Subcritical water extraction of tannins, phenolics and fiber from Canadian faba bean hull and green pea pod

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

In
Food Science and Technology

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Abstract

Processing of pulses such as faba bean and green pea provides 15% hull and 30% pod as by-products, respectively, which are good sources of bioactives such as tannins, phenolics, and carbohydrates. In this study, subcritical water (SCW) was used as an environmentally friendly extraction technique to remove bioactives from faba bean hull and green pea pod. Extracts were obtained using a semi continuous SCW extraction system. SCW extractions of faba bean hull and green pea pod were performed at 100-200℃ and 50-100 bar for 40 min. Particle size (0.5 mm) and flow rate (5 mL/min) were maintained constant. Also, solid-liquid (S-L) extraction was performed using water, 70% acetone, and 70% ethanol with a solid to solvent ratio of 2:20, 2:30, and 2:40 w/v at 50℃ and 70℃ within 3h. For both systems, temperature was the most important process parameter. Pressure had no significant effect on the SCW extraction of bioactives. For faba bean hull, the highest removal of total tannins (~73.6 mg tannic acid/g hull), and condensed tannins (42.3 mg catechin/g hull) were obtained at 160℃ and 50/100 bar. Also, the highest total phenolics (~44.4 mg gallic acid/g hull) were removed at 120℃. Total tannin removed using SCW (~73.6 mg tannic acid/g hull) was 3.4 times more than the one obtained by water at 70℃ and atmospheric pressure (~22 mg tannic acid/g hull).

For green pea pod, the highest removal of total tannins (~12.9 mg tannic acid/g pea pod), and total phenolics (~56.6 mg gallic acid/g pea pod) were obtained at 180℃ and 50 bar. SCW removed more total tannins (12.96 mg tannic acid/g pea pod) than water at 70℃ and atmospheric pressure (3.6 mg tannic acid/g pea pod). Furthermore, 70% acetone was the most efficient solvent for the total tannin removal at 70℃ and atmospheric pressure using the S-L extraction.

These two by-products have in common a major component of dietary fiber (faba bean hull: 79.29±0.04% DW, and green pea pod: 55.10±0.05 % DW). For green pea pod, SCW extraction at 200℃ and 50 bar decreased fiber values to the lowest content of 3.25±0.02% DW soluble fiber and 45.09±0.16% DW insoluble fiber. For faba bean hull, SCW at 200°C and 50 bar led to a faba bean hull solid residue containing the lowest total dietary fiber (~71.14% DW), soluble fiber (4.36% DW), and insoluble fiber (65.71% DW). However, using the S-L extraction with acetone or ethanol was obtained 5.6-5.9% DW soluble fiber and 78.29-80.77% DW insoluble fiber in the residue. The SCW extraction is an environmentally friendly method better than the S-L extraction for the removal of bioactives such as tannins, phenolics, and carbohydrates from faba bean and green pea by-products.

Keywords: Subcritical water extraction, Faba bean hull, Green pea pod, Bioactives.

ACKNOWLEDGEMENTS

It has been a great privilege to spend a couple of years in the Department of Agriculture, Food and Nutritional Science at University of Alberta, and this experience will be useful and invaluable to me. I would like to thank many people, who helped me during my master thesis journey. Foremost, my sincere gratitude must go to my supervisor, Dr. Marleny Aranda Saldaña, for providing me the vision, encouragement, and guidance throughout my research. I would like also to extend my special thanks to my committee member, Dr. Nadir Erbilgin for his support and helpful hints and suggestions. I also thanks Jun Gao for his help with fiber analysis.

A special thank is extended to my lab colleagues for providing a productive environment for learning and growing. I take the opportunity to thank Angelica, Eduardo, Raquel, Carla, Yujia, and Idaresit for their constant help and suggestions.

Foremost, I am forever grateful to my husband, Masoud, who helped me throughout the difficult times, and all the emotional support, entertainment and caring. I also want to thank my parents and sisters who always supported me. Lastly, and most importantly, I would like to thank Alberta Pulse Growers and Natural Sciences and Engineering Research Council of Canada for providing the funds to carry out this thesis research.

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NOMENCLATURE

Symbols and abbreviations

T: Temperature

P: Pressure

US: United States

UK: United Kingdom

FAO: Food and Agriculture Organization

CVD: Cardiovascular disease

DW: Dry weight

Na: Symbol of chemical element Sodium

Ca: Symbol of chemical element Calcium

K: Symbol of chemical element Potassium

Cu: Symbol of chemical element Copper

Zn: Symbol of chemical element Zinc

Fe: Symbol of chemical element Iron

Mn: Symbol of chemical element Manganese

Mg: Symbol of chemical element Magnesium

LDL: Low-density lipoprotein

GC: Gas chromatography

GT: Gallotannins

EGT: Ellagitannins

L-DOPA: L-3,4-dihydroxy phenylalanine

PD: Parkinson’s disease

EDTA: Ethylenediamine tetra acetic acid

SCW: Subcritical water

SWE: Subcritical water extraction

PHW: Pressurized hot water

NCW: Near-critical water

HCW: Hot compressed water

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

SOD: Superoxide dismutase

TNT: Trinitrotoluene

PLE: Pressurized liquid extraction

Chapter 1: Introduction

1.1. Rationale

Direct and indirect outcome of employing non-usable parts of plants capture scientist's attention toward plants by-products to produce an economic return. Making effort to maximize the use of by-products is the recent general mission in food processing plants. For this purpose, top-grade processes and technologies are proposed to improve the quality of materials in line with customers' needs.

Subcritical water (SCW) extraction technique is one of the modern technologies that has been used in food and biomass studies (Kulkarni, Suzuki, & Etoh, 2008). At the beginning, this technology was used for the extraction of organic pollutants such as polycyclic aromatic hydrocarbons from environmental solids (Hawthorne, Yang, & Miller, 1994). Now, recent studies have shown its potential for separation of bioactives and phytochemicals from food (He et al., 2012) and non-food (Hassas-Roudsari Chang, Pegg, & Tyler, 2009) matrices. For SCW, water is in its liquid form at temperatures above 100 °C and below 374 °C and pressure below 22 MPa.

In the subcritical region, water has specific range of physical properties such as viscosity, dielectric constant and density, which enhances mass transfer due to high diffusivity and high extraction efficiency (Toor, Rosendahl, & Rudolf, 2011). Subcritical water extraction studies on by-product of cinnamon bark (Pramote, Nucha, Suched, Parinda, & Prasong, 2012), apple pomace (Wijngaard & Brunton, 2010), pomegranate seed residues (He et al., 2012), defatted rice bran (Chiou, Neoh, Kobayashi, & Adachi, 2012), and potato peel (Singh & Saldaña, 2011) are some examples of extracting phenolics in which physical properties changes benefit the

extraction. In these studies, increase in temperature (>120℃) and pressure (>5 MPa) has led to decrease in polarity and dielectric constant. The dielectric constant decreases because of hydrogen bonds breakage and radical formation in subcritical water conditions (Cheigh, Yoo, Ko, Chang, & Chung, 2015; Watanabe et al., 2004), providing proper conditions for the release of organic compounds from the initial matrix into the solvent media (Shitu, Izhar, & Tahir, 2015). Formation of ion product of water and free radicals initiate other chemical reactions. This benchmark has been applied for the hydrothermal conversion of lignocellulosic waste under subcritical conditions (Zhao, Lu, Chen, Zhang, & Wang, 2014).

Moreover, subcritical water has the benefit of taking less time compared with maceration (Vongsak et al., 2013) and Soxhlet extraction (Ruiz-Montañez et al., 2014), which are widely used conventional techniques. Another hindrance of conventional techniques is the necessity of using large quantities of organic solvents, which produce toxic organic waste (Azmir et al., 2013). But, subcritical water extraction technique is considered eco-friendly and green as it provides the opportunity of using pure water or mixtures of water with less volume of toxic solvents (Abdelmoez, Nage, Bastawess, Ihab, & Yoshida, 2014). Therefore, subcritical water extraction technology has gained increasing attention for food and biomass research areas. One of the areas of focus is production of value added products of legumes (Haldar, 2013) and agro-food by-products, such as corn straw, rice bran, corn shell, potato, durian and mango peel (Shitu, Izhar, & Tahir, 2015; Singh & Saldaña, 2011).

Faba bean (*Vicia faba L.*) belongs to a large group of flowering plants with large seeds, which grows in green long pods. This plant has a bushy structure with tapering leaves, yielding about 25 to 50 pods per plant (Duc, 1997). Faba bean originated in Mediterranean and Middle Eastern countries (Saxena, 1991). In 1972, production of faba bean started from Western parts of

Canada. Later, in 1997, faba bean started to grow commercially in St. Jean region of Quebec (Munro & Small, 1997). The popularity of faba bean cultivation extended from Canada (Saxena, 1991) to the United States (Bean, 1999). Faba bean protein content of 24–30% db made it a suitable source of protein for livestock feeding (Small, 1999). Moreover, faba bean advantages for crop rotation systems, such as biological N_2 fixation of crop systems, diversification of crops to decrease diseases and lowering fossil energy consumption encouraged farmers to grow faba bean as a cover crop in different parts of Alberta (Jensen, Peoples, & Hauggaard-Nielsen, 2010).

The main application of faba bean has been for food and feed purposes (Suso, Bebeli, & Palmer, 2015). Nevertheless, genetic modifications and breeding systems on faba have shown different level of nutritional value for this crop (Crépon et al., 2010). For instance, some faba bean provide less nutritional value due to the presence of tannin, vicine and convicine in the cotyledons (Olaboro, Marquardt, Campbell, & Fröhlich, 1981). Further, low tannin content cultivars (0.1 g/kg DW condensed tannin) have high crude protein (319 g/kg DW), which makes them suitable for feed and food purposes (Crépon et al., 2010). The edible part of faba bean is mainly the faba bean seed in most places. However, southern regions of Europe, have faba bean seed cultivars with mean tannin content of 5-10 g/kg dry matter, which limits their consumption as food (Duc, Marget, Esnault, Le Guen, & Bastianelli, 1999). Faba bean cultivars need to have protein content of more than 350 g/kg to be used as a pig diet (Fekete, Willequet, Gâtel, Quemere, & Grosjean, 1985). In such cases, these cultivars can be used for other application purposes. The remained parts of faba bean could be the entry in unit operations for value added compounds production for the pharmaceutical, tanning, cosmetic or polymer composites industries. Also, removing the undesirable compounds like tannins from high tannin faba bean

cultivars (6.6 g/kg DW condensed tannin) not only boost the energy level of digestibility but also adjust the protein digestibility of faba bean for pigs and poultry (Crépon et al., 2010).

Like faba bean, green pea botany structure includes edible seeds, which are covered by a tender green pod. These two plants belong to legume family *Fabaceae*.

Green pea *Pisum sativum var. sativum L* is a cool season annual plant, which is found in green pea, sugar or snow pea, snap or sugar snap pea (Olivier & Annandale, 1998). In the middle ages, green pea was consumed as one of the main ingredients in staple food of Middle Eastern, North Africans and Europeans. India, Myanmar, Brazil, the United States and Mexico are the largest international pea producers (Janzen, Brester, & Smith, 2014). The United States is the main consumers of green pea (Messina, 1999). In western part of Canada, field pea and grass pea are the most common cultivars with protein content of 22-25% (field pea) and 20-30% (grass pea) (Wang, 2017). As explained by Gatel and Grosjean (1990), dark-flowered cultivars of green pea and faba bean are less used as protein sources in feed due to the presence of condensed tannins (Gatel & Grosjean, 1990; Jansman, Verstegen, Huisman, & Van den Berg, 1995; Alzueta, Treviño, & Ortiz, 1992). Another study showed the effect of tannin-rich faba bean hulls (varieties Brunette and Minica) on amino acid, starch and lipid digestion level (Jansman et al., 1995).

Aside from being food and feed supply, agronomic value of green pea is to interrupt disease and pest cycles to help soil aggregation and water retention as well as balance of microbial diversity and activity (Chen et al., 2006).

Therefore, domestic production of faba bean and green pea and their benefits to the agriculture, food and health sectors requires increasing research to foster knowledge and create positive economic impact of these cultivars. Also, the dearth of information about tannin

extraction from these pulses and the evaluation of tannin biochemistry in food and feed studies indicate the importance of having a green approach for tannin removal from pulses. This thesis focus on removal of tannins and fiber using subcritical water extraction technology. The use of this technology provides the possibility of adding more value to faba bean hull and green pea pod.

1.2 Hypothesis

Subcritical water technology will be used effectively to hydrolyze and remove tannins and soluble fiber from pulse biomass.

1.3 Thesis objectives

The main objective of this research was to use subcritical water as a green technology to obtain tannins and soluble fiber from faba bean hull and green pea pod. Since tannins have complex chemistry, identification of specific tannin structures present in faba bean hull and green pea pod benefits the knowledge of these pulses. Additionally, use of subcritical water extraction for soluble fiber removal fulfill the need of food and polymer industry for suitable composites reinforced with vegetable fibers. In addition, it can provide nutrition and health benefits. Due to the lack on studies of faba bean hull and green pea pod, the specific objectives are:

- To extract tannins and fiber from faba bean and green pea pod using subcritical water extraction technique,
- To evaluate specific process parameters that influence tannin and soluble fiber extraction performance from pulse by-products using subcritical water extraction technology, and
- To compare the S-L extraction method with the subcritical water extraction method based on the removal yield of bioactives from faba bean hull and green pea pod.

Chapter 2: Literature Review

2.1 . Pulse industry

Legumes are food sources with two to three times more protein content than cereals (Reddy, Pierson, Sathe, & Salunkhe, 1985). According to the Food and Agriculture Organization (FAO), pulses are a type of legumes with a pod containing a set of one to twelve seeds (Tiwari & Singh, 2012). These edible legumes belong to the Leguminosae plant family with dicotyledonous seed (Ambigaipalan et al., 2011). This family of pulses has a rich nutritional composition of 17-30 % protein (7.7 g/0.5 cup serving), 11-33% fiber (~7 g/0.5 cup serving), 50-65% carbohydrates, 0.8-1.5% fat, and 2-3.5% minerals (Havemeier, Erickson, & Slavin, 2017). In 2010, the US Department of Agriculture determined the consumption of 2.5-3.5 cups of cooked pulses for a weekly diet (Marshall, 2011). However, in the Canadian food guideline, the recommended consumption amount of cooked legumes is 175 mL (0.75 cup). Taking a half-cup serving of legume can substitute one serving of vegetables, and a three-quarter-cup serving provides the nutritional fact of one serving of meat. Another benefit of having pulses in the diet is their low energy block of 1.3 kilocalories per gram (McCrory, Hamaker, Lovejoy, & Eichelsdoerfer, 2010).

Moreover, clinical research trials have identified the positive effect of eating beans, peas, lentils and chickpeas on obesity, diabetes and cardiovascular diseases (Tosh & Yada, 2010). However, the presence of anti-nutrients in pulses inhibit proteases, resulting in poor protein digestibility (Jaffé, 1950; Kakade, Simons, Liener, & Lambert, 1972). Those anti-nutrients are phytates (Griffiths & Jones, 1977), trypsin and chymotrypsin, oxalate, lectin, saponins (El-Adawy, 2002; Sandberg, 2002; De Almeida Costa, Da Silva Queiroz-Monici, Reis, & De Oliveira, 2006; Wang et al., 2010) and tannins (Reddy, Subhani, Khan, & Kumar, 1985).

In addition to the nutritional benefit, growing these legumes can adjust the amount of transferred atmospheric nitrogen into the soil by symbiotic process in the presence of *Rehizobium* bacteria (Hamdi, 1982). As well, pulses are commonly used in crop rotations to control weeds and diseases.

The statistics of world pulse production was about 61.5 million tonnes in 2009 (Tiwari & Singh, 2012). This number reached 72.2 million tonnes in 2014 with a contribution of India as the first producer with 18.4 million tonnes. After India, the main global pulse producers are China, Brazil, Canada, Myanmar and Australia (Joshi & Rao, 2017). Canada total pulse production was reported as 5.7 and 5.9 million tonnes in 2010 and 2015, respectively (Government of Canada, 2014). These statistical data refer to the production of beans, lentils, peas and chickpeas as the top four major categories of pulses (Maskus, 2010). As shown in Fig. 2.1, beans (46%) and peas (26%) together account for more than half of the pulse production. Canada pulse production is distributed among Saskatchewan (79.3% of the total pulse production), Ontario (38.4%), Manitoba (32.1%) and Alberta (18.8%) (Government of Canada, 2014). Another pulse commodity that is growing in Canada is faba bean.

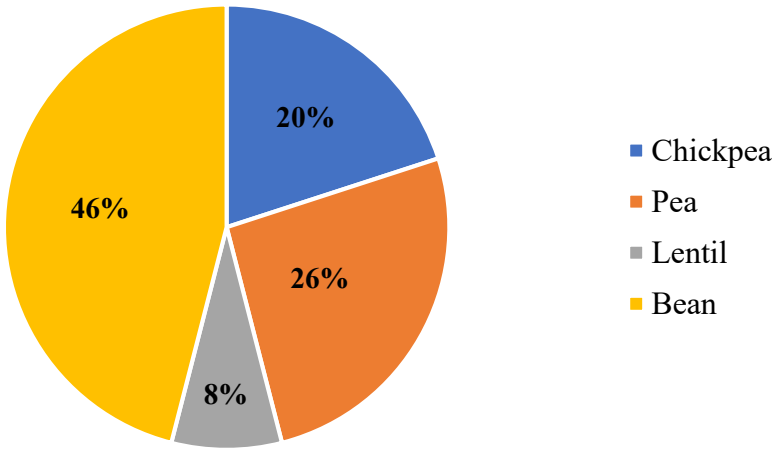


Fig. 2.1. Global pulse production in 2006-2007 (Adapted from Sharon, 2007).

2.2 . Faba bean

2.2.1. Production and significance

Cultivation of faba bean started 8000 years ago, near the East and Mediterranean regions (Zohary, Hopf, & Weiss, 2012). There are approximately 2.6 million ha of faba bean grown worldwide, mostly in China, Ethiopia and the European Union such as United Kingdom (100,000 ha), France (78,000 ha), Spain (56,000 ha) and Italy (45,000 ha) (Jensen, Peoples, & Hauggaard-Nielsen, 2010).

In Canada, major production of faba bean has been reported for Alberta, Manitoba and Saskatchewan. In 2014, cultivated faba bean areas for Alberta, Saskatchewan and Manitoba were around 6210, 4345 and 438 ha, respectively (Alberta Agriculture and Forestry, 2015). In 2013, research trials in southern Alberta have reported faba bean yields up to 10,575 kg ha^{-1} (157 bushels $acre^{-1}$) (Strydhorst & Olson, 2013).

In the Mediterranean regions and Latin America, faba bean is mostly used for food purpose. However, the United States of America and northern Europe produce faba bean only for livestock pasture, hay, and silage (Singh et al., 2012; Singh, Bharati, & Pedpati, 2013). Not only faba bean is a rich source of protein for Chinese and Mediterranean consumers but also provides 75% of daily protein intake in the Egyptian diet (Singh, Bharati, & Pedpati, 2013). The high content of protein in faba bean has influenced an increase of its cultivation. Moreover, its nitrogen fixing ability, adaptability to different soil environments and good performance under different atmospheric changes makes faba bean an excellent pulse crop (Singh, Bharati, & Pedpati, 2013). Faba bean like other pulses also play a role in green manuring (Wani, McGill, Haugen-Kozyra, Robertson, & Thurston, 1994). Field trials at University of Saskatchewan in 2012 demonstrated the resistance of faba bean against humidity compared to the Meteor pea (*Pisum sativum L.*) cultivar. There was also a difference of about double in the production yield of faba bean compared with pea under the same humidity conditions and area (Follow, 2008).

