ABSTRACT

PROPERTIES OF SOY PROTEIN ISOLATE FILMS FORTIFIED WITH UNDERUTILIZED QUINOA STALKS AND LEAVES

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Quinoa (Chenopodium Quinoa Wild) stalk and leaf are abundant and underutilized materials. Quinoa stalk and leaf extracts were used to modify properties of soy protein isolate films thus extending the application of films. Quinoa stalk cellulose (QSC) was extracted by NaOH and HCl. QSC was added in film forming solution then the solution was casted into film. QSC can be incorporated into soy protein isolate (SPI) films to improve their tensile strength. This was because of the possible interaction of hydrogen bonding between QSC and SPI macromolecules. However, the color of soy protein isolate films was affected by QSC. Quinoa leaf extract (QLE) was rich in phenolic compounds. QLE and gallic acid were used as fillers to modify edible SPI films. Ethanol/water extracted QLE, contributed to the improved mechanical, antioxidant, and antimicrobial properties of SPI films. Hydrogen bonding was the major interaction between phenolic compounds and SPI macromolecules. This contributed to reinforcing the mechanical properties. The antioxidant and antimicrobial properties of SPI films were significantly improved by adding QLE. QSC and QLE both can increase the mechanical properties of SPI films, and QLE added films had also showed improved antioxidant and antimicrobial capacities.

Key words: quinoa stalk and leaf, cellulose, phenolic compounds, soy protein isolate film, mechanical properties, antioxidant properties, antimicrobial properties
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List of Abbreviation

AAE: ascorbic acid equivalent

ABTS: 2,2-azino-bis-(3-ethylbenzthiazolin-6-sulfonate) diammonium salt

ACE: angiotensin converting enzyme

AHA: alpha-hydroxy acids

AMD: age-related macular degeneration

ANOVA: one-way analysis of variance

BHA: butylated hydroxyanisole

BHT: butylated hydroxytoluene

CE: catechin equivalent

DART-MS: real time mass spectrometry

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DOCA: desoxycorticosterone

DW: dry weight

E. coli: Escherichia coli

EAB: elongation at break

EtOAc: ethyl acetate

EU: European union

FRAP: ferric ion reducing antioxidant power

FTIR: fourier transform infrared

GA: gallic acid

GAE: gallic acid equivalent
GC: gas chromatography
GC-µECD: GC method with micro-electron capture detection
GC-MS: GC with mass spectrometry
HPLC: high performance liquid chromatography
HPLC-DAD: HPLC with diode array detection
LCUV: Chromatography-UV detection
MRLs: maximum residue limits
ORAC: Oxygen radical absorbance capacity
PV: peroxide value
QSC: quinoa stalk cellulose
QLE: quinoa leaf extract
QuEChERS: quick easy cheap effective rugged and safe
SPI: Soy Protein Isolate
TAE: tannic acid equivalent
TBARS: thiobarbituric acid reactive substance
TE: trolox equivalent
TEAC: trolox equivalent antioxidant capacity
TS: tensile strength
TPC: total phenolic compounds
TPTZ: 2,4,6-tripyridyl-s-triazine
UPLC-MS: ultra-performance liquid chromatography coupled with tandem mass spectroscopy
Chapter 1: Literature Review

Section 1.1 Extraction and Utilization of Functional Phytochemicals from Food Waste

Abstract

Food waste materials produced during the harvesting and processing of various food products and beverages are mostly discarded in landfills along with potentially value-adding phytochemicals. The phytochemical content can be extracted from food waste and has been shown to possess antioxidant, antimicrobial, and antifungal properties. A large volume of processing byproducts could be extracted for active compounds to be used in ameliorating shelf life or in the bio-fortification of food products, such as protein-rich, carbohydrate-rich, fat-rich foods, vegetables, and fruits. In addition, food wastes have also been investigated for their utilization in coatings and films for food packaging. This review examines recent literature regarding extraction of food waste and their potential applications in the food industry.
1.1.1 Introduction

The processes involved in bringing foods from farm to fork result in the generation of large volumes of biomass waste. Approximately 30 – 60 % of all plants grown for food become wasted during the harvesting, drying, processing, evaluation, packaging, and even at the point of consumption of various food products (Parfitt, Barthel, Macnaughton 2010). The gathered food waste contains added value that can be extracted in the form of phytochemicals. A plethora of fruits and vegetables could be processed to become ready-to-eat food products as well as juices and wine resulting in the generation of significant amount processing residues. For example, grape pomace, rich in polyphenols, is the liquid-solid waste produced from wine manufacturing and grape juice processing. It accounts for 13% (w/w) of the total mass of grapes used on a dry weight basis (Nishiumi and others 2012; Torres and others 2002). The production of canned peanuts is even more inefficient where only 40% of the total peanut plant is utilized (Constanza and others 2012). A number of food waste extracts contain phytochemicals known to possess empirical biological functions as proven in vitro and in vivo experiments (Adetoro and others 2013; Afzal and others 2013; Dimpfel 2013; Elfalleh and others 2011; Haque, Ansari, Rashikh 2013; He and others 2012; Yu and Ahmedna 2013). In some cases, the plant waste contains equal or higher levels of certain phytochemicals than any other part of the original plant (Chougui and others 2013; Vattem and Shetty 2002). The discarding of potentially usable waste is quite common in most food industries including coffee (Cruz and others 2012), potato fries and chips (Wu and others 2012), olive oil
(Frankel and others 2013), fish products (Ferraro and others 2013) and orange juice (Grohman and others 2013), only to name a few. Although a certain amount of food processing waste is usually converted to animal feed, the discarded waste can be rich in polyphenols and other compounds possessing significant antioxidant and/or antimicrobial activities (Agourram and others 2013; Constanza and others 2012; Ferraro and others 2013; Li Chaofeng and others 2013; Roopchand and others 2013; Taing and others 2012; Yu and Ahmedna 2013). Grape skin and seed were reported to have anti-mutagenic and anti-carcinogenic properties, as well as anti-lipogenic, anti-aging, antioxidant and antimicrobial characteristics (Yu and Ahmedna 2013). Fish processing by-product extracts were found to have antioxidant and antihypertensive activities (He and others 2012). Pomegranate peel was reported to have antioxidant, anti-inflammatory, anti-allergic, and anticancer properties (Ismail, Sestili, Akhtar 2012). Producers may have an incentive to utilize otherwise worthless, and even costly, byproducts and extract value from large amounts of waste materials in the form of bioactive compounds with potential to ameliorate shelf life or in the bio-fortification of other food products.

1.1.2 Food waste extract compounds and extraction methods

Water

Water is commonly used in extracting phytochemical compounds from food waste as a polar inorganic solvent. Fruit peels and seeds which contain large quantity of phenolics are the main byproducts from fruit juice or jam processing. Fruit peel discarded during processing must be lyophilized or air/sun dried prior to extraction as
in the case of pomegranate, mango, citrus peels, and peanut skins (Lyu and Lee 2013; Singh, Murthy, Jayaprakasha 2002; Taing and others 2012; Yu, Ahmedna, Goktepe 2005), while liquid nitrogen has been used prior to grinding grape pomace (Nishiumi and others 2012). A few examples dealing with extracted material are provided below. Dried mature citrus peels extracted using distilled water was reported to have a crude extract yield of 38.3% (w/w, dry weight (DW)), and contain many flavonoids (i.e., hesperidin, naringin, nobiletin) and alkaloid (synephrine), as reported by Lyu and Lee (2013). The most commonly method used to quantify total phenolic content (TPC) in food waste extract is the Foli-Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate) (Samad and others 2013; Vattem and Shetty 2002). In one study, pomegranate peel and seed was powdered and extracted using distilled water and resulted in a 3% (w/w, DW) tannic acid equivalents (TAE) phenolics in both peel and seed (Edison and Sethuraman 2013; Singh, Murthy, Jayaprakasha 2002). Grape seed extract, prepared by distilled aqueous extraction, also yielded 42.1% (w/w, DW) catechin equivalents phenolic compounds (Cheah and others 2009; Cheah and others 2013; Singh, Murthy, Jayaprakasha 2002). The total phenolic and flavonoid content of winter melon seeds aqueous extracts were 0.008 (w/w, DW) gallic acid equivalents (GAE) and 0.05% (w/w, DW) catechin equivalents, respectively (Samad and others 2013).

Individual compounds found in various waste products can be identified by using chromatographic techniques such as high performance liquid chromatography (HPLC). Food waste extracts are generally concentrated and filtered prior to being
injected into the HPLC apparatus (Singh, Murthy, Jayaprakasha 2002; Yu, Ahmedna, Goktepe 2005). Peanut skin aqueous extract not only contains large quantities of dietary fiber and phytochemicals, but also a wide variety of phenolics. This can be demonstrated by comparing retention times and absorbance spectra with authenticated standards chemicals using HPLC. In such analyses, the detection at 280 nm is used for phenolic acids (i.e., caffeic, chlorogenic, ferulic and coumaric acids), and 360 nm for flavonoids (i.e., catechins and procyanidins) and stilbenes (i.e., resveratrol) (Ma and others 2013; Yu, Ahmedna, Goktepe 2005). Lotus seed water extract was also found to be rich in flavonoid, such as myricetin, quercetin, kampferol, and isohamnetin (Chen and others 2012). Black cumin seeds (Nigella sativa), extracted by distilled water, was found to be a good source of minerals, vitamins, and various functional compounds, such as alkaloids and tannins (Mahmoud and Torchilin 2013). HPLC analysis of pomegranate peel extract revealed various phytochemicals such as ellagic tannins, ellagic acid and GA (Singh, Murthy, Jayaprakasha 2002). The water extract of ginkgo biloba leaf was also found to contain flavonoids (i.e., kaempferol, quercetin and isorhamnetin) and terpene lactones (i.e., bilobalide, ginkgolide) (Haruyama and Nagata 2013).

**Ethanol**

Ethanol (pure or in an aqueous solution) is typically used in extracting antioxidant compounds from food waste, as it is considered both efficient and safe at extracting phytochemicals from plant materials. Total phenolic and flavonoid content in the ethanolic extract of grape seed were reported to be 13 mg GAE/g and 21.6 mg
catechin equivalents (CE)/g dry seed, respectively (Krishnaswamy and others 2013). Ethanol extracts from the leaves of the Ipecacuanha (*Tylophora indica*) plant (mostly grown in Southern India) was found to contain phenanthrene–indolizidine alkaloids (Kathuria and others 2013). Aqueous ethanol extract of peanut skin was reported to be rich in proanthocyanidins (Constanza and others 2012; Khaliq and others 2013). Beetroot pomace, a by-product of the sugar industry, was extracted using 50% (v/v) ethanol with 0.5% acetic acid and found to contain various phytochemicals, especially phenolics, such as catechin, epicatechin, ferulic, protocatechuic, vanillic, p-coumaric, p-hydroxybenzoic, caffeic, and syringic acids (Vulic and others 2013). Ethanol extract of quinoa leaves (agriculture waste in quinoa production) contains large amount of phenolics, including ferulic acid (762.3 µg/g, DW), sinapinic acid (193.5 µg/g, DW), GA (162.9 µg/g, DW), rutin (62.1 µg/g, DW), kempferol (46 µg/g, DW), chlorogenic acid (37.6 µg/g, DW), vanillic acid (22.7 µg/g, DW), syringic acid (18.7 µg/g, DW), p-coumaric acid (33.3 µg/g, DW), as reported by Gawlik-Dziki and others (2013).

**Methanol**

Methanol extract of pomegranate peel contains 44% phenolics TAE, which is much higher than can be obtained by water extraction (3% phenolics) (Singh, Murthy, Jayaprakasha 2002). Grape pomace (grape skin and seed) extracted by methanol containing 1% acetic acid, was found to be very rich in polyphenols, mainly procyanidins, oligomeric proanthocyanidins, anthocyanins (Casassa and others 2013; Charradi and others 2013; Nishiumi and others 2012). Pomegranate seeds also contain anthocyanidins and flavonols (Mongkholkhajornsilp and others 2005;
Total anthocyanin in pomegranate peel ranged from 6.4 – 8.4% cyanidin-3-glucoside equivalent dry weight (DW) (Elfalleh and others 2011). The tocopherol (α-tocopherol, γ-tocopherol, δ-tocopherol) contents of pomegranate peel anthocyanin were reported to be 1.66%, 1.07%, and 0.27% (w/w, DW), respectively (Elfalleh and others 2011). Another example is olive oil processing byproducts (olive oil mill wastewater, olive pomace, filter cake), which contain various phenolic compounds, especially simple phenols, hydroxy-benzoic acid, and flavonoids (Frankel and others 2013). Citrus peel extracted by methanol was also found to contain various phytochemicals, such as tangeretin, nobiletin, tetramethylscutellarein, sinensetin, hexamethyl-o-quercetagetin, and heptamethoxyflavone (Green and others 2013).

The main flavonoids of grape pomace extracted by methanol are quercetin 3-O-glucoside (179 mg/kg, DW) and quercetin 3-O-glucuronide (130 mg/kg, DW), and isorhamnetin 3-O-glucoside (63.8 mg/kg, DW) (Amico and others 2004). Grape pomace methanol extract also contains small amounts of kaempferol, kaempferol 3-O-glucoside, quercetin, laricitrin 3-O-glucoside, and myricetin 3-O-glucoside (Amico and others 2004). Malvidin 3-O-glucoside (64.6 mg/kg, DW) is the main anthocyanin of grape pomace extract determined by HPLC-UV at 530 nm (Amico and others 2004). Peonidin 3-O-glucoside (18.7 mg/kg) and petunidin 3-O-glucoside (10.7 mg/kg) are more abundant than other anthocyanins (Amico and others 2004).

Other solvents
Hexane is an organic non-polar solvent that can also be used in extracting components from food waste. It is most used commonly to defat food waste materials before extraction with other solvents (Mahmoud and Torchilin 2013). For example, hexane and methanol were used together in a sequential Soxhlet extraction of mango fruit peel, followed by methanol extraction (Taing and others 2012). Hexane can dissolve carotenoids and other highly non-polar compounds, while methanol can dissolve polyphenols (Taing and others 2012).

Acetone is yet another solvent that can be used to extract various phytochemicals from food waste (Cheng and others 2012; Michael, Salib, Eskander 2013; Panyathep and others 2013). Acetone (50% v/v) extract of wine residue had a crude extract yield of 23.9% (w/w, DW), which was similar to material obtained with 50% (v/v) ethanol and (50% v/v) methanol extracts 22.7 and 26.3 (% w/w DW), respectively (Cheng and others 2012). However, much phenolic compounds were found in grape peel, where total phenolic of acetone extract was 67.5% (gallic acid equivalents (GAE) w/w, DW). This was higher than ethanol extraction (64.9 GAE w/w, DW) and methanol extraction (57.8 GAE w/w, DW) (Cheng and others 2012). Dried longan seeds have been extracted with 80% acetone, and were found to be rich in antioxidant polyphenolic compounds such as corilagin, GA, and ellagic acid (Panyathep and others 2013). Gallocatechin (162.2 mg/g, DW) was the main flavan-3-ols in ethyl acetate extract of brewery waste stream (Barbosa-Pereira and others 2013). The crude brewery waste stream also contains large amount of protocatechuic acid (16.7 mg/g, DW), epigallocatechin (50.6 mg/g, DW), catechin (41.3 mg/g, DW), epicatechin (17.2 mg/g,
DW), ferulic acid (39.5 mg/g, DW), naringenin (5.1 mg/g, DW), apigenin (7.1 mg/g, DW), isoquercetin (15.9 mg/g, DW), quercetin (15.1 mg/g, DW), kaempferol (14.7 mg/g, DW), and acetosyringone (14.6 mg/g, DW) (Barbosa-Pereira and others 2013).

Avocado peel acetone extract contains large amount of catechins (7.519 mg/g, DW), hydroxybenzoic acids (0.225 GAE mg/g, DW), hydroxycinnamic acids (599.4 chlorogenic acid equivalents mg/100 g, DW), flavonols (3.611 rutin equivalents mg/g, DW), and procyanidins (134.843 catechin equivalents mg/g, DW) (Rodriguez-Carpena and others 2011). Chloroform/methanol/water (2.5:5:1, v/v/v) solvent extracted pumpkin seeds contain large amount of \( \gamma \)-tocopherol (0.1081 mg/g, DW), but only little \( \alpha \)-tocopherol (0.0074 mg/g, DW), \( \beta \)-tocopherol (0.00068 mg/g, DW) (Veronezi and Jorge 2012).

1.1.3 Antioxidant activity of food waste extracts and their application to food and food packaging

Polyphenolic extracts are excellent electron and proton donors, and their intermediate radicals are quite stable due to electron delocalization phenomena and due to the lack of positions attackable by \( \text{O}_2 \) (Banon and others 2007). Many analytical methods are used for testing antioxidant activity of food waste extract, including DPPH assay, ABTS assay, ORAC assay, FRAP assay, TEAC assay, etc (Agourram and others 2013; Babbar and others 2011; Kanatt, Arjun, Sharma 2011; Rodriguez-Rodriguez and others 2012; Savatovic and others 2012; Silva and others 2013; Yang and others 2011; Zhang, Li, Wang 2010). DPPH is a stable free radical, and can be reduced by an antioxidant compound, the color of DPPH will change to
yellow due to losing its chromophore. DPPH assay is widely used to screen antioxidant components within the primary extracts because it is simple, rapid, sensitive and a reproducible procedure (Savatovic and others 2012). The ORAC measures the scavenging capacities in food waste extract samples in vitro, by testing the oxidative degradation of the fluorescent molecule after it is mixed with free radical generators. The ABTS assay is another method to test antioxidant capacity of food waste extract. ABTS\(^+\), generated by reacting ABTS with potassium persulfate, has a relatively stable blue-green color. The color can be reduced by antioxidant compounds to a degree that is in proportion to their antioxidant concentration or activity. Reducing power test is another method that can be used to measure the antioxidant activity of food waste extract. In this assay, the presence of antioxidants in the samples would result in the reduction of the Fe\(^{3+}\)/ferricyanide complex to its ferrous form and this can then be detected at 700 nm. A simple, automated test measuring the ferric reducing ability of plasma, the FRAP assay, is presented as a developed method for assessing food waste extract antioxidant capacity (Benzie and Strain 1996). Commonly more than one assay has been used in evaluating the antioxidant capacity of food waste extracts in order to assess various radical-scavenging mechanisms (Agourram and others 2013).

Using the DPPH assay, the antioxidant activity of tomato pomace was found to be the highest (879 mg trolox equivalent (TE) /100 g DW), followed by broccoli stems, cabbage cut-offs, and cauliflower cut-offs (512, 284, 200 mg TE/100 g DW) (Savatovic and others 2012). Based on the antioxidant capability test of food waste
extracts, the IP values could be subdivided into three groups: high (≥50%), moderate (20–50%), and low (≤20%) (Agourram and others 2013). Many fruit and vegetable waste extracts were found to have high antioxidant activity bases on the DPPH assay. Examples include pomegranate peel (95.62%), hazelnut skin (92.90%), cornelian cherry seeds (77.44%), dog rose pulp (74.37%), marcs (residues obtained after pressing) from red grape (65.98%), apple peel (63.44%), and marcs from white grape (58.34%) (Agourram and others 2013). Some food waste extracts were also shown to have moderate antioxidant activity, such as dog rose seeds (45.40%), pomegranate pomace (24.91%), and potato peel (20.34%). Moreover, the total antioxidant activity of the pomace of the above mentioned fruits and vegetables was significantly higher than their pulp (Agourram and others 2013). Quercetagetin (a flavonol) was isolated from citrus peel at a concentration of 20 µmol/L; the DPPH activity was 91%. This was much higher than the ascorbic acid (46.2%) at the same concentration (Yang and others 2011). The intracellular reactive oxygen species scavenging activity of quercetagetin (86.3% at a concentration of 10 µmol/L) was quite higher than Vitamin C, which is known as a high antioxidant functional chemical (17.9% at the concentration of 35.5 µmol/L) (Yang and others 2011).

Kanatt, Arjun, & Sharma have found that the antioxidant activity of legume hull extract can be principally attributed to its phenolic composition (Kanatt, Arjun, Sharma 2011). The IC_{50} value (concentration of sample required to scavenge 50% of the free radicals) of pigeon pea hull extract was only 21.3 µg/mL, which means that the extract had relatively high antioxidant properties (Kanatt, Arjun, Sharma 2011).
Moreover, total phenolics and DPPH activities were strongly correlated. This indicates that the antioxidant capacity was due to the phenolic compounds in the hull extracts (Kanatt, Arjun, Sharma 2011). For extract of grape pomace, the IC$_{50}$ of DPPH scavenging activity was 53 µg/mL (Rodriguez-Rodriguez and others 2012). Pure water extract of pineapple stem waste showed the highest DPPH activity (IC$_{50}$ of 164.2 µg/mL) followed by pure methanol extract (335.2 µg/mL) and 50% (v/v) methanol extract not clear what the difference between the 2 methanols mentioned (335.5 µg/mL) (Upadhyay and others 2012). In their study, the highest DPPH activity of distilled water extract of pineapple stem waste was strongly related to the high TPC. Because the TPC was 279 mg GAE/100 g, it was concluded that this level was higher than found in the pineapple fruit and pulp (Upadhyay and others 2012). In another food waste extract investigation, methanol extract was superior to other extracts, such as acetone extract, and similar to α-tocopherol with regard to scavenging abilities (Zhang, Li, Wang 2010).

