, resulted in the liberation of the polymer chains with the possibility to react with the fibre in the kiwifruit juice.

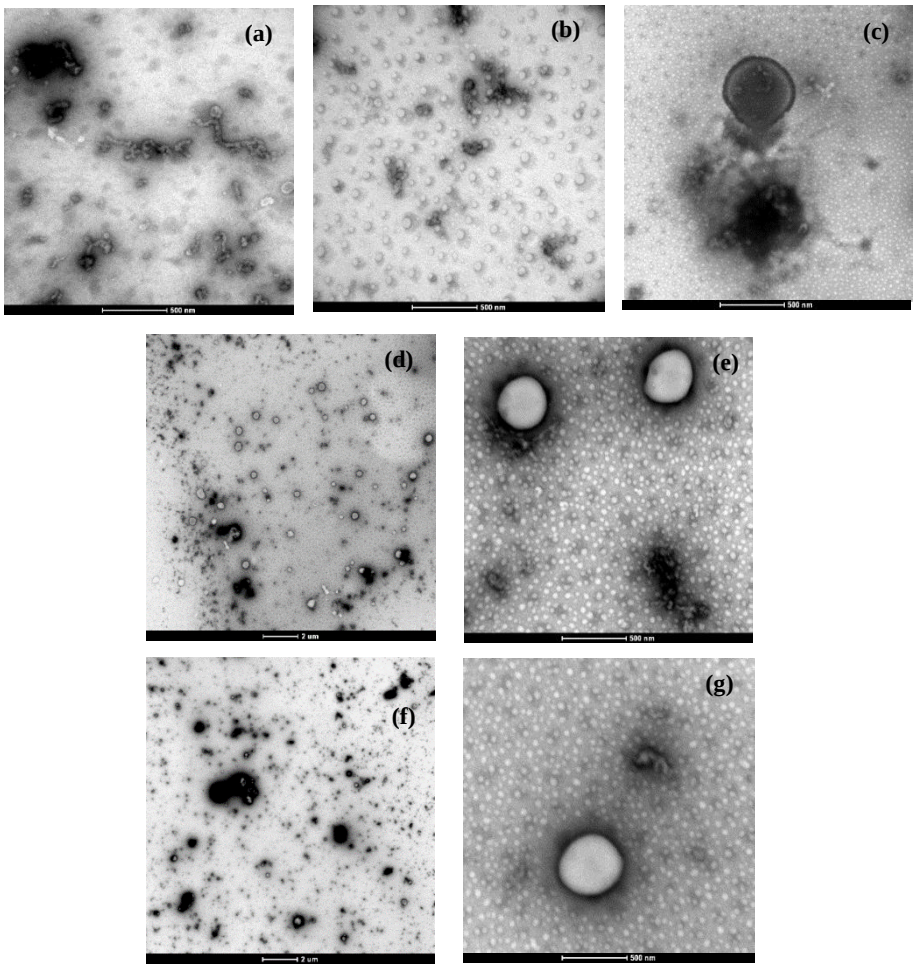


Figure 4.9 Transmission electron micrographs of the kiwifruit juice fortified with (a) no liposomes, (b) free green tea waste extract (GTWE), (c) empty liposomes, (d) and (e) GTWE-loaded liposomes without pasteurisation, and (e) and (f) GTWE-loaded liposomes with pasteurisation. Magnification: 6000× for (d) and (f) and 43000× for (a), (b), (c), (e), and (g).

4.3.4 Chemical stability of catechins measured using HPLC

As shown in Figure 4.10, in the case of the kiwifruit juice containing GTWE-loaded liposomes, pasteurisation slightly affected the concentration of total catechins in GTW during the 28 days of juice storage. On the contrary, pasteurisation played a crucial role in the degradation of natural and free GTW catechins in the control and fortified

kiwifruit juice samples, respectively. The unpasteurised kiwifruit juice could maintain the same quantity of the natural catechins (about 29.12-32.45 ppm) during entire storage period. While the pasteurised kiwifruit juice showed a higher amount of the catechins (40.50 - 49.24 ppm) than unpasteurised kiwifruit juice until Day 14, but catechin concentration drastically decreased to 11.67 and 3.63 on Day 21 and 28, respectively.

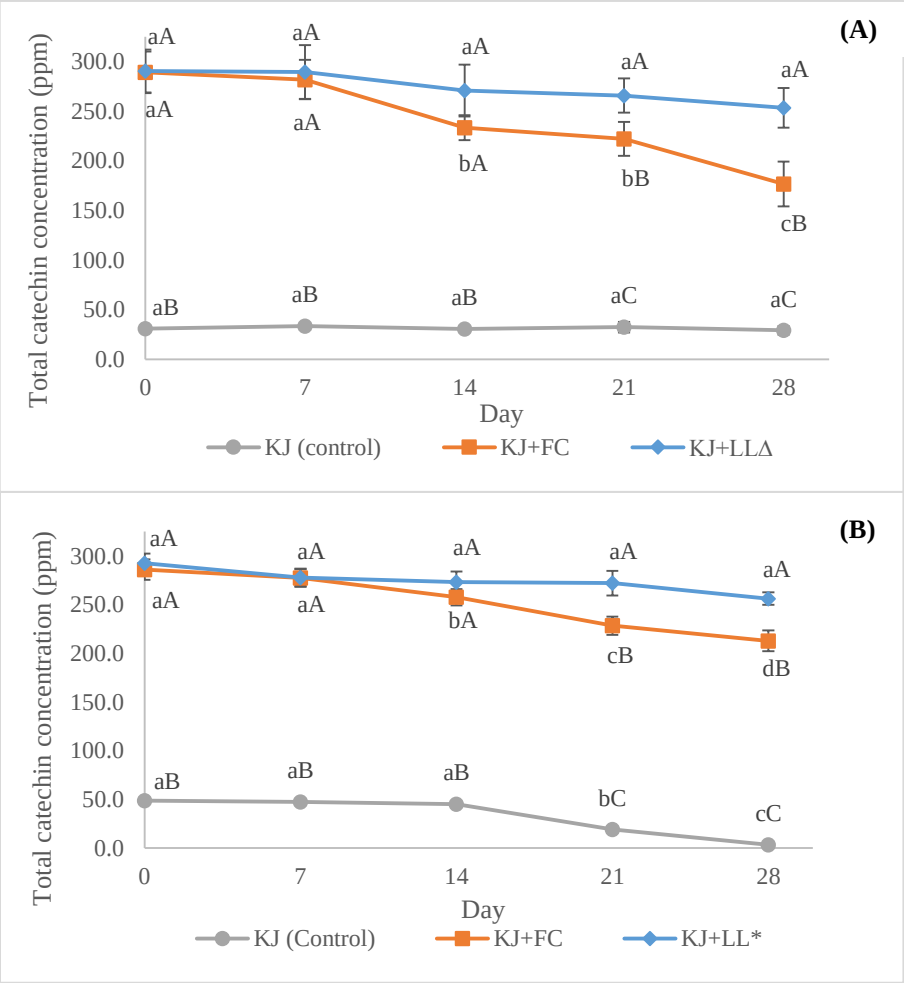


Figure 4.10 Total catechin concentration (ppm) of control kiwifruit juice vs unpasteurised (A) and (B) pasteurised kiwifruit juice samples, analysed by high-performance liquid chromatography (HPLC). KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free green tea waste (GTWE); KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice enriched with GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes. Different lowercase letters indicate significant (p<0.05) differences among the same kiwifruit juice samples for different storage time. Different uppercase letters stand for statistically significant (p<0.05) differences among different kiwifruit juice samples at the same storage time.

In terms of the kiwifruit juice containing free GTW catechins, a steadily decrease was observed from Day 0 to Day 28 in the case of both pasteurised and unpasteurised samples, except for the unpasteurised kiwifruit juice on Day 28 that presented a drastic decrease for free GTW catechins. These results indicated that the liposomal technology could protect GTW catechins from the pasteurisation process of the kiwifruit juice until 28 days of storage while the kiwifruit juice samples enriched with free GTW catechins showed a continuously decreasing trend over time. However, the natural GTW catechins in kiwifruit juice treated with pasteurisation were mostly degraded after 14 days of storage, but the unpasteurised sample could remain the same quantity until the end of the storage period.

Regarding the discrepancy of the values for total catechins between the pasteurised and unpasteurised control kiwifruit juice samples during storage observed in this study, Tembo et al. (2017), who investigated the influence of thermal process (72 °C, 15 s) on epicatechins in the pasteurised baobab juice kept in the refrigeration storage (6°C) for 60 days, reported that the pasteurisation could enhance the quantity of epicatechins in the juice about 10% and 70% on Days 0 and 28 of storage, respectively, compared with the unpasteurised juice kept under the same condition. In the case of the current study, after Day 28, the level of epicatechins gradually decreased until Day 60. The reasons for this phenomenon originated from the impact of the pasteurisation on the loss of polyphenols−dietary fibre bonds, and the epimerisation and the deterioration of polyphenols. The complex of polyphenols−dietary fibre could be broken by high-temperature process leading to the release of polyphenols from the dietary fibre (Saura-Calixto & Díaz-Rubio, 2007). Accordingly, the thermal process affected the acceleration

of catechins epimerisation from epimerised catechins (i.e., EC, ECG, EGC, and EGCG) to non-epimerised catechins (i.e., (±)-catechins (C), (−)-catechin gallate (CG), (−)-gallocatechin (GC), and (−)-gallocatechin-3-gallate (GCG)) at the C-2 position (Ananingsih et al., 2013). Moreover, the non-epimerised catechins rapidly degraded because of their poor stability (Xu et al., 2004).

The reason for the decrease of total catechins during storage in the kiwifruit juice containing free GTWE treated by pasteurisation was related to the influence of pasteurisation on the stability of phenolic compounds. In this regard, Igual et al. (2010) studied the total phenols of grapefruit juice after pasteurisation (95 °C, 10 s) and stored at 4 °C for two months. The results demonstrated that the total phenols of pasteurised grapefruit juice were decreased constantly (from about 70 to about 50 mg GAE/100 ml on Days 0 and 25, respectively) and remained stable until Day 60. This may agree with the decrease of the total catechins content values of the kiwifruit juice samples noticed during the current study. Therefore, it can be concluded that pasteurisation could substantially decrease the total catechins of the kiwifruit juice during storage and under refrigeration conditions. Therefore, low-temperature pasteurisation or non-thermal processing may be better options for pasteurisation of such a functional food containing green tea catechins.

In terms of the stability of the liposomes after thermal treatment, Feng et al. (2019) investigated the impact of pasteurisation (65°C, 30 min) on low-methoxyl pectin-coated liposomes, entrapping EGCG and resveratrol in orange juice using the thiobarbituric acid reactive substance (TBARS) assay. TBARS values could measure lipid peroxidation, which was determined by the detection of malondialdehyde (MDA),

so it could be implied that the higher levels of MDA are detected, the more liposomes are degraded. The results demonstrated that the effect of pasteurisation did not considerably influence on the stability of liposomes, because the TBARS values of pasteurised orange juice samples, kept at 4°C for 0, 10, and 20 days, presented a slight increase during storage (approximately 4 μM per 10 days). Moreover, the leakage percentage of the EGCG liposomes was 5.69 and 13.53% on day 10 and 20, respectively, which may correspond with the decrease in the total catechins content values observed during the current study (Figure 4.10).

4.3.5 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical assay

Although all samples showed a slight reduction of the DPHH antioxidant activity during the 28 days of storage (Figure 4.11), the kiwifruit juice samples supplemented with the GTWE-loaded liposomes had significantly (p>0.05) higher DPPH free radical scavenging ability than both control kiwifruit juice and the kiwifruit juice enriched with free GTW catechins. Focusing on the kiwifruit juice samples fortified with GTWE-loaded liposomes, the unpasteurised sample showed a significantly (p<0.05) greater DPPH values than the pasteurised sample until Day 14, while the DPPH results of both samples on Day 28 were not significantly (p>0.05) different. These results are consistent with the study of Kim et al. (2020), who investigated the influence of storage temperature on the antioxidant activity of green tea catechins using DPPH assay. In their study (Kim et al., 2020), DPPH radical scavenging activity (%) of green tea powder kept at 4°C was not significantly (p>0.05) different until Day 14 (>80% and 75.42% on Day 0 and Day 14, respectively), but a significant decrease was observed after Day 21.

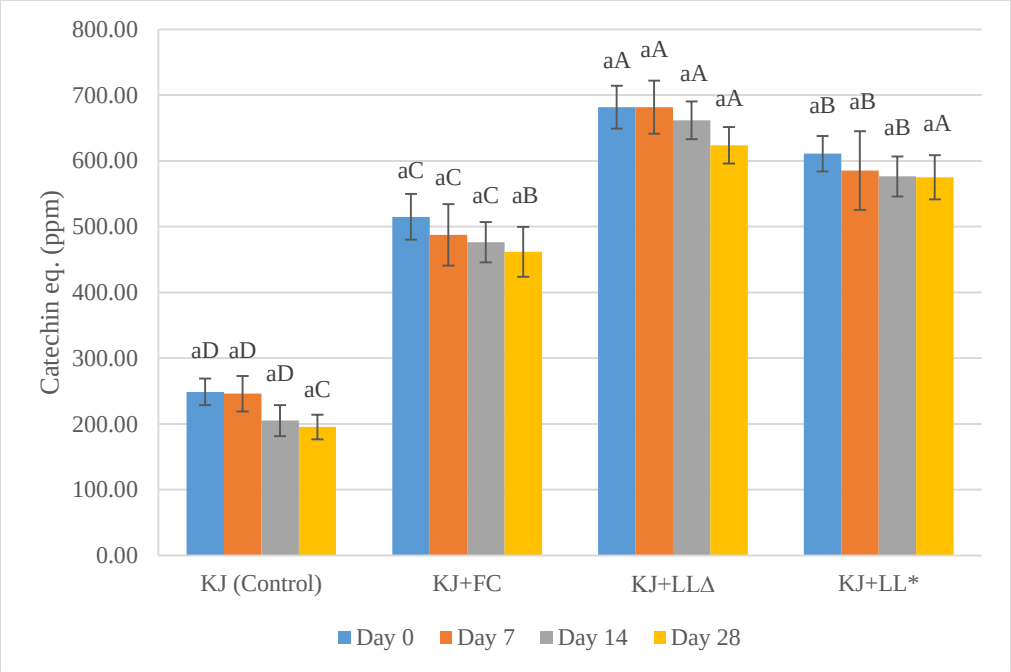


Figure 4.11 DPPH (2,2-diphenyl-1-picryl-hydrazyl) antioxidant activity of kiwifruit juices during 28 days of storage, expressed as catechins equivalent (ppm). KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free green tea waste extract (GTWE); KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice enriched with GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes. Different lowercase letters indicate significant (p<0.05) differences among the same kiwifruit juice samples for different storage time. Different uppercase letters stand for statistically significant (p<0.05) differences among the different kiwifruit juice samples at the same storage time.