2.2.2. Faba bean structure

Structure and composition of faba bean influence color, texture, flavor, and nutritional value, as well as its acceptance as a food in the market. As shown in Fig. 2.2, faba bean structure consists of large ovary seeds (edible) and the pod (outer non-edible cover).

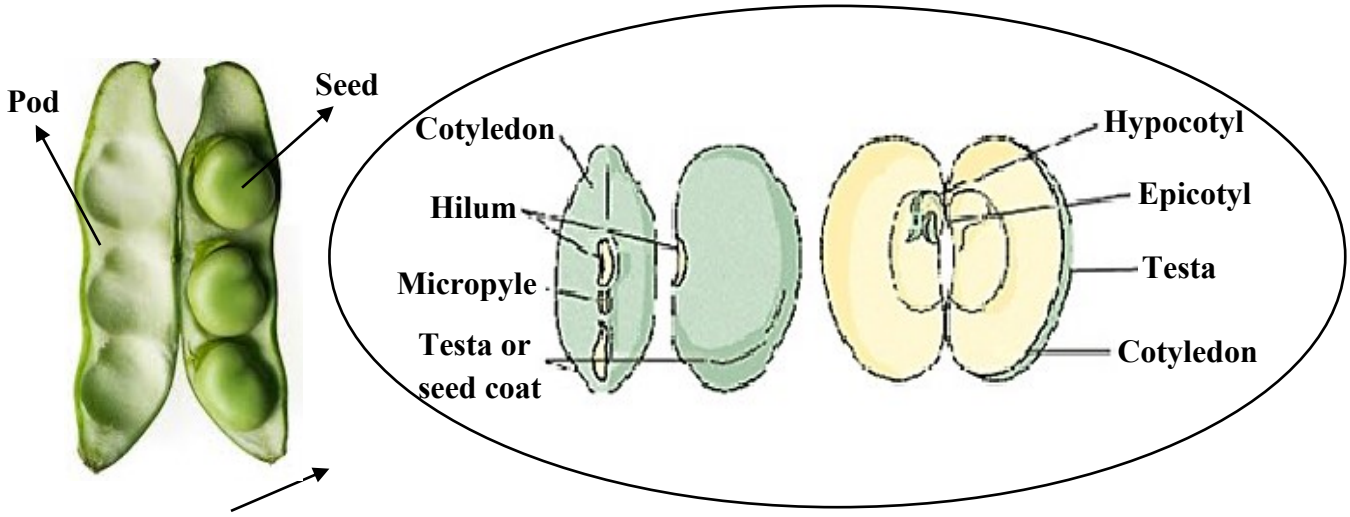


Fig. 2.2. Faba bean pod and seed structure (Adapted from Duc et al., 1999).

2.2.2.1. Faba bean pod

Faba bean is a green legume that grows in a long pod. The beans grow on bushy plants ranging from 60-180 cm in height with tapering leaves, yielding from 25 to 50 pods per plant. Immature pods have a white, velvety interior that turns brown to black and become tough and hard at maturity stage. Each pod contains 3-4 seeds of different range of weights (0.35-0.80 g) and color (low tannin variety with white seeds, and high tannin variety with brown seeds). Faba bean seeds can be found in yellow, green, brown, black, or violet color. The anatomy of this plant includes flowers in white, brown, or violet of approximately 2-3 cm long (Duc, 1997). Large leaves, green hollow stem, and flowers at the base of the leaves are the specific characteristics of faba plant. One quarter of the flowers form the faba bean pods (Patrick & Stoddard, 2010). Faba bean has a tap-root system bearing clusters of lobed nodules where the nitrogen fixing bacteria, *Rhizobium leguminosarum,* exists. A faba bean plant can have more than 1000 nodules that are up to one centimeter in length.

2.2.2.2. Faba bean seed coat

Faba bean seed consists of three parts: an embryo, stored food and a seed coat. Evaluation of tannin concentration of faba bean seed coat showed high amounts for the violet-colored seed coats and low amounts for the white-colored seed coats (Elias, Fernandez, & Bressani, 1979). Faba bean seeds display large genetic variation in seed coat color and pattern (spotted, and marbled), hilum color and cotyledon color (yellow or green) (Duc, 1997).

2.2.3. Faba bean composition and properties

Table 2.1 shows the proximate compositional analysis of Canadian, Spanish and Egyptian faba beans. Faba bean seed also has dietary fiber (soluble: 3-6 g/100 g raw seed and insoluble: 20-28 g/100 g raw seed), minerals (Na: 297, Ca: 220, K: 748, Cu: 2.5, Zn: 11.7, Fe: 6.6, Mn: 2.3 and Mg: 281 mg/100 g dry matter) (Wang et al., 2010) and B group vitamins (Chavan, Kute, & Kadam, 1989). Protein (24.3-39.7% of seed dry matter) and carbohydrates (42.4-70.9% of seed dry matter) are the main components of faba bean. Globulins (79%), albumins (7%), and glutelins (6%) are the main proteins of faba bean (Hossain & Mortuza, 2006). Faba seed coat is mainly composed of carbohydrates (91.8%), protein (5.1%), and ash (3.6%).

TABLE 2.1. Proximate compositional analysis of faba bean.

Origin	***Canadian*[a]**	***Egyptian*[a]**	***Middle eastern*[b]**	***Spanish*[c]**	***European Spring var.*[d]**	***European* Winter var.[d]**	***Persian*[e]**
Whole seed							
Moisture (%)	10.1	9.9	7.1-7.6	nd	nd	nd	nd
Crude protein (%)	28	24.8	31.8-39.7	31.4	25.5-35.5	24.3-29.9	31.2
Ash (%)	3.2	3.5	nd	3.4	3.1-4.8	3.4-4.4	2.9
Lipid (%)	0.9	0.9	1.5-2.1	1.1	1.2-2.0	1.3-1.7	1.0
Carbohydrate (%)	66	70.9	42.4-47.3	53.2-55.7	nd	nd	57.4
Cotyledon							
Moisture (%)	10.1	9.9	nd	nd	nd	nd	nd
Crude protein (%)	30.5	28.4	nd	nd	nd	nd	nd
Ash (%)	3.2	3.5	nd	nd	nd	nd	nd
Lipid (%)	1	1	nd	nd	nd	nd	nd
Carbohydrate (%)	63.3	67.2	nd	nd	nd	nd	nd
Seed coat							
Moisture (%)	10.4	9.8	nd	nd	nd	nd	nd
Crude protein (%)	5.1	5	nd	nd	nd	nd	nd
Ash (%)	2.7	3.6	nd	nd	nd	nd	nd
Lipid (%)	0.1	0.1	nd	nd	nd	nd	nd
Carbohydrate (%)	91.8	91.4	nd	nd	nd	nd	nd

[a]Means of 10 samples of faba beans of Canadian cultivars (Ackerperle, Diana, Herz Freya, MP-79-1, 74-RM 925, UMFB-925) grown at the University of Manitoba and Egyptian cultivars (Balady, Giza1 and Giza 2) grown at several locations in Egypt (Youssef, Bushuk, Murray, Zillman, & Shehata, 1982) [b]Renia Blanka cultivar (Alghamdi, 2009), [c]*Vicia faba L.* cultivar (Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, Zapata-Revilla, & Tenorio-Sanz, 2010; Chavan, Kute, & Kadam., 1989), [d]Eden (1968), [e]Bhatty (1974), and nd: not determined.

Table 2.2 shows the distribution of different carbohydrates in faba bean. Sucrose, raffinose, stachyose, and verbascose are carbohydrates responsible for flavor and prebiotic nature of faba bean. Sucrose is the predominant carbohydrate in mature faba bean that contributes with 0.02-5.23% (Pritchard, Dryburgh, & Wilson, 1973; Lattanzio et al., 1986; Quemener, 1988; Frias et al., 1996). Also, faba bean has water soluble oligosaccharides such as raffinose, stachyose, and verbascose (Sosulski & Cadden, 1982). These oligosaccharides behave as prebiotics as they can reach to the large intestine without being digested or absorbed in the intestinal tract (Tosh & Yada, 2010).

TABLE 2.2. Composition of sugar, oligosaccharides and inositols in faba bean flour (% dry matter)(Adapted from Sosulski, Elkowicz, & Reichert, 1982).

Faba bean flour	Sucrose (%)	Raffinose (%)	Stachyose (%)	Verbascose (%)	Galactinol (%)	Galacto pinitol isomers (%)
Hull free flour	2.00	0.22	0.67	1.45	0.22	0.17
Protein fraction	1.35	0.33	1.37	3.96	n.r	0.32
Starch fraction	2.72	0.25	0.48	0.44	0.20	0.08

n.r: not reported

Dietary fiber is the major fraction of beans and pods from pulse by-products with a potential market for fiber rich products and ingredients (Redondo-Cuenca, Villanueva-Suárez, & Mateos-Aparicio, 2008; Mateos-Aparicio, Redondo-Cuenca, Villanueva-Suárez, & Zapata-Revilla, 2008). Table 2.3 summarizes fiber content of pulses such as dry green bean, chickpea, lentil and pea. There are no reports on total dietary fiber from faba bean hull or green pea pods.

TABLE 2.3. Dietary fiber content of different pulses.

Pulse	Total dietary fiber (%)	Insoluble fiber (%)	Soluble fiber (%)	Reference
Common beans (*P. vulgaris)*	23–32	20–28	3–6	Granito et al. (2002); Kutos et al. (2003); Perez-Hidalgo et al. (1997)
Chickpeas *(C. arietinum)*	18–22	10–18	4–8	Dalgetty and Baik (2003); Perez-Hidalgo et al. (1997); Rincón et al. (1998)
Lentils *(L. culinaris)*	18–20	11–17	2–7	Dalgetty and Baik (2003); Perez-Hidalgo et al. (1997)
Peas *(P. sativum)*	14–26	10–15	2–9	Borowska et al. (1996); Dalgetty and Baik (2003); Martín-Cabrejas et al. (2003)
Pea pod *(Pisum sativum L.)*	58.6	54.4	4.2	Mateos-Aparicio et al. (2010)
Okara from soybean *(Glycine max L.)*	54.3	50.1	4.2	Mateos-Aparicio et al. (2010)
Broad bean pod *(Vicia faba L.)*	40.1	30.8	9.3	Mateos-Aparicio et al. (2010)

The presence of starch and fiber in pulses keep the level of LDL-cholesterol low enough to prevent any risk of heart attack and stroke (Cho & Dreher, 2001; Hoover & Sosulski, 1991). For example, faba bean has rapid digestible starch (2.5%), slow digestible starch (76.3%), and resistant starch (11.0%) (Ambigaipalan et al., 2011). Besides, insoluble fiber of pulses regulates the food digestion process.

Pulses contain antinutritional compounds such as tannins, and vicine-convicine (Rizzello et al., 2016). Condensed tannins are the first antinutritional components located in the testa of faba bean (Griffiths & Jones, 1977). Tannins provide a bitter flavor to faba bean, and vicine-convicine aglycone derivatives cause the rare genetic disorder favism (Crépon et al., 2010). Vicine (1.94%

dry matter) and convicine (0.83% dry matter) contents of faba bean have been quantified using gas liquid chromatography (Pitz & Sosulski, 1979; Pitz, Sosulski, & Rowland, 1981). The absence of tannins in a "zero-tannin" faba bean cultivar increases the protein digestibility in mono-gastric animals, while low contents of vicine and convicine in "low vicine-convicine" faba beans improve feed value in poultry and reduce favism disease in humans (Crépon et al., 2010).

2.2.4. Uses of faba bean and its by-products

Faba bean is commonly used as food, livestock feed and forage/silage. Seeds are the edible part that provide a protein supply to Mediterranean (Youssef, Hamza, El-Aal, Shekib, & El-Banna, 1986) and South American consumers (Hacıseferoğulları, Gezer, Bahtiyarca, & Mengeş, 2003). Recently, Ali et al. (2014) reported that compared with other pulses, faba bean is a less consumed crop by humans in western countries but more consumed food in Africa, parts of Asia and Latin America (Ali, Awadelkareem, Gasim, & Yousif, 2014).

Several studies have shown antinutritional components in pulses used as animal feed (Makkar, Becker, Abel, & Pawelzik, 1997) and pesticides. A few of them are saponins, tannins, flavonoids, alkaloids, trypsin (protease) inhibitors, oxalates, phytates, hemagglutinin (lectins), cyanogenic glycosides, coumarins, which might be deleterious to health without proper processing (Soetan & Oyewole, 2009). For example, phytic acids cannot be destroyed by cooking in boiling water, causing diarrhea (Emire, Jha, & Mekam, 2013). Also, adverse effect of high molecular weight (>5000 Da) condensed tannins on absorption of iron, zinc and copper due to the insolubility of these elements was reported for cooked pulses (Tiwari, Gowen, & McKenna, 2011). Such high molecular weight tannin compounds lost its protein precipitation capacity and fermentability, and become insoluble in the colon (Serrano, Puupponen-Pimiä,

Dauer, Aura, & Saura-Calixto, 2009). Therefore, removal of these antinutritional components is required before consumption as food or feed (Soetan & Oyewole, 2009).

Tannins are phenolic compounds widely found in different pulses (Champ, 2002; Saura-Calixto, 1988), promoting good health, owing to anti-carcinogenic and anti-microbial properties (Chung, Wong, Wei, Huang, & Lin, 1998). For example, tannin extract from faba seed coat prevents intestinal D-glucose transport during the *in vivo* experiments on rats (Barcina, Alcalde, Ilundain, & Larralde, 1984). In another study, tannins have been reported as blood glucose lowering agents (Etuk, Opara, Okeudo, Esonu, & Udedibie, 2012). However, high dosage of tannins in animal feed led to protein degradation, which increases amino acid flow to the small intestine, resulting in a decrease of protein digestibility (Lee, Choi, Kim, Amanullah, & Kim, 2016). Also, gall nuts (50-70%), treripod (65%), chestnut wood (30%), myrobalan fruit (30-35%), and sumac leaves (20-35%) are rich sources of tannins that have been used in the leather industry (Kipnis, Levenko, Strakhov, & Shifrin, 1972; Prabhu & Bhute, 2012). Mostly, hydrolysable tannins such as gallotannins (GT) and ellagitannins (EGT) have been used in the leather process (Falcão & Araújo, 2013).

Another potential application of faba bean is in the preparation of novel food ingredients, additives and nutritional supplements due to its well-balanced amino acid composition (Gueguen & Cerletti, 1994). It has been reported that high protein content of lupins (*Lupinus albus L*., 34.7%), peas (*Pisum sativum L.,* 23.4%) and broad beans *(Vicia faba L.,* 32.5% w/w dry matter) make them suitable candidates for this application (Makri, Papalamprou, & Doxastakis, 2005). For this purpose, solubility of a protein is its main attribute, which greatly influences other properties, such as emulsification, gelation and foaming ability of a food ingredient.

Previously, it was found similarity among water solubility (%) of protein isolates obtained from pea, broad bean and soy bean. The minimum solubility was obtained at a pH range of 4.0-6.0 while the maximum solubility was obtained at pH of 8.0-9.0. However, oil and water absorption capacity of broad bean *Vicia faba L*. (Oil: 1.6±0.2%, water: 1.8±0.1%) was similar or higher than soybean (Oil: 1.3±0.1%, water: 1.1±0.1%) (Braga Fernandes, Goncalves, & Lefebvre, 1989). Moreover, preparation of foam using 1% (w/v) protein isolate from pea, broad bean and soybean at pH values of 5.5 or 7.0 revealed the foam stability order of pea>broad bean>lupin in retaining air for a period of 30 min (Makri, Papalamprou, & Doxastakis, 2005). Soybean meal was traditionally used in the diet of animals, producing milk and meat, but recently there is an increased substitution of alternative plant proteins instead of soybean meal. For example, heat-processed flaked faba beans were used for Reggiana breed dairy cows. Dehulling, flaking or extrusion not only decreased tannin content and protein degradation in the rumen but also increased insoluble protein fraction in faba bean (Volpelli et al., 2010).

Reported data for faba bean and pea have shown an average crude protein content of 30.8 and 24.9% dry matter, which is close to 38.7% dry matter in lupin. Moreover, similar quantities of lysine, methionine and cysteine in faba bean (0.87, 0.67, 0.57% dry matter), pea (0.85, 0.76, 0.67% dry matter) and lupin (0.87, 0.81, 0.84% dry matter) (Link, Weber, & Duc, 2005) have brought the possibility of replacing soy bean with faba bean, pea and lupin for animal feed (Adamidou et al., 2011).

2.3. Green pea

Green pea is also called common pea, dry pea, field pea and garden pea (Ratnayake et al., 2001). Pea (*Pisum sativum L.*) is the second largest legume after common bean (*Phaseolus vulgaris L.*), which is cultivated in Canada, south west Asia, Europe, Ethiopia, and North west

India (Maxted & Ambrose, 2001; Kumari et al., 2013). It is an annual cold season crop that is in the family Leguminosae (Singh et al., 2010; Shereena & Salim, 2006). It is classified as legume having pods with a single ovary that splits along two margins when dried. A temperature range of 12–18 °C with a relatively humid climate is the optimum growth condition for this crop. Peas are different in height, color of flowers (blue, purple, and white), size (3-4 mm, and 6-8 mm), shape of seeds, color and texture of seed coat and cotyledon (yellow and green). Pea seeds are rich in protein (23-25%), slowly digestible starch (50%), soluble sugars (5%), riboflavin and niacin (Smýkal et al., 2012).

2.3.1. Production and significance

Green pea originally grew in Southeast Asia while the major pea producers are Canada, Russian Federation, United States, India, France and Ethiopia (Food and Agriculture Organization, 2015).

Canada is the second world producer with 3.96 kilotonnes green pea per year (25% of four total world producers) and the largest exporter with 2.78 kilotonnes green pea per year, sending to other countries without any further processing (40% of total world exports) (Agriculture and Agri-Food Canada, 2015; Statistics Canada, 2011). Canada is the leader on pea production with 28% of the total yield, followed by France and Russia with 14% and 10%, respectively (Smith & Jimmerson, 2005). Only 10% of Canadian pea production is used as a food, including the whole and cracked seeds, which are used in stews, soups and canned products (Raghunathan, Hoover, Waduge, Liu, & Warkentin, 2017). Besides being used as a popular vegetable, peas can be dried and consumed as snacks or milled for making soups, flour, and canned products like mushy peas. Moreover, dried peas are fed to pigs and poultry as a source of protein (19.34-27.3%), containing

four main amino acids: glutamic acid (3.85%), aspartic acid (2.46%), arginine (2.35%), and lysine (1.61%) (Wrigley, Corke, Seetharaman, & Faubion, 2015).

Since Europe established using pea in the feed market in 1985, farmers increased pea production from 74,400 ha in 1985 to 1,345,000 ha in 2014 (Agriculture and Agri-Food Canada, 2015). In Canada, Saskatchewan, Alberta and Manitoba produce 79, 18, and 2% of green pea, respectively.

In Europe and North America, peas are mostly used in the food industry as cereal flours in food products. Asian and South Americans consume whole or cracked pea seeds. Moreover, their fiber and starch fractions were used for high fiber bread making, adhesive and paper production (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001). Recently, a new bio-based adhesive derived from mimosa tannin extract or soy bean a low molecular mass lignin and tannin without incorporating any synthetic resin was reported (Mansouri et al., 2011; Navarrete et al., 2010). In another study, corn starch-quebracho tannin-based resin 20% (containing 15% corn starch and 5% quebracho tannin) was used instead of phenol-formaldehyde in the fabrication of plywood (Moubarik, Pizzi, Allal, Charrier, & Charrier, 2009). Corn starch-quebracho tannin-based resin had better water resistance and low formaldehyde emission level compared to the commercial phenol-formaldehyde resin (Moubarik, Allal, Pizzi, Charrier, & Charrier, 2010).