Very high TEAC values were detected in pomegranate peel, hazelnut skin, cornelian cherry seeds, dog rose pulp, marcs from red grape, apple peel, marcs from white grape (around 0.7 µM trolox equivalent/g dry extract weight) (Agourram and others 2013). Endoproteases mixture enzyme (trypsin- and chymotrypsin-like) extract of grape pomace had an ORAC value of 4238.9 nmol TE/mg dry extract weight (Rodriguez-Rodriguez and others 2012). DPPH and ABTS assays both showed that lotus rhizome knot and lotus leaf treatments significantly increased radical scavenging (Huang and others 2011).
Protein-rich foods

Food waste extracts can enhance the quality of meat products, such as pork, beef, and chicken by preventing or retarding protein and lipid oxidation. Extracts obtained from food waste have been successfully added to raw meat at the grinding and mixing stages (Kanatt, Arjun, Sharma 2011; Rodriguez-Rodriguez and others 2012; Utrera and others 2012). Thiobarbituric acid-reactive substance (TBARS) has been the most widely used method for testing the oxidative rancidity in food products (Kanatt, Arjun, Sharma 2011; Rodriguez-Rodriguez and others 2012; Utrera and others 2012). Acetone water solvent (70:30, v/v) extracts from peel and seed of avocado were mixed with raw pork meat patties. The peel and seed extracts were found to effectively inhibit lipid and protein oxidative reactions in the patties (Rodriguez-Carpena and others 2011; Rodriguez-Rodriguez and others 2012). The extracts inhibited the formation of TBARS from 72% to 91% (Rodriguez-Carpena and others 2011). In other research, both pomegranate pomace extract treated chicken and untreated chicken meat showed an increase in TBARS during chilled storage (Kanatt, Arjun, Sharma 2011). However, oxidative rancidity was less in chicken chilly and also in lollipop containing pomegranate pomace extract during storage (as compared to that of unfortified chicken) (Kanatt, Arjun, Sharma 2011). Fresh beef patties showed low oxidation levels when treated with green tea extract and grape seed extract (Banon and others 2007). Besides, the two extracts showed similar antioxidant effect on the beef patties (Banon and others 2007). The ethanol extract of potato peel, which contains large amounts of phenolic compounds, was also very
effective in retarding lipid and protein oxidation in minced horse mackerel. It resulted in low levels of peroxide value, volatiles, and carbonyl compounds and protected against the loss of α-tocopherol, tryptophan and tyrosine residues (Farvin, Grejsen, Jacobsen 2012). Frozen mackerel was also fortified with a combination of antioxidant compounds from dry hyssop, prunella, lemon balm, and rosemary extracts. The fortified mackerel showed a decrease in cholesterol oxide products, including 7-α-hydroxy-cholesterol, 7-β-hydroxy-cholesterol and 7-keto-cholesterol (Lebovics and others 2009). Therefore, potato peel, and plant extracts can be used as functional ingredients in raw mackerel products due to their contribution to improve antioxidant properties (Farvin, Grejsen, Jacobsen 2012; Lebovics and others 2009).

The sensory quality of lotus rhizome knot and lotus leaf extracts treated meat was not lower than the results of control group, although the extracts affected color appearance of the samples. Therefore, lotus rhizome knot and lotus leaf waste extracts were considered acceptable by the panelists (Huang and others 2011). The utilization of green tea extract and grape seed extract did not significantly influence the sensory characteristics of cooked beef patties, therefore demonstrating an attractive alternative of using food waste extract in food products (Banon and others 2007).

Avocado extracts, containing phytochemicals such as catechins, chlorogenic acid, and procyanidins, were found to be very efficient in preventing lipids and proteins oxidation in protein-rich food products through the aforementioned antioxidant mechanisms (Rodriguez-Carpena and others 2011). Those avocado phytochemicals
may have protected proteins against oxidation by inhibiting the oxidation of particular amino acid residues such as tryptophan and lysine (Utrera and others 2012).

**Carbohydrate-rich foods**

Grape seed extract, with lower thermal degradation, showed stronger antioxidant capacities than the bulk extract when added to cookies (Davidov-Pardo and others 2012). Bread fortified with grape seed extract had three to four times stronger antioxidant activity than the control bread, in which a decrease in the quantity of N-(carboxymethyl) lysine was observed. Overall, a strong antioxidant activity was achieved by the high concentration of grape seed extract (Davidov-Pardo and others 2012).

**Fat-rich foods**

Lipid oxidation is very critical in affecting the quality of fat-rich foods. Food waste extracts have been shown to enhance quality of oils, such as sunflower, wheat germ, and fish. The pods of the tara have always been used in the leather industry because of their environmentally friendly tannins (Romero, Fernandez, Robert 2012). Tara pods polyphenol extract showed a strong antioxidant capacity in protecting regular and high-oleic sunflower oil under accelerated storage conditions (Romero, Fernandez, Robert 2012). Another example is fish oil, to which strawberry leaf extract was added. Peroxide value (PV) is the most commonly measured and informative indicator for primary oxidation products. Strawberry leaf extract decreased the PV value of fish oil during the storage (Raudoniute and others 2011). Cheese was
wrapped with a red algae film containing grapefruit seed extract; the wrapped cheese showed lower peroxide and thiobarbituric acid values than the non-fortified food (Shin and others 2012).

1.1.4 Antimicrobial and antifungal properties of food waste extracts and their application

Several research papers have reported the influence of food waste extracts on microorganisms, including gram-positive bacteria, gram-negative bacteria, and fungus (Agourram and others 2013; Butkhup and Samappito 2011; Cheng and others 2012; Oliveira and others 2013; Upadhyay and others 2012). Gram-positive bacteria are more sensitive to antimicrobial compounds than gram-negative bacteria (Cheng and others 2012). This is because gram-negative bacteria have a single membrane and a less structurally complex cell wall, which facilitate the penetration of lipophilic compounds, including some antimicrobial compounds (Cheng and others 2012; Oliveira and others 2013). Nevertheless, gram-negative bacteria not only have two cell membrane layers, but also lipopolysaccharides (in their outer membrane), which provide strong hydrophilicity and also a strong barrier against antimicrobial compounds (Butkhup and Samappito 2011; Oliveira and others 2013).

The inhibition effect of food waste extracts on pathogenic, food spoilage, and common bacteria found in food has been investigated (Agourram and others 2013). Tannins, which are present in various fruit and vegetable peels, have been showed to inhibit bacteria adhesions, enzymes, cell envelope transport proteins, and
modification of morphology. Pomegranate peel extracts were found to inhibit the growth of eleven bacterial species, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. However antimicrobial activity of the seed extracts was very limited compared with the peel extract (Agourram and others 2013). Grape peel extract did not show any antimicrobial activity against several food spoilage and pathogenic bacteria, such as *S. aureus*, *Bacillus cereus*, *E. coli* and *P. aeruginosa*, while the grape seed extract had high antimicrobial activity against the above-mentioned bacteria (Oliveira and others 2013). This may be because of the higher content of phenolic compounds, such as GA in grape seed extract than in the grape skin extract (Baydar, Ozkan, Sagdic 2004; Oliveira and others 2013). Apple peel extract affected the growth of some microorganisms, including *Bacillus cereus*, *P. aeruginosa*, *E. coli*, and *Salmonella spp*. However, potato peel extract did not have any antimicrobial capacity. *Staphylococcus marcescens*, *Staphylococcus xylosus*, and *Lactobacillus sakei* were found to be the most resistant bacteria while *Staphylococcus aureus* was the most sensitive (Agourram and others 2013). The extract of mao luang seeds (from Euphobiaceae family) have been used as animal feed or fertilizer in Northeastern Thailand and were discarded as a potential food addative. Recently, the seed extracts were found to inhibit the growth of all gram-positive bacteria except *Streptococcus thermophilus*, but less inhibitory to some gram-negative bacteria such as *E. coli*, *Salmonella typhoi* (Butkhup and Samappito 2011).
Grape pomace extract, extracted by hexane (using Soxhlet apparatus), was inhibitory activity to *Bacillus cereus*, and this property was not found in grape pomace easily extracted by ethanol and methanol (Oliveira and others 2013). The lipid fraction from grape pomace, can be easier extracted by hexane, and may present antimicrobial behavior due to the presence of sterol components (lipophilic). Grape pomace extract showed higher inhibition of gram-positive bacteria (*S. aureus* and *Bacillus cereus*), compared to gram-negative bacteria (*E. coli* and *P. aeruginosa*) (Oliveira and others 2013). Similarly, the antimicrobial activity against bacteria (*Bacillus cereus* and *Brevibacterium ammoniagense*) may be due to the present of phenolic compounds in pineapple stem waste extracts (Upadhyay and others 2012). Only a few food waste extracts were found to inhibit the growth of gram-negative bacteria. The waste extract of pineapple stem obtained by using pure methanol was able to inhibit the growth of gram-negative bacteria *E. coli* (Upadhyay and others 2012). Methanol extracts (100% and 50%, v/v), and water extract of pineapple stem waste showed inhibition toward three fungi, *Colletotrichum gloeosporiodes*, *Colletotrichum acutatum*, and *Sclerotinia sclerotiorum* (Upadhyay and others 2012). Methanol aqueous (50% v/v) grape seed extracts exhibited higher antimicrobial activity against *S. aureus* than the pure ethanol and pure water extracts (Cheng and others 2012). This may be because of differences in the quantity of the phenolics (i.e. higher total phenolic content in the case of pinot meunier grape seeds) and the type of phenolics (higher catechin compounds in the case of pinot noir grape seeds) extracts. However, the role of individual compounds especially those of anthocyanins
is not widely known. Overall, anthocyanin found in grape skin extract showed good minimum inhibitory concentration values. Malvidin chloride had a lower antimicrobial activity against gram-positive or gram-negative bacteria compared with GA. Pinot noir wine residue extracts showed more inhibition against *S. aureus* and *Candida albicans* than pinot meunier wine residue extracts. This may be because of the presence of more flavanols and anthocyanins in pinot noir extracts compared to those of pinot meunier extracts. Various phenolic compounds showed different antimicrobial capacity. For example, catechin showed more antimicrobial than other phenolics (Cheng and others 2012). Hexane was shown to be a better solvent to extract more antimicrobial phytochemicals from different food waste. Aqueous extract of husk from coconut (*Cocos nucifera*) was active against 17 of the tested bacterial and 37 of the *Vibrio* isolates; while the n-hexane extract showed antimicrobial activity against 21 of the test bacteria and 38 of the test *Vibrio* species (Akinyele and others 2011).

**Protein-rich foods**

The quality of fresh meat products has been always limited by microbial spoilage. Fresh meat products have a shelf life of around a week under refrigeration, depending on hygiene and preservation conditions (Banon and others 2007). For example, chicken chilly samples treated with grape pomace extract lasted for 20 days while untreated samples spoiled within seven days of storage. Also the treated samples showed that *S. aureus* growth was delayed for 12 days compared to the untreated groups (Kanatt, Arjun, Sharma 2011). Grape pomace extract (0.1% w/w) were found to extend the shelf life of fresh chicken by 14 days (Kanatt, Arjun, Sharma
Grape seed extract would be mainly effective against gram-positive bacteria, with GA as the main active component (Banon and others 2007). Green tea extract would inhibit *E. coli, S. aureus, Staphylococcus epidermidis* and *Streptococcus mutans* (Banon and others 2007).

**Carbohydrate-rich foods**

Carbohydrate-rich foods are usually kept at room temperature, which can provide optimum temperature for the growth of microorganisms. Protein of amaranth seeds is always treated as by-products of the starch production (Rizzello and others 2009). The addition of the water-soluble amaranth seeds extract to gluten free sliced breads delayed the growth of *Penicillium roqueforti* by 21 days. When used for wheat flour bread making, the water-soluble amaranth seeds extract had the same effect on fungal contamination. Overall, combination of natural preservatives with sourdough fermentation is strongly desirable for extending the shelf life of baked goods (Rizzello and others 2009).

**Vegetables and fruits**

Grapefruit seed extract, as well as a mixture of grapefruit seed extract with citric acid were found to have antimicrobial activity, when added into slices/juice of cucumber and lettuce (Xu and others 2007b). The extract inhibited the growth of *Salmonella spp.* and *L. monocytogenes*. The antimicrobial capacity was higher when combining grapefruit seed extract with citric acid (Xu and others 2007b). Moringa oleifera leaf extract was shown to inhibit both gram-positive and gram-negative
bacteria after adding it to fruit juice (Arabshahi-Delouee and Urooj 2007). Fruits were also protected by what is considered food waste extract when used as a fortified packaging film (Jang, Shin, Song 2011b). Strawberries were packed with grapefruit seed extract fortified rapeseed protein–gelatin film (Jang, Shin, Song 2011b). The film was found to inhibit pathogeneses, like *E. coli O157:H7*, and *Listeria monocytogenes*, total aerobic bacteria, yeast, and molds. However, when added into slices of cucumber and lettuce, grapefruit seed extract significantly reduced the overall acceptability as compared with the untreated samples. The treated samples had unacceptable sensory quality at approximately 2 days for cucumber (whole and fresh-cut pieces), and 6 days for lettuce (Xu and others 2007b). Although several parameters, such as solubility, stability and acceptability need to be taken into account before using food waste extract, the moringa oleifera leaf extract was shown to significantly change (improved / decreased) the overall acceptability of fruit juices (Arabshahi-Delouee and Urooj 2007).

**Food packaging**

Food waste extracts were also useful when added into food coatings and films (Moradi and others 2011b; Yerlikaya, Gokoglu, Topuz 2010). Grape seed extract fortified chitosan film protected sausages and showed significant lower growth of *Listeria monocytogenes* than the control sample (Moradi and others 2011b). The main reason for this was the diffusion of active compounds from the film into meat at the early stages and during the storage. The populations of aerobic, mesophile and lactic acid bacteria gradually increased during storage at 4 °C. Released phenolic
compounds of grape seed extract mainly influenced the rate of microorganism growths. Grape seed extract more effectively inhibited aerobic mesophiles compared to lactic acid bacteria. The authors indicated that the different trends are the results of factors such as type of meat, test microorganism, film preparation method, bacterial inoculation methods, and particularly nature of the chitosan used (Moradi and others 2011a; Moradi and others 2011b). The purpose of incorporating antimicrobial compounds into an edible film, instead of applying them directly onto the meat surface (i.e., by spraying or dipping), was to extend delivery of the antimicrobials during meat storage rather than delivering them in a one time single massive dose (Moradi and others 2011b). Cheese, is another example of fat-rich food. When wrapped with red algae film containing grapefruit seed extract; it had low growth of pathogenic bacteria, like E. coli O157:H7, and L. monocytogenes (Shin and others 2012).

### 1.1.5 Future directions

Although several foods were already found to be fortified by food waste extracts, more categories of food products (especially with food waste extracts) should be investigated. Grape skin and seed extracts have been shown to deliver strong antioxidant properties in prepared lamb meat (Jeronimo and others 2012) and bread (Davidov-Pardo and others 2012), but these extracts may also be able to enhance other protein-rich/carbohydrate-rich foods, such as beef/pork patties, and cookies. Overall, more categories of food waste extracts should be evaluated and later added to various other foods. Additional concentrations of food waste extracts should be
experimented with in order to optimize the best combination used in food. Further studies are needed to evaluate, for example, the effect of different dietary levels of grape pomace concentrate over the live chicken’s complete growth period (Sayago-Ayerdi and others 2009). More factors should be studied and explored in order to find the best condition to enhance food quality. Studies are needed to prove the effectiveness of this approach under normal food processing conditions. Further studies should be undertaken in order to elucidate the underlying mechanisms responsible for processes such as the oxidative stability of meat by grape seed extract. The impact of extracts on the sensory properties of the treated foods should be elucidated in upcoming studies. More exploration of the compounds of food waste extracts should be preformed to gain knowledge of the function better. Further studies are also required to fully comprehend the extracts interaction mechanisms of compounds (Utrera and others 2012).

1.1.6 Conclusion

Food waste materials from harvesting and processing are generated in large quantities. Some extracts contain important active compounds such as phytochemicals, phenolic, and alkaloids. Some of the extracts were shown to have relatively high antioxidant and antimicrobial activity. When food waste extracts are added back to food products, directly or indirectly (food packaging films and coatings), they can help protect the quality in various protein/carbohydrate/fat rich foods, vegetables, and fruits. This literature review points to the numerous possibilities of
recovering food waste in a more economic method to protect food and enhance quality.
Section 2: The use of Fruit and Vegetable Waste in Food Industry, Medicine, and Cosmetology, and Quality Control of the Waste

Abstract

The roots, stems, leaves, peel and pulp waste of fruits and vegetables can be used as a source of many value-added products, as in food industry, as well as in other areas, such as medicine and cosmetology.

In food industry, plant waste materials have a great potential. As consumers frequently compare aspects of color, flavor and odor for acceptance, the use of natural waste materials to improve these components in food products will increase likelihood for a sales purchase. Flavor extracts can be obtained from plant wastes through the use of juices, essential oils, distillates, isolates and extracts in a variety of different plant peels, leaves or seeds. Volatile constituents from these waste compounds are also found to have the ability to improve odor when added to food products. Pigments in plant waste materials such as those present in fruits and vegetables provide bright colors that can be adapted into products for the development of high quality foods and optimum consumer appeal.

Medical use of the extracts of peel and pulp of many fruits and vegetables is based on properties of phytochemicals they contain. The phytochemicals have been shown to provide hypotensive, hypoglycemic, anticancer, antioxidant, antimicrobial and anti-ulcerogenic effects. They are also used in cosmetology to combat the effects of photoaging and as compounds of sunscreens and SPF cosmetics.
It is very important to use safe and pesticide-free fruit and vegetable wastes. Most pesticide residues are found in fruit and vegetables peels, which are waste from food processing industry. Agricultural waste, including plant leaves, stems, and roots, also contains certain amount of pesticides. Food processing procedures, such as washing, peeling, and boiling can remove most of pesticides. Organic foods are grown without any synthetic pesticides, which makes them more attractive to consumers, so the use of organic fruit and vegetable waste offers an advantage.

These and other aspects of fruit and vegetable waste use are discussed in further detail in the current review.

1.2.1 Introduction

Natural color and flavors are increasing in importance due to consumers’ growing interest in health and safety. Flavor is considered as an essential factor that determines our food selection for consumption. Developing high quality flavors is crucial in processing for the final sale of premium foods. Also, consumers associate color with certain foods. Color is an aesthetic attribute and an indicator of quality; raw foods such as plant peels contain natural pigments that are capable of being used as colorants. Plant waste material can also be incorporated into food products to provide certain odors. Overall, extracts and peels from plant waste products are a method of adapting natural colors, flavors and odorants into foods.

Fruits and vegetables contain many phytochemicals which include flavonoids (i.e. quercetin and apigenin), anthocyanidins, norepinephrine and serotonin, triterpenes, amino acids, alkaloids, carotenoids, and other substances, which provide
hypotensive, hypoglycemic, anticancer, antioxidant, antimicrobial and anti-ulcerogenic effects (Dunjic and others 1993; Horinaka and others 2006; Naseri and others 2008; Pari and Maheswari 1999). Cosmetological use of the extracts of fruit and vegetable waste is based on their alpha-hydroxy acids content (Rivers 2008) and the use of anthocyanins and flavonoids (Korac and Kambholja 2011).

Before utilizing fruit and vegetable wastes, pesticide residues should be removed. Pesticide residues in commercial food products have been studied for years, and for each type of pesticide the maximum residue limits (MRLs) have been established by various organizations, even when the limits have only small differences. Recently, the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method has been accepted by laboratories all over the world (Liang and others 2014; Zhu and others 2014). Waste from organically grown fruits and vegetables make them more attractive to consumers, as no synthetic pesticides are used, but microorganisms and environmental contamination should be of concern in regard to organic fruits and crops, including use of their waste.

1.2.2 Flavor production from plant waste materials

Flavor is considered as the combination of two components, taste and odor. Flavors in food products are affected by many human sensations including temperature and texture. There are a variety of different plant materials that can contribute to flavor development. Plant materials have been used as flavorings or seasoning to improve food products. For example plants have been adapted used as spices and condiments are usually aromatic and pungent owing to the presence of
varying types of essential oils. There are two categories of flavor; the compound type and physical form. The compound type involves esters, aldehydes, ketones, ethers, alcohol, sulphur, acids, nitrogen compounds and lactones. The physical form is the fruit juice, essential oils, distillates, isolates and extracts. Natural flavors are not always consistent or stable in flavor strength; these products are often costly in comparison to synthetic flavours.

Salem (2003) states that many types of branched aldehydes and alcohols are essential flavor compounds for food products. Orange is a very unique fruit flavor that contains these compounds. Baldwin (2002) found that oxygenated terpenoids like linalool, neral, and geranial are important in the orange flavor. Rose oil has been able to be produced through the extraction of the fresh rose flower or wastes using enzymes (Rumyantseva 1981). The product is able to be added to foods for enhancement of flavor. Mishra and others (2006) found that the extraction of essential oils from the peels on mandarin fruits can be achieved by enzyme pre-treatment with hydrodistillation and cold press extraction. The peels produced up to 15% more essential oil using enzymes then the control. Shaw and Wilson (1976) discovered that flavedo, outer peel extracts, from lemon, lime and a lemon-lime hybrid contain many extracts that can be added to products such as fruit juices or mouthwashes as flavourings. Each of the oils was extracted using hexane with filtration. The lemon and lime extracts are favourable to consumers when added to beverage products as flavourings. The main difference between the lemon and lime in comparison to the hybrid, is that this hybrid contains thymol and thymol ether.
Thymol and thymol ether are compounds that have a medicinal flavor therefore making it unacceptable to add to food products however acceptable in products such as mouthwashes. Moshonas and Shaw (1974) state that tangerine peel oil contains these compounds in weaker concentrations.