The reasons for the reduction of antioxidant activity during storage were related to the epimerisation and stability of catechins. As reported by Xu et al. (2004), the epimerised catechins, consisting of EC, ECG, EGC, and EGCG, had higher antioxidant activity than non-epimerised catechins such as C, CG, GC, and GCG and the epimerisation process was accelerated by heat treatment including pasteurisation. Therefore, the high temperature could be a cause of the changes in the structures of catechins from epimerised to non-epimerised forms resulting in the reduction of antioxidant activity. Rashidinejad et al. (2013) presented the influence of catechins added to a low-fat hard cheese on the levels of antioxidant activity of this food. The cheese

containing different concentrations of catechins (125, 250, and 500 ppm) was kept at 8°C and examined on Day 0, 30, and 90 by the DPPH method. The results of all concentrations showed an increasing tendency of antioxidant activity during three months of storage, resulting in the release of antioxidants from cheese regardless of the change in the catechin structure. It was speculated that almost all catechins added to the cheese were stable at 8°C during the 90 days of storage (Rashidinejad et al., 2013). These findings correspond with the discrepancy of total antioxidant activity between the pasteurise and unpasteurised kiwifruit juice, as well as the stability of GTW catechins during 28 days of storage, presented in this study. Nevertheless, the behaviour of catechins in a beverage system such as the kiwifruit juice experimented in this study can be different than that in a complex food matrix such as the cheese experimented by Rashidinejad et al. (2013).

The discrepancy between antioxidant activity of the kiwifruit juice fortified with free GTWE and GTWE-loaded liposomes originated from the stability improvement of catechins due to the nanoencapsulation technology, as well as the synergistic effect between catechins and vitamin C (present in kiwifruit juice). Dube et al. (2010) reported that the half-initial concentration degradation of free green tea catechins was faster than encapsulated catechin in the period of 8 and 24 h. The kinetics of both catechins were also measured by *k* values represented as a degradation rate constant. The degradation rate of free catechin was -0.09 and -0.11 %/h comparing to -0.03 and -0.02 %/h for the encapsulated catechin and EGCG, respectively. Toro-Uribe et al. (2019) investigated the liposomal stability of procyanidins (extracted from cocoa) by measuring total antioxidant activity (DPPH method). Procyanidins are originated from the formation of catechin and

epicatechin monomers and are usually found in cocoa, tea, and apple. The results of DPPH indicated that the encapsulated procyanidins gained about 91.31% DPPH reduction; whereas, free procyanidins received only about 44.83% DPPH reduction during 8 h of the simulated duodenum digestion. This led to the speculation that the encapsulation technique successfully delivered procyanidins to duodenum with high antioxidant activity while over half of free procyanidins were degraded.

In terms of the synergistic effect between catechins and vitamin C, Dube et al. (2010) compared the degradation of the individual catechin (control) with the combination of catechin and vitamin C. The control and the mixture of catechin and vitamin C were prepared in phosphate buffer (pH 7.4) and the remaining catechins were observed over time. The remaining catechins in the control was about 67.5 and 19.2% at 6 and 24 h, respectively, which was substantially lower than the remaining catechins in the mixture of catechin and vitamin C (79.1 and 38.1 % at 6 and 24 h, respectively). In an almost similar experiment, Saucier and Waterhouse (1999) studied the synergistic effect of vitamin C and (+)-catechin. The results showed that the combination of (+)-catechin and vitamin C gained significantly ($p<0.05$) higher antioxidant capacity than that of either (+)-catechin or vitamin C alone, meaning that the synergistic effect of these compounds could enhance the total antioxidant capacity.

4.3.6 Total phenolic content

Folin-Ciocâlteu method was carried out in this study for the determination of total phenolic content (TPC), which was necessary for the confirmation of the results received by HPLC analysis and DPPH assay. The total phenolic content of all kiwifruit juice

samples, including control, was decreased during the storage period (Figure 4.12). The TPC results from the unpasteurised kiwifruit juice fortified with GTWE-loaded liposomes possessed the highest values with statistical significance at every storage point (i.e., Day 0, 7, 14, and 28), ranging from about 289.4 to about 357.83 ppm of catechins equivalent followed by the pasteurised kiwifruit juice fortified with GTWE-loaded liposomes. These results are in agreement with the finding of Zhao and Shah (2014), who presented the effect of storage time on the TPC concentration of fermented soymilk enriched with green tea extract. The TPC concentration of fermented soymilk containing green tea extract continuously decreased from 4.31 to 3.71%, meaning that the tea phenolics were stable for 28 days of storage at 4°C.

The decrease of TPC in all kiwifruit juice samples during storage is associated with the decrease in the compounds that may not be antioxidant but still interact with the Folin-Ciocâlteu reagent, as well as the initial degradation of the compounds such as vitamin C that contribute to the antioxidant activity of kiwifruit juice. Some compounds such as amino acids, reducing sugars, and vitamin C) can interact with the Folin-Ciocâlteu reagent and as they degrade over the storage period, the TPC values measured for the kiwifruit samples also decrease. It has been reported that about 70-80% of vitamin C degraded within 8 h after the squeeze, even when it was kept in the refrigerated condition (8°C) (Sapei & Hwa, 2014). Besides, the previous research showed that the proper storage temperature and time, which were 4°C for 28 days, could not have a negative effect on the TPC results in green tea polyphenols (Kevers et al., 2007; Klimczak et al., 2007; Piljac-Žegarac et al., 2009). For these reasons, in this experiment, the drastic reduction of TPC on Day 0 to 7 was possibly resulted from the degradation of

other compounds in kiwifruit juice (e.g., reducing sugars and vitamin C), while the TPC reduction for the rest of storage period mostly resulted from the decrease of GTW catechins.

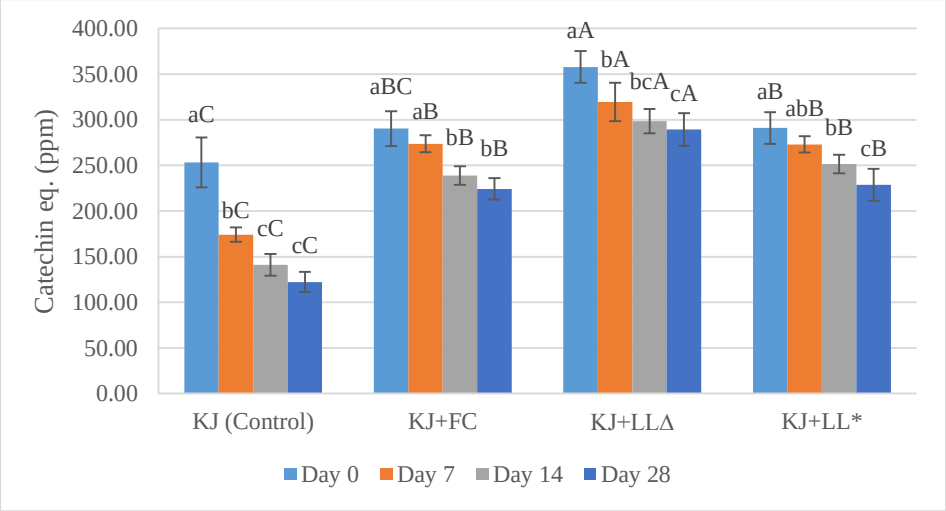


Figure 4.12 Total phenolic content (TPC) of kiwifruit juices during 28 days of storage expressed as catechins equivalent (ppm). KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free GTW catechins; KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice containing GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes. Different lowercase letters indicate significant (p<0.05) differences among the same kiwifruit juice samples for different storage time. Different uppercase letters stand for statistically significant (p<0.05) differences among different kiwifruit juice samples at the same storage time.

The mean correlation coefficients (R^2) among the values obtained using HPLC analysis, DPPH assay, and TPC are presented in Table 4.5. DPPH assay showed a strong correlation with TPC in the case of all points of storage periods, which could be assumed that phenolic compounds extracted from GTW, were responsible for the DPPH antioxidant activity. The mean correlation coefficients between HPLC analysis and DPPH were high, indicating that total catechins also played an essential role in the DPPH antioxidant activity. However, a lower correlation between HPLC analysis and TPC was found on Day 0, due to the different principles of analytical methods. HPLC was

implemented to identify, quantify, and isolate catechins that were categorised as a part of phenolic compounds (Danilo, 2011; Du et al., 2012; Lorenzo & Munekata, 2016). On the contrary, as it was mentioned earlier in this section, TPC measured not only the phenolic compounds, but also other compound such as vitamins (e.g., vitamin C) and reducing sugars that could interfere with the determination of TPC values (Sánchez-Rangel et al., 2013). Therefore, the mean correlation coefficient between HPLC and TPC on Day 0 was low because of the overrated TPC values. After Day 7, strong correlations were observed, which may relate to the completion of the degradation of vitamin C (i.e., no more vitamin C was available to react with the reagent). Sapei and Hwa (2014) reported that the vitamin C in strawberry juice (40-70 mg/100 g FW) decreased about 70-80% within 8 hours after the squeeze, although it was kept in the refrigerated condition (8°C). To compare, kiwifruit juice contains around 92.7-105.4 mg/100 g FW vitamin C, so at least 70-80% of vitamin C would be decreased after 7 days of storage, which significantly resulted in the decrease of TPC values and increasing the correlation between TPC and other methods of analysis.

Table 4.5 The mean correlation coefficient (R^2) of three different methods for determining total antioxidant activity, total phenolic content, and total catechins in the kiwifruit juice enriched with catechin-loaded nanoliposomes.

Storage period	HPLC*/DPPH▲	HPLC*/TPC■	TPC■/DPPH▲
Day 0	0.93	0.69	0.86
Day 7	0.92	0.95	0.98
Day 14	0.96	0.95	0.99
Day 28	0.99	0.93	0.96

* High-Performance Liquid Chromatography (HPLC) analysis
▲ 2,2-diphenyl-1-picryl-hydrazyl radical assay (DPPH)
■ Total phenolic content (TPC).

4.3.7 pH

According to the results shown in Figure 4.13, three unpasteurised samples including control kiwifruit juice, kiwifruit juice fortified with free GTW catechins, and the kiwifruit juice containing GTWE-loaded liposomes behaved almost similarly in terms of pH change. The pH dramatically increased from 3.30 to 3.58 within seven days and then remained stable until the end of storage. In terms of the pasteurised kiwifruit juice containing GTWE-loaded liposomes, the pH continuously increased from 3.32 to 3.57 during 14 days of storage and then maintained unchanged until the end of the storage period. These results are in agreement with the finding of Lyu et al. (2018), who reported a significant increase in pH for pasteurised and unpasteurised kiwifruit puree stored at 4°C. They also explained the effect of pasteurisation on the different tendencies of the elevated pH.

The thermal process may accelerate the release of organic acids and the ionisation of the kiwifruit cells contributed to an increase in acidity so that the increase in pH of the pasteurised kiwifruit juice containing GTWE-loaded liposomes was slower than the unpasteurised sample. Rashidinejad, Birch, Sun-Waterhouse, et al. (2016) reported that the pH of low-fat cheeses fortified with 125 and 250 ppm of free (unencapsulated) green tea catechins was not found significantly different ($p>0.05$) than that of the control cheese, while at 500 ppm concentration, the pH of the fortified cheese significantly ($p<0.05$) decreased. These researchers (Rashidinejad, Birch, Sun-Waterhouse, et al., 2016) stated that the reason for the decrease in pH of the cheese due to the addition of a high concentration of catechins (i.e., 500 ppm) was the fact that catechins are acidic in nature, while they may also contribute to the acceleration of lactic acid production in

cheese. Nevertheless, in another study from the same authors (Rashidinejad, Birch, Sun-Waterhouse, et al., 2016), it was found that liposomal encapsulated catechin or green tea extract at 500-1000 ppm did not affect the pH of a full-fat cheese.

In the case of the current study, where 280 ppm of catechins in form of GTWE-loaded liposomes was incorporated into the kiwifruit juice, there was no sign of the pH alteration related to the addition of GTWE-loaded liposomes. Therefore, GTWE-loaded liposomes were considered as the efficient and suitable delivery system for GTWE and its incorporation into the functional kiwifruit juice.

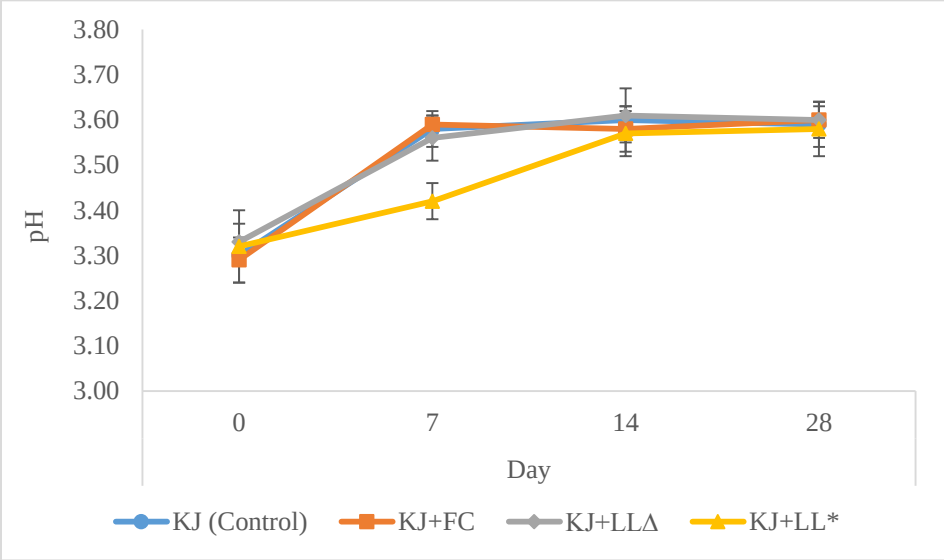


Figure 4.13 The pH of different kiwifruit juices (i.e., control, containing empty liposomes, containing free green tea waste extract (GTWE), and containing GTWE-loaded liposomes) during 28 days of storage at 4°C. KJ: control kiwifruit juice; KJ+EL: kiwifruit juice fortified with empty liposomes; KJ+FC: kiwifruit juice containing free GTWE; KJ+LL$^{\Delta}$: unpasteurised kiwifruit juice enriched with GTWE-loaded liposomes; KJ+LL*: pasteurised kiwifruit juice containing GTWE-loaded liposomes.