2.3.2. Green pea structure, seed and pod

Most legumes including pea and faba bean undergo industrial process, in which seeds are separated from the pods (testa) before preparing fresh, canned, or frozen food (Schieber, Stintzing, & Carle, 2001). Green pea is classified as a dicotyledon positioned in an enlarged ovary/pod to accommodate the seeds inside (Fig. 2.3). Green pea has a closed pod with a rough inner membrane, having a length of 2 to 10 cm. Within the pod, it has 5-12 round seeds which can be yellow, green, beige, reddish orange, brown, reddish blue, dark violet to almost black, or spotted (Pavek, 2012). Pea pod has a green color due to the presence of chlorophyll pigments. These pigments absorb energy from light and contribute to photosynthesis to provide nutrition supply for the seeds (Postiglione, 1976).

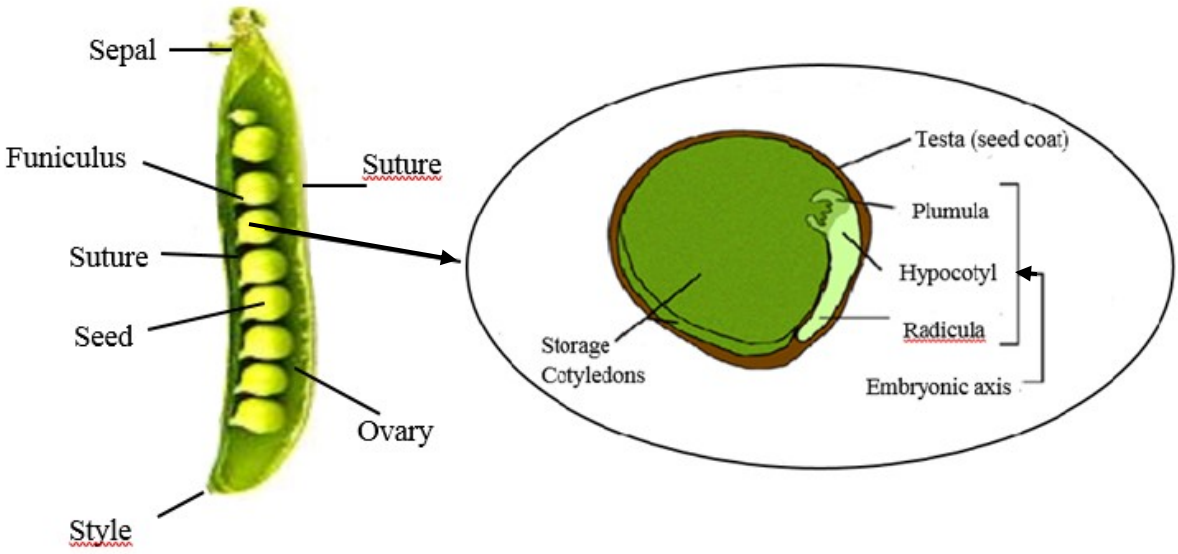


Fig. 2.3. Green pea pod and seed structure (Adapted from Dardick & Callahan, 2014; Finch-Savage & Leubner-Metzger, 2006).

2.3.3. Green pea composition and properties

Table 2.4 shows the compositional analysis of green pea pod in which carbohydrates, total dietary fiber and protein are the main components. Pea pod is rich in dietary fiber (58.0% dry matter), glucose (11.9% dry matter), and sucrose (7.9% dry matter) (Mateos-Aparicio et al., 2010). Proximate composition analysis of green pea pod shows similar composition to green bean pod, and okra (Table 2.4). Green pea seeds have high nutritional value due to the protein and carbohydrate contents (Berrios ,Morales, Cámara & Sánchez-Mata, 2010).

TABLE 2.4. Compositional analysis of green pea pod.

Composition (%)	**Pea pod**	**Bean pod**	**Okra**
Protein	10.8±0.3[a]	13.6±0.2[b]	33.4±0.3[c]
Fat	1.3±0.2[a]	1.3±0.5[a]	8.5±0.3[b]
Moisture	nd	nd	nd
Ash	6.6±0.5[b]	6.3±0.1[b]	3.7±0.2[a]
Total carbohydrates	nd	nd	nd
LMWC	22.7±0.2[b]	26.6±0.5[c]	3.9±0.2[a]
Starchyose + raffinose	nd	1.5±0.1[a]	1.4±0.1[a]
Sucrose	7.9±0.3[c]	6.1±0.2[b]	0.6±0.1[a]
Glucose	11.9±0.6[b]	13.3±0.5[c]	0.2±0.0[a]
Galactose	0.8±0.1[c]	0.3±0.0[b]	0.2±0.0[a]
Arabinose	0.9±0.2[a]	1.3±0.1[b]	1.0±0.1[a]
Fructose	1.2±0.1[b]	4.1±0.3[c]	0.1±0.0[a]
Starch	3.7±0.1[b]	11.7±0.2[c]	0.5±0.0[a]
Dietary fiber (DF)	58.6±1.2[b]	40.1±1.0[a]	54.3±2.3[b]
Insoluble DF	54.4±1.6[b]	30.8±1.2[a]	50.1±2.9[b]
Soluble DF	4.2±0.6[a]	9.3±0.6[b]	4.2±1.8[a]
Total tannin	nd	nd	nd

LMWC: low molecular weight carbohydrates, nd: not determined (Adapted from Mateos-Aparicio et al., 2010). [a-c]Letters indicate significant difference between mean±standard deviations in each row.

2.3.4. Uses of pea and pea by-products

In agriculture, pea plant is a host for *Rhizobium* to fix nitrogen in the soil bringing economic value in the cropping system. Like other pulses, peas are mainly used as food in confectionery and snacks or milled to produce split peas for making flour, canned products, and soups. Moreover, as a forage plant, pea is used as a green manure crop to make hay and silage. In the feed industry, peas are used particularly in the diet of pigs and poultry due to their protein content.

Pea hull fiber (80-88% total dietary fiber and 2-10% soluble dietary fiber) has been used in high fiber wheat bread to increase water retention capacity and dough resistance (Wang, Rosell, & de Barber, 2002). At industrial scale, pea starch containing high amylose percentage was used to obtain flexible films with good mechanical and gas barrier properties (Sun, Sun, & Xiong, 2013) Also, pea pod waste has shown suitable amounts of cellulose (26%), hemicellulose (20.5%), lignin (3.92%), crude protein (20.2%) to be used for butanol production (Leite, de Jesus, Schmiele, Tribst, & Cristianini, 2017). Green pea pod, a by-product, can be used to make cellulases (Sharma, Rawat, Bhogal, & Oberoi, 2015).

2.4. Tannins

2.4.1. History of classification, structures and properties

Tannins are polyphenols, either cross-linked flavonoids or large polymeric structures of flavonoid and phenolic acids. Fossen & Andersen (2006) reported more than 7,000 flavonoids as the most studied group of polyphenols (Fossen & Andersen, 2006).

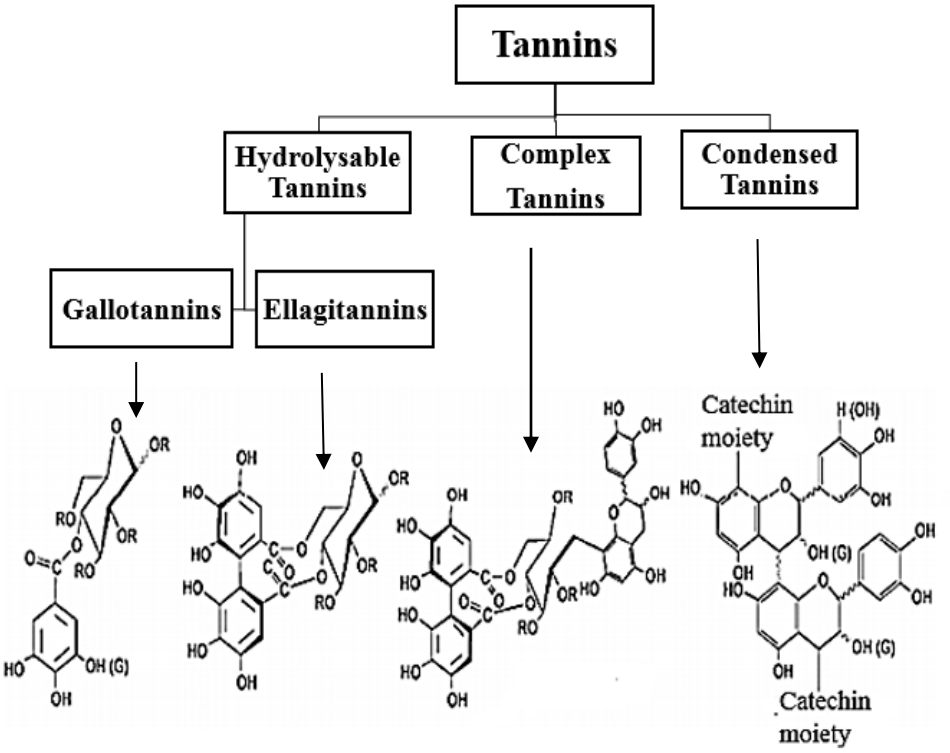


Fig 2.4. Tannin classification, G: galloyl moiety, R: other substitution groups (Adapted from Khanbabaee & van Ree, 2001).

The fundamental structure of a flavonoid is a $C_6{-}C_3{-}C_6$ unit, which consists of two phenyl rings and a heterocycle ring (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; Clifford & Brown, 2005). Tannin compounds are divided into hydrolysable, complex, and condensed tannin (proanthocyanidins) as shown in Fig. 2.4. Hydrolysable tannins are sub-divided into gallotannins and ellagitannins.

Tannins can also be classified as hydrolysable and condensed tannins (Porter, 1989). Tannin molecular weight range was believed to be between 500 and 3,000 Da (Naczk, Nichols, Pink, & Sosulski, 1994) but recent accepted molecular weight for proanthocyanidins is as high as 20,000 Da (Cheynier, 2012). Tea and chocolate, fruits (i.e., berries, raspberries, grape seeds), legumes (i.e., dry beans and sorghum), nuts (i.e., hazelnuts, pecans, pistachios, almonds) and spices (i.e.,

cinnamon and curry) are common natural sources of tannins (Gu et al., 2004; De Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000; Amarowicz, Troszyńska, & Shahidi 2005).

Tannins are known for their complex structures made of one or more aromatic rings attached to several hydroxyl groups. Tannins have special affinity to bind and precipitate proteins and form complexes with starch, cellulose and minerals (Vermerris & Nicholson, 2008).

Tannins are responsible for several bioactive functions such as antioxidant activity, antimicrobial, antiviral and anti-inflammatory properties (Ignat, Volf, & Popa, 2011).

2.4.1.1. Hydrolysable tannins

Hydrolysable tannins can be divided into two groups: gallotannins and ellagitannins. Gallotannins are made of a sugar molecule esterified by different number of gallic acid moieties. For example, isolated gallotannins from plants have a polyol residue derived from D-glucose (Khanbabaee & van Ree, 2001).

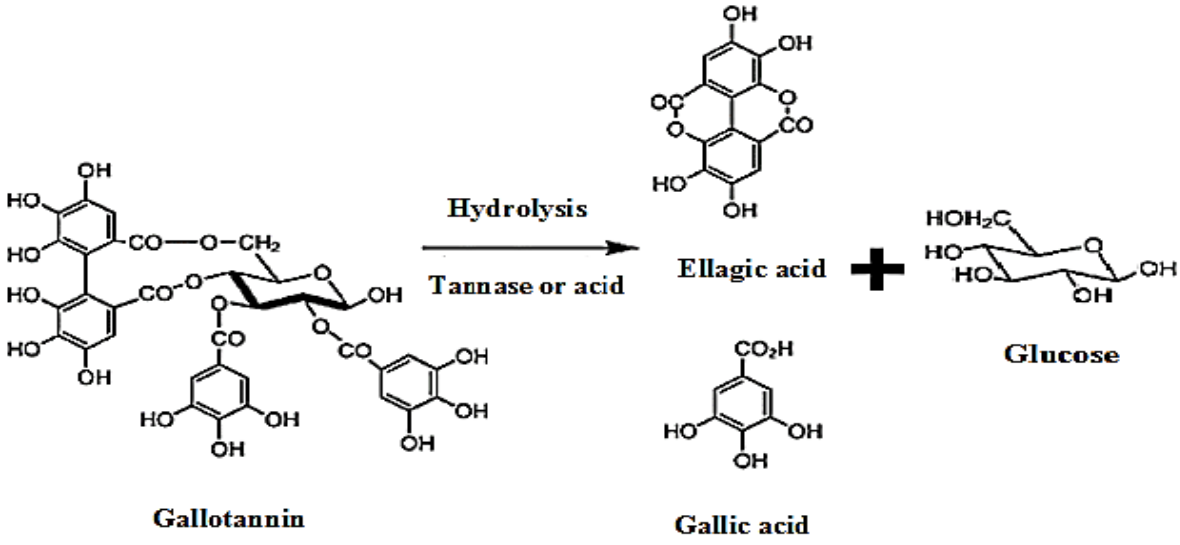


Fig. 2.5. Hydrolysis of hydrolysable tannin into its main components (Molyneux et al., 2007).

Both gallotannin and ellagitannin yield gallic acid and ellagic acid after hydrolysis. Hydrolysable tannins have gained interest because of their nutraceutical potential. Among hydrolysable tannins, gallotannins and ellagitannins show different biochemical properties that result in various health benefits such as anti-diabetic, anti-mutagenic, and anti-microbial (Olivas-Aguirre et al., 2014).

Ellagitannins are present in significant amounts in many berries, including strawberries (63 mg ellagic acid/100g), red raspberries (47 mg ellagic acid/100g), black raspberries (90 mg ellagic acid/100g), wild raspberries (270 mg ellagic acid/100g) (Landete, 2011; Zafrilla, Ferreres, & Tomás-Barberán, 2001), blackberries (150 mg ellagic acid/100g), and nuts such as walnuts (59 mg ellagic acid/100g) (Fukuda, Ito, & Yoshida, 2003), pistachio, cashew nut, chestnuts, oak acorns (Cantos et al., 2003; Mokhtarpour et al., 2014) and pecans (Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos, 2007). They are also abundant in pomegranate (121.1 mg ellagic acid/100g) (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000), and muscadine grapes (36-91.2 mg ellagic acid/100g) (Lee, Johnson, & Talcott, 2005). Also, recent studies show that ellagitannins were found in walnut, pomegranate, oak wine, and berries (Sepúlveda et al., 2018).

2.4.1.2. Condensed tannins

Condensed tannins or proanthocyanidins include the oligomers and polymers composed of favan-3-ol nuclei (Schofield, Mbugua, & Pell, 2001). Condensed tannins are made of at least two linked catechin units C_4 with C_8 or C_4 with C_6. There is no carbohydrate core in the structure of condensed tannins, but they are a range of polymers (Mangan, 1988). Since hydrolysable tannins undergo condensation reaction, condensed tannins are known as proanthocyanidins. Condensed

tannins produce anthocyanidins under acid catalyzed oxidation reaction. Cyanidin (from procyanidin) and delphinidin (from prodelphinidin) are common anthocyanidin (Fig. 2.6).

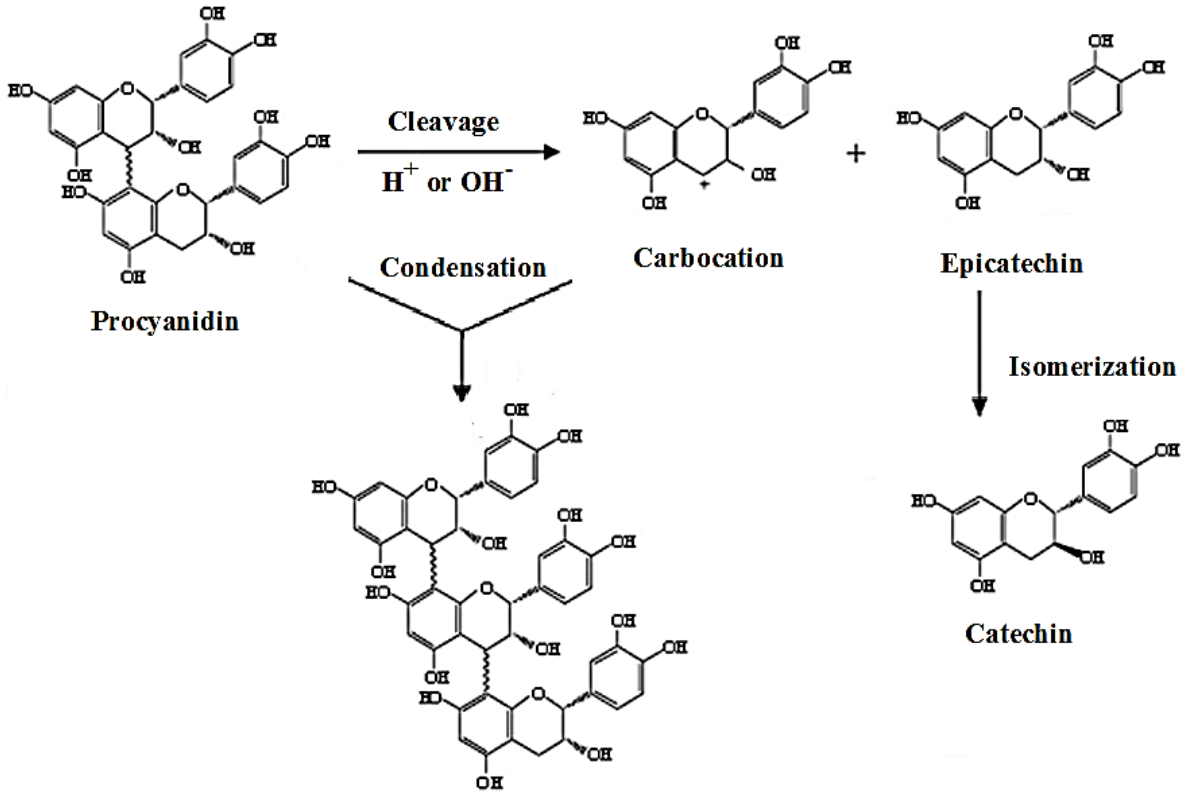


Fig. 2.6. Condensation reaction of proanthocyanidins (Adapted from Jiang et al., 2015)

Condensed tannins extracted from Mexican plants (Mexican blueberry, cuautecomate fruit, garambullo fruit, aubergine, coffee pulp and residues of black grapes) were equilibrated to pH values of 2-12, being stable at pH of 4-8 and 10-70 ℃. Beyond these ranges, they oxidized to a brownish color (García, Aguilera, Contreras-Esquivel, Rodríguez, & Aguilar, 2008). Although tannin sources are widely available, the diversity of their chemical structures and similarity of their behavior in chemical reactions make their identification challenging (Hagerman, Zhao, & Johnson, 1997).