The oils from the leaves of lemons contains a large range of components such as limonene, $\beta$-pinene, geranial and many others (Lota and others 2002). Lime leaf oils also were found to exhibit similar traits and many compounds that were present in lemon leaf oils. Lemon and lime leaf oils can also vary by cultivar, species and origin as some contain more or less sweetness and concentration of certain compounds (Huang and Pu 2000).

Hamden and others (2013) identified waste products in mandarins, such as peels and leaf oil, can be adapted for flavourings in the food industry. The oils produced from leaves of Cleopatra Mandarins from Egypt include linalool, sabinene, terpinen-4-ol and E-(\(\beta\))-ocimene. The peel of the mandarins mainly produced limolene. Attaway and others (1967) extracted oils from grapefruit leaves and found linalool, sabinene, ocimene and a variety of other flavor compounds of value are present in grapefruit. The compounds percentages varied over time as the cultivar was analysed every other week from March to January during the season from mature trees.

Luo and others (2013) discovered banana peel waste can be added to the diet of sea urchins for improving the species’ flavor. Sea urchins are most commonly fed
kelp, however when fresh banana peel was added to the diet in place of kelp the
flavor of the sea urchins became much sweeter.

1.2.3 Addition of plant materials for odor

As mentioned in the previous section, odor is often considered a component of
flavor. Odorants are recognized by humans as chemical compounds which are
volatile. These compounds have the potential to be inhaled through the air into the
olfactory epithelium of the nasal cavity. An odorant has to maintain certain molecular
traits to be recognized by sensory processes. Therefore, a significant vapor pressure
as well as lipophilicity is required to allow for it to be carried to the olfactory system.
Also, a certain level of water solubility is needed to permeate small layers of mucus to
be received by olfactory receptors.

Vanillin has also has been able to be extracted from the green pods using
enzymes. This process produces glucovanillan and ethanol soluble aroma
compounds (Ruiz-Teran, Perez-Amador, Lopez-Munguia 2001). Hamdan and others
(2013) reported 48 compounds that are volatile constituents in Cleopatra mandarin
peels and 81 in Cleopatra mandarin leaf oil can be an added to improve odor
properties foods; however some of these compounds are in trace amounts. Also as a
pomegranate fruit is processed into juice, a large section is left over as a by-product.
This can involve the peel, leaves and seeds. The peel of a pomegranate is abundant
in classes of phenolics known as punicalagins. Lansky and Newman (2007)
pomegranate seed oil has a conjugated fatty acid, punicic acid that makes up
approximately 65% to 80% of the oil from pomegranate seeds. Pomegranate leaves and mesocarp contain many polyphenols such as punicalagin and ellagitannin which provide the flavor of the fruit. Fischer and others (2011) found that the pomegranates contain 20.3 g/kg of polyphenols in the dried mesocarp in comparison to 10.5 g/kg in the dried peel. Interestingly, the fresh pomegranate mesocarp only provided 4.2 g/kg and the peel produced 4.5 g/kg. Therefore, the dried mesocarp has the ability to provide the most polyphenol as a flavour component.

### 1.2.4 Natural color pigmentation

In the food industry, there has been an increased usage of food additives, specifically food colorants, which has led to a lot of controversy. The color pigments present in fresh fruit and vegetables are vivid in nature. However as the plant cell is disturbed or destroyed through heavy processing, the pigments will experience detrimental physical and chemical conditions. These conditions can initiate degradation. A food product that is colored can improve the appetite and enjoyment for the consumer. However, consumers can be quite distrustful of added colors to foods that provide certain aesthetic properties, these additive are also used to hide faults such as breakdown in flavor, nutritional and microbiological features. Color is essential in the “first-buy” sales as well as toward the impression of natural content. Preference of color is based on psychology; it further influences the flavour and aroma of a food (Timberlake and Henry 1986).
Some common methods for the extraction of color pigments from plants have used enzymes. Plants contain many color pigments, which are able to be accessed for the use in many products. For example, materials such as plant wastes that are rich in these pigments that have been obtained by enzyme-assisted extraction are grapes, strawberry, marigold, safflower, beetroot, alfalfa, chilli, and red cherries. The majority of color pigments are produced from the groups are known as chlorophyll, betalins, anthocyanins, carotenoids and flavones.

Press cakes are solid materials leftover after pressing a product to extract the liquids. The method of juice processing produces wastes in press cake residues, these wastes are composed of the stem, seeds and skins. Blueberry press cakes consists of high concentrations of antioxidants such as polyphenols, and anthocyanins, which make it a potential source of natural colorings and nutraceuticals. Pomegranate leaves, peel and mesocarp also have polyphenols, which contribute to their red colors (Fischer, Carle, Kammerer 2011). Polyphenols can be extracted from these waste products and use as food colorants. Recently, Cam and others (2013) discovered pomegranate peels and seeds can be incorporated into ice cream to create a more intense yellow color. However, as the oil and seeds are slightly acidic this will decrease the pH values. Gastaldi and others (1996) states that the pH of ice cream is very important for milk protein structure. A low pH in an ice cream could cause the destabilization of casein micelles as well as coalescence of the micelles. Kammerer and others (2007) discovered that black carrot and elderberry have the ability to be added as a concentrate to canned strawberries to improve the stability of
the color over a long storage time period. Irradiated beetroot extract also can be added to food as a natural source of color. Recently, irradiated red beetroot has been used in Brazilian cream cheese to create a red or purple color and remain stable (Goncalves and others 2010). Also Gonclaves and others (2010) found that beetroot extract could be added to strawberry ice cream to provide as a source of red color.

1.2.5 Medical use of fruit and vegetable waste

Hypotensive effect of quercetin

Naseri and others (2008) studied vasorelaxant and hypotensive effects of the hydroalcoholic extract of onion (*Allium cepa*) in rats. Hypertension in rats was induced by high-fructose diet. It was shown that onion extract reduced aorta contractions in a concentration-dependent manner, which could be due to the inhibition of calcium influx. After three weeks of use, the extract also reduced hypertension caused by fructose, which could be possibly explained by the presence of a flavonol quercetin in the extract, antioxidant activity, and inhibition of vascular smooth muscle cells $\text{Ca}^{2+}$ influx.

Patil, Pike, and Yoo (1995) suggested that genetic and storage factors an affect quercetin content in onion. In their research, total quercetin content in yellow, pink, and red onions varied from 54 to 286 mg/kg fresh weight. White onions contained trace amounts of total quercetin. Free quercetin content in almost all the onions tested was low.
There are a few papers describing the effect of quercetin in decreasing blood pressure, as well as reduction of coronary heart disease and stroke. In the studies conducted by Edwards and others (2007), individuals (both men and women) with prehypertension and stage 1 hypertension participated in a randomized, double-blind, placebo-controlled, crossover study. In this study, the efficacy of 730 mg quercetin/day for 28 day versus a placebo was tested. It was shown that after quercetin administration for prehypertensive patients their blood pressure did not change, while it was reduced in stage 1 hypertensive patients (systolic, diastolic, and mean arterial pressures).

Vegetables and herbs that are known to be high in quercetin are radish (leaves), dill, lovage, rumex, and coriander (Bhagwat and others 2005; Justesen and Knuthsen 2001). A study aimed at the optimization of extraction of quercetin from radish (*Raphanus sativus*) was performed by Vangalapati and others (2014) who used Soxhlet extractor. In this study, methanol was found to be the best solvent for the extraction of Quercetin from the leaves of radish. In the optimized conditions of the extraction (methanol as a solvent, soaking time 1 day, extraction time 1 hour, 200 particle mesh size, solvent percentage 80% (v/v), 1:1 ratio of hexane with methanol, pH 6.0), the highest concentration of quercetin obtained was 45 µg/mL.

Hertog and others (1992) proposed a rapid method of quantitative determination of quercetin, as well as of four other flavonoids, in freeze-dried vegetables and fruits (lettuce, endive, cranberry, onion, leek), based on RP-HPLC with UV detection, performed after acid hydrolysis of parent glycosides.
Justesen and others (1998) used HPLC with a photo-diode array and mass spectrometric detection to perform quantitative analysis of quercetin and other flavonols, flavones and flavanones which were analysed as aglycones, obtained after acid hydrolysis of freeze-dried fruits and vegetables (apples, oranges and pears were peeled, and the peel was analyzed separately from the pulp). The highest level of quercetin was found in red and yellow onion (45 ± 21 and 34 ± 7 mg/100 g fresh weigh respectively). The content of quercetin in apples was 2.0 ± 0.4 mg/100 g, and it was found only in the skin (average value 16 mg/100 g fresh weight). The pulp of the apples did not contain quercetin.

Balasuriya and Rupasinghe (2012) studied antihypertensive properties of flavonoid-rich apple peel extract. Quercetin-3-O-glucuronic acid was found to be the most effective inhibitor of angiotensin converting enzyme (ACE) among all the flavonoids and metabolites studied. This property can be used in prevention of the conversion of angiotensin I into angiotensin II and related to this raising of blood pressure.

The role of quercetin and other flavonoids contained in apple peel (proanthocyanidins, anthocyanins), as well as apple pectin, in lowering blood pressure was confirmed by Kaur and Baluja (2013): apple flavonoids inhibit ACE, improve endothelial function of blood vessels, prevent oxidation of low density lipoprotein and prevent atherosclerosis.
Anticancer activity of quercetin

Quercetin was also shown to have an anticancer activity. In the studies by Williamson and others (1996), the bioactivities of the two flavonoid glycosides purified from onions (quercetin-3,4’-diglucoside and quercetin-4’-glucoside) were compared to quercetin aglycone, and also to flavonoid glycosides, rutin (quercetin-3-rutinoside) and isoquercitrin (quercetin-3-glucoside). The most effective inducer of the anticarcinogenic phase II marker enzyme, quinone reductase, in Hepalclc7 cells of mice, was found to be quercetin aglycone. Of all the glycosides, only quercetin-4’-glucoside could induce activity of quinone reductase.

Activity of quercetin against fibromyalgia

An efficacy of quercetin in the treatment of fibromyalgia was suggested by Lucas and others (2006). Fibromyalgia can be associated with an increased secretion of corticotrophin-releasing hormone and substance from neurons in specific muscle sites, which stimulates local mast cells to release proinflammatory and neurosensitizing molecules, and quercetin. These compounds have anti-inflammatory and mast cell inhibitory properties, and can be potentially used together with other treatment modalities.

Apigenin extraction and its anticancer effects

Miean and Mohamed (2001) studied the flavonoid content of 62 edible tropical plants. The highest flavonoid (specifically apigenin) content was observed in onion leaves, followed by Semambu leaves, bird chili, and black tea. A similar study,
conducted by Croizer and others (1997) on commercial tomatoes, onions, lettuce and celery, places lettuce in the lead for flavonoid content. The latter study used HPLC to analyze the level of flavonoids, as well as suggests that cooking the vegetables lowered the flavonoid content.

Onions, as well as parsley, celery, garlic, bell pepper, Chinese cabbage, guava and oranges, also contain apigenin, flavones, or 4′, 5, 7,-trihydroxyflavone (Horinaka and others 2006; Patel, Shukla, Gupta 2007). Apigenin has been shown to exert remarkable anti-inflammatory, antioxidant and anti-carcinogenic activity (Choudhury and others 2013; Patel, Shukla, Gupta 2007). In the review by Patel and others (2007), the cancer chemopreventive effects of apigenin are discussed. According to Choudhury and others (2013), apigenin induced apoptosis and cell death in lung epithelium cancer (A549) cells, alone and synergistically with curcumin.

In the studies by Horinaka and others (2006), apigenin was shown to remarkably induce expression of Death Receptor 5 and synergistically work with exogenous soluble recombinant human tumor necrosis factor–related apoptosis-inducing ligand to induce apoptosis (programmed cell death) in malignant tumor cells.

Thin layer chromatography analysis of parsley seed flavonoids showed that the major flavonoid is apigenin, which manifested the cytotoxic effect in mice with mammary adenocarcinoma. Significant reduction in tumor volume and growth rate inhibition were evident after 24 days of oral administration of apigenin; stimulation of immune system by apigenin was demonstrated histopathologically (Alol, Al-Mzaien, Hussein 2012).
In the research conducted by Justesen & Knuthsen (2001), the amount of apigenin in parsley varied from 510 to 630 mg/100 g fresh weight. Its quantification in herbs was performed by HPLC after acid hydrolysis. In celery leaves, the content of apigenin is approximately 40 times higher than its level in the stalks (Justesen, Knuthsen, Leth 1998).

**Green method of extraction of phenolic compounds from mango peel**

Tunchaiyaphum and others (2013) studied new, green methods of extraction of bioactive components from peels of mango (*Mangifera indica* L. cultivar Phalun). Mango peels are known to contain high amounts of phenolic compounds. For their extraction, the method of subcritical water extraction was used. The amount of phenolic compounds obtained from mango peels using the method of subcritical water extraction was 1.5 times higher than that using soxhlet extraction (extraction time 60 min). It was also shown that the maximum total phenolic compounds (35.96 mg GAE/g DW) was observed at 90 min. Also, the total amount of phenolic compounds was the highest at 180 °C (30.62 mg GAE/g DW), due to the increased solubility of phenolic compounds in water with higher temperature. With temperatures above 180 °C, the total phenolic content decreased, which could be explained by thermal degradation of phenolic compounds.

**Anthocyanins and their properties**

Anthocyanins have been shown to prevent the progression of liver damage related to non-alcoholic fatty liver disease. In a recent review prepared by Valenti and others
three independent mechanisms of anthocyanin activity were suggested: inhibition of lipogenesis by reducing sterol regulated element binding protein 1c (Srebp1c), promotion of lipolysis by induction of the activity of β/δ peroxisomes proliferator activated receptor (PPAR), and reduction of oxidative stress.

According to Valenti and others (2013), the six anthocyanidins commonly found in fruit and vegetables are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. Elderberries have the highest content of cyanidin (485.3 mg/100 g); black currant and bilberries are especially rich in delphinidin (87.9 and 97.6 mg/100 g, respectively); cultivated blueberries have the highest content of malvidin (67.6 mg/100 g); black raspberries are rich in pelargonidin (16.7 mg/100 g); cranberries contain increased levels of peonidin (49.2 mg/100 g); and the highest amount of petunidin can be found in bilberries (42.7 mg/100 g). Of all the vegetables tested, radicchio had the highest content of cyanidin (127 mg/100 g); raw eggplant is especially rich in delphinidin (85.7 mg/100 g); black beans contain 10.6 mg/100 g of malvidin and 15.4 mg/100 g of petunidin; radishes have 63.1 mg/100 g of pelargonidin; and red onion has 2.1 mg/100 g of peonidin. Anthocyanins have low absorption and are rapidly metabolized, but their regular intake reduces the risks of cardiovascular disease and cancer.

Bridgers and others (2010) describe a method of extraction of anthocyanins from industrial purple-fleshed sweet potatoes. Enzymatic hydrolysis and laboratory extractions were conducted on the vegetable. Operating variables such as temperature, solvent and solid loading variations were tested and observed in the
experiment. The most effective extraction trial was performed at 80 °C, using acidified methanol at 3.3% (w/v) as the solid loading, yielding 186.1 mg of anthocyanins per 100 grams of fresh purple-washed sweet potatoes. This experiment has shown that effective production of anthocyanins from vegetables can be achieved using various methods of extraction and that these methods may be used on a large industrial scale.

Chandrasekhar and others (2012) examined another method of extraction of anthocyanins, using red cabbage as the source. The extraction process involved the usage of 50% (v/v) ethanol and acidified water. At the end of the experiment, a yield concentration of anthocyanins of 390.6 mg/L was obtained. Furthermore, the process was extended to purify the obtained extract.

Phytochemicals in banana waste and their anti-ulcerogenic properties

Health benefits of bananas were described by several authors. Imam and Akter (2011) compiled a review of studies of phytochemical and pharmacological properties of Musa paradisiaca L. and Musa sapientum L., which are common species of bananas. It was reported that the fruits of these species had been used in the treatment of diarrhea, dysentery, intestinal lesions in ulcerative colitis, diabetes, hypertension, cardiac diseases, and some other conditions. The unripe pulp of plantain (M. sapientum var. paradisiaca) was found to contain leucocyanidin, quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside. According to Ghani (2003), fruit pulp of M. paradisiaca and M. sapientum has norepinephrine, serotonin, tannin, indole compounds, starch, iron, crystallisable and non-crystallisable sugars,
vitamin C, B-vitamins, albuminoids, and mineral salts. Acyl steryl glycosides and steryl glycosides were isolated from the fruits of *M. paradisiaca* (Ghoshal 1985). Bananas are shown to contain triterpenes (Ragasa and others 2007). Both pulp and peel of *M. paradisiaca* also contain arginine, aspartic acid, glutamic acid, leucine, valine, phenylalanine, and threonine (Ghani 2003).

An anti-ulcerogenic activity of banana is believed to be due to its pectin and phosphatidylcholine content, which strengthens the mucous-phospholipid layer of the gastric mucosa (Dunjic and others 1993). According to Lewis and others (1999), leucocyanidin, a flavonoid in the pulp of unripe banana (*Musa sapientum* L. var. *paradisiaca*), protects the gastric mucosa from aspirin-induced erosions.

**Antioxidant and antibacterial properties of phytochemicals in banana waste**

The studies of Sahaa and others (2013) demonstrated an antioxidant and antibacterial activity of *Musa sapientum* var. *sylvesteris in vitro*. In these studies, sun-dried and powdered plant material was extracted with methanol by cold extraction process, the extracts were concentrated and further stored until used. The extracts were found to contain various types of phytochemical active compounds: alkaloids, flavanoids, steroids, saponins, glycosides, phenols, and proanthrocyanidines. Antioxidant activity of the extracts was confirmed using the DPPH radical scavenging method. The studies showed hydrogen peroxide scavenging activity, reducing activity and hydrogen peroxide induced hemolytic inhibition activity of the extract. In addition, the binding affinity to the receptors of erythrocytes and ability to prevent agglutination were found, which suggests possible
use of the extracts of *M. sapientum var. sylvestris* as antiviral therapeutics. In an antibacterial assay, broad spectrum antibacterial activities of the extracts against *V. mimicus, S. typhi, S. dysentry, S. aureus and B. serus*, in a dose-response manner, were confirmed.

The research by Yin and others (2008) showed the preventative role of a single banana meal on plasma oxidative stress and improvement in the resistance to oxidative modification of low density lipoprotein in healthy individuals.

Mokbe and Hashinaga (2005) discussed antibacterial and antioxidant properties of the peel of banana fruits (*Musa*, cv. Cavendish, which is a subgroup of triploid (AAA) cultivars). The fresh green and yellow banana peel was treated with 70% and sequentially partitioned with chloroform and ethyl acetate (EtOAc). The EtOAc and water-soluble fractions of green peel had high antimicrobial and antioxidant activity, respectively. Antioxidant activity of water extracts was found to be comparable to those of synthetic antioxidants. When tested against Gram-negative and positive bacterial species, the isolated components β-sitosterol, malic acid, succinic acid, palmatic acid, 12-hydroxystearic acid, glycoside, the D-malic, and 12-hydroxystearic acid showed the highest antibacterial activity.

**Hypoglycemic properties of phytochemicals in banana waste**

Ojewole and Adewunmi (2003) demonstrated a hypoglycemic effect of methanolic extract of the green fruits of *M. paradisiaca*. The extract induced significant,
dose-related reductions in the blood glucose concentrations in diabetic and normal mice.

Pari and Maheswari (1999) studied of *M. sapientum* L. in alloxan-induced diabetic rats. According to the authors, *M. sapientum* is used in Indian folk medicine for the treatment of diabetes mellitus. Oral administration in rats of the chloroform extract of the flowers of *M. sapientum* for 30 days resulted in a significant reduction in blood glucose, glycosylated haemoglobin and an increase in total haemoglobin. 0.15, 0.20 and 0.25 g/kg were administered, and in case of 0.25 g/kg the effect was highly significant.

Usha and others (1989) researched on the effect of dietary fiber from banana on metabolism of carbohydrates in the liver of rats. Animals fed isolated dietary fiber from *M. paradisiaca* had lower levels of fasting blood glucose and higher concentration of liver glycogen. Activity of such enzymes as glycogen phosphorylase, glucose-1-phosphate, uridyl transferase, glycogen synthase, glucose-6-phosphatase, and glucose-6-phosphate dehydrogenase was higher, while activity of phosphoglucomutase, as well as of some glycolytic enzymes, such as hexokinase and pyruvic kinase, was lower. The activity of fructose 1-6 diphosphatase was not affected. The effect of banana dietary fiber on the enzyme activity was explained by the higher concentration of bile acids produced in the liver.

Mallick and others (2006) found that the use of methanolic extract of the root of *M. paradisiaca* in streptozotocin-induced diabetic rat resulted in a significant remedial
effect on blood glucose level as well as on the quantity of liver and skeletal muscle glycogen.

