Integrated approach for valorisation of polyphenols in spent black tea: extraction, microencapsulation, and development of functional packaging film

（紅茶殻ポリフェノール類高付加価値化のための統合的アプローチ：

抽出、マイクロカプセル化、機能性包装フィルムの開発）

Hokkaido University　Graduate School of Agriculture

Frontiers in Production Sciences　Doctor Course

Disanayakage Surakshi Wimangika Rajapaksha

Abstract

In a circular economy, the utilization of food manufacturing waste for obtaining functional ingredients has received great attention. Spent black tea (SBT) is an abundant waste generated after beverage manufacturing process and considered as a potential and underutilized source of polyphenols with high antioxidant power. Moreover, the studies which utilized the SBT for the recovery of polyphenols and their subsequent use as a natural antioxidant in the food packaging have not been reported to date. Hence, the main objectives of the research are: (1) to develop a method for recovering phenolic compounds from SBT by integrating the processes of subcritical solvent extraction (SSE) and microencapsulation using different wall materials by spray drying technique (2) to assess the potential of recovering polyphenols in SBT by SSE at pilot-plant scale to propose a suitable scale-up method, and (3) to develop and characterization of a functional food film incorporating SBT extract.

The first study evaluated the SSE for recovery of polyphenols in SBT, and microencapsulation to improve the stability of obtained extract. Optimization of extraction conditions was carried out by response surface methodology for the best recovery of antioxidant phenolic compounds. Two variables [temperature (°C) and ethanol concentration (%)] were used to design the optimization model using central composite inscribed. Extraction temperature of 180°C and ethanol concentration of 71% were optimal for the highest yield of total polyphenols (126.89 mg gallic acid equiv./g SBT) and 2,2-diphenyl-1-picrylhydrazyl scavenging activity (69.08 mg gallic acid equiv./g SBT). The extract was encapsulated using pectin, sodium caseinate, and a blend of these compounds (ratio 1:1) as wall materials by spray drying. The wall material significantly influenced encapsulation efficiency, particle size, morphology, thermal stability, crystallinity, and storage stability. The blend of wall materials produced an amorphous powder with the

highest phenolic retention (94.28%) in the accelerated storage at 45°C for 40 days. The microcapsules prepared with sodium caseinate were smaller with lowest mean diameter and highest thermal stability than the other types of materials. Obtained microencapsulates have potential use in different food systems to enhance their antioxidant property.

By exploiting the lab-scale knowledge, a pilot-scale process on semi-continuous SSE of polyphenols from SBT was developed. Treatment of SBT with ethanol-water (50% w/w) as solvent at 125 °C and 0.3 MPa achieved a significantly higher yield of polyphenols (80.82 g GAE/kg black tea) with antioxidant activity (64.20 g GAE/kg black tea), compared to hot water extraction. SSE improved the soluble matter content in extracts. UV-visible spectra of extracts from SSE detected an additional absorption peak at 360 nm. Based on the results of LC-MS, theaflavin-3,3'-digallate was the most abundant polyphenol from a total of 12 compounds to be extracted by SBT with 50% ethanol. The obtained results suggested that evaluated SSE could be used as a scale-up extraction method to recover polyphenols from SBT.

Moreover, the potential of using SBT extract as an active ingredient in food packaging was evaluated. Free or microencapsulated forms of SBT, using a pectin–sodium caseinate mixture as a wall material, were incorporated in a cassava starch matrix and films developed by casting. The effect of incorporating SBT at different polyphenol contents (0.17% and 0.34%) on the structural, physical, and antioxidant properties of the films, the migration of active compounds into different food simulants and their performance at preventing lipid oxidation were evaluated. The results showed that adding free SBT modified the film structure by forming hydrogen bonds with starch, creating a less elastic film with antioxidant activity (173 and 587 µg(GAE)/g film). Incorporating microencapsulated SBT improved the mechanical properties of active films and preserved their antioxidant activity (276 and 627 µg(GAE)/g film). Encapsulates significantly enhanced the release of antioxidant

polyphenols into both aqueous and fatty food simulants. Both types of active film exhibited better barrier properties against UV light and water vapour than the control starch film and delayed lipid oxidation up to 35 d. This study revealed that starch film incorporating microencapsulated SBT can be used as a functional food packaging to protect fatty foods from oxidation.

Subcritical solvent extraction was an efficient method for extracting phenolic compounds from SBT. Microencapsulation turned the extracted polyphenols into valuable food ingredient with improved stability. The SBT extract had the potential to be incorporated into cassava starch in developing packaging films with improved antioxidant activity, which allows the potential application in the prevention of lipid oxidation of fatty foods.

Acknowledgement

I feel great pleasure to express my deep sense of gratitude to Associate Professor Naoto Shimizu under whose supervision and guidance this work has been carried out. I am deeply indebted to him for his great supervision, valuable comments and directing me to reach the goal of the doctoral degree. Not only that but also his encouragement during research period, creating me a good learning environment and care during my stay in Japan is highly appreciated.

I sincerely thank Professor Kazunori Iwabuchi in the Laboratory of Agricultural Biosystem Engineering and Distinguished Professor Noboru Noguchi in the Laboratory of Vehicle Robotics for their careful review of my thesis.

I am also grateful to technical staff, present and past lab mates in my laboratory, Agricultural Biosystem Engineering, for their valuable support during my study. Moreover, I would be grateful to the Izumi Food Machinery Co., Ltd. (Japan) and the staff for their great support to conduct the pilot-scale extractions of my research. I gratefully acknowledge the help of Prof. Kiyonori Takahashi, Research Institute for Electronic Science, Hokkaido University for providing the X-ray diffraction facility. Also, I wish to thank the Global Facility Centre of Hokkaido University for providing different analytical facilities to succeed in this study.

I would like to show my gratitude to the Japan Student Services Organization (JASSO), Reservation Program for Monbukagakusho Honors Scholarship and Otsuka Toshimi Scholarship Foundation in Japan for granting me financial support during my doctoral study.

At last, but not least I am thanking my parents, my husband Heshan Kodithuwakku and my son for their love, care and support on me which kept me focus on my study.

Table of Contents

List of Figures

List of Tables

Chapter 1

General Introduction

1.1 Food processing waste (FPW)

As the world population is increasing alarmingly, food production and their consumption rise on demand. Correspondently, food loss or wastage is continuing to increase. According to the FAO estimation, 1.3 billion tons of food, about one-third of the annual production for human use, is globally lost or wasted every year.

Though the huge amount of food waste caused during production and postharvest of the food products (75%), waste generated from food processing has been reached up to 140 billion tons per year (Panzella *et al*., 2020). Disposal these waste raises serious management problems, both environmental and economic point of view. Therefore, the sustainable and efficient utilization of food processing waste (FPW) has become a matter of high research priority in the recent times. In this case, FPW can be utilized as materials for biorefining and production of high value-added products in agriculture and food industry. Generally, FPW can be recognized as a source of bioactive compounds particularly, natural antioxidants.

Polyphenols as a major group of natural antioxidants can be recovered from these cheap sources of waste biomasses and turned into valuable food ingredients. The basic monomer in polyphenols is phenolic ring and further, it classified as phenolic acids and phenolic alcohols. These compounds are known for protecting the human health, in preventing cardiovascular disease, osteoporosis, neurogenerative disease, cancer, and diabetes mellitus. These effects have been attributed due to their ability to act as potent antioxidants and scavengers of reactive oxygen species. Hence, these properties open into usage of recovered polyphenols as functional supplements in food and pharmaceutical industry. Recently, many research focused on the potential to utilize FPW as natural sources for extracting antioxidants since, artificial sources may contain unfavorable health effects.

FPW could be defined as any part of food or drink products that are disposed of during the processing or manufacturing. Among these, plant-based drink manufacturing industry (fruits, vegetables, tea, and coffee) discards considerable content of polyphenols through by-products or residues. After initial usage or extraction, the discarded fraction or residue left after remains non-extractable polyphenols which associated with macromolecules and having a potential to recover through efficient extraction technique.

1.2 Production of waste from tea beverage processing

Besides water, any portable liquids which may quench the thirst are considered beverages and when the source of the drinks are plants, can categorized as plant-based beverages. Recently, the intake of these plant-based beverages has been increased globally due to their functional benefits to humans. Moreover, owing to increasing world population, consumption of commercial beverages also has grown 3.6% per year. As a result, content of by-products generated during beverages processing has been increasing.

Tea, a beverage with a variety of flavours and health benefits and their consumption second only to packaged water by volume in 2021 with approximately 297 billion litres liquid consumed around the world (https://www.worldteanews.com, accessed 15/11/2021). It is produced by processing of the leaves of *Camellia synensis*. Depending on the extent of fermentation and oxidation of tea leaves, different types of teas including black tea, green tea, oolong tea, yellow tea, and white tea, etc. are available in the market. The global tea production was approximately 6.1 million metric tons of tea in 2019 and Indonesia, India, China, Sri Lanka and Kenya were rated as the world's largest tea-exporting countries (https://www.statista.com; accessed on 18.08.2021). From total tea production, black tea accounts for 78% in globally and it has been predicted an increase by 2.2 % annually over the next decade to reach 4.4 million tonnes in 2027. Moreover, owing to existing health benefits of black tea, the segment will continue to hold a major share of the global tea market.

With increasing of tea production, consumption way has been diversified from home preparation to more convenient instant tea powder, tea bags and ready-to-drink (RTD) tea industry. Since tea beverages are considered as close substitute for water, tea drinking is mostly popular among all age groups. Owing to the increasing the consumption, amount of tea residues left after preparation of tea is rising annually, approximately, it is more than 0.12 million tons annually all over the world (Mukhtar *et al*., 2018).

Majority of these tea residues are usually dumped into landfills or incinerated, except a small percentage that is used as agricultur al feedstock, turned into compost, adsorbents for heavy metal and bioenergy generation (Debnath *et al*., 2021).

1.3 Production process of black tea leaves

The steps involved in the production of black tea include plucking, withering, rolling, fermentation and drying (Figure 1.1).

Figure 1.1 An overview of processing and preparation of black tea. (Reprinted and modified from Chen *et al*., 2020).

First fresh tea leaves are harvested by manually or by machine. Generally, only the bud and the two young leaves below it is plucked and then carried to the tea factory. In the tea factory, Leaves are placed on withering troughs and hot air is blown from bottom in order to evaporate the moisture. The period varies from 8 to 12 hours depending on the leaf condition. During the rolling process, the leaves are bruised and twisted, then broken into small pieces using rolling or distorting machines. When withered Leaves are distorted by rolling, it produces conventional orthodox tea and CTC tea is produced after distorting of non withered leaves using modern machine. This process helps to wring out the juice from leaves and expose for oxidation.

Fermentation is the most crucial step of processing black tea which cause to certain chemical and biochemical changes. The rolled or macerated leaves are spread out on tracks or on factory floor. Thereby, the leaf takes up its copper-red to brown colour with development of characteristic flavor and color. Due to oxidation of catechin, high molecular weight polyphenols such as theaflavin, thearubigins and theabrownins are developed. The ratio of theaflavin and thearubigins content is 1:10 is considered as the ideal point for measuring the rate of fermentation. On the high point of the fermentation, drying of tea leaves are done to halt the fermentation process by destroying the enzymes. The final moisture content of the leaves is between 5-6%.

1.4 Polyphenols in black tea and their health benefits

Tea infusion is normally prepared by steeping of dried tea leaves in hot water for 5-10 minutes. Since it is a gentle form of preparation, high molecular weight hydrophobic polyphenols in tea may not pass with hot water infusion and remain in tea residue. Therefore, a variety of phenolic compounds at a considerable level can be founds in spent tea leaves.

Amount and types of polyphenols available in tea are varied between different tea types. Fresh tea leaves contain higher quantity of catechin. Due to fresh tea leaves are processed

swiftly by steaming, rolling, and drying to produce green tea, it contains higher quantity of catechin than other tea types. The mostly abundant 4 types of catechin in green tea are (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG) and (−)-epigallocatechin-3-gallate (EGCG).

Complete fermentation of black leads to combine some of the catechins and form complex theaflavins which offer characteristic taste and color to black tea, large polymeric compounds like thearubigins and other flavonoids (Khan and Mukhtar, 2019). From total flavonoids in black tea, thearubigins accounts for the majority 60%, and theaflavins accounts for 10% (Zhang *et al*., 2019). Due to their higher molecular weights, thearubigins have not been as well characterized chemically and biochemically. The major theaflavins in black tea are theaflavin (TF1; 18%), theaflavin-3-gallate (TF2A; 18%), theaflavin-3′-gallate (TF2B; 20%), theaflavin-3, 3′-digallate (TF3; 40%) (Figure 1.2). In addition to the already mentioned compounds, black tea infusion contains flavonols, phenolic acids, alkaloids, and amino acids (Table 1.1).

Table 1.1: Composition of black tea infusion

Component	Weight (%)
Catechins	3-10
Theaflavins	3-6
Thearubigins	12-18
Flavonols	6-8
Phenolic acids and despites	10-12
Amino acids	13-15
Methylxanthines	8-11
Carbohydrates	15
Protein	1
Mineral matter	10
Volatiles	< 0.1

Adapted from (Łuczaj and Skrzydlewska, 2005).

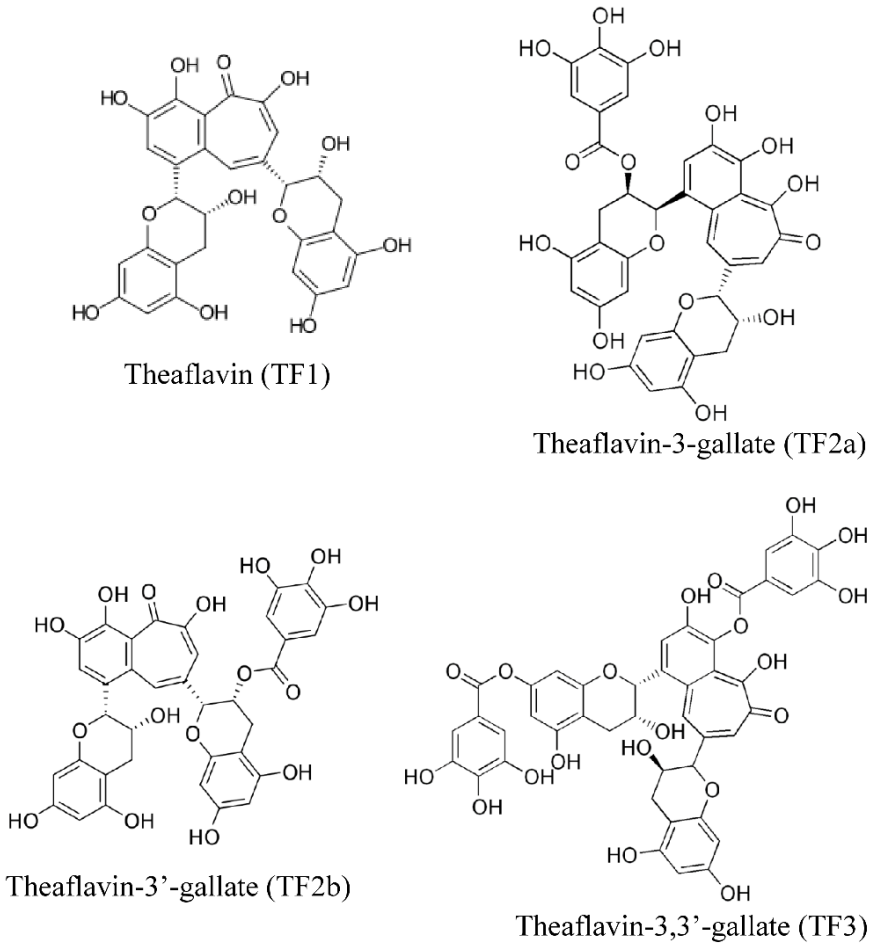

Figure 1.2 Major theaflavins in black tea.

Numerous animal and clinical studies have been documented mainly on health benefits of green and black. Epigallocatechin-3-gallate (EGCG) is the major catechin component in green tea, which offer the preventive effects on cardiovascular disease, cancer, obesity, diabetes mellitus, oxidative stress/inflammation, neurodegenerative diseases, and allergic diseases (Wang *et al*., 2018; Xing *et al*., 2019; Sun *et al*., 2018). The polymeric polyphenols available in black tea have poor systemic bioavailability in the small intestine and pass into the colon where they are subject to metabolise by gut microbiota; however, theasinensins, theaflavins from black tea have attributed in several biological activities such as anticancer activities, antioxidant activities, anti-cardiovascular activities, antimicrobial activities, anti-hyperglycemic activities, and anti-obesity activities.

1.5 Extraction of polyphenols

The conventional extraction techniques include infusion, decoction and maceration. Due to existing drawbacks such as large volume of solvents is required, labor-intensive long extraction times, and low yields of these extraction techniques led to study modern and green extraction techniques. Extraction of phenolic compounds which covalently bound to the cell wall structure or to other macromolecules, is difficult using simple extraction methods, especially in tea and coffee. Hence, the applications of modern and green extraction processes are become more interest to overcome the limitations of conventional techniques. Several modern extraction techniques exist for the recovery of phenolic compounds from FPW, the most used are ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), subcritical water extraction (SWE) and pressurized liquid extraction (PLE).

1.5.1 Ultrasound assisted extraction (UAE)

UAE uses ultrasonic energy (20 kHz-100 MHz frequency), and it makes cavitation bubbles. The technique is based on the propagation of ultrasound pressure waves and resulting cavitation forces, where bubbles can explosively collapse and generate localized pressure causing plant cell rupture and facilitating the leaching of extractable intracellular substances into the solvent. (Pagano *et al*., 2021). This technique has been used in both static and dynamic mode. Under static conditions, that is in closed vessels, with no solvent refreshing, or in a dynamic mode, in which fresh solvent is supplied in continuously (Panzella *et al*., 2020). the effects of extraction microenvironment conditions such as temperature, solvent to material ratio, time, frequency, intensity, and power in UAE determine the biochemical properties and yield of the extracts.While conventional extraction techniques are often time, solvent and energy consuming, ultrasound-assisted extraction provides advantages from an industrial perspective. Ultrasound as an extraction technique has shown commercial scale application, with high returns on capital investment.

1.5.2 Microwave assisted extraction (MAE)

MAE is a non-conventional extraction technique which uses the electromagnetic waves to change the cell structure. The MAE reduces extraction time, solvent consumption, and improves extraction yield. The introduction of electric and magnetic field leads to dipole moment between solvent and sample. The principle is that once polar molecules present in a substance are hit by the electromagnetic beams, they make dipolar rotations, and consequently the alternative action of polar molecules creates friction between them, which thermal energy is produced in a closed environment. It leads the disruption of cell wall's structure and diffusion from solid to liquid phase. Various factor such as extraction time, power, solvent composition, preleaching time, solvent : raw materials ratio, pH, particle size, and sample moisture influence on the process of MAE (Kala *et al*., 2016). Applicability of this technique has been evaluated for the recovery of various classes of bioactive compounds (quinones, polyphenols, alkaloids, saponins, terpenoids, etc.) with different polarity.

1.5.3 Pulsed Electric Field (PEF)

The pulsed electric field (PEF) is a novel extraction technique that includes the application of high voltage pulses (usually 20-80 kV/cm in continuous mode and 100-300 V/cm in batch mode) to food product or raw material placed between electrodes. Food materials are placed between two electrodes and a high-voltage electric field. The principle of extraction by PEF is the electroporation due to dielectric disruption of cell membrane. The ruptured membrane loses its structural functionality, and the plant material is extracted.

1.5.4 Subcritical solvent extraction (SSE)

Subcritical solvent extraction is a technique using moderate temperature and pressure to maintain the solvent in a liquid state above its atmospheric boiling point, but below its critical point. Higher temperature of the solvents causes the decreasing of viscosity and

improving the diffusion rate. It facilitates the mass transfer efficiency of phenolics, resulting in higher extraction yields.

The SSE method is required a less time and volume than conventional extraction methods. It also reduces or eliminates degradation of the bioactive components. The solvents widely used to extract bioactive compounds are water, ethanol, methanol, and carbon dioxide (CO_2) under subcritical conditions (Figure 1.3).

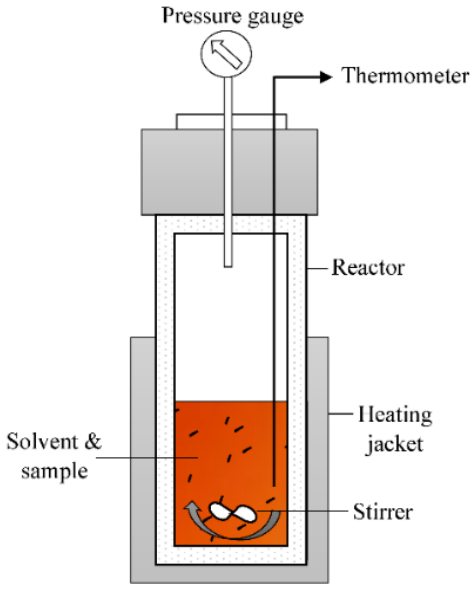

Figure 1.3 Apparatus for subcritical solvent extraction.

When using the subcritical water as a solvent, the technique is known as subcritical water extraction (SWE) or pressurized hot water extraction. The SWE is a novel green extraction method, and it is generally performed using water at various temperatures below their critical point (100–374 °C) under sufficient pressures (0.1–22.1 MPa) (Figure 1.4). Applied pressure maintains the liquid state of water at high temperatures. Once the temperature rises to 250 °C from room temperature, the dielectric constant of water reduces from 80ε to nearly 25ε, behaving similarly to standard organic solvent such as ethanol and methanol, thereby improving the capacity of the phenolic extraction. The subcritical conditions change the properties of water, thus improve the ability of dissolving the analyte.

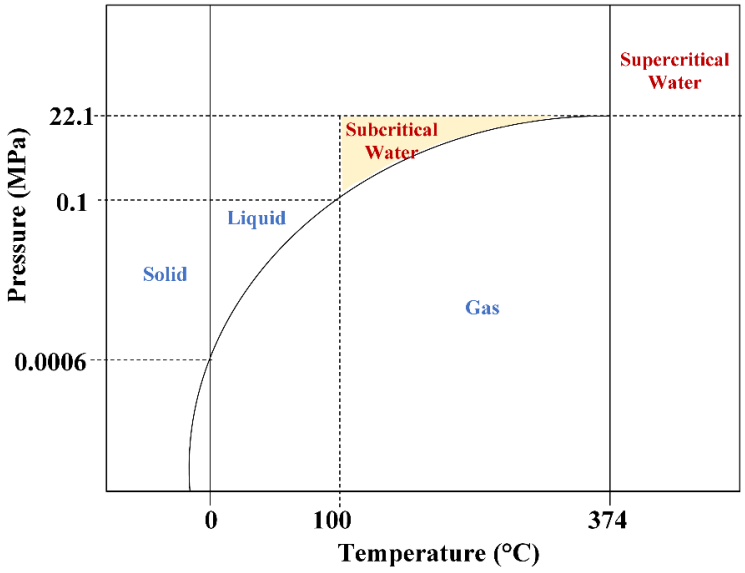

Figure 1.4 Water phase diagram as a function of pressure and temperature.

The SWE allows the higher extraction rates, reducing extraction time, reduction of organic solvent consumption and the extraction of phytochemicals without changing their chemical integrity. Recently, it has been widely explored this technique for extraction of phenolic compounds from waste biomass.

Instead of pure water, different mixtures of water and food grade organic solvents (ethanol, methanol) also can be used as the solvent of SSE. This technique is known as pressurized liquid extraction (PLE). The PLE is almost alike SWE and works at high pressures (>0.1 MPa) and temperatures, typically between 60 and 250 °C. Ethanol is not expensive and possesses GRAS status. There are some advantages in using an ethanol-water mixture: contribute to the formation of a moderately polar medium that fasten the extraction of phenolics, ethanol improves the solubility of solutes while water helps desorption of solutes from matrix. Further, the addition of ethanol lowers the boiling point thereby, improves the extraction efficiency (Pagano *et al.*, 2021). The technique of SWE can promote the unwanted reactions such as caramelization and Maillard reaction at elevated temperatures. As a

consequence, some potential human carcinogens such as acrylamide and hydroxymethylfurfural (HMF) can be produced during SWE. However, use of ethanol as a co-solvent in PLE disfavour the production of HMF (Mariotti-Celis *et al*., 2018). Factors such as temperature, pressure, solvent concentration, solvent: raw materials ratio and extraction time influence on the extraction yield of PLE.

1.6 Encapsulation of phenolic compounds

Encapsulation can be defined as a process to entrap an active compound within a carrier material to produce encapsulates with functional properties. Depending on the size of the encapsulates, it can be classed as micro or nano-encapsulation. In the encapsulation process one or mixture of active materials are coated by one or mixture of materials. Active material is termed as "core material" and coated material is termed as "wall or carrier material". Core materials can be in the form of solid, liquid or gas. Food and pharmaceutical industry use the encapsulation for different purposes; 1) to protect the reactive core material from adverse environment 2) for the convenience of handling, 3) the controlled release and targeted delivery of the core material, 4) mask the taste of the core material and 5) dilute the effect of active material (Shahidi and Han, 1993).

Recently, research on encapsulation of phenolic compounds has become more interesting due to their applications in functional food and nutraceutical industry. However, vulnerability of polyphenols to different environmental conditions in the food processing and storage or in the gastrointestinal tract such as temperature, light, oxygen, pH and enzymes limit the activity and potential health benefits of the active compounds. Further, some polyphenols have bitter or astringency taste and lower solubility in water. Thus, encapsulation of polyphenols can alleviate these drawbacks and can improve their stability, target delivery, controlled release, and bioavailability. The encapsulation can be classified mainly into two categories; are chemical and physical encapsulations.

1.6.1 Chemical encapsulation methods

1.6.1.1 Coacervation

Coacervation is the separation into two liquid phases in colloidal systems and subsequent deposition the newly formed coacervate phase around the active ingredient suspended or emulsified in the same reaction media (Fang and Bhandari, 2010). Coacervation can be classified into to types: simple and complex coacervation. In simple coacervation, one polymer is formed and in complex coacervation, two or more polymers with opposite charges are used. Both processes form polymer-rich solution to encapsulate the active materials.

1.6.1.2 Molecular inclusion

Molecular inclusion is referred as a molecular association between core and wall and cyclodextrins (CDs) are considered as the most appropriate wall material. Cyclodextrins are cyclic oligosaccharides and available three types: α-, β- and γ-cyclodextrins. β-cyclodextrin which has a medium cavity diameter (6.5-6.0 Å) is the most commonly used type molecule for inclusion encapsulations (Santos *et al*., 2017). This molecule has an amphiphilic nature with hydrophobic center and hydrophilic outer surface. This character facilitates the encapsulation of less polar molecules through a hydrophobic interaction.