One of the largest sources of tannins are legumes but their chemical nature is still unknown. One study reported proanthocyanidin tannins such as falavan-3-ols either as 2,3-*trans*-flavan-*3*-ols (e.g. gallocatechin or catechin) or 2,3-*cis*-flavan-*3*-ols (e.g. epigallocatechin or epicatechin) (Jin et al., 2012). Also, flavan-3,4-diols (leucocyanidin and leucodelphidin) were identified as the main condensed tannins in horse bean seed coat (Martin-Tanguy, Guillaume & Kossa, 1977). Moreover, Merghem et al. (2004) identified the presence of (+)-gallocatechin-4-phloroglucinol, (−)-epigallocatechin-4-phloroglucinol, (+)-gallocatechin, (−)-epicatechin-4-phloroglucinol, (+)-catechin-4-phloroglucinol, (+)-catechin and (−)-epicatechin in faba seed coat extracted with an acetone/water mixture of 70:30 v/v, containing 0.2 mg/L sodium metabisulphite to prevent oxidation (Merghem, Jay, Brun, & Voirin, 2004).

In 1980, Cansfield's group used methanol extraction and ether precipitation method to fractionate tannin content of faba beans. In that study, the highest and the least polymerized fractions had 0.9-10 mg condensed tannin/g seed coat and 0.7-2.1 mg condensed tannin/g seed coat, respectively (Cansfield, Marquardt, & Campbell, 1980). Moreover, dry bean seed coat is the main source of tannins whereas tannin concentration in bean cotyledon is low or negligible (Ma & Bliss, 1978). Another study also confirmed that seed coat of other legumes like cow pea had mostly tannins located between the outer integument and the aleurone layer (Lattanzio et al., 2005).

Table 2.5 shows tannins in various plant sources such as sorghum grain, berries, pomegranate, plant leaves, green tea and carob pod. Sorghum has condensed tannins, causing weight loss in children and lowering digestability and protein efficiency in rats. Also, the presence of prodelphinidins in plant leaves prevents protein and cellulose degradation in rumen and help to control bloat. A recent study showed that the addition of condensed tannin and ellagitannin to

commercial young Lambrusco red wine could stabilize wine's color without affecting wine reactivity towards salivary proteins (Picariello et al., 2018). Moreover, extruded sorghum cereals containing tannins increased the total antioxidant capacity and superoxide dismutase in patients with chronic kidney disease (Lopes et al., 2018). Also, mimosa condensed tannins showed inhibitory effect on ruminal metabolism and biohydrogenation compared to chestnut hydrolysable tannins (Costa et al., 2018).

TABLE 2.5. Tannin source and type with its nutritional benefit.

Plant source	**Tannin**	**Tannin type**	**Nutritional benefit**	**Reference**
Sorghum *L.Moench* (grain)	Catechin	Condensed	Weight loss in children, Lower DMD & PER in rats.	Asquith & Butler (1986); Maxson et al. (1973)
Onobrychis viciifolia L. (leaves)	Prodelphinidin	Condensed	Protection of leaf protein from rumen degradation, bloat control	Osbourn et al. (1971); Jones & Mangan (1977)
Lotus pedunculatus L. (leaves)	Proanthocyanidin	Condensed	Protection of leaf protein from rumen degradation, less weight gain in sheep, inhibit carbohydrates digestion	Barry & Manley (1986)
Acacia nilotica (leaves)	(+)-Catechin mono and digallates	Condensed Hydrolysable	Precipitation of leaf proteins, inhibition of rumen fermentation	Self et al. (1986)
Camellia sinensis (green tea)	(-)- Epicatechin, (-)-Epigallocatechin gallate	Hydrolysable	Percipitation of milk and other dietary proteins	Bradfield & Bate-Smith (1950)
Leucaena Leucocephala (leaves)	Gallotannin, Catechin	Hydrolysable & Condensed	Inhibition of digestibility	D'Mello & Fraser (1981)

TABLE 2.5. Continue.

Quercus robur (leaves, callus)	Gallotannin, Ellagitannin Catechin	Hydrolysable & Condensed	Inhibition of tryptic digestion of protein	Feeny (1969); Haddock et al. (1982)
Lespedeza cuneate (leaves)	Prodelphinidin	Condensed	Inhibition of rumen fermentation of cellulose and proteins	Bell et al. (1965)
eratonia siliqua (carob pods)	Galloyl-D-glucose, Flavan-3-ol-gallate	Hydrolysable	Depression of growth in rats and chickens, inhibition of rumen fermentation	Tamir & Alumot (1970); Joslyn et al. (1968); Haddock et al. (1982)
Pulses	Total tannin	Condensed & hydrolysable tannin	Lowering blood glucose and hormonal responses to starchy foods Decreasing blood lipid and cancer risk	Champ, (2002)
Carob pod	Tannin	Condensed tannin	Making complex with proteins and fiber compounds and cause overestimation of dietary fiber	Saura-Calixto, (1988)
Vicia faba seed	Polyphenolics & tannin	Condensed tannin	*In vivo* inhibition of D-glucose across rat small intestine	Barcina et al. (1984)
Pomegranate juice	Hydrolysable tannin	Ellagic acid	Reducing prostate tumor growth and prostate-specific antigen in mice, inhibition the proliferation of human cancer cells	Seeram et al. (2006)
Edible berries	Hydrolysable tannin	Ellagic acid	Protection against cancers of the colon, lung, and esophagus	Nile & Park (2014)

DMD: dry matter digestibility, and PER: Protein efficiency ratio.

2.4.2. Tannin uses

Different tannin compounds can be used in the process of leather production based on the type and application of the final leather, availability and cost. In 2015, the FAO reported 1.85 billion square meter of annual leather production worldwide accounting for C$130 billion dollars as an exchange value for the leather industry (FAO, 2015). Mainly, a large part of the produced leather is used in footwear, gloves, bags, clothes, furniture and cars (Covington & Covington, 2009; Laurenti, Redwood, Puig, & Frostell, 2016). But, the production of leather can cause environmental problems bringing human and animal health issues.

The role of tannins is to prevent animal hides from decaying, making them resistant to water, and keep them flexible and durable. To date, chromium (III) salts are the current tanning agents in 80-90% of worldwide leather production (Guillén et al., 2011). Statistics illustrates that the chemical compounds, which are consumed in the process of one metric ton raw-hide yields 200 kg of leather along with 800 kg of solid waste. This solid waste includes tanned solid waste (250 kg), non-tanned solid waste (350 kg) and the rest (200 kg) disposed into the wastewater stream. Also, 45-50 m^3 water and high quantity of chemical reagents (total 400 kg) such as sodium chloride, lime, sodium sulfide, sulfuric acid and basic chromium sulfate are consumed for the processing of one metric ton hide into leather (Kanagaraj, Velappan, Babu, & Sadulla, 2006). Thus, these wastes are potential threats to public health and the environment (Famielec & Wieczorek-Ciurowa, 2011). Instead of chromium (III) salts, tanning agents extracted from vegetables can provide similar tanning functions and avoid the production of toxic waste (Achabou & Dekhili, 2013). Also, mixing these tannins with minerals (Aluminum, titanium, zirconium, chromium) or aldehydes (formaldehyde, glutaraldehyde) (Covington, 1997) provide stiffness, and UV resistance to the leather. In the United Kingdom, oak bark tanning process took

approximately 3-6 months to tan the hides. To meet the increasing demand for leather production with less time-consuming processes, other new sources of vegetable tannins are needed. One vegetable tannin currently commercialized is Valonea extracted from acorn cups of the oak tree (Onem, Gulumser, Renner, Oelbermann, & Yesil-Celiktas, 2015). Also, oak bark has been extensively used in Europe as a vegetable source for leather making (Falcão & Araújo, 2018).

Myrobalan, the dried fruit of an Indian tree, quebracho extract from heartwoods of *Schinopsis brentzii* from Argentine, wattle or mimosa extract from bark of mimosa, *algarobilla* (a fruit bearing pod), valonea (the acron cups of the bearded oak *Quercus*) and sumac from the leaves of *Rhus sp.* are some of these sources (Haslam, 1989). Among them, the dried extracts of mimosa, chestnut bark (Krisper, Tišler, Skubic, Rupnik, & Kobal, 1992), and quebracho wood were used for manufacturing high-priced leather products (Seigler, Seilheimer, Keesy, & Huang, 1986). The tanning process is based on the diffusion of tannins into the animal hide and interactions of tannin with the collagen available in the animal hide that stabilizes the hide for the tanning process (Maier, Oelbermann, Renner, & Weidner, 2017).

Moreover tannins are widely distributed in non-usable parts of fruits and vegetables. In this way, leather-making process could become environmentally friendly.

2.4.2.1. Tannin in human health and nutrition

To date, tannic acid and its polymer structures have been introduced as lowering factors of feed intake, growth rate, protein digestibility and feed efficiency as tannins form complex with starch, protein and digestible enzymes (Chung et al., 1998). For example, it has been shown the negative effect of tannins on carbohydrate absorption in sorghum (Blakeslee & Wilson, 1979). Likewise, affinity of tannin molecules towards carbonyl groups in protein structures might form cross links with peptides and make them indigestible (Salunkhe, Jadhav, Kadam, Chavan, &

Luh, 1983). These types of interferences in absorption of nutrients due to the presence of tannins emphasize the low nutritional value of foods rich in tannins. Moreover, association of esophageal cancer with consumption of raw betel nuts (21-30% catechin tannin) and herbal teas rich in gallic acid or tannic acid (5-5000 μM) were reported (Chung et al., 1998). In some studies, oral cancer in the throat and the esophagus were attributed to betel chewing (Morton, 1972). However, other studies reported anticarcinogenic role of tannin consumption. There was a negative relation between stomach cancer (Stocks, 1970) and gastric cancer (Kono, Ikeda, Tokudome, & Kuratsune, 1988) with tea consumption. Tannin-containing plant extracts have been used as diuretics for treating diarrhea in Chinese and Japanese medicinal remedies, astringent for stomach and duodenal tumors, and anti-inflammatory, antiseptic, and haemostatic pharmaceuticals (Takuo Okuda, 1991).

Administration of quercetin to rodents has shown anticarcinogenic effect against skin and colon cancer (Leighton et al., 1992; Chung et al., 1998). Gallic acid could impede production of destructive nitrosamine. A significant inhibition effect was demonstrated with consumption of ellagic acid on colon, liver, lung, and tongue cancer in rats, mice and humans (Das, Bickers, & Mukhtar, 1985; Maas, Galletta, & Stoner, 1991). Also, ellagic acid is consumed as a food additive, providing antioxidant activity in some countries, like Japan (Sepúlveda et al., 2018). In Northern Europe, berries are the tannin source for daily use and the oak bark tannin has been consumed in traditional remedies without any toxic effect (Paaver, Matto, & Raal, 2010).

Another positive effect of condensed tannins is their role as antimicrobial agents. An example is the inhibitory role of tannic acid and propyl gallate against food borne bacteria, and aquatic bacteria (Chung et al., 1998). This inhibitory role is induced by tannin capability in quenching electrons from electron transfer chain surrounding the bacterial membrane. Thus, instability of

electron flow disorganizes the normal oxidative phosphorylation and stop the bacterial growth. The antimicrobial nature of tannic acid, gallic acid, and propyl gallate were used in the production of catfish fillets with a shelf life of 15 days (Maqsood, & Benjakul, 2010; Vattem & Shetty, 2005). Further, tannin provided other physiological functions such as lowering blood pressure and lipid serum, stimulation of blood clotting and immune response regulations (Bhargava & Westfall, 1969; Yugarani, Tan, & Das, 1993).

It has been found that faba bean leaf and flower is a source for L-DOPA (L-3,4-dihydroxy phenylalanine). The L-DOPA is a glucoside that has a similar structure to vicine-convicine (Bjerg, Eggum, Jacobsen, Olsen, & Sorensen, 1984), which was proposed as an ingredient for Parkinson's disease synthetic medicine (Ray & Georges, 2010). The amount of L-DOPA in flowers and leaf tissues ranged from 27.8-63.5 (mg/g dry matter) and 18.2-48.7 (mg/g dry matter), respectively (Hu et al., 2015).

The importance of dietary fiber in pulses is related to their beneficial effect in controlling heart disease, diabetes, obesity, and reduction of colon, rectal and breast cancer (Marlett, McBurney, & Slavin, 2002). Consumption of pulse fiber and resistant starch could play a controlling role to manage blood sugar (Lehmann & Robin, 2007). Pulses with a low glycemic index have proved to help glycemic control in type 1 diabetic patients (Lafrance, Rabasa-Lhoret, Poisson, Ducros, & Chiasson, 1998; Thorne, Thompson, & Jenkins, 1983). The main reason is the poor digestibility of starch in pulses, which delays postprandial glucose level change, insulin response and the glycemic index (Jenkins, Wolever, Taylor, Barker, & Fielden, 1980). Moreover, tannin presence in faba bean might be used to complement the potential demands in nutrition, cosmetics and pharmaceutical industries (Aires, Carvalho, & Saavedra, 2016).

2.4.2.2. Legume as a fiber source

Cellulose, hemicellulose, lignin, pectin and various gums are common fibers found in plant cell walls (Fig. 2.7). Since the digestion mechanism of such fiber compounds in the human and animal body is different, they are classified into two categories according to their solubility: soluble fiber and insoluble fiber (Marlett, McBurney, & Slavin, 2002).

Soluble fibers are water-soluble pectins that originate from fruits, guar gum, beans and cereals. While insoluble fibers include cellulose, polysaccharides, and lignin (Saura-Calixto, Pérez-Jiménez, & Goñi, 2009). Whole grains, legumes, vegetables, nuts, seeds, and fruits are natural food grade sources of dietary fiber (Turner & Lupton, 2011). Among them, pulses are considered as low glycemic index sources because they are rich in total fiber as well as in resistant starch (Messina, 2014).

Although dietary fibers are indigestible in the human stomach and small intestine, their consumption is recommended due to the benefits for health maintenance and disease prevention. Such benefits include lowering blood cholesterol level (Brown, Rosner, Willett, & Sacks, 1999), insulin level, and normalization of blood sugar (Ou, Kwok, Li, & Fu, 2001; Qi, Rimm, Liu, Rifai, & Hu, 2005). Dietary fiber content also relates to its therapeutic effect on diabetes (Anderson & Ward, 1979; Jenkins, Wolever, Taylor, Barker, & Fielden, 1980) and hyperlipidemia (Anderson et al., 1984). For these benefits, there are specific recommendations for fiber daily intake by the American dietetic association of 20-35 g fiber per day for adults, which is equal to 10-13 g of dietary fiber per 1000 kcal (Williams, Bollella, & Wynder, 1995). Moreover, dietary fibers can play prebiotic role to improve the growth and activity of beneficial bacteria in human digestive tract by acting as food for the beneficial human intestinal microflora (Slavin, 2013).

As observed in Fig. 2.7, dietary fiber includes soluble polysaccharides, non-cellulosic polysaccharides, cellulose, and lignin (Anderson & Bridges, 1988). Previous studies removed dietary fibers from plants and used them as functional non-meat ingredients in beef patties, fat replacer, texture modifier in meat products to prevent quality changes of frozen meat products after the freezing/thawing processes (Kim, Miller, Lee, & Kim, 2016).

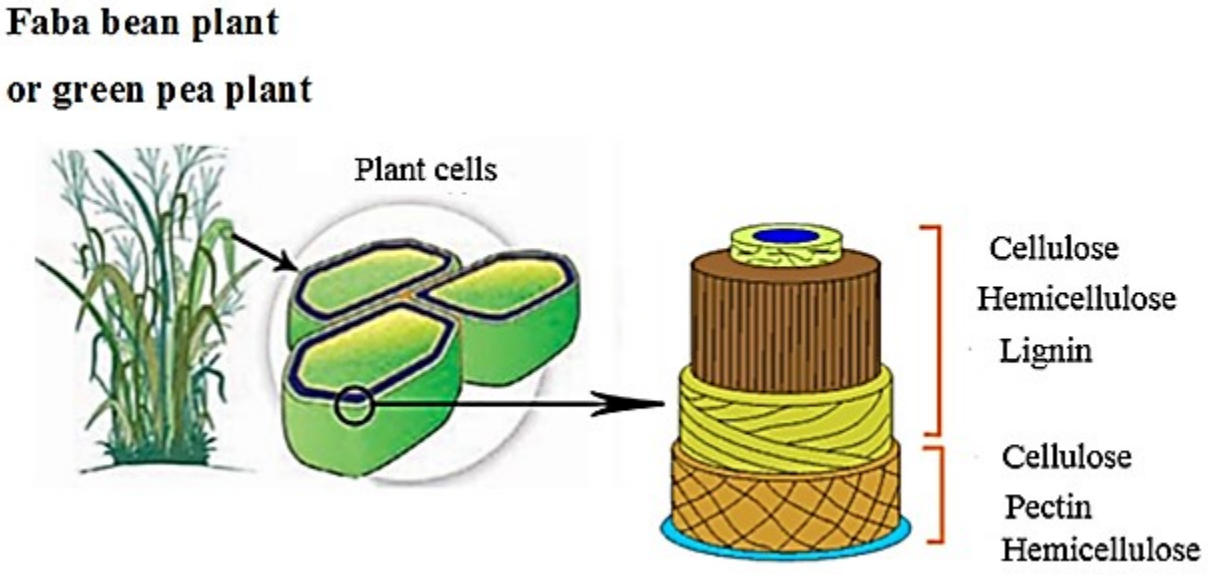


Fig. 2.7. Internal view of a plant cell wall with fiber components.

β-Glucan is one of the soluble fibers in oat, barley, algae, and mushroom. Guar gum is a galactomannan, a combination of galactose and mannose, found in most leguminous seeds.

Pectin is another common fiber found in the primary cell wall and intracellular layer of plant cells. For example, the peels of apple and citrus fruit contain 0.5–3.5% of pectin. Among insoluble fibers are cellulose, hemicellulose, chitin, chitosan, and lignin (Mudgil & Barak, 2013). According to recent studies, not only fruit and vegetables but also their by-products are potential sources of dietary fiber. Pea and broad bean pods are two examples of dietary fiber sources containing cellulose and xyloglucans. In broad bean pod, the main monomers are glucose, uronic

acid and xylose, indicating the presence of cellulose attached to pectin. Likewise, high amount of xylose in pea pod shows the occurrence of xyloglucans and xylans (Table 2.6).

TABLE 2.6. Sugar composition of pea pod and broad bean pod.

Legume	Glucose (%)	Uronic acid (%)	Arabinose (%)	Galactose (%)	Xylose (%)
Pea pod	42-43	9	2	4	39-41
Broad bean pod	40-45	16–24	13–14	13–14	8

Adapted from Mateos-Aparicio, Redondo-Cuenca, & Villanueva-Suárez (2012) and Guillon & Champ (2002).

2.5.Extraction methods

Extraction is a process used to separate the desired compounds from the mixture of a solid matrix. The separation process depends on the permeability of the target compound through the complex structure of the main matrix. For example, to diffuse out water soluble components of low molecular weight compounds like phenolics from fruit and vegetable matrices, the osmotic barrier of tissue should be broken (Cassano, 2017). Grinding using a mill breaks the cell structure for better extraction. Also, heating disintegrates the tissues to aid the transfer of desired compounds from inside to the solvent media. However, precautions need to be taken when applying heat to avoid any degradation of target labile compounds. Degradation can also occur due to the change of pH (Amendola, De Faveri, & Spigno, 2010; Chethan & Malleshi, 2007). For pH sensitive compounds, the use of a specific buffer system is essential to control degradation during the extraction. Therefore, proper pre-treatment of the sample before extraction is needed either for separation of the target substance from the matrix or to eliminate the effect of interferences in the matrix. For this reason, non-polar solvents are used to separate fats, waxes, chlorophyll, and carotenoids from plant matrices. Similarly, unwanted interfering

elements should be removed from the matrix. Some divalent cations can remain intact and initiate a crosslink with other compounds; therefore, chelating agents like ethylenediamine tetra acetic acid (EDTA) could be used (Vassil, Kapulnik, Raskin, & Salt, 1998).