Antihypertensive properties of phytochemicals in banana waste

Osim & Ibu studied the effect of papain (*Musa paradisiaca*) on rats with hypertension caused by administration of desoxycorticosterone acetate (DOCA). The consumption of plantain diet lowered the mean arterial blood pressure to control values. Also, DOCA administration to rats previously fed with plantain diet produced no significant change in the mean arterial blood pressure when compared with control.

Use of carotenoids in ophthalmology

Carotenoids, which can be found in many fruits and vegetables, seem to play an important role in prevention of eye diseases and vision deterioration, and this role is discussed by many authors. Lutein and zeaxanthin are the only carotenoids found in the lens (Yeum and others 1995). Macular pigment of eyes is characterized by the presence of stereoisomers of two carotenoids, lutein and zeaxanthin (Bone and others 1993). Age-related macular degeneration (AMD) is triggered by many factors, involving oxidative stress. Macular pigment protects against AMD, and administration of supplements containing meso-zeaxanthin was shown to be effective at raising macular pigment density, which may be used in protection against AMD (Bone and others 2007).
Gao and others (2011) studied the role of lutein and zeaxanthin in reducing the risk of senile cataract. H$_2$O$_2$ is one of the physiologically relevant oxidants in the lens and in the aqueous humor of the eye. Exposure of human lens epithelial cells to H$_2$O$_2$ increases levels of oxidized proteins, lipid peroxidation, and DNA damage. It was shown that pre-incubation with lutein, zeaxanthin, or $\alpha$-tocopherol significantly reduced the damage in cells.

Sommerburg and others (1998) measured the content of different carotenoids and their isomers in homogenates of various fruits and vegetables, two fruit juices, and egg yolk. For the measurements, HPLC using a single column with an isocratic run, and a diode array detector were used. Egg yolk and maize (corn) were found to contain the highest mole percentage (% of total) of lutein and zeaxanthin. Of all the vegetables tested, maize (corn) had the highest amount of lutein, while the orange pepper had the highest content of zeaxanthin.

**Antioxidant properties of phytochemicals in carrot waste**

The most important source of dietary carotenoids in many countries is carrots. Shyamala and Jamuna (2010) studied the nutritional content and antioxidant properties of pulp waste from carrots (*Daucus carota*). Carrot pulp wastes were found to contain $4.00 \pm 0.02$ mg/100 g of total carotenes, and $3.92 \pm 0.61$ mg/100 g of beta-carotene. To study the antioxidant potential of the extracts of pulp wastes, free radical scavenging capacity using DPPH, reducing power and total antioxidant activity by phosphomolybdenum method were determined. At 20 mg/g concentration, carrot extracts had 40.8% activity, which was higher in methanol extract compared to
ethanol or aqueous extracts. The reducing power was higher in methanol extract as well. Methanol extract had the highest polyphenol content in carrot pulp waste (250 mg TAE/100 g of sample), but the total antioxidant activity was higher in its aqueous extract (4.5 g ascorbic acid/g of sample). The nutritional analysis of the composition of carrot pulp waste indicates that it is a good source of dietary fibre, including the soluble fibre.

Zhang and Hamauzu (2003) analyzed different tissues of carrots to determine the content of phenolic compounds and their antioxidant properties. Carrots contained mainly hydroxycinnamic acids and derivatives, among which chlorogenic acid was a major hydroxycinnamic acid, the range of which varied from 42.2% to 61.8% of total phenolic compounds. The highest phenolic content was observed in peel; it was less in the phloem, and lowest in the xylem of the carrot. Carrot peel provided the highest amount - 54.1% - of total phenolics in 100 g fresh weight of carrots (phloem 39.5 and xylem 6.4% respectively). Antioxidant and radical scavenging properties were also the highest in carrot peel, and the lowest in xylem.

Antioxidant properties of phytochemicals in pomegranate waste

A few articles describe the antioxidant activity of pomegranate (*Punica granatum*) peel and seeds. Singh and others (2002) performed extraction of antioxidant-rich fractions from pomegranate peels and seeds using ethyl acetate, methanol, and water. The extracts were further tested for their antioxidant properties *in vitro*. Antioxidant activity of the methanol extract of peels was 83 and 81% at 50 ppm, using the beta-carotene-linoleate and DPPH (1,1-diphenyl-2-picryl hydrazyl)
model systems, respectively. The antioxidant activity of the methanol extract of seeds at 100 ppm was 22.6 and 23.2% using the same model systems, respectively. The methanol extract of the peel was tested for lipid peroxidation, hydroxyl radical scavenging activity, and human low density lipoprotein oxidation at 100 ppm, and it showed 56, 58, and 93.7% inhibition, respectively.

Chidambara Murthy and others (2002) also studied the antioxidant activity of pomegranate peel extract in vivo. Dried pomegranate peels were powdered, extracted with methanol and then fed to albino rats, followed by carbon tetrachloride. The amounts of various enzymes (catalase and peroxidase), as well as lipid peroxidation, were subsequently studied. Treatment of rats with carbon tetrachloride decreased the levels of enzymes, while lipid peroxidation increased. The use of methanolic extract of pomegranate peel before the treatment with carbon tetrachloride preserved the enzymes, so their values were comparable with the control; the lipid peroxidation was decreased. Histopathological studies confirmed the protective effect provided by methanolic extract of pomegranate peel.

**Antimicrobial and antioxidant properties of phytochemicals in potato peel**

Al-Weshahy and Rao has recently (2012) published an extensive review on the use of potato peel as a source of important antioxidant nutraceuticals. Singh and Rajini (2004) studied free radical scavenging activity of a freeze-dried aqueous extract of potato peel. Potato peel powder demonstrated strong inhibition of lipid peroxidation of rat liver homogenate caused by the FeCl₂–H₂O₂ system, and also inhibited deoxyribose oxidation in a concentration-dependent manner. Antioxidant
activity of potato peel powder was also shown in the DPPH radical assay system. Besides, potato peel had strong reducing power, superoxide scavenging ability and ferrous ion chelating potency.

Significant antibacterial activity of potato peel was shown against *P. aeruginosa* and *Clavibacter michigenensis* (Deviprasad & Pushpa 2007).

**Fruit waste and peel as the source of pectin**

Sen and others (2014) studied the role of fruit waste pectin in establishment of probiotic bacteria, such as *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. Probiotic bacteria were inoculated into MRS broth, incubated for 2 hours, then diluted and plated on MRS agar. After that, they were incubated for 48 hours and enumerated; and the experiment was replicated five times. The samples of pectin from apple, lemon, orange and commercial pectin were tested at pH 2, 6, 7 and 8, respectively with the bacterial culture. At all the pH levels, *L. acidophilus* had the higher number of colonies in comparison to *B. bifidum*. The highest growth of *B. bifidum* was observed in apple pectin at pH 2 and in orange pectin at pH 6, 7 and 8 at dilution $10^{-4}$. *L. acidophilus* grew better in apple pectin at pH 2, 7 and 8 and orange pectin at pH 6 at dilution $10^{-7}$. The authors concluded that fruit and vegetable waste can be utilized in enhancing the growth of probiotic bacteria.

**1.2.6 Use of fruit and vegetable waste in cosmetology**
Treatments of photoaged skin using alpha-hydroxy acids obtained from fruit waste

Alpha-hydroxy acids (AHA) are naturally occurring acids that fall under the general carboxylic acid group. AHAs are found in several foods. Glycolic, lactic, citric, and malic acids are found in sugar cane, milk, citrus fruits, and apples respectively (Stoker 2013). Alpha-hydroxy acids act on epidermal and dermal levels of human skin, and promote cellular renewal (Vanscott and Yu 1984). As a result, they are widely used as anti-aging skincare ingredients (Rivers 2008).

Ditre and others (1996) have conducted a study that examined the effects of alpha-hydroxy acids on human photoaged skin. A group of patients applied a test lotion containing glycolic, lactic, or citric acids at a 25% concentration to one forearm and a placebo lotion to the other forearm for an average period of six months. At the end of the study, biopsy samples of the forearms were collected and processed. Specimens from the forearms treated with alpha-hydroxy acids were concluded to be 25% thicker, suggesting that treatment with AHAs has reversed the effects of photoaging skin.

While alpha-hydroxy acids have been used extensively in cosmetics, the dermal and systematic safety of these ingredients is still questioned. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers limits the usage of glycolic acid to a level under 4% and a pH ≥ 3.8. Lactic acid cannot be present in cosmetics at a concentration greater than 2.5% with a pH ≥ 5.0 (SCCNFP 2004).
Citric acid has been widely used in dermatology and cosmetics along with other alpha-hydroxy acids as it improves the appearance of photoaged skin and corrects the disorders of keratinization, the production and conversion of keratin. There is still little known about the specific effects of alpha-hydroxy acids on the epidermis and the changes in skin due to the application of the acid (Bernstein and others 1997).

Citric acid is a commonly used cosmetics substance that is found in citric fruits. Torrado and others (2011) describes the production of citric acids from orange peel wastes by solid-state fermentation of *Aspergillus niger* in Erlenmeyer flasks. The concentration of the fungi ranged between $0.5 \times 10^3$ to $0.7 \times 10^8$ spores/g dry orange peel, with the bed varying from 1.0 to 4.8 g of dry orange peel. Furthermore, to investigate the effects of operating variables, moisture ranged from 50% to 100% of the maximum water retention capacity of the material. The conditions for the most effective production of citric acid yielded an acid concentration of 193 mg/g dry orange peel. The addition of methanol was detrimental to the production of citric acid. Overall, the results of the study suggest that the production of citric acid from dry orange peel by solid-waste fermentation may have future applications in industry.

Glycolic acid is an odorless, colorless, hygroscopic molecule that is capable of penetrating human skin. As a result, it has many applications in medicine, cosmetology and dermatology (Farznurfariza 2012). There are several effective ways of obtaining glycolic acid from natural sources. Firdaus (2012) studied the effectiveness of the methods of production of glycolic acid. Glycolic acid was extracted from various sources using an ultrasonic homogenizer; the operating
variables included the concentration of ethylene glycol, temperature, and time. The study has shown that fresh banana peels contain the highest concentration of glycolic acid, at 0.914 M. The most effective concentration of variables were temperature at 70 °C, solvent concentration at 1 M and the extraction time of 50 min. This method of production of glycolic acid is not only fast and safe, but also helps to manage the waste in the market and individual households.

Several studies conducted by the FDA examined the effect of alpha-hydroxy acids (AHA) on the sensitivity of human skin to ultraviolet radiation. These studies suggest that application of lotions containing AHA increases the sensitivity of human skin to UV light. After four weeks of applications, the volunteer’s skin was 18 percent more susceptible to reddening due to UV. The studies were not able to explain the mechanisms of how exactly AHA affects sensitivity of human skin. The increase in human skin sensitivity due to the application of AHA did not cause significant increase in damage of direct UV light to human skin. As a result, FDA continues to review the data available on the effects of AHA on human skin. The current advice of the FDA is to use sun protection if applying AHA frequently. It is required of manufacturers to warn consumers of the presence of alpha-hydroxy acids on labels to prevent any damage to the skin.

The use of anthocyanins and apigenin in SPF cosmetics and sunscreens

Nowadays, manufacturers of cosmetics and dermatological products offer a wide range of sun-blocking lotions, creams, and sprays. Sunscreens are offered in two main types: physical and chemical. Physical sunscreens usually use zinc oxide and
titanium dioxide as the main active component, as both provide protection from ultraviolet light. Chemical sunblocks are composed of several types of chemicals, as individual chemicals block only a narrow region of UV light. Most effective sunblocks are a combination of both physical and chemical active ingredients. Anthocyanidins are commonly used in herbal- and fruit- based sunscreens. Many plants, especially those that originate from areas exposed to large amounts of direct sunlight, contain natural substances that block potentially damaging ultraviolet light (Korac and Khabhola 2011).

Another substance that can potentially be used in sunscreens and SPF cosmetics is apigenin, a common flavonoid found in fruits and vegetables. When added to cosmetics and dermatological products under correct conditions, apigenin demonstrates anti-inflammatory activity. It is also effective in preventing carcinogenesis due to ultraviolet light (Korac and Khabhola 2011).

Fruits and vegetables with a high level of antioxidants are often considered for the production of SPF cosmetics. Li and others (2006) analysed the methods of extraction of antioxidants from pomegranate peel. A mixture of ethanol, methanol and acetone was used to effectively obtain antioxidants from dry pomegranate peel.

1.2.7 Pesticide control in fruit and vegetable waste

Pesticide residues have been studied since the beginning of 1990s, because high consumption of pesticides is harmful to human health. For example, organophosphorus and carbamate exert anticholinesterase activity in the nervous system.
system (Caldas and others 2006). The main route of human’s exposure to pesticides is through their digestive system; therefore, regulatory organizations have clearly made public the limitations of pesticide residues in food products, especially fruits and vegetables (Rawn and others 2008). For example, maximum residue limits (MRLs) of pesticides in different agricultural products and food have been established by the above-mentioned organizations and agencies to ensure the safety (Chen and others 2013; Zhu and others 2014). The example is imidacloprid, of which the MRL in mango in the European Union is 0.2 mg/kg (Bhattacherjee and Bhattacherjee 2013). However, the limit of the same pesticide might be different in other countries. For example, MRLs of trifloxystrobin in citrus are 0.02–2 mg/kg in the European Union (EU), while 0.05–10 mg/kg in Japan; the MRLs of kresoxim-methyl is 0.05 mg kg⁻¹ in the EU but 0.03 mg/kg in Japan (Zhu and others 2014). Another example is clothianidin: the MRLs are 3, 1, and 0.05 mg/kg, respectively, in Japan, Korea, and the United States (Li and others 2012a). An exception is forchlorfenuron in citrus fruit, for which no MRLs are established in some countries (Chen and others 2013), although in the EU it is limited in citrus to 0.01 mg/kg.

Three classes of insecticides used in agriculture are organophosphorus, organochlorine, and pyrethroid insecticides (Zhou and others 2011). Pesticides are sprayed during the agricultural product growth and they may remain in food for quite a long period of time. For instance, the concentrations of imidacloprid in mango peel and pulp, when sampled 1 month after utilization, were 1.21 and 0.565 mg kg⁻¹, respectively. However, the residues were not detected either in pulp or in peel after
nearly 3 months after utilization (Bhattacherjee and Bhattacherjee 2013). Therefore, the choice of detection method for pesticides in food and the sampling order are very important. Collection of samples starts at fruit development stage and continues up to harvest (Bhattacherjee and Bhattacherjee 2013). At least 6 replicate samples are taken from minimum 8–12 lots in order to be able to analyze a relative 95% range of sampling uncertainty within 50% (Farkas and others 2014).

1.2.8 Methods for pesticide testing in fruit and vegetable waste

The rapid direct analysis in Real Time Mass Spectrometry (DART-MS) method is used for efficient and sensitive testing of pesticide levels in apple, oranges, lemon, grapefruit, tomato, and potato peel (Farré and others 2010). Pesticides are measured by the following methods: anilinopyrimidine fungicides in tomato and courgette by gas chromatography (GC) (Amvrazi and others 2009); clothianidin in tomato by gas chromatography-mass spectrometry (GC-MS) (Li and others 2012a); diethofencarb and pyrimethanil in apple peel by HPLC (Liang and others 2014; Zhou and others 2011); fungicides in grapes by high performance liquid chromatography with diode array detection (HPLC-DAD) (de and others 2006), in apples, grape and orange by the gas chromatographic method with micro-electron capture detection (GC-µECD); famoxadone trifloxystrobin and fenhexamid residues in tomato and grape by GC (Likas and others 2007; Zhu and others 2014); pesticides in apples and peaches, cabbages and tomatoes by ultra-performance liquid chromatography coupled with tandem mass spectroscopy (UPLC-MS) (Liang and others 2014).
Recently, the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method with simple procedures has been accepted by pesticide-testing laboratories all over the world (Liang and others 2014; Zhu and others 2014). QuEChERS can be used for testing a wide range of pesticides, including highly polar, highly acidic and basic ones. The method is based on extraction with acetonitrile and partitioning after the addition of a salt mixture. The final extract in acetonitrile is then directly amenable to an LC-based analysis (Zhu and others 2014). The QuEChERS method with Chromatography-UV detection (LCUV) can be used to determine residues of kresoxim-methyl and trifloxystrobin in citrus (Zhu and others 2014); the QuEChERS method combined with UPLC-MS/MS can be used to test anilinopyrimidine fungicides, including mepanipyrim, in fresh vegetables or fruits (Liang and others 2014).

In one study, 36 pesticide residue levels have been tested in large amounts of fruits and vegetables, and the average levels of various pesticides in the samples were in the range 0.002 - 2 mg/kg. It was reported that only a small proportion of all fruits and vegetables had exceeded the limitation while the average levels of pesticide residues were under the MRLs limit (Hill and others 2002). In one of the papers it was published that such procedures as washing, peeling, boiling, and juicing are able to significantly decrease the total residue of pesticides in food, especially peeling (Han and others 2013; Li and others 2012b). Peeling reduced the level of chlorpropham in tubers by 91-98% and washing removed residues by 33-47%; washing and peeling combination can decrease the level of chlorpyrifos by 93.4% (Han and others 2013; Lentza-Rizos, Lentza-Rizos, Balokas 2001). This means that
most pesticides are retained in fruit and vegetables peel. Hence, it is important to pay attention to the commercial by-product safety, i.e., tomato skin removal should be considered in decreasing pesticides residue when it is applied for lycopene production (Han and others 2013), as well as citrus fruits are always eaten after removing the peel where pesticides are mostly detected (Chen and others 2013).

Agricultural waste, such as leaves, stems, and roots of fruits and vegetables, also contain certain amount of pesticides. Different parts of plant are contaminated in the following sequence: leaves - stalks - roots (Barriada-Pereira and others 2004). This may be the result of the cell structure of leaves, made of long-chain polyesters, which are able to accumulate lipophilic compounds; contamination from the soil environment can be transferred from the root system to leaves (Marco and others 2007). Pesticide composition in mango leaves depends on such factors as the development of the leaf waxy cuticle, tree condition, physicochemical properties of soil, and environmental conditions (Marco and others 2007).

The effective removal method of pesticide residues from food products before foods are sold has been researched considerably (Ikeura and others 2012). Ozone is known as a strong oxidizing agent, which is used for such purposes as deodorization, bleaching and decomposition of organic compounds, sterilization and virus inactivation. Organic acids, soluble sugars, lycopene and some other micro compounds of food have been shown not to be affected by ozone treatment in low concentrations (Ikeura and others 2012; Kusvuran and others 2012; Tzortzakis and others 2007). Many researchers have reported that ozone treatment can decrease
levels of mancozeb, azinophos-mythyl, captan, and formetanate hydrochloric acid residues on apple (Hwang and others 2001; Ong and others 1996); methyl-parathion, parathion, diazinon and cypermethrin on vegetable surface (*Brassica rapa*) (Wu and others 2007); fenitrothion on vegetables and fruits (lettuce, tomatoes, and strawberries) (Ikeura and others 2012); fenhexamid, cyprodinil, and pyrimethanil on grape (Karaca and others 2012; Kusvuran and others 2012).

### 1.2.9 Waste from organically grown foods and vegetables

Organic food products are grown without using any synthetic pesticides, herbicides, fungicides, readily soluble mineral fertilizers, and also sewage and waste compost, which makes consumers believe that organic food contains lower levels of pesticides (Gennaro and Quaglia 2003; Hoefkens and others 2009). Recently, pyrethrins, rotenone, copper salts and sulfur came to be used in organic farming for the control of pests and plant diseases (Lairon and Lairon 2010; Moore and others 2000), although the use of some of them is controversial (Pottorff 2010). About 94% of raw organic food samples were lower in pesticides than the detection level, including above 97.4% of organic farming products in Italy (Tasiopoulou and others 2007) and about 97.2% of organic samples in the Danish market (Poulsen and others 2003). At the same time, pesticide residues were found in 17–50% of conventional foods in Sweden (Bourn and others 2002; Lairon and Lairon 2010). However, the residues of pesticides in conventional food products are mostly below MRLs (Gennaro and Quaglia 2003).
Besides pesticide residues, there are several other chemical hazards associated with not only conventional foods, but also organic foods, i.e., chemical environmental contaminants detected both in organic and conventional foods. Also, productive animal manure and other animal wastes are often used as a fertilizer or soil nutrient, which causes concerns related to the risk of contamination of food by pathogens (especially *E. coli*), when manure is not treated properly (Gennaro and Quaglia 2003). Even worse, the use of less pesticides and fungicides might make organic food easier to be contaminated by microorganisms than conventional foods (Hoefkens and others 2009).