1.6.1.3 Cocrystallization

Encapsulation by cocrystallization utilizes the sucrose for wall matrix to encapsulate active ingredients. In this process, the active component is introduced to the supersaturated sucrose syrup under vigorous agitation to attain crystallization that provides nucleation and entrapment of active ingredient into the sucrose. This process converts the crystalline structure of sucrose to agglomerated, irregular, and porous crystals to provide considerable void space and increased surface area for the incorporation of active ingredient (Kaur *et al*., 2021). This method can improve the solubility, wettability, homogeneity, dispersibility,

hydration, anticaking, stability and flowability of the encapsulated materials. In addition, the core materials in a liquid form can be converted to a dry powdered form without additional drying.

1.6.2 Physical encapsulation methods

1.6.2.1 Freeze drying

Freeze drying, also known as lyophilization. It is suitable for thermosensitive and unstable molecules since the minus temperature is employed to freeze the feed solution. It is a dehydration operation at minus temperature and low pressure consisting in eliminating water by sublimation of the formed ice crystals. A pump is used to create a vacuum condition during the drying. Therefore, it is considered as expensive drying technology due to the high energy consumption for completion of the drying process (24-48 h). Following the drying process, amorphous cake can be obtained and milling or grinding can turn it into smaller size particles. The factor such as the core material concentration, nature and composition of the wall materials, chamber pressure, freezing rate and temperature during the sublimation should be considered for obtaining the quality particles by freeze drying technique (Rezvankhah *et al*., 2020).

1.6.2.2 Fluidized bed coating

Fluidized bed coating is an encapsulation technique by which coated particles are produced by spraying an encapsulating agent onto a fluidized powder bed. The powder particles which suspended by an air stream are coated with shell materials. The factors such as solid circulating rate and nozzle atomization pressure, humidity, coating feed rate and temperature influence on the agglomeration and film forming of the particles and on the coating efficiency (Coronel-Aguilera and Martín-González, 2015). The different fluidized bed coating methods are available: (1) top-spray (2) bottom-spray and (3) tangential spray. The main advantageous of this technique involve higher shelf life, masking of off-taste, ease of

handling, controlled release, and improved esthetics, taste, and color (Gadkari and Balaraman, 2015).

1.6.2.3 Spray drying

Spray drying is the most widely used technique for encapsulation owing to its advantageous such as simple, fast, reproducible, and scalable drying technology. Spray drying can convert the fluid material containing coating agent and phenolic compounds into solid materials in a short period of time. Due to the rapid evaporation of liquid, active phenolic compounds are exposed to short thermal contact time, thus the bioactivity of final product is not negatively affected.

Acquired solid material is a fine powder with entrapped polyphenols in a wall matrix. The resulting particles are more or less spherical, with a size distribution between 10 and 100 micrometers (Munin and Edwards-Lévy, 2011). Due to the rapid evaporation of liquid, active phenolic compounds are exposed to short thermal contact time, thus the bioactivity of final product is not negatively affected. Spray drying consists of four main stages, which include atomization of feed solution, drying medium and spray contact, drying of feed and separation of product from air (Murugesan *et al*., 2012). The schematic diagram of a spray dryer is shown in Figure 1.5.

The parameters such as wall:core material ratio, atomization pressure, feed flow rate, feed viscosity, feed surface tension, inlet air temperature and drying air flow rate influence on the final quality of the spray dried product (Kaderides *et al*., 2015).

Selecting a suitable wall material is crucial for efficient for spray drying. Since the wall material must be soluble in water at an acceptable level, limited number of wall materials available, the most common biopolymers such as maltodextrins, starches, corn syrup solids, and acacia gums have all been widely used as encapsulating agents. Among these biopolymers, pectin is potential promising wall material, because it has several benefits like

being emulsion stabilizer, gelling properties, binding abilities, solubility and have a hydrophobicity (Rehman *et al*.,2019).

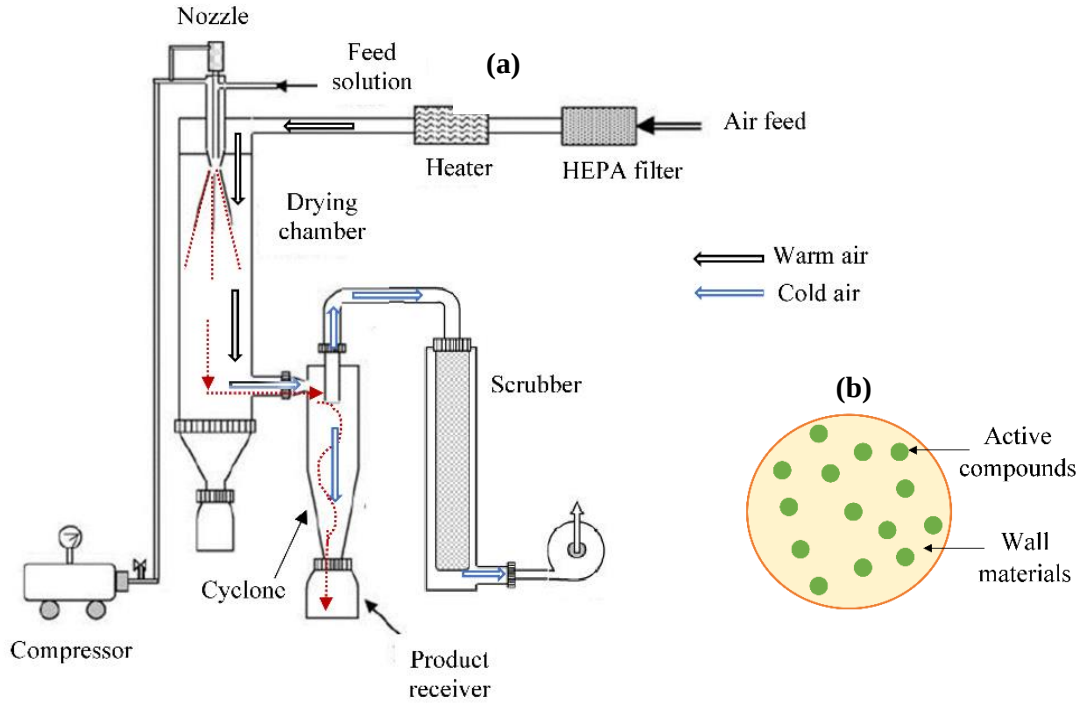

Figure 1.5: Schematic diagram of the spray dryer (a), Illustration of matrix type microencapsulates produced by spray drying (b).

The use of dairy proteins as wall materials in microencapsulation has been explored with great interest because of their well-known functional properties. Among dairy proteins, sodium caseinate offers several physical and functional properties such as amphiphilic character, emulsifying characteristics, thermal stability, and water solubility. Sodium caseinate has a smaller mass and higher water solubility than casein micelles (average molar mass and diameter range 1200-4700 kDa, 50 to 120 nm, respectively) (Lucey *et al*., 2000) (Figure 1.6).

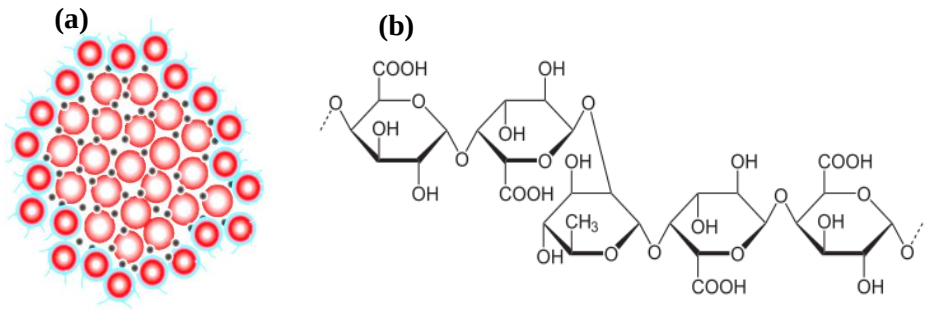

Figure 1.6 Structure of the casein micelle; hydrophobic submicelles (red spheres), calcium phosphate bridging bonds (black dots), κ-caseins at the surface (blue) (a) and Chemical structure of pectin (b). (Figure 1.6a reprinted from Hege *et al*., 2020).

1.7 Applications of microcapsules in the food industry

Micro/nano capsules produced using different encapsulation techniques have been applied in food industry, represent a solution to the drawbacks of direct incorporation of polyphenols into food products. It facilitates i) protection of core material from degradation by reducing its reactivity (ii) increasing the compatibility and solubility with the host material (iii) controlling the release of core materials at a particular time (iv) increasing the bioavailability of encapsulated compounds (v) separating the components in a mixture (Haponska *et al*., 2020). The foods included microcapsules as a functional ingredient are produced to add or modify the health benefits, such as antimicrobial, antioxidant, anticancer, and anti-inflammatory etc. In addition to direct addition of microcapsules into food materials, their incorporation into the packaging materials has been interesting due to offering additional benefits such as, prevention of lipid oxidation in food stuff and acting as indicator for food spoilage while providing bioactive compounds to foods.

1.7.1 Active or functional food packaging

Active packaging is design to provide an intentional functionality to the packaging system beyond their role of packaging. The antioxidant compounds in packaging can improve the antioxidant activity of food packaging systems. Both primary and secondary antioxidants can be incorporated into active packaging materials to improve the shelf life of foods. Primary antioxidants are free radical scavengers, which can donate hydrogen to reactive free radicals (*e.g.*, $L^{\cdot}$, $LO^{\cdot}$, $LOO^{\cdot}$, *etc.*) and form stabled structure that not to cause further initiation or propagation reactions in the lipid oxidation process. Secondary antioxidants can prevent oxidative reaction by chelating metals, screening UV light, scavenging oxygen, and quenching singlet oxygen (Tian *et al*., 2013).

Active packaging system is composed mainly three main components: active substance, packaging material and food. When it has a direct contact between packaging material and food component, it refers as a package-food system (Figure 1.7). In this system, the migration of antioxidants compounds take place by diffusion in the polymer structure, transfer from packaging materials to food surface. However, the package-food system of films incorporating encapsulated antioxidant compounds includes one pre-step: migration from microcarrier to film matrix.

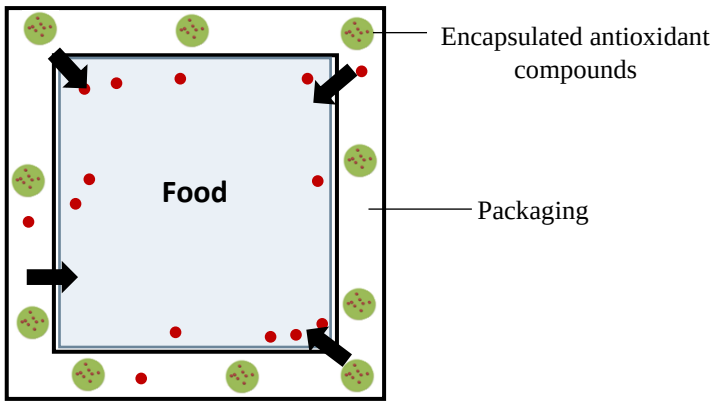

Figure 1.7 Schematic diagram of the package-food system.

Since the lipid oxidation of foods are generally induced from surface of the foodstuff, incorporation of antioxidant substances into the packaging system has been deeply studied by recent researchers. However, still it has a possibility to deteriorate and lose the activity of antioxidant compounds in film due to reactions with light, oxygen. Therefore, encapsulation of antioxidant prior to addition into the film matrix can protect the stability of antioxidant compounds while processing and storage.

1.8 Problem statement of research, hypothesis, and objectives

Spent black tea (SBT) is a waste generated after beverage manufacturing process, which disposed into incineration and land filling except for a small percentage that is used as agricultural feedstock or turned into compost. Moreover, consumption of black tea derived products such as bottled tea drinks, instant tea powder, tea seed oil, and tea extracts have raised the spent tea generation. Since the brewing of black tea is performed in mild conditions using hot water, considerable amount of polyphenols are likely to remain in spent tea leaves without leaching into the infusion. Therefore, the SBT can be considered as a potential and underutilized source of polyphenols with high antioxidant power. The antioxidant phenolic compounds have not only nutritional and health benefits to humans but also, they can maintain the shelf life of foods by preventing lipid oxidation process. However, studies which have utilized the SBT for recovery of antioxidant phenolic compounds, are relatively limited. Moreover, exploration of recovered polyphenols as a natural alternative of artificial antioxidants in the food packaging have not been reported to date. Therefore, there is a need testing and development of efficient methods for their extraction and determine the feasibility of using recovered polyphenols from spent black tea waste.

Therefore, **the hypothesis of this research** is that; sizable quantity of polyphenols is available in the SBT, and integrated approach of subcritical solvent extraction and microencapsulation can turn them to a valuable functional ingredient in food packaging.

Hence, **the main objectives of the research** are:

1. to develop a method for recovering phenolic compounds from SBT by integrating the processes of subcritical solvent extraction and microencapsulation using different wall materials by spray drying technique
2. to assess the potential of recovering polyphenols in SBT by subcritical solvent extraction at pilot-plant scale to propose a suitable scale-up method
3. To develop and characterization of a functional food film incorporating SBT extract

Chapter 2

Valorisation of Spent Black Tea by Recovery of Polyphenols: Subcritical Solvent Extraction and Microencapsulation

2.1 Introduction

Besides water, black tea is the most consumed beverage in the world. It is produced by a process that consists of withering, rolling, fermenting, and drying the leaves of Camellia synensis. During the fermentation process, the tea leaves oxidize to produce multimeric polyphenols with high antioxidant potency (Łuczaj & Skrzydlewska, 2005). Of the different varieties of tea produced worldwide, green, black, oolong, yellow, white, and dark, 78% consists of black tea (Kosińska & Andlauer, 2014; Weerawatanakorn *et al*., 2015).Because of its convenience of consumption, ready-to-drink (RTD) or bottled tea is produced commercially in most parts of the world, in particular, Japan. As a consequence, a large amount of spent black tea (SBT), the residues after manufacturing this tea product, is generated annually (Kondo, Hirano, Kita, Jayanegara, & Yokota, 2018). These used tea leaves mostly become waste with only a small percentage used as feedstock or turned to compost (Sagar, Pareek, Sharma, Yahia, & Lobo, 2018). As the conditions used for tea brewing are mild, a significant amount of polyphenols with a high antioxidant power is retained in SBT (Abdeltaif, SirElkhatim, & Hassan, 2018). Hence, utilizing food manufacturing waste such as SBT is a sustainable and economically attractive way to recover antioxidant phenolic compounds but an efficient method for extracting polyphenols from SBT has not yet been developed and tested.

Several techniques have been investigated for extracting phytochemicals from food waste (Sagar *et al*., 2018; Yanagida, Shimizu, & Kimura, 2005). Of these techniques, subcritical solvent extraction (SSE) is a greener and faster method (Munir, Kheirkhah, Baroutian, Quek, & Young, 2018), which uses a pressurized liquid kept below its critical point (374°C for

water) and above its boiling point (100°C for water). These conditions allow fluids to remain in a liquid state due to the applied pressure and it creates low polar water with equivalent to organic solvents at ambient temperature (Shimizu, Ushiyama, & Itoh, 2019; Zhang, Baroutian, Munir, & Young, 2017; Zhang & Wolf 2019). This technique facilitates rapid extraction without the loss or changing the chemical integrity of thermolabile compounds (Essien, Young, & Baroutian, 2020). Combining subcritical water with an organic solvent such as ethanol and methanol has also been used to improve the yield, extraction time and solubility of compounds (Kwon & Chung, 2015; Pronyk & Mazza, 2009). Response surface methodology (RSM) is commonly used for optimizing the process parameters for the extraction of phytochemicals. This is a useful mathematical and statistical tool for defining the effect of independent variables and their interactions on a particular response, such as the yield.

However, effectiveness of polyphenols mainly depends on their stability, bioactivity, and bioavailability. The unsaturated bonds in the molecular structure of polyphenols make them vulnerable to oxidants, light, and heat, thus reducing their activity (Kailaspathy, 2015). Therefore, protecting phenolic compounds by encapsulation following their extraction would be a better way to maintain the structural integrity of polyphenols until their industrial application. The microencapsulation of phenolic extracts not only preserves them but also produces a powdered product that is convenient for food application. At present, spray drying is the most widely used technique for the microencapsulation of polyphenols and other heat labile compounds because of its short thermal contact time, cost-effectiveness, and suitability for industrial application.

As well as the technique used for microencapsulation, selecting a coating or wall material is also crucial for efficient spray drying (Ushiyama & Shimizu, 2018). Of the different types of wall material, polysaccharides and protein agents are commonly used either alone or in

combination because of their distinct properties. Pectin is a polysaccharide with strong film-forming, gelling, and binding abilities. Its ability to form stable dispersions at low concentrations facilitates microencapsulation by spray drying (Rehman *et al*., 2019). Sodium caseinate is the salt of casein, a major milk protein fraction. Generally, milk proteins act as effective film-formers and emulsifiers while polysaccharides act as filler materials (Augustin & Oliver, 2014).

Ultimately, encapsulated SBT would exhibit important properties that would facilitate the shelf life of polyphenols because the coating materials can act as a barrier against adverse environmental conditions.

Therefore, the objective of the present study is to develop a method for recovering phenolic compounds from SBT by integrating the processes of SSE optimization and the subsequent encapsulation of SBT using different wall materials by spray drying. The encapsulated powder using pectin, sodium caseinate, and a mixture of these compounds as wall materials will be characterized to evaluate their encapsulation efficiency, morphology and size, thermal stability, crystallinity, and storage stability.

2.2 Materials and Methodology:

Low grown unblended black tea was supplied by Nawa withana Kanda tea factory in Sri Lanka, gallic acid by Sigma-Aldrich (Shanghai, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH) by Sigma-Aldrich (Taufkirchen, Germany), Folin and Ciocalteu phenol reagent and casein sodium salt from bovine milk by Sigma-Aldrich (St Louis, MO, USA), and pectin from citrus, sodium carbonate, ethanol, and methanol by Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Distilled water was used in all the experiments. All other chemicals and solvents used were analytical grade.

2.2.1 Preparation of spent black tea

Black tea leaves (20 g) were brewed in 1000 mL of boiling water (100 °C) for 6 min. The infusion was then filtered using a tea strainer and the residue was dried in an air-drying oven at 45 °C overnight.

2.2.2 Subcritical solvent extraction

Subcritical solvent extraction was performed using an organic synthesizer (Chemi-station PPV 3000, Tokyo Rikakikai Co. Ltd, Tokyo, Japan) with an agitator and an 11-ml reactor with a maximum temperature and pressure of 200°C and 5 MPa, respectively. For each experimental run, 0.5 g of SBT was mixed with 10 ml of solvent at a solid: solvent ratio of 1 g: 20 ml. The extraction reactor was filled and then purged three times with nitrogen gas to remove the atmospheric oxygen present in the reactor vessel, and then, an initial pressure of 2.0 MPa was applied. The heating control was adjusted to obtain the desired temperature, which was then maintained for 10 min. During extraction, the agitation speed was kept at 17 g to prevent any local overheating and to increase the mass transfer. The extraction process was conducted in at various ethanol concentration (0%–100%) and temperature (100°C–180°C) ranges, based on the RSM design given in Table 1. After the extraction, the reactor was immediately cooled by placing it in a container of cold water. The extracts were filtered through filter paper (6 μm) under vacuum after the vessel pressure reached the initial pressure, and then, the filtrate was lyophilized. The lyophilized powder of SBT extract was stored at 4°C until further analysis.

Table 2.1 Coded levels for process variables used in the experimental design (central composite inscribed (CCI))

Independent variables	Coded levels				
	-α (-1)	Low (-0.7)	Medium (0)	High (+0.7)	+α (+1)
Temperature ˚C	100	112	140	168	180
Ethanol Concentration %	0	15	50	85	100

2.2.3 Determination of total phenolic content

The total phenolic content (TPC) of the dried extract was measured colorimetrically using the Folin–Ciocalteu (FC) method described by Dranca and Oroian (2016) with little modifications. Briefly, the dried extract obtained was diluted with a dilution factor of 100, and then, a 1.0-ml aliquot of the extract in triplicate was transferred into a test tube and mixed thoroughly with 5.0 ml of FC reagent diluted 1:10 with distilled water. After keeping for 3 min, 5.0 ml of sodium carbonate (7.5%, w/v) was added and mixed. The mixtures were then allowed to stand for 1 hr in the dark before measuring the absorbance using a UV–Vis spectrophotometer (JASCO V-560, JASCO corporation, Tokyo, Japan) at 756 nm against the blank. Gallic acid was used as the standard for preparation of the standard curve (7.812–250 μg/ml, R2 = .998). The TPC values were expressed as milligrams of gallic acid equivalent/g (dry weight) material (mg GAE)/g SBT).

2.2.4 Determination of antioxidant activity

The scavenging capacity of SBT extract towards 2,2-diphenyl-1-picrylhydrazyl free radicals (DPPH) was measured using a slightly modified method of Brand-Williams *et al*. (1995). For that purpose, 0.1 mM DPPH solution was prepared. DPPH reagent and properly diluted liquid extracts (dilution factor; 70) mixed (2.9 mL + 0.1 mL) and incubated at room temperature for 20 min. Absorbance was further measured at 517 nm with UV–vis spectrophotometer **(**JASCO V-560, JASCO corporation, Tokyo, Japan). Gallic acid was used as the standard for preparation of standard curve (3.90-62.5 μg/mL, $R^2 = 0.997$). DPPH scavenging capacity was expressed as milligrams of gallic acid equivalent/g (dry weight) material (mg GAE)/g SBT).

2.2.5 Response surface methodology design

The study was conducted as a two-factor full factorial experiment with the influence of two independent variables (temperature and ethanol concentration) on the responses (Total

phenolic content and DPPH scavenging capacity) being evaluated (Table 2.2). The CCI design consisted of 13 experiments using 5 centres, 4 axial and 4 factorial points.

Table 2.2 Central composite design – inscribed (CCI) matrix and results

Run	Process variables – real and (coded) values		Responses	
	Temperature (°C)	Ethanol concentration (%)	Extraction yield (mg GAE/g SBT)	
			TPC	DPPH activity
1	112 (-0.7)	15 (-0.7)	77.72	35.88
2	180 (+1)	50	124.43	58.78
3	100 (-1)	50 (0)	88.42	43.61
4	112 (-0.7)	85 (+0.7)	78.00	44.40
5	168 (+0.7)	85 (+0.7)	105.98	67.68
6	168 (+0.7)	15 (-0.7)	79.11	44.36
7	140 (0)	100 (+1)	61.36	45.87
8	140 (0)	0 (-1)	42.19	28.35
9	140 (0)	50 (0)	87.14	40.55
10	140 (0)	50 (0)	90.62	45.36
11	140 (0)	50 (0)	89.50	45.51
12	140 (0)	50 (0)	95.58	43.61
13	140 (0)	50 (0)	93.10	42.14

Abbreviations: TPC, total phenolic content; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability; GAE, gallic acid equivalent.

The experimental data obtained were fitted to a second order polynomial model of the form:

$y = b_o + \sum_{i=1}^{n}(b_i x_{i)+} \sum_{i=1}^{n}(b_{ii} x^2{}_{ii)+} \sum_{ij=1}^{n}(b_{ij} x_i x_j)$,

where y is the predicted values of TPC or DPPH scavenging capacity; x_i, the coded levels of the design variables (Temperature and ethanol concentration); b_o, a constant; b_i, the linear effect; b_{ii}, the quadratic effect; and b_{ij}, interaction effects.

The statistical significance of differences between the mean values of variables was determined at the 5% probability level ($P < 0.05$) and the data was analysed by ANOVA. Minitab 19.1.1 software (Minitab Inc., State College, PA, USA) was used to generate the surface plots and the optimized conditions. All assays for characterizing the SBT extract were performed in triplicate.

2.2.6 Process of encapsulation

2.2.6.1 Preparation of feed solutions

The coating materials (3 g) were dissolved in 100 mL of distilled water at 90 °C then stirred until a clear dispersion was achieved. Three coating materials were evaluated: 100% pectin (PE), 100% sodium caseinate (SCN) and a 50%:50% combination of pectin and sodium caseinate (PE+SCN). The prepared PE solution was kept at room temperature while the SCN and PE+SCN solutions were kept in a refrigerator overnight to allow complete hydration to occur.

The next day, SBT extract concentrated by a rotary evaporator (N-1210 and SB-1300 water bath, EYELA Tokyo Rikakikai Co., Ltd, Tokyo, Japan) was added dropwise to the prepared biopolymer solutions heated to 40 °C with magnetic stirring at 800 rpm for 20 min. The prepared feed solutions were sonicated for 20 min then homogenized (HERACLES-16g, Koike Precision Instruments, Tokyo, Japan) with stirring for 30 min before further processing. The feed solution contained 20 g of the carrier solution and 1 g of the concentrated SBT extract. All the prepared feed solutions were then spray dried.

2.2.6.2 Measurement of viscosity

Before spray drying, the viscosity of all feed solutions was measured using a Sine-wave Vibro Viscometer SV-10 (A&D Co. Ltd., Tokyo, Japan). All measurements were carried out at room temperature. Each experiment was performed three times and the average value was taken as the final value.

2.2.6.3 Spray drying conditions

The liquid feeds were spray dried using a laboratory scale spray dryer OSK 55MO102 (Osaka Seimitsu Kikai Co. Ltd, Osaka, Japan). The values of the operational parameters established for the drying process were: solid concentration, 3% (g/g); inlet air temperature, 140 °C; outlet air temperature, 85 ± 3 °C; atomization pressure, 0.4 MPa; and feed flow rate, 5 mL/min. The spray nozzle diameter was 0.5 mm. The same conditions were used for all feed solution formulations, and each experiment was performed in duplicate. SBT extract with no added biopolymer coating material was spray dried under similar conditions to the other samples. The resulting powders were packed in zip-lock bags covered with aluminium foil then stored in a refrigerator until further evaluation.

2.2.7 Characterization of powders

2.2.7.1 Encapsulation efficiency

The encapsulation efficiency (EE%) of the powders was calculated as:

$$EE\% = \frac{TPC - SPC}{TPC} \times 100,$$

where, TPC is the total phenolic content and SPC is the surface phenolic content (Kaderides and Goula, 2019).

TPC was determined by dissolving 10 mg of the sample in 4 mL of ethanol and methanol followed by thorough agitation and sonication for 40 min to completely break down the microencapsulates. Then the solution was filtered through a 0.45-μm filter. SPC was measured by washing a powder sample (10 mg) into a filter paper (0.45 μm) using 4 mL of ethanol and methanol.

2.2.7.2 Morphology and particle-size analyses

The morphology of the particles was examined using scanning electron microscopy (SEM, JSM-6301F, JEOL Ltd., Tokyo, Japan) at a beam voltage of 10 kV and a working distance

of 39 mm. From the micrographs, the particle diameter was calculated using Image J open source software (imagej.net). For measuring the size distribution, 100 particles were counted.