Since some food components are thermally sensitive and vulnerable to chemicals, a suitable extraction method should be chosen to prevent loss of nutritional compounds, lead to high yield, use short time and require low energy consumption. These considerations hinder prolonged heating, stirring, and use of large volumes of solvents (Chemat et al., 2017). These shortcomings motivated researchers to apply different sustainable extraction methods for separation of components from a complex matrix. Normally, knowing the structure and main components of the selected matrix is required to suggest the best separation procedure. For solvent extraction, a suitable solvent is mixed with homogenized ground sample. Extraction time and temperature are selected, considering the nature and solubility of the compound in the selected solvent. The best solvent provides the best extraction of the desired compound with minimal decomposition, isomerization or polymerization. Also, stability of the compound after extraction contribute to the selection of the extraction method.

2.5.1. Conventional extraction

The most common conventional extraction methods are Soxhlet, ultrasound assisted solvent extraction (Chen et al., 2014; González-Centeno et al., 2014), and microwave assisted solvent extraction (Chupin et al., 2015).

The Soxhlet method only works at the boiling point of the solvent where there is less surface tension and viscosity, and the solvent can easily penetrate into the matrix (Markom, Hasan, Daud, Singh, & Jahim, 2007). However, extraction using the Soxhlet method requires boiling temperatures of the solvent for a long time which is not compatible with the nature of thermal

sensitive compounds (De Castro & Garcıa-Ayuso, 1998). For this method, the type of solvent and physical characteristics of the matrix, like particle size, are the two main factors influencing the time and extraction efficiency (Wang & Weller, 2006). Moreover, a large quantity of waste organic solvents remains after Soxhlet extraction.

Another concern related to traditional extraction methods is the use of considerable volumes of petrochemical solvents (e.g. methanol, acetone, chloroform, and n-hexane). Therefore, safety cautions should be considered to deal with the solvent toxicity. The solvent consumption depends on the chemical structure and physicochemical property of the target component. Boiling aqueous methanol, aqueous acetone, or acidic methanol are mostly used for condensed tannins (Bate-Smith, 1977), with low recovery up to 30% (Martin & Martin, 1982: Bate-Smith, 1977; Swain, 1979). Ether has been shown to be a good solvent for separation of condensed tannins in combination with the Sephadex LH-20 and G-50 chromatography (Salunkhe et al., 1983). However, the use of Sephadex LH-20 for condensed tannin has been reported as a tedious process in which methanol or 70% acetone is used for washing low molecular tannins (Butler, Hagerman, & Price, 1980). Price et al. (1978) stated that acidic methanol with HCl is the best solvent for the extraction of condensed tannins from sorghum (variety not mentioned) (Price, Van Scoyoc, & Butler, 1978).

Acidic methanol was used for hydrolysable tannins at pH<3 as above this pH range (pH=5-6), methanolysis of depside bonds (aromatic ester bonds) occurs. Moreover, Hagerman (1988) found that for the leaves of oak and lyophilized maple using 70% aqueous acetone was more efficient compared with methanol or 1% HCl in methanol (Inoue & Hagerman, 1988). But, no quantitative estimates for the recovery of hydrolysable tannins from such plants were reported when aqueous acetone was used (Inoue & Hagerman, 1988).

To achieve the best results in the extraction of hydrolysable tannins from plant tissue, it is recommended to use fresh samples. If dried samples are used, freeze drying is preferred over air drying to prevent loss of water soluble components (Okuda, Yoshida, & Hatano, 1989).

Selection of a suitable solvent for the extraction depends also on the molecular weight of the compound. Methanol is mostly used for low molecular weight compounds. Also, extraction of 10-20% w/w tannic acid from galls of *Q. infectoria* plant obtained at 45℃ dissolving 100 g sample in 500 mL acetone within 24 h (Basri & Fan, 2005).

Microwave assisted extraction of nutraceuticals initiate internal and homogenous heating, promoting changes of plant cell wall (Wang & Weller, 2006). Variables such as temperature, time, and solvent and microwave irradiation power are involved in the extraction. Among these factors, temperature (67℃) and polarity of the solvent were significant and showed a positive effect on microwave assisted extraction of phenolic compounds from plants. The main advantages of the use of microwave assisted extraction are the considerable reduction of time to 30 min, and solvent consumption (15 g/125 mL) and increased purity of the plant extract (Asghari, Ondruschka, & Mazaheritehrani, 2011). Also, microwave power of 225W within 120 sec at temperature of 60-68℃ resulted in removal of 407 mg tannic acid /g grape seed using methanol as solvent was compared to microwave power of 150 W and 300W within 20-200 sec (Hong et al., 2001). It was demonstrated that using microwave power of 500W, a solid to solvent ratio of 1/35 g/mL, at 30°C within 15 min 128.65 mg/g tannin could remove from Chinese herb *Agrimonia pilosa Ledeb* (Jin, Wang, & Chen, 2010).

In the tanning process of leather making, ultrasound power has been used as a novel technique to improve the mechanism of natural dye extraction by rupturing the cell wall and transporting of the released dye in to the external medium (Sivakumar, Vijaeeswarri, & Anna, 2011).

As an example, ultrasound power of 100 W had increased mass transfer of the solid-liquid extraction of natural dyes from Avaram bark (*Cassia auriculata*). Sivakumar et al. (2014) reported that the total natural dye extract in ultrasound assisted extraction was 1.6-fold higher than the total extract obtained using a magnetic stirring process (Sivakumar et al., 2014).

Ultrasound power of 20–100W has been used for the extraction of natural dyes from myrobalan nut or wattle bark (Sivakumar, Verma, Rao, & Swaminathan, 2007) (Sivakumar et al., 2009) and natural dyes as alternatives to synthetic dyes from Beetroot, Green wattle bark, Marigold flowers, Pomegranate rinds, 4'o clock plant flowers and Cocks Comb flowers in leather making (Sivakumar et al.,2009; Sivakumar, Vijaeeswarri, & Anna, 2011).

Due to long extraction times and the use of toxic solvents for conventional extraction, subcritical water extraction is a potential method to prevent imperfections of other extraction methods.

TABLE 2.7. Tannin extraction and analysis from different sources.

Matrix (g)	**S-L ratio**	**Extraction**	**Analysis**	**Yield (%)**	**Target compound**	**Reference**
Cow pea seed coat (0.1 g)	NR	acetone:water 70:30 v/v, t:30 min, T:30 ℃, centrifuge 12000g 5 min	Condensed Tannin: modified vanillin method (Burns,1971) ABS read at 500 nm	89.2 g catechin/100g seed coat	Lignin and Tannin	Morrison et al. (1995)
Sorghum grain (0.2 g)	10 mL	methanol:water 1:1 v/v	Modified vanillin -HCl	42% of catechin equivalent	Tannin	Price,Van Scoyoc, & Butler (1978)
Tannin and non-tannin sorghum grain (1g)	1:50 w/v	50 mL methanol t:20-28 h	Modified vanillin-HCl method of Price, Van Scoyoc, and Butler (1978) Fluorescence detection	Tannin: 4.53 g catechin/100 g Total Polyphenols: 1.34 g catechin/100 g	Tannin and total phenol	Adetunji, Duodu, & Taylor (2015)
Sorghum grain (2g)	25 mL acetone:water 70:30 v/v 1:20 w/v	95% ethanol two-dimensional TLC on silica gel	Spectrophotometry, Paper chromatogram, Sephadex LH-20 column	NR	Condensed Tannin	Strumeyer & Malin (1975)
Forage legume plants (0.34g)	3 mL acetone:water 70:30 v/v with ascorbic acid (1g/L)	Solvent extraction	Butanol/HCl & Vanillin/HCl	0.15-18.7 % DM	Condensed Tannin	Terrill et al. (1992)
Faba bean (5g)	5:1 w/v bean:water T: 21 °C t:12 h	Dehulling: T:21◦C, t:12 h extrusion: T: 140 and 180 °C	Folin Denis (Swain & Hillis, 1981) Vanillin method	Tannin in whole beans were 1.55% (FD) and 0.67% vanillin	Tannin content	Van der Poel,Gravendeel, & Boer (1991)
Low-tannin faba bean (0.2g)	8mL ethanol 80% (v/v)	Solvent extraction T:50 ℃ T:1 h	Solid-phase micro-extraction technique Gas chromatography-mass spectrometry	TP=5.5-41.8 mg catechin/g	Phenolics, Phytic acid, and phytates	Oomah, Razafindrainibe, & Drover (2014)

TABLE 2.7. Continue.

Faba bean shoot (0.05g) Roots (0.1g)	Hydrolysis:4 mL 62.5% methanol and 1 mL of 8 M HCl Reflux, T: 90 ◦C t:2 h	Acid hydrolysis 50% methanol, 1.6 M HCl	HPLC solvent A: 99.5% water and 0.5% acetic acid Solvent B:100% acetonitrile Flow rate:0.3 mL/min	NR	Flavonoid aglycones (kaempferol, luteolin, and quercetin)	Li et al. (2012)
Beach pea (*Lathyrus maritimus* L.), green pea and grass pea (1-2 g)	1:40 and 1:20 w/v T=40 °C	Acid hydrolysis 70% acetone, containing 1% concentrated HCl	vanillin-HCl assay	Beach pea: 11.6% Indian grass pea:1.54%, Canadian grass pea:109 % green pea:72 %	Condensed tannin	Chavan, Shahidi & Naczk (2001)
Tea leaves	NR	T:100-200°C P:100 bar t:10 min	Subcritical water extraction HPLC Solvent A: acidified water (2% acetic acid) Solvent B: methanol–water–acetic acid (90:8:2)	0.62, 3.31 mg/g	Catechin & epicatechin	Piñeiro, Palma, & Barroso (2004)
***Terminalia chebula* Retz fruit (1g)**	NR	T: 120–220°C F: 2–4 mL/min P: 40 bar	Subcritical water extraction	14.72, 5.38, and 5.86 mg/g	Gallic acid, Ellagic acid, & Corilagin	Rangsriwong et al. (2009)
***(Viciafaba L)*, seed coat (16.5 g)**	16.5:300 w/v	Maceration 13h, second maceration for 30 min in 150 mL ethanol, rotary-evaporation at 50°C and made up volume with saline solution (0.9% NaC1) to 160m	Spectrophotometry, analytical method was not reported	0.28±0.01 mg/mL 0.18±0.01 mg/mL	Catechin, & anthocyanogens	Barcina et al. (1984)
Pomegranate Rind (1 g)	1:20 w/v Solvent: water	Pre-cut dried powder T:90°C, t:45 min pH:11	UV-Visible Spectrophotometer	26% w/w	Total tannin	Prabhu & Bhute (2012)

TABLE 2.7. Continue.

Pomegranate juice	Soluble solid 15.5%	Hand pressed juice	Folin-Ciocalteu Uv-visible Spectrophotometer at 660 nm	1.21*	Ellagic Acid	Gil et al. (2000)
Pomegranate peel (*Punica granatum L.)*	0.2 mm 1:8 w/v Solvent: water, ethanol, & acetone	Maceration T: 60 ◦ C, t:62 min pH:6	Condensed tannin: acidic ferrous Hydrolysable tannin: KIO3 method Uv-visible Spectrophotometer at 550 &530 nm	Hydrolysable tannin: 579.54 mg tannic acid/g Condensed tannin: 18.64 mg cyanidin/g	Condensed & hydrolysable tannin	Ben-Ali et al. (2018)
***Averrhoa bilimbi* fruits (5.3 g) and leaves (5.5 g)**	125 mL acetone/water 7:3 v/v	Magnetic stirring, 1 h, vacuum drying of solvent at 30°C	Liquid chromatography–mass spectrometry analysis	0.8 g/100g fruit, 1.3 g/100 g	Proanthocyanidins	Ramsay & Mueller-Harvey (2016)
Strawberry & raspberry (1 g)	1:100 w/v acetone and water 80:20 v/v	Total 3 extractions, every 4 h with 100 ml solvent t:12 h	Hydrolysis of extract at 100°C with an equal volume of 4 N trifluoroacetic acid	strawberry (0.63 mg/g) & raspberry (1.5 mg/g)	Ellagic acid	Daniel et al. (1989)
Berries fruit (*Fragaria & Rubus Species)* (5 g)	5:40 w/v	vigorous vortexing of samples with ethyl acetate (4 × 10 mL)	20 mL extract evaporated to dryness with a rotary evaporator, dissolved in 1 mL of methanol, and analyzed with LC-DAD	0.9-2.7 mg/g	Ellagic acid	Määttä-Riihinen et al. (2004)
Walnuts (Juglans regia L.) (10 kg)	Crushed walnuts, 5 mm, 70% ethanol	T: room temperature, t:24 h concentration of extract with hexane (1: 1×3), EtOAc (1:1×3), and n-BuOH (1:1×3), successively	^{1}H and ^{13}C NMR	NR	Hydrolysable tannins, Glansrins A–C	Fukuda, Ito, & Yoshida (2003)

TABLE 2.7. *Continue.*

Muscadine grapes (*Vitis rotundifolia*) (5 g)	Methanol/water 10% v/v	Waters C18 Sep-Pak cartridges and hand-packed Sephadex LH-20 cartridges	HPLC-ESI-MS	0.36-0.91 mg/g	Ellagic Acid Conjugates	Lee, Johnson, & Talcott (2005)
Pecan kernel (1 g)	20 mL Acetone/water 70:30 v/v	Defatted & dissolved insolvent, centrifuged at 18,000 g, supernatants flushed with nitrogen	vanillin assay	34±1.3 mg/g	Condensed tannin	Villarreal-Lozoya, Lombardini, & Cisneros-Zevallos (2007)
Pistachio hull (0.2 g)	<0.5 mm 10 mL70% aqueous acetone 0.2:10 w/v	Solvent extraction, t:12 h, centrifuge at 3000×g at 4°C	Non- tannin compounds: Folin-Ciocalteu Tannin compounds: PVPP	0.31%	Total tannin	Mokhtarpour et al. (2014)
Almond seed (1 g)	Acetone 80% 1:10 w/v	Defatted with hexane in a Soxhlet, solvent extraction, T:50°C, t:30 min	Modified vanillin Uv-visible Spectrophotometer at 660 nm	28.3 ± 0.6 **	Total tannin	Amarowicz, Troszyńska, & Shahidi (2005)
Eucalyptus (*E. globulus*) bark & chestnut (*C. sativa*) shell	Bark: 1:10 w/v Shell: 1:15 w/v ethanol–water 80/20 v/v methanol–water 50/50 v/v	Soxhlet, t:2 h, boiling point of the solvent,	Folin-Ciocalteu UV-visible Spectrophotometer at 760 nm	Chestnut: 55.8% Eucalyptus: 18.3%	Total phenolics	Vázquez et al. (2008)
Pine bark (1 g)	1:10 w/v Ethanol: (5,10, & 15) % v/v	Solid-liquid extraction, 2h, adittives:NaOH (0.5, 1.0 & 1.5%, w/v); formic acid (0.5, 1.0 & 1.5%) v/v T: room temperature	Condensed tannin: vanillin-H_2SO_4 Hydrolysable tannin: reaction with KIO_3	34.8 gallic acid equivalent 62.8 catechin monohydrate	Condensed & hydrolysable tannin	Seabra et al. (2018)

TABLE 2.7. Continue.

Spruce (Picea abies Karst) (2g)	<50 µm Hot water (22mL)	PHWE system Sample+ 1g diatomaceous earth in 22 mL water T:90°C, P:100 bar, t:20 min	Folin-Ciocalteu method	Total tannin: 0.03-0.05%	Total tannin	Ding et al. (2017)
Chestnut Bark	350 mg in 20 mL methanol	T: room temperature, t:30 min, vortex 1 min, sonicate using ultrasonic bath (35 kHz)	Folin–Ciocalteu UV-visible spectrophotometer at 750 nm HPLC	Total tannin: 4.75-16.73%	Total tannin	Comandini et al. (2014)
Oak tree (*Quercussp.*) acorn cups (valonea) **(5 g)**	0.25-1.5 mm Solvent/feed: 100 w/w, H2O/CO2 binary system	Supercritical CO2 T:80°C, P:100 bar t: 3 h, 2 h 10 min pressurization & 50 min depressurization	Titration with potassium permanganate ($KMnO_4$)	Total tannin: 34.73±0.02%	Total tannin	Onem et al. (2015)
Myrobalan **(20 g)**	1:6 w/v 120 mL distilled water	ultrasonic power 80 W, T:40°C, t:2 h	Gravimetry	Extraction efficiency: 90%	Natural dye	Sivakumar et al. (2007)
Sumac (*R. coriaria L.*) leave	2-4 mm	counter-current extraction procedure with a four-vessel T:45°C, t:60 min without agitation	Folin–Ciocalteu UV-visible spectrophotometer at 760 nm	23.3mg tannic acid/g leaves	Total tannin	Zalacain et al. (2003)
Carob pod **0.01-0.02 (g)**	<0.3 mm 10 mL water	Extract obtained after enzymatic removal of proteins and fiber T:100 t:3 h	Acid butanol method UV-visible spectrophotometer at 550 nm	Condensed tannin: 17.1±0.4%	Condensed tannin	Saura-Calixto (1988)

S-L: solid-liquid, NR: not reported, FD: Folin Denis, and PVPP: polyvinyl polypyrrolidone, T: temperature, F: flowrate, P: pressure, t: time, DM: dry matter, TP: total phenolic, *mg/mL, **A_{500}/g, NaOH: sodium hydroxide, PHWE: pressurized hot water extraction, KHz: kilohertz.

2.5.2. Subcritical water (SCW) extraction

Recently, consumers demand motivates researchers to invest time on using environmentally friendly techniques to remove desirable value-added compounds from a matrix. Such value-added compounds providing antioxidant, anti-cancer and anti-inflammatory properties from agricultural wastes or by-products could be used in the food, cosmetic and pharmaceutical areas. Moreover, subcritical water extraction has gained prominence to convert organic waste or unused resources to value-added compounds such as saccharides, fatty acids, phenolic compounds, amino acids and proteins (Koomyart et al., 2014). Also, this green extraction method was used for simultaneous removal of inorganic elements such as Ba, Ca, Cu, Fe, Mg, Mn, Na, Pb, Sr, and Zn from lichen and algae samples (Matusiewicz & Ślachciński, 2014).

Pressurized hot water (PHW), near-critical water (NCW) and hot compressed water (HCW) describe water at subcritical conditions (Fig. 2.8). At ambient conditions (T= 295 K and P= 0.1 MPa), water is a polar solvent with a density of 1000 kg m^{-3}, a dielectric constant ε of 79.73 and an ionic product Kw of 10^{-14}. At elevated temperatures, thermodynamic properties of water and aqueous solutions significantly change. These changes include variation of water properties such as dielectric constant, conductivity, ionic product and hydrogen bond network (Galkin & Lunin, 2005). Other property changes that occur in water include viscosity, heat capacity, diffusion coefficient and density, influencing mass transfer of compounds into water. For example, Strezov & Evans (2014) reported that subcritical water is a preferable solvent for hydrophobic compounds due to the lower dielectric constant of subcritical water compared to ambient water (Carr, Mammucari, & Foster, 2011; Evans, Strezov, & Evans, 2014). Also, compared with ambient water, subcritical water has three orders of magnitude higher ion product (H^+ and OH^- values) that act as catalysts for hydrolysis of biomass without adding any acid or base (Meillisa,

Woo, & Chun, 2015). According to the below equation, at room temperature and atmospheric pressure, the $(H_2O)_{i+1}$ consist of 100 molecules associated with an infinite network of hydrogen bonds.

$$(H_2O)_i + H_2O \underset{}{\overset{K_i(T)}{\rightleftharpoons}} (H_2O)_{i+1}$$

where, i = 1, 2, ... , ∞ and K_i is the equilibrium constant for association during the formation of hydrogen equilibrium constant (Heremans, 1996). Therefore, elevated temperatures decrease the bonding, thermal energy is released into the environment. For subcritical water, the hydrogen bonds in water break, leading to the decrease in the dielectric constant, changes in the dynamic viscosity and an increase in the self-diffusion coefficient of water to favor compounds transfer into water.