### 1.2.10 Conclusion

Recently, consumers have developed a strong interest in purchasing healthier foods; therefore, the food industry is placing higher importance on developing products with a focus on natural ingredients. Plant waste products have the ability to be optimized in this pursuit. Peel, seeds and leaves from plant waste can be added to improve flavor, odor and color. The use of these compounds will attract consumers as they adapt natural pigments, oils and materials from plants that are not synthetic and carcinogenic. Natural remedies and cosmetics are also becoming more attractive for the consumers. Pesticide residues have been regulated efficiently by the organizations in the past few years, and test methods have significantly improved. Although pesticides are quite widely used in growing of conventional food, their residues usually remain below the MRL before sale, or organic food wastes can be
used, so consumers may become more interested in purchasing products containing extracts obtained from pesticide-free green label fruits and vegetables.
Chapter 2: QSC Fortified SPI Film

Abstract

Quinoa stalk is rich in cellulose. Cellulose derivatives were found to enhance the tensile strength of SPI films. Quinoa stalk cellulose was added to SPI film to enhance the mechanical properties. Based on tensile strength (TS), elongation at break (EAB), and Fourier Transform Infrared Spectroscopy (FTIR) analysis of QSC fortified SPI films, the strong interactions between QSC and SPI were confirmed and hydrogen bonding was the major bond forming. The 5% and 10% QSC fortified SPI films had relatively high TS, which indicated that QSC can enhance the mechanical properties of SPI films. However, the color of high concentrations (5% and 10%) QSC fortified SPI films were different from the pure SPI films, and consumer acceptability tests should be applied before its utilization.
2.1 Introduction

Quinoa (Chenopodium Quinoa Wild) has been planted in South America for centuries, in areas such as the Andean region, and recently more studies were conducted about growing quinoa in Europe, Asia, North America and Africa (Bhargava, Shukla, Ohri 2006; Gómez-caravaca and others 2012). Quinoa belongs to the family Amaranthaceae, and its preparation/cooking method is similar to wheat and rice as a pseudocereal (Gómez-caravaca and others 2012). Quinoa can grow in a wide range of climates, altitude, and soil, and possesses desirable functional features and high nutrition values (contains all amino acids human need) (Bhargava, Shukla, Ohri 2006; Gómez-caravaca and others 2012; Miranda and others 2013; Vega-Galvez and others 2010). Quinoa seeds are rich in protein (up to 16.7%). The two essential amino acids, lysine (5–8%) and methionine (2.4–5.1%), are normally limiting factors in other grains (Fischer and others 2013; Gonzales, Gonzales-Castaneda, Gasco 2013). Quinoa seeds also contain various other compounds, including dietary fiber, vitamins (riboflavin, thiamine, α- and γ-tocopherol), minerals, folic acid, and various antioxidant compounds, such as carotenoids, flavonoids (Bhargava, Shukla, Ohri 2006; Fischer and others 2013). In addition, quinoa seeds are also gluten-free grain, which can be used as an alternative to gluten-containing grains, such as wheat, rice, or corn (Alvarez-Jubete, Arendt, Gallagher 2009; Miranda and others 2013). The essential fatty acids in quinoa seeds include oleic, linoleic, and α-linolenic (Palombini and others 2013). Another functional compound of quinoa seeds is saponin (represents the unpleasant bitter flavor), which
has antimicrobial activity and cholesterol-reducing function (Gómez-caravaca and others 2012).

Cellulose is the main compound and structural material of cell wall of plant stalk, husk, leaves, and usually the agriculture underutilized part of various plants. The utilization of cellulose has already been extended to various fields, such as agriculture, construction, food packaging, energy resources, animal feed, drug industry, and biomaterials (Luo and others 2010). Quinoa stalk cellulose (QSC) is edible, nontoxic, renewable, abundant, biocompatible, biodegradable, hydrophilicitive, and good film forming. It has non-water soluble and can even be used as dietary fiber (Wang and others 2012). QSC macromolecules consist of linear glucopyranose linked by β-(1,4)-linkages. They have a relatively large molecular weight because of the abundant hydrogen bonding inside, and have a high aspect ratio and large interface area (Su and others 2010b; Tavera Quiroz and others 2013; Wang, Cao, Zhang 2006). Cellulose and their derivatives have already been researched and used to improve mechanical properties of soy or whey protein films, and increase permeability characteristics in corn flour or gelatin blended film (Luo and others 2010; Wang, Cao, Zhang 2006).

There has been an increasing interest in studying the utilization of biodegradable and edible food packaging material to reduce the usage of petroleum-based non-degradable plastic packaging materials that caused the so-called white pollution (Wang and others 2012; Wang, Cao, Zhang 2006). Biopolymers were studied mostly due to their renewable, biocompatible, non-toxic, low cost, and biodegradable
properties (Luo and others 2008; Luo and others 2010; Wang and others 2012; Zhou and others 2008). Besides, biopolymers were always used as the base material of biodegradable or edible film, such as cellulose, starch, polysaccharides, lipids, and especially proteins (Luo and others 2010; Wang, Cao, Zhang 2006; Zhou and others 2008).

SPI, the abundant underutilized part of soybean oil industry, has been already studied as a food additive, environmentally friendly adhesives, biodegradable materials, biomedical plastics, and edible or non-edible packaging (Luo and others 2008; Luo and others 2010; Su and others 2012; Wang and others 2012; Wang, Cao, Zhang 2006). SPI has a relatively higher content of protein than other soy protein products, which make it good for film forming (Su and others 2012). Besides, when it is dry, SPI film has better water, gas, and oil barrier than some other synthetic films (Luo and others 2010; Wang and others 2012). Mechanical properties of food packaging materials, including films and coatings, are very important factors, so that food quality is maintained by using good integrity and barrier packaging during handling, shipping and storage (Chana-Thaworn, Chanthachum, Wittaya 2011; Wang and others 2012; Wang and others 2013). However, the low mechanical properties of pure SPI films limit their utilization (Luo and others 2010; Su and others 2012). In addition, sensory characteristics are also very important in consumer acceptance of various fortified SPI film (Su and others 2012). Various fillers, used to modify SPI films, must interact with SPI and have the ability to enhance properties of films (Su and others 2010b). The tensile strength (TS) of SPI films was found to be enhanced
by polysaccharides (Galus and others 2012), transglutaminase (Tang and others 2005), sodium dodecyl sulfate (Rhim and others 2002), or montmorillonite (Lee and Lee 2010), type-B-bovine-bone (Cao, Fu, He 2007; Galus and others 2012), long chain PVA molecule (Su and others 2008), cellulose derivatives (Zhou and others 2008). Cellulose, the lowest cost and abundant material, added to SPI film was shown to increase the mechanical properties (Jensen 2012; Lodha and Netravali 2002; Su and others 2007; Su and others 2010b; Wang and others 2012; Wang, Cao, Zhang 2006; Wu and others 2009).

Some other additives and process conditions also influence the mechanical properties of SPI films. The mechanical properties, including tensile strength (TS), elongation at break (EAB), and Young’s modulus, of SPI films were very different under various humidity conditions (Chen and Zhang 2005). This is because the water was absorbed and also acted as plasticizer in SPI films which resulted in the changing of microstructure and mechanical properties of (Chen and Zhang 2005). In addition, the processing methods also influenced the mechanical properties of the films (Galus and others 2012).

The addition of plasticizers (small molecules) was also found to reduce the intermolecular forces and improve the macromolecular mobility of the SPI polymers, which resulted in better physical properties, such as the extension and flexibility characteristics (Tavera Quiroz and others 2013). In that research, glycerol was used as a plasticizer for SPI films. Glycerol unit has three hydroxyl groups and they can also interact with SPI by hydrogen bonding (Guerrero and others 2010). This
hydrogen bonding can decrease the inter- and intra- molecular interactions inside protein macromolecules and improve their motion ability, which improve the flexibility and extension of SPI films (Guerrero and others 2010). The higher glycerol content, the lower the mechanical properties, less stable, small crystal structure, and less crystallinity of the cellulose blended SPI films (Su and others 2010b). Films were too brittle to test the TS with less than 30% glycerol (w/w, based on SPI), and too sticky with higher than 50% glycerol (w/w, based on SPI) (Guerrero and others 2010).

The objective of this study was to test the mechanical properties of SPI films fortified by QSC.

2.2 Materials

SPI Supro 760 was bought from Solae (St. Louis, MO, USA). Glycerol, 95% ethanol, sodium hydroxide (pellets), hydrochloride acid, and vacuum grease were bought from Fisher Scientific Canada (Ottawa, ON, CA).

2.3 Methods

2.3.1 QSC Extraction

The QSC extraction method was similar to a previously published method with some modifications (Jensen 2012). Quinoa stalk were ground using a coffee grinder and filtered with a 1 mm sieve (Model 3, Thomas Wiley, Swedesboro, NJ, USA). The quinoa stalk powder was weighed and soaked in distilled water (1:10, w/w) and stirred for 3 hrs at room temperature. Excess water was removed by using a filter bag before the quinoa stalk material was added to 17.5 wt% of sodium hydroxide solution.
kept at 90 °C for 2 h (3:10, w/w). Then the quinoa stalk powder was washed through filtering against running water overnight, followed by checking the pH of the slurry to be 7.0 ± 0.5 using a pH meter (Accumet XL60, Fisher Scientific, Ottawa, ON, CA). The quinoa stalk was added into 1M HCl at 90 °C for 2 h (3:10, w/w) followed by washing overnight and the pH checking process as described in the step. Quinoa stalk were then placed back into an alkaline treatment using NaOH at 2 wt% and kept at 90 °C for 2 hours along with washing overnight and the pH checking process. Then quinoa stalk solutions were homogenized for 5 min using a rotary homogenizer (PowerGen 1000, Fisher Scientific, Ottawa, ON, CA) to liberate and partially suspend the QSC in water. The suspended mixture was then fed through a high-pressure homogenizer for 20 passes at 4000 to 6000 PSI to obtain microfibrillated fibers (QSC solutions) (Model 15MR Laboratory Homogenizer, APV Gaulin, Concord, ON, CA). QSC concentration (2%) was determined by drying the solutions at 50 °C overnight.

2.3.2 Preparation of QSC Fortified SPI Film Forming Solutions

4 g (10% w/w based on film forming solutions) SPI powder was mixed with 5 g ethanol (95%) to create a homogeneous protein slurry prior to adding 2 g glycerol. Then QSC solutions and distilled water were added as outlined in Table 2-1. The solutions were homogenized at medium speed for 10 min (Agitator, Arrow Engineering Co. Inc., Hillside, NJ, USA). The pH of the solutions were adjusted to pH 12 using 1M NaOH. The film forming solutions were warmed in a water bath at 80 °C for 30 min while using minimum speed to cotinuosly stir continuously, at the end the solutions changed from cloudy to transparent. The solutions were filtered by going
through two layers of cheesecloth before the next procedure. This was done to remove the bubbles in solutions, which may cause a weak point during TS test.

Table 2-1. Quinoa stalk cellulose (QSC) and water content of different quinoa stalk cellulose concentrations used in modification of soy protein isolate film.

<table>
<thead>
<tr>
<th>QSC concentration (%)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>QSC Solution (mL)</td>
<td>N/A</td>
<td>1.8</td>
<td>3.6</td>
<td>18.2</td>
<td>36.4</td>
</tr>
<tr>
<td>Distilled Water (mL)</td>
<td>40</td>
<td>38.2</td>
<td>36.4</td>
<td>21.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>
2.3.3 Film Casting

The surface of the horizontal, glass-topped hot plate was wiped with a thin layer of vacuum grease. Excess grease was then removed using 95% ethanol and Kimwipes (Kimberly-Clark, Irving, TX, USA). This was done to help removal of the actual film later on. Film forming solutions were poured onto the plate at 82 °C, which was controlled by a water bath. The solutions were dried for around 30 min. Then the film was peeled off from the plate and stored in an environmental chamber at 25 °C and 57% relative humidity (using saturated NaBr solution).

2.3.4 Film Thickness

Film thickness was tested using a digital micrometer (Testing Machines Inc., New Castle, DE). Three representative rectangular strips were cut (2.5 x 9 cm) from each film using a sample cutter (JDC Precision Sample Cutter, Thwing-Albert, West Berlin, NJ, USA), and each film was replicated for 3 times. Five representative spots for one strip of each film were tested and the average was used as thickness in calculating TS of films.

2.3.5 Mechanical Properties

TS is the force required when the film reach the break point. Percentage elongation at break (EAB) is the ratio of maximum changed length to initial length at the breakage of the film. Three representative rectangular strips were cut (2.5 x 9 cm) from each film using a sample cutter (JDC Precision Sample Cutter, Thwing-Albert, West Berlin, NJ, USA), and three films were replicate for each film type. All film strips
were hung over in sealed glass jars at 57% relative humidity (NaBr saturated solution) at 25 °C for at least 24 hrs before the tensile test. The test was preceded using the Instron Universal Testing Machine (Model 1122, Instron, Norwood, MA, USA) according to the ASTM standard method D 882-90 (ASTM 1990). The initial grip separation was set at 50 mm and mechanical crosshead speed was 100 mm/min. Film samples were suspended vertically between two pneumatic grippers and stretched until failure while the displacement and force data were automatically recorded by the software.

2.3.6 FTIR Analysis

Different QSC fortified SPI films were conditioned at 57% humidity levels at least 24 h before analyzing using a Fourier transform infrared spectrometer (IR Prestige-21, Shimazu Corporation, Kyoto, JP), equipped with an attenuated total reflection (ATR) sampling cell (MIRacle Single Reflection, Pike Technologies, Madison, WI, USA) with a wavenumber range from 4000 to 400 cm⁻¹.

2.3.7 Color Values

$L^*$, $a^*$, and $b^*$ values were determined by using the portable Minolta CR-300 (Minolta, Osaka, JP). Films were cut into small pieces (1 cm × 1 cm) and put on a Minolta calibration plate (CR-300) until the values were recorded.

2.3.8 Statistical analysis
All data are presented as means ± one standard deviation. One-way analysis of variance (ANOVA) was used to determine differences between treatment groups. A value of $P < 0.05$ was considered to be statistically significant.
2.4 Results and Discussion

2.4.1 Mechanical Properties of QSC Fortified SPI Films

TS and EAB are both very important factors of mechanical properties used to determining the utilization of films. TS is the maximum tensile stress sustained by the sample at the breaking point during the tensile test (Chana-Thaworn, Chanthachum, Wittaya 2011). As shown in Figure 2-1, the TS was decreasing when the QSC concentration was below 1% \((P < 0.05)\), and then increasing when the QSC concentration was above 5% \((P < 0.05)\). The highest TS were seen at the 5% and 10% QSC fortified SPI film (no significant difference between these two concentrations of QSC fortified SPI films). The TS of 1% and 10% QSC fortified SPI films, which were stirred overnight was lower than the ones stirred for 1 hour \((P < 0.05)\).

The mechanical properties of QSC fortified SPI films are depended on the microstructure and interactions between all compounds in the network, including inter- and intra- molecular interactions and distribution of compounds (Bos and van Vliet 2001; Chambi and Grosso 2006; Sinz 2006; Wang and others 2013). The SPI macromolecule has abundant amine, amide, carboxyl, and hydroxyl groups and has hydrogen bonds, disulfide bonds, dipole actions, charge charge, hydrophobic bonds, and dipole-dipole bonds (Lodha and Netravali 2002; Wang and others 2012). When the SPI molecules are denatured during pH changing and heating process, some disulfide bonds are disrupted and sulfhydryl and hydrophobic bond are formed between QSC and SPI molecule (Su and others 2010b). Cellulose chains consist of
anhydroglucose units that have three hydroxyl groups (Lodha and Netravali 2002; Su and others 2007; Su and others 2010b; Wang and others 2012). New hydrogen bonding between QSC and SPI macromolecule (–NH2 (in arginine and lysine), –NH– (in proline and histidine), –OH (in tyrosine, threonine, and serine), –COOH (in glutamic acid), and peptide bonds) are also formed during the drying process (Su and others 2010b). The hydrogen bonding interaction was formed between the hydroxyl groups of QSC and the amidogen and carboxyl groups of protein polymer at the interfaces in cellulose fortified SPI film (Lodha and Netravali 2002; Su and others 2007; Su and others 2010b; Wang and others 2012; Wang, Cao, Zhang 2006; Wu and others 2009), or gelatin film (Bai and others 2013; Nishiumi and others 2012). This strong interfacial interaction between QSC and SPI could enhance the TS. The TS of QSC fortified SPI film was improved also because the molecular entanglements between QSC and SPI molecules (Su and others 2010b). The new bond formed in Maillard reactions between reducing sugars and amino acids also contribute to the enhancement of TS of blended films (Ajandouz and others 2008; Su and others 2010b; Yasir and others 2007). The 0.5% and 1% QSC fortified SPI films showed slightly lower TS probably be caused by the quite low concentration of cellulose which might disrupt the cross link of SPI matrix by the hydrogen bonding which resulted in decreased TS.

In films fortified with different QSC contents the TS increased with the increasing of QSC content above 5%, which was consistent with cellulose nanofibrous mats reinforced SPI films (Jensen 2012). This meant more cellulose fiber interacts with SPI
per unit cross-section area of the composite will results stronger mechanical properties of films (Jensen 2012). Based on the theory, the force was transferred to the cellulose fiber so that the film will resist more strength when stretching the film (Jensen 2012). In various contents of QSC fortified SPI films, the TS was highest when the QSC was 10%, which was because of the abundant hydrogen bond between QSC and SPI. This was consistent with other research (Hoque, Benjakul, Prodpran 2011; Luo and others 2008; Mu and others 2012; Su and others 2007; Zhou and others 2008).

However, the decreased TS of cellulose fortified protein based films was found when further increase the concentration of cellulose, because the gradually increased aggregates of cellulose on structure of crystalline domains caused stiffness of cellulose, and the fillers disperse irregularly in the films which may led to the broken of hydrogen bonds and micro-phase separation between the cellulose and protein (Luo and others 2008; Wang and others 2012; Wang, Cao, Zhang 2006; Zhou and others 2008).

As shown in Figure 2-2, the EABs of all QSC fortified SPI films were lower than the control SPI film ($P < 0.05$). Only 1% QSC fortified SPI film stirred 1 h showed higher EAB than the one stirred overnight ($P < 0.05$). This might be because of the disturbance of the original ordered structure of SPI by QSC macromolecules. Hydrolysis of proteins were also happens due to the very high pH. Therefore, the resulting films became stiffer with the addition of cellulose based on the other research (Wang and others 2012; Zhou and others 2008). The glycerol decreased the
interaction between the protein macromolecule and improved the SPI chain mobility, flexibility, extensibility, and process ability, and results in different crystalline structure, glass transition behavior, and microstructure (Chen and Zhang 2005; Guerrero and others 2010; Su and others 2007). In QSC fortified SPI films, the glycerol molecule penetrates in the SPI and cellulose macromolecules and weak the hydrogen bond between them (Su and others 2010b).
Figure 2-1. Tensile strength (MPa) of different concentration (0.5 – 10 wt%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films

* means significantly different from control SPI film at $P < 0.05$.

** means significantly different from 1% QSC fortified SPI film solutions stir overnight at $P < 0.05$.

*** means significantly different from 10% QSC fortified SPI film solutions stir overnight at $P < 0.05$. 
Figure 2-2. Elongation at break (%) of different concentration (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films

* means significantly different from control SPI film at $P < 0.05$.

** means significantly different from 1% QSC fortified SPI film solutions stir overnight at $P < 0.05$. 
2.4.2 FTIR spectrum of QSC fortified SPI films, pure QSC and SPI powder

The FTIR spectrum absorbance peak wavenumber was affected by the different compounds, because each compound had its typical peak, and the interactions between each other, in this case, water, glycerol, SPI, and QSC have affected the peak wavenumber. The interactions between QSC polymer and SPI macromolecules and different concentration of QSC had resulted the different peak wavenumbers and intensity of each peak in FTIR spectrum compared to the pure cellulose and pure SPI.

As shown in Figure 2-3(a), the broader peak at around 3273 cm\(^{-1}\) wavenumber vibration found in pure SPI and all blended films, was attributed to hydrogen bonding the free \(-\text{OH} \text{ and } \text{-NH}\) bond formed with carbonyl group of the peptide linkage inside SPI macromolecules, and the hydrogen bonding between \text{-OH}, \text{-NH}, and \text{-CO} of amino acids (peptide and carboxyl groups) in SPI protein and water content (\text{-OH}), this wavenumber is also found in other protein based films (Guerrero and others 2010; Karnnet, Potiyaraj, Pimpan 2005; Luo and others 2010; Schmidt, Giacomelli, Soldi 2005; Su and others 2008; Su and others 2010b; Su and others 2012).

The peaks at 846 cm\(^{-1}\), 923 cm\(^{-1}\), 991 cm\(^{-1}\) wavenumber vibration, found in pure SPI and all films, were attributed to the three C–C skeletons of glycerol. The 1039 cm\(^{-1}\), found in all QSC fortified SPI films and control SPI film except pure SPI and QSC, was attributed to C–O linkage in C\(_1\) and C\(_3\) of glycerol (Guerrero and others 2010). The 1117 cm\(^{-1}\) was attributed to C-O in C2 in glycerol found in spectrums of any QSC fortified SPI films and control SPI films (Guerrero and others 2010). No
shifts of above characteristic peaks were observed after combining the glycerol and SPI in all fortified films compared to the pure SPI. This means no covalent interactions between glycerol and SPI, which was consistent with findings found by other sources (Guerrero and others 2010).