2.2.7.3 Thermogravimetric analysis

Thermogravimetric analyses (TGA) were carried out using a Rigaku TG 8120 thermogravimetric analyser (Rigaku Corp., Austin, TX, USA). Approximately 5 mg of the sample were placed in an aluminium pan with an empty pan used as a reference. The samples were heated from 25 to 600 °C at 10 °C/min under an argon atmosphere.

2.2.7.4 Crystallinity of powders

The crystallinity of the encapsulated phenolic extract was evaluated using a Rigaku Rint-Ultima III X-ray diffractometer (Rigaku Corp.) with Cu-Kα radiation generated at 40 kV/40 mA at a wavelength of 0.154187 nm. The scanning range was 5-40 °2θ at a speed of 2 °2θ/min. The degree of crystallinity was calculated as described by Ahmadian *et al*. (2019):

$$CD = \frac{I_{net}}{I_{total}} \times 100,$$

where CD is the degree of crystallinity (%); I_{net}, the crystalline intensity of peaks; and I_{total}, the overall intensity.

2.2.7.5 Accelerated storage stability study

Glass vials containing three types of microencapsulated powders loaded with SBT extract (20 mg) were stored in an incubator at 45 °C for 40 days. After given periods (0, 20 and 40 d), samples were collected from each batch then their retained phenolic content was determined.

2.2.8 Statistical analysis

Statistical analyses were carried out using Minitab 19.1.1. (Minitab Inc., State College, PA, USA) to determine of significant differences ($P < 0.05$) between encapsulated samples, one-

way analysis of variance (ANOVA) and Turkey′s multiple comparison test were used. The results were reported as the mean value of three repeated experimental data.

2.3 Results and Discussion

2.3.1 Model fitting

The central composite inscribed design was used to optimize the factors (ethanol concentration and temperature) of SSE for extracting the polyphenols. Analysis of variance (ANOVA) showed a significant ($P < 0.05$) model F-value with a non-significant lack of fit for all responses. The coefficient of determination (R^2) showed a good fit with the experimental data (0.96 for TPC and 0.93 for DPHH activity) with little variation around the mean (Table 2.3). Therefore, it was assumed that the selected model could be used for optimizing the extraction of phenolics.

Table 2.3 Regression coefficients (β), coefficient of determination (R^2) and F-test value of the predicted second order polynomial models for TPC and DPPH activity

Regression coefficients		
	TPC	DPPH activity
Intercept		
β_o	266.1	141.2
Linear		
β_1	-3.18^{***}	-1.65^{***}
β_2	$+0.72^{**}$	-0.14^{***}
Quadratic		
β_{11}	$+0.01^{**}$	$+0.01^{**}$
β_{22}	-0.01^{***}	$-0.001^{n.s.}$
Interaction		
$\beta_1\beta_2$	$+0.01^{*}$	$+0.003^{*}$
R^2	0.96	0.93
F value (model)	36.70	21.06
F value (lack of fit)	4.16	3.73

Abbreviations: TPC, total phenolic content (mg GAE/g SBT); DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability (mg GAE/g SBT); R^2, Coefficient of determination.

Note: Level of significance $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s.non-significant at $p > 0.05$.

The linear, quadratic and interactive effects of ethanol concentration (%) and temperature (°C) significantly influenced ($P < 0.05$) the TPC and DPPH scavenging capacity of the SBT extracts. The regression coefficient (β) values of the identified variables were obtained by multiple linear regression (Table 2.3).

2.3.2 Effect of variables on total phenolic content

A three-dimensional surface graph was plotted of the results for the total phenolic content (TPC) of the SBT extracts (Figure 2.1a). The surface describes the variation of TPC as a function of the variables, over the range of values studied. This shows that raising the temperature up to a maximum of 180 °C increased the phenolic content. This effect of temperature on TPC, confirmed in previous studies (Vergara-Salinas *et al*., 2012; Syahariza *et al*., 2017), was possibly caused by the enhanced solubility of the compounds and their increased mass transfer rate. However, ethanol concentration had a greater effect on phenolic content than temperature. The level of phenolics extraction did not increase linearly with increasing ethanol concentration but there was an optimum point after which the TPC decreased. This was confirmed by the most significant p-value ($P < 0.0001$) of the quadratic term for ethanol concentration. It can also be seen that pure water (ethanol concentration 0%) even at a subcritical temperature was not suitable for extracting phenolic compounds but using water, combined with an organic solvent can help to give better results. This phenomenon could have been caused by the creation of a reduced polar medium through using ethanol as the co-solvent (Mussatto *et al*., 2011; Kwon and Chung, 2015). Nevertheless, polyphenols present in plant tissues have been found to be bound to proteins/polysaccharides by hydrogen and hydrophobic bonds. Therefore, this caused the yield using water extraction to be low, or possibly water alone cannot cleave hydrogen bonds

(Miralai *et al*., 2008). However, ethanol can precipitate polysaccharides and expel them from the solution (Xu *et al*., 2014).

The interactive effect of two variables also showed a positive effect on the TPC. The polynomial equation obtained for the apparent phenolic content was:

$$Y_{TPC} = 266.1 - 3.178\ X_1 + 0.716\ X_2 + 0.01143\ X_1^2 - 0.01454\ X_2^2 + 0.00665\ X_1X_2$$

2.3.3 Effect of variables on antioxidant activity

The antioxidant activity was determined by the DPPH assay. Like TPC, DPPH scavenging capacity was plotted as a response surface diagram (Figure 2.1b). The DPPH values ranged between 36.78 and 75.61 mg GAE/g SBT with the lowest value observed for the conditions of 0% ethanol concentration and 100 °C temperature.

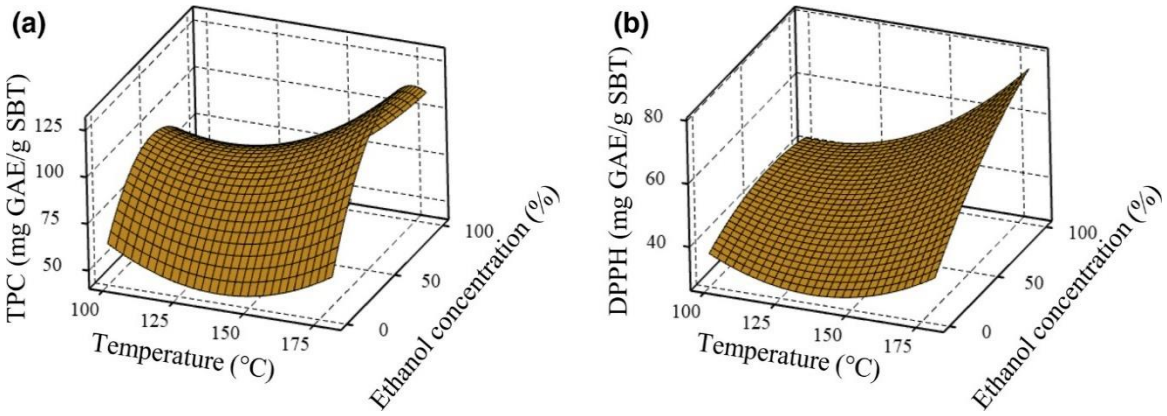

Figure 2.1 Response surface plots illustrating the effect of temperature and ethanol concentration on (a) the total phenolics content and (b) the DPPH antioxidant activity of spent black tea extract.

When comparing this plot with the surface plot of TPC, the patterns of the variation in responses with maximized regions were different. However, the values for TPC and DPPH scavenging capacity were significantly positively correlated ($r = 0.759$, $P < 0.01$) (Figure 2.2). The ANOVA revealed that the linear terms for temperature and ethanol concentration

were most significantly affected by the responses of the antioxidant activity of the SBT extract.

The quadratic term for temperature and the interactive effect of both variables demonstrated the positive effect on the responses as shown by the following equation:

$$Y_{AO} = 141.2 - 1.652\ X_1 - 0.143\ X_2 + 0.0061X_1^2 - 0.0017X_2^2 + 0.0037\ X_1X_2$$

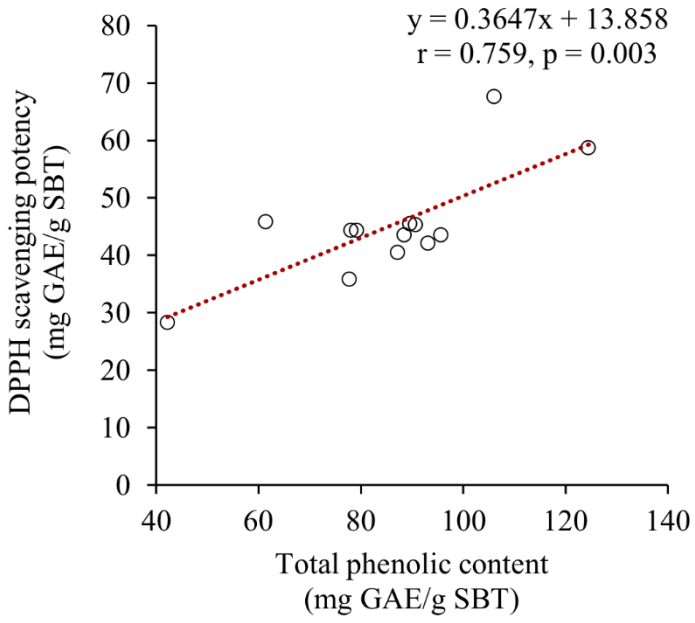

Figure 2.2 Correlation between DPPH scavenging activity and total phenolic content of SBT.

2.3.4 Optimization of extraction process and experimental validation

The SSE was optimized to yield an extract with a high content of phenolic compounds and a high antioxidant activity. A graphical optimization based on the effect of the two factors on the responses was conducted using the highest desirability level (Figure 2.3). This shows that under the optimal conditions (180 °C temperature and 71% ethanol concentration), a

TPC of 126.89 mg GAE/g SBT and DPPH activity of 69.08 mg GAE/g SBT can be obtained. These optimal conditions were used later for validation and the results obtained for TPC (127.15 ± 1.67 mg GAE/g SBT) and DPPH activity (71.31 ± 3.40 mg GAE/g SBT) were very close to those predicted.

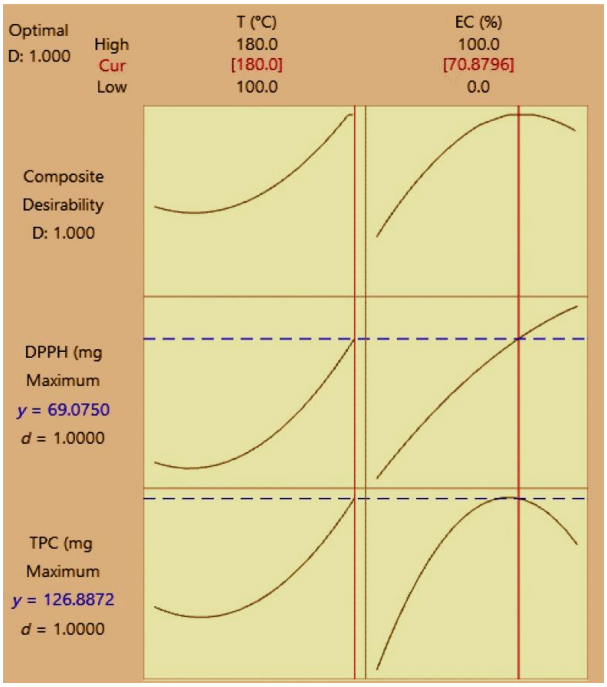

Figure 2.3 Optimization plot for the responses of total phenolic content. T: temperature, EC: ethanol concentration.

Under the optimal conditions, the phenolic content and antioxidant activity of the raw and spent black tea were compared (Figure 2.4). The results showed that only 33% of the antioxidant phenolic compounds had been lost during tea brewing while the remaining 67% could be recovered using the optimized conditions for SSE. The optimal values of TPC from SSE in the present study were considerably higher than that (91.06 mg GAE/g SBT) reported in a previous study that used the maceration-mediated liquid-liquid extraction of SBT (Mukhtar *et al*., 2018).

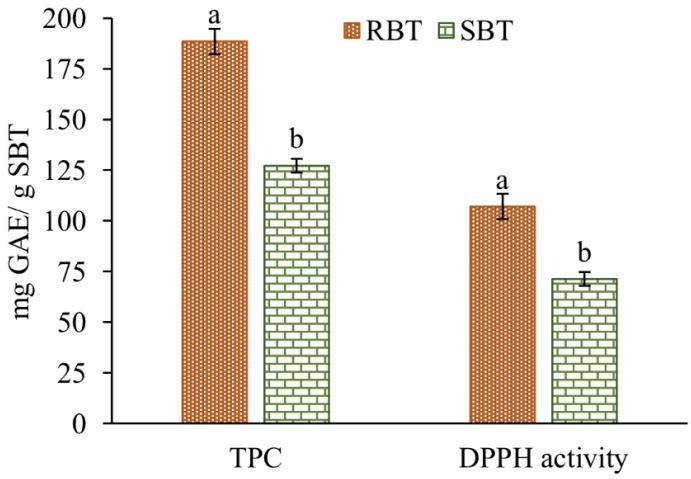

Figure 2.4 Comparison of the extract from raw black tea (RBT) and spent black tea (SBT). Obtained under optimal conditions of 180 °C temperature and 71% ethanol concentration.

2.3.5 Microencapsulation and microparticle characterization

2.3.5.1 Particle size analysis

The particle size and size distribution are important characteristics of powder that can affect their storage and handling. To evaluate the effect of the type of wall material, the average particle diameter and size distribution were measured (Table 2.4).

Table 2.4 Effect of different wall materials on the viscosity of the feed solutions before spray drying, average particle diameter and encapsulation efficiency of spray dried spent black tea powders

Sample	Viscosity of feed solution (mPa.s)	Mean particle diameter (μm)	Encapsulation efficiency (%)
PE	$66.30^{c} \pm 1.96$	$4.61^{a} \pm 2.10$	$75.77^{b} \pm 1.023$
SCN	$2.37^{a} \pm 2.10$	$3.90^{b} \pm 1.52$	$60.07^{c} \pm 0.002$
PE + SCN	$19.35^{b} \pm 2.30$	$4.50^{ab} \pm 1.45$	$81.56^{a} \pm 1.998$

Note: Different letters in the same column indicate a statistically significant difference ($p < 0.05$) between mean values. Values represent the mean ± SD of three individual runs. Wall materials: PE, Pectin; SCN, Sodium caseinate; and PE + SCN, Pectin and sodium caseinate.

This revealed that the type of wall material significantly influenced the diameter of the particles ($P < 0.05$). The standard deviation values of the powder diameter measurements are related to the distribution of particles, with lower values indicating a more homogenous distribution. The microcapsules with SCN exhibited the lowest mean diameter (3.90 μm). The average particle size increased as the amount of pectin in the coating increased. Ahmadian *et al*. (2019) observed the same effect on the particle size with pectin. Particle size is related to the viscosity of the feed solution used for spray drying (Table 2.4). The solution with pectin possessed the highest viscosity and sodium caseinate the lowest. The particles with the mixture of wall materials exhibited the narrowest size distribution (1.45 μm) so were of a more uniform size than particles coated with the other two wall materials. This could have been caused by the heating of the pectin-sodium caseinate blend during its preparation, thus decreasing the polydispersity index by forming a more compact and dense particulate structure through re-arranging the pectin molecules on the sodium caseinate surface (Liang and Luo, 2020).

2.3.5.2 Encapsulation efficiency

The encapsulation efficiency (EE%) of SBT powder with coatings varied between 60.06%-81.55%, thus confirming the successful entrapment of phenolic compounds within the wall materials (Table 2.4). The results also revealed that the type of coating agent used for encapsulation had an important role in retaining phenolic compounds in the carrier matrix ($P < 0.05$). The best result was achieved by the sample with SCN + PE (1:1 mass/mass). Pectin offers the advantageous as a protective carrier and its capability of interacting with hydrophobic molecules (Rehman *et al*., 2019). However, carbohydrates such as pectin mostly lack the interfacial functionality (Livney, 2010). Hence, it is better to combine polysaccharides with surface active biopolymer like caseinate to achieve successful encapsulation (Hogan *et al*., 2001). Nevertheless, milk protein such as sodium caseinate can

bind with polyphenols, especially with catechin, with this mechanism being promoted by a pre-heating treatment (Shpigelman *et al*., 2010; Haratifar and Corredig, 2014).

2.3.5.3 Morphology

SEM was used to observe the microstructure of the encapsulated SBT extracts (Figure 2.5). Figure 2.5 shows that encapsulating SBT with wall materials effectively changed the external structure of the particles. The SBT powder with no coating materials exhibited an agglomerated and disordered structure while the encapsulated SBT powder formed apparently spherical particles with surface depressions. A similar observation was reported by Yinbin *et al*., (2018) when spray drying plum extract with different encapsulating agents.

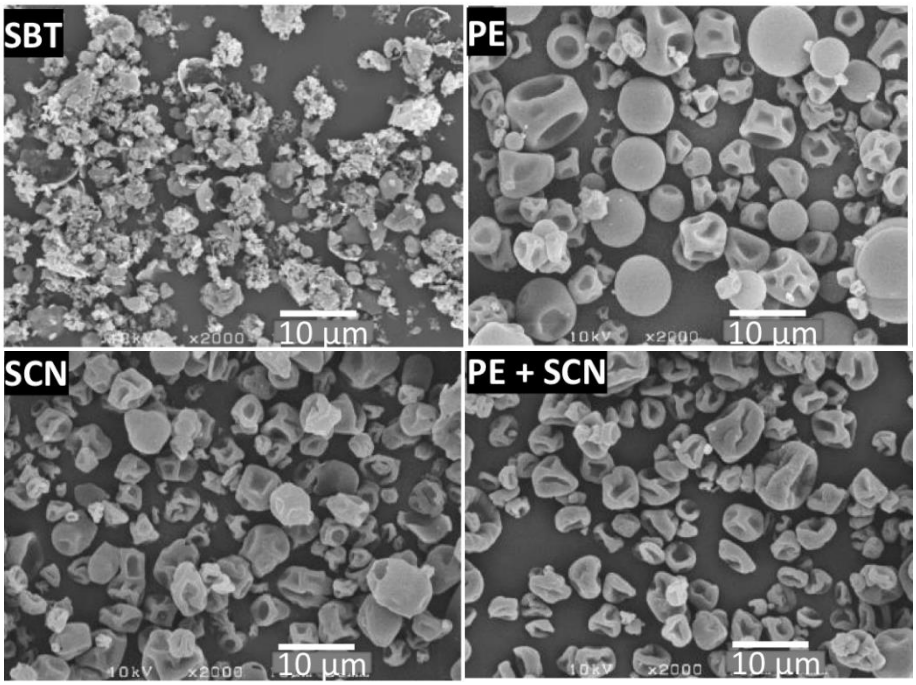

Figure 2.5 Micrographs spray dried powders loaded with spent black tea extract. SBT: phenolic powder spray dried with no encapsulating agents, PE: Pectin, SCN, Sodium caseinate, PE + SCN: Pectin and sodium caseinate. Magnification 2000 ×.

The powder coated with pectin appeared more spherical than that coated with other two wall materials investigated. However, the surface of most of the microparticles was wrinkled. This could have been affected by the lower inlet drying temperature (140 °C). Microparticles produced at the higher drying temperatures exhibited a smoother surface with less shrinkage (Shamaei *et al.*, 2017; Yingngam *et al.*, 2018). Particles with a coating of pectin and a combination of pectin and sodium caseinate exhibited fewer cracks and fissures which would have reduced the retention of polyphenols through contact with the external air and adverse heat conditions.

2.3.5.4 Thermal stability

The thermogravimetric (TG) and derivative thermogravimetric (DTG) curves obtained from microparticles allow the visualization of their thermal degradation behaviour (Figure 2.6).

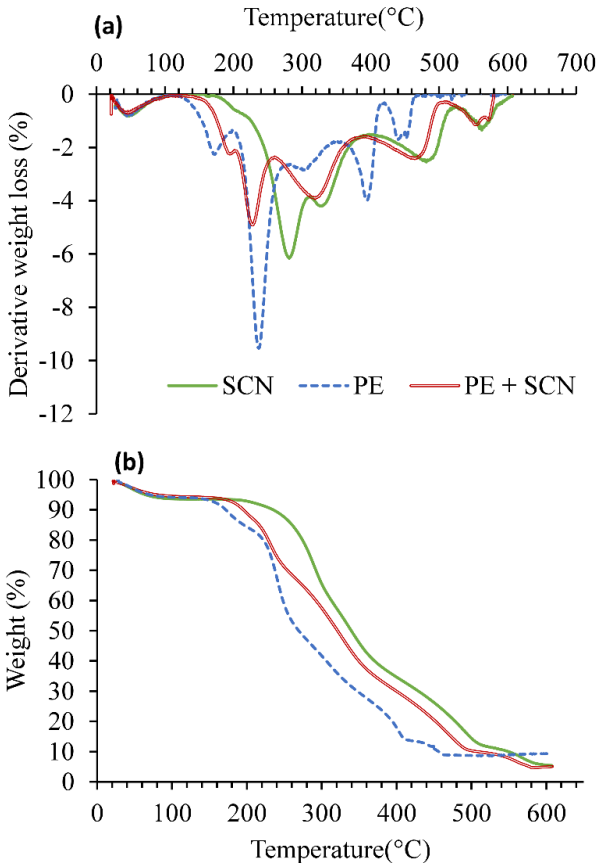

Figure 2.6 DTG (a) and TGA (b) thermographs of microencapsulated SBT powders using pectin (PE), sodium caseinate (SCN) and SCN+PE as wall materials.

All the tested samples exhibited multistage decomposition. The first stage of mass loss occurring between 30 and 100 ℃ could have been caused by dehydration from the microencapsulated particles. The onset temperature for the second stage of decomposition of samples was observed in TGA curves at 120 ℃ for SBT with pectin wall material, at 150 ℃ for the combination of wall materials and at 210 ℃ for the sample with sodium caseinate wall material. Thus, sodium caseinate as a wall material provided greater thermal stability than the other two materials. This was possibly because, with its flexible and disordered structure, it is less sensitive to changes in temperature (McClements, 2018). Figure 2.6a shows that the rate of weight loss during decomposition was lowest in the particles coated with a combination of wall materials possibly because of the enhanced electrostatic interaction between pectin and sodium caseinate (Chang *et al*., 2017). Pectin is an anionic polysaccharide that can interact with casein mainly through electrostatic, steric or covalent interactions.

2.3.5.5 Crystallinity

The XRD patterns of the encapsulated SBT extracts coated with three polymer mixtures are shown in Figure 2.7. The results of XRD were evaluated after smoothing by using Match! Software (Crystal Impact, Bonn, Germany). Normally, a crystalline fraction diffracts X-ray coherently according to Bragg′s Law to give a sharp peak while an amorphous fraction diffracts incoherently to give a diffuse halo (Chung, 2009). Thus, in Figure 2.7, the XRD patterns with their wide bases indicate the amorphous structure all the obtained microcapsules. The degree of crystallinity of the microparticles coated with pectin, sodium caseinate and the blend of pectin-caseinate was 5.75 ± 0.8%, 6.17 ± 0.4% and 4.8 ± 0.9% respectively. These results show that all coating treatments produced samples containing mostly an amorphous phase with the highest proportion (95.2%) in the particles coated with

a mixture of wall materials. These results agreed with XRD results in previous studies on pectin (Hosseinnia *et al*., 2017) and sodium caseinate (Pan *et al*., 2013). Forming of an amorphous structure enhances the rate of dissolution and solubility compared with a crystalline material thereby increasing the bioavailability of the active material (Kanaujia *et al.*, 2015).

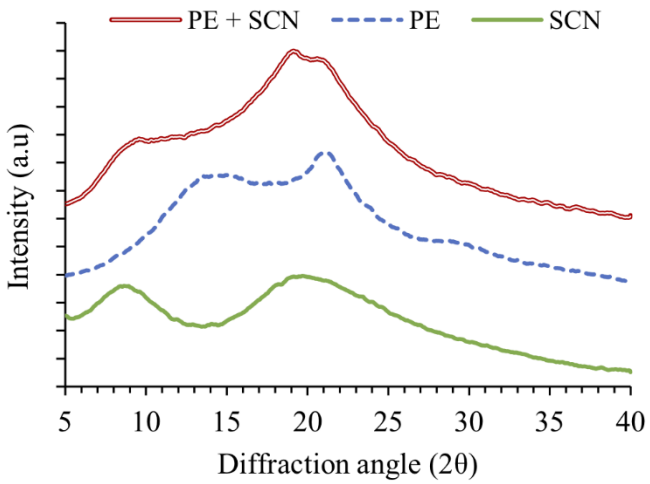

Figure 2.7 X-ray diffraction pattern of microencapsulated SBT powders using pectin (PE), sodium caseinate (SCN) and SCN+PE as wall materials.

2.3.5.6 Accelerated Storage stability

The stability of the encapsulated SBT extract was evaluated (Figure 2.8). Irrespective of the type of wall material, the coatings preserve the core polyphenols than that of SBT extract without coatings.

After 40 d of storage, the SBT extract with no coatings retained only 59.79% of its phenolic content, but the encapsulated powder contained 87.00% phenolic content on average for the three types of wall material. Similar results reported by Zheng *et al*. (2011) and Tsali and Goula, (2018), who evaluated phenolic extracts and their microcapsules from by bayberry and grape pomace, respectively.

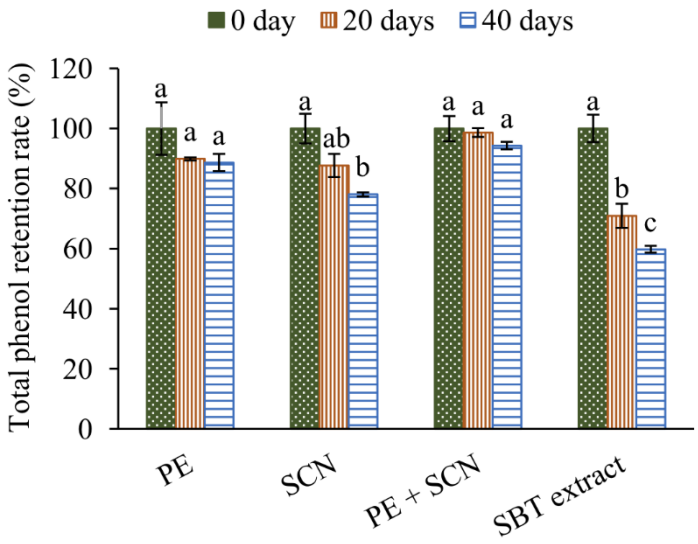

Figure 2.8 Changes in the phenolic content of SBT extract and microencapsulated powder with different wall materials. Different letters in each coating material indicated a statistically significant difference ($p < .05$). Values represent the mean (n = 3).