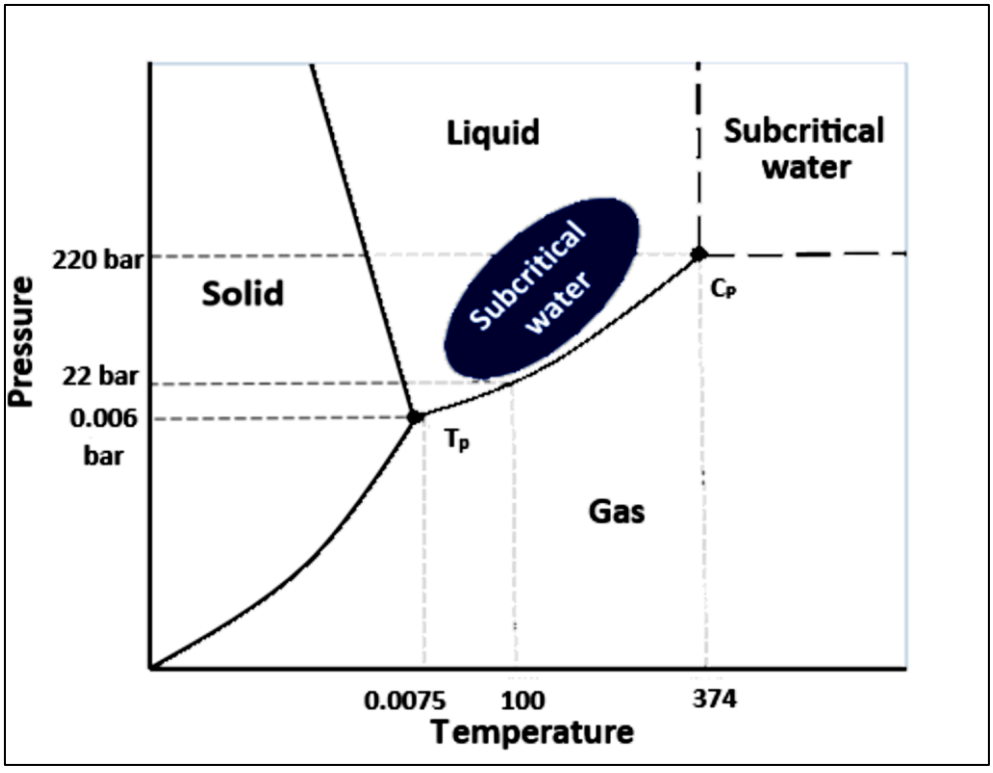


Fig. 2.8. Thermodynamic phase diagram of water (Asl & Khajenoori, 2013).

Using water, a green and safe solvent instead of other toxic, fire-hazardous and explosive solvents make subcritical water extraction unique. Also, the temperature and pressure are adjustable so that the properties of water provide the condition for a specific organic reaction. For example, acidified subcritical water containing 4% (v/v) HNO_3 at pH<7 has been used to extract pollutants from coal at 150 ℃ and 100 bar (Wang, Li, Li, & Wang, 2007). In another study, subcritical water was used for the removal of explosive organic compounds such as trinitrotoluene (TNT), cyclonite, and hexogen from contaminated soils.

Also, antioxidant capacity of oil from sunflower dried seed powder increases by hydrolysis of ester and glycoside bonded antioxidants using subcritical water at 60-160 °C, 30 bar, and 5-120 min at a solvent ratio of 1/20 g/mL compared to Soxhlet extraction for 4 h using the same material and solvent ratio (Ravber, Knez, & Škerget, 2015). Moreover, subcritical water technology was used to produce drug nano-particles of prednisolone (Chen et al., 2015). In this way, those particles with poor solubility in water become more soluble when increasing temperature. Also, a spherical shape is ideal for nano drugs, which is attainable at elevated temperatures using subcritical water. In fact, supersaturation occurs during subcritical water at high temperatures and make these particles smaller (Chen et al., 2015). Physical changes and decrease in polarity followed by less dissolved polar compounds speed up the subcritical water extraction of polyphenolic compounds (Aliakbarian, Fathi, Perego, & Dehghani, 2012; Carr, Mammucari, & Foster, 2011; Singh & Saldaña , 2011).

Subcritical water extraction can be selective depending on the range of temperature used. Low temperatures favor polar compounds and elevated temperatures favor non-polar compounds.

Different studies have shown that subcritical water is more suitable for the extraction of polar compounds without the use of organic solvents and slightly non-polar compounds.

At subcritical water conditions, raise in temperature reduces the dielectric constant of water, leading change in hydrogen bond network of water. Therefore, water start to behave as non-polar solvent, which enables water to trap top and bottom hydrophobic faces of cellulose structure for better solvation. Another consequence is the breakage of crystalline cellulose structure (Tolonen, Penttilä, Serimaa, Kruse, & Sixta, 2013). Also, specific hydrolytic properties of SCW such as increased ionization constant and release of OH^- and H^+ (H_3O^+) ions promotes the break-down of bigger molecules into small molecules (Plaza et al., 2010). For example, subcritical water extraction has been used for producing seasoning with shrimp-like flavor at 160 and 180℃ (Koomyart et al., 2014).

Subcritical water extraction has also been used to separate small concentrations of Cd and Zn in plants such as Virginia tobacco leaves, tea leaves, spinach leaves, poplar leaves, marine sediment and soil samples (Maurí-Aucejo, Arnandis-Chover, Marín-Sáez, & Llobat-Estellés, 2007; Moreda-Piñeiro et al., 2006). Subcritical water extraction of phenolics has been reported from apple pomace and citrus peel (Wang, Chen, & Lü, 2014), cacao husk (Prado et al., 2014), pomegranate residues, barley and lupin hull (Ciftci & Saldaña, 2015), potato peel (Singh & Saldaña, 2011), rice bran (Pourali, Asghari, & Yoshida, 2010), and flax shives (Kim & Mazza, 2006).

One of the challenges occurs in applying subcritical water technology is the possibility of matrix degradation at high temperatures. Degradation of matrix components rely on molecule tendency to undergo hydrolysis, oxidation, methylation, isomerization and other reactions, depending on the molecule structure, temperature and the duration of contact between compound

and subcritical water. Among these reactions, hydrolysis particularly depends on temperature. For example, complete hydrolysis of phenoxy acids and acetylsalicylic acid initiated at 120°C and 160°C, respectively (Chienthavorn, Pengpumkiat, Noomhorm, & Smith, 2007; Smith, Chienthavorn, Wilson, Wright, & Taylor, 1999). However, most degradations occur at 220°C and above, which is close to the maximum temperature (300°C) for the dissociation constant of water (Kritzer, 2004).

2.5.3. Extraction of tannins using pressurized fluids

Pressurized liquid extraction (PLE) uses a liquid solvent at elevated temperature (100-200℃) and pressure (5-200 bar) to increase the solubility and mass transfer mechanisms (Saldaña & Valdivieso-Ramirez, 2015). No unique extraction method is found for all diverse types of hydrolysable tannins. The most efficient solvent reported for gallic acid and ellagic acid was water at 100℃ and 100 bar compared to 60℃ and 100 (Price, Van Scoyoc, & Butler, 1978). Similarly, corilagin has the same solubility in water at subcritical condition owing to a decrease in the polarity of water at subcritical condition, resulting corilagin content of (4.11%) in pressurized water which was 39% higher than what obtained using Soxhlet extraction (2.96%) (Markom et al., 2007). In the extraction of catechin (0.57-1.82 mg/g) and epicatechin (0.65-3.31 mg/g) from tea leaves and grape seed, pressurized liquid extraction improved the solubility of these compounds (Piñeiro, Palma, & Barroso, 2004). The extraction of catechin and epicatechin from tea leaves using pressurized ethanol and methanol (T=100 ℃ , P=101 bar, F=0.3 mL/min, and 10 min) was more efficient than ultrasonic (power of 200W and 10 min) or magnetic stirring (T=60℃ and 10 min). The extraction yields of catechin and epicatechin using as pressurized methanol (C=1.90 mg/g, EC=0.72 mg/g), pressurized ethanol (C=0.59 mg/g, EC=0.25 mg/g),

ultrasonic (C= 0.93 mg/g, EC= 0.46 mg/g) and magnetic stirring (C=0.85 mg/g, EC=0.44 mg/g) were reported by Piñeiro et al. (2004).

PLE improves the imperfections of time, solvent consumption and low polarity CO_2 for supercritical fluid extraction (Markom, Hasan, & Daud, 2010).

Subcritical water has been identified as an effective solvent, catalyst and reactant for hydrolytic conversions and extractions. When water is the pressurized liquid, it can be used efficiently for a wide range of molecules with different polarities. Extraction of corilagin and ellagic acid from *Phyllanthus niruri*, a herbal plant at 100℃, and 100-150 bar resulted in high yields (41.1 mg/g) (Markom et al., 2007). Water polarity has an indirect relation with temperature. Flow rate is another parameter in a dynamic system, influencing the contact of solvent with the target component. Providing well suited flow rate and time for an adequate interaction between solvent and target compound influence the results. For example, flowrates of 0.5-1.5 mL/min were used for the extraction of hydrolysable tannins from *Phyllanthus niruri* (Markom et al., 2007). They have found that a decrease in water flow rate from 3.0 to 1.5 mL/min increased contact time and extraction efficiency, resulting better equilibrium and mass transfer within a longer residence time. Using low flow rates, the solvent capability to contact the target compound is better, requiring less volume of water.

TABLE 2.8. Physicochemical properties of water at different conditions.

Property	**Normal water**	**Subcritical water**		**Supercritical water**	
Temperature (°C)	25	250	350	400	400
Pressure (MPa)	0.1	5	25	25	50
Density, ρ (g cm^{-3})	1	0.8	0.6	0.17	0.58
Dielectric constant, ε ($F.m^{-1}$)	78.5	27.1	14.07	5.9	10.5
Ionic product, pK_w	14	11.2	12	19.4	11.9
Heat capacity C_p (kJ kg^{-1} K^{-1})	4.22	4.86	10.1	13	6.8
Dynamic viscosity, η (mPa s)	0.89	0.11	0.064	0.03	0.07

Adapted from Toor, Rosendahl, & Rudolf (2011), and Mustafa & Turner (2011).

Solvent is a crucial factor in the extraction of desired compounds from a matrix. Solvent ability to solubilize compounds, with minimum co-extraction of other matrix components, make a good separation of the desired compounds. Likewise, polarity of the solvent should be close to that of the compounds of interest. For example, It was reported that sonication of 0.4 g grape skin in 4 mL solvent within 20 min at room temperature showed that acetone (8 mg/g skin) was better than ethanol (4 mg/g skin) for extraction of condensed tannins based on HPLC analysis and characterization of the length of tannin polymers (Downey & Hanlin, 2016). In another study, proanthocyanidins have been extracted from grapes within 60 min using 50% (v/v) ethanol, 50% acetonitrile and water without reporting the yields (Sarneckis et al., 2006).

Also, different volumes of water were mixed with methanol, ethanol, propanol, acetone, ethyl acetate, and dimethylformamide to extract phenolics from fruits, eggplant and berries (Antolovic

h, Prenzler, Robards, & Ryan, 2000; Luthria & Mukhopadhyay, 2006; Zadernowski, Naczk, & Nesterowicz, 2005). Different mixtures of ethanol and water were used for the extraction of phenolics such as rutin (20%) and chlorogenic acid (2%) from buckwheat. Both high temperature and high ethanol concentration reduced enzymatic activity, which led to a better stability of rutin in solution by extraction with 30% ethanol at 60°C for 2h (Hinneburg & Neubert, 2005). Naczk et al. (1992) indicated that Soxhlet extraction of 1 g canola meal at 50℃ for 12 h in 10 mL 70% (v/v) acetone/water mixture provided the optimal condition to obtain 0.32% tannin yield (Naczk, Shahidi, & Sullivan, 1992). Comparison of methanol and acetone and their mixtures with water revealed that water content of 0-10% was favorable for phenolic removal than tannin removal. Also, using methanol and acetone at a solvent to water ratio of 50:50 (v/v) resulted in 81-88% phenolics. Addition of 1% HCl to 70% aqueous methanol and 70% aqueous acetone increased the yield of phenolics from 0.4% and 0.07% to 1.08% and 1.01%, respectively (Table 2.9). Also, the effect of solvent on extraction of condensed tannins from beach pea (10.2%), grass pea (1.04%), and green pea (0.059%) using 70% acetone was better than using water (0.2%, 0.04%, and 0.015%, respectively) (Chavan, Shahidi, & Naczk, 2001).

TABLE 2.9. Effect of solvent on the extraction of total phenolics and tannins from different sources.

Plant (g)	Solvent	Solid/solvent ratio (w/v)	Solvent/water ratio (v/v %)	Phenolics (%)	Tannins (%)
Canola meal (1g) *	Acetone	1:10	100	0.07	0.00
	Acetone/water	1:10	90/10	0.69	0.16
	Acetone/water	1:10	80/20	0.77	0.32
	Acetone/water	1:10	70/30	0.81	0.32
	Acetone/water	1:5	50/50	0.81	0.26
	70% Acetone+1% HCl	1:5	70/30	1.01	0.22
	Methanol	1:5	100	0.40	0.04
Canola meal (1g) *	Methanol/water	1:5	90/10	0.61	0.09
	Methanol/water	1:5	80/20	0.65	0.19
	Methanol/water	1:10	70/30	0.87	0.24
	Methanol/water	1:5	50/50	0.88	0.24
	Methanol+1% HCl	1:10	100	0.89	0.07
	70% Methanol+1% HCl	1:10	70/30	1.08	0.23
Grape skin (0.4 g) **	Acetone/water	1:10	70/30	nd	0.80
	Acetone/water	1:10	50/50	nd	0.75
	Acetone/water	1:10	30/70	nd	0.71
	Ethanol/water	1:10	70/30	nd	0.30
	Ethanol/water	1:10	50/50	nd	0.45
	Ethanol/water	1:10	30/70	nd	0.37
Green pea (1-2 g) **	70% Acetone+1% HCl	1:40	70/30	nd	72.00
	70% Methanol +1% HCl	1:40	70/30	nd	69.8
	70% Acetone	1:40	70/30	nd	58.8
	70% Methanol	1:40	70/30	nd	52.7
Beach pea (1-2 g) **	70% Acetone+1% HCl	1:40	70/30	nd	11.6
	70% Methanol +1% HCl	1:40	70/30	nd	4.54
	70% Acetone	1:40	70/30	nd	10.2
	70% Methanol	1:40	70/30	nd	0.92

TABLE 2.9. Continue.

Pine bark (1 g) ***	5% Ethanol	1:10	5/100	33.30	60.60
	COOH+Na_2SO_3	1:10	5/100	14.60	35.00
	Ethanol+Na_2SO_3	1:10	5/100	34.00	66.70
Carob pod (0.6 g) ∴	100% Acetone	(1:10) ×2	100/0	0.2	0.03
	70% Acetone	(1:10) ×2	70/30	1.95	0.04
	70% Methanol	(1:10) ×2	70/30	1.25	0.03
Lentile (0.5 g) ∵	70% Acetone	1:10	70/30	0.24	0.12
	70% Methanol	1:10	70/30	0.25	0.17
	50% Acetone	1:10	50/50	0.66	0.59
	70% Acetone+1% HCl	1:10	70/30	0.75	0.87
Black tea (0.2 g) ǂ	80% Acetone	1:10	80/20	13.02	nd
	80% Ethanol	1:10	80/20	7.73	nd
	50% Ethanol	1:10	50/50	10.43	nd
	80% Methanol	1:10	80/20	7.7	nd
Mate tea (0.2 g) ǂ	80% Acetone	1:10	80/20	12.85	nd
	80% Ethanol	1:10	80/20	8.58	nd
	50% Ethanol	1:10	50/50	12.11	nd
	80% Methanol	1:10	80/20	9.46	nd

Total phenolics and tannins data expressed as trans-sinapic acid and catechin equivalents, respectively, *(Naczk, Shahidi, & Sullivan, 1992), **Downey & Hanlin (2016),***Seabra et al. (2018), ∴Avallone et al. (1997), ∵Xu & Chang (2007), ǂTurkmen, Sari, & Velioglu (2006), nd: not determined.

2.5.4. Extraction of fibers using pressurized fluids

Agricultural by-products are rich in fiber compounds such as cellulose, hemicellulose and lignin. Thermochemical liquefaction using sub/supercritical fluids has reported as a method of converting fiber compounds in biomass into energy (Prado et al., 2014). Extraction of β-glucans from waxy barley using pressurized hot water (155°C, 18 min and 50 bar) compared to conventional extraction (55°C, 3 h, and ambient pressure) resulted in an increase of the

molecular weight three times better (200 kDa vs. 55 kDa) (Benito-Román, Alonso, & Cocero, 2013). Also, subcritical water was successfully used to obtain ~80% cellulose from sweet blue lupin hull at 180°C, 50 bar, 5 mL/min, and pH=5 (Ciftci & Saldaña , 2015). Moreover, subcritical water has been used for separation of hemicellulose and lignin from triticale straw with 73-78% hemicellulose removal at 165°C, 110 bar , and 115 mL/min. Whereas the optimum condition for cellulose removal (65%) from triticale straw was at 165°C, 110 bar , and 165 mL/min (Pronyk & Mazza, 2011). Also, due to the high cellulose and hemicellulose content of water hyacinth, subcritical water at 165°C, and 50 bar was used within 30 min to obtain 68.2% cellulose (Thi, Ong, Thi, & Ju, 2017).

Fiber has carbohydrate-based polymer structure so hot water is the most common solvent to extract them. At subcritical conditions, hydrogen bonds between water molecules become loose and the dielectric constant of water favors solubility and reactive selectivity for polar compounds, facilitating the extraction of polysaccharides such as pectin, cellulose and hemicellulose. For example, subcritical water extraction of apple pomace (130-170°C) and citrus peel (100-140°C) for 5 min yielded 21.95% and 16.68% pectin, respectively (Wang, Chen, & Lü, 2014). Also, Tanaka et al. (2012) used a semi-continuous extraction system (T=160-320◦C, F= 2.1, 3.5, and 7.0 mL/min, and P= 200 bar) to obtain 78% pectin from Citrus junos peel (Tanaka et al., 2012).

2.6. Advantages of SCW extraction method over conventional methods

The most important merit of SWE relates to the use of water as an environmentally friendly extraction medium, which is non-toxic, and non-flammable. Also, from the waste disposal perspective, SWE is cost-effective, owing to its safe and clean life cycle. Subcritical water

extraction technique is free from any organic solvent that brings the necessity of a solvent removal step after the extraction (Chemat et al., 2017). Compare with other solvents, subcritical water can be a tunable solvent due to its dielectric constant sensitivity to change of temperature. At subcritical conditions, temperature and pressure are increased to 250 °C and 50 bar, respectively and the dielectric constant of water changes to ε = 27, which is close to the range of solvents such as methanol ε=33, ethanol ε=24, acetone ε=20.7, and acetonitrile ε = 37 (Fig. 2.9).