As shown in Figure 2-3(b), the hydroxyl, amide, and characteristics group-absorbance of pure SPI powder and cellulose powder are correspondingly found in the blended films, similar to the study by Luo and others (2008). The band at around 1631 cm⁻¹ in the control SPI film was related to the amide I (–C=O stretching), and this wavenumber is similar to the other studies which were in the range 1623 – 1655 cm⁻¹ (Chen and Zhang 2005; Guerrero and others 2010; Luo and others 2008; Luo and others 2010; Schmidt, Giacomelli, Soldi 2005; Su and others 2007; Su and others 2012; Tian 2012; Wang and others 2012; Wu and Zhang 2001a; Wu and others 2009). In the QSC fortified SPI films, the amide I peak shifted to 1645 – 1649 cm⁻¹, this means the interaction as hydrogen bonding between hydroxyl group of cellulose and amide, carbonyl groups of SPI (Luo and others 2008; Luo and others 2010). This hydrogen bonding interrupt the original hydorgen bond inside SPI so the typical peak of SPI protein shifted to a higher wavenumber. The 1241 – 1472 cm⁻¹ was C-N stretching and N-H bending (amide I) in soy protein spectrum (Su 2009). The band at around 1525 cm⁻¹ was related to the amide II (–NH stretching of protein), and this wavenumber is similar to the other studies which is in the range 1530 –1551 cm⁻¹ (Guerrero and others 2010; Luo and others 2010; Su and others 2010b; Tian 2012; Wang and others 2012; Wu and others 2009). In the QSC fortified SPI films,
the amide II peak shifted to 1541 cm\(^{-1}\), which indicates the interaction as hydrogen bonding between hydroxyl group of QSC and amide II. This hydrogen bonding interrupt the original hydorgen bond inside SPI so the typical peak of SPI protein shifted to a higher wavenumber. In addition, the intensity of peak around 1541 cm\(^{-1}\) (from 0.20117 to 0.199353) was decreasing slightly with the increase concentration of QSC, which agrees with the other sources (Luo and others 2010). The band of control SPI film at 1234 cm\(^{-1}\) was related to the amide III (–NH, –CN banding vibration stretching in protein), and this wavenumber is similar to the other studies in the range 1230 – 1278 cm\(^{-1}\) (Guerrero and others 2010; Schmidt, Giacomelli, Soldi 2005; Su and others 2007; Wu and Zhang 2001a; Wu and others 2009). In the QSC fortified SPI films, the amide III peak shifted to 1238 cm\(^{-1}\), this also verify the interaction as hydrogen bonding between hydroxyl group of QSC and amide III (–NH).

As shown in Figure 2-3(c), the peak only shows in pure QSC spectrum, at 898 cm\(^{-1}\), is characteristic absorbance of glucose units of cellulose (Luo and others 2008; Luo and others 2010; Subirade and others 1998). The peak wavenumber at around 1020 cm\(^{-1}\), only showed in pure QSC spectrum, was assigned to –CH vibrations bending attached to the aromatic ring (Huang and others 1995; Wu and Zhang 2001b). These peaks were not found in the QSC fortified SPI films which might because they were covered by other peaks or –CH in QSC interact with SPI and formed other peaks in blended films spectrum.

As shown in Figure 2-3 (a, b, c), the intensity are of bands 3273, 1645-1649, and 846 cm\(^{-1}\) increased and then decreased after adding 5% QSC with the increasing
cellulose in the blended films. This observation agrees with the other study, and it might be caused by the different concentrations QSC used in films (Su and others 2010a).

As shown in Figure 2-3(b), the intensity of peak around $1541 \text{ cm}^{-1}$ is increasing with the increase of QSC content in blended films, and this means there is an interaction between QSC and SPI (Luo and others 2008). The $1241 - 1472 \text{ cm}^{-1}$ found in QSC fortified SPI films was $\text{–CN}$ vibration because the $\text{–OH}$ groups in QSC and amino groups in SPI were consumed and combined during the heating procedure when making the film forming solutions (Su and others 2010b).

In addition, the Maillard reaction was one of the major interactions between QSC and SPI. The peaks around $1278 - 1296 \text{ cm}^{-1}$ and around $1645 - 1647 \text{ cm}^{-1}$ were $\text{–CN}$ stretching vibration due to the Maillard reaction (Aaslyng, Larsen, Nielsen 1999; Ajandouz and others 2008; Alaiz, Hidalgo, Zamora 1999; Su and others 2008; Su and others 2010b). These two bands did not show in this case, might due to the covering by other big peaks, but Maillard reaction did occur in QSC reinforced SPI films because of the color change of films.

In conclusion, the shift of bands, new band peak wavenumbers, and change of intensity of peaks all showed the hydrogen bonding interactions between QSC and SPI in the blended films (Wu and others 2009).
Figure 2-3. Fourier transform infrared (FTIR) spectroscopy spectra of different concentrations (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films, pure quinoa stalk cellulose, and soy protein isolate powder.
Figure 2-3(a). Fourier transform infrared (FTIR) spectroscopy spectra (from 2400 – 4000 cm$^{-1}$) of different concentration (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films, pure quinoa stalk cellulose, and soy protein isolate powder.
Figure 2-3(b). Fourier transform infrared (FTIR) spectroscopy spectra (from 1200 – 2400 cm\(^{-1}\)) of different concentration (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films, pure quinoa stalk cellulose, and soy protein isolate powder.
Figure 2-3(c). Fourier transform infrared (FTIR) spectroscopy spectra (from 700 – 1200 cm⁻¹) of different concentration (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films, pure quinoa stalk cellulose, and soy protein isolate powder.
2.4.3 Color Test Results

Color, influencing consumer acceptability, is one of the important factors of food biopolymer packaging films (Sivarooban, Hettiarachchy, Johnson 2008; Su and others 2012). Su and others (2012) reported that color was affected by pH, cross-linkage degree of interaction between QSC and SPI, plasticizer (glycerol) addition, thermal treatment, and the drying process. They also mentioned that the color of protein-based films was affected mostly by protein concentration and other fillers, instead of treatments and reactions. The protein content was consistent in all films. As shown in Figure 2-4, 0.5% and 1% QSC did not change the color of SPI films, while more cellulose (5% and 10% QSC) decreased the $L^*$ color value (lightness), which was consistent with the other research (Su and others 2012). 10% QSC fortified SPI films had higher $a^*$ value (greenness).

From the appearance of films, films conditioned at 57 % relatively humidity did not changed basically, only a little more sticky were found in all films. This is due to the movement of glycerol (small molecule) from internal to the surface. The colors of all QSC/SPI films were getting brown after several days which indicated the Maillard reaction between sugars and amino acids in the blended films, this observation agrees with Su and others (2010b). The Maillard reaction, was also a factor significantly influencing the color of protein-based films and products. It can be very complex (Su and others 2012). The color of SPI films changed with high cellulose concentrations, and this could influence consumer acceptability (Su and others 2012).
Figure 2-4. Color determination ($L^*$, $a^*$, $b^*$) of different concentrations (0.5 – 10%) of quinoa stalk cellulose (QSC) fortified soy protein isolate (SPI) films

* means significantly different from control SPI film at $P < 0.05$. 
2.5 Conclusion

Based on TS and FTIR of all QSC fortified SPI films, the interactions between QSC and SPI were confirmed and hydrogen bonding was the major bond forming. Based on the above tests, the 10% QSC fortified SPI films had the highest TS but lowest EAB, which may limit its utilization in food packaging. While 0.5% QSC fortified SPI films had the lowest TS and relatively high EAB. However, the color of high content of QSC fortified SPI films was different from the pure SPI films, and consumer acceptability tests should be assessed before its utilization by the food industry.
Chapter 3 Quinoa Leaf Extract Fortified SPI Films

Abstract

Quinoa leaves were reported to be rich in phenolic compounds. Quinoa leaf extract (QLE) was added to SPI films to enhance the properties. The major interaction between the QLE and SPI macromolecules was hydrogen bonding. This bond contributed to the fortified mechanical properties. Besides that, QLE fortified SPI films showed significant ($P < 0.05$) higher antioxidant, and antimicrobial properties compared to control SPI films. However, in the microbiology test as an example of real application test, QLE reinforced SPI films did not show decreased population of *E.coli T36* in packed ground beef samples, compared to the control SPI films and blank groups (without any package). In conclusion, QLE were found to fortify mechanical and antioxidant properties of SPI films with further research on the antimicrobial properties and test on real food.
3.1 Introduction

Quinoa (*Chenopodium Quinoa Wild*) has been planted in South America for centuries (e.g., Andean region) and more recently trials were conducted in Europe, Asia, North America and Africa (Bhargava, Shukla, Ohri 2006; Gómez-caravaca and others 2012). Quinoa belongs to the Amaranthaceae family. Its cooking method is similar to wheat and rice as a pseudocereal (Gómez-caravaca and others 2012). Quinoa can grow in a wide range of climates, altitudes, and soils, and it has high nutrition values (contains all amino acids human need) and desirable functional properties (Bhargava, Shukla, Ohri 2006; Gómez-caravaca and others 2012; Miranda and others 2013; Vega-Galvez and others 2010). Quinoa is rich in protein. The two essential amino acids, lysine (5 – 8%) and methionine (2.4 – 5.1%), are normally limited in other grains (Fischer and others 2013; Gonzales, Gonzales-Castaneda, Gasco 2013). Quinoa also contains compounds, such as dietary fiber, vitamins (riboflavin, thiamine, α- and γ-tocopherol), minerals, folic acid, and antioxidants, such as carotenoids, flavonoids (Bhargava, Shukla, Ohri 2006; Fischer and others 2013).

In another important selling point is the fact that quinoa is a gluten-free grain, which can be used as alternatives to grain, such as wheat, rice, or corn (Alvarez-Jubete, Arendt, Gallagher 2009; Miranda and others 2013). Its fatty acid composition includes oleic, linoleic, and α-linolenic is also important for human nutrition (Palombini and others 2013). Another functional compound of quinoa is saponin, which has an unpleasant bitter flavor but provides antimicrobial activity and cholesterol-reducing functions (Gómez-caravaca and others 2012). Fortunately, the bitter problem with
saponins can be removed by genetic methods, which also makes the quinoa plant easy to adapt to different climates and environments (Palombini and others 2013). Quinoa seeds were found to have excellent antioxidants, antiallergic, anti-inflammatory, antiviral, and anticarcinogenic activities, and cardiovascular protective properties (Gómez-caravaca and others 2012). Phenolic compounds, including phenolics, flavonoids, isoflavone, flavones, anthocyanin, catechin, isocatechin, carotenoids, phytates, isothiocyanates, phytosterols, phytoesterogens, and organosulphur etc, were major phytochemical components responsible for the above properties, especially antioxidant and free radical scavenging properties (Arunachalam and Parimelazhagan 2014; Chen and others 2012; Kunyanga and others 2012; Oboh, Raddatz, Henle 2008; Sroka and Cisowski 2003).

Free radicals were produced in aerobic organisms respiration system during energy production process (Arunachalam and Parimelazhagan 2014; Packer and Packer 1999). Free radicals were reacted with biomolecules, including protein, lipid, and result in protein and lipid peroxidation, which will lead to structural damage, tissue injury, and gene mutation (Arunachalam and Parimelazhagan 2014; Mantle, Eddeb, Pickering 2000). In addition, phenolic compounds were found to protect food products (Arunachalam and Parimelazhagan 2014; Zin and others 2007). Antioxidants were found to delay or inhibit the oxidation of lipids and proteins and other biomolecules (Arunachalam and Parimelazhagan 2014; Shahidi 2004; Suganya Tachakittirungrod, Siriporn Okonogi, Sombat Chowmentpohn 2007). They can prevent the formation of new free radicals, convert existing free radicals to less
harmful molecules, and prevent radical chain reactions (Arunachalam and Parimelazhagan 2014; Rodriguez and others 2007).

Phenolic compounds extracted from plants leaves, barks, roots, fruits, and seeds, such as phenol, flavonoids, and tannins were demonstrated to exhibit potential antioxidant, antimicrobial, anticancer, anti-obesity, anti-diabetic and anti-hypertensive, anti-mutagenic, anti-inflammatory, anti-allergic, antithrombotic, antiviral, and anti-carcinogenic activities (Arunachalam and Parimelazhagan 2014; Kunyang and others 2012; Sowndhararajan and Kang 2013). Natural antioxidants were used in the food industry as nutraceuticals, bio-pharmaceuticals, as well as food additive due to consumer preference, because adverse antioxidants may contain toxicity (Sowndhararajan and Kang 2013).

However, few researchers have focused on the agriculture under-utilized part of quinoa plant, such as leaves and stalks. Quinoa leaves were treated as food underutilized part since though they are edible and contain large amounts of functional ingredients (Gawlik-Dziki and others 2013). QLE was identified as being rich in phenolic compounds, such as ferulic acid (762 µg/g, dry weight (DW)), sinapinic acid (193 µg/g, DW), and gallic acid (GA) (163 µg/g, DW) (Pasko and others 2009), and these compounds were relatively high compared to well-characterized sources (Gupta and Prakash 2009; Kraujalis and others 2013). The quinoa leaves were found to contain high antioxidant activity compared to some other green leafy vegetables which can be attributed to the high phenolic compounds (Gupta and Prakash 2009; Kraujalis and others 2013). These phenolic compounds extracted from
plants might be suitable to replace synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which might be carcinogenic and toxic (Lagha-Benamrouche and Madani 2013).

Plant leaves extract was found to contain antimicrobial properties indicating its protection to food product (Garcia-Ruiz and others 2012; Gonzalez-Rompinelli and others 2013). Pure phenolic compounds were found to inhibit the growth of enological lactic acid bacteria in wine production (Campos, Couto, Hogg 2003; Garcia-Ruiz and others 2009; Garcia-Ruiz and others 2011; Garcia-Ruiz and others 2012; Gonzalez-Rompinelli and others 2013). Aqueous extracts of leaves from various plants, such as *Callistemon vininalis, Garcinia mangostana* tissues, had stronger antimicrobial activity against gram-positive bacteria (i.e. *Listeria monocytogenes, Staphylococcus aureus*), than against gram-negative bacteria (i.e. *E. coli* O157:H7, *Salmonella typhimurium*) (Palakawong and others 2013; Salem and others 2013; Srivastava and others 2003).

The condition of phenolic compounds extraction process, such as the type of solvent used, should also be determined before the application of the underutilized part extract. Water and ethanol were the safest, cheapest, traditionally used, and most environmentally friendly and accessible solvents than organic solvents traditionally used to extract plant bioactive, such as methanol and acetone (Vuong and others 2013). There were some studies researching the optimized extraction condition: 1g plant underutilized part extracted using 25 mL ethanol solution (50%, v/v) repeated twice (Gawlik-Dziki and others 2013). Similar procedures were used in this
research. Polyphenols are sensitive to heat, light, and oxygen, so the extraction condition and storage conditions are very strict to get the maximum yield (Vuong and others 2013). Regulatory requirements may be less stringent for potential food applications as processing aids or ingredients in sanitizers, but high costs in comparison with conventional products remain due to the value of raw materials, the complexity of extraction technologies, or the need to remove solvent residues (Palakawong and others 2013).

3.2 Materials

Quinoa (Chenopodium Quinoa Wild.) leaf samples were harvested in the area of Guelph, Ontatio, Canada: sample A (grown Sep.2011-Oct.2012); sample B (grown Sep.2011-Oct.2012); sample C (grown Sep.2012-Oct.2013); sample D (grown Sep.2012-Oct.2013). Each grown at a different location. Soy protein isolate (SPI) was purchased from Solae (Supro 760 St. Louis, MO, USA). Glycerol, ethanol, sodium hydroxide (pellets), gallic acid, Folin-Ciocalteu reagent, NaCO₃, methanol, trolox solution, and vacuum grease were bought from Fisher Scientific (Ottawa, ON, CA). A fortified growing medium for microbial testing (Modified Oxford Medium, GA46-H14122 Hardy Dx, Lansing, MI, USA), Tryptone soya agar with kanamycin monosulfate (TSA-K) agar (Tryptone Soya Agar, Oxoid CM0131) and monosulfate (Kanamycin, 194531) were from MP Biomedicals (Solon, OH, USA).

The objective of this study is to test the mechanical, antioxidant, and antimicrobial properties of SPI films fortified by QLE.
3.3 Methods

3.3.1 Quinoa Leaf Extraction

The QLE was produced following the procedure of Chen and others (2013) and Gawlik-Dziki and others (2013) with some modifications. Quinoa leaves were dried at 50 °C oven overnight and then ground with coffee grinder for 1 min prior to mixing with a 50% ethanol/water solution. A ratio of 1:25 quinoa leaf powder to ethanol solution was used. The whole slurry was stirred for 5 hrs and then centrifuged at 2000 xG for 15 min at room temperature. This was repeated three times and the supernatant was collected and combined with other 2 supernatants before filtering through a filter paper (Whatman #1). Then, the ethanol and most of water were removed using a reduce pressure evaporator (Buchi Rotary Evaporators & Neslab RTE-100 bath, St. Louis, MO, USA) at 50 °C followed by freeze-drying (Virtis Genesis Freeze Dryer 25 ES, Gardiner, NY, USA) for approximately 2 days. The extract yields were 34.0% (QLE A), 26.0% (QLE B), 12.8% (QLE D), 20.1% (QLE C); i.e., weight of extract divided by weight of quinoa leaf powder. The dried QLE was collected and kept at 4 °C before using. Reducing sugar content (tested using the phenol-sulphuric acid assay) showed 31.00% QLE A (w/w), 12.97% QLE B (w/w), 10.85% QLE C (w/w), and 13.86% QLE D (w/w).

3.3.2 TPC Determination of QLE

Total soluble phenolic contents of QLE were tested using Folin-Ciocalteu regent (GA as a standard phenolic compound) based on Shukla’s research with some
modifications (Shukla and others 2012). Series concentration of GA solutions was prepared. Using a 96 cells plate, folin-Ciocalteu reagent was added to all GA solutions and QLE cells. The microplate was swirled and rested for 10 min. NaCO₃ was added and microplate was rested again for at least 30 min. Then the absorption at 765 nm was tested using the microplate reader. The total phenolic content (TPC) of QLE was evaluated by comparing absorption of QLE with the GA standard curve.

3.3.3 Preparation of QLE Fortified SPI Film Forming Solutions

4 g (10% w/w based on film forming solutions) soy protein isolate (SPI) powder was mixed with 5 g ethanol (95%) to create a homogeneous protein slurry prior to adding 2 g glycerol and followed by mixing with 40 mL distilled water to facilitate dispersion. The solutions were homogenized at medium speed for 10 min (Agitator, Arrow Engineering Co. Inc., Hillside, NJ, USA). The pH of the solutions were adjusted to pH 12 using 1M NaOH. The film forming solutions were warmed in a water bath at 80 ºC for 30 min and stirred at low speed continuously, as the solutions changed from opaque to more transparent. The solutions were cooled to room temperature by stirring in a cool water bath for 10 min and then a certain amount of GA solutions (0.5%, w/w) (except for the pH 7 film) or QLE solutions were added according to Table 3-1. The solutions were filtered through two layers of cheesecloth. This was done to remove bubbles in the solutions, which may cause weak points during tensile test. For the pH 7 films, pH of the protein solutions were adjusted from pH 12 to approximately pH 7 by adding 0.325 M HCl under constant stirring after heating. GA or QLE solutions were added after pH adjustment followed by casting procedures.
<table>
<thead>
<tr>
<th>Name of Films</th>
<th>GA solution(^a) (mL)</th>
<th>QLE solution(^b) (mL)</th>
<th>pH</th>
<th>Stir time (min)</th>
<th>Drying time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPI control film pH12</td>
<td>N/A</td>
<td>N/A</td>
<td>12</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>5GA SPI film pH7</td>
<td>1</td>
<td>N/A</td>
<td>7</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>10GA SPI film pH7</td>
<td>2</td>
<td>N/A</td>
<td>7</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>5GA SPI film pH12</td>
<td>1</td>
<td>N/A</td>
<td>12</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>10GA SPI film pH12</td>
<td>2</td>
<td>N/A</td>
<td>12</td>
<td>30</td>
<td>80</td>
</tr>
<tr>
<td>5GA SPI film pH12</td>
<td>1</td>
<td>N/A</td>
<td>12</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>5GA SPI film pH12 dry over night</td>
<td>1</td>
<td>N/A</td>
<td>12</td>
<td>30</td>
<td>Overnight</td>
</tr>
<tr>
<td>SPI control film pH7</td>
<td>N/A</td>
<td>N/A</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE A(^c) SPI film</td>
<td>N/A</td>
<td>4.665</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE B(^c) SPI film</td>
<td>N/A</td>
<td>5.950</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE D(^c) SPI film</td>
<td>N/A</td>
<td>7.225</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE C(^c) SPI film</td>
<td>N/A</td>
<td>7.765</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE A(^c)&amp;GA SPI film</td>
<td>1</td>
<td>4.665</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE B(^c)&amp;GA SPI film</td>
<td>1</td>
<td>5.950</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE C(^c)&amp;GA SPI film</td>
<td>1</td>
<td>7.225</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>QLE D(^c)&amp;GA SPI film</td>
<td>1</td>
<td>7.765</td>
<td>7</td>
<td>120</td>
<td>80</td>
</tr>
</tbody>
</table>

Table 3-1. Film ingredients table of optimization of phenolic-rich quinoa leaf extract (QLE) fortified soy protein isolate (SPI) films

\(^a\)Gallic acid (GA) (0.5% w/w) was also dissolved into distilled water first then added to film forming solutions.

\(^b\)QLE solutions concentrations were 40 mg/mL obtained by dissolving freeze dried QLE into distilled water. Total phenolic content (TPC) of QLE equaled to TPC of 5 mg GA were added to the film forming solutions.

\(^c\)Each QLE extract was added using total phenolic content equals to 5 mg gallic acid.
3.3.4 Film Casting

The surface of the horizontal, glass-topped hot plate was wiped with a thin layer of vacuum grease. Excess grease was then removed using 95% ethanol and Kimwipes (Kimberly-Clark, Irving, TX, USA). This was done to help removal of the actual film later on. Film forming solutions were poured onto the plate at 82 °C, which was controlled by a water bath. The solutions were dried for around 30 min. Then the film was peeled off from the plate and stored in an environmental chamber at 25 °C and 57% relative humidity (using saturated NaBr solution).