This effective encapsulation from using a wall material enhances the shelf life of polyphenols in their extracted form by avoiding the damage caused by exposure to oxygen, high temperature and humidity etc. during storage.

Particles coated with the combination of wall materials provided a greater level of preservation of the core polyphenols compared with other two types. These results emphasize the effectiveness of blending pectin with sodium caseinate for use as a wall material as confirmed by the observations on morphology, encapsulation efficiency and crystallinity.

2.4 Conclusions

Subcritical solvent extraction with ethanol as a co-solvent was an efficient method for extracting phenolic compounds from spent black tea. The linear and combined interaction of temperature and ethanol concentration had a significant effect on the total phenolic

content and the antioxidant activity of the SBT extract. The optimal extraction conditions were achieved at 180 °C temperature and at an ethanol concentration of 71%. For microencapsulation, the greatest entrapment and preservation of antioxidant phenolic compounds were obtained by particles with a blend of pectin and caseinate blend as wall material. Using sodium caseinate was more appropriate for producing a more thermally-stable microcapsules with the finest and smallest particles thus enhancing the handling of the powder. The proposed model based on a central composite inscribed design could be used for the subcritical solvent extraction of phenolic compounds in spent black tea, and the subsequent microencapsulation could enhance the stability of the polyphenols. Therefore, antioxidant phenolic compounds can be effectively recovered from spent black tea a food manufacturing waste product with subsequent microencapsulation turning it into valuable food ingredient.

Chapter 3

Pilot-Scale Extraction of Polyphenols from Spent Black Tea by Semi-Continuous Subcritical Solvent Extraction

3.1 Introduction

Black tea [*Camellia sinensis* (L.) O. Kuntze (family: *Theaceae*)] is a popular herbal beverage and contains numerous types of polyphenols that are beneficial to human health. The crucial step in the manufacture of black tea which is called fermentation leads to the formation of these polyphenols. Therefore, the hot water infusion produced from tea leaves can be considered as a therapeutic beverage for consumers interested in health and wellbeing, in addition to its role as a thirst quencher. Numerous studies have analysed black teas for the presence of antioxidant polyphenols, which offer health benefits such as anti-ageing, anti-diabetic, and anti-cancer effects, and preventative effects against cardiovascular and gastrointestinal diseases (Zhang, Qi, & Mine, 2019). As a result of the perceived benefits of tea consumption, world tea production has increased to 5.73 million tons in 2016, of which black tea accounts for 78% (Falla, Demasi, Caser, & Scariot, 2021).

Black tea infusion is prepared by steeping dried tea leaves in hot water for 5–10 min. After brewing, spent tea leaves become a waste product that requires disposal. However, the mild conditions of brewing are not sufficient to extract all the available polyphenols in tea and appreciable amounts of polyphenols remain in spent black tea leaves. These remaining polyphenols exist as non-extractable polyphenols (NEPPs), which may complex with protein and cell wall polysaccharides (Durazzo, 2018). In particular, the ready-to-drink (RTD) tea industry disposes of huge amounts of SBT, estimated to be more than 0.12 million tons annually all over the world (Mukhtar, Mushtaq, Akram, & Adnan, 2018). RTD iced tea is ready prepared tea generally consumed cold and available as a powder format or as RTD tea bottles. Because of the convenience of its consumption, the global bottled tea market is

projected to show a compound annual growth rate of nearly 4% through to 2027 (Tea and Coffee Trade Journal, 2019).

In the system of circular economy, valorisation of food by-products can be used as a source for bioactive compounds, particularly antioxidant polyphenols for further application as functional ingredients in the food industry (Rajapaksha & Shimizu, 2021). Also, the potential and feasible utilization of black tea waste is of high research endeavour. In this context, the recovery of phenolic compounds from SBT requires an efficient and scalable extraction method. Our previous study evaluated the potential of hot pressurised liquid extraction under subcritical conditions (subcritical solvent extraction; SSE) for laboratory-scale recovery of polyphenols from SBT and optimised the processing conditions (Rajapaksha & Shimizu, 2020). SSE is an environmentally friendly method (Shimizu, Ushiyama, & Itoh, 2019), which uses a pressurised liquid kept below its critical point (374 °C for water) and above its boiling point (100 °C for water). The SSE technique promotes the extraction of active compounds without changing their chemical integrity, with reduced solvent volume and extraction times (Liang, Nielsen & Christensen, 2020). For scale-up of this extraction methodology into the design of an industrial plant, a pilot-scale extraction system must be tested. To the best of our knowledge, pilot-plant-scale data for the extraction of polyphenols from SBT using SSE or scale-up studies have not been reported to date. Hence, the aim of the work reported here was to assess the potential of recovering antioxidant polyphenols by SSE at pilot-plant scale to propose a suitable scale-up method. Furthermore, non-extracted polyphenols that remained in SBT after hot water extraction were identified.

3.2 Materials and Methodology

Black tea (expiration date: 09/09/2021) was supplied by Mitsui Norin (Tokyo, Japan). Gallic acid was supplied by Sigma-Aldrich (Shanghai, China), 2,2-diphenyl-1-picrylhydrazyl

(DPPH) by Sigma-Aldrich (Taufkirchen, Germany), Folin–Ciocalteu phenol reagent by Sigma-Aldrich (St Louis, MO, USA), and sodium carbonate, ethanol, and methanol by Fujifilm Wako Pure Chemical (Osaka, Japan). Distilled water was used in all the experiments. All other chemicals and solvents used for liquid chromatography–mass spectrometry (LC–MS) were HPLC grade.

3.2.1 Extraction procedure

The prior knowledge on laboratory-scale use of SSE was considered in the development of a pilot-scale process (Rajapaksha & Shimizu, 2020). In addition, maximum pressure limit of the pilot-scale reactor and the safety matters also determined the process temperature and ethanol concentration of SSE in pilot-scale experiment. Pilot-scale semi-continuous SSE was conducted in a 200-L extractor (TEX0513) as shown in Figure 3.1.

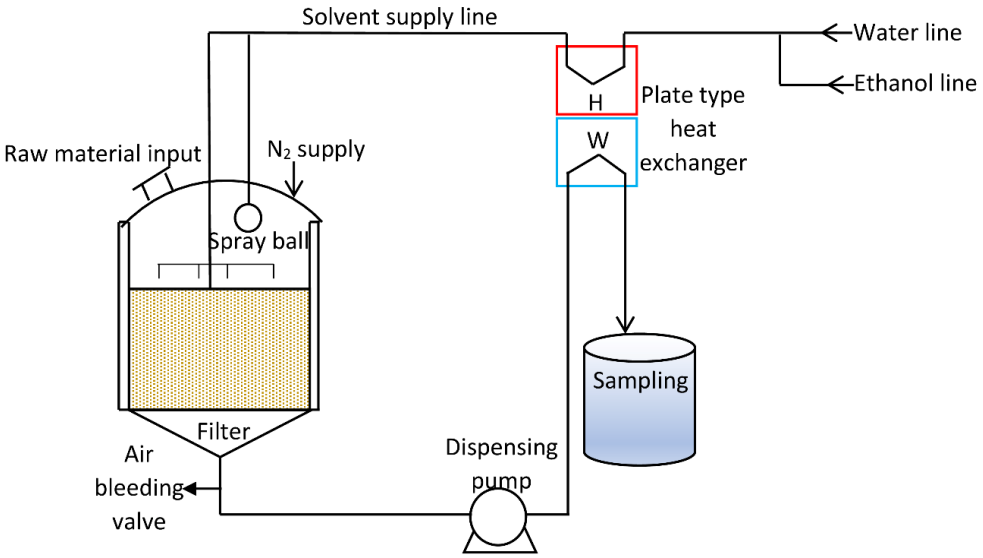

Figure 3.1 Schematic diagram of the hot pressurised extraction equipment at pilot-plant scale.

As given in Table 3.1, three main extraction trials (T1–T3) were conducted. The first extraction was performed with raw black tea (5 kg) in 1:1 ethanol–water mixture (100 kg, 200 kg/h) at 125 °C (SSE: subcritical solvent extraction). Before extraction, the reactor was

purged with N_2 gas to pressurise the inside up to 0.3 MPa (0.25–0.35 MPa). To achieve a semi-continuous process, extracts were expelled in 15-kg fractions while aqueous ethanol was supplied continuously.

Table 3.1 Experimental trials and their conditions

No.	Source	Extraction conditions	Symbol
T1	BT	125 °C, 50% aqueous ethanol, 0.3 MPa	T1
T2	BT	Hot water, 90 °C	HWE
	SBT	125 °C, 50% aqueous ethanol, 0.3 MPa	T2 et
T3	BT	Hot water, 90 °C	HWE
	SBT	125 °C, 40% aqueous ethanol, 0.3 MPa	T3 et

Abbreviations: BT, black tea; SBT, spent black tea.

The second and third experimental runs were carried out to extract the phenolic compounds from SBT. First, raw black tea (5 kg) was extracted with hot water at 90 °C (125 kg, 300 kg/h) and hot water extracts (HWE) were collected for analysis. After removal of HWEs, the remaining SBT leaves were treated by SSE, which using an ethanol-water mixture (T2, 50% ethanol; T3, 40% ethanol) with a solid-to-solvent ratio of 1:20 (100 kg, 200 kg/h) at 125 °C and 0.3 MPa. The extracts were then expelled as 15 kg fractions while the extraction solvent was supplied continuously. To cool the extractor, an extra 20 kg of water was supplied at the end of the ethanolic extraction and the residual liquid was drained. Temperature and pressure in the reactor were recorded during the extraction. The collected extracts were stored at −20 °C until further analysis.

3.2.2 Determination of total solids content and brix

Extract (1 mL) was evaporated to dryness in a laboratory oven at 105 °C for 24 h until constant weight was obtained. The dry weight was recorded to give the total solids (TS) content (g/mL). The total soluble solids in homogenized extracts were determined by a

digital refractometer (RX–5000α–Plus, Atago, Japan) with an accuracy of ±0.010% at temperature of 25°C and were expressed in brix values (0–100 %).

3.2.3 Analysis of total phenolic content

The total phenolic content (TPC) of the liquid extract was measured using the Folin–Ciocalteu (FC) method. Briefly, the obtained extract was diluted (1:100) with solvent, and then a 1.0 mL aliquot of the extract (performed in triplicate) was transferred into a test tube and mixed thoroughly with 5.0 mL of FC reagent diluted (1:10) with distilled water. After 3 min, 5.0 mL of sodium carbonate solution (7.5%, w/v) was added and mixed. The mixtures were then allowed to stand for 1 h in darkness before measuring the absorbance using a UV–Vis spectrophotometer (V-560, JASCO, Tokyo, Japan) at 756 nm against a blank. Gallic acid was used as the standard for preparation of the standard curve (7.812–250 µg/mL, R^2 = 0.998). The TPC values were expressed as grams of gallic acid equivalent per kilogram (dry weight) material (g GAE/kg).

3.2.4 Analysis of antioxidant activity

Antioxidant activity (AA) was determined by measuring the DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging activity using the method of Brand-Williams *et al*., (1995) with slight modification. Fresh DPPH reagent (2.9 mL, 0.1 mM) and diluted (1:70) liquid extract (0.1 mL) were mixed and incubated at room temperature for 20 min before the absorbance was measured at 517 nm (V-560, JASCO). Gallic acid was used as the standard for preparation of the standard curve (3.90–62.5 µg/mL, R^2 = 0.997). DPPH scavenging capacity was expressed as grams of gallic acid equivalent per kilogram (dry weight) of material (g GAE/kg).

3.2.5 Liquid chromatography–mass spectrometry analysis

Liquid chromatography–mass spectrometry (LC–MS) analysis of extracts was performed using a Nexera-XR liquid chromatograph (Shimadzu, Kyoto, Japan) coupled to a mass

spectrometer (LTQ-Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA). Extract (5 µL) was injected into an Inert Sustain AQ-C18 column (1.9 µm, 2.1 × 100 mm, GL Sciences, Tokyo, Japan) maintained at 35 °C. Aqueous formic acid (0.1%, solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used as mobile phase. Gradient elution was performed at 150 µL/min using the following program: 0% B 0 min, 0% B 2 min, 95% B 20 min, 95% B 24 min, 20% B 25 min, 20% B 30 min.

Electrospray ionisation (ESI) was used in negative mode. Other instrument settings: source voltage, 4.0 kV; nitrogen sheath gas flow rate, 30 L/ min; auxiliary gas flow rate, 10 L/min; sweep gas flow rate, 0 L/min; capillary temperature, 300 °C; capillary voltage, 30 V; tube lens voltage, 80 V. All extracts were evaluated in FTMS mode, and the mass range was acquired by full range acquisition covering *m*/*z* 100–1800. Samples were typically diluted (1:50) with solvent A. Data analysis was achieved using XCalibur software v2.0.7 (Thermo Fisher Scientific).

3.2.6 Statistical analysis

Statistical analyses were carried out using Minitab 19.1.1. (Minitab, State College, PA, USA) to determine significance of difference. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare and identify significant differences ($p < 0.05$) between group means. The results were reported as the mean value of three repeated experimental trials.

3.3 Results and Discussion

3.3.1 Study of temperature and pressure during extraction

The preheated water at 90 °C was supplied to the reactor for HWE. Temperature inside the reactor during HWE showed some fluctuation between 70 °C and 83 °C (see Figure 3.2a), which could be due to the heat transfer into the biomass and reactor wall through conduction.

Upon the removal of extract, the outlet temperature increased, although that level was maintained at around 80 °C. During SSE, four phases were observed.

The first phase started with the supply of water–ethanol mixture and the heating and pressurisation of solvent (see Figure 3.2b). The second phase began when the system reached maximum temperature and pressure, allowing the removal of 15 kg extracts. At the beginning of the second phase, the pressure was maintained around 0.3 MPa but it decreased gradually, possibly because of a slight reduction in temperature.

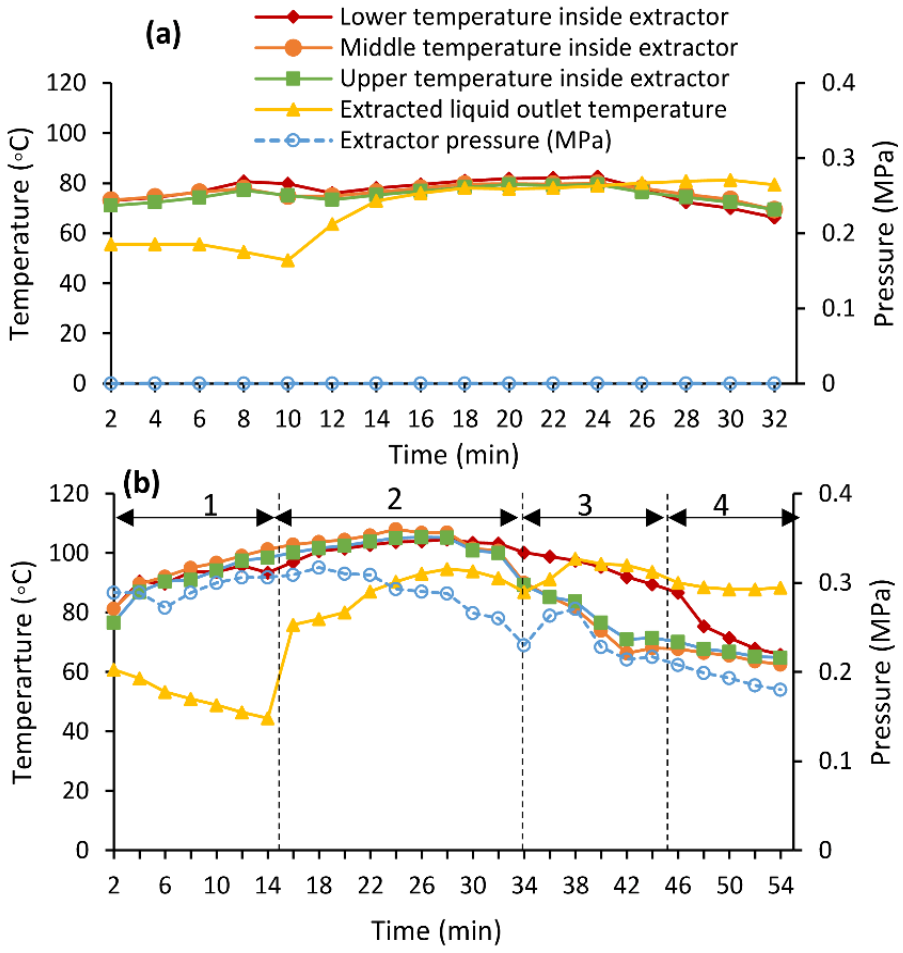

Figure 3.2 Temperature and pressure variation during the HWE (a) and SSE (b). HWE, hot water extraction; SSE, subcritical solvent extraction.

However, this semi-continuous process can minimise the degradation of antioxidant phenolic compounds by shortening the overall extraction time (Asofiei *et al*., 2019). During the third phase, the temperature and pressure decreased as the solvent supply was turned off and the extractor was cooled by water showering. The fourth phase included draining of all extracts after the water shower.

3.3.2 Total solids content and brix value

TS content and the brix value of all fractions for the three main extractions are shown in Figure 3.3. The TS content of extracts represents both soluble and insoluble matter, while brix is used to analyse the percentage of soluble solids in the solution.

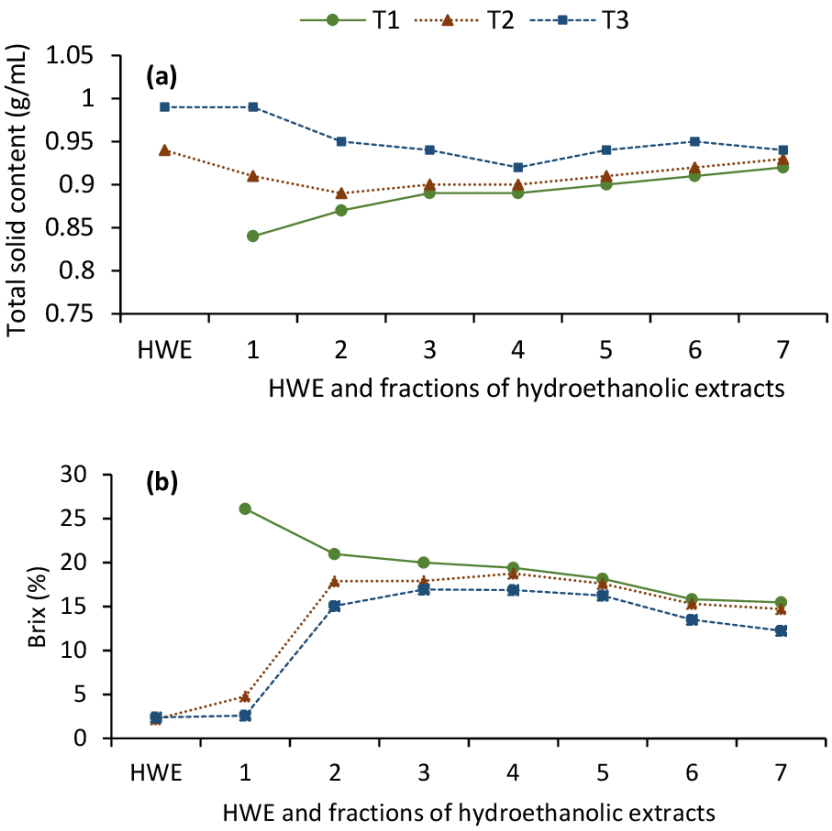

Figure 3.3 Total solids content (a) and brix value (b) of HWE and all fractions of hydroethanolic extracts of T1, T2, and T3.

The TS values were lower in all fractions of the T1 extract and were higher in the T2 and T3 samples. In contrast to the trend in TS content, the highest brix value was observed in T1 sample, while T3 showed the lowest value. These results reflect the trend of higher soluble solids in T1 extract than insoluble material, and T3 contained higher content of insoluble material than T1 and T2. Moreover, the HWEs showed lower brix and higher TS content than other ethanolic fractions, which indicates a higher content of insoluble particles in HWEs. From T2 and T3, the ethanolic fractions of T2 (T2 et) contained higher levels of soluble solids. Soluble matters would be expected to include sugars, polyphenols, phenolic acids, lignans, alkaloids, and soluble amino acids, have more affinity to hydroethanolic mixtures than pure water. Thus, it is noteworthy that the SSE technique could facilitate the mass transfer from SBT, and that the ethanol–water mixture enhanced the solubility of compounds in SBT. This finding is consistent with the prior result showing that increased yields of soluble solid was obtained from green tea extracts obtained by different extraction techniques (Das *et al*., 2019).

3.3.3 Analysis of homogenized fractions of the extracts

3.3.3.1 Total phenolic content and antioxidant activity

The quantitative analyses of TPC and AA of the HWEs and homogenous samples from T1, T2 et, and T3 et were performed (see Figure 3.4a,b). The extract of T1 from raw black tea had the highest values of TPC and AA, owing to the presence of polyphenols not extracted previously. HWE removed less than half of the antioxidant polyphenols from T1 and a large amount remained in the SBT. SSE carried out as the second extraction using 50% hydroethanolic solvent at 125 °C and 0.3 MPa (T2 et) extracted significantly higher amounts of polyphenols and antioxidant content (80.82 and 64.20 g GAE/kg black tea, respectively) ($p < 0.05$). Lowering the ethanol concentration from 50% to 40% caused decreased polyphenol and antioxidant yields. This result was consistent with our previous work, which

showed that increased temperature and increased ethanol concentration favoured greater recovery of polyphenols. This was attributed to the creation of a moderately polar medium and a greater ability of ethanol to establish intermolecular interactions with polyphenols (Rajapaksha and Shimizu, 2020). Moreover, a solvent of ethanol–water can enhance the extraction efficiency by reducing the polarity even at the temperature as 125 °C, thus increasing the solubility and diffusivity of phenolics at high temperatures (Allcca-Alca *et al*., 2021).

3.3.3.2 UV-visible absorption spectrum

UV-vis spectra of the extracts were recorded from 200 to 600 nm and are shown in Figure 3.4c.

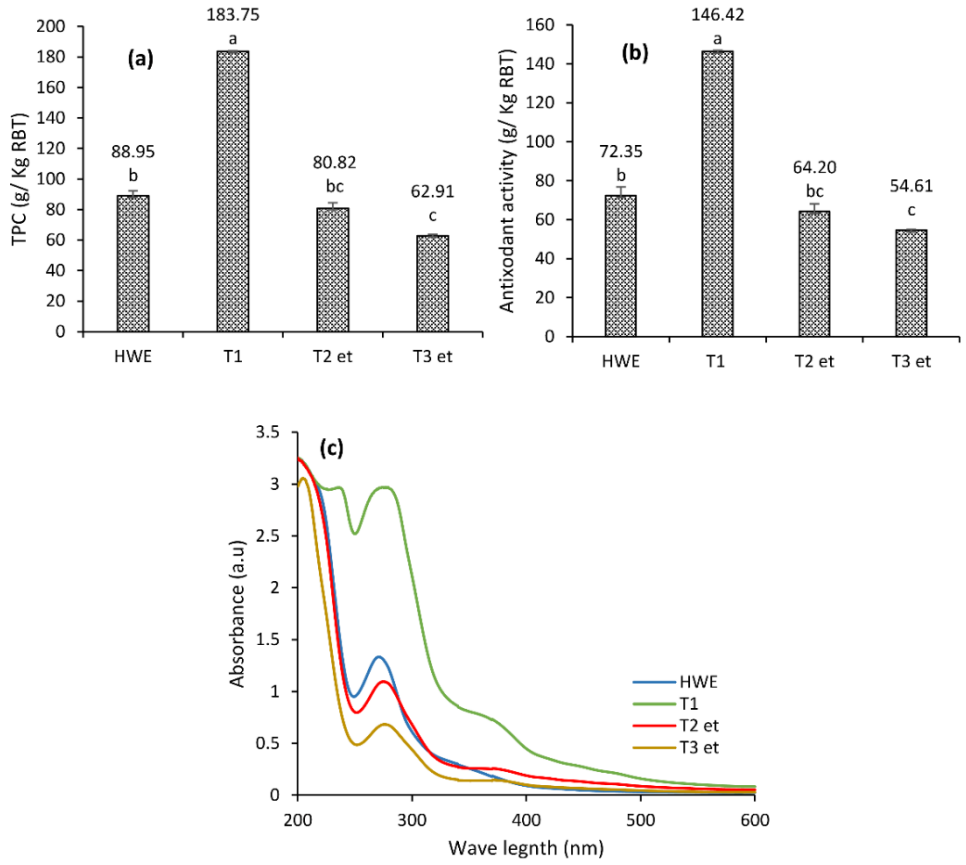

Figure 3.4 Total phenolic content (TPC) (a), antioxidant activity (b), and UV-visible absorption spectra of hot water extract (HWE) and hydroethanolic extracts (T1, T2 et, T3 et) (c). Bars marked with different letters (a–c) are significantly different ($p < 0.05$).

All extracts showed a maximum absorption at 280 nm, which likely corresponds to the polyphenols in black tea, in particular theaflavin and catechin derivatives, phenolic acids, and alkaloids. In addition, extracts from SSE showed another absorption peak at 360 nm. This result is confirmed by Figure 3.5, which shows a reddish-brown colour in the extract, likely caused by thearubigins in black tea (Uchida *et al*., 2016).

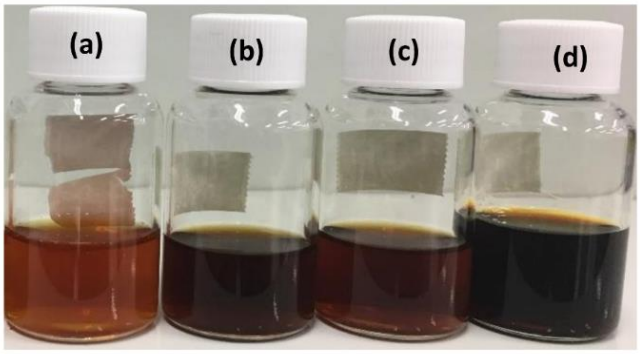

Figure 3.5 Visual appearance of HWE (a), T2 et (b), T3 et (c), and T1 (d).

3.3.4 Total phenolic content and antioxidant activity of T2

TPC and AA content of HWE and each fraction (15 kg) of T2 et were measured for the T2 extract. As presented in Table 3.2, the content varied significantly from one fraction to the next. The maximum amount of phenolic content was obtained from the second fraction of the ethanol–water extract and the TPC values were in accordance with the AA values in same fractions. Rupturing of cell walls facilitates solvent access and leads to extraction of more polyphenols in the initial fractions.