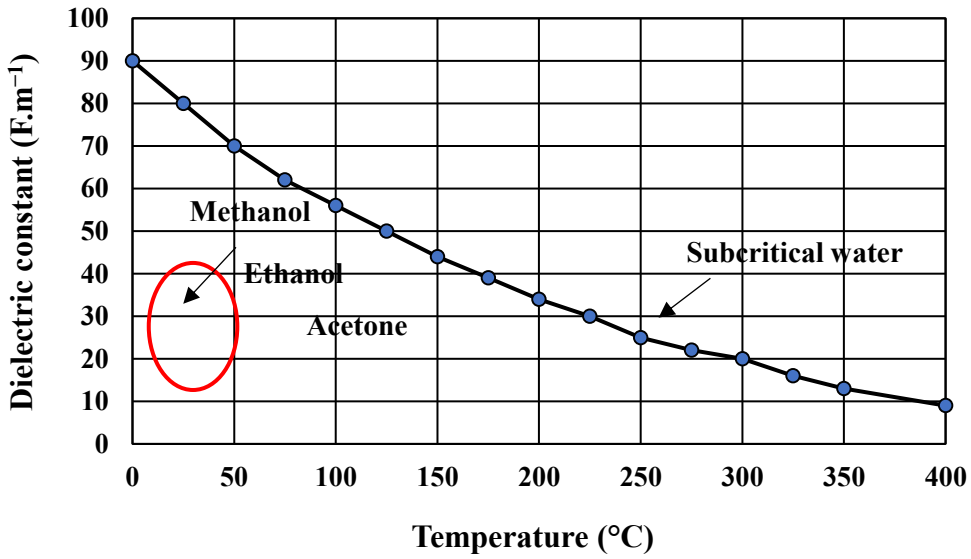


Fig. 2.9. Changes in dielectric constant of water compared to organic solvents (Adapted from Herrero, Cifuentes, & Ibañez, 2006).

Subcritical water can extract both polar and non-polar compounds whereas in conventional extractions, non-polar or semi-polar solvents are required to solubilize hydrophobic compounds (Teo et al., 2010).

Unlike conventional solvent extraction, SWE is very easy to apply, requiring less time, reducing error possibilities. Extraction can also be easily controlled to achieve selective extraction of polar, moderately polar, and non-polar compounds by adjusting the dielectric

constant of water using extraction parameters such as temperature, pressure and co-solvent (Liang & Fan, 2013). One of the most important aspects of subcritical water extraction relates to the use of short times. Also, subcritical water extraction is preferred over traditional solvent extraction (Table 2.10)

TABLE 2.10. Advantages of SCW extraction compared to other extraction methods.

Extraction method	**Extraction conditions**			
	Solvent	**Time (min)**	**Advantages**	**Disadvantages**
SCW extraction	Water	60	Fast, time and solvent efficient, green	Selective design, thermal degradation at elevated temperatures Require training
Pressurized fluid extraction	Water+organic solvents	60	Fast, high yields, time efficient, less damage to thermolabile compounds	Require training sophisticated instrumentation
High pressure processing assisted by temperature	Water	1-60	Uniform heating, enzyme & bacterial spore's inactivation	High cost, require specific packaging, limited range of temperature
Traditional solvent extraction	Noxious solvents	>60	Simple design	Time and solvent consuming

Chapter 3: Subcritical water extraction of bioactives for valorization of green pea pod

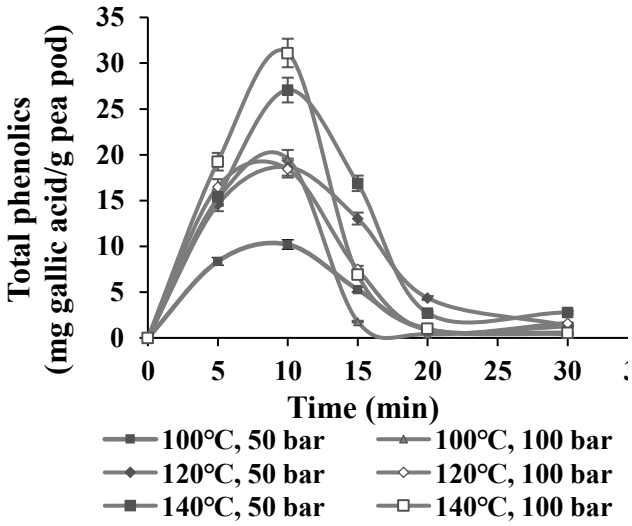
Abstract

There is no study reported on extraction of phytochemicals, mainly tannins, phenolics and carbohydrates, from green pea pod. Removal of these phytochemicals from pea pod biomass is critical to avoid interference with the food chain. In this study, subcritical water (SCW) was used as a green extraction technique to valorize the snap pea pod *Pisum sativum* var. *macrocarpon*. Liquid extracts of green pea pod were obtained using a semi continuous SCW extraction system. Extraction conditions were optimized using different combinations of temperature (100-200℃) and pressure (50-100 bar) for 40 min. Particle size (0.5 mm), flow rate (5 mL/min) and solid-liquid ratio (1:20 w/v) were maintained constant. In addition, conventional solid-liquid (S-L) extraction was used at 50℃ and 70℃ during 3 h with an agitation power of 100 rpm using water, acetone/water (70/30, v/v) and ethanol/water (70/30, v/v). Kinetics of phenolic removal showed a maximum rate of extraction (25.5 mg gallic acid/g pea pod) in the first 10 min. The trend reached to a constant level of less than 5 mg gallic acid/g pea pod after 40 min. Total tannins, phenolics, and carbohydrates were determined using spectrophotometric methods. The highest total phenolics (56.59±0.16 mg gallic acid/g pea pod) and total tannins (12.96±4.45 mg tannic acid/g pea pod) were obtained at 180℃ and 50 bar using SCW extraction. The results showed maximum carbohydrates removal of 402.8±7.33 mg glucose equivalent/g pea pod at 140℃ (100-140℃ at both pressures investigated). The use of SCW enhanced extraction of total tannins compared to water at 70℃ and atmospheric pressure, 12.96 and 3.6 mg tannic acid/g pea pod, respectively. On the other hand, organic solvent mixtures such as acetone/water (70/30, v/v) removed the highest content of total tannins (5.45±0.17 mg tannic acid/g pea pod) at 70℃

compared with water and ethanol/water (70/30, v/v). Fiber content of green pea pod showed 5.59±0.03% DW soluble fiber, and 49.50±0.02% DW insoluble fiber. Using SCW extraction at 180℃ and 50 bar decreased these values to 4.23±0.01% DW soluble fiber and 46.77±0.10% DW insoluble fiber. Comparison of conventional S-L extraction for total tannins (3.6±0.28 mg tannic acid/g pea pod) with SCW extraction (12.96±4.45 mg tannic acid/g pea pod) indicates the benefit of SCW shorter time (40 min) for total tannin removal. This emerging environmentally friendly technology can be used to promote the removal of phytochemicals from green pea pod.

Subcritical water extraction

Green pea pod



Kinetics of phenolic extraction from green pea pod using SCW.

Keywords: Green pea pod, Subcritical water extraction (SCW), Phenolics, Tannins, Fibers.

3.1. Introduction

Phytochemicals obtained from natural sources such as fruits and vegetables are increasingly gaining importance over synthetic compounds due to their broad distribution in the plant kingdom, their abundancy in daily usages, and their corresponding health-promoting properties. Green pea *Pisum sativum var. sativum L* is one of these sources found in three types of edible peas (green pea, sugar or snow pea and snap or sugar snap pea) (Anurag, Manjunatha, Jha, & Kumari, 2016). In 2017, total Canadian pea production was estimated around 4.1 million tonnes. Peas are reported as relatively inexpensive and highly nutritious due to the high content of fiber (5.3% soluble and 20.3% insoluble) and protein (essential amino acids such as tryptophan and lysine), low content of sodium and fat, and an excellent source of complex carbohydrates (68.4%) (Wang, 2017). Also, green peas leftover after shelling peas are pods which contain high crude protein content of 19.8% to be used as animal feed (Dhillon et al., 2017). However, presence of phytochemicals such as condensed tannins was reported for mature beach pea seed (7-11%) and premature beach pea pod (9.13%), mature beach pea pod (2.05%), green pea seed (0.07%) and grass pea seed (0.11%) after extraction using 70% acidified acetone (Chavan, Shahidi, & Naczk, 2001).

The use of petrochemical solvents for extraction is a traditional method for removal of tannins from plants and vegetables. Chavan et al. (2001) reported condensed tannins content of beach pea (11.6%), green pea (0.07%) and grass pea (0.11%) using methanol or acetone. The highest yield of condensed tannin was obtained using 70% acetone, containing 1% concentrated HCl (Chavan et al. 2001). Also, solvents such as acetone, ethanol, and less often methanol have

been used for separation of polar polyphenols from plant by-products (Soural et al., 2015). But, traditional extraction using solvents is not environmentally friendly and it is time consuming.

Subcritical water (SCW) extraction is considered a clean technology to convert biomass like green pea pod into valuable ingredients that can be further used in food, pharmaceutical, and tannin industries. One of the advantages of SCW is reduced time, less solvent consumption and minimal sample preparation, like homogenization. Abundant availability of green pea pod, approximately 70% of total production from three million metric tons of green pea and the lack of study on removal of useful phytochemicals (carbohydrates, phenolics and tannins) from green pea pod were motivation of using green pea pod as the matrix. Also, removal of tannin from the pod helps to improve the nutritional value of pods to be used as animal feed. However, no literature data is available on phenolics, tannins, and fiber removal from green pea pod using subcritical water extraction. Therefore, the main objective of this chapter was to remove tannins, phenolics, and carbohydrates using SCW extraction from green pea pod.

3.2. Materials and methods

3.2.1. Sample preparation

Fresh Mann's Sugar Snap Peas were purchased from the Canadian Superstore supermarket (Edmonton, AB, Canada). Green pea pods were manually separated from the seeds and freeze dried at -43℃ for 4 days. The dried green pea pods were then milled using a Retsch mill (ZM 200, Burlington, ON, Canada) and sieved to obtain particles of 0.5 mm. The ground samples were packed in plastic zip lock to prevent moisture and oxygen transfer, labeled and stored at 4℃ for further analysis.

3.2.2. Chemicals

Chemical reagents, such as sulphuric acid (97%, ACS reagent), ethanol (99.9%, HPLC grade), acetone (99.9%, HPLC grade), sodium carbonate anhydrous (≥97%, ACS grade), Folin-Ciocalteau's phenol reagent (2N), Folin-Denis' reagent (99% purity), gallic acid (99.9% purity), D-(+)-glucose (99% purity), tannic acid (98% purity), vanillin (99% purity), and (+)-catechin hydrate (minimum 98%) were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). Glass beads (2.3 mm) and glass wool were purchased from Fisher Scientific Co. Ltd (Toronto, ON, Canada). Total dietary fiber kit containing α-amylase (thermostable, 3000 units/mL), protease (50 mg/mL, 350 Tyrosine units/mL), and amyloglucosidase (3,300 units/mL) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland)

3.3. Proximate compositional analysis

All proximate compositional analysis was carried out at least in duplicate.

3.3.1. Moisture content

A gravimetric method (AOAC, 2000) was used to determine moisture content of green pea pod. Three sets of 2 *g* freeze dried green pea pod were weighed with an analytical balance (Mettler Toledo, Mississauga, ON, Canada). Then, samples were spread into dried and pre-weighed aluminum containers with specifications of 50 mm diameter×23 mm depth. The containers were kept in a warm air oven (Model 655G, Fisher Scientific IsoTemp® oven, Toronto, ON, Canada) to dry at 105℃ for 3h. Then, the containers were transferred into the desiccators for cooling, and the weight of containers with the dried samples were recorded. The moisture content was calculated using eq. (3.1).

$$\text{Moisture content (\%)} = 100\times [\,(W_b - W_a)/W_b] \qquad (3.1)$$

where, W_b = Weight (g) of the sample before drying, and W_a= Weight (g) of the sample after drying.

3.3.2. Ash content

Ash content of the green pea pod was determined following the AOAC 923.3 method (AOAC, 2000). First, two identical porcelain crucibles were cleaned, dried and pre-weighed. Then, approximately 1 *g* of sample powder was weighed. Crucibles containing samples were kept inside desiccators until the muffle furnace (Model F-A1730, Thermolyne Corporation, Dubuque, IA, USA) reached 550°C. Then, samples were transferred to the muffle furnace and were incinerated for 15 h at 550°C. Crucibles were removed from the muffle furnace and cooled inside the desiccators. Weights of crucibles with incinerated samples were recorded. Ash content was calculated according to the following equation:

$$\text{Ash content (\%w/w)} = \frac{(Wca - Wc)}{(Wcs - Wc)} \times 100 \qquad (3.2)$$

where, W_{ca}: Weight of crucible and ash,W_{cs}: Weight of crucible and sample, and W_c: weight of crucible.

3.3.3. Protein content

The Leco TruSpec nitrogen analyser (Model FP-428, Leco instruments Ltd., Mississauga, ON, Canada) was used for the measurement of protein content. This instrument quantifies protein content based on the content of nitrogen in the sample. To prepare the sample for protein quantification analysis, approximately 0.1 g of sample was poured into an aluminum foil cone and pressed to form a pellet. Ethylene diamine tetra acetic acid (EDTA) and corn-starch were used as a control for the protein content. Pellets of standards were prepared exactly as described for the samples. Then, control and samples were placed into the loading head. To avoid any atmospheric gas entry during the loading step, the system was sealed, and samples were purged. In the presence of pure oxygen, combustion of

standard and samples occurred inside a furnace at 950°C. The instrument was calibrated with corn-starch control. The Leco analyzer reported the nitrogen content in percent, which is multiplied by a factor of 6.25 to obtain protein content of the unknown sample.

$$\text{Protein content (\%)} = \frac{N \times 6.25}{W_a} \times 100 \qquad (3.3)$$

where, N is the nitrogen content and W_a is the weight of sample after drying.

3.3.4. Fat content

For the fat content, a Goldfisch extraction unit (Labconco Co., Kansas, MO, USA) was used following the AOAC standard method. Freeze-dried green pea pod (2 *g*) was first weighed into cellulose extraction thimbles (25 mm I.D×80 mm length, Whattman International Ltd., Maidstone, England). Samples were covered with a 'small' amount of glass wool to minimize sample loss. Then, 40 mL petroleum ether was added under the fume hood to the sample inside the thimble. A blank was prepared with only 40 mL petroleum ether in a similar timble and run through the entire extraction process. For each sample, a clean dry extraction beaker was used and pre-weighted. The extraction thimbles were then attached to the unit and kept at temperature of 60°C for 5h so that lipid and lipid soluble compounds, such as chlorophyll, volatile oils, and resins could have enough time to contact with the solvent. In this method, the organic solvent refluxed through the sample to remove soluble material. At the end of 5 h, heaters were turned off and beakers were put under the hood for cooling. Once the remaining of the petroleum ether evaporated, extraction beakers were placed inside a forced-air oven (Model 655G, Fisher Scientific Iso Temp® oven, Toronto, ON, Canada) at 110°C for 30 min to remove any moisture. Weight of thimbles were recorded after cooling down in a desiccator.

The equation used for the calculation of fat content was:

$$\text{Fat content (\%)} = [W_f / W_s] * 100 \qquad (3.4)$$

where, W_f: weight of fat, and W_s: weight of sample.

3.3.5. Carbohydrates

Proximate compositional analysis results for the percentage of moisture, ash, protein, and fat contents were added and the final value deducted from 100% to calculate the total carbohydrate content.

$$\text{Total carbohydrates (\%)} = 100\% - (\text{moisture \%} + \text{ash \%} + \text{protein \%} + \text{fat \%}) \qquad (3.5)$$

3.3.6. Starch content

The starch content of the green pea pod was measured using the Megazyme total starch protocol (Fig 3.1). Ground freeze dried green pea pod (0.5 g) was measured in duplicate and transferred to the 50 mL test tubes. Since green pea pod has carbohydrates, 5 mL ethanol 80% was added to each tube. Then, tubes were incubated in a water bath at 80-85℃ for 5 min. Another 5-min incubation was used after mixing the content with a vortex mixer. Then, the tubes were centrifuged at 1000 *xg* for 10 min. Supernatants were discarded and 10 mL more ethanol 80% added to re-suspend the pellet. This step was followed by the vortex and centrifuge step at 1000 *xg* for another 10 min. Then, 3 mL of thermostable alpha-amylase (300 Units) was added to the tubes. Tubes were incubated in boiling water for 6 min. After 2 min and 4 min, the content was mixed using a vortex. Then, tubes were cooled down to 50℃. Sodium acetate buffer (4 mL) and 0.1 mL amyloglucosidase reagent were added and mixed. The new mixture was incubated for 30 min at 50℃ with an alternative vortex every 10-15 min. As the starch content of green pea pod is more than 20%, 25 mL of water was added to dilute the content to 32.1 mL. The resulting solution was transferred into 2 mL micro centrifuge tubes in order to centrifuge at 1000 *xg* for 10 min. Then, 3 mL GOPOD reagent was added to 1 mL of each

solution in glass tubes. The blank was prepared mixing 0.1 mL distilled water and 3 mL GOPOD. Also, 0.1 mL glucose standard solution was added to 3 mL GOPOD to prepare glucose controls. All samples, controls and blank were incubated in a water bath at 50℃ for 20 min before reading the absorbance at 510 nm. Equation 3.6 was used to calculate the starch content. The absorbance of glucose control was approximately 1.10±0.02.

$$\text{Starch (\%)} = (A_s - A_b) * F * D * (1/1000) * (100/W_s) * (162/180) \qquad (3.6)$$

where, A_s: Absorbance of the sample, A_b: Absorbance of the blank, W_s: sample weight, D: volume correction for the dilution that is equal to 32.1/0.1, (1/1000): Conversion coefficient, (100/W): beta glucan content as a percentage of sample, (162/180): conversion coefficient for anhydrous glucose, and

$$F: \frac{100\ \mu g\ of\ glucose}{Average\ absorbance\ of\ three\ 100\ \mu g\ of\ glucose\ standard}$$

3.3.7. Determination of total dietary fiber

The contents of total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) in green pea pod were measured using the Megazyme total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland). This procedure is the modification of protocols explained in AOAC method 991.43 (1995). Briefly, 1 g of sample was treated with 50 µL heat stable α-amylase, followed by 100 µL protease and then 200 µL amyloglucosidase to digest starch and protein into their basic monomers. Then, the insoluble fraction (Residue 1) was washed with 95% ethanol and acetone to obtain the soluble fiber, which was dried. The filtrates and water washing solution's weight were adjusted to 80 g and mixed with 95% ethanol (~320 mL) to precipitate soluble fibers at room temperature. Then, the precipitate was separated by applying a

vacuum filtration system and sequential washing of the residues with 20 mL of 78% ethanol, 95% ethanol, and acetone, respectively. Both soluble (Residue 1) and insoluble (Residue 2) fractions were dried overnight at 100℃, weighed and corrected for protein and ash contents using the equations:

$$\text{Dietary fiber (\%)} = \frac{\frac{R_1+R_2}{2} - P - A - B}{\frac{m1+m2}{2}} * 100 \qquad (3.7)$$

where, R_1: residue weight of sample 1; R_2: residue weight of sample 2 (duplicate of sample 1); m_1 and m_2: sample weight 1 and 2; A: Ash weight of residue 1; P: protein weight from residue 2, and B: blank. For the blank:

$$B = \frac{B_{R_1}+B_{R_2}}{2} - B_P\text{-}B_A \qquad (3.8)$$

where, B_R: blank residue; B_A: blank ash from BR_2; and B_P: blank protein from BR_1.