4.3.8 Sedimentation behaviour of different kiwifruit juices

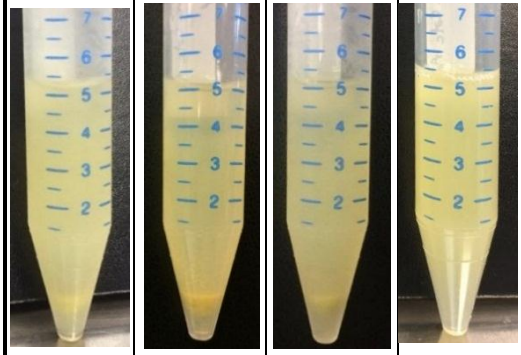
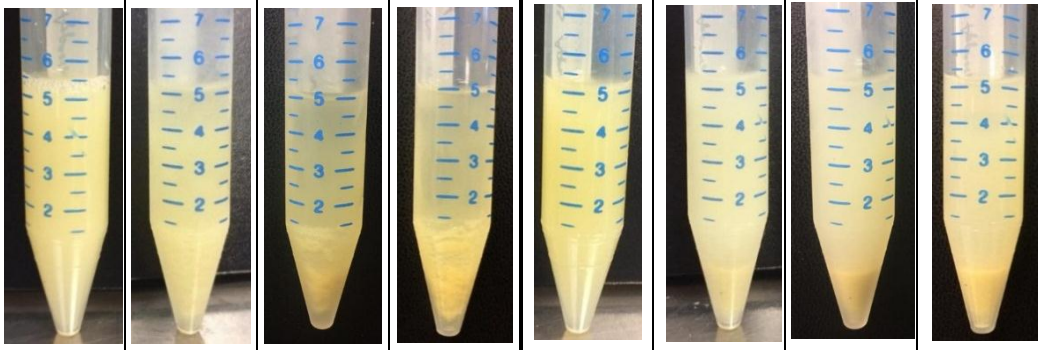
Cloud stability is one of the quality parameters reflecting the shelf-life of fruit juices and the degradation of cloud stability could occur by the application of processes

such as heat treatment and the activity of PME (Goodner et al., 1999). Nevertheless, it cannot be concluded whether the cloud loss is a defect in fruit juice products or not; because, some customers have a positive perception that the sedimentation is a sign of 'natural processing' representing the freshness of fruit juices (Timmermans et al., 2011). According to a systematic search in the Google Scholar and Web of Science (on 2 September 2020), there was no customer survey studying the customer perception related to the sedimentation of kiwifruit juice. Even so, there are various techniques and processes designed for the modification of the sedimentation in such beverages, which are out of the scope of the current study as they fall under new product development (NPD) not functional food manufacture as such. However, the author though it would be an opportunity to investigate a solution for decreasing a degree of sedimentation that was accidentally found in the kiwifruit juice samples in this experiment (Table 4.6), in order to expand the intellectual horizon.

Table 4.6 The physical stability (appearance) of kiwifruit juice samples; containing empty liposomes (KJ+EL), free green tea waste extract (GTWE; KJ+FC), and GTWE-loaded liposomes (KJ+LL) during 28 days of storage at 4°C.

Sample name	**Unpasteurised**				**Pasteurised**			
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28
KJ+EL								

Table 4.6 (Continued) The physical stability (appearance) of kiwifruit juice samples; containing empty liposomes (KJ+EL), free green tea waste extract (GTWE; KJ+FC), and GTWE-loaded liposomes (KJ+LL) during 28 days of storage at 4°C

Sample name	Unpasteurised				Pasteurised			
	Day 0	Day 7	Day 14	Day 28	Day 0	Day 7	Day 14	Day 28
KJ+FC								
KJ+LL								

Chitosan has been used as a stability enhancer for liposomes (especially for anionic liposomes), owing to its advantages such as non-toxicity, biodegradability, and biocompatibility (Cuomo et al., 2018; Liu et al., 2017). Based on the information available in the literature, chitosan is positively charged with having a potential to coat around the anionic liposomal membrane and form a protective polyelectrolyte layer to improve the stability of liposomes (Cuomo et al., 2018; Tan, Feng, et al., 2016). Chitosan-coated liposomes (CCL) have been studied for the delivery of several bioactive compounds, focusing on the development of liposomal stability and the sustainability of

bioactive compounds release. Nevertheless, there appears to be lack on evidence related to the benefit of chitosan-coated liposomes on the decrease of sedimentation in fruit juice products. Therefore, the experiment was conducted to elucidate the potential of chitosan-coated liposomes on the prevention of the possible sedimentation by measuring the precipitate weight ratio (PWR).

As can be seen from Table 4.6, the unpasteurised kiwifruit juice samples gained the highest total catechin contents with some sedimentation after seven days of storage. Chitosan-coated liposomes were incorporated into the unpasteurised kiwifruit juice in order to maintain the total catechin contents, as well as to decrease the sedimentation (determined by PWR). According to Table 4.7, a slight increase in the PWR was found in the kiwifruit juice enriched with empty liposomes (about 3%), compared with control kiwifruit juice (about 2.82%). The kiwifruit juice fortified with GTWE-loaded liposomes gained the highest PWR (about 3.82%), followed by the kiwifruit juice fortified with chitosan-coated liposomes (about 3.09%). These results are lower than those reported by Kaur et al. (2007) and Oke et al. (2010), who studied the PWR of tomato juice. A possible explanation for the discrepancy of PWR values is associated with different types of fruit juices and sample preparation methods. Thus, it can be concluded that the addition of the chitosan-coated liposomes affected the reduction of sedimentation in the kiwifruit juice sample; however, the PWR results were not the only factor in proving that the decrease in the sedimentation resulted from the property of the chitosan-coated liposomes. To further understand the physicochemical properties of the kiwifruit juice enriched with the chitosan-coated liposomes, the particle size, zeta potential, total

antioxidant activity, and morphology were investigated and presented in Section 4.3.9 below.

Table 4.7 The effect of chitosan-coated liposomes on the improvement of sedimentation in kiwifruit juice samples measuring by the precipitate weight ratio (PWR, %).

Sample name	Precipitate weight ratio (PWR, %)*
Kiwifruit juice (control)	2.82±0.07^{c}
Kiwifruit juice + empty liposomes (KJ+EL)	3.00±0.07^{bc}
Kiwifruit juice + GTWE-loaded liposomes▲ (KJ+LL)	3.82±0.23^{a}
Kiwifruit juice + chitosan-coated liposomes▲ (KJ+CCL)	3.09±0.18^{b}

a-c: values with different superscripted letters within the same column are significantly different (p<0.05).
*Values are mean of three replications (n=3 ± SD)
▲ The pasteurisation condition was 80°C for 10s.

4.3.9 The behaviour of the chitosan-coated liposomes in the functional kiwifruit juice

Primary liposomes, mostly produced from phosphatidylcholine (PC), have abundant advantages as described above; however, they still have limitations such as susceptibility during food processing, leakage of the encapsulated compounds, decrease of surface charges, and aggregation. Hence, based on the literature suggestions (Cuomo et al., 2018; Laye et al., 2008; Liu et al., 2017; Tan, Xie, et al., 2016; Taylor et al., 2005), adding another layer to the primary liposomes and manufacturing secondary liposomes is one of the efficient methods to enhance the stability and application of primary liposomes (Taylor et al., 2005).

Polysaccharides are the renowned natural coating materials for bioactive nanoencapsulation in pharmaceutical and food applications (Tan, Xie, et al., 2016). Chitosan is a natural polysaccharide produced by the deacetylation of chitin and has been used as a stability enhancer for anionic liposomes, based on the theory of the electrostatic

deposition (Cuomo et al., 2018; Liu et al., 2017; Tan, Xie, et al., 2016). Chitosan is positively charged, which gives it the potential to adsorb to the PC liposomal membrane and form a protective polyelectrolyte layer, as the second layer liposomes (Mun et al., 2006).

In this study, based on the information available in the literature (Cuomo et al., 2018; Laye et al., 2008; Liu et al., 2017; Tan, Xie, et al., 2016; Taylor et al., 2005), it was hypothesised that a secondary liposome could be more suitable for the incorporation of GTWE into a kiwifruit juice product, due to fewer stability challenges. However, there was a concern with regards to the possible interactions between the positively charged polymers used for the secondary layer of the liposomes and the negatively charged fibre in the kiwifruit juice. Nevertheless, there is no robust evidence supporting that the positively charged secondary liposomes are not suitable for the kiwifruit juice application or any food applications as such. Therefore, it was decided to test this hypothesis and the chitosan-coated liposomes (CCL) were incorporated into the kiwifruit juice. The other hypothesis that the author could not complete its testing (due to the experimental disruptions caused by COVID-19) was that the incorporation of a three-layer liposome in kiwifruit juice would result in even better outcomes in terms of the chemical stability of catechins, besides better colloidal stability of the native particles in the kiwifruit juice. Such liposomes (Three-layer liposomes) would consist of a negatively charged primary layer (e.g., soy lecithin), a positively charged secondary layer (e.g., chitosan), and another negatively charged tertiary layer as the final coating (e.g., gum arabic and pectin). Theoretically, in such a three-layer system, due to the electrostatic interactions between the two opposite charges (positive and negative), they attract each other and make a

robust liposomal structure (Forest et al., 2015; Verma & Stellacci, 2010; Zhao et al., 2011). The negatively charged tertiary liposomes with specific properties have already been manufactured in the previous investigations (Ghaffari et al., 2006; Ghaffari et al., 2008; Gibis et al., 2014). These systems once incorporated into kiwifruit juice, where most of the colloidal system is negatively charged, should be stable with no significant interaction with the beverage matrix.

4.3.9.1 Particle size and zeta potential of the secondary liposomes compared with the primary liposomes

The particle size of chitosan-coated liposomes ranged from about 431.84 to about 443.14 nm, which was significantly higher than that of the primary liposomes (about 184.27 to 187.78 nm) ($p<0.05$). The particle size of the primary liposomes was similar to the values found in the study of Rashidinejad et al. (2014), who entrapped green tea catechins and EGCG with the same methodology as that in this experiment. The particle size of chitosan-coated liposomes was also in agreement with the finding of Laye et al. (2008) who investigated the effect of chitosan concentration on the particle size of liposomes. However, Gibis et al. (2012) and Gibis et al. (2014) presented a smaller particle size of chitosan-coated liposomes, ranging from 100 to 150 nm. Such differences could be due to the application of a different process in their study, where the liposomes were prepared by microfluidisation that is more efficient in size reduction of the manufactured particles. Nonetheless, it is expected that with the addition of chitosan, the particle size of liposomes increases, since chitosan adsorbs to the surface of soy lecithin bilayer by electrostatic attraction and bridging flocculation, and thus, it forms the second layer that results in a thicker wall (Mun et al., 2006; Panya et al., 2010).

The zeta potential of the primary liposomes ranged from about -25.23 to -20.53 mV, while the opposite zeta potential was seen in the case of the chitosan-coated liposomes, ranging from about +26.65 to +33.65 mV during 28 days of storage. The opposite values were resulted from the influence of coating material on the surface charge of liposomes. Because, the soy lecithin liposomes contain negative charges while the chitosan-coated liposomes present positive charges, due to the adsorption of chitosan around the soy vesicle (liposome) membrane (Cuomo et al., 2018; Tan, Feng, et al., 2016). Gibis et al. (2014) reported similar results for the soy lecithin liposomes, but they gained higher zeta potential for the chitosan-coated liposomes, which is due to different conditions of their experiment than what was practised in the current study.

4.3.9.2 Behaviour of the secondary liposomes in kiwifruit juice

4.3.9.2.1 Particle size and zeta potential

As shown in Table 4.8, the size of the particles in the kiwifruit juice containing chitosan-coated liposomes was significantly higher than that of the control juice (approximately 2.90 µm) at any point of the storage period, especially on Day 28 (around 22.62 µm). This might be attributed to the presence of calcium ion (Ca^{2+}), pectin methylesterase (PME) activity, and the electrostatic attraction between chitosan-coated liposomes and pectin polysaccharides. Wicker et al. (2002) reported the effect of PME and calcium ion on the clarification of orange juice. The cloud stability of orange juice stored at 4°C was eight days for the juice containing PME and calcium ion and 13 days for the control juice. The addition of PME and calcium ion could increase the particle size of the orange juice up to 30 µm (D[4, 3]-Volume weighted mean) after eight days. Therefore, the presence of PME and calcium ion (approximately 34 mg/100 g FW) in

kiwifruit juice might be one of the responsible factors for increasing the size of the particles in the kiwifruit juice fortified with chitosan-coated liposomes. Chitosan-coated liposomes contain positive charges while pectin in kiwifruit juice contains negative charges; thus, they can interact with each other by electrostatic force (Sorrivas et al., 2006; Zhou et al., 2018). This reaction can be considered as another cause of the increase in particle size observed since Day 0, which could be clearly explained by TEM micrographs in Section 4.3.9.2.2 (Figure 4.14).

Table 4.8 The particle size (D [4, 3]-volume weighted mean (μm)) and zeta potential (mV) of kiwifruit juice containing chitosan-coated liposomes during the 28 days of storage at 4°C.

Analytical methods/storage period	**Day 0**	**Day 7**	**Day 14**	**Day 28**
Particle size (D [4, 3]-volume weighted mean (μm))				
Control kiwifruit juice	2.85 ± 0.04^{aB}	2.90 ± 0.05^{aB}	2.88 ± 0.04^{aB}	2.91 ± 0.02^{aB}
Kiwifruit juice containing chitosan-coated liposomes	3.23 ± 0.07^{bA}	3.34 ± 0.08^{bA}	3.27 ± 0.11^{bA}	22.62 ± 1.71^{aA}
Zeta potential (mV)				
Control kiwifruit juice	-8.80 ± 0.46^{aA}	-8.30 ± 0.55^{aA}	-8.45 ± 0.46^{aA}	-9.06 ± 0.79^{aA}
Kiwifruit juice containing chitosan-coated liposomes	-8.98 ± 0.46^{aA}	-8.77 ± 0.49^{aA}	-8.11 ± 0.52^{aA}	-8.88 ± 0.66^{aA}

Different lowercase superscripts indicate significant ($p<0.05$) differences among the same kiwifruit juice samples for different storage time. Different uppercase superscripts stand for statistically significant ($p<0.05$) differences among different kiwifruit juice samples at the same storage time.

The zeta potential of the kiwifruit juice containing chitosan-coated liposomes was almost as same as the control juice throughout the storage period (Table 4.8). This might be due to the fact that the concentration of chitosan-coated liposomes added in the kiwifruit juice was not high enough to influence the surface charge of the particles in the juice. In other words, it is possible that the negative charges of kiwifruit juice particles

dominated the positive charges of chitosan. Therefore, there was no significant difference between the zeta potential of the control kiwifruit juice and the kiwifruit juice containing chitosan-coated liposomes. These results are partially in line with the finding of Rashidinejad, Birch and Everett (2016) who measured the zeta potential of catechin, EGCG, and green tea extract, added to the milk fat globules dispersions at different concentrations (125, 250, 500, and 1000 ppm). Based on their study (Rashidinejad, Birch, & Everett, 2016), in the case of the low concentration of catechins, the zeta potential of milk fat did not significantly change ($p>0.05$). For example, the zeta potential of milk fat fortified with catechins in the concentration of 125, 250, and 500 ppm ranged from about -12.70 to -12.67 mV, which was not significantly different ($p>0.05$) from the pure milk fat (control dispersion; -12.67 mV), but the milk fat containing 1000 ppm catechins gained a surface charge of -13.34 mV, which was significantly ($p<0.05$) higher than the control. Based on this evidence, the concentration of chitosan-coated liposomes in the current study may have not been high enough to change the zeta potential of the kiwifruit juice.