The content of TPC and AA decreased in later fractions because of the removal of most of the polyphenols in the initial fractions. However, the first hydroethanolic fraction had low levels of TPC and AA, probably because of mixing of solvent with the remaining in HWE. The first four T2 et fractions (60 kg of extracts) extracted almost 75% of the phenolic content. Thus, it is noteworthy that 60 kg of aqueous ethanol would be sufficient to extract more than

75% of misspent polyphenols from 5 kg of black tea; in other words, the solvent-to-solid ratio of 12:1 is better than 20:1. Use of this result will lead to efficient extraction in terms of cost and environmental considerations.

Table 3.2 Total phenolic content and antioxidant activity of HWE and ethanol–water fractions in T2 extract

Fraction	TPC μg/ g solid extract	AA μg/ g solid extract
HWE	36.78 ± 0.86^{a}	30.20 ± 1.94^{a}
1	49.29 ± 0.83^{b}	54.20 ± 0.61^{b}
2	68.39 ± 0.72^{c}	66.98 ± 1.15^{c}
3	34.36 ± 0.24^{c}	38.60 ± 0.45^{d}
4	24.19 ± 0.08^{d}	28.88 ± 0.09^{d}
5	15.44 ± 0.11^{e}	19.41 ± 0.09^{e}
6	12.61 ± 0.01^{f}	12.90 ± 0.73^{ef}
7	12.49 ± 0.05^{f}	14.49 ± 0.13^{f}

Note: $^{a–f}$Means within the same line with different letters differ significantly ($p < 0.05$). All data are expressed as the mean ± standard error of triplicate measurements. Abbreviations: TPC, total phenolic content; AA, antioxidant activity.

3.3.5 Phenolic composition in HWE, T2 et, and T1

The overview of the relative abundance of the major polyphenols available in the extracts is presented in Figure 3.6 and their fragmented ions, retention times, and tentative identifications are summarised in Table 3.3. While HWE reflects normal tea infusion, T2 et extract reflects the residues that remain in SBT leaves. Qualitative analysis showed that HWE could extract relatively few compounds, mainly theasinensin C, theasinensin-gallate, theaflavin and apigenin and kaempferol linked with carbohydrate moieties. After SSE treatment of SBT leaves, the collected T2 et extracts contained some non-polar polyphenols, such as galloyl esters of theaflavin and hydrophobic fractions of high molecular weight theasinensin. The remaining polyphenols in T2 et can be considered to be NEPPs, which are an understudied fraction of polyphenols. NEPPs mostly occur as conjugates with

macromolecules such as polysaccharides and proteins. Because of their presence in food by-products, they are mostly neglected as waste (Pérez-Jiménez and Saura-Calixto, 2018). Theaflavin-33'-digallate (TF3) was identified as the most abundant polyphenol in T2 et extract but was not detected in HWE. TF3 is the major theaflavin found in black tea and is formed from the co-oxidation of (−)-epicatechin gallate and (−)-epigallocatechin gallate (EGCG) during black tea production (Pan *et al*., 2018). This result indicates that TF3 has a greater ability to solubilize in the hydroethanolic solvent at high temperature and pressure than in hot water. A similar observation was reported by Friedman *et al*., (2006). In addition to TF3, theasinensin A was relatively abundant in SBT leaves. Theasinensin and theaflavin have been known as the catechin dimers that serve as precursors of thearubigins (TR). Recent studies have proposed that TRs are generated by oxidative cascade-type reactions of catechin (Drynan *et al*., 2012; Kuhnert *et al*., 2010). In addition, malic acid, gallic acid, epigallocatechin-gallate, quercetin-3-*O*-rutinoside, epicatechin-gallate, and epitheaflagalline-3-gallate were tentatively identified as abundant polyphenols in T2 et and T1 extracts.

In the total ion spectrum of T1 extract, 18 phenolic compounds were identified including the polyphenols identified in HWE and T2 et. These results confirmed that the extractable polyphenol fraction from black tea using hot water was significantly less than the fraction obtained by SSE. As mentioned above, most of the NEPPs in SBT were inaccessible to the solvent during the HWE process, probably because of hydrophobic interactions between NEPPs and matrix compounds. These hydrophobic interactions can be weakened by increasing the temperature and pressure in SSE because they are impeded by the decrease of ionic strength (Domínguez-Rodríguez *et al*., 2017). In addition, decreased viscosity and surface tension in solvents facilitates the solubility and diffusivity of phenolics, resulting in higher extraction yields for a variety of NEPPs.

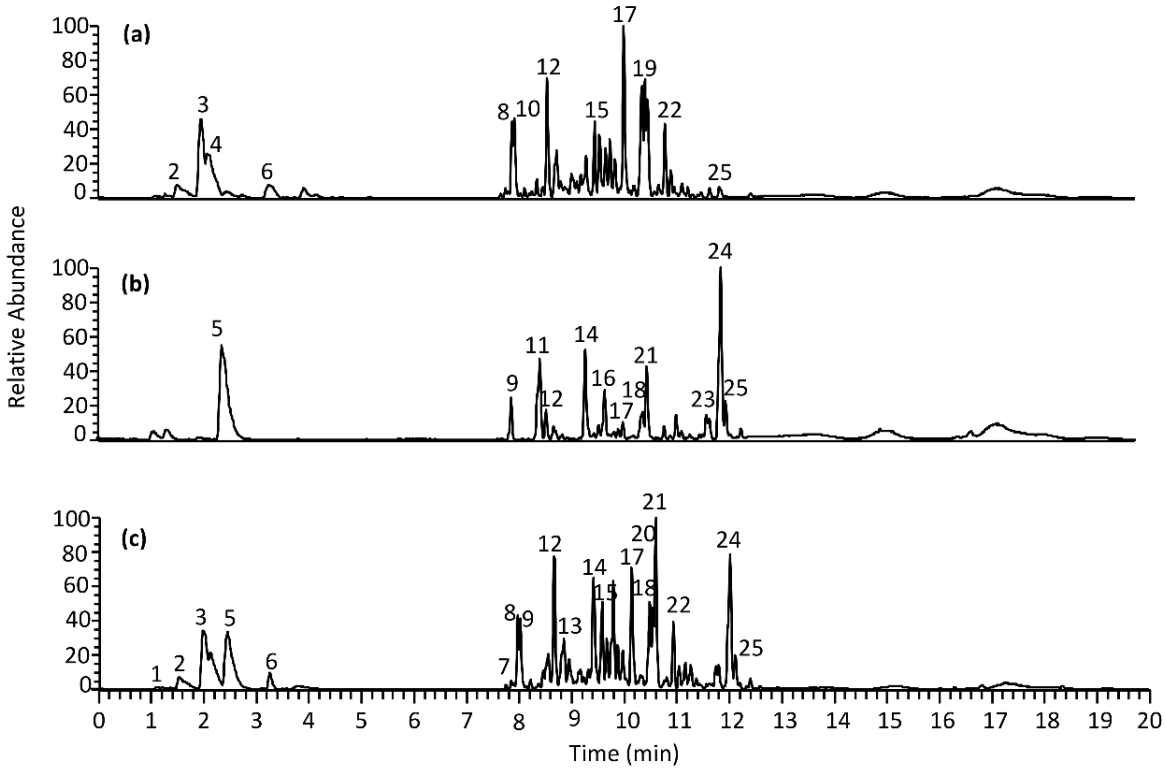

Figure 3.6 Total ion chromatograms obtained at negative MS ionisation for HWE (a), T2 et (b), and T1 (c) extract samples. Peaks identification: 1. Caffeic acid 2. Caffeic acid derivative 3. Quinic acid 4. Unknown 5. Malic acid 6. Unknown 7. Gallic acid hexoside 8. Unknown 9. Gallic acid 10. 5-O-Galloylquinic acid 11. Unknown 12. Theasinensin-gallate 13. Galloyl-HHDP-hexoside 14. Theasinensin A 15. Theaflavin 16. Epigallocatechin-gallate 17. Theasinensin C 18. Quercetin-3-O-rutinoside 19. Apigenin-6, 8-C- dihexoside 20. P2 (EGCG digallate dimer) 21. Epicatechin-gallate 22. Kaempferol-3-O-glucoside 23. Epitheaflagalline-3-gallate 24. theaflavin-3 3'-digallate 25. Theaflavin-3-gallate.

Table 3.3 Mass spectrometric data for tentatively identified compounds

No.	Rt (min)	Observed [M-H$^+$] (m/z)	Fragments (m/z)	Assignment	Samples
1	1.10	178.9899	135	Caffeic acid	T1
2	1.53	356.9989	179	Caffeic acid derivative	HWE, T1
3	1.99	190.9998	85, 93	Quinic acid	HWE, T1
4	2.12	386.9999	-	Unknown	HWE
5	2.43	132.8937	115, 73, 87	Malic acid	T2 et, T1
6	3.29	173.0116	-	Unknown	HWE, T1
7	7.86	331.0330	169, 125	Gallic acid hexoside	T1
8	7.99	609.0637	-	Unknown	HWE, T1
9	8.00	168.9044	125	Gallic acid	T2 et, T1
10	8.04	343.0604	191, 169	5-*O*-Galloylquinic acid	HWE
11	8.55	160.9064	-	Unknown	T2 et
12	8.67	760.9925	609, 591	Theasinensin-gallate	HWE, T2 et, T1
13	8.86	633.0733	301, 463, 275	Galloyl-HHDP-hexoside	T1
14	9.42	912.8475	761, 743, 591	Theasinensin A	T2 et, T1
15	9.59	562.9991	545, 425, 407	Theaflavin	HWE, T1
16	9.80	459.0021	331, 169, 305	Epigallocatechin-gallate	T2 et
17	10.15	609.1007	532, 952	Theasinensin C	HWE, T2 et, T1
18	10.53	609.0012	609, 463, 301	Quercetin-3-*O*-rutinoside	T2 et, T1
19	10.56	593.1317	473, 503	Apigenin-6, 8-*C*-dihexoside	HWE
20	10.61	882.7050	713, 543	P2 (EGCG digallate dimer)	T1
21	10.61	441.0357	289, 169	Epicatechin-gallate	T2 et, T1
22	10.95	447.0540	285	Kaempferol-3-*O*-glucoside	HWE, T1
23	11.75	551.0112	399, 295, 261, 169	Epitheaflagalline-3-gallate	T2 et
24	12.03	866.9273	715, 697, 527	theaflavin-3 3'-digallate	T2 et, T1
25	12.12	714.9858	563, 545, 501	Theaflavin-3-gallate	HWE, T2 et, T1

3.4 Conclusions

In the present study, applicability on pilot-scale extraction of polyphenols from spent black tea by semi-continuous subcritical solvent extraction (SSE) was evaluated. SSE led to increased diffusivity and solubility of phenolics. The use of 1:1 ethanol–water solvent at 125 °C and 0.3 MPa extracted significantly higher phenolic content from spent black tea than hot water extraction (HWE). In addition, a variety of non-extractable polyphenols in spent black tea leaves, which remained after HWE, were extracted by SSE.

Chapter 4

Development and Characterization of Functional Starch-Based Films Incorporating Free or Microencapsulated Spent Black Tea Extract

4.1 Introduction

Lipid oxidation or oxidative rancidity, one of the main causes of food deterioration alongside microbial spoilage, generates harmful compounds in foods as well as degrading their colours and nutrients (Vieira *et al*., 2017). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), and propyl gallate have often been used to overcome rancidity. However, undesirable side-effects associated with these synthetic antioxidants have directed scientific studies towards investigating natural antioxidants (Caleja *et al*., 2017; Pires *et al*.,2017). Therefore, phenolic compounds originating from plants, the main category of natural antioxidants, have become of growing scientific interest. The large amount of waste arising from food manufacturing is a potential source of underused polyphenols which possess antioxidant power (Dueñas and García-Estévez, 2020; Kumar *et al*., 2021). In this sense, using this waste is a sustainable and economically attractive way of obtaining natural antioxidants.

Tea has been a popular beverage since ancient times. One type, black tea, is consumed throughout the world and contains many types of polyphenols such as theaflavin, thearubigin, and catechin which are responsible for its antioxidant activity (Łuczaj and Skrzydlewska, 2005; Butt *et al*., 2014). Tea is prepared by infusing tea leaves in boiling water for a short time. After black tea is brewed during the industrial beverage manufacturing process, the remaining residue, spent black tea (SBT), is usually discarded as waste. SBT still contains a significantly high and recoverable quantity of antioxidant phenolic compounds and our previous study has shown that subcritical water extraction with ethanol as a co-solvent is an efficient process for extracting phenolic compounds from SBT

(Rajapaksha and Shimizu, 2020). This method, subcritical solvent extraction (SSE), uses a pressurized mixture of water and ethanol in the liquid state above its boiling point to enhance the solubility of the compounds and the mass transfer rate during the extraction process, thereby improving the extract yield while decreasing the time and solvent consumption during the process.

The phenolic extract from SBT can be incorporated directly into foods or in food packaging to control lipid oxidation. However, adding phenolic extracts directly to food may neutralize their antioxidant activity after they react with the food and reduce its quality. As the lipid oxidation process is induced from the surface of the foodstuff, designing a functional food film incorporating phenolic extracts has recently received more research attention (Ceballos *et al*., 2020; Valdés García *et al*., 2020). Antioxidants incorporated into a film can, not only prevent oxidative damage in fatty foods, but also act as a functional additive by migrating from the packaging into the food product. However, the stability of polyphenols can deteriorate during food processing and storage because of their sensitivity to oxygen, light, and heat. Encapsulating phenolic extracts in various types of wall material can efficiently reduce this deterioration during the preparation of the films. Encapsulation can also help to regulate the kinetics of the active compounds as they are released into food products while maintaining the physical properties of the film.

Spray drying is the most widely used technique for encapsulating active and heat-labile compounds because of its short thermal contact time and suitability for industrial application. The type of wall material is also crucial when producing microencapsulates by spray drying (Ray *et al*., 2016). Of the various types of wall material, conjugated mixtures of protein and polysaccharides, such as sodium caseinate and pectin, are of great interest because of their complementary effects on the stability of the core material (Baracat *et al*., 2012; Nooshkam and Varidi, 2020). Sodium caseinate is derived from casein, the principal

protein in the milk of bovine and other ruminant animals. Its emulsifying properties combined with its heat stability promote its suitability as a wall material for encapsulation (Augustin *et al*., 2011). Pectin is a negatively charged plant polysaccharide which has a wide range of food applications including as a protective carrier because of its gel-forming and stabilization properties. In our recent study, polyphenols obtained from SBT were successfully encapsulated in a mixture of pectin and sodium caseinate to produce a functional food ingredient (Rajapaksha and Shimizu, 2020). These encapsulated polyphenols can be used as stable active compounds in different food applications. However, few studies have reported on the application of these prepared functional microencapsulates to food products and active food packaging incorporating SBT extract microencapsulates has not yet been developed to the best of our knowledge. For producing food packaging, the use of biobased polymers rather than synthetic material is gaining popularity because they are biodegradable, cheap, abundant, and edible. Starch from a variety of plant sources is considered as one of the most promising biopolymers. In particular, cassava starch has been reported to be an excellent raw material for food packaging because of its characteristics of being odourless, tasteless, colourless, non-toxic, and with a high amylopectin content and high viscosity (dos Santos *et al*., 2018; Luchese *et al*., 2018; Qin *et al*., 2019; Lim *et al*., 2020). It has also been used as a carrier for antioxidants and antimicrobials in active food packaging and improved the physical properties of the film (Piñeros-Hernandez *et al*., 2017; Luchese *et al*., 2017; Yun *et al*., 2019).

Hence, the present study aimed to develop a functional film incorporating SBT extract and to investigate the effect of incorporating free and encapsulated forms of the extract on the physico-chemical properties and migration behaviour of active compounds into food simulants. The performance of antioxidants in preventing lipid oxidation of soybean oil will also be studied.

4.2 Materials and Methodology

The cassava starch was obtained from a local supermarket in Sapporo (Hokkaido, Japan). Unblended black tea was supplied by New Vithanakande Tea Factory (Pvt) Ltd., (Ratnapura, Sri Lanka). Glycerol, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin and Ciocalteu phenol reagent, and casein sodium salt from bovine milk were provided by Sigma-Aldrich (St. Louis, MO, USA), and pectin from citrus, sodium carbonate, magnesium nitrate, ethanol, and methanol were provided by Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Distilled water was used in all experiments. All other chemicals and solvents used were of analytical grade.

4.2.1 Preparation of spent black tea powders

4.2.1.1 Preparation of spent black tea

The SBT was produced as described previously (Rajapaksha and Shimizu, 2020). Briefly, black tea leaves were brewed in boiling water for 6 min (2 g/100 mL), then filtered to obtain the residue. The SBT was prepared after air-drying the filtered residue in an oven at 45 °C.

4.2.1.2 Extraction and encapsulation of polyphenols

The antioxidant phenolic compounds from SBT were extracted by subcritical solvent extraction (SSE) using the conditions optimized in a previous study (Rajapaksha and Shimizu, 2020). Briefly, a mixture of pure water and ethanol (71% concentration) and SBT (20 mL/g) were mixed in an 11-mL reactor with an agitator (Chemi-station PPV 3000, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The extraction reactor was purged with nitrogen gas and 2.0 MPa of initial pressure was applied. The sample in the reactor was then heated to 180 °C and maintained there for 10 min. During extraction, the agitation speed was kept at 1000 rpm to prevent any local overheating and to increase the mass transfer. After the extraction, the reactor was cooled in a cold-water bath; then, the sample was filtered through filter paper (6 μm) to recover the SBT extract. The recovered SBT extract was concentrated

using a rotary evaporator (N-1210 and SB-1300 water bath, EYELA Tokyo Rikakikai Co., Ltd., Tokyo, Japan), then stored at 4 °C until encapsulation.

The SBT extract was microencapsulated as described previously (Rajapaksha and Shimizu, 2020). A 50%:50% combination of pectin and sodium caseinate was used as the wall or coating material. First, the coating material was dissolved in distilled water at 90 °C (3 g/100 mL), then stirred until a clear dispersion was obtained. The polymer solution was then kept in a refrigerator overnight to allow complete hydration. On the next day, the concentrated SBT extract was added dropwise to the prepared biopolymer solution (1 g SBT extract: 20 g solution) after it had been heated to 40 °C; then, the mixed solution was stirred for 20 min. The prepared feed solutions were sonicated for 20 min, then homogenized for 30 min (HERACLES-16g, Koike Precision Instruments, Tokyo, Japan). The samples with a 3% solids concentration were then spray-dried with a laboratory-scale spray dryer OSK 55MO102 (Osaka Seimitsu Kikai Co. Ltd., Osaka, Japan) using a spray nozzle (diameter 0.5 mm), an inlet air temperature of 140 °C, an outlet air temperature of 85 °C, an atomization pressure of 0.4 MPa, and a feed flow rate of 5 mL/min. The non-encapsulated SBT extract was spray dried under similar conditions to the other samples with each experiment performed in duplicate. The resulting SBT extract powders with or without encapsulation were packed in zip-lock bags, covered with aluminium foil, then stored in a refrigerator until further use.

4.2.2 Preparation of the films

The films were prepared by the solvent casting process, as described in previous studies (Stoll *et al*., 2016; Piñeros-Hernandez *et al*., 2017; Talón *et al*., 2019). Cassava starch control films were produced by blending 4.0 g of starch, 1.2 g of glycerol, and 84.0 g of distilled water. Active starch films were prepared by replacing the starch component in the formulations with the same amount of free or encapsulated SBT extract powder with

predetermined phenolic content by Folin–Ciocalteu method (Rajapaksha and Shimizu, 2020) (total phenolics content of powders: 359.90 and 17.14 µg of gallic acid equivalent/mg, respectively), to obtain films with two different concentrations of total polyphenols (Table 4.1). The total solids concentration of all the control and active films were set at 4% (*m*/*m*). First, an aqueous starch dispersion was heated at 95 °C for 30 min under constant stirring until the starch was gelatinized. Glycerol was then added to the solution at a total solid: glycerol ratio of 10:3 (*m*/*m*) and agitated for 30 min. After cooling the solutions to 40 °C in a cold-water bath, the free and microencapsulates of SBT were added to the starch solution, then homogenized for 15 min. The film-forming dispersions (FFD) obtained were degassed by sonication then poured into polypropylene Petri dishes while maintaining the amount of FFD in the dish constant at 0.28 g/cm^2. After drying at 35 °C for 24 h, the films were conditioned at 25 °C and 53% relative humidity over saturated Mg $(NO_3)_2$ before characterization.

Table 4.1 Formulations of starch films incorporating free or encapsulated SBT extracts

	Types	**Polyphenols %**	**Starch (g)**	**SBT/SBT$_{en}$ (g)**	**Glycerol (g)**	**Water (g)**
Control films	S		4.000	-	1.2	84.0
Films with SBT extract	SBT 0.17%	0.17%	3.981	0.0190	1.2	84.0
	SBT 0.34%	0.34%	3.962	0.0380	1.2	84.0
Films with encapsulated SBT extract	SBT$_{en}$ 0.17%	0.17%	3.600	0.4000	1.2	84.0
	SBT$_{en}$ 0.34%	0.34%	3.200	0.8000	1.2	84.0

4.2.3 Measurement of viscosity of film-forming dispersions

The viscosity of all the FFD was measured using a Sinewave Vibro Viscometer SV-10 (A&D Co. Ltd., Tokyo, Japan).

All measurements were carried out at room temperature in triplicate, and the average value was taken as the final value.

4.2.4 Characterization of the films

4.2.4.1 Fourier transform infrared (FT-IR) spectroscopy of powders and films

The preliminary structures of the SBT and SBT_{en} powders were characterized using an FTIR spectrophotometer (JASCO FTIR660plus, JASCO Corp.). The samples were prepared by pressing a mixture of the powder sample and KBr into pellets. The functional groups of the films were identified by the FTIR spectrophotometer equipped with an attenuated reflection accessory, ATR (Spectrum 100, PerkinElmer Co., Ltd., Shelton, CT, USA). A spectral resolution of 4 cm^{-1} was used and 32 scans were acquired for each spectrum in the range of 650–4000 cm^{-1}. This experiment was performed in a room at 23 °C and 50% relative humidity.

4.2.4.2 Scanning electron microscopy (SEM)

The surface morphology of the films was observed using a field emission scanning electron microscope (SEM, JSM-6301F, JEOL Ltd., Tokyo, Japan). The samples were dried at 35 °C for 12 h, mounted on aluminium stubs using double-sided carbon tape, then sputter-coated with gold. The micrographs were captured at an accelerating voltage of 10 kV.

4.2.4.3 Film thickness and tensile properties

The thickness of the films was measured by a digital micrometer (ABSOLUTE Digimatic Micrometer, Mitutoyo Corp., Kawasaki, Japan) to the nearest 0.001 mm. The average thickness was based on measurements at three random positions on the film.

The tensile properties, tensile strength (TS), elongation at break (%EAB), and Young's modulus (YM) of the films were analysed using a universal testing machine system (AG-100kNXplus, Shimadzu Corp., Kyoto, Japan) according to the ISO 527-3 (2018) test method (Babaghayou *et al*., 2018). All the films were initially cut into a dumbbell shape using a

cutter (JIS K7113 No.1½, Shimadzu Corp.) and preconditioned for 5 d at 23 °C and 50% relative humidity before the mechanical testing. The initial grip separation and the testing speed were set at 50 mm and 50 mm/min, respectively. The results are based on three measurements for each formulation.

4.2.4.4 Water vapour transmission rate (WVTR)

The water vapour transmission rate of the samples was analysed as described previously (Akhter *et al*., 2019), with minor modifications. First, the film samples were equilibrated in a desiccator containing a saturated solution of NaCl (75% relative humidity). The films were then fixed using elastic bands over the top of a weighing bottle containing 5.0 g of anhydrous $CaCl_2$, then weighed immediately (W_1). All the bottles were then placed in a chamber for 24 h (75% relative humidity at 25 °C) then weighed again (W_2). The WVTR was calculated as follows:

$$\text{WVTR (g mm/m}^2\text{ 24 h)} = \frac{(W2-W1) \times \text{Film thickness}}{\text{Area} \times \text{Holding time}}$$

4.2.4.5 Light transmission

The light transmission of the films was measured using a UV-Vis spectrophotometer (JASCO V-560, JASCO Corp.) in the wavelength range of 200–800 nm. First, the films were cut into rectangular strips (3.5 cm × 0.5 cm), then placed directly into a cuvette to allow the light beam to pass through the films.

The analyses were performed in triplicate and an empty cell was used as the reference. The film transparency for visible light was determined at 600 nm (Loo and Sarbon, 2020). The percentage transparency was calculated as follows:

$$\text{Transparency value} = (\log T_{600})/x$$

where T is the fractional transmission at 600 nm and x is the thickness of the film sample (mm).

4.2.4.6 DPPH radical scavenging assay

The antiradical effect of the films was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method (Dou *et al*., 2018) with slight modifications. First, a piece of film (30 mg) was completely dissolved in distilled water, then 3 mL of ethanol was added. The resulting solution was centrifuged at 6000× *g* for 10 min. Two millilitres of the supernatant and 5 mL of 0.1 mM DPPH solution were mixed, kept in the dark for 30 min, then the absorbance was measured at 517 nm using a UV–vis spectrophotometer (JASCO V-560, JASCO Corp.). Gallic acid was used as the standard for preparation of the standard curve (0.5–6.0 μg/mL, R^2 = 0.99). The DPPH scavenging capacity was expressed as micrograms of gallic acid equivalent/g (dry weight) film (μg GAE)/g film).

4.2.4.7 Migration test

Pieces of film sample (2 cm × 2 cm) were placed in 8 mL of water (representing an aqueous food) and 95% ethanol (representing a fatty food) in separate glass vials. The vials were then purged with nitrogen gas before closing and kept at 25 °C for 7 d. The migration of the antioxidant compounds into each food simulant was then tested using the DPPH method, as described earlier (Section 3.5.6). The results were expressed as micrograms of gallic acid equivalent/g (dry weight) film (μg GAE)/g film).

4.2.4.8 Peroxide value (PV) of soybean oil

Soybean oil (5 mL) was poured into dark glass vials, then sealed with films of each formulation (S, SBT 0.17%, SBT 0.34%, SBT_{en} 0.17% and SBT_{en} 0.34%). The sealed vials containing the oil were then placed upside-down to ensure the oil was in contact with the films. An open dark glass vial containing 5 mL of soybean oil was used as a control. All vials were stored at 27 ± 2 °C and 50 ± 5% relative humidity under fluorescent light, then samples were taken after 0, 7, 14, and 35 d of storage to determine the PV of the oil. The peroxide value of the soybean oil was determined by iodometric titration. The oil sample (1

g) was mixed with 10 mL of glacial acetic acid and chloroform (3:2, *v*/*v*) in an Erlenmeyer flask. The mixture was shaken vigorously to dissolve the oil completely, then mixed with 0.5 mL of saturated potassium iodide solution. The mixture was then kept in the dark for 1 min before dilution with 30 mL of distilled water and titrated against 0.01 M sodium thiosulphate in the presence of a starch indicator (5 mL). The volume of sodium thiosulphate consumed was recorded and the PV expressed as millimolar equivalents of free iodine per kilogram of oil (meq of oxygen/kg). All the analyses were performed in triplicate.