3.3.5 Film Thickness

Film thickness was tested using a digital micrometer (Testing Machines Inc., New Castle, DE, USA). Three representative rectangular strips were cut (2.5 x 9 cm) from each film using a sample cutter (JDC Precision Sample Cutter, Thwing-Albert, West Berlin, NJ, USA), and each film was replicated for 3 times. Five representative spots for one strip of each film were tested and the average was used as thickness in calculating tensile strength (TS) of films.

3.3.6 Mechanical Properties

TS is the force required when the film reach the break point. Percentage EAB is the ratio of maximum changed length to initial length at the breakage of the film. Three representative rectangular strips were cut (2.5 x 9 cm) from each film using a sample cutter (JDC Precision Sample Cutter, Thwing-Albert, West Berlin, NJ, USA), and three films were replicate for each film type. All filmstrips were hung over in
sealed glass jars at 57% relative humidity (NaBr saturated solution) at 25 °C for at least 24 hrs before tensile test. The test was preceded using the Instron Universal Testing Machine (Model 1122, Instron, Norwood, MA, USA) according to the ASTM standard method D 882-90 (ASTM 1990). The initial grip separation was set at 50 mm and mechanical crosshead speed was 100 mm/min. Film samples were suspended vertically between two pneumatic gripers and stretched until failure while the displacement and force data were automatically recorded by the software.

3.3.7 Color Values

L*, a*, and b* values were determined by using the chroma meter (Minolta CR-300, Minolta, Osaka, JP). All resulting films were cut into small piece (1 cm × 1 cm) and put on the calibration plate (Minolta, CR-300) and then values were recorded.

3.3.8 FTIR Analysis

Different QLE B and/or GA fortified SPI films were conditioned at 57% relative humidity levels before analyzing using a Fourier transform infrared spectrometer (IR Prestige-21, Shimazdu Corporation, Kyoto, JP), equipped with an attenuated total reflection (ATR) sampling cell (MIReacle Single Reflection, Pike Technologies, Madison, WI, USA) using a wavenumber range from 4000 to 700 cm⁻¹.

3.3.9 Antioxidant Properties

DPPH assay
The QLE and GA fortified SPI films were dissolved in water at 50 °C for 4 hrs before antioxidant capacity determination. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay used was modified from the paper (Herald, Gadgil, Tilley 2012). A series of trolox concentrations was made and tested to draw a standard curve. DPPH working solution was made by dissolving DPPH in methanol. Only 225 µL pure methanol was added to the blank wells in the 96-well plate. 25 µL methanol and 200 µL DPPH working solution was added to the control wells. 25 µL QLE and/or GA fortified SPI films dissolving solutions and 200 µL DPPH working solutions were added to the wells. The plate was then sealed and incubated at room temperature for 6 hrs in a dark environment before reading the absorbance at 517 nm, using a plate reader. The results are expressed as trolox equivalents (TE) µmol/g film.

FRAP assay

The resulting SPI films were dissolved in water at 50 °C for 4 hrs before antioxidant capacity determination using FRAP assay (Benzie and Strain 1996). The working solutions included 300 mM pH 3.6 acetate buffer (3.1 g sodium acetate and 16 mL glacial acetic acid), 10mM 2,4,6-tripyridyl-s-striazine (TPTZ) solution (0.03123 g in 10 mL HCl), and 20 mM FeCl₃-6H₂O solution (0.05406 g FeCl₃-6H₂O in 10 mL H₂O). The fresh working solutions were prepared by mixing acetate buffer, TPTZ solution, and FeCl₃-6H₂O solution as ratio of 10:1:1 and then warmed at 37 °C before using. A series of ascorbic acid standard solution concentrations was made and tested to draw the standard curve. 10 µL QLE fortified SPI films dissolving solutions or standard ascorbic acid solution were added to wells followed by adding
300 µL FRAP solution. Plates were incubated at room temperature for 6 hrs in a dark environment before reading the absorbance at 593 nm. The result was expressed as ascorbic acid equivalent (AAE) µmol/g film.

3.3.10 Antimicrobial Properties

Test_1

The method used is based on the research of Song, Shin, Song (2012) on Listeria inoculated. This is one of the most virulent food-borne pathogens, which was cultured at 30 °C for 24 hrs in a 50 mL conical tube using Listeria enrichment broth. Then 0.1 mL Listeria inoculated solution was plated onto a bacterial growth medium (Fortified Oxford Medium, GA46-H14122 Hardy Dx, Lansing, MI, USA). Ten mm round piece of films were cut and placed onto the inoculated plates with tightly attached. Each film was tested in 3 replicates. All plates were stored at 4 °C for 3 hrs in order to allow the diffusion of phenolic compounds from the films to the medium. Inhibited zone, measured in millimeter by a caliper; accurated to 0.05 mm, was determined after incubated all plates at 37 °C incubator (THELCO Laboratory Incubator, Hanover, MA, USA) for 48 hrs.

Test_2

Extra lean ground beef fresh was purchased from a local grocery store. Samples were inoculated with a level of 6.0 log CFU/g of E.coli T36 on the surface of 10 g ground beef samples, which was then placed into 47 mm diameter petri dish. Different concentrations of QLE fortified SPI films were prepared and placed on the
surface of the beef, which was already compressed to fit in the petri dish. The beef samples were stored at 4 °C for a certain time (2 hrs, 3 days, 5 days, 7 days, and 10 days) before being counted. The beef samples were stomached using a stomacher (STOMACHER Lab-Blender 400) for 30 sec then inoculated to the TSA-K agar (Tryptone Soya Agar, Oxoid CM0131, Solon, OH, USA) with monosulfate (Kanamycin, 194531, MP Biomedicals, Solon, OH, USA) and incubated at 37 °C incubator (THELCO Laboratory Incubator, Hanover, MA, USA) then counted after 24 hrs.

3.3.11 Statistical analysis

All data are presented as means ± standard deviation. One-way analysis of variance (ANOVA) was used to determine differences between treatment groups. A value of P<0.05 was considered to be statistically significant.
3.4 Results and Discussion

3.4.1 Yield and Total Phenolic Content of QLE

The antioxidant activity showed correlation with total phenolic content (Alhakmani, Kumar, Khan 2013). Plant phenolics were reported to be the major group of compounds acting as primary antioxidants or free radical scavengers. Therefore, it is common to determine the total phenolic content in plant extracts (Alhakmani, Kumar, Khan 2013).

As shown in the Table 3-2, QLE A was found to have the highest TPC (i.e. 26.8 mg GAE), followed by QLE B (i.e. 21.0 mg GAE), QLE D (i.e. 17.3 mg GAE), and QLE C (i.e. 16.1 mg GAE). The results suggest that the TPC varied significantly ($P < 0.05$) from one plant to another even though they were the same basic grain. QLE C and D had lower extract yields and this reduced TPC might have been because the measured QLE C and QLE D leave had some husk of quinoa while QLE B and QLE A did not. TPC of *Moringa oleifera* flowers extracted by an ethanol solution (70%, v/v) was 44.35 mg tannic acid equivalents (TAE) (Alhakmani, Kumar, Khan 2013), and knotweed leaf was 24.62 mg TAE (Azlim Almey and others 2010). Our results were different from them could be that the different levels of TPC may be attributed to the plants category, extraction procedure, solvent category, and solvent concentration. Extracting solvent category significantly affected the yield of phenolic content of several extracts (Azlim Almey and others 2010; Sun and Ho 2005). Although methanol was found to be more efficiency and is easier to evaporate, compared to
water and ethanol, in extracting phenolic compounds in food and plant underutilized part (Azlim Almey and others 2010; Perez, Calderon, Croci 2007; Yang and others 2007), the cost of methanol should be considered before its commercial application. Besides, color measurement of Folin-Ciocalteu reagent (i.e., reaction was non-specific with phenol), there was a chance that other components, such as ascorbic acid could react with Folin-Ciocalteu reagent (Azlim Almey and others 2010).
Table 3-2. Yield of Extract (%, DW of raw leaf) and total phenolic content (gallic acid equivalent (GAE) mg/g, DW of extract) of quinoa leaf extract (QLE) A, B, C, and D.

<table>
<thead>
<tr>
<th>QLE Categories</th>
<th>Yield of Extract (%&lt;sub&gt;DW of raw leaf&lt;/sub&gt;)</th>
<th>Total phenolic content (GAE mg/g, DW of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QLE A</td>
<td>34.0±0.4&lt;sup&gt;*&lt;/sup&gt;</td>
<td>26.8±0.4&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>QLE B</td>
<td>26.0±0.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>21.0±0.6&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>QLE C</td>
<td>20.1±0.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>16.1±0.1&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>QLE D</td>
<td>12.8±0.3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>17.3±0.3&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* means all QLE type fortified SPI film had different yield of extract value from the other at ($P < 0.05$).

** means all QLE type fortified SPI film had different total phenolic content value from the other QLE type group ($P < 0.05$).
3.4.2 Mechanical Properties of GA, QLE Fortified SPI Films

As discussed in the Chapter 2, tensile strength (TS) is an important mechanical property of food packaging films. The interaction of the basic materials like protein, plasticizers (e.g., glycerol), water, fillers (antioxidant and antimicrobial), determine the TS of the films (Sivaroooban, Hettriachchy, Johnson 2008).

Figures 3-1 and 3-2 show that the TS and EAB of all pH 7 GA fortified SPI films (both 5 mg GA and 10 mg GA concentration) are significant lower than for the pH 12 control groups ($P < 0.05$). The 5 mg GA fortified SPI film forming solutions stirred for either 2 hrs, or stirred overnight have similar EAB (Figure 3-2). While the GA fortified SPI film stirred overnight have significant lower TS than the ones stirred for 2 hrs ($P < 0.05$) (Figure 3-1). This phenomenon could have occurred because the 2 hrs stirring time allowed for interactions between SPI and GA, but further stirring inversely interrupted the interactions inside the SPI matrix and between GA and SPI, and at the same time protein was hydrolysed due to the long time exposure to very high pH. For the 10 mg GA fortified SPI films and those with added 5 mg GA had similar TS and EAB under pH 7 and pH 12. This might be due to the hydrogen bonding between SPI and GA was saturated. These results are consistent with Nuthong, Benjakul, Prodpran (2009).

As shown in Figures 3-1 and 3-2, TS of GA fortified SPI films (pH 7) are not different from that of control pH 7 SPI films ($P > 0.05$). This is not consistent with the other pure phenolic acids, such as ferulic acid, tannic acid, caffeic acid, which
increased the TS of protein based films even when used at different concentrations (Cao, Fu, He 2007; Nuthong, Benjakul, Prodpran 2009; Orliac and others 2002; Ou and others 2005; Prodpran, Benjakul, Phatcharat 2012). The increased TS values of GA fortified SPI films were attributed to the hydrogen bonding between GA and SPI macromolecules. These interactions were dependent on the type and structure of phenolics, the concentrations of each phenolic compounds, and protein structure, temperature, etc (Sivarooban, Hettiarachchy, Johnson 2008; Zhang and others 2010). The similar TS and EAB (P > 0.05) between 5 mg GA fortified SPI films (pH12 ones) and control films are not consistent with Nuthong, Benjakul, Prodpran (2009), such as 1-5% caffeic acid and tannic acid decreased the EAB of films. Because phenolic acids, such as caffeic acid and tannic acid molecules, contain several functional groups and can increase the chain length by polymerizing which result in decreased EAB (Nuthong, Benjakul, Prodpran 2009).

In conclusion, the optimized concentration and forming condition of GA fortified SPI films are 5 mg/4g SPI and stir time 2 hrs. The pH 12 blended films can be used for environmentally friendly food packaging with significant stronger mechanical properties than pure SPI films but the films are not edible due to the high pH. The TS were still lower than of the QSC fortified SPI films and this limits the application of phenolic compounds fortified SPI films (pH12). The pH 7 GA fortified SPI films can be used as edible food packaging.
Figure 3-1. Tensile strength (MPa) at different pH, gallic acid (GA) concentrations, stir time, and drying time of gallic acid fortified soy protein isolate (SPI) films.

5 mg GA: concentration of GA used was 0.125% (w/w SPI).

10 mg GA: concentration of GA used was 0.25% (w/w SPI).

* means significantly different from control SPI film (unfortified with GA, pH 12, no stir time) at $P < 0.05$.

** means significantly different from 5 mg GA, pH12, stir 2 hrs GA fortified SPI film at $P < 0.05$. 
Figure 3-2. Elongation at break (%) at different pH, gallic acid (GA) concentrations, stir time of gallic acid fortified soy protein isolate (SPI) films.

5 mg GA: concentration of GA used was 0.125% (w/w SPI).

10 mg GA: concentration of GA used was 0.25% (w/w SPI).

* means significantly different from control SPI film (unfortified with GA, pH 12, no stir time) at $P < 0.05$. 
Figures 3-3 and 3-4 show that TS of most QLE fortified SPI films are increased due to the strong interactions between QLE and SPI molecules. This is consistent with the addition of some other food plants’ underutilized part extracts, such as grape seed extract, cinnamon extract, clove extract, star anise extract, and vegetable tannins, which were found to increase the TS of protein based protein-based films (Gomez-Guillen and others 2007; Hoque, Benjakul, Prodpran 2011; Sivarooban, Hettiarachchy, Johnson 2008; Song, Shin, Song 2012). Phenolic compounds reacted with protein and led to stiffer and stronger intermolecular cross-links and enhanced the interaction between protein chains within SPI films (Nuthong, Benjakul, Prodpran 2009; Sivarooban, Hettiarachchy, Johnson 2008). Not all plant and food extracts can reinforce the mechanical properties of packaging films. Some plant extracts, such as from blueberry, sunflower seed, ferulic acid, caffeic acid, and catechin, didn’t influence the TS of protein (such as, porcine plasma, fish myofibrillar protein, sunflower protein, or SPI) based films (Nuthong, Benjakul, Prodpran 2009; Prodpran, Benjakul, Phatcharat 2012; Salgado and others 2012; Zhang and others 2010). Furthermore, in some other research, phenolic-rich plant extracts or pure phenolics, such as from grape seed, murta leaf, tannin, vitamin E, and ferulic acid were found to decrease the TS of starch or protein based films due to the much higher concentrations used (Corrales, Han, Tauscher 2009; Gomez-Guillen and others 2007; Mathew and Abraham 2008; Ou and others 2005; Prodpran, Benjakul, Phatcharat 2012; Song, Shin, Song 2012; Zhang and others 2010). This was because of their effects on weakening or reduction of the intermolecular interactions between the
protein molecules (Corrales, Han, Tauscher 2009; Jang, Shin, Song 2011a; Song, Shin, Song 2012).

It has been reported that hydroxyl groups of phenolic compounds react with polar groups of hydrogen acceptor, in proteins, by hydrogen bonds (Hoque, Benjakul, Prodpran 2011; Prodpran, Benjakul, Phatcharat 2012; Rattaya, Benjakul, Prodpran 2009; Sivarooban, Hettiarachchy, Johnson 2008). Phenolic compounds had hydrophobic groups, which interacted with hydrophobic district protein region by hydrophobic bonding (Arcan and Yemenicioglu 2011; Hoque, Benjakul, Prodpran 2011; Kanatt and others 2012). Phenolic compounds in seaweed extracts were incorporated with gelatin protein through protein polyphenol hydrophobic interactions and hydrogen bonds (Rattaya, Benjakul, Prodpran 2009). Different extracts (e.g., oregano, rosemary) also showed the modification of gelatin films (Gomez-Estaca and others 2009). Borage extract showed the same (Gomez-Estaca and others 2009; Hoque, Benjakul, Prodpran 2011). Catechin and GA molecules contain large amount of –OH groups, and this bond can creat a stronger network within proteins macromolecules (Arcan and Yemenicioglu 2011).

Phenolic compounds influencing the EAB of SPI films were believed to be the major group of compounds in extracts, such as cyanidin, petunidin, malvidin, and delphinidin derivatives obtained from blueberry extract and picatechin and catechin in grape seed extract (Sivarooban, Hettiarachchy, Johnson 2008; Wang and others 2012; Zhang and others 2010). Grape seed extract, anthocyanin-rich red raspberry extract increased the EAB of protein or starch films (Corrales, Han, Tauscher 2009;
Song, Shin, Song 2012; Wang and others 2012); and unlike from some other extracts, such as grape seed extract, blueberry extract, rosemary extract (Corrales, Han, Tauscher 2009; Gomez-Estaca and others 2009; Sivarooban, Hettiarachchy, Johnson 2008; Song, Shin, Song 2012; Wang and others 2012; Zhang, Li, Wang 2010). This may be caused by the formation of cross-links with high molecular weights phenolic components in grape seed extract (Sivarooban, Hettiarachchy, Johnson 2008; Song, Shin, Song 2012). The grape seed extract, including predominantly epicatechin, catechin, gentistic acid, and syringic acid polyphenols, can facilitate protein inter-chain interactions and stabilize the film structure (Sivarooban, Hettiarachchy, Johnson 2008). QLE is better than some other pure phenolic compounds such as the GA (used in this research), ferulic acid, tannic acid, caffeic acid, and catechin. Because the EAB of protein films were not changed by adding the above phenolics (Gomez-Estaca and others 2009; Prodpran, Benjakul, Phatcharat 2012; Wang and others 2012). In conclusion, the influence of antioxidant compounds on the EAB of protein-rich films was very different.
Figure 3-3. Tensile strength (MPa) of quinoa leaf extract (QLE) A, B, C, and D and/or gallic acid (GA) fortified soy protein isolate (SPI) films.

* means significantly different from control SPI film at $P < 0.05$. 
Figure 3-4. Elongation at break (%) of quinoa leaf extract (QLE) A, B, C, and D and/or gallic acid (GA) fortified soy protein isolate (SPI) films.

* means significantly different from control SPI film at $P < 0.05$. 
3.4.3 FTIR Spectrum of QLE Fortified SPI Films

FTIR spectroscopy at 4000 – 700 cm\(^{-1}\) is used to examine the differences between the secondary structure of GA and QLE B fortified SPI films as shown in Figure 3-5. The wavenumber of each typical band is changed after adding phenolic compounds in to the protein film, and this indicates the interactions between phenolic compounds and protein macromolecules, as shown in Figure 3-5.

Figure 3-5(c) shows that the similar bands caused by glycerol were also found in the range 846 – 850 cm\(^{-1}\) (C-C skeleton of glycerol), 922 – 923 cm\(^{-1}\) (C-C skeleton of glycerol), 993 cm\(^{-1}\) (C-C skeleton of glycerol), 1039 – 1041 cm\(^{-1}\) (C-O in C1 and C3), and 1107 – 1109 cm\(^{-1}\) (C-O in C2, and this peak is not found in QSC fortified SPI films). As shown in Figure 3-5(b), the typical bending vibrations of SPI protein were in the range 1633 – 1635 cm\(^{-1}\) (represent amide I, -C=O stretching, hydrogen bonding coupled with –COO), 1539 –1541 cm\(^{-1}\) (represent amide II, bending vibration of N-H groups and stretching vibrations of C-N groups), and 1234 – 1238 cm\(^{-1}\) (represent amide III, vibrations in plane of C-N and N-H groups of bond amide or vibrations of CH2 groups of glycine). As shown in Figure 3-5(b), the shift to a lower wavenumber of amine I, II and III peaks is observed from 1635, 1541 and 1238 cm\(^{-1}\) in the control SPI film to 1633, 1539 and 1236 cm\(^{-1}\) in the SPI films fortified by quinoa GA and/or QLE, respectively. These are small shifts which was consistent to Aewsiri and others (2009). As shown in Figure 3-5(a), the peak in the range 3273 – 3275 cm\(^{-1}\) is related to the hydrogen bonding inside SPI, and between water and SPI macromolecules. This agrees with the material presented in Chapter 2 and other research SPI films.
(Aewsiri and others 2009; Guerrero and others 2010; Hoque, Benjakul, Prodpran 2011; Karnnet, Potiyaraj, Pimpan 2005; Schmidt, Giacomelli, Soldi 2005; Su and others 2008; Su and others 2010b).

As shown in Figure 3-5(a), the amide A peak shifts from 3273 cm\(^{-1}\) in control SPI films to a higher wavenumber at 3275 cm\(^{-1}\) (amide A, representative of NH-stretching, coupled with hydrogen bonding) in the SPI films fortified by QLE, and this is consistent with Hoque, Benjakul, Prodpran (2011). Besides, the amide A peak amplitude increased by adding GA and QLE in the SPI films (Abs. 0.201 – 0.206) compared to control films (Abs. 0.196). 2931 cm\(^{-1}\) was amide B (–CH stretching and NH\(_3\)) and amplitude was increased in the phenolic-rich SPI films (Abs. 0.087 – 0.090) compared to control groups (Abs. 0.086), which disagreed with Hoque, Benjakul, Prodpran (2011).

In other research, the wavenumbers at 690 and 655 cm\(^{-1}\) were related to the C–C bonds in the aromatic and –OH group in phenol, respectively (Aewsiri and others 2009). A shift of peaks to lower wavenumbers is associated with a lower molecular order. These are not shown in our case since the wavenumber below 700 cm\(^{-1}\) was not detectable. Results showed that star anise extract or caffeic acid induced interaction between phenolic compounds and NH\(_2\) of gelatin or porcine-plasma protein molecules and this led to crosslinking of gelatin (Hoque, Benjakul, Prodpran 2011; Nuthong, Benjakul, Prodpran 2009).
In conclusion, the result indicated the incorporation of phenolic compounds with a -OH group into SPI macromolecule.
Figure 3-5. Fourier transform infrared (FTIR) spectroscopy spectra of quinoa leaf extract (QLE B) and gallic acid (GA) fortified soy protein isolate films.