4.3.9.2.2 Morphology

According to Figure 4.14, the chitosan-coated liposomes were attached to the fibre in kiwifruit juice, possibly due to the electrostatic attractions between the positively charged chitosan-coated liposomes and negatively charged pectin. The morphology of chitosan-coated liposomes and fibre seen in the current study is close to that has been reported by Mady et al. (2009), as well as the findings presented by Sorrivas et al. (2006), who reported the morphology of chitosan-coated liposomes and apple fibre, respectively.

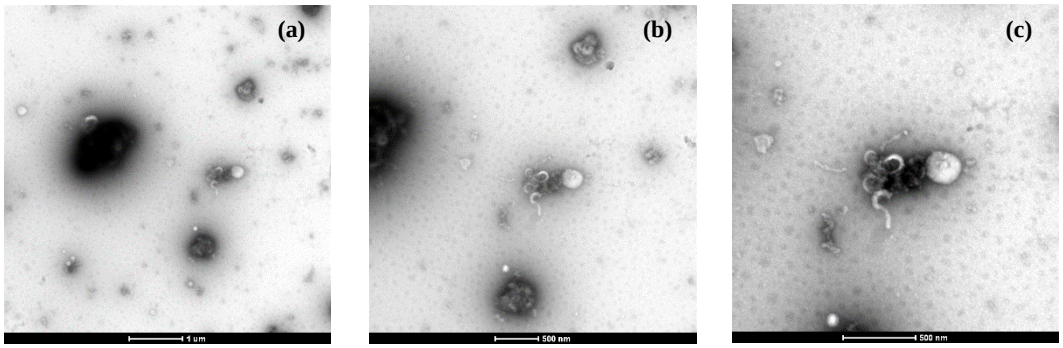


Figure 4.14 Transmission electron micrographs of the kiwifruit juice containing chitosan-coated liposomes at different magnification; (a) 16500×, (b) 26500×, and (c) 43000×.

4.3.9.2.3 Total phenolic content and total antioxidant activity

As can be seen from the results presented in Table 4.9, the kiwifruit juice containing chitosan-coated liposomes showed significantly higher TPC value than both the control kiwifruit juice and the kiwifruit juice fortified with free GTW catechins ($p<0.05$) on Day 0. However, from Day 7 until the end of the storage period (i.e., Day 28), the TPC values of the kiwifruit juice containing chitosan-coated liposomes were not significantly different ($p>0.05$) than the kiwifruit juice fortified with free GTW catechins, although still higher than the TPC value of control juice.

In terms of the total antioxidant activity analysed by DPPH assay, like TPC values, on Day 0, the kiwifruit juice containing chitosan-coated liposomes presented significantly higher values than both the control and the kiwifruit juice fortified with free GTW catechins ($p<0.05$). However, there was no significant difference between the kiwifruit juice containing chitosan-coated liposomes and the kiwifruit juice fortified with free GTW catechins after 7 days of storage. These values are lower than those reported in Sections 4.3.5 and 4.3.6, because of the difference in the type of liposomes that showed different stability after their incorporation into the kiwifruit juice. It could be presumed

that soy lecithin liposomes (primary liposomes) had more potential to protect GTW catechins in kiwifruit juice than chitosan-coated liposomes. Possibly, the repulsion force between soy lecithin liposomes and fibre in kiwifruit juice could protect the aggregation and precipitation processes, leading to better stability of the primary liposomes. On the other hand, the interactions between chitosan in the case of the secondary liposomes and fibre in kiwifruit juice resulted in the instability of such particles. Therefore, it can be said that in the case of this study, in terms of the protection of GTW catechins in kiwifruit juice, the Single-layer (soy lecithin) liposomes were more efficient than Double-layer (chitosan-coated) liposomes. Nonetheless, this cannot be generalised, meaning that the chitosan-coated liposomes could be more efficient for the incorporation of some other bioactives and into some other food formulations. In this study, only one hypothesis was tested and that was based on the information available in the literature. As it was stated previously, the second experiment (i.e., the incorporation of a three-layer liposomal system), although out of the scope of this study, could not be completed due to the experimental disruption caused by Covid-19 pandemic.

Table 4.9 Total phenolic content and total antioxidant activity of kiwifruit juice containing chitosan-coated liposomes during the 28 days of storage at 4°C.

Analytical methods/storage period	Day 0	Day 7	Day 14	Day 28
Total phenolic content (catechin equivalent (ppm))				
Kiwifruit juice (control)	253.30 ± 27.40^{aB}	174.09 ± 7.83^{bB}	141.16 ± 11.94^{cB}	122.29 ± 10.90^{cB}
Kiwifruit juice + free GTW catechins	290.37 ± 19.11^{aB}	273.59 ± 9.28^{aA}	238.90 ± 10.06^{bA}	224.24 ± 11.83^{bA}
Kiwifruit juice + chitosan-coated liposomes	359.86 ± 30.65^{aA}	263.99 ± 9.39^{bA}	226.44 ± 8.07^{cA}	226.12 ± 11.36^{cA}
Total antioxidant activity (Catechin equivalent (ppm))				
Kiwifruit juice (control)	248.64 ± 20.28^{aC}	245.90 ± 26.90^{aB}	205.07 ± 23.46^{aB}	195.30 ± 18.85^{aB}
Kiwifruit juice + free GTW catechins	514.90 ± 34.60^{aB}	487.30 ± 46.70^{aA}	476.20 ± 30.80^{aA}	461.50 ± 37.90^{aA}
Kiwifruit juice + chitosan-coated liposomes	555.08 ± 26.58^{aA}	488.21 ± 11.56^{bA}	480.24 ± 10.27^{bA}	472.67 ± 18.35^{bA}

*Different lowercase letters indicate significant ($p<0.05$) differences among the same kiwifruit juice samples for different storage time. Different uppercase letters stand for statistically significant ($p<0.05$) differences among the different kiwifruit juice samples at the same storage time.

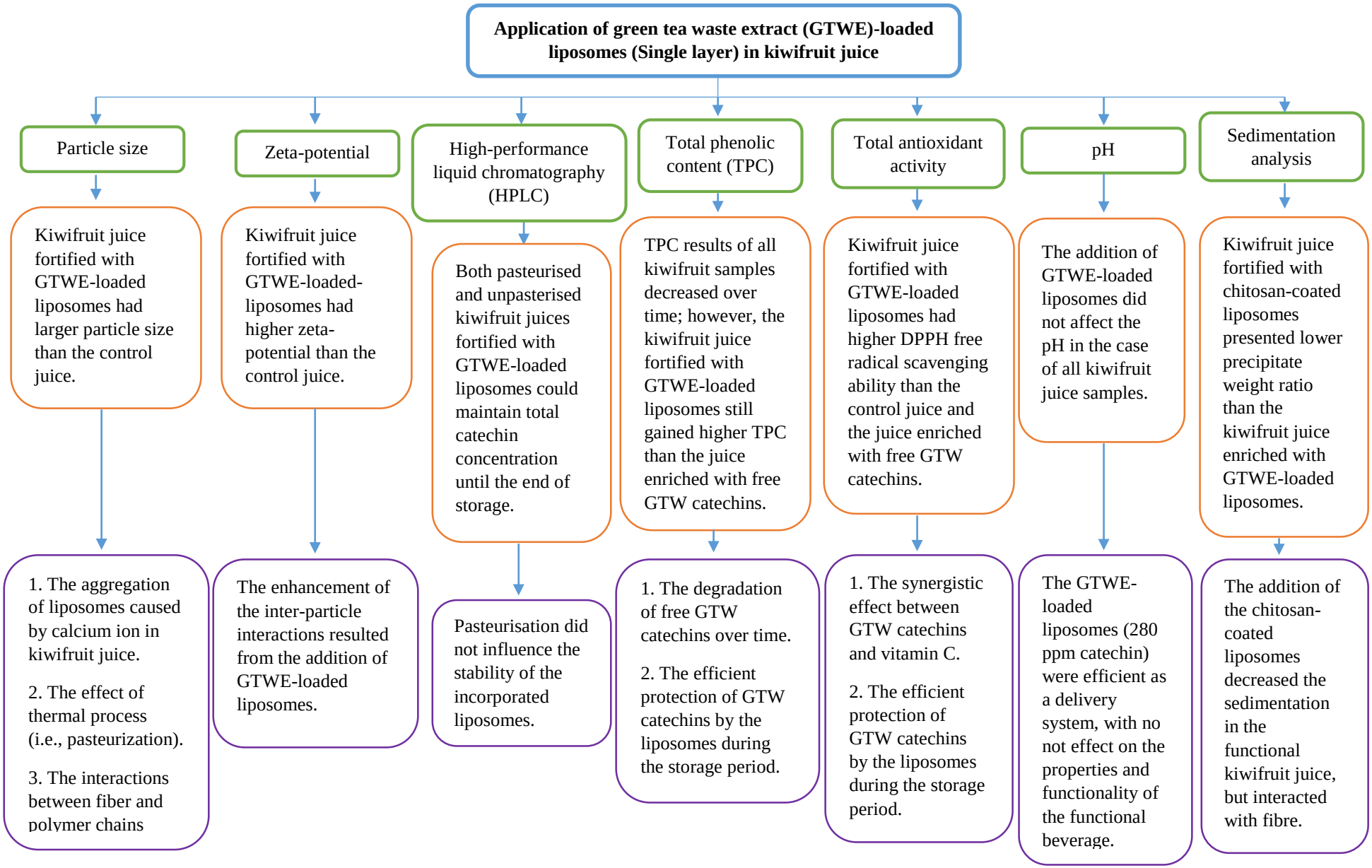


Figure 4.15 A summary flowchart for the results and discussion of the application of GTWE loaded-liposomes (Single-layer) in the kiwifruit juice section. ▭ : Section name, ▭ : Evaluation factors, ▭ : Results, and ▭ : Discussions

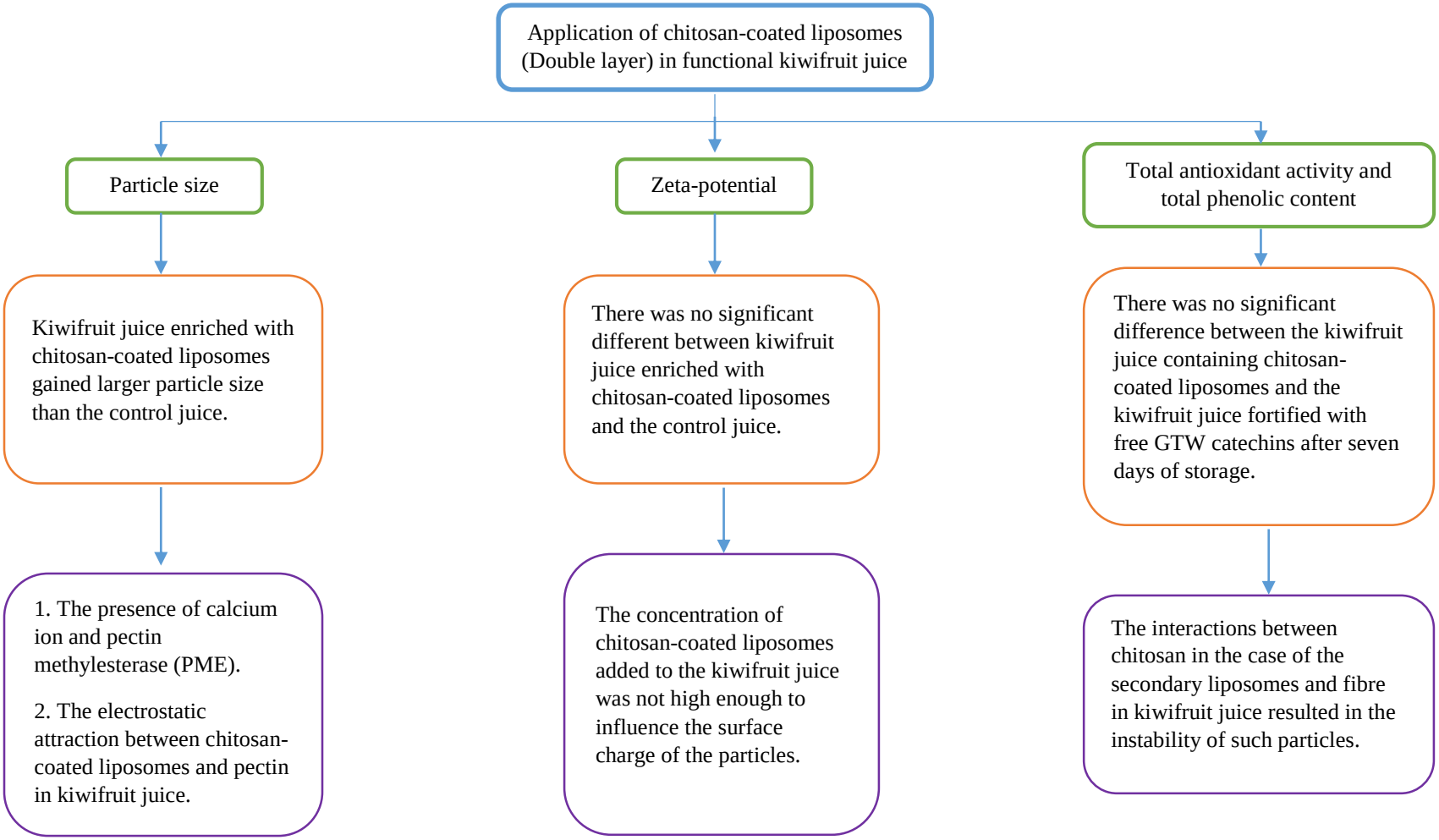


Figure 4.16 A summary flowchart for the results and discussion of the application of chitosan-coated liposomes (Double-layer) in the kiwifruit juice section. ▭ : Section name, ▭ : Evaluation factors, ▭ : Results, and ▭ : Discussions

Chapter 5. Conclusions, recommendations, and future directions

5.1. Conclusions

The extraction of catechins from green tea waste (GTW) was investigated by applying various extraction techniques and solvent ratios. Based on the findings, it can be concluded that hot water extraction provided the highest extraction yield compared with both ultrasonic-assisted and ethanol extractions at the ratio of 1:50. Total catechins from hot water extraction also showed the highest catechin concentration in the case of every ratio (i.e., 1:100, 1:50, or 1:20).

The liposomal technology was implemented to entrap green tea waste extract (GTWE), where various properties of soy lecithin (Single-layer) liposomes were investigated using the corresponding techniques. The Single-layer liposomes possessed a high encapsulation efficiency (~70 %), encapsulation yield (~75 %), and loading capacity (~37 %). They could also maintain their original particle size (~44 nm) and zeta potential (>-35 mV) during the entire 28 days of storage. TEM micrographs revealed a homogenous dispersion of these liposomes, reflecting their advanced physical stability. Therefore, the manufactured soy lecithin liposomes containing GTWE were used for the fortification of a kiwifruit juice, in order to boost its phenolic content and antioxidant activity and conveniently deliver green tea catechins in a functional beverage matrix.