4.2.5 Statistical analysis

Statistical analyses were carried out using Minitab 19.1.1. (Minitab Inc., State College, PA, USA) to determine the significance of differences of factors and levels. One-way analysis of variance (ANOVA) and Tukey′s multiple comparison test were used to compare and identify significant differences ($p < 0.05$) between group means. The results were reported as the mean value of three repeated experimental data.

4.3 Results and Discussion

4.3.1 Fourier transform infrared (FTIR) analysis of films and SBT extract powders

FTIR spectroscopy is used for analysing the functional bonds and intermolecular interactions between compounds by identifying their molecular vibrations. The infrared spectra of free and encapsulated spent black tea extract powders (SBT, SBT_{en} respectively) are shown in Figure 4.1a.

The FTIR spectrum of SBT extract reflects the main functional groups in polyphenols, amino acids, and alkaloids. The broad band at 3318 cm^{-1} is related to the O–H and N–H stretching modes in tea extract (Moosa *et al*., 2015; Brza *et al*., 2020). The peaks observed at 2922, 2858, and 1034 cm^{-1} have been attributed to C–H stretching, O–H stretching in alkanes and carboxylic acid, and C=C bond stretch in aromatic rings, respectively (Ali *et al*., 2018). The 1234 cm^{-1} band probably arises from the C–O group in polyols such as

hydroxyflavones and catechins (Rengga *et al*., 2017). The absorption bands at 1630 and 1530 cm^{-1} have been attributed to amide I (C=O stretching) and amide II (N–H bending) in the amino acids present in black tea (Thummajitsakul *et al*., 2020).

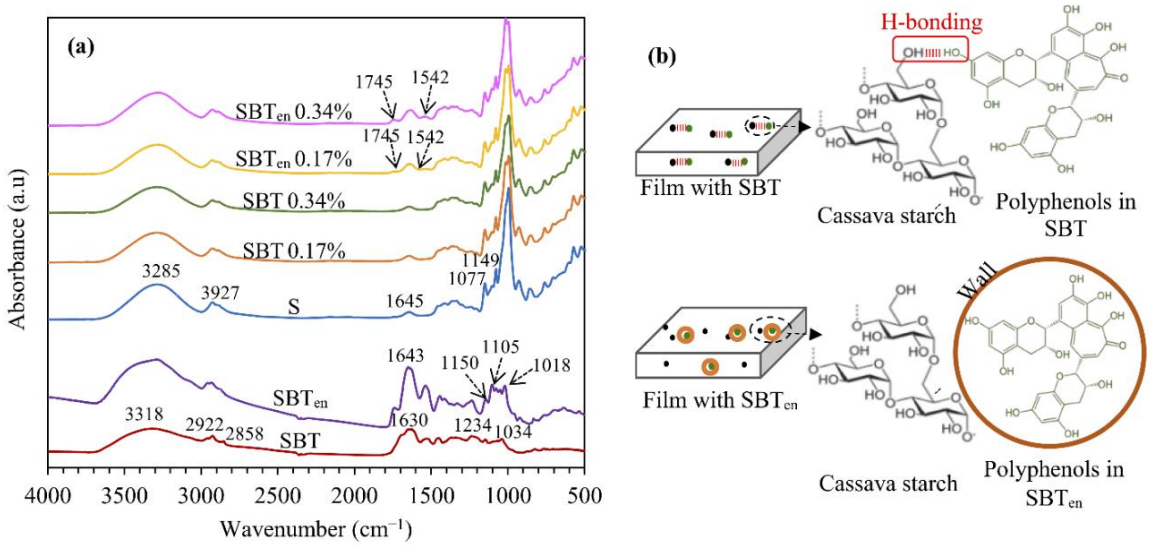

Figure 4.1 FTIR spectra of spray-dried SBT and SBT_{en}, starch-based films without (S), and with SBT or SBT_{en} (a); Schematic illustration of interaction between starch and SBT polyphenols in active films with SBT or SBT_{en} (b).

For SBTen powder, a strong peak observed at 1643 cm−1 could be caused by the migration of the –COO stretching vibration at 1630 cm−1 from the spectrum of pectin and the C=O band at 1648 cm−1 from the spectrum of casein (Figure 4.2). These spectral changes have been documented as characteristics of the pectin–casein bonding which may be created due to the Maillard conjugation (Ghazi, 1999; Ren *et al*., 2019; Abd El-Salam and El-Shibiny, 2020).

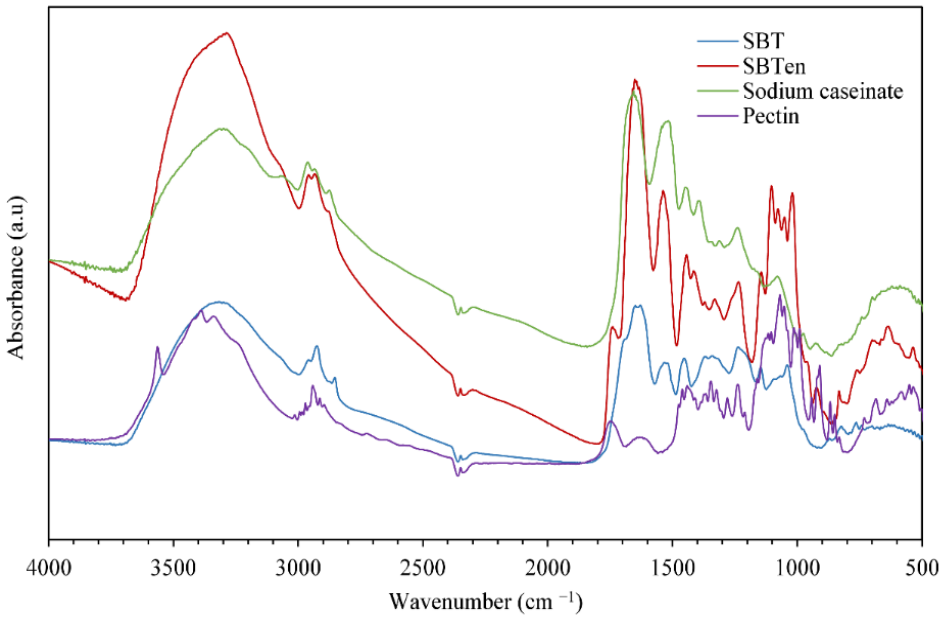

Figure 4.2 FTIR spectra of SBT, SBT_{en}, sodium caseinate and pectin powers.

The results from X-ray diffraction and thermal stability in our previous study have also confirmed the formation of a pectin–caseinate complex (Rajapaksha and Shimizu, 2020). Moreover, emerged new peaks at 1105 and 1018 cm^{-1} in SBT_{en} presented a band at 1150 cm^{-1} (C–O stretching) in both SBT and SBT_{en} and a mild shift in the peak of O–H stretching at 3290 cm^{-1}, and indicated that conjugation had occurred between the polyphenols and sodium caseinate–pectin during the encapsulation process (Figure 4.2). This result was consistent with Jin *et al.*'s study (Jin *et al.*, 2018), which revealed FTIR spectra of conjugation between tea polyphenols, pectin, and soy protein.

In the IR spectra of all films (Figure 4.1a), the wide band between 3000 and 3600 cm^{-1} associated with O–H stretching and the peak at 2927 cm^{-1} could be attributed to the C–H from alkyl groups (Lei *et al.*, 2019). The peaks at 1149 and 1077 cm^{-1} were attributed to C–O bond stretching of the C–O–H group. It has been reported that the vibrational band at

around 1645 cm^{-1} is related to the O–H bending of the adsorbed water in the amorphous regions of cassava starch (Edhirej *et al*., 2017). In films incorporating SBT_{en}, this peak overlapped with C=O stretching (amide I; at 1635 cm^{-1}) in the sodium caseinate wall materials. The films with SBT_{en} exhibited bands at 1542 cm^{-1} (amide II) and 1745 cm^{-1}, corresponding to typical peaks for SBT_{en} powder, thus confirming the successful incorporation of the encapsulated powder.

After adding intact SBT extract to the film, a flattening and a slight red shift of the O–H stretching band from 3285 cm^{-1} to 3291 cm^{-1} can be observed, indicating that there was no chemical interaction, but hydrogen bonds between active groups of the starch matrix and the phenolic hydroxy groups in the SBT. In contrast, the related peak in films with SBT_{en} showed less flattening than with SBT and shifted to the lower wavenumber. This behaviour indicated the higher availability of O–H in SBT_{en} films than in the other formulations owing to having unbound surface O–H groups in SBT_{en} film (Nebahani and Jaisingh, 2020).

The ratio of the intensities of the peaks at 3300 and 1149 cm^{-1} (I_{3300}/I_{1149}), associates to the stretching vibration of 'C–O' in the 'C–O–H' group, was also calculated to compare the number of hydroxyl groups available in the different formulations. The films containing SBT_{en} exhibited higher ratios indicating a higher number of available hydroxyl groups and the film containing SBT showed a lower number than the other formulations (Piñeros-Hernandez *et al*., 2017). It can thus be presumed that phenolic hydroxyl groups can interact with the cassava starch matrix by creating hydrogen bonds, but encapsulating SBT in wall materials can interrupt these bonds, as illustrated in Figure 4.1b.

4.3.2 Morphology of control and active Films

The effect of incorporating free and biopolymer-encapsulated SBT extract into starch films on their superficial morphology was analysed using scanning electron microscopy (SEM) images (Figure 4.3). The cassava starch films exhibited a smooth, homogenous, and flat

surface, but adding SBT generated the rough surface on films, which interfered with the regular starch matrix. This damaging of matrix could be ground for the poor mechanical properties of the active films.

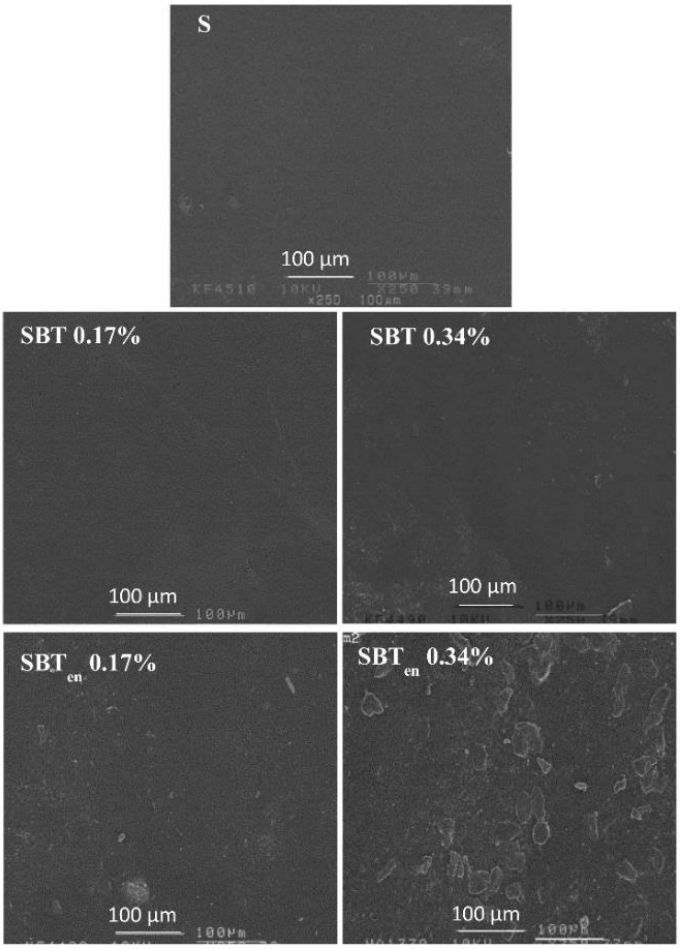

Figure 4.3 SEM micrographs of the surface of starch films incorporating free or encapsulated SBT extracts: control film (S) and active films incorporating SBT or SBT_{en} at 0.17% or 0.34%.

Films incorporating the free SBT extract showed no significant differences in their surfaces regardless of the polyphenol concentration, suggesting that the SBT extract had spread evenly throughout the starch matrix and interacted via hydrogen bonding during the processing of the film. Similar results have been observed during the development of active

starch films incorporating tea polyphenols (Feng *et al*., 2018). After adding SBT_{en}, white particles were observed on the surface of the films, possibly related to the partial dissolving of SBT_{en} resulting in the entrapment of SBT extract within the core of the microencapsulates. The SBT_{en} 0.34% films exhibited a very rough surface with a less homogenous surface, suggesting that the polymer chains had been interrupted by the microcapsules. This phenomenon has also been reported where undissolved particles were observed on the surface of cassava starch films incorporating anthocyanin microencapsulates (Stoll *et al*., 2016).

4.3.3 Tensile properties, thickness, Young's modulus, and viscosity of film-forming solutions

The mechanical behaviour of films is related to their internal structure and can be described in terms of tensile strength (TS), elongation at break (EAB), Young's modulus (YM), and film thickness. For the different film formulations, Table 4.2. shows the mean values of their mechanical parameters and Figure 4.4, their stress–strain curves.

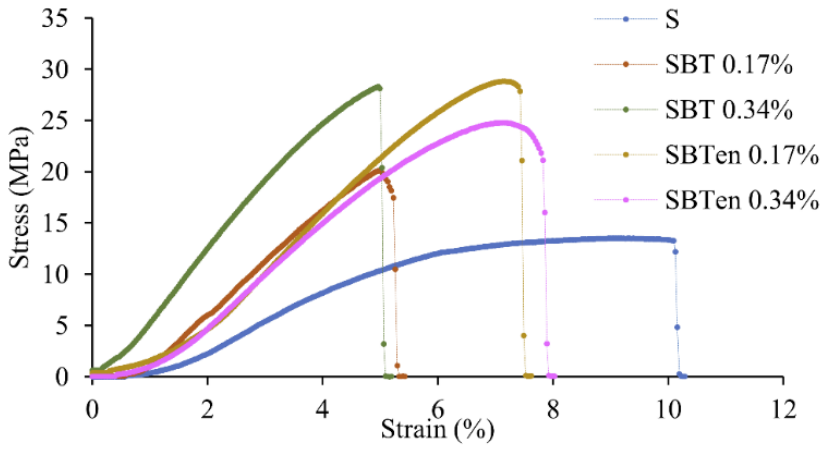

Figure 4.4 Stress–strain curves of starch films incorporating free or encapsulated SBT extracts: control film (S) and active films incorporating SBT or SBT_{en} at 0.17% or 0.34%.

Incorporating free and encapsulated SBT decreased the thickness of the active films associated with the decreasing starch mass. The control film with the highest mass of cassava starch exhibited the highest thickness influenced by the viscosity of the film-forming dispersion (FFD) (Table 4.2), an important parameter to be evaluated for the film casting process. This can affect the spread ability, thickness, uniformity of the casting layer, and the mechanical properties of the biopolymer film (Manshor *et al*.,2019). Table 4.2 shows that the tensile strength (TS) of the films incorporating SBT was significantly higher than that of the control film ($p < 0.05$) with the film incorporating 0.34% SBT exhibiting the highest value of 25.33 MPa. Simultaneously, the strain at break of the SBT films decreased significantly. This behaviour can be attributed to the generation of hydrogen bond interactions between the black tea extract and the cassava starch molecules. The spent black tea extract comprises polyphenols with hydroxyl groups, particularly thearubigins, theaflavin, and catechin, which influence their linking with the functional groups in cassava starch. Similar results have been found in starch films incorporating tea polyphenols and rosemary extracts (Piñeros-Hernandez *et al*., 2017; Feng *et al*., 2018). The incorporation of encapsulated SBT into film also significantly increased both the value of TS and Young's modulus (YM) ($p < 0.05$). This may have been caused by the microencapsulates acting as a reinforcement filler in the starch matrix, thus developing a less continuous microstructure. However, the values of TS and YM in films with SBT_{en} were lower than those with free SBT at 0.34%, possibly influenced by the sodium caseinate and pectin used as wall materials in the microencapsulates. High levels of rigidity and brittleness are seriously disadvantageous limitations in active biobased packaging films and thus affect their use in the food industry. Consequently, incorporating encapsulated SBT into the starch matrix can overcome these disadvantages by increasing the elasticity and positively affecting the mechanical behaviour of the film.

Table 4.2 Viscosity of FFD, thickness, mechanical properties, and water vapor transmission rates (WVTR) of starch films incorporating free or encapsulated SBT extracts

Film	Viscosity of FFD (mPa.s)	Thickness (mm)	Tensile Strength (MPa)	Young's Modulus (MPa)	WVTR (g mm^2/m^2 24 h)
S	195.5 ± 2.5 ^{a}	0.1133 ± 0.0036 ^{a}	13.43 ± 0.141 ^{b}	224.45 ± 86.3 ^{c}	0.61 ± 0.19 ^{a}
SBT 0.17%	119.0 ± 5.0 ^{b}	0.1002 ± 0.0120 ^{ab}	17.54 ± 4.535 ^{ab}	231.94 ± 63.7 ^{c}	0.29 ± 0.04 ^{b}
SBT 0.34%	104.3 ± 5.1 ^{bc}	0.0957 ± 0.0028 ^{ab}	25.33 ± 3.706 ^{a}	1282.39 ± 84.4 ^{a}	0.54 ± 0.12 ^{a}
SBT_{en} 0.17%	90.5 ± 1.5 ^{bc}	0.0.0906 ± 0.0043 ^{b}	25.17 ± 4.578 ^{a}	955.05 ± 74.0 ^{b}	0.38 ± 0.05 ^{ab}
SBT_{en} 0.34%	82.5 ± 3.5 ^{c}	0.0862 ± 0.0032 ^{b}	23.47 ± 5.301 ^{ab}	1088.19 ± 72.34 ^{ab}	0.52 ± 0.13 ^{a}

Note: Starch films incorporating free or encapsulated SBT extracts: control film (S) and active films incorporating SBT or SBT_{en} at 0.17% or 0.34%. Different letters in the same column (a, b, c) indicate a statistically significant difference ($p < 0.05$) between mean values. Values represent the mean ± standard deviation of three individual runs.

4.3.4 Water vapour transmission rate

The water vapor transmission rate (WVTR) is a critical property of packaging films which affects the moisture level of the food products by preventing the loss or gaining moisture. The WVTR of the different film formulations with free and encapsulated SBT was assessed (Table 4.1). The results indicated that the significant effect on WVTR of adding SBT at 0.17% could be attributed to the formation of hydrogen bonds between the starch and polyphenols, as discussed previously. This can reduce the quantity of hydrophilic functional groups, thus lowering the WVTR value. However, this value increased again with the addition of SBT at a concentration of 0.34% owing to the hygroscopic nature of phenolic extracts. The WVTR of a film can be influenced by several factors such as the hydrophobic/hydrophilic nature of the materials used for the preparation of film, the

presence of cracks or voids, and steric hindrance and tortuosity in the structure (Moghadam *et al*., 2020). The WVTR values of active films with encapsulated SBT increased, but not above those of the control and the SBT 0.34% films, possibly because of the water-soluble nature of pectin in an aqueous environment (Chen *et al*., 2010) and the hydrophilic groups of sodium caseinate in the wall of the microencapsulates (Chew *et al*., 2018). However, the decrease in the WVTR indicated the adequate cohesion of the polymeric chain formed, thus illustrating that strong intermolecular interactions had created barriers to the diffusion of water vapor through the matrix, whereas the presence of microcapsules as fillers can also create a tortuous path for water molecules to pass through.

4.3.5 Light transmission and transparency of films

The light transparency of packaging materials is an important parameter, because it affects the quality of the protection to foods while also influencing its appearance and attractiveness. Figure 4.5a shows that the control film based only on cassava starch was clear and transparent, but adding SBT or SBT_{en} made the films slightly brown in colour. The control film (S) exhibited the highest transparency value (−1.783), but after adding the SBT and SBT_{en} powder, the transparency of the resulting active films (average: −2.98 and −3.15, respectively) decreased significantly ($p < 0.05$).

Light can catalyse many reactions causing food to deteriorate, influencing lipid oxidation, off-flavour development, the generation of undesired colours, and the degradation of nutritional compounds. UV light, with a higher energy than visible light, has a great potential for breaking chemical bonds. Therefore, blocking out UV light is a desirable property for a packaging film, particularly when used on foods with a high lipid content which are prone to photosensitized oxidation.

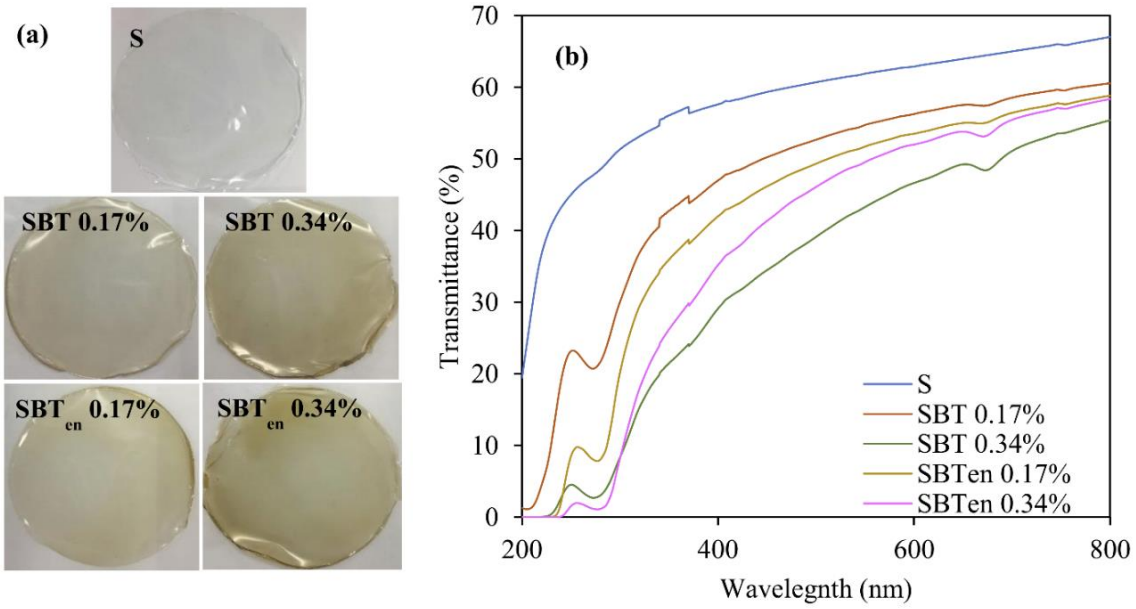

Figure 4.5 The visual appearance (a) and light transmittance (b) of the control film (S) and starch films incorporating free or encapsulated SBT extracts at 0.17% or 0.34%.

The wavelength of ultraviolet (UV) light ranges from 10 to 380 nm and visible light from 400 to 780 nm (Plakett and Siro, 2012); thus, the transmission of UV and visible light was determined at a wavelength between and 200 to 800 nm (Figure 4.5b). All the active films provided a lower transmission of UV light than the control film (S), possibly due to the fact that UV light was absorbed after incorporating the phenolic extracts into the starch matrix. It was shown to increase the obstruction to UV light with increasing SBT or SBT_{en} content. This behavior agreed with previous studies on active cassava starch film incorporating Chinese berry anthocyanins (Yun *et al*., 2019), and cassava starch/chitosan active film with Pitanga leaf extract (Chakravartula *et al*., 2020). In the case of the SBT_{en} 0.34% film, the light transmission in the range of 400–800 nm was 35–58%, whereas for the SBT 0.34% film, it was 29–55%, possibly because the polymeric wall materials in SBT_{en} had covered the active compounds.

4.3.6 Antioxidant content of films and their migration into food simulants

Biopolymer films incorporating antioxidant compounds are manufactured for active food packaging applications. The incorporated antioxidant compounds can help reduce oxidative reactions in foods and thus significantly increase their shelf life. The DPPH radical scavenging activity assay is commonly used to evaluate the activity of antioxidant compounds in food by quantifying their ability to quench the DPPH radical. The dark purple colour of DPPH disappears when it is reduced to its non-radical form. The degree of fading of the free radical scavenger can be quantified by measuring the absorbance at 517 nm. The antiradical effects of all active film formulations measured by DPPH are shown in Table 4.3.

The results showed that the concentration of polyphenols and encapsulation significantly affected the antiradical effect of the films ($p < 0.05$). The total antioxidant activity of the SBT_{en} 0.34% film was the highest at 630 µg GAE/g film, whereas that of the SBT 0.17% film was significantly lower than that of the other formulations at 173 µg GAE/g film. This can be attributed to the loss of antioxidant polyphenols during the preparation and drying of films incorporating free SBT. However, the microencapsulation of polyphenols could preserve the antioxidant activity of the films, which agreed with a report on the use of microencapsulation for preserving green tea polyphenols during different food applications (Massounga *et al*., 2018). Incorporating a higher level of polyphenols (0.34%), whether encapsulated or free, also significantly increased the antioxidant activity of the films.

The migration or release test is important for providing information on the affinity of food products for active materials, thus making it feasible to select the most suitable active material for each type of food. The release completely depends on the compatibility of the antioxidant compound with the food product or food simulant (Gómez-Estaca *et al*., 2014). Various studies have quantified the compounds which are released into recommended food

simulants such as water (representing aqueous foods) and 95% ethanol (representing fatty foods) (Adilah *et al*., 2018; Fasihnia *et al*., 2020). The results of the present study showed a significantly higher release of antioxidant compounds from films incorporating SBT microcapsules (SBT_{en}) into water than into 95% ethanol (Table 4.3, Figure 4.6a).

Table 4.3 Antioxidant content and their migration into food simulants (water and 95% ethanol), expressed in terms of μg (gallic acid equivalent; GAE)/g film

Film	Total Antioxidant μg (GAE)/g Film	Migration (Water) μg (GAE)/g Film	Migration (95% Ethanol) μg (GAE)/g Film
SBT 0.17%	173.14 ± 6.88 ^{d}	9.84 ± 3.00 ^{c}	10.85 ± 7.22 ^{c}
SBT 0.34%	587.06 ± 6.98 ^{b}	30.03 ± 4.00 ^{c}	53.31 ± 5.11 ^{b}
SBT_{en} 0.17%	276.13 ± 6.88 ^{c}	105.63 ± 10.11 ^{b}	35.08 ± 4.44 ^{bc}
SBT_{en} 0.34%	629.70 ± 20.80 ^{a}	391.22 ± 24.40 ^{a}	118.53 ± 5.12 ^{a}

Note: Starch films incorporating free or encapsulated SBT extracts: control film (S) and active films incorporating SBT or SBT_{en} at 0.17% or 0.34%. Different letters in the same column (a, b, c, d) indicate a statistically significant difference ($p < 0.05$) between mean values. Values represent the mean ± standard deviation of three individual runs.