5GA: concentration of GA used was at 0.125% (dry weight g/g SPI).

10GA: concentration of GA used was at 0.25% (dry weight g/g SPI).
Figure 3-5(a). Fourier transform infrared (FTIR) spectroscopy spectra (from 2200 – 4000 cm\(^{-1}\)) quinoa leaf extract (QLE B) and gallic acid (GA) fortified soy protein isolate films.

5GA: concentration of GA used was at 0.125% (dry weight g/g SPI).

10GA: concentration of GA used was at 0.25% (dry weight g/g SPI).
Figure 3-5(b). Fourier transform infrared (FTIR) spectroscopy spectra (from 1200 – 2200 cm$^{-1}$) of phenolic-rich quinoa leaf extract (QLE B) and gallic acid (GA) fortified soy protein isolate films.

5GA: concentration of GA used was at 0.125% (dry weight g/g SPI).

10GA: concentration of GA used was at 0.25% (dry weight g/g SPI).
Figure 3-5(c). Fourier transform infrared (FTIR) spectroscopy spectra (from 700 – 1200 cm$^{-1}$) of phenolic-rich quinoa leaf extract (QLE B) and gallic acid (GA) fortified soy protein isolate films.

5GA: concentration of GA used was at 0.125% (dry weight g/g SPI).

10GA: concentration of GA used was at 0.25% (dry weight g/g SPI).
3.4.4 Color Test of QLE Fortified SPI Films

Color is an important factor to a film because film appearance influences consumer acceptability (Kunte and others 1997; Sivarooban, Hettiharachchy, Johnson 2008). From the visual appearance, the control SPI film was clear and transparent, while the phenolic compounds and QLE fortified films had more yellow-green color. This is consistent with report by Prodpran, Benjakul, Phatcharat (2012).

In color tests, the parameters used to describe the films are \( L^* \) (lightness), \( a^* \) (greenness), and \( b^* \) (yellowness). Previous reports showed that the addition of plant extracts or pure phenolic compounds changed the color of films. The reportes described that grape seed extract, herb extract, clove extract, star anise extract, anthocyanin-rich red raspberry, tannic acid, caffeic acid, and ferulic acid changed the color because of the phenolic acid and flavonoids compounds in them (Corrales, Han, Tauscher 2009; Hoque, Benjakul, Prodpran 2011; Nuthong, Benjakul, Prodpran 2009; Sivarooban, Hettiharachchy, Johnson 2008; Wang and others 2012). Different extracts and different concentrations fortified SPI films had different levels effect on colors. Figure 3-6 shows that the GA, and/or QLE, or the combination fortified SPI films had lower \( L^* \) value, lower \( a^* \) and \( b^* \) value than control SPI films, which is consistent to Nuthong, Benjakul, Prodpran (2009). Changes in lightness, redness and yellowness may because of the color components, such as chlorophylls, remaining in the extracts, and later responsible to the color changed films (Hoque, Benjakul, Prodpran 2011; Koca, Karadeniz, Burdurlu 2007; Nuthong, Benjakul, Prodpran 2009; Rattaya, Benjakul, Prodpran 2009). However, some previous study reported that \( L^* \) value
increased, $a^*$ value and $b^*$ value decreased in the protein based films fortified by various plant extract and pure phenolic compounds (Prodpran, Benjakul, Phatcharat 2012; Rattaya, Benjakul, Prodpran 2009). This means the different plant extracts had different effect on $L^*$, $a^*$, $b^*$ color values of fortified films.

In conclusion, the color of SPI films was influenced by the concentrations and type of individual phenolic compounds. This agrees with previous work (Prodpran, Benjakul, Phatcharat 2012; Sivarooban, Hettiarachchy, Johnson 2008; Wang and others 2012). The color acceptability to customers of plant extract and phenolic compounds fortified SPI films should be tested before real applications (Sivarooban, Hettiarachchy, Johnson 2008).
Figure 3-6. Color determination ($L^*, a^*, b^*$) of quinoa leaf extract (QLE) A and B and/or gallic acid (GA) fortified soy protein isolate (SPI) films

5GA: concentration of GA used was at 0.125% (w/w SPI).

10GA: concentration of GA used was at 0.25% (w/w SPI).

* means significantly different from control SPI film at $P < 0.05$. 
3.4.5 Antioxidant Properties of QLE Fortified SPI Films

DPPH assay

The antioxidant activity of edible film used as a primary food packaging is because the film contact the food products and antioxidants can migrate into the food to enhance its quality without influencing the integrity of food products (Kanatt and others 2012). Since the antioxidant capacity of food is determined by a different antioxidants present (i.e., each with different action mechanisms). Due to the synergistic interactions; it is necessary to combine more than one method in order to determine the overall antioxidant capacity (Ruiz-Navajas 2013). To complement the evaluation of antioxidant activity of the QLE and GA fortified SPI films samples, both FRAP and DPPH assays were performed (Figure 3-7).

DPPH is a compound that consists of a nitrogen free radical, which is easily quenched by a proton radical scavengers of hydrogen donating antioxidants and are subsequently transformed into a nonradical form (DPPH-H) (Ruiz-Navajas 2013). Figure 3-7 shows that the SPI control films had certain antioxidant properties, which were similar to pure sunflower protein or pure gelatin films previously shown to have antioxidant properties (Gomez-Estaca and others 2009; Gomez-Guillen and others 2007; Moradi and others 2012; Salgado and others 2012). The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Binsan and others 2008). The different antioxidant values between pure sunflower
and soybean films were due to their different chemical composition (Salgado and others 2012).

It can be seen from Figure 3-7, QLE and GA provided antioxidant properties in the edible films. This is similar to the other phenolic-rich plant and food extract, such as murta extract, grape seed extract, borage extract, rosemary extract, oregano extract, mint extract, pomegranate peel extract, Zataria multiflora Boiss essential oil, red and white ginseng extract (Gomez-Estaca and others 2009; Gomez-Guillen and others 2007; Kanatt and others 2012; Moradi and others 2012; Norajit, Kim, Ryu 2010; Salgado and others 2012; Siripatrawan and Noipha 2012). The degree of antioxidant power provided to edible films is generally proportional to the amount of the antioxidant added (Gomez-Estaca and others 2009; Gomez-Guillen and others 2007; Moradi and others 2012). QLE A and QLE D, are natural sources of antioxidant extracts, represent a promising approach (higher antioxidant activity) to enhance the antioxidant power of edible films than pure phenolics, such as GA (Gomez-Estaca and others 2009).

As in the case of DPPH the antioxidant capacity is concentration dependent. Even with the low concentrations of QLE used in SPI films, much higher antioxidant activity was decreased in fortified films in control films. This is not consistent to the other research which reported which showed no antioxidant activity with low content of herb extract fortified chitosan films (Ruiz-Navajas and others 2013).

Moradi and others (2012) and Ruiz-Navajas and others (2013) found that antioxidant properties of protein films were increased by adding Zataria multiflora
boiss essential oil, grape seed extract, thymus morocleri herb extract, and thymus piperella extract. The increased concentrations of the above extracts showed increased antioxidant capacity of the fortified films. Those films showed high antioxidant activity because of the phenolic acids in the extracts, such as terpenoids in the essential oils (Ruiz-Navajas and others 2013). The antioxidative activity of phenolic acids depends on the number and position of hydroxyl groups bound to the aromatic ring (Sroka and Cisowski 2003). The phenolic and terpenoid compounds in essential oils were closely associated with their antioxidant function, mainly because of the redox properties exerted by various possible mechanisms: free radical scavenging activity, hydrogen donors, transition metal chelating activity, and/or singlet-oxygen-quenching capacity (Liyana-Pathirana and Shahidi 2006; Ruiz-Navajas and others 2013). Again, there was a high correlation between antioxidant activity and TPC of the edible films (Baltrusaityte, Venskutonis, Ceksteryte 2007; Contreras-Calderon and others 2011; Ruiz-Navajas and others 2013; Ruiz-Navajas and others 2011).

FRAP assay

Ferric reducing antioxidant power (FRAP) generally measures the reducing capacity of the ferric ion and has been correlated to the radical scavenging capacity. The FRAP of SPI films fortified with different quinoa leaf or pure GA fortified SPI films at various levels is shown in Figure 3-7.

The control SPI film without QLE extract or GA showed some antioxidant capacity (268.45 ± 5.85 µmol AAE/g film), attributed to the amino acids content (e.g., glycine
and proline), this agrees with results and reported for pure chistosan or fish gelatin film (Gomez-Estaca and others 2009; Ruiz-Navajas and others 2013). As shown in Figure 3-7, the FRAP of SPI films fortified with 5GA, 10 GA, QLE A, QLE B, QLE C, and QLE D are higher than that of the control gelatin ($P < 0.05$). Similar modification was also found in murta leaf extract added to gelatin films (Gomez-Guillen and others 2007; Ruiz-Navajas and others 2013). Gomez-Guillen and others (2007) found that higher concentration of phenolic compounds fortified films had higher antioxidant capacity. All QLE fortified SPI films showed higher FRAP values than 5GA or 10GA, which might be because of the various phenolics compounds, or other antioxidants components present in the extract.

The result was generally in agreement with those of DPPH radical scavenging activities, except for the QLE B fortified SPI and QLE D one (Aewsiri and others 2009). DPPH values were much lower than control film ($P < 0.05$) while FRAP value were higher than control film ($P < 0.05$), and this was inconsistent with the other research (Oboh, Raddatz, Henle 2008). A marked increase ($P < 0.05$) in FRAP values of SPI film was observed in SPI film fortified with QLE A, B, C, and D, and GA, in comparison with the control SPI film. The enhanced antioxidant was especially showed in the combination of GA and QLE fortified SPI films, and this was consistent the Aewsiri and others (2009), who found oxidized phenolic acid, ferulic acid, tannic acid can enhance the antioxidant of protein films. The antioxidative activity of phenolic acids depends on the number and position of hydroxyl groups bound to the
aromatic ring (Sroka and Cisowski 2003). Even oxidized phenolic acid fortified antioxidant properties of SPI films (Gomez-Guillen and others 2007).

As regards to QLE fortified SPI film, even when low concentrations of phenolic compounds was added into SPI films, significant antioxidant activity was observed in SPI films. This observation disagreed with the other research where no enhanced antioxidant activity of SPI films was reported (Ruiz-Navajas and others 2013). The presence of carbohydrates in QLE, either in the form of single sugars, polysaccharides or even polysaccharide conjugates, may also contribute to their reducing and radical scavenging capacities (Chen and Liu 2008; Chen and others 2012; Salgado and others 2012). This antioxidant activity could be related to the presence, in the edible films, of bioactive compounds such as phenolics acids of terpenoids coming from the essential oils (Ruiz-Navajas and others 2013).

The phenolic and terpenoid compounds present in the EOs are closely associated with their antioxidant function mainly due to their redox properties which can be explained by various possible mechanisms: free-radical scavenging activity, hydrogen donors, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (Ruiz-Navajas and others 2013).

Phenolic compounds in QLE may also react noncovalently with proteins via hydrogen-bonding, ionic and hydrophobic interactions (Salgado and others 2012). For gelatin films, phenolic compound rich extracts may interact with gelatin and cause lower antioxidant capacity (Salgado and others 2012). However, phenolic compounds in QLE fortified SPI films were mostly free of phenolic compounds, which did not
interact with SPI proteins (Salgado and others 2012). The antioxidant activity of phenolic compounds could not be decreased by interactions with proteins (Salgado and others 2012).

The lower antioxidant capacity of QLE fortified SPI films, as compared to other QLE fortified SPI films, can be attributed not only to the lower absolute content of total phenolic compounds, but also to a greater degree of protein-phenolic interaction in the starting protein concentrate (Salgado and others 2012).

When manufacturing QLE fortified SPI films in industry, QLE should be protected due to the high sensitive to oxygen and temperature. In addition, when the QLE fortified SPI films used as primary food package materials, they should be protected by secondary package as well. Because the films are very sensitive to environment humidity.
Figure 3-7. 2,2-diphenylpicrylhydrazyle (DPPH) value (trolox equivalent (TE) µmol/g film) and ferric reducing antioxidant power (FRAP) value (ascorbic acid equivalent (AAE) µmol/g film) of quinoa leaf extract (QLE) and/or gallic acid (GA) fortified soy protein isolate (SPI) films.

5GA: concentration of GA used was at 0.125% (w/w SPI)

10GA: concentration of GA used was at 0.25% (w/w SPI)

* means significantly different from control SPI film at \( P < 0.05 \).
3.4.6 Antimicrobial Properties of QLE Fortified SPI films

Test_1

Inhibitory activity was measured based on the clear zone surrounding a circular film disk. If there is no clear zone, it is assumed that there is no inhibition (Maizura and others 2007).

Increasing evidence showed that films fortified with phenolic compound enhanced the antimicrobial properties of the films. For example, Song (2012) reported that the barley bran protein-gelatin film containing 1% grape seed extract, the inhibition zones for *E. coli O157:H7* and *Listeria monocytogenes* were 17.08 and 21.39 mm, respectively, compared to the control film (10 mm) (Song, Shin, Song 2012). Galangal extract had antimicrobial activity against *E. coli O157:H7* (gram-negative) and *Listeria monocytogenes* (gram-positive), *S. typhimurium*, and *Botrytis cinerea* (fungi) (Mayachiew and Devahastin 2010; Reagor and others 2002; Sivarooban, Hettiarachchy, Johnson 2008; Song, Shin, Song 2012; Xu and others 2007a). In addition, the inhibition zone for *E. coli O157:H7* and *Listeria monocytogenes* increased with the increasing concentration of grape seed extract (Mayachiew and Devahastin 2010; Sivarooban, Hettiarachchy, Johnson 2008; Song, Shin, Song 2012). Figure 3-8 shows that GA (0.125%) and in combination with QLE B fortified SPI films had significant higher antimicrobial activity (*P < 0.05*) than control SPI films or QLE B fortified films. This is consistent with the above research. But the
above results are much higher than results in the present study. This is because they use higher phenolic compounds concentration compared to QLE B fortified SPI films.

The phenolic compounds were the major ingredients in plant extracts believed to be responsible for the antimicrobial activities (Lin, Labbe, Shetty 2004; Sivarooban, Hettiarachchy, Johnson 2008). The phenolic compounds potentially disturbed the function of the bacterial wall and membranes, and also may have inhibited bacteria enzyme, which caused retardation of growth and multiplication of bacteria (Sivarooban, Hettiarachchy, Johnson 2008). The inhibitory effect of phenolic compounds from plant extracts was potent on gram-positive bacteria (Puupponen-Pimia and others 2005; Salgado and others 2012; Sivarooban, Hettiarachchy, Johnson 2008; von Staszewski, Pilosof, Jagus 2011). The different antimicrobial activity might be due to different intermolecular interactions between the QLE B and SPI films, different microstructure of the films as well as different degrees of film swelling (Mayachiew and Devahastin 2010). In the present study, the lack of antimicrobial activity may be attributed to interactions between phenolic compounds and proteins. Similarly, the antimicrobial effect of the polyphenols (from green tea infusions) in the presence of whey proteins increased with the reduction of whey protein concentration (von Staszewski, Pilosof, Jagus 2011).
Figure 3-8. Inhibition zones (mm) of quinoa leaf extract (QLE) B and/or gallic acid (GA) fortified soy protein isolate (SPI) films to *Listeria inoculated*.

5GA: concentration of GA used was at 0.125% (w/w SPI).

10GA: concentration of GA used was at 0.25% (w/w SPI).

* means significantly different from control SPI film at $P < 0.05$.

** means significantly different from QLE & GA fortified SPI film at $P < 0.05$. 
Increasing evidence showed that phenolic compounds fortified films had lower microbial populations in fresh meat and fruits. *Listeria monocytogenes*, *brochothrix thermosphacta*, and *E. coli* O157:H7 in raw meat were decreased by packing with phenolic compounds fortified protein based films (Corrales, Han, Tauscher 2009; Ku, Hong, Song 2008a; Song, Shin, Song 2012). The population of coliform bacteria, *E. coli* O157:H7, and *Listeria monocytogenes* in salmon, turkey frankfurter were also found to be inhibited by grape seed extract (different concentrations from 0.5% to 5%) fortified barley bran protein-gelatin films than control groups during refrigerated storage (Baydar, Ozkan, Sagdic 2004; Gadang and others 2008; Hoque, Benjakul, Prodran 2011; Jang, Shin, Song 2011a; Song, Shin, Song 2012). Pure phenolic compounds, such as catechin and GA, were also reported to show antimicrobial activity to pathogen population, including *E. coli* T36 and *Listeria inoculated*, of films and films packed sausage sample (Anastasiadi and others 2009; Arcan and Yemenicioglu 2011; Ku, Hong, Song 2008a; Ku, Hong, Song 2008b; Saucier and Waterhouse 1999). However, the marginal effectiveness of various phenolic-rich extracts fortified protein based films on food in the above research should be taken into consideration (Ku, Hong, Song 2008a; Song, Shin, Song 2012). In the present study, QLE B and/or GA fortified SPI films packed extra lean ground beef did not have significantly low *E. coli* T36 population compared to the blank group (Figure 3-9). As shown in Figure 3-9, GA (0.25% w/w), QLE B, and their combination fortified SPI
films packed beef samples shows significantly higher population of *E. coli T36* than control SPI films packed ones in Day 10 (*P* < 0.05).

The mechanism of the inhibitory to the bacteria of phenolic compounds was due to the inhibition of bacteria enzymes and weakening of bacterial cell wall and membranes (Song, Shin, Song 2012). For foods packed grape seed extract fortified films, the water-soluble phenolic compounds were transferred into the surface of food products, and inhibited the growth of bacteria (Song, Shin, Song 2012). The migration depended on nature, structure, and features of films, of phenolic compounds from extract fortified films to the food surface (Corrales, Han, Tauscher 2009). In the present study, no significant difference was observed between QLE B and/or GA fortified SPI films packed ground beef and blank meat. This may be because the strong bond between QLE B and SPI macromolecules that led to the low migration of phenolic compounds from films to the meat.
Figure 3-9. Microbiology test (E. coli T36 log CFU/g) of quinoa leaf extract (QLE) B and/or gallic acid (GA) fortified soy protein isolate (SPI) films.

Blank group: ground beef samples were not packed by any film.

5GA: concentration of GA used was 0.125% (w/w SPI).

10GA: concentration of GA used was 0.25% (w/w SPI).

* means significantly different from control SPI film at $P < 0.05$. 
3.5 Conclusion

QLE was found to enhance various properties of SPI films. The mechanical properties, including TS and EAB, of SPI film were enhanced by QLE. The major interaction between the QLE and SPI macromolecules was hydrogen bonding. QLE fortified SPI film showed significant ($P < 0.05$) higher antioxidant, and antimicrobial properties (test_1) compared to control SPI films. In the antimicrobial test_2, QLE reinforced SPI films did not show significant different, from the control SPI films and blank groups. However, the antimicrobial properties can be determined in other research when higher concentration of phenolic-rich extract used, which means that higher concentration of QLE can be used to modify the antimicrobial properties of SPI films. In conclusion, QLE were found to fortify mechanical and antioxidant properties of SPI films with further research on the antimicrobial properties and test on real food.
Chapter 4 Conclusions and Future Studies

In conclusion of Chapter 2, based on TS and FTIR of all QSC fortified SPI films, the interactions between QSC and SPI were confirmed and hydrogen bonding was the major bond forming. Based on the above tests, the 10% QSC fortified SPI films had the highest TS but lowest EAB, which may limit its utilization in food packaging. While 0.5% QSC fortified SPI films had the lowest TS and relatively high EAB. However, the color of high content of QSC fortified SPI films was different from the pure SPI films, and consumer acceptability tests should be assessed before its utilization by the food industry.

In conclusion of Chapter 3, QLE was found to enhance various properties of SPI films. The mechanical properties, including TS and EAB, of SPI film were enhanced by QLE. The major interaction between the QLE and SPI macromolecules was hydrogen bonding. QLE fortified SPI film showed significant ($P < 0.05$) higher antioxidant, and antimicrobial properties (test_1) compared to control SPI films. In the antimicrobial test_2, QLE reinforced SPI films did not show significant different, from the control SPI films and blank groups. However, the antimicrobial properties can be determined in other research when higher concentration of phenolic-rich extract used, which means that higher concentration of QLE can be used to modify the antimicrobial properties of SPI films. In conclusion, QLE were found to fortify mechanical and antioxidant properties of SPI films with further research on the antimicrobial properties and test on real food.
This study has found that QSC can fortify the mechanical properties of SPI films, and the films are environmentally friendly. QLE can fortify the mechanical, antioxidant, and antimicrobial properties of edible SPI films. Therefore, the QLE fortified SPI films can be used as primary food packaging materials attached to food directly.

QLE concentration used to fortify SPI films need to be optimized to get enhanced properties (mechanical, antioxidant, and antimicrobial properties). The antimicrobial properties of SPI films are very important due to the food safety concern. Other nature edible antimicrobial compounds can also be added to fortify the antimicrobial properties of SPI films. And sensory evaluation and customer acceptability should be tested before apply the edible films in industry.
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