Kiwifruit juice was chosen as the suitable delivery vehicle for GTWE-loaded liposomes, due to the potential synergistic effect between catechins and vitamin C. Even though the addition of GTWE-loaded (Single-layer) liposomes increased the particle size and zeta potential of the kiwifruit juice, the TEM micrographs illustrated the separation

of GTWE-loaded liposomes and kiwifruit fibre molecules, indicating their stability in the juice. Additionally, the fortification of GTWE-loaded liposomes could increase the total phenolic content, total antioxidant activity, and total catechin concentration (analysed by TPC, DPPH, and HPLC, respectively) of the kiwifruit juice, without any alteration of its pH. High correlations were found between HPLC/DPPH, HPLC/TPC, and TPC/DPPH, meaning that all applied analytical methods were suitable for assessing the phenolic contents, antioxidant activity, and catechin content of the kiwifruit juice fortified with GTWE-loaded liposomes.

Based on the evidence from the literature and the hypothesis that an additional (i.e., second) layer could result in further improvement of the properties of liposomes and their behaviour in the kiwifruit juice, the Single-layer liposomes were coated with chitosan to manufacture Double-layer liposomes. However, the results showed that the chitosan-coated liposomes could not protect GTWE as efficiently as the soy lecithin liposomes when incorporated into kiwifruit juice, meaning that null hypothesis (*H*0) was confirmed. TEM images of the kiwifruit juice containing these liposomes showed that the attraction of chitosan layer and kiwifruit fibre molecules increased the size of the particles in this beverage. Additionally, the total phenolic content and total antioxidant activity were lower, when compared with the primary (Single-layer) liposomes. This was possibly due to the leakage of the encapsulated catechins from the sedimented liposomes (caused by the interactions between chitosan and fibre) and their consequent degradation.

All in all, the findings of this research confirmed the efficient utilisation of GTW for the extraction of its high-value bioactive compounds (i.e., catechins) using a green (hot water), industrially-relevant, simple, and efficient method, and their subsequent

incorporation into a kiwifruit juice for the enhancement of its functionality. This research adds value to the available knowledge around the valorisation of GTW that is currently known as a low-value by-product and the use of its extract as a high-value ingredient for the creation of functional food products.

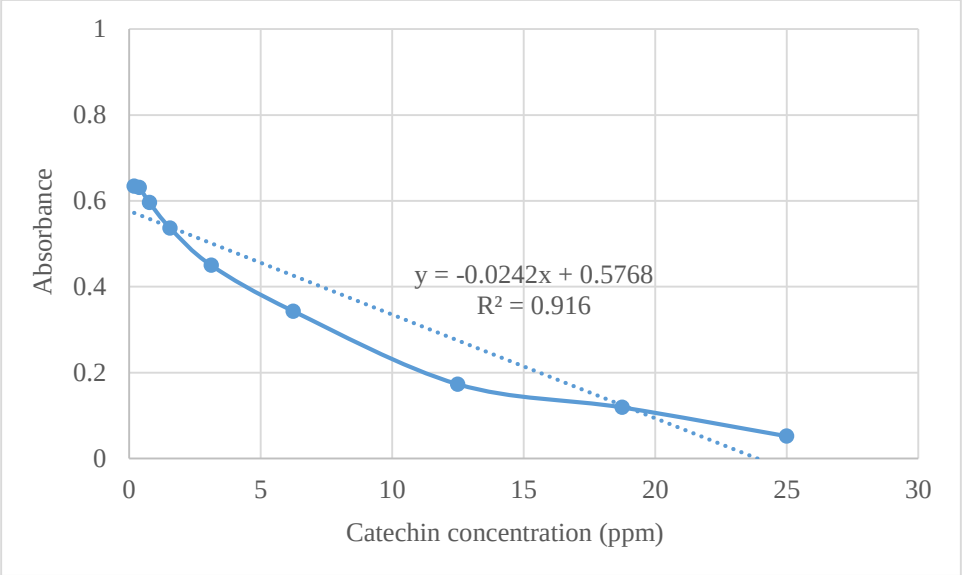
5.2. Recommendations and future directions

The developed functional kiwifruit juice as the outcome of this study can fulfil the adequate intake of catechins that present health-promoting properties such as strengthening the immune system and the prevention of chronic diseases and disorders (e.g., cardiovascular disease, cancer, obesity, and diabetes). Nevertheless, future development should be focused on the tertiary (Three-layer) liposomes containing catechins from GTW and their incorporation into kiwifruit juice, which could not be completed during the current investigation. Along these lines, the interactions between catechins from GTW and the components of kiwifruit juice (e.g., actinidin, fibre, sugar, minerals, and vitamins) can be studied at the molecular level.

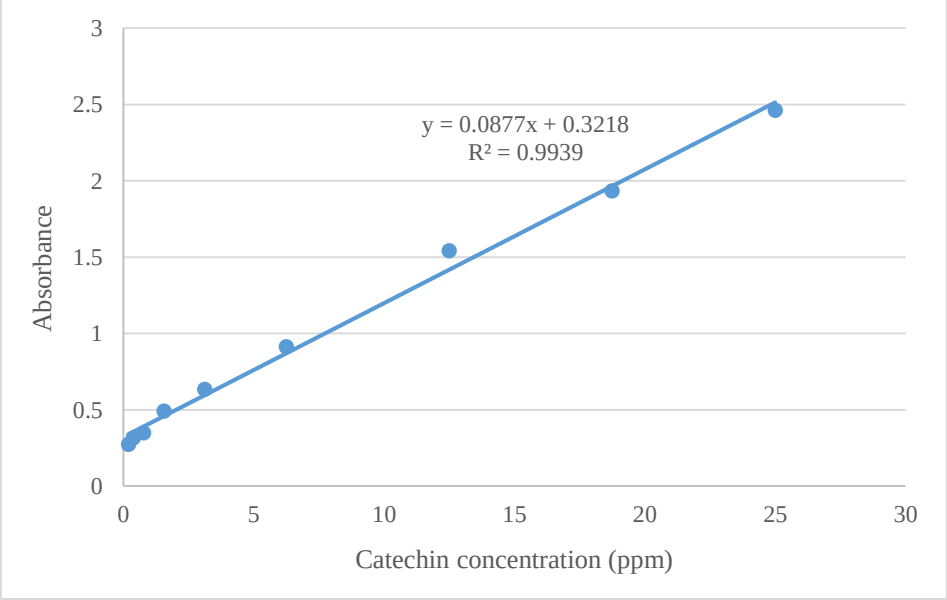
Additionally, the *in vitro* release of catechins from liposomes, along with the assessment of their bioaccessibility, bioavailability, and toxicity, is yet to be studied. While in this study, the effect of only one pasteurisation method on the stability of GTWE incorporated into the kiwifruit juice was experimented, the effect of other pasteurisation methods/heat treatments is yet to remain unknown. Finally, there appear to be unclear regulations (or in the case of some countries, lack of regulations) around the functional food products containing encapsulated bioactives and the corresponding health claims. Therefore, this needs to be addressed when the development of functional kiwifruit juice containing liposomal encapsulated catechins is considered.

Appendices

Appendix 1 A calibration curve of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical assay.



Appendix 2 A calibration curve of the Folin-Ciocâlteu method**.**



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Appendix 4 Supportive pictured taken during various experimentations.

Figure 1 The physical appearance of dried green tea waste obtained from Ghuizhou Eight Grams Tea and Agricultural Development Ltd. (Qiannan, China).

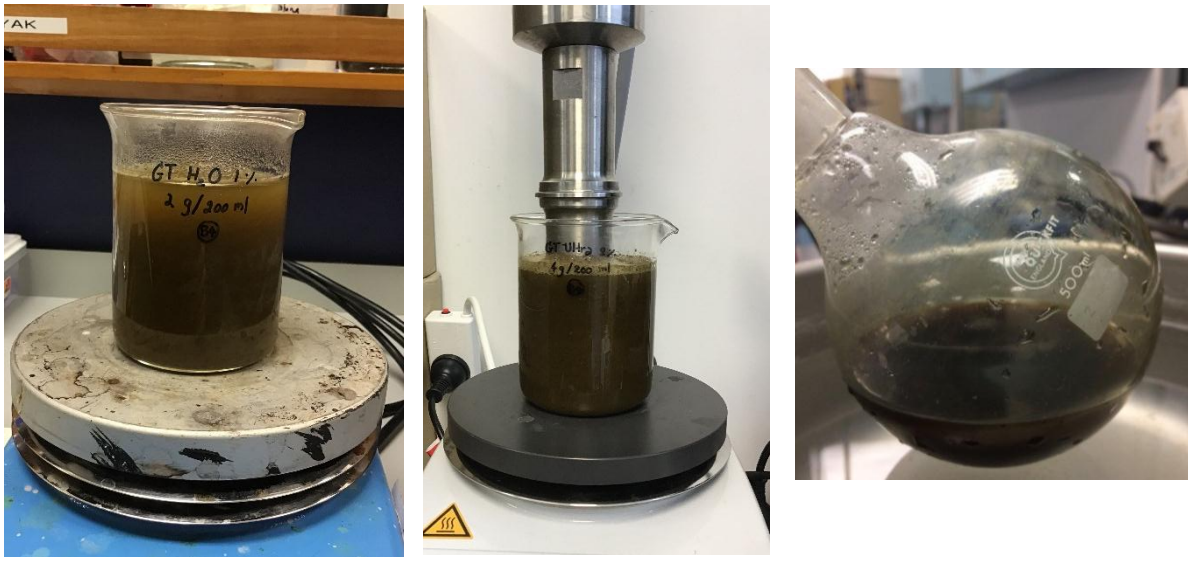

Figure 2 The green tea waste mixture extracted by hot water (Left), ultrasound-assisted (Middle), and ethanol (Right) extraction methods. The hot plate (Left and middle) and water bath (Right) were applied to increase the mixture temperature in order to achieve the target temperature. A digital thermometer was used to monitor the temperature.

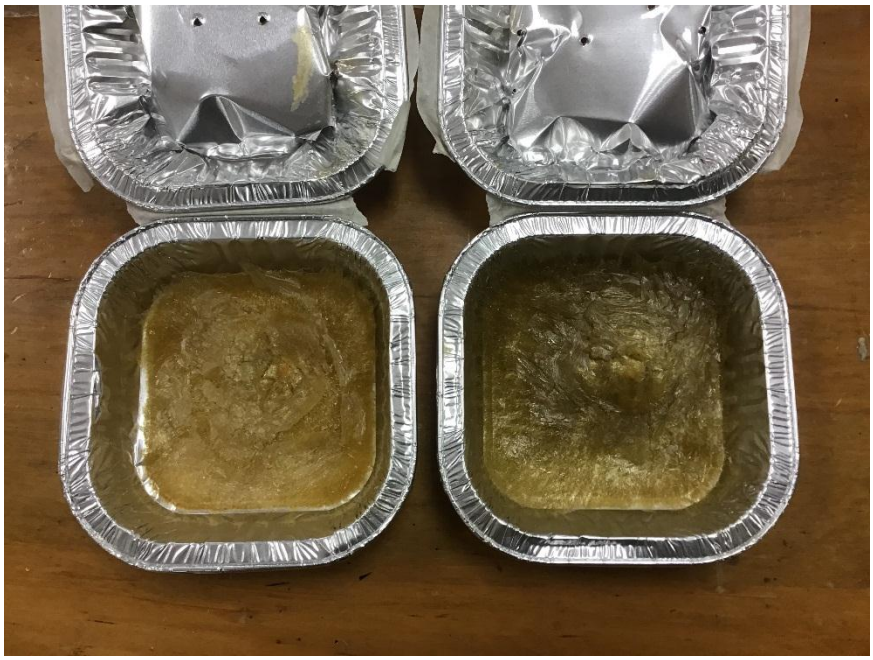

Figure 3. The physical appearance of green tea waste extract after freeze drying process. Left: Hot water extraction; Right: Ultrasound-assisted extraction.

Figure 4. The physical appearance of green tea waste extracted by ethanol.

Figure 5. The physical appearance of kiwifruit juice before filtration (Left) and after filtration (Right).

References

Adlercreutz, H., & Mazur, W. (1997). Phyto-oestrogens and Western diseases. *Annals of Medicine, 29*(2), 95-120.

Afroz Bakht, M., Geesi, M. H., Riadi, Y., Imran, M., Imtiyaz Ali, M., Ahsan, M. J., & Ajmal, N. (2019). Ultrasound-assisted extraction of some branded tea: Optimization based on polyphenol content, antioxidant potential and thermodynamic study. *Saudi Journal of Biological Sciences, 26*(5), 1043-1052.

Ahmad, N., Ahmad, R., Naqvi, A. A., Alam, M. A., Ashafaq, M., Samim, M., Iqbal, Z., & Ahmad, F. J. (2016). Rutin-encapsulated chitosan nanoparticles targeted to the brain in the treatment of Cerebral Ischemia. *International Journal of Biological Macromolecules, 91*, 640-655.

Ahn-Jarvis, J. H., Parihar, A., & Doseff, A. I. (2019). Dietary Flavonoids for Immunoregulation and Cancer: Food Design for Targeting Disease. *Antioxidants, 8*(7). https://doi.org/10.3390/antiox8070202

Alaimo, M. H., Farrell Jr, H. M., & Germann, M. W. (1999). Conformational analysis of the hydrophobic peptide αs1-casein. *Biochimica et biophysica Acta-Protein Structure and Molecular Enzymology, 1431*(2), 410-420.

Ambrose, J. A., & Singh, M. (2015). Pathophysiology of coronary artery disease leading to acute coronary syndromes. *F1000Prime Reports, 7*, 7-8.

Ananingsih, V. K., Sharma, A., & Zhou, W. (2013). Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International, 50*(2), 469-479.

Bandyopadhyay, P., Ghosh, A. K., & Ghosh, C. (2012). Recent developments on polyphenol–protein interactions: effects on tea and coffee taste, antioxidant properties and the digestive system. *Food and Function, 3*(6), 592-605.

Barenholz, Y., Gibbes, D., Litman, B., Goll, J., Thompson, T., & Carlson, F. (1977). A simple method for the preparation of homogeneous phospholipid vesicles. *Biochemistry, 16*(12), 2806-2810.

Beekwilder, J., Hall, R. D., & De Vos, C. (2005). Identification and dietary relevance of antioxidants from raspberry. *Biofactors, 23*(4), 197-205.

Bettuzzi, S., Brausi, M., Rizzi, F., Castagnetti, G., Peracchia, G., & Corti, A. (2006). Chemoprevention of human prostate cancer by oral administration of green tea catechins in volunteers with high-grade prostate intraepithelial neoplasia: a preliminary report from a one-year proof-of-principle study. *Cancer Research, 66*(2), 1234-1240.

Boeing, H., Bechthold, A., Bub, A., Ellinger, S., Haller, D., Kroke, A., Leschik-Bonnet, E., Müller, M. J., Oberritter, H., & Schulze, M. (2012). Critical review: vegetables and fruit in the prevention of chronic diseases. *European Journal of Nutrition, 51*(6), 637-663.

Bravo, L. (1998). Polyphenols: Chemistry, Dietary Sources, Metabolism, and Nutritional Significance. *Nutrition Reviews, 56,* 317-333.

Cai, Y. J., Ma, L. P., Hou, L. F., Zhou, B., Yang, L., & Liu, Z. L. (2002). Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes. *Chemistry and Physics of Lipids, 120*(1-2), 109-117.