The three main factors affecting the migration of active compounds into food simulants are the liquid diffusion into the film network, the solubility of the film in the simulant, and the diffusion of the film into the simulant (Adilah *et al*., 2018). The liquid diffusion into the film matrix also depends on the polarity of the simulant, as indicated by the swelling degree of the film. Figure 4.6b,c shows that the swelling degree of all the active films in water was higher than those in ethanol and that the films incorporating encapsulated SBT exhibited a higher swelling degree in water than those incorporating free SBT. This could be the effect of the hydrophilic nature of the films, thus making them swell more in water than in ethanol. In particular, films incorporated the encapsulated SBT were more hydrophilic than the film

incorporated free SBT, and thus led to the higher migration of antioxidant compounds into water.

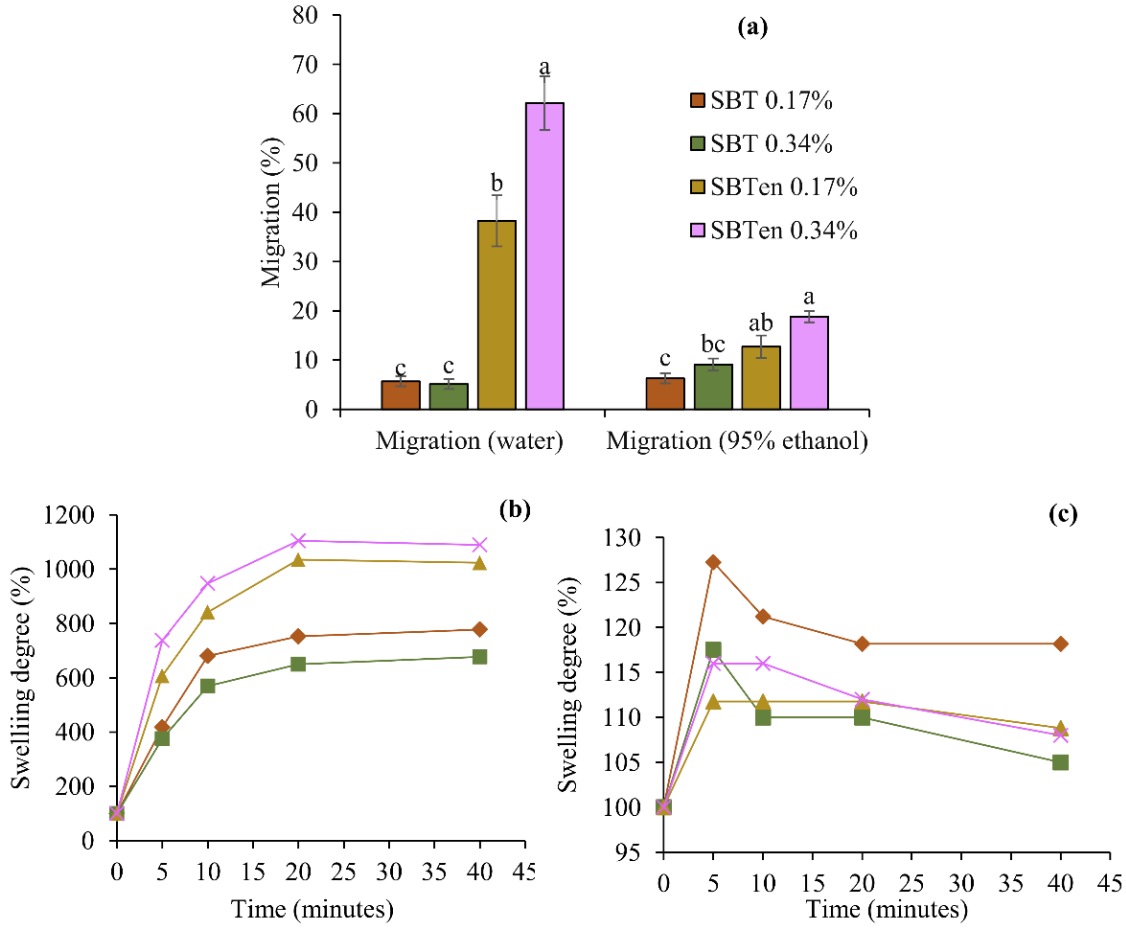

Figure 4.6 Migration percentage of antioxidant compounds into water and 95% ethanol (a), Swelling degree in water (b) and Swelling degree in 95% ethanol (c) of starch films incorporating free or encapsulated SBT extracts at 0.17% or 0.34%. Different letters in each food simulant (a, b, c) indicated a statistically significant difference ($p < 0.05$).

In contrast, films with free SBT released more active compounds into 95% ethanol than into water, possibly because of the greater affinity and swelling of SBT films in 95% ethanol (Figure 4.6c). This could also be attributed to the hydrophobic nature of polyphenols in their

free form, but when covered with a polymer wall material, the resulting microcapsules exhibited a more hydrophilic nature. Even though the release from SBT films into 95% ethanol was higher than into water, it was always lower than that from the SBT_{en} films. This behaviour could also be attributed to the presence of hydrogen bonds between the polyphenols and starch molecules in the SBT film, but their absence in the SBT_{en} films promotes the release of microcapsules. Thus, active films incorporating encapsulated SBT released more into both types of food simulant, but after release, the hydrophilic nature of the microcapsules seemed to limit the complete release of active compounds more into ethanol than into water. A similar observation has been reported for the release of microencapsulated eugenol from thermoprocessed starch films (Talón *et al*., 2019).

4.3.7 Effect of antioxidant activity on preventing lipid oxidation

Lipid oxidation is a detrimental process in food systems, because it can reduce the nutritional value and sensory quality of foods and produces toxic compounds hazardous to human health. The degree of lipid oxidation is commonly measured by evaluating the peroxide value (PV) of foods. This is also an important test for measuring the effectiveness of active packaging films placed in direct contact with foods. The PV determines the concentration of hydroperoxide, the primary oxidation products of foods, with a high peroxide value indicating a higher level of lipid oxidation or food spoilage (Wang *et al*., 2019). In the present study, the PV of soybean oil samples in contact with the different formulations of films was measured during storage for 35 d. The soybean oil in the open vial reached the highest PV of 68.4 ± 3.04 (meq O2/Kg) after 35 d of storage (Figure 4.7) because of its exposure to light and oxygen. The all-active films exhibited a lower PV than the open control sample and the sample sealed with the starch film. As the concentration of polyphenols increased, the PV of the oil decreased. This suggested that adding SBT extract to the film in either the free or encapsulated form could retard the formation of peroxides due to lipid

oxidation. Theoretically, lipid oxidation can be induced by oxygen in the presence of initiators such as heat, free radicals, light, and metal ions (Laguerre *et al*., 2007). Thus, the polyphenols in the SBT extract could scavenge the free radicals in oil by donating hydrogen from the phenolic hydroxyl group, which helped to stop the chain of radical propagation.

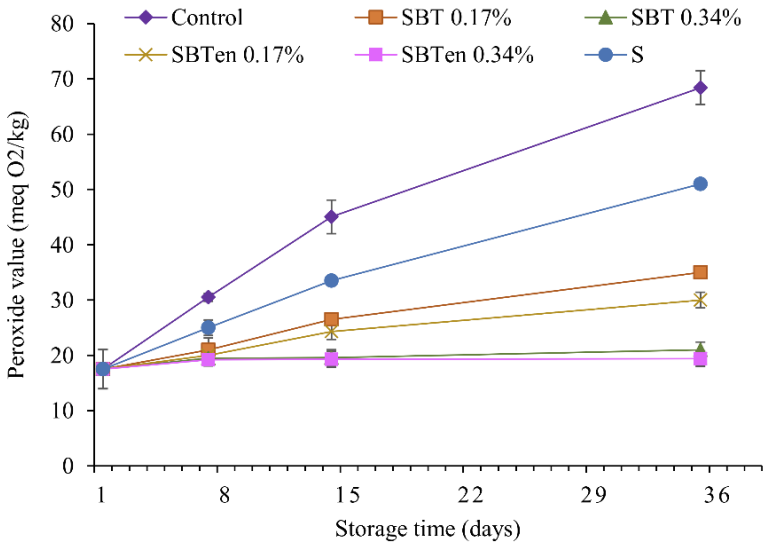

Figure 4.7 The changes of peroxide value of soybean oil during 35 days of storage at 27 °C. open glass vial (control) and starch films without and with incorporating free or encapsulated SBT extracts at 0.17% or 0.34%.

The PV of oil sealed with SBT_{en} films was lower than that of oil sealed with SBT films, possibly because of its higher release of antioxidant compounds. However, the PV of oil samples sealed with either SBT or SBT_{en} films at 0.34% were similar, possibly because the lower transparency of the SBT films at 0.34% would remove the initiators needed for oil oxidation.

4.4 Conclusions

In the present study, a functional film has been developed using free and microencapsulated SBT extract as the active ingredient. The results showed that adding SBT either in the free

or encapsulated form significantly affected the mechanical, barrier (water and light), and antioxidant properties of the films. Non-encapsulated SBT extract could modify the starch matrix by forming hydrogen bonds with cassava starch, thus inducing a reduction in the mechanical properties of the film and in antioxidant migration from the film. The film incorporating microencapsulated SBT extract exhibited a heterogenous film surface and enhanced physical properties compared with the SBT film. The microencapsulation of SBT protected the antioxidant compounds during the film processing and caused a significant increase in the migration of the active compounds into both the aqueous and fatty food simulants. The SBT_{en} 0.34% film exhibited the highest free radical scavenging activity and prevented lipid oxidation of soybean oil samples for more than 35 d.

Chapter 5

General Discussion and Conclusions

5.1 General Discussion

In recent past, a lot of researchers have explored extraction, characterization, and bioactivity evaluation of phenolic compounds in agricultural and food processing waste. Non-conventional extraction techniques such as subcritical water extraction (SWE) and pressurized liquid extraction (PLE) have been used independently for recovery of bioactive compounds (polyphenols). However, the comparison of both SWE and PLE is necessary for showing the effect of each method on the extraction efficiency. This research has used the subcritical solvent extraction (SSE) technique which used both pure water (SWE) and mixture of ethanol:water (PLE) at subcritical conditions as the solvents, further it compared the effect of both extractions on the yield of antioxidant polyphenols in SBT

This research has compared the effect of both extractions, which on the yield of antioxidant polyphenols in SBT. High temperature and pressure held during both extractions facilitated bursting of cells and enhanced the diffusion of phenolics to the solvents. Nevertheless, it increases the solubility of extractable compounds by releasing of phenolics which covalently bound to the matrix and reduce the solvent's viscosity and interfacial tension, thus enhancing mass transfer. The dielectric constant of a solvent is a measure of its polarity, and it effects on the selectivity of extractable compounds. Lab-scale experimental results of the subcritical solvent extraction has shown that using of mixture of ethanol:water at subcritical conditions increases the yield of total phenolic content and DPPH activity of the SBT extract than using subcritical water as solvent. It has shown that hydroethanolic mixtures are more efficient than pure solvents in the extraction of amphiphilic or moderately polar molecules, such as polyphenols. It can be due to development of intermediate polarity in solvent, which is similar to those of phenolic compounds. Higher soluble matter content in extracts of SSE than hot water extracts during the pilot-scale study confirmed these higher diffusion and

mass transfer of extractable phenolics. Furthermore, coextraction of compounds such as water-soluble proteins, peptides, carbohydrates, and organic acids other than phenolics may be occurred when increasing the water concentration in the extraction solvent or when using pure water.

Lab-scale experiment of SSE optimization provided the most efficient processing conditions for obtaining higher phenolic yield and, the effect of solvent on the extraction of compounds. However, not only the optimal conditions in lab-scale, but maximum pressure of the pilot-scale reactor and other safety matters also determined the process temperature and ethanol concentration of SSE in pilot-scale experiment. Pilot-scale study provided the extraction yields and extract composition of SSE which ensure the reproducibility at large scales, and thus facilitate their industrial application in the fields of food or pharmaceutical. However, pilot-scale SSE presents some limitations; maximum limits of pressure, ethanol concentration and temperature are comparatively lower than lab-scale, high investment and operating cost.

Extraction of polyphenols promotes their exposure to deleterious environmental factors such as oxygen and light. Therefore, until their application as functional ingredients in food industry, stability should be maintained, and their bioactivity is needed to preserve. In this study, microencapsulation by spray drying technique has been used to keep the stability of phenolic extract obtained from SBT and polysaccharide and protein biopolymers were used as the wall or carrier materials. It showed that biopolymeric complexes using proteins and polysaccharides as carriers can efficiently encapsulate, protect and deliver phenolic compounds, thereby, produce high-added value biological compounds. This powder of SBT extract can be used as an alternative to artificial food additives and for the development of functional foods that aim to maintain better health. However, prior to application in food

industry, scale-up study is required for explore the feasibility this method on stabilizing the bioactivity of phenolic compounds.

Inclusion of phenolic extracts of SBT into the packaging materials and their effect on prevention of lipid oxidation of oil has been evaluated during this study. Lipid oxidation is a process which involves various reactions that produce several physical and chemical alterations in food stuff. The antioxidant activity of phenolics can delay or prevent this lipid oxidation due to their redox properties that make them act as free radical scavengers, metal chelators, ultraviolet (UV) absorbers, oxygen scavengers, and singlet oxygen quenchers. The present study revealed that inclusion of SBT extract with or without encapsulation can retard the lipid oxidation of soybean oil. Hence, the active films with SBT extracts regardless of their formation can be used to package liquid oils or foods rich in polyunsaturated fatty acids. The major drawback of extract incorporation into films is reducing of mechanical properties, which limit their potential for application. However, incorporation of encapsulated form of SBT extract could increase the mechanical properties of active films. Although some properties of the active films need to be improved, these kind of studies for application of phenolics are required to exploited due to their delivery of bioactivity in environmentally friendly manner.

5.2 General Conclusions and Future Recommendations

In a circular economy, the utilization of food manufacturing waste for obtaining functional ingredients has received great attention. Spent black tea (SBT), an abundant residue from tea beverage manufacturing, has been studied as a low-cost material for the extraction of antioxidant polyphenols. Process optimization for subcritical solvent extraction (SSE) was evaluated as a novel greener method for the recovery of polyphenols from SBT. The optimal conditions enhanced the extraction of phenolic compounds with a high antioxidant capacity using hydroethanolic solvents in a short time of extraction. It is a reproducible method

allowing the extraction on a large scale. The SBT extracts from pilot-scale SSE showed a comparatively similar total polyphenol content and antioxidant activity as first brewing with hot water, and it comprised a variety of non-extractable polyphenols. However, latter fractions of pilot-scale SSE provided a significant decrease in the phenolic content, thus it reveals that low solvent-to-solid ratio could be better than 20:1. Further experiment for selecting optimal solvent-to-solid ratio in lab-scale would be resolved this. Moreover, further analysis of free amino acids, theaflavin: thearubigins ratio and quantification of phenolic compounds including caffeine will ease the better identification of all extracts from SSE and hot water.

Microencapsulation turned the polyphenols into valuable food ingredient with improved stability. The blend of pectin and caseinate as wall material created the greatest entrapment and preservation of antioxidant phenolic compounds. For SBT extraction before the microencapsulation, this study was used the optimal conditions of lab-scale SSE. However, for the large-scale production of SBT microencapsulates, the optimal conditions in pilot-scale SSE can be suggested to use for the extraction of phenolic compounds.

The phenolic extract of SBT has a better potential to be incorporated into cassava starch in developing edible functional packaging films with improved antioxidant activity, which allows the potential application in the prevention of lipid oxidation of fatty foods. Incorporation of free form of SBT extract into film caused the formation of hydrogen bonds between starch matrix and polyphenols, thus reduced the mechanical properties of film. The incorporation of the microencapsulated form of SBT extract caused to improvement in the mechanical properties of active films while preserving the antioxidant activity of polyphenols. However, further studies are required to increase the mechanical properties of functional starch films and in-depth studies to test the active potential of these films in more food packaging systems should be explored more in future.

References

Abd El-Salam, M. H., & El-Shibiny, S. (2020). Preparation and potential applications of casein–polysaccharide conjugates: a review. Journal of the Science of Food and Agriculture, 100(5), doi:1852-1859. 10.1002/jsfa.10187.

Abdeltaif, S. A., SirElkhatim, K. A. & Hassan, A. B. (2018). Estimation of phenolic and flavonoid compounds and antioxidant activity of spent coffee and black tea (processing) waste for potential recovery and reuse in Sudan. Recycling, 3(2), 27. doi.org/10.3390/recycling3020027.

Adilah, Z. M., Jamilah, B., & Hanani, Z. N. (2018). Functional and antioxidant properties of protein-based films incorporated with mango kernel extract for active packaging. Food Hydrocolloids, 74, 207-218. doi:10.1016/j.foodhyd.2017.08.017.

Ahmadian, Z., Niazmand, R. & Pourfarzad, A. (2019). Microencapsulation of Saffron Petal Phenolic Extract: Their Characterization, In Vitro Gastrointestinal Digestion, and Storage Stability. Journal of Food Science, 84(10), 2745-2757. doi:10.1111/1750-3841.14807.

Akhter, R., Masoodi, F. A., Wani, T. A., & Rather, S. A. (2019). Functional characterization of biopolymer based composite film: Incorporation of natural essential oils and antimicrobial agents. International journal of biological macromolecules, 137, 1245-1255. doi:10.1016/j.ijbiomac.2019.06.214.

Ali, A., Bilal, M., Khan, R., Farooq, R., & Siddique, M. (2018). Ultrasound-assisted adsorption of phenol from aqueous solution by using spent black tea leaves. Environmental Science and Pollution Research, 25(23), 22920-22930. doi:10.1007/s11356-018-2186-9.

Allcca-Alca, E. E., León-Calvo, N. C., Luque-Vilca, O. M., Martínez-Cifuentes, M., Pérez-Correa, J. R., Mariotti-Celis, M. S., & Huamán-Castilla, N. L. (2021). Hot Pressurized Liquid Extraction of Polyphenols from the Skin and Seeds of Vitis vinifera L. cv. Negra Criolla Pomace a Peruvian Native Pisco Industry Waste. Agronomy, 11(5), 866. doi.org/10.3390/agronomy11050866.

Asofiei, I., Calinescu, I., Trifan, A., & Gavrila, A. I. (2019). A semi-continuous process for polyphenols extraction from sea buckthorn leaves. Scientific reports, 9(1), 1-7. doi.org/10.1038/s41598-019-48610-6.

Augustin, M. A. & Oliver, C. M. (2014). Use of milk proreins for encapsulation of food ingredients. In: Microencapsulation in the food industry: a practical implementation guide (Edited by A. G. Gaonkar, N. Vasisht, A. R. Khare and R. Sobel). Pp. 211-226. Acadamic Press.

Augustin, M. A., Oliver, C. M., & Hemar, Y. (2011). Casein, caseinates, and milk protein concentrates. In: *Dairy ingredients for food processing: Chandan/Dairy Ingredients for Food Processing* (edited by R. C. Chandan and A. Kilara). 1, pp. 161-178. Blackwell Publishing Ltd: Ames, IA, USA.

Babaghayou, M. I., Mourad, A. H. I., Lorenzo, V., Chabira, S. F., & Sebaa, M. (2018). Anisotropy evolution of low density polyethylene greenhouse covering films during their service life. Polymer Testing, 66, 146-154. doi:10.1016/j.polymertesting.2018.01.007.

Baracat, M. M., Nakagawa, A. M., Casagrande, R., Georgetti, S. R., Verri, W. A., & de Freitas, O. (2012). Preparation and characterization of microcapsules based on biodegradable polymers: pectin/casein complex for controlled drug release systems. AAPS pharmscitech, 13(2), 364-372. doi:10.1208/s12249-012-9752-0.

Bora, A. F. M., Ma, S., Li, X., & Liu, L. (2018). Application of microencapsulation for the safe delivery of green tea polyphenols in food systems: Review and recent advances. Food Research International, 105, 241-249. doi:10.1016/j.foodres.2017.11.047.

Boye, J.I. (2015). Nutraceutical and functional food processing technology. Pp. 318. John Wiley & Sons, Hoboken, USA.

Brand-Williams, W., Cuvelier, M. E. & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30. doi.org/10.1016/S0023-6438(95)80008-5.

Brza, M. A., Aziz, S. B., Anuar, H., Ali, F., Dannoun, E. M., Mohammed, S. J., ... & Al-Zangana, S. (2020). Tea from the drinking to the synthesis of metal complexes and fabrication of PVA based polymer composites with controlled optical band gap. Scientific reports, 10(1), 1-17. doi:10.1038/s41598-020-75138-x.

Butt, M. S., Imran, A., Sharif, M. K., Ahmad, R. S., Xiao, H., Imran, M. H. A. R., & Rsool, H. A. (2014). Black tea polyphenols: a mechanistic treatise. Critical reviews in food science and nutrition, 54(8), 1002-1011. doi:10.1080/10408398.2011.623198.

Caleja, C., Barros, L., Antonio, A. L., Oliveira, M. B. P., & Ferreira, I. C. (2017). A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits. Food chemistry, 216, 342-346. doi:10.1016/j.foodchem.2016.08.075.

Ceballos, R. L., Ochoa-Yepes, O., Goyanes, S., Bernal, C., & Famá, L. (2020). Effect of yerba mate extract on the performance of starch films obtained by extrusion and compression molding as active and smart packaging. Carbohydrate Polymers, 244, 116495. doi:10.1016/j.carbpol.2020.116495.

Chakravartula, S. S. N., Lourenço, R. V., Balestra, F., Bittante, A. M. Q. B., do Amaral Sobral, P. J., & Dalla Rosa, M. (2020). Influence of pitanga (Eugenia uniflora L.) leaf extract and/or natamycin on properties of cassava starch/chitosan active films. Food Packaging and Shelf Life, 24, 100498. doi:10.1016/j.fpsl.2020.100498.

Chang, C., Wang, T., Hu, Q., Zhou, M., Xue, J. & Luo, Y. (2017). Pectin coating improves physicochemical properties of caseinate/zein nanoparticles as oral delivery vehicles for curcumin. Food Hydrocolloids, 70, 143-151. doi.org/10.1016/j.foodhyd.2017.03.033.

Chen, P. H., Kuo, T. Y., Kuo, J. Y., Tseng, Y. P., Wang, D. M., Lai, J. Y., & Hsieh, H. J. (2010). Novel chitosan–pectin composite membranes with enhanced strength, hydrophilicity and controllable disintegration. Carbohydrate Polymers, 82(4), 1236-1242. doi:10.1016/j.carbpol.2010.06.057.

Chen, Y., Zeng, L., Liao, Y., Li, J., Zhou, B., Yang, Z., & Tang, J. (2020). Enzymatic reaction-related protein degradation and proteinaceous amino acid metabolism during the black tea (Camellia sinensis) manufacturing process. *Foods*, *9*(1). 66.https://doi.org/10.3390/foods9010066.

Chew, S. C., Tan, C. P., & Nyam, K. L. (2018). Microencapsulation of refined kenaf (Hibiscus cannabinus L.) seed oil by spray drying using β-cyclodextrin/gum arabic/sodium caseinate. Journal of food engineering, 237, 78-85. doi: 10.1016/j.jfoodeng.2018.05.016.

Chung, H. F. (1999). Paint and pigment industry. In: Industrial applications of X-ray diffraction (edited by F. H. Chung & D. K. Smith). Pp. 513-521. Taylor & Francis, CRC Press.

Coronel-Aguilera, C. P., & San Martín-González, M. F. (2015). Encapsulation of spray dried β-carotene emulsion by fluidized bed coating technology. *LWT-Food Science and Technology*, *62*(1), 187-193. https://doi.org/10.1016/j.lwt.2014.12.036.

Das, P. R., Kim, Y., Hong, S. J., & Eun, J. B. (2019). Profiling of volatile and non-phenolic metabolites—Amino acids, organic acids, and sugars of green tea extracts obtained by different extraction techniques. Food chemistry, 296, 69-77. doi.org/10.1016/j.foodchem.2019.05.194.

Debnath, B., Haldar, D., & Purkait, M. K. (2021). Potential and sustainable utilization of tea waste: A review on present status and future trends. Journal of Environmental Chemical Engineering, 106179. https://doi.org/10.1016/j.jece.2021.106179.

Domínguez-Rodríguez, G., Marina, M. L., & Plaza, M. (2017). Strategies for the extraction and analysis of non-extractable polyphenols from plants. Journal of Chromatography A, 1514, 1-15. doi.org/10.1016/j.chroma.2017.07.066.

dos Santos Caetano, K., Lopes, N. A., Costa, T. M. H., Brandelli, A., Rodrigues, E., Flôres, S. H., & Cladera-Olivera, F. (2018). Characterization of active biodegradable films based on cassava starch and natural compounds. Food packaging and shelf life, 16, 138-147. doi:10.1016/j.fpsl.2018.03.006.

dos Santos, C., Buera, P., & Mazzobre, F. (2017). Novel trends in cyclodextrins encapsulation. Applications in food science. *Current Opinion in Food Science*, *16*, 106-113. https://doi.org/10.1016/j.cofs.2017.09.002.

Dou, L., Li, B., Zhang, K., Chu, X., & Hou, H. (2018). Physical properties and antioxidant activity of gelatin-sodium alginate edible films with tea polyphenols. International journal of biological macromolecules, 118, 1377-1383. doi: 10.1016/j.ijbiomac.2018.06.121.

Dranca, F. & Oroian, M. (2016). Optimization of ultrasound-assisted extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC) from eggplant (Solanum melongena L.) peel. Ultrasonics sonochemistry, 31, 637-646. doi.org/10.1016/j.ultsonch.2015.11.008.

Drynan, J. W., Clifford, M. N., Obuchowicz, J., & Kuhnert, N. (2012). MALDI-TOF mass spectrometry: avoidance of artifacts and analysis of caffeine-precipitated SII thearubigins from 15 commercial black teas. Journal of agricultural and food chemistry, 60(18), 4514-4525. doi.org/10.1021/jf205125y.

Dueñas, M., & García-Estévez, I. (2020). Agricultural and food waste: Analysis, characterization and extraction of bioactive compounds and their possible utilization. doi:10.3390/foods9060817.

Durazzo, A. (2018). Extractable and Non-extractable polyphenols: An overview. In *Non-extractable Polyphenols and Carotenoids: Importance in Human Nutrition and Health* (edited by F. Saura-Calixto & J. Pérez-Jiménez), pp. 37-45. The Royal Society of Chemistry.