Chang, K. (2015). World tea production and trade Current and future development. *Food and Agriculture Organization Of The United Nations, Rome*.

Chao, Y. C., & Chiang, B. H. (1999). The roles of catechins and caffeine in cream formation in a semi-fermented tea. *Journal of the Science of Food and Agriculture, 79*(12), 1687-1690.

Charalampopoulos, D., Wang, R., Pandiella, S., & Webb, C. (2002). Application of cereals and cereal components in functional foods: a review. *International Journal of Food Microbiology, 79*(1-2), 131-141.

Charlson, F. J., Moran, A. E., Freedman, G., Norman, R. E., Stapelberg, N. J., Baxter, A. J., Vos, T., & Whiteford, H. A. (2013). The contribution of major depression to the global burden of ischemic heart disease: a comparative risk assessment. *BioMed Central, 11*(1), 250.

Charlton, A. J., Baxter, N. J., Khan, M. L., Moir, A. J., Haslam, E., Davies, A. P., & Williamson, M. P. (2002). Polyphenol/peptide binding and precipitation. *Journal of Agricultural and Food Chemistry, 50*(6), 1593-1601.

Chen, I. J., Liu, C. Y., Chiu, J. P., & Hsu, C. H. (2016). Therapeutic effect of high-dose green tea extract on weight reduction: A randomized, double-blind, placebo-controlled clinical trial. *Clinical Nutrition, 35*(3), 592-599.

Chen, Y., Li, W., Qiu, S., Vladmir, C., Xu, X., Wang, X., Nian, X., Chen, Q., Wang, Q., Tu, P., Zhang, L., Yan, S., Li, K., Chen, J., Wu, H., Wang, X., Wang, X., Liu, J., Cai, M., Wang, Z., Wang, B., & Sun, Z. (2020). Tea consumption and risk of diabetes in the Chinese population: a multi-centre, cross-sectional study. *British Journal of Nutrition, 123*(4), 428-436.

Chen, Z. Y., Zhu, Q. Y., Tsang, D., & Huang, Y. (2001). Degradation of green tea catechins in tea drinks. *Journal of Agricultural and Food Chemistry, 49*(1), 477-482.

Chen, Z., Bertin, R., & Froldi, G. (2013). EC50 estimation of antioxidant activity in DPPH. assay using several statistical programs. *Food Chemistry*, *138*(1), 414-420.

Cheynier, V. (2005). Polyphenols in foods are more complex than often thought. *The American Journal of Clinical Nutrition, 81*(1), 223S-229S.

Choung, M. G., Hwang, Y. S., Lee, M. S., Lee, J., K., S. T., & Jun, T. H. (2014). Comparison of extraction and isolation efficiency of catechins and caffeine from green tea leaves using different solvent systems. *International Journal of Food Science & Technology, 49*(6), 1572-1578.

Chow, H. S., Hakim, I. A., Vining, D. R., Crowell, J. A., Ranger-Moore, J., Chew, W. M., Celaya, C. A., Rodney, S. R., Hara, Y., & Alberts, D. S. (2005). Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clinical Cancer Research, 11*(12), 4627-4633.

Clifford, M., & Scalbert, A. (2000). Ellagitannins, occurrence in food, bioavailability and cancer prevention. *Journal of the Science of Food and Agriculture, 80*, 1118-1125.

Clifford, M. N. (1999). Chlorogenic acids and other cinnamates–nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 79*(3), 362-372.

Cuomo, F., Cofelice, M., Venditti, F., Ceglie, A., Miguel, M., Lindman, B., & Lopez, F. (2018). In-vitro digestion of curcumin loaded chitosan-coated liposomes. *Colloids and Surfaces B: Biointerfaces, 168*, 29-34.

Dag, D., & Oztop, M. H. (2017). Formation and Characterization of Green Tea Extract Loaded Liposomes. *Journal of Food Science, 82*(2), 463-470.

Damodaran, S. (2017). *Food proteins and their applications*. Routledge.

Danilo, C. (2011). *Handbook of HPLC* (2nd ed.). CRC Press.

Dawes, H. M., & Keene, J. B. (1999). Phenolic composition of kiwifruit juice. *Journal of Agricultural and Food Chemistry, 47*(6), 2398-2403.

de Leeuw, J., de Vijlder, H. C., Bjerring, P., & Neumann, H. A. (2009). Liposomes in dermatology today. *Journal of the European Academy of Dermatology and Venereology, 23*(5), 505-516.

de Mejia, E. G., Ramirez-Mares, M. V., & Puangpraphant, S. (2009). Bioactive components of tea: cancer, inflammation and behavior. *Brain, Behavior, and Immunity, 23*(6), 721-731.

Dons, F., Sessa, M., & Ferrari, G. (2019). Encapsulation of bioactive compounds. *Applications of Encapsulation and Controlled Release*, 405.

Doyon, M., & Labrecque, J. (2008). Functional foods: a conceptual definition. *British Food Journal*. *110*(11), 1133-1149.

Drummond, L. (2013). The composition and nutritional value of kiwifruit. *Advances in Food and Nutrition Research, 68*, 33-57.

Du, G., Li, M., Ma, F., & Liang, D. (2009). Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. *Food Chemistry, 113*(2), 557-562.

Du, G. J., Zhang, Z., Wen, X. D., Yu, C., Calway, T., Yuan, C. S., & Wang, C. Z. (2012). Epigallocatechin Gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients, 4*(11), 1679-1691.

Dube, A., Ng, K., Nicolazzo, J. A., & Larson, I. (2010). Effective use of reducing agents and nanoparticle encapsulation in stabilizing catechins in alkaline solution. *Food Chemistry, 122*(3), 662-667.

Ellench, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fiber and fiber–rich by products of food processing; characterisation, technological functionality any Commercial applications. *A review. Food Chemistry, 124*, 411-421.

Ersus, S., & Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (Daucus carota L.) by spray drier. *Journal of Food Engineering, 80*(3), 805-812.

Feng, S., Sun, Y., Wang, P., Sun, P., Ritzoulis, C., & Shao, P. (2019). Co-encapsulation of resveratrol and epigallocatechin gallate in low methoxyl pectin-coated liposomes with great stability in orange juice. *International Journal of Food Science & Technology, 55*(5), 1872-1880.

Ferguson, A., & Ferguson, L. (2003). Are kiwifruit really good for you?, *Acta Horticulturae*. *610*, 131-138

Ferlay, J., Colombet, M., Soerjomataram, I., Mathers, C., Parkin, D. M., Pineros, M., Znaor, A., & Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International Journal of Cancer, 144*(8), 1941-1953.

Forest, V., Cottier, M., & Pourchez, J. (2015). Electrostatic interactions favor the binding of positive nanoparticles on cells: A reductive theory. *Nano Today, 10*(6), 677-680.

Frémont, L. (2000). Biological effects of resveratrol. *Life sciences, 66*(8), 663-673.

Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Millard, M., & Dumoulin, E. (2006). Encapsulation of oil in powder using spray drying and fluidised bed agglomeration. *Journal of Food Engineering, 75*(1), 27-35.

Gadkari, P. V., & Balaraman, M. (2015). Catechins: Sources, extraction and encapsulation: A review. *Food and Bioproducts Processing, 93*, 122-138.

German, B., Schiffrin, E. J., Reniero, R., Mollet, B., Pfeifer, A., & Neeser, J. R. (1999). The development of functional foods: lessons from the gut. *Trends in Biotechnology, 17*(12), 492-499.

Ghaffari, A., Oskoui, M., Helali, K., Bayati, K., & Rafiee-Tehrani, M. (2006). Pectin/chitosan/Eudragit® RS mixed-film coating for bimodal drug delivery from theophylline pellets: Preparation and evaluation. *Acta Pharmaceutica, 56*(3), 299-310.

Ghaffari, A., Reza, A. M., Reza, M. H., Oskoui, M., Bayati, K., & Rafiee-Tehrani, M. (2008). Mechanistic analysis of drug release from theophylline pellets coated by films containing pectin, chitosan and Eudragit® RS. *Drug Development and Industrial Pharmacy, 34*(4), 390-402.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40*(9), 1107-1121.

Giangrieco, I., Proietti, S., Moscatello, S., Tuppo, L., Battistelli, A., La Cara, F., Tamburrini, M., Famiani, F., & Ciardiello, M. A. (2016). Influence of geographical location of orchards on green kiwifruit bioactive components. *Journal of Agricultural and Food Chemistry, 64*(48), 9172-9179.

Gibis, M., Rahn, N., & Weiss, J. (2013). Physical and oxidative stability of uncoated and chitosan-coated liposomes containing grape seed extract. *Pharmaceutics, 5*(3), 421-433.

Gibis, M., Vogt, E., & Weiss, J. (2012). Encapsulation of polyphenolic grape seed extract in polymer-coated liposomes. *Food and Function, 3*(3), 246-254.

Gibis, M., Zeeb, B., & Weiss, J. (2014). Formation, characterization, and stability of encapsulated hibiscus extract in multilayered liposomes. *Food Hydrocolloids, 38*, 28-39.

Golay, A., & Ybarra, J. (2005). Link between obesity and type 2 diabetes. *Best Practice & Research Clinical Endocrinology & Metabolism, 19*(4), 649-663.

Goodner, J., Braddock, R., Parish, M., & Sims, C. (1999). Cloud stabilization of orange juice by high pressure processing. *Journal of Food Science, 64*(4), 699-700.

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, A. (2014). What is a bioactive compound? A combined definition for a preliminary consensus. *International Journal of Nutrition and Food Sciences, 3*, 174-179.

Gul, K., Singh, A. K., & Jabeen, R. (2016). Nutraceuticals and Functional Foods: The Foods for the Future World. *Critical Reviews in Food Science and Nutrition, 56*(16), 2617-2627.

Guldiken, B., Gibis, M., Boyacioglu, D., Capanoglu, E., & Weiss, J. (2018). Physical and chemical stability of anthocyanin-rich black carrot extract-loaded liposomes during storage. *Food Research International, 108*, 491-497.

Guo, J., Yuan, Y., Dou, P., & Yue, T. (2017). Multivariate statistical analysis of the polyphenolic constituents in kiwifruit juices to trace fruit varieties and geographical origins. *Food Chemistry, 232*, 552-559.

Hara, Y. (2001). *Green Tea: Health Benefits and Applications*. CRC press.

Haratifar, S., & Corredig, M. (2014). Interactions between tea catechins and casein micelles and their impact on renneting functionality. *Food Chemistry, 143*, 27-32.

Hashimoto, T., Kumazawa, S., Nanjo, F., Hara, Y., & Nakayama, T. (1999). Interaction of tea catechins with lipid bilayers investigated with liposome systems. *Bioscience, Biotechnology and Biochemistry, 63*(12), 2252-2255.

Hayashi, N., Ujihara, T., & Kohata, K. (2005). Reduction of catechin astringency by the complexation of gallate-type catechins with pectin. *Bioscience, Biotechnology and Biochemistry, 69*(7), 1306-1310.

Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wähälä, K., Deyama, T., Nishibe, S., & Adlercreutz, H. (2001). In vitro metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *Journal of Agricultural and Food Chemistry, 49*(7), 3178-3186.

Henning, S. M., Fajardo, L. C., Lee, H. W., Youssefian, A. A., Go, V. L., & Heber, D. (2003). Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity. *Nutrition and Cancer, 45*(2), 226-235.

Hidalgo, M., Sánchez-Moreno, C., & de Pascual-Teresa, S. (2010). Flavonoid–flavonoid interaction and its effect on their antioxidant activity. *Food Chemistry, 121*(3), 691-696.

Ho, C. T., Lin, J. K., & Shahadi, F. (2009). *Tea and Tea Products: Chemistry and Health-Promoting Properties*. CRC press.

Hofmann, T., Glabasnia, A., Schwarz, B., Wisman, K. N., Gangwer, K. A., & Hagerman, A. E. (2006). Protein binding and astringent taste of a polymeric procyanidin, 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose, castalagin, and grandinin. *Journal of Agricultural and Food Chemistry, 54*(25), 9503-9509.

Howard, M., Merken,, & Beecher, G., R. (2000). Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review. *Journal of Agricultural and Food Chemistry, 48*, 577-599.

Hussain, S., Anjali, K. P., Hassan, S. T., & Dwivedi, P. B. (2018). Waste tea as a novel adsorbent: a review. *Applied Water Science, 8*(6). https://doi.org/10.1007/s13201-018-0824-5

Igual, M., Contreras, C., Camacho, M. M., & Martínez-Navarrete, N. (2013). Effect of Thermal Treatment and Storage Conditions on the Physical and Sensory Properties of Grapefruit Juice. *Food and Bioprocess Technology, 7*(1), 191-203.

Igual, M., García-Martínez, E., Camacho, M. M., & Martínez-Navarrete, N. (2010). Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chemistry, 118*(2), 291-299.

Iwasa, K. (1976). Physiological aspects of catechin biosynthesis in tea plants. *Japan Agricultural Research Quarterly, 10*, 89-93.

Jafari, S., He, Y., & Bhandari, B. (2006). Nano-emulsion production by sonication and microfluidization—a comparison. *International Journal of Food Properties, 9*(3), 475-485.

Jöbstl, E., O'Connell, J., Fairclough, J. P. A., & Williamson, M. P. (2004). Molecular model for astringency produced by polyphenol/protein interactions. *Biomacromolecules, 5*(3), 942-949.

Kanakis, C., Hasni, I., Bourassa, P., Tarantilis, P., Polissiou, M., & Tajmir-Riahi, H.A. (2011). Milk β-lactoglobulin complexes with tea polyphenols. *Food Chemistry, 127*(3), 1046-1055.

Kara, Ş., & Erçelebi, E. A. (2013). Thermal degradation kinetics of anthocyanins and visual colour of Urmu mulberry (Morus nigra L.). *Journal of Food Engineering, 116*(2), 541-547.

Kartsova, L., & Alekseeva, A. (2008). Effect of milk caseins on the concentration of polyphenolic compounds in tea. *Journal of Analytical Chemistry, 63*(11), 1107-1111.

Kaur, C., George, B., Deepa, N., Jaggi, S., & Kapoor, H. (2007). Viscosity and quality of tomato juice as affected by processing methods. *Journal of Food Quality, 30*(6), 864-877.

Kaur, S., & Das, M. (2011). Functional foods: An overview. *Food Science and Biotechnology, 20*(4), 861-875.

Kennedy, M., List, D., Lu, Y., Foo, L., Robertson, A., Newman, R., & Fenton, G. (1999). Kiwifruit waste and novel products made from kiwifruit waste: uses, composition and analysis. In *Analysis of plant waste materials* (pp. 121-152). Springer.

Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J., & Pincemail, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. *Journal of Agricultural and Food Chemistry, 55*(21), 8596-8603.

Kim, J. M., Kang, J. Y., Park, S. K., Han, H. J., Lee, K. Y., Kim, A. N., Kim, J. C., Choi, S. G., & Heo, H. J. (2020). Effect of storage temperature on the antioxidant activity and catechins stability of Matcha (Camellia sinensis). *Food science and biotechnology, 29*(9), 1261-1271.

Kim, M., Hagiwara, N., Smith, S., Yamamoto, T., Yamane, T., & Takahashi, T. (1994). Preventive effect of green tea polyphenols on colon carcinogenesis. ACS Publications.

Klimczak, I., Małecka, M., Szlachta, M., & Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis, 20*(3-4), 313-322.

Ko, S. H., Choi, S. W., Ye, S. K., Cho, B. L., Kim, H. S., & Chung, M. H. (2005). Comparison of the antioxidant activities of nine different fruits in human plasma. *Journal of medicinal food, 8*(1), 41-46.

Koch, W., Kukula-Koch, W., Czop, M., Helon, P., & Gumbarewicz, E. (2020). The Role of Extracting Solvents in the Recovery of Polyphenols from Green Tea and Its Antiradical Activity Supported by Principal Component Analysis. *Molecules, 25*(9). https://doi.org/10.3390/molecules25092173

Koch, W., Kukula-Koch, W., Komsta, L., Marzec, Z., Szwerc, W., & Glowniak, K. (2018). Green Tea Quality Evaluation Based on Its Catechins and Metals Composition in Combination with Chemometric Analysis. *Molecules, 23*(7). https://doi.org/10.3390/molecules23071689

Kopjar, M., Lončarić, A., Mikulinjak, M., Šrajbek, Ž., Šrajbek, M., & Pichler, A. (2016). Evaluation of antioxidant interactions of combined model systems of phenolics in the presence of sugars. *Natural product communications, 11*(10), 1445-1448.

Koynova, R., & Caffrey, M. (1995). Phases and phase transitions of the sphingolipids. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1255*(3), 213-236.

Kurahashi, N., Sasazuki, S., Iwasaki, M., Inoue, M., Tsugane, S., & Group, J. S. (2008). Green tea consumption and prostate cancer risk in Japanese men: a prospective study. *American Journal of Epidemiology, 167*(1), 71-77.

Kuriyama, S., Shimazu, T., Ohmori, K., Kikuchi, N., Nakaya, N., Nishino, Y., Tsubono, Y., & Tsuji, I. (2006). Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *The Journal of the American Medical Association, 296*(10), 1255-1265.

Lasch, J., Weissig, V., & Brandl, M. (2003). Preparation of liposomes. *Liposomes: a practical approach* (No. 264). Oxford University Press.

Laye, C., McClements, D. J., & Weiss, J. (2008). Formation of biopolymer-coated liposomes by electrostatic deposition of chitosan. *Journal of Food Science, 73*(5), N7-15.

Lee, B. L., & Ong, C. N. (2000). Comparative analysis of tea catechins and theaflavins by high-performance liquid chromatography and capillary electrophoresis. *Journal of chromatography A, 881*(1-2), 439-447.

Lees, M. (2003). *Food authenticity and traceability*. CRC Press.

Leo, M., L., Nollet, & Fidel, T. (2013). *Food Analysis by HPLC* (3rd ed.). CRC Press.

Lernoud, J., Potts, J., Sampson, G., Garibay, S. V., Lynch, M., Voora, V., Willer, H., & Wozniak, J. (2017). The State of Sustainable Markets 2017-Statistics and Emerging Trends.

Li, D., Zhao, Y., Wang, X., Tang, H., Wu, N., Wu, F., Yu, D., & Elfalleh, W. (2020). Effects of (+)-catechin on a rice bran protein oil-in-water emulsion: Droplet size, zeta-potential, emulsifying properties, and rheological behavior. *Food Hydrocolloids, 98*, https://doi.org/10.1016/j.foodhyd.2019.105306

Lin, D. Q., Brixius, P. J., Hubbuch, J. J., Thommes, J., & Kula, M. R. (2003). Biomass/adsorbent electrostatic interactions in expanded bed adsorption: a zeta potential study. *Biotechnology and Bioengineering, 83*(2), 149-157.

Liu, W., Tian, M., Kong, Y., Lu, J., Li, N., & Han, J. (2017). Multilayered vitamin C nanoliposomes by self-assembly of alginate and chitosan: Long-term stability and feasibility application in mandarin juice. *LWT - Food Science and Technology, 75*, 608-615. https://doi.org/10.1016/j.lwt.2016.10.010

Lončarić, A., Pablo Lamas, J., Guerra, E., Kopjar, M., & Lores, M. (2018). Thermal stability of catechin and epicatechin upon disaccharides addition. *International Journal of Food Science & Technology, 53*(5), 1195-1202.

Lorenzo, J. M., & Munekata, P. E. S. (2016). Phenolic compounds of green tea: Health benefits and technological application in food. *Asian Pacific Journal of Tropical Biomedicine, 6*(8), 709-719.

Lun Su, Y., Leung, L. K., Huang, Y., & Chen, Z.Y. (2003). Stability of tea theaflavins and catechins. *Food Chemistry, 83*(2), 189-195.

Lyu, X., Peng, X., Wang, S., Yang, B., Wang, X., Yang, H., Xiao, Y., Baloch, A. B., & Xia, X. (2018). Quality and consumer acceptance of radio frequency and traditional heat pasteurised kiwi puree during storage. *International Journal of Food Science & Technology, 53*(1), 209-218.

Ma, T., Lan, T., Geng, T., Ju, Y., Cheng, G., Que, Z., Gao, G., Fang, Y., & Sun, X. (2019). Nutritional properties and biological activities of kiwifruit (Actinidia) and kiwifruit products under simulated gastrointestinal in vitro digestion. *Food and Nutrition Research, 63*. https://doi.org/10.29219/fnr.v63.1674

Ma, T., Lan, T., Ju, Y., Cheng, G., Que, Z., Geng, T., Fang, Y., & Sun, X. (2019). Comparison of the nutritional properties and biological activities of kiwifruit

(Actinidia) and their different forms of products: towards making kiwifruit more nutritious and functional. *Food and Function, 10*(3), 1317-1329.

Madene, A., Jacquot, M., Scher, J., & Desobry, S. (2006). Flavour encapsulation and controlled release - a review. *International Journal of Food Science and Technology, 41*(1), 1-21.

Mady, M. M., Darwish, M. M., Khalil, S., & Khalil, W. M. (2009). Biophysical studies on chitosan-coated liposomes. *European Biophysics Journal, 38*(8), 1127-1133.

Mahfoudhi, N., Ksouri, R., & Hamdi, S. (2016). Nanoemulsions as potential delivery systems for bioactive compounds in food systems: preparation, characterization, and applications in food industry. In *Emulsions* (pp. 365-403). Academic Press.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727-747.

Marčelja, S., & Wolfe, J. (1979). Properties of bilayer membranes in the phase transition or phase separation region. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 557*(1), 24-31.

Marsanasco, M., Márquez, A. L., Wagner, J. R., del V. Alonso, S., & Chiaramoni, N. S. (2011). Liposomes as vehicles for vitamins E and C: An alternative to fortify orange juice and offer vitamin C protection after heat treatment. *Food Research International, 44*(9), 3039-3046.

Matsui, T., Ebuchi, S., Kobayashi, M., Fukui, K., Sugita, K., Terahara, N., & Matsumoto, K. (2002). Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the α-glucosidase inhibitory action. *Journal of Agricultural and Food Chemistry, 50*(25), 7244-7248.

Mérillon, J. M., & Ramawat, K. G. (2019). *Bioactive Molecules In Food*. Springer.

Muller, R. H., Mader, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*, 161-177.

Mun, S., Decker, E. A., & McClements, D. J. (2006). Effect of molecular weight and degree of deacetylation of chitosan on the formation of oil-in-water emulsions stabilized by surfactant–chitosan membranes. *Journal of colloid and interface science, 296*(2), 581-590.

Muzaffar, S., Ahmad, M., Wani, S. M., Gani, A., Baba, W. N., Shah, U., Khan, A. A., Masoodi, F. A., Gani, A., & Wani, T. A. (2016). Ultrasound treatment: effect on physicochemical, microbial and antioxidant properties of cherry (Prunus avium). *The Journal of Food Science and Technology, 53*(6), 2752-2759.

Nakagawa, H., Mustafa, G., Takii, K., Umino, T., & Kumai, H. (2000). Effect of dietary catechin and Spirulina on vitamin C metabolism in red sea bream. *Fisheries science, 66*(2), 321-326.

Nechuta, S., Shu, X. O., Li, H. L., Yang, G., Ji, B. T., Xiang, Y. B., Cai, H., Chow, W. H., Gao, Y. T., & Zheng, W. (2012). Prospective cohort study of tea consumption and risk of digestive system cancers: results from the Shanghai Women's Health Study. *American Journal of Clinical Nutrition, 96*(5), 1056-1063.

New, R. R. (1990). *Liposomes: A Practical Approach.* IRL at Oxford University Press.

Normington, K. W., Baker, I., Molina, M., Wishnok, J. S., Tannenbaum, S. R., & Puju, S. (1986). Characterization of a nitrite scavenger, 3-hydroxy-2-pyranone, from Chinese wild plum juice. *Journal of Agricultural and Food Chemistry, 34*(2), 215-217.

Ognjenovic, J., Stojadinovic, M., Milcic, M., Apostolovic, D., Vesic, J., Stambolic, I., Atanaskovic-Markovic, M., Simonovic, M., & Velickovic, T. C. (2014). Interactions of epigallo-catechin 3-gallate and ovalbumin, the major allergen of egg white. *Food Chemistry, 164*, 36-43.

Oke, M., Jacob, J. K., & Paliyath, G. (2010). Effect of soy lecithin in enhancing fruit juice/sauce quality. *Food Research International, 43*(1), 232-240.

Ostro, M. J. (1987). Liposomes. *Scientific American, 256*, 102-111.

Ozkan, G., Franco, P., De Marco, I., Xiao, J., & Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. *Food Chemistry, 272*, 494-506.

Panya, A., Laguerre, M., Lecomte, J., Villeneuve, P., Weiss, J., McClements, D. J., & Decker, E. A. (2010). Effects of chitosan and rosmarinate esters on the physical and oxidative stability of liposomes. *Journal of Agricultural and Food Chemistry, 58*(9), 5679-5684.

Park, Y. S., Im, M. H., Ham, K.-S., Kang, S. G., Park, Y. K., Namiesnik, J., Leontowicz, H., Leontowicz, M., Katrich, E., & Gorinstein, S. (2013). Nutritional and pharmaceutical properties of bioactive compounds in organic and conventional growing kiwifruit. *Plant Foods for Human Nutrition, 68*(1), 57-64.

Pasrija, D., & Anandharamakrishnan, C. (2015). Techniques for Extraction of Green Tea Polyphenols: A Review. *Food and Bioprocess Technology, 8*(5), 935-950.

Pastoriza, S., Mesias, M., Cabrera, C., & Rufian-Henares, J. A. (2017). Healthy properties of green and white teas: an update. *Food and Function, 8*(8), 2650-2662.

Pelloux, J., Rusterucci, C., & Mellerowicz, E. J. (2007). New insights into pectin methylesterase structure and function. *Trends in Plant Science, 12*(6), 267-277.

Perva-Uzunalić, A., Škerget, M., Knez, Ž., Weinreich, B., Otto, F., & Grüner, S. (2006). Extraction of active ingredients from green tea (Camellia sinensis): Extraction efficiency of major catechins and caffeine. *Food Chemistry, 96*(4), 597-605.

Petelska, A. D., & Figaszewski, Z. A. (2002). Effect of pH on the interfacial tension of bilayer lipid membrane formed from phosphatidylcholine or phosphatidylserine. *Biochimica et Biophysica Acta, 1561*(2), 135-146.

Phillips, G. O., & Cui, S. W. (2011). An introduction: Evolution and finalisation of the regulatory definition of dietary fibre. *Food Hydrocolloids, 25*(2), 139-143.

Piljac-Žegarac, J., Valek, L., Martinez, S., & Belščak, A. (2009). Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage. *Food Chemistry, 113*(2), 394-400.

Rashidinejad, A., Birch, E. J., & Everett, D. W. (2016). A novel functional full-fat hard cheese containing liposomal nanoencapsulated green tea catechins: manufacture and recovery following simulated digestion. *Food and Function, 7*(7), 3283-3294.

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2013). Effects of catechin on the phenolic content and antioxidant properties of low-fat cheese. *International Journal of Food Science & Technology*, *48*(12), 2448-2455.

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2014). Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. *Food Chemistry*, *156*, 176-183.

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2016). Effect of liposomal encapsulation on the recovery and antioxidant properties of green tea catechins incorporated into a hard low-fat cheese following in vitro simulated gastrointestinal digestion. *Food and Bioproducts Processing*, *100*, 238-245.

Rashidinejad, A., Loveday, S. M., Jameson, G. B., Hindmarsh, J. P., & Singh, H. (2019). Rutin-casein co-precipitates as potential delivery vehicles for flavonoid rutin. *Food Hydrocolloids*, *96*, 451-462.

Ré, M. I., Santana, M. H. A., & d'Ávila, M. A. (2019). Encapsulation technologies for modifying food performance. *Applications of Encapsulation and Controlled Release*, 267.

Rice-Evans, C. (1999). Implications of the mechanisms of action of tea polyphenols as antioxidants in vitro for chemoprevention in humans. *Proceedings of the Society for experimental Biology and Medicine*, *220*(4), 262-266.

Richardson, D. P., Ansell, J., & Drummond, L. N. (2018). The nutritional and health attributes of kiwifruit: a review. *European Journal of Nutrition*, *57*(8), 2659-2676.

Rizvi, S. I., & Zaid, M. A. (2001). Insulin-Like Effect Of (–) Epicatechin On Erythrocyte Membrane Acetylcholinesterase Activity In Type 2 Diabetes Mellitus. *Clinical and Experimental Pharmacology and Physiology*, *28*(9), 776-778.

Rizvi, S. I., Zaid, M. A., Anis, R., & Mishra, N. (2005). Protective role of tea catechins against oxidation-induced damage of type 2 diabetic erythrocytes. *Clinical and Experimental Pharmacology and Physiology*, *32*(1-2), 70-75.

Robards, K., & Antolovich, M. (1997). Analytical Chemistry of Fruit Bioflavonoids: A Review. *Analysis*, *122*, 11R–34R.

Roccaro, A. S., Blanco, A. R., Giuliano, F., Rusciano, D., & Enea, V. (2004). Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrobial Agents and Chemotherapy, 48*(6), 1968-1973.

Rocha, S., Generalov, R., Pereira, M. d. C., Peres, I., Juzenas, P., & Coelho, M. A. (2011). Epigallocatechin gallate-loaded polysaccharide nanoparticles for prostate cancer chemoprevention. *Nanomedicine, 6*(1), 79-87.

Rodriguez-Nogales, J. M., & Delgadillo, A. (2005). Stability and catalytic kinetics of microencapsulated β-galactosidase in liposomes prepared by the dehydration–rehydration method. *Journal of Molecular Catalysis B: Enzymatic, 33*(1-2), 15-21.