Edhirej, A., Sapuan, S. M., Jawaid, M., & Zahari, N. I. (2017). Effect of various plasticizers and concentration on the physical, thermal, mechanical, and structural properties of cassava-starch-based films. Starch-Stärke, 69(1-2), 1500366. doi:10.1002/star.201500366.

Essien, S. O., Young, B. & Baroutian, S. (2020). Recent advances in subcritical water and supercritical carbon dioxide extraction of bioactive compounds from plant materials. Trends in Food Science & Technology, 97, 156-169. doi.org/10.1016/j.tifs.2020.01.014.

Falla, N. M., Demasi, S., Caser, M., & Scariot, V. (2021). Phytochemical Profile and Antioxidant Properties of Italian Green Tea, a New High Quality Niche Product. Horticulturae, 7(5), 91. doi.org/10.3390/horticulturae7050091.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols–a review. *Trends in Food Science & Technology*, *21*(10), 510-523. https://doi.org/10.1016/j.tifs.2010.08.003.

Fasihnia, S. H., Peighambardoust, S. H., Peighambardoust, S. J., Oromiehie, A., Soltanzadeh, M., & Peressini, D. (2020). Migration analysis, antioxidant, and mechanical characterization of polypropylene-based active food packaging films loaded with BHA, BHT, and TBHQ. Journal of Food Science, 85(8), 2317-2328. doi:10.1111/1750-3841.15337.

Feng, M., Yu, L., Zhu, P., Zhou, X., Liu, H., Yang, Y., ... & Chen, P. (2018). Development and preparation of active starch films carrying tea polyphenol. Carbohydrate polymers, 196, 162-167. doi:10.1016/j.carbpol.2018.05.043.

Friedman, M., Levin, C. E., Choi, S. H., Kozukue, E., & Kozukue, N. (2006). HPLC analysis of catechins, theaflavins, and alkaloids in commercial teas and green tea dietary supplements: comparison of water and 80% ethanol/water extracts. Journal of food science, 71(6), C328-C337. doi.org/10.1111/j.1750-3841.2006.00090.x.

Gadkari, P. V., & Balaraman, M. (2015). Catechins: Sources, extraction and encapsulation: A review. *Food and Bioproducts Processing*, *93*, 122-138. https://doi.org/10.1016/j.fbp.2013.12.004.

Ghazi, A. (1999). Extraction of β-carotene from orange peels. Food/Nahrung, 43(4), 274-277. 10.1002/(SICI)1521-380343:4<274::AID-FOOD274>3.0.CO;2-S.

Global production and exports of tea from 2004 to 2019. (2021). Retrieved from https://www.statista.com/statistics/264183/global-production-and-exports-of-tea-since-2004/. Accessed on August 18, 2021.

Gómez-Estaca, J., López-de-Dicastillo, C., Hernández-Muñoz, P., Catalá, R., & Gavara, R. (2014). Advances in antioxidant active food packaging. Trends in Food Science & Technology, 35(1), 42-51. doi: 10.1016/j.tifs.2013.10.008.

Haponska, M., Luczak, M., Nowak, P., Bajek, A., Tylkowski, B., & Tsibranska, I. (2020). 5. Polyphenol encapsulation–application of innovative technologies to improve stability of natural products. In *Microencapsulation* (pp. 109-130). De Gruyter. https://doi.org/10.1515/9783110642070-005.

Haratifar, S. & Corredig, M. (2014). Interactions between tea catechins and casein micelles and their impact on renneting functionality. Food chemistry, 143, 27-32. doi.org/10.1016/j.foodchem.2013.07.092.

Hege, J., Palberg, T., & Vilgis, T. A. (2020). Interactions of different hydrocolloids with milk proteins. *Journal of Physics: Materials*, *3*(4), 044003.10.1088/2515-7639/aba2b7.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D. & O'Sullivan, M. (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. International Dairy Journal, 11(3), 137-144. doi.org/10.1016/S0958-6946(01)00091-7.

Hosseinnia, M., Khaledabad, M. A. & Almasi, H. (2017). Optimization of Ziziphora clinopodiodes essential oil microencapsulation by whey protein isolate and pectin: A comparative study. International Journal of Biological Macromolecules, 101, 958-966. doi.org/10.1016/j.ijbiomac.2017.03.190.

Jin, B., Zhou, X., Liu, Y., Li, X., Mai, Y., Liao, Y., & Liao, J. (2018). Physicochemical stability and antioxidant activity of soy protein/pectin/tea polyphenol ternary nanoparticles obtained by photocatalysis. International journal of biological macromolecules, 116, 1-7. doi.org/10.1016/j.ijbiomac.2018.04.164.

Kaderides, K. & Goula, A.M. (2019). Encapsulation of pomegranate peel extract with a new carrier material from orange juice by-products. Journal of Food Engineering, 253, pp.1-13. doi.org/10.1016/j.jfoodeng.2019.02.019.

Kaderides, Kyriakos, Athanasia M. Goula, and Konstantinos G. Adamopoulos. "A process for turning pomegranate peels into a valuable food ingredient using ultrasound-assisted extraction

and encapsulation." *Innovative Food Science & Emerging Technologies* 31 (2015): 204-215. https://doi.org/10.1016/j.ifset.2015.08.006.

Kala, H. K., Mehta, R., Sen, K. K., Tandey, R., & Mandal, V. (2016). Critical analysis of research trends and issues in microwave assisted extraction of phenolics: Have we really done enough. *TrAC Trends in Analytical Chemistry*, *85*, 140-152. https://doi.org/10.1016/j.trac.2016.09.007.

Kanaujia, P., Poovizhi, P., Ng, W. K. & Tan, R. B. H. (2015). Amorphous formulations for dissolution and bioavailability enhancement of poorly soluble APIs. Powder Technology, 285, 2-15. doi.org/10.1016/j.powtec.2015.05.012.

Kaur, P., Elsayed, A., Subramanian, J., & Singh, A. (2021). Encapsulation of carotenoids with sucrose by co-crystallization: Physicochemical properties, characterization and thermal stability of pigments. *LWT*, *140*, 110810. https://doi.org/10.1016/j.lwt.2020.110810.

Khan, N., & Mukhtar, H. (2019). Tea polyphenols in promotion of human health. *Nutrients*, *11*(1), 39. https://doi.org/10.3390/nu11010039.

Kondo, M., Hirano, Y., Kita, K., Jayanegara, A. & Yokota, H. O. (2018). Nutritive evaluation of spent green and black tea leaf silages by in vitro gas production characteristics, ruminal degradability and post-ruminal digestibility assessed with inhibitory activity of their tannins. Animal Science Journal, 89(12), 1656-1662. doi.org/10.1111/asj.13106.

Kosińska, A., & Andlauer, W. (2014). Antioxidant capacity of tea: effect of processing and storage. In Processing and impact on antioxidants in beverages (Edited by Victor Preedy). Pp. 109-120. Academic Press.

Kuhnert, N., Drynan, J. W., Obuchowicz, J., Clifford, M. N., & Witt, M. (2010). Mass spectrometric characterization of black tea thearubigins leading to an oxidative cascade hypothesis for thearubigin formation. Rapid Communications in Mass Spectrometry, 24(23), 3387-3404. doi.org/10.1002/rcm.4778.

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics Sonochemistry, 70, 105325. doi:10.1016/j.ultsonch.2020.105325.

Kwon, H. L. & Chung, M. S. (2015). Pilot-scale subcritical solvent extraction of curcuminoids from Curcuma long L. Food Chemistry, 185, 58-64. doi.org/10.1016/j.foodchem.2015.03.114.

Laguerre, M., Lecomte, J., & Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Progress in lipid research, 46(5), 244-282. doi:10.1016/j.plipres.2007.05.002.st.

Lei, Y., Wu, H., Jiao, C., Jiang, Y., Liu, R., Xiao, D., ... & Li, S. (2019). Investigation of the structural and physical properties, antioxidant and antimicrobial activity of pectin-konjac glucomannan composite edible films incorporated with tea polyphenol. Food Hydrocolloids, 94, 128-135. doi:10.1016/j.foodhyd.2019.03.011.

Liang, L. & Luo, Y. (2020). Casein and pectin: Structures, interactions, and applications. Trends in Food Science & Technology, 97, 391-403. doi.org/10.1016/j.tifs.2020.01.027.

Lim, W. S., Ock, S. Y., Park, G. D., Lee, I. W., Lee, M. H., & Park, H. J. (2020). Heat-sealing property of cassava starch film plasticized with glycerol and sorbitol. Food Packaging and Shelf Life, 26, doi:100556. 10.1016/j.fpsl.2020.100556.

Livney, Y.D. (2010). Milk proteins as vehicles for bioactives. Current opinion in colloid & interface science, 15(1-2), 73-83. doi.org/10.1016/j.cocis.2009.11.002.

Loo, C. P., & Sarbon, N. M. (2020). Chicken skin gelatin films with tapioca starch. Food Bioscience, 35, 100589. doi:10.1016/j.fbio.2020.100589.

Lucey, J. A., Srinivasan, M., Singh, H., & Munro, P. A. (2000). Characterization of commercial and experimental sodium caseinates by multiangle laser light scattering and size-exclusion chromatography. *Journal of Agricultural and Food Chemistry*, *48*(5), 1610-1616. https://doi.org/10.1021/jf990769z.

Luchese, C. L., Garrido, T., Spada, J. C., Tessaro, I. C., & de la Caba, K. (2018). Development and characterization of cassava starch films incorporated with blueberry pomace. International journal of biological macromolecules, 106, 834-839. doi:10.1016/j.ijbiomac.2017.08.083.

Luchese, C. L., Spada, J. C., & Tessaro, I. C. (2017). Starch content affects physicochemical properties of corn and cassava starch-based films. Industrial Crops and Products, 109, 619-626. doi:10.1016/j.indcrop.2017.09.020.

Łuczaj, W. & Skrzydlewska, E. (2005). Antioxidative properties of black tea. Preventive medicine, 40(6), 910-918. doi.org/10.1016/j.ypmed.2004.10.014.

Manshor, N. M., Jai, J., Hamzah, F., & Somwangthanaroj, A. (2019, November). Rheological properties of film solution from cassava starch and kaffir lime oil. In Journal of Physics: Conference Series (Vol. 1349, No. 1, p. 012045). IOP Publishing. doi:10.1088/1742-6596/1349/1/012045.

Mariotti-Celis, M. S., Martínez-Cifuentes, M., Huamán-Castilla, N., Vargas-González, M., Pedreschi, F., & Pérez-Correa, J. R. (2018). The antioxidant and safety properties of spent coffee ground extracts impacted by the combined hot pressurized liquid extraction–resin purification process. *Molecules*, *23*(1), 21. https://doi.org/10.3390/molecules23010021.

McClements, D. J. (2018). Encapsulation, protection, and delivery of bioactive proteins and peptides using nanoparticle and microparticle systems: A review. Advances in colloid and interface science, 253, 1-22. doi.org/10.1016/j.cis.2018.02.002.

Miralai, S., Khan, M. M. & Islam, M. R. (2008). Replacing artificial additives with natural alternatives. In: Nature Science and Sustainable Technology Research Progress (edited by M. R. Islam). Pp. 45-76. Nova Publishers, In, Newyork, USA.

Moghadam, M., Salami, M., Mohammadian, M., Khodadadi, M., & Emam-Djomeh, Z. (2020). Development of antioxidant edible films based on mung bean protein enriched with pomegranate peel. Food Hydrocolloids, 104, 105735. doi: 10.1016/j.foodhyd.2020.105735.

Moosa, A. A., Ridha, A. M., & Allawi, M. H. (2015). Green synthesis of silver nanoparticles using spent tea leaves extract with atomic force microscopy. International Journal of Current Engineering and Technology, 5(5), 3233-3241. http://inpressco.com/category/ijcet.

Mukhtar, B., Mushtaq, M., Akram, S. & Adnan, A. (2018). Maceration mediated liquid–liquid extraction of conjugated phenolics from spent black tea leaves extraction of non-extractable phenolics. Analytical Methods, 10(35), 4310-4319. doi:10.1039/C8AY01350K.

Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics*, *3*(4), 793-829. https://doi.org/10.3390/pharmaceutics3040793.

Munir, M. T., Kheirkhah, H., Baroutian, S., Quek, S. Y. & Young, B. R. (2018). Subcritical water extraction of bioactive compounds from waste onion skin. Journal of Cleaner Production, 183, 487-494. doi.org/10.1016/j.jclepro.2018.02.166.

Murugesan, Ramesh, and Valérie Orsat. "Spray drying for the production of nutraceutical ingredients—a review." *Food and Bioprocess Technology* 5.1 (2012): 3-14. https://doi.org/10.1007/s11947-011-0638-z.

Mussatto, S. I., Ballesteros, L. F., Martins, S. & Teixeira, J. A. (2011). Extraction of antioxidant phenolic compounds from spent coffee grounds. Separation and Purification Technology, 83, 173-179. doi.org/10.1016/j.seppur.2011.09.036.

Nebahani, L. & Jaisingh, A. (2020). Polymer Science and Innovative Applications. In: *Chemical Analysis of Polymers: Materials, Techniques, and Future Developments* (edited by M.A.A. Almaadeed, D. Ponnamma & M.A. Carignano), 1, pp. 69–117. Elsevier: Cambridge, MA, USA.

Nooshkam, M., & Varidi, M. (2020). Maillard conjugate-based delivery systems for the encapsulation, protection, and controlled release of nutraceuticals and food bioactive ingredients: A review. Food Hydrocolloids, 100, 105389. doi:10.1016/j.foodhyd.2019.105389.

Pagano, I., Campone, L., Celano, R., Piccinelli, A. L., & Rastrelli, L. (2021). Green non-conventional techniques for the extraction of polyphenols from agricultural food by-products: A review. *Journal of Chromatography A*, 462295. https://doi.org/10.1016/j.chroma.2021.462295.

Pan, H., Li, J., Rankin, G. O., Rojanasakul, Y., Tu, Y., & Chen, Y. C. (2018). Synergistic effect of black tea polyphenol, theaflavin-3, 3′-digallate with cisplatin against cisplatin resistant human ovarian cancer cells. Journal of functional foods, 46, 1-11. doi.org/10.1016/j.jff.2018.04.037.

Pan, K., Zhong, Q. & Baek, S. J. (2013). Enhanced dispersibility and bioactivity of curcumin by encapsulation in casein nanocapsules. Journal of agricultural and food chemistry, 61(25), 6036-6043. doi.org/10.1021/jf400752a.

Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., & Napolitano, A. (2020). Bioactive phenolic compounds from agri-food wastes: An update on green and sustainable extraction methodologies. *Frontiers in Nutrition*, *7*, 60. https://doi.org/10.3389/fnut.2020.00060.

Pérez-Jiménez, J., & Saura-Calixto, F. (2018). Fruit peels as sources of non-extractable polyphenols or macromolecular antioxidants: Analysis and nutritional implications. Food Research International, 111, 148-152. doi.org/10.1016/j.foodres.2018.05.023.

Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., & Goyanes, S. (2017). Edible cassava starch films carrying rosemary antioxidant extracts for potential use as active food packaging. Food hydrocolloids, 63, 488-495. doi:10.1016/j.foodhyd.2016.09.034.

Pires, M. A., Munekata, P. E., Villanueva, N. D., Tonin, F. G., Baldin, J. C., Rocha, Y. J., ... & Trindade, M. A. (2017). The antioxidant capacity of rosemary and green tea extracts to replace the carcinogenic antioxidant (BHA) in chicken burgers. Journal of Food Quality, 2017. 1–6, doi:10.1155/2017/2409527.

Plakett, D. & Siro, I. (2012). Developments in Packaging Materials. In *Emerging food Packaging Technologies: Principles and Practice* (edited by K.L. Yam & D.S. Lee).1, pp.237–359. Woodhead Publishing Limited: Philadelphia, PA, USA.

Pronyk, C. & Mazza, G. (2009). Design and scale-up of pressurized fluid extractors for food and bioproducts. Journal of Food Engineering, 95(2), 215-226. doi.org/10.1016/j.jfoodeng.2009.06.002.

Qin, Y., Liu, Y., Yong, H., Liu, J., Zhang, X., & Liu, J. (2019). Preparation and characterization of active and intelligent packaging films based on cassava starch and anthocyanins from Lycium ruthenicum Murr. International journal of biological macromolecules, 134, 80-90. doi:10.1016/j.ijbiomac.2019.05.029.

Rajapaksha, D. S. W., & Shimizu, N. (2020). Valorization of spent black tea by recovery of antioxidant polyphenolic compounds: Subcritical solvent extraction and microencapsulation. Food Science & Nutrition, 8(8), 4297-4307. doi.org/10.1002/fsn3.1726.

Rajapaksha, S. W., & Shimizu, N. (2021). Development and Characterization of Functional Starch-Based Films Incorporating Free or Microencapsulated Spent Black Tea Extract. Molecules, 26(13), 3898. doi.org/10.3390/molecules26133898.

Ray, S., Raychaudhuri, U., & Chakraborty, R. (2016). An overview of encapsulation of active compounds used in food products by drying technology. Food Bioscience, 13, 76-83. doi:10.1016/j.fbio.2015.12.009.

Rehman, A., Ahmad, T., Aadil, R. M., Spotti, M. J., Bakry, A. M., Khan, I. M., Zhao, L., Riaz, T. & Tong, Q. (2019). Pectin polymers as wall materials for the nano-encapsulation of bioactive compounds. Trends in Food Science & Technology, 90, 35-46. doi.org/10.1016/j.tifs.2019.05.015.

Ren, J. N., Hou, Y. Y., Fan, G., Zhang, L. L., Li, X., Yin, K., & Pan, S. Y. (2019). Extraction of orange pectin based on the interaction between sodium caseinate and pectin. Food chemistry, 283, 265-274. doi:10.1016/j.foodchem.2019.01.046.

Rengga, W. D. P., Yufitasari, A., & Adi, W. (2017). Synthesis of silver nanoparticles from silver nitrate solution using green tea extract (Camelia sinensis) as bioreductor. Jurnal Bahan Alam Terbarukan, 6(1), 32-38. doi:10.15294/jbat.v6i1.6628.

Rezvankhah, A., Emam-Djomeh, Z., & Askari, G. (2020). Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: A review. *Drying Technology*, *38*(1-2), 235-258. https://doi.org/10.1080/07373937.2019.1653906.

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M. & Lobo, M. G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. Comprehensive Reviews in Food Science and Food Safety, 17(3), 512-531. doi.org/10.1111/1541-4337.12330.

Shahidi F, Han XQ. 1 Encapsulation of food ingredients. Crit Rev Food Sci Nut. 1993; 33: 501–47. https://doi.org/10.1080/10408399309527645.

Shamaei, S., Seiiedlou, S. S., Aghbashlo, M., Tsotsas, E. & Kharaghani, A. (2017). Microencapsulation of walnut oil by spray drying: Effects of wall material and drying conditions on physicochemical properties of microcapsules. Innovative Food Science & Emerging Technologies, 39, 101-112. doi.org/10.1016/j.ifset.2016.11.011.

Shimizu, N., Ushiyama, T. & Itoh, T. (2019). The Hydrolysis Mechanism of Inulin and Its Hydrolysate in the Reaction Field by the Hot Compressed Water. Environmental Control in Biology, 57(4), 87-92. doi.org/10.2525/ecb.57.87.

Shpigelman, A., Israeli, G. & Livney, Y.D. (2010). Thermally-induced protein–polyphenol co-assemblies: beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG. Food Hydrocolloids, 24(8), 735-743. doi.org/10.1016/j.foodhyd.2010.03.015.

Stoll, L., Costa, T. M. H., Jablonski, A., Flôres, S. H., & de Oliveira Rios, A. (2016). Microencapsulation of anthocyanins with different wall materials and its application in active biodegradable films. Food and bioprocess technology, 9(1), 172-181. doi:10.1007/s11947-015-1610-0.

Sun, H., Chen, Y., Cheng, M., Zhang, X., Zheng, X., & Zhang, Z. (2018). The modulatory effect of polyphenols from green tea, oolong tea and black tea on human intestinal microbiota in vitro. *Journal of food science and technology*, *55*(1), 399-407. https://doi.org/10.1007/s13197-017-2951-7.

Syahariza, Z.A., Torkamani, A.E., Norziah, H.M., Mahmood, W.A.K. & Juliano, P. (2017). Optimisation of pressurised liquid extraction for antioxidative polyphenolic compound from Momordica charantia using response surface methodology. International Journal of Food Science & Technology, 52(2), 480-493. doi.org/10.1111/ijfs.13304.

Talón, E., Vargas, M., Chiralt, A., & González-Martínez, C. (2019). Eugenol incorporation into thermoprocessed starch films using different encapsulating materials. Food Packaging and Shelf Life, 21, 100326. doi:10.1016/j.fpsl.2019.100326.

Tea and Coffee Trade Journal. (2019). Bottled tea rises in popularity. Retrieved from https://www.teaandcoffee.net/news/23359/bottled-tea-rises-in-popularity/. Accessed August 3, 2021.

Thummajitsakul, S., Samaikam, S., Tacha, S., & Silprasit, K. (2020). Study on FTIR spectroscopy, total phenolic content, antioxidant activity and anti-amylase activity of extracts and different tea forms of Garcinia schomburgkiana leaves. LWT, 134, 110005. doi:10.1016/j.lwt.2020.110005.

Tian, F., Decker, E. A., & Goddard, J. M. (2013). Controlling lipid oxidation of food by active packaging technologies. *Food & function*, *4*(5), 669-680. 10.1039/C3FO30360H.

Tsali, A. & Goula, A. M. (2018). Valorization of grape pomace: Encapsulation and storage stability of its phenolic extract. Powder Technology, 340, 194-207. doi.org/10.1016/j.powtec.2018.09.011.

Uchida, K., Ogawa, K., & Yanase, E. (2016). Structure determination of novel oxidation products from epicatechin: thearubigin-like molecules. Molecules, 21(3), 273. doi.org/10.3390/molecules21030273.

Ushiyama, T. & Shimizu, N. (2018). Microencapsulation using spray-drying: The use of fine starch solution for the wall material. Food Science and Technology Research, 24(4), 653-659. doi.org/10.3136/fstr.24.653.

Valdés García, A., Juárez Serrano, N., Beltrán Sanahuja, A., & Garrigós, M. C. (2020). Novel antioxidant packaging films based on poly (ε-caprolactone) and almond skin extract: Development and effect on the oxidative stability of fried almonds. Antioxidants, 9(7), 629. doi:10.3390/antiox9070629.

Vergara-Salinas, J. R., Pérez-Jiménez, J., Torres, J. L., Agosin, E. & Pérez-Correa, J. R. (2012). Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (Thymus vulgaris). Journal of Agricultural and Food Chemistry, 60(44), 10920-10929. doi.org/10.1021/jf3027759.

Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological implications of lipid oxidation products. Journal of the American Oil Chemists' Society, 94(3), 339-351. doi:10.1007/s11746-017-2958-2.

Wang, J., Tang, L., Zhou, H., Zhou, J., Glenn, T. C., Shen, C. L., & Wang, J. S. (2018). Long-term treatment with green tea polyphenols modifies the gut microbiome of female sprague-dawley rats. *The Journal of nutritional biochemistry*, *56*, 55-64. https://doi.org/10.1016/j.jnutbio.2018.01.005.

Wang, S., Xia, P., Wang, S., Liang, J., Sun, Y., Yue, P., & Gao, X. (2019). Packaging films formulated with gelatin and anthocyanins nanocomplexes: Physical properties, antioxidant activity and its application for olive oil protection. Food Hydrocolloids, 96, 617-624. doi:10.1016/j.foodhyd.2019.06.004.

Weerawatanakorn, M., Hung, W. L., Pan, M. H., Li, S., Li, D., Wan, X., & Ho, C. T. (2015). Chemistry and health beneficial effects of oolong tea and theasinensins. Food Science and Human Wellness, 4(4), 133-146. doi.org/10.1016/j.fshw.2015.10.002.

World Tea News. (2018). Retrieved from https://www.worldteanews.com/Features/tea-consumption-second-only-packaged-water. Accessed November 15, 2021.

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. https://doi.org/10.1021/acs.jafc.8b06146.

Xu, J., Yue, R. Q., Liu, J., Ho, H. M., Yi, T., Chen, H. B. & Han, Q. B. (2014). Structural diversity requires individual optimization of ethanol concentration in polysaccharide precipitation. International Journal of Biological Macromolecules, 67, 205-209. doi.org/10.1016/j.ijbiomac.2014.03.036.

Yanagida, T., Shimizu, N. & Kimura, T. (2005). Extraction of wax from banana leaves as an alternative way of utilizing agricultural residues. Japan Journal of Food Engineering (Japan), 6(1), 29-35.

Yinbin, L., Wu, L., Weng, M., Tang, B., Lai, P. & Chen, J. (2018). Effect of different encapsulating agent combinations on physicochemical properties and stability of microcapsules loaded with phenolics of plum (Prunus salicina lindl.). Powder Technology, 340, 459-464. doi.org/10.1016/j.powtec.2018.09.049.

Yingngam, B., Tantiraksaroj, K., Taweetao, T., Rungseevijitprapa, W., Supaka, N. & Brantner, A. H. (2018). Modeling and stability study of the anthocyanin-rich maoberry fruit extract in the fast-dissolving spray-dried microparticles. Powder Technology, 325, 261-270. doi.org/10.1016/j.powtec.2017.10.059.

Yun, D., Cai, H., Liu, Y., Xiao, L., Song, J., & Liu, J. (2019). Development of active and intelligent films based on cassava starch and Chinese bayberry (Myrica rubra Sieb. et Zucc.) anthocyanins. RSC advances, 9(53), 30905-30916. doi:10.1039/C9RA06628D.

Zhang, H., Qi, R., & Mine, Y. (2019). The impact of oolong and black tea polyphenols on human health. Food Bioscience, 29, 55-61. doi.org/10.1016/j.fbio.2019.03.009.

Zhang, J. & Wolf, B. (2019). Physico-Chemical Properties of Sugar Beet Pectin-Sodium Caseinate Conjugates via Different Interaction Mechanisms. Foods, 8(6), 192. doi.org/10.3390/foods8060192.

Zhang, J., Wen, C., Zhang, H., Duan, Y. & Ma, H. (2020). Recent advances in the extraction of bioactive compounds with subcritical water: A review. Trends in Food Science & Technology, 95, 183-195. doi.org/10.1016/j.tifs.2019.11.018.

Zhang, Z., Baroutian, S., Munir, M. T. & Young, B. R. (2017). Variation in metals during wet oxidation of sewage sludge. Bioresource Technology, 245, 234-241. doi.org/10.1016/j.biortech.2017.08.164.

Zheng, L., Ding, Z., Zhang, M. & Sun, J. (2011). Microencapsulation of bayberry polyphenols by ethyl cellulose: Preparation and characterization. Journal of Food Engineering, 104(1), 89-95. doi.org/10.1016/j.jfoodeng.2010.11.031.