Rojas, J., & Buitrago, A. (2019). Antioxidant Activity of Phenolic Compounds Biosynthesized by Plants and Its Relationship With Prevention of Neurodegenerative Diseases. In *Bioactive Compounds* (pp. 3-31). Woodhead Publishing

Ruozi, B., Belletti, D., Tombesi, A., Tosi, G., Bondioli, L., Forni, F., & Vandelli, M. A. (2011). AFM, ESEM, TEM, and CLSM in liposomal characterization: a comparative study. I*nternational Journal of Nanomedicine, 6*, 557-563.

Saklar, A., Sena, Karadeniz, B., Sagcan, N., Bahar, B., Us, A. A., & Alasalvar, C. (2018). Optimizing the extraction parameters of epigallocatechin gallate using conventional hot water and ultrasound assisted methods from green tea. *Food and Bioproducts Processing, 111*, 37-44.

Salminen, W. F., & Russotti, G. (2017). Synergistic interaction of ascorbic acid and green tea extract in preventing the browning of fresh cut apple slices. *Journal of food processing and preservation, 41*(5), https://doi.org/10.1111/jfpp.13192

Sampson, H. A. (2004). Update on food allergy. *Journal of allergy and clinical immunology, 113*(5), 805-819.

Samra, M. A., Chedea, V. S., Economou, A., Calokerinos, A., & Kefalas, P. (2011). Antioxidant/prooxidant properties of model phenolic compounds: Part I. Studies on equimolar mixtures by chemiluminescence and cyclic voltammetry. *Food Chemistry, 125*(2), 622-629.

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods, 5*(21), 5990-5999.

Sanders, M. E. (1998). Overview of functional foods: emphasis on probiotic bacteria. *International Dairy Journal, 8*(5-6), 341-347.

Sano, J., Inami, S., Seimiya, K., Ohba, T., Sakai, S., Takano, T., & Mizuno, K. (2004). Effects of green tea intake on the development of coronary artery disease. *Circulation Journal, 68*(7), 665-670.

Sapei, L., & Hwa, L. (2014). Study on the Kinetics of Vitamin C Degradation in Fresh Strawberry Juices. *Procedia Chemistry, 9*, 62-68.

Saucier, C. T., & Waterhouse, A. L. (1999). Synergetic activity of catechin and other antioxidants. *Journal of Agricultural and Food Chemistry, 47*(11), 4491-4494.

Saura-Calixto, F., & Díaz-Rubio, M. E. (2007). Polyphenols associated with dietary fibre in wine. *Food Research International, 40*(5), 613-619.

Scalbert, A., Johnson, I., T, , & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition, 81*, 215S–217S.

SCENIHR. (2007). Opinion on the scientific aspects of the the existing and proposed definitions relating to products of nanoscience and nanotechnologies. *Health & Consumer Protection Directorate-General, European Commission*.

Sibele, S. F., Michele, S. C., Myriam, d. l. M., & Salas, M. (2019). Bioactive Compounds as Ingredients of Functional Foods: Polyphenols, Carotenoids, Peptides From Animal and Plant Sources New. In *Bioactive Compounds* (pp. 129-142). Woodhead Publishing.

Sims, I. M., & Monro, J. A. (2013). Fiber: composition, structures, and functional properties. *Advances in Food and Nutrition Research, 68*, 81-99.

Sorrivas, V., Genovese, D., & Lozano, J. (2006). Effect of pectinolytic and amylolytic enzymes on apple juice turbidity. *Journal of Food Processing and Preservation, 30*(2), 118-133.

Stalmach, A., Mullen, W., Steiling, H., Williamson, G., Lean, M. E., & Crozier, A. (2010). Absorption, metabolism, and excretion of green tea flavan-3-ols in humans with an ileostomy. *Molecular Nutrition & Food Research, 54*(3), 323-334.

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., & Velickovic, T. C. (2013). Binding affinity between dietary polyphenols and β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chemistry, 136*(3-4), 1263-1271.

Sui, W., Xiao, Y., Liu, R., Wu, T., & Zhang, M. (2019). Steam explosion modification on tea waste to enhance bioactive compounds' extractability and antioxidant capacity of extracts. *Journal of Food Engineering, 261*, 51-59.

Sun-Waterhouse, D., Bekkour, K., Wadhwa, S. S., & Waterhouse, G. I. N. (2013). Rheological and Chemical Characterization of Smoothie Beverages Containing High Concentrations of Fibre and Polyphenols from Apple. *Food and Bioprocess Technology, 7*(2), 409-423.

Sun, Y., Zhong, L., Cao, L., Lin, W., & Ye, X. (2015). Sonication inhibited browning but decreased polyphenols contents and antioxidant activity of fresh apple (malus pumila mill, cv. Red Fuji) juice. *Journal of Food Science and Technology, 52*(12), 8336-8342.

Suzuki-Sugihara, N., Kishimoto, Y., Saita, E., Taguchi, C., Kobayashi, M., Ichitani, M., Ukawa, Y., Sagesaka, Y. M., Suzuki, E., & Kondo, K. (2016). Green tea catechins prevent low-density lipoprotein oxidation via their accumulation in low-density lipoprotein particles in humans. *Nutrition Research, 36*(1), 16-23.

Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry, 120*(2), 599-606.

Taiyo, K. (2020). *Sunphenon product specification*. Retrieved 5 May 2020 from https://taiyokagakuindia.com/sunphenon/

Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science & Emerging Technologies, 19*, 29-43.

Tan, C., Feng, B., Zhang, X., Xia, W., & Xia, S. (2016). Biopolymer-coated liposomes by electrostatic adsorption of chitosan (chitosomes) as novel delivery systems for carotenoids. *Food Hydrocolloids, 52*, 774-784.

Tan, C., Xie, J., Zhang, X., Cai, J., & Xia, S. (2016). Polysaccharide-based nanoparticles by chitosan and gum arabic polyelectrolyte complexation as carriers for curcumin. *Food Hydrocolloids, 57*, 236-245.

Taylor, T. M., Davidson, P. M., Bruce, B. D., & Weiss, J. (2005). Liposomal nanocapsules in food science and agriculture. *Critical Reviews in Food Science and Nutrition, 45*(7-8), 587-605.

Tembo, D. T., Holmes, M. J., & Marshall, L. J. (2017). Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi. *Journal of Food Composition and Analysis, 58*, 40-51.

Tenore, G. C., Campiglia, P., Giannetti, D., & Novellino, E. (2015). Simulated gastrointestinal digestion, intestinal permeation and plasma protein interaction of white, green, and black tea polyphenols. *Food Chemistry, 169*, 320-326.

Thompson, A. K., & Singh, H. (2006). Preparation of Liposomes from Milk Fat Globule Membrane Phospholipids Using a Microfluidizer. *Journal Dairy Science, 89*, 410-419.

Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L., der Plancken, I. V., Hendrickx, M. E., & Matser, A. M. (2011). Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: Impact on overall quality attributes. *Innovative Food Science & Emerging Technologies, 12*(3), 235-243.

Tiwari, B. K. (2015). Ultrasound: A clean, green extraction technology. *Trends in Analytical Chemistry, 71*, 100-109.

Tomas-Barberan, F., & Clifford, M. (2000). Dietary hydroxybenzoic acid derivatives and their possible role in health protection. *Journal of the Science of Food and Agriculture, 80*, 1024-1032.

Toro-Uribe, S., López-Giraldo, L. J., Alvarez-Rivera, G., Ibáñez, E., & Herrero, M. (2019). Insight of Stability of Procyanidins in Free and Liposomal Form under an in Vitro Digestion Model: Study of Bioaccessibility, Kinetic Release Profile,

Degradation, and Antioxidant Activity. *Journal of Agricultural and Food Chemistry, 67*(7), 1990-2003.

Trigo, J. P., Alexandre, E. M. C., Saraiva, J. A., & Pintado, M. E. (2020). High value-added compounds from fruit and vegetable by-products - Characterization, bioactivities, and application in the development of novel food products. *Critical Reviews in Food Science and Nutrition, 60*(8), 1388-1416.

Tunick, M. H., Van Hekken, D. L., Cooke, P. H., & Malin, E. L. (2002). Transmission electron microscopy of mozzarella cheeses made from microfluidized milk. *Journal of Agricultural and Food Chemistry, 50*(1), 99-103.

Valls, J., Millan, S., Marti, M. P., Borras, E., & Arola, L. (2009). Advanced separation methods of food anthocyanins, isoflavones and flavanols. *Journal of Chromatography A, 1216*(43), 7143-7172.

Verma, A., & Stellacci, F. (2010). Effect of surface properties on nanoparticle–cell interactions. *small, 6*(1), 12-21.

Verma, S., & Hussain, M. E. (2017). Obesity and diabetes: An update. *Diabetes and Metabolic Syndrome, 11*(1), 73-79.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*(3), 701-705.

Wang, H., Provan, G. J., & Helliwell, K. (2003). HPLC determination of catechins in tea leaves and tea extracts using relative response factors. *Food Chemistry, 81*(2), 307-312.

Wang, J., Li, H., Chen, Z., Liu, W., & Chen, H. (2016). Characterization and storage properties of a new microencapsulation of tea polyphenols. *Industrial Crops and Products, 89*, 152-156.

Wang, L., Gong, L.H., Chen, C. J., Han, H. B., & Li, H. H. (2012). Column-chromatographic extraction and separation of polyphenols, caffeine and theanine from green tea. *Food Chemistry, 131*(4), 1539-1545.

Wang, R., & Zhou, W. (2004). Stability of tea catechins in the breadmaking process. *Journal of Agricultural and Food Chemistry, 52*(26), 8224-8229.

Wang, Y., Liu, J., Chen, F., & Zhao, G. (2013). Effects of molecular structure of polyphenols on their noncovalent interactions with oat beta-glucan. *Journal of Agricultural and Food Chemistry, 61*(19), 4533-4538.

Ward, C., & Courtney, D. (2013). Kiwifruit: taking its place in the global fruit bowl. In *Advances in Food and Nutrition Research* (Vol. 68, pp. 1-14). Academic press.

Warrington, I. J., & Weston, G. C. (1990). *Kiwifruit: science and management*. Ray Richards.

Weiskirchen, S., & Weiskirchen, R. (2016). Resveratrol: How Much Wine Do You Have to Drink to Stay Healthy? *Advances in Nutrition, 7*(4), 706-718.

WHO. (2017). *Cardiovascular disease (CVDs)*. World Health Organization. Retrieved 18 April 2020 from https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

WHO. (2018). *Diabetes*. World Health Organization. Retrieved 13 April 2020 from https://www.who.int/health-topics/diabetes#tab=tab_1

Wicker, L., Ackerley, J., & Corredig, M. (2002). Clarification of juice by thermolabile Valencia pectinmethylesterase is accelerated by cations. *Journal of Agricultural and Food Chemistry*, *50*(14), 4091-4095.

Willson, K. C., & Clifford, M. N. (1992). *Tea Cultivation to Consumption*. Chapman & Hall.

Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C., & Yamamoto, K. (2011). Interaction of dietary polyphenols with bovine milk proteins: molecular structure–affinity relationship and influencing bioactivity aspects. *Molecular Nutrition & Food Research, 55*(11), 1637-1645.

Xing, L., Zhang, H., Qi, R., Tsao, R., & Mine, Y. (2019). Recent Advances in the Understanding of the Health Benefits and Molecular Mechanisms Associated with Green Tea Polyphenols. *Journal of Agricultural and Food Chemistry, 67*(4), 1029-1043.

Xu, J. Z., Yeung, S. Y., Chang, Q., Huang, Y., & Chen, Z. Y. (2004). Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. *British Journal of Nutrition, 91*(6), 873-881.

Xu, Y. Q., Chen, G. S., Du, Q. Z., Que, F., Yuan, H. B., & Yin, J. F. (2014). Sediments in concentrated green tea during low-temperature storage. *Food Chemistry, 149*, 137-143.

Xu, Y.-Q., Hu, X.-F., Zou, C., Shi, J., Du, Q.-Z., Teng, B.-T., & Yin, J.-F. (2017). Effect of saccharides on sediment formation in green tea concentrate. *LWT - Food Science and Technology, 78*, 352-360.

Ye, J., Fan, F., Xu, X., & Liang, Y. (2013). Interactions of black and green tea polyphenols with whole milk. *Food Research International, 53*(1), 449-455.

Yoder, S. C., Lancaster, S. M., Hullar, M. A., & Lampe, J. W. (2015). Gut microbial metabolism of plant lignans: influence on human health. In *Diet-Microbe Interactions in the Gut* (pp. 103-117). Elsevier.

Yuerong, L., & Bee, R. (1995). Effect of Carbohydrates and Quenching on Black TeaCream Formation. *Journal of Zhejiang Agricultural University*(5), 05.

Yuksel, Z., Avci, E., & Erdem, Y. K. (2010). Characterization of binding interactions between green tea flavanoids and milk proteins. *Food Chemistry, 121*(2), 450-456.

Zhang, L., Mou, D., & Du, Y. (2007). Procyanidins: extraction and micro- encapsulation. *Journal of the Science of Food and Agriculture, 87*(12), 2192-2197.

Zhao, D., & Shah, N. P. (2014). Antiradical and tea polyphenol-stabilizing ability of functional fermented soymilk–tea beverage. *Food Chemistry, 158*, 262-269.

Zhao, F., Zhao, Y., Liu, Y., Chang, X., Chen, C., & Zhao, Y. (2011). Cellular uptake, intracellular trafficking, and cytotoxicity of nanomaterials. *small, 7*(10), 1322-1337.

Zhen, Y. S., Chen, Z. M., Cheng, S. J., & Chen, M. L. (2002). *Tea: Bioactivity and Therapeutic Potential*. Taylor & Francis.

Zheng, Q., Chen, K., Zhong, Y., Han, C., Zhang, Z., & Zheng, J. (2015). Determination and analysis of main components of four kinds of tea and their tea wastes. *Guangdong Agricultural Sciences, 42*, 14-20.

Zheng, X. X., Xu, Y. L., Li, S. H., Liu, X. X., Hui, R., & Huang, X. H. (2011). Green tea intake lowers fasting serum total and LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials. *American Journal of Clinical Nutrition, 94*(2), 601-610.

Zhou, F., Xu, T., Zhao, Y., Song, H., Zhang, L., Wu, X., & Lu, B. (2018). Chitosan-coated liposomes as delivery systems for improving the stability and oral bioavailability of acteoside. *Food Hydrocolloids, 83*, 17-24.

Zhu, D., Shen, Y., Wei, L., Xu, L., Cao, X., Liu, H., & Li, J. (2020). Effect of particle size on the stability and flavor of cloudy apple juice. *Food Chemistry, 328*, 126967.

Zokti, J., Badlishah, A., Baharin, S., Abdulkarim, S., & Abas, F. (2016). Microencapsulation of green tea extracts and its effects on the phys-ico-chemical and functional properties of mango drinks. *International Journal of Basic & Applied Sciences, 16*, 16-32.

Zou, L. Q., Peng, S. F., Liu, W., Gan, L., Liu, W. L., Liang, R. H., Liu, C. M., Niu, J., Cao, Y. L., Liu, Z., & Chen, X. (2014). Improved in vitro digestion stability of | (-)-epigallocatechin gallate through nanoliposome encapsulation. *Food Research International, 64*, 492-499.