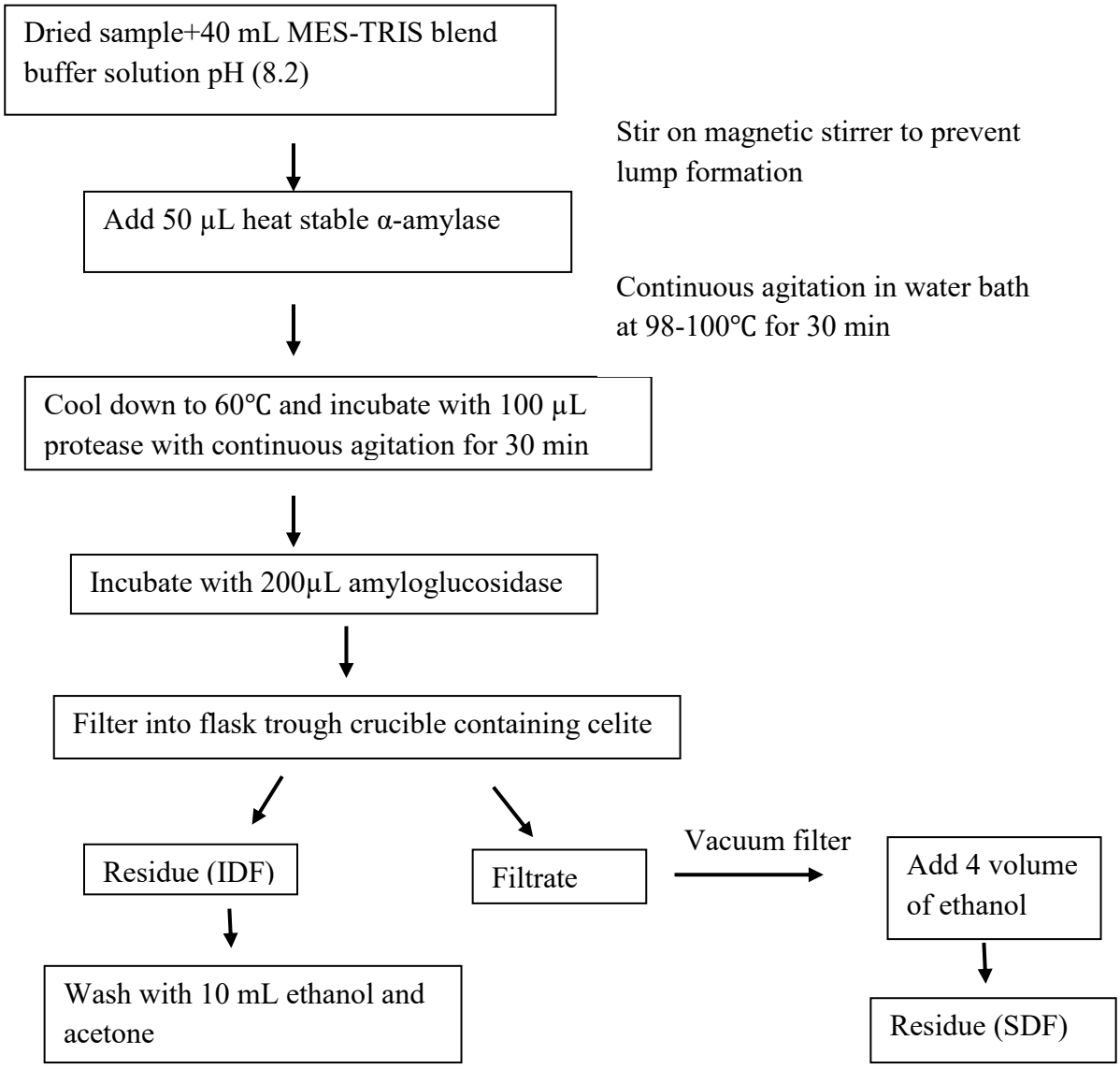


Fig. 3.1. Analytical method for fiber analysis.

3.4. Extraction methods

In this study, solid-liquid extraction with three different solvents (acetone, ethanol and water) was performed as the traditional extraction method. The main purpose was to evaluate the effect of using different organic solvents on extraction of phenolics and tannins. Also, subcritical water extraction technique was used as a new green extraction method.

3.4.1. Conventional solid-liquid extraction

The extraction was performed using 2 g of freeze dried and ground green pea pod and 40 mL of a selected solvent mixture. In this study, solid-liquid extraction was done in duplicate for each solvent media. Selected solvents were pure water, 70% ethanol-water and 70% acetone-water mixtures. The tubes were kept under agitation for 3 h at 50℃ and 70℃. Then, the tubes were centrifuged at 1560 *xg* for easy separation of the supernatant from the solid residue. Finally, the obtained extracts were stored at -18℃ for further analysis.

3.4.2. Subcritical water extraction (SWE)

A semi-continuous flow type subcritical water system (Ciftci & Saldaña, 2015) was used for the subcritical water extraction (Fig. 3.2). Basically, the unit is comprised of a water reservoir, a high pressure pump (Model 305 pump, GILSON Inc, Guelph, ON, Canada), a convection oven (Binder drying oven, ED 115, USA), a stainless steel reactor of 2.54 cm diameter × 10 cm length with a heating band (Trutemp, Edmonton, AB, Canada), two K-type thermocouples (Trutemp, Edmonton, AB, Canada), a temperature controller (Thermomart, Toronto, ON, Canada), a digital pressure gauge (DPI 104, GE-Druck, Calgary, AB, Canada), a pressure relief valve (RVP, Parker Autoclave Engineers, Erie, PA, USA), a back pressure regulator (26-1700 Series, Tescom, Elk River, MN, USA) and a cooling system (Swagelok Valve and Fitting Inc, Edmonton, AB, Canada).

For a typical extraction, green pea pod (2 g) and glass beads (25 g) were mixed and filled into the high-pressure reactor. Two filters of 2.54 cm diameter ×2.9 cm thickness, 20 μm (Mac Master-Carr, Aurora, OH, USA) were placed at the inlet and outlet of the reactor. Temperature and pressure were monitored by digital temperature and pressure controllers, respectively. When the temperature of the system reached the desired work temperature, a flow of sonicated distilled

water was delivered with the HPLC pump at a flow rate of 5 mL/min to the preheating section and then into the reactor. The experiments using a sample with particle size of 0.5 mm were performed at temperatures of 100-200℃ and pressures of 50 and 100 bar. The extracts were collected in vials every 5 min for 40 min and stored at -18 °C for further analysis.

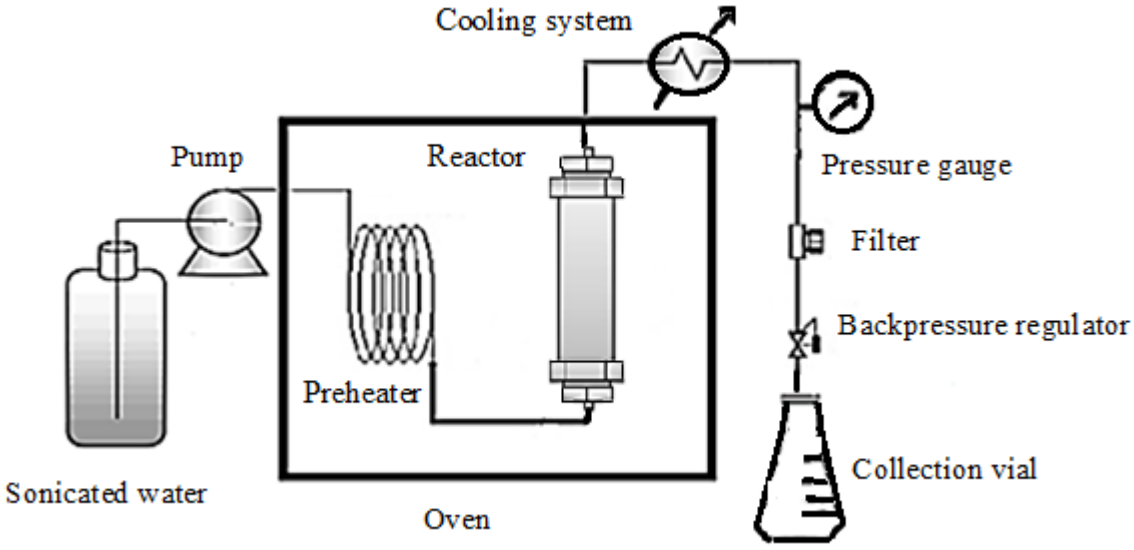


Fig. 3.2. Subcritical water extraction unit.

3.5. Analysis of liquid extracts

3.5.1. Total tannin content

Liquid extracts were analyzed for total tannin content using the method reported by Kyamuhangire et al. (2006). Briefly, 250 µL of 10% Folin-Denis reagent and 500 µL of saturated Na_2CO_3 (7.5%) solution was added to 3.5 mL of the liquid extract. The mixture was vortexed for 15 seconds and incubated for 30 min. Then, the absorbance was read at 700 nm using a spectrophotometer (Jenway 6320D Visible Spectrophotometer, Edmonton, Canada). Tannic acid was used as the standard by preparing standard solutions of 0.1-0.9 mg/mL. The

concentration of tannins in the liquid extracts was expressed as milligram of tannic acid per gram of sample (Fig. A1).

3.5.2. Total phenolic content

Total phenolic content of green pea pod extract was determined by a colorimetric method using the Folin-Ciocalteau reagent as described by Sarkar et al. (2014). Briefly, 40 μL of sample was dispersed in 3160 μL of Milli-Q water and then 200μL of Folin-Ciocalteau reagent was added. The solution was vortexed and let to react for 6 min. Saturated calcium carbonate solution (600 μL) was then added and vortexed. The samples were incubated in darkness for 2 h. The absorbance of samples was measured at 765 nm within 1.5 mL plastic cuvettes in a spectrophotometer (6320D, Jenway, Bibby Scientific Ltd, Dunmw, Essex, UK). Standard solutions of gallic acid (0.05-0.7 mg/mL) were prepared for the calibration curve. All measurements were done in triplicates, including the blank (Fig. A2).

3.5.3. Total carbohydrate content

Total carbohydrate was measured following the method described by Dubois et al. (1956). Briefly, 1 mL of liquid extract was mixed with 0.5 mL phenol (4%) and 2.5 mL of sulfuric acid (96%). After each addition, the mixtures were vortexed for approximately 2 min. Then, the tubes were incubated in a water bath at 20 ℃ for 20 min. D-(+)-glucose standard solutions of 0.05-0.6 mg/L were prepared for the calibration curve (Fig. A3). The absorbance of the hap samples, blank and the standards were measured at 490 nm using a spectrophotometer (Genova MK3, New Malden, Surrey, UK). The results were expressed as milligrams of glucose equivalent per gram of green pea pod.

3.5.4. Hemicellulosic sugar content

The neutral sugar composition of green pea pod was determined based on the procedure reported by Englyst et al. (1987) and Englyst (1989). Briefly, prior to neutral sugar determination, starch and protein were enzymatically removed from green pea pod raw sample using α-amylase and protease, respectively. Then, 50 mg of purified sample was hydrolyzed with 12M H_2SO_4 at 100°C for 2h and Myo-inositol was added as the internal standard prior to monosaccharides derivatization to alditol acetates. Finally, alditol acetates were analyzed by gas chromatography (GC) coupled with mass spectrometry (5975C Electron Impact/Chemical Ionization (EI/CI), Mass selective detector (MSD) Agilent, USA), using a SPB-17 30m×0.25mm×0.25μm capillary column.

3.5.5. pH measurements

The pH of the extracts was measured at room temperature (22°C) using an Excel XL50 pH meter (Fisher Scientific Accumet, Brightwaters, NY, USA). The electrodes for the pH measurements were a liquid-filled probe and a four-cell conductivity nominal constant k=1. The uncertainties for the pH and conductivity measurements were ±0.05 and ±10 μs/cm, respectively.

3.5.6. Data analysis

The statistical analysis was performed to evaluate the differences among the extraction treatments. Two-way analysis of variance (ANOVA) and the significant difference of the data at $p<0.01$ was carried out using Minitab version 18.0 (Minitab Inc., State College, PA, USA) at 95% confidence interval.

3.6. Results and discussion

3.6.1. Compositional analysis of green pea pod

Green pea pod had 64.28% total carbohydrates, 55.10±0.05% fiber and 19.65±0.06% protein as its major components (Table 3.1). Similarly, pea pod and green bean pod had fiber contents of 58.6±1.2% DW and 40.1±1.0% DW, respectively. It was reported that Canadian green peas contain 10.8% moisture, 21.9% protein, 47.9% starch, 17.7% fiber and 2.9% ash on dry basis (Wang, 2017). Also, protein contents of pea pod (10.8±0.3 % DW) and green bean pod (13.6±0.2% DW) were reported as the second important component after fiber (Mateos-Aparicio et al., 2010). Iqbal et al. (2006) also reported carbohydrates (62.2% DW) and protein (24.9% DW) as major components of green pea seed. Iqbal et al. (2006) reported protein content of 24.9±0.03% DW and total fiber content of 25.6% DW in green pea seed. Soluble and insoluble fiber content of green pea pod was higher than values obtained for green pea seed (Table 3.1). Total phenolic content of green pea pod 5.6±0.16% DW was in the range of previous reported total phenolic content of legume pods (6.39% for *C. gaumeri* pod and 4.4% for *P. piscipula* pod, Ortiz-Domínguez et al., 2017).

Table 3.*1.* Proximate composition of green pea pod, pea pod, green bean pod, and green pea seed.

Component	Plant type			
	Green pea pod (This study)	**Pea pod°**	**Green bean pod°**	**Green pea seed ×**
Moisture **(%)**	11.1±0.4	nd	nd	7.8±0.1
Protein **(%)**	19.6±0.1	10.8±0.3	13.6±0.2	24.9±0.0
Fat **(%)**	0.9±0.4	1.3±0.2	1.3±0.5	1.5±0.0
Ash **(%)**	3.9 ± 0.2	6.6±0.5	6.3±0.1	3.6±0.0
Total CHO **(%)**	64.28	nd	nd	62.2
Starch **(%)**	3.1±0.3	3.7±0.1	11.7±0.2	49.5±0.0
Dietary fiber **(%)**	55.1±0.1	58.6±1.2	40.1±1.0	25.6*
Soluble fiber **(%)**	5.6±0.0	4.2±0.6	9.3±0.6	5.3*
Insoluble fiber **(%)**	49.5±0.0	54.4±1.6	30.8±1.2	20.3*
Total tannins **(%)**	1.3±4.5	nd	nd	nd
Total phenolics **(%)**	5.6±0.2	nd	nd	nd

°Mateos-Aparicio et al. (2010), ×Iqbal et al. (2006), *AOAC (2011), CHO: carbohydrates, nd: not determined. Data reported as mean±standard deviation between replicates.

Hemicellulosic sugars of green pea pod

Hemicellulosic sugars found in raw green pea pod are glucose (18.53±0.09% DW), galactose (9.71±0.04% DW), and xylose (4.97±0.001% DW) (Table 3.2). According to Tosh et al. (2013), smaller values of glucose (5.60±0.40% DW), galactose (0.90±0.10% DW), and xylose (2.10±0.20% DW) were reported for carob pod. Mateos-Aparicio et al. (2010) showed that for green bean pod, the most abundant sugars were glucose (13.3±0.50% DW), sucrose (6.1±0.20% DW) and fructose (4.1±0.30% DW). Also, pea pod had smaller values for glucose (11.90±0.60% DW) and fructose (1.20±0.10% DW) compared to green bean pod (Mateos-Aparicio et al., 2010). Reddy et al. (1984) showed that green pea seed has arabinose

(5.58±0.11% DW), glucose (2.81±0.37% DW), galactose (2.55±0.04% DW), sucrose (2.3-2.4% DW), and mannose (2.12±0.40% DW) (Table 3.2).

Table 3.2. Hemicellulosic sugars (% DW) in various pulses.

Carbohydrates (%)	**Green pea pod (This study)**	**Carob pod****	**Pea seed***
Glucose **(%)**	18.53±0.09	5.60±0.40	2.81±0.37
Fructose **(%)**	0.27±0.01	0.10±0.00	nd
Sucrose **(%)**	nd	nd	2.3-2.4
Xylose **(%)**	4.97±0.001	2.10±0.20	1.03±0.06
Arabinose **(%)**	1.96±0.01	1.20±0.10	5.58±0.11
Mannose **(%)**	1.47±0.002	0.40±0.05	2.12±0.40
Rhamnose **(%)**	1.71±0.042	0.10±0.00	0.85±0.09
Galactose **(%)**	9.71±0.04	0.90±0.10	2.55±0.04
Raffinose **(%)**	nd	nd	0.3-0.9
Stachyose **(%)**	nd	nd	2.2-2.9
Verbascose **(%)**	nd	nd	1.7-3.2

*Reddy et al. (1984); Tosh et al. (2013), ** Saura-Calixto (1988), nd: not determined, Data reported as mean±standard deviation between replicates.

3.6.2. Total phenolic extraction by subcritical water

Fig. 3.3a shows total phenolic extraction using subcritical water extraction at temperatures of 100°C, 120°C, and 140°C, a constant water flow rate of 5 mL/min and a constant pressure of 50 and 100 bar up to 30 min. The maximum rate of phenolic extraction occurred in the first 10 min followed by a constant trend from 20 min to 30 min (Fig. 3.3b). At all temperatures investigated, a similar kinetic trend was obtained for phenolic extraction. However, the amount of phenolic removed at 140°C was higher than those obtained at 100°C and 120°C. As previously stated, physico-chemical properties of subcritical water such as surface tension, self-ionization, wetting property and mass transfer rate change at temperatures higher than 100°C, leading to better removal of solubilized components in water (Plaza & Turner, 2015). Moreover, the increase in the phenolic content removal at 140°C could be related to the decrease in water polarity (low dielectric constant), initiating an increase in the solubilization capability of total phenolic compounds. A similar behavior was observed for the cumulative trend of phenolic extraction at both pressures, indicating the solubility region up to 10 min and mass transfer region up to 30 min (Fig. 3.3b).

Total phenolic content was determined for green pea pod extracts obtained at 100°C-200°C and pressures of 50 bar and 100 bar within 40 min. , the total phenolic content of green pea pod extract is significantly influenced by temperature (Fig. 3.3c). The maximum removal of phenolics (54 mg gallic acid/g green pea pod) was obtained at 180°C and 50 bar. However, pressure had no significant effect at 100°C to 160°C, but had a significant effect at 180°C and 200°C. Kumazawa et al. (2002) reported total phenolic content of 19.2% from carob pod using the Folin-Ciocalteu method. Recently, total phenolic content of unroasted and roasted carob pods was reported at 120-150°C as 20–52% (Rodríguez-Solana, Dantas, & Romano, 2017). In this study, the highest yield of phenolic

removal using subcritical water was 5.65% DW (Fig. 3.3c). Carole et al. (2018) reported saponins (0.067%), flavonoids (0.052%), phenols (0.049%), and steroids (0.036%) after solvent extraction of green pea seed with a solid to solvent ratio of 1:50 w/v in 80% methanol at room temperature (Carole, Olajide, & Hassan, 2018). Also, Parikh & Patel (2018) reported total phenolic content of 6.59% DW in green pea seed after three times extraction using 80% aqueous methanol at pH 2 (Parikh & Patel, 2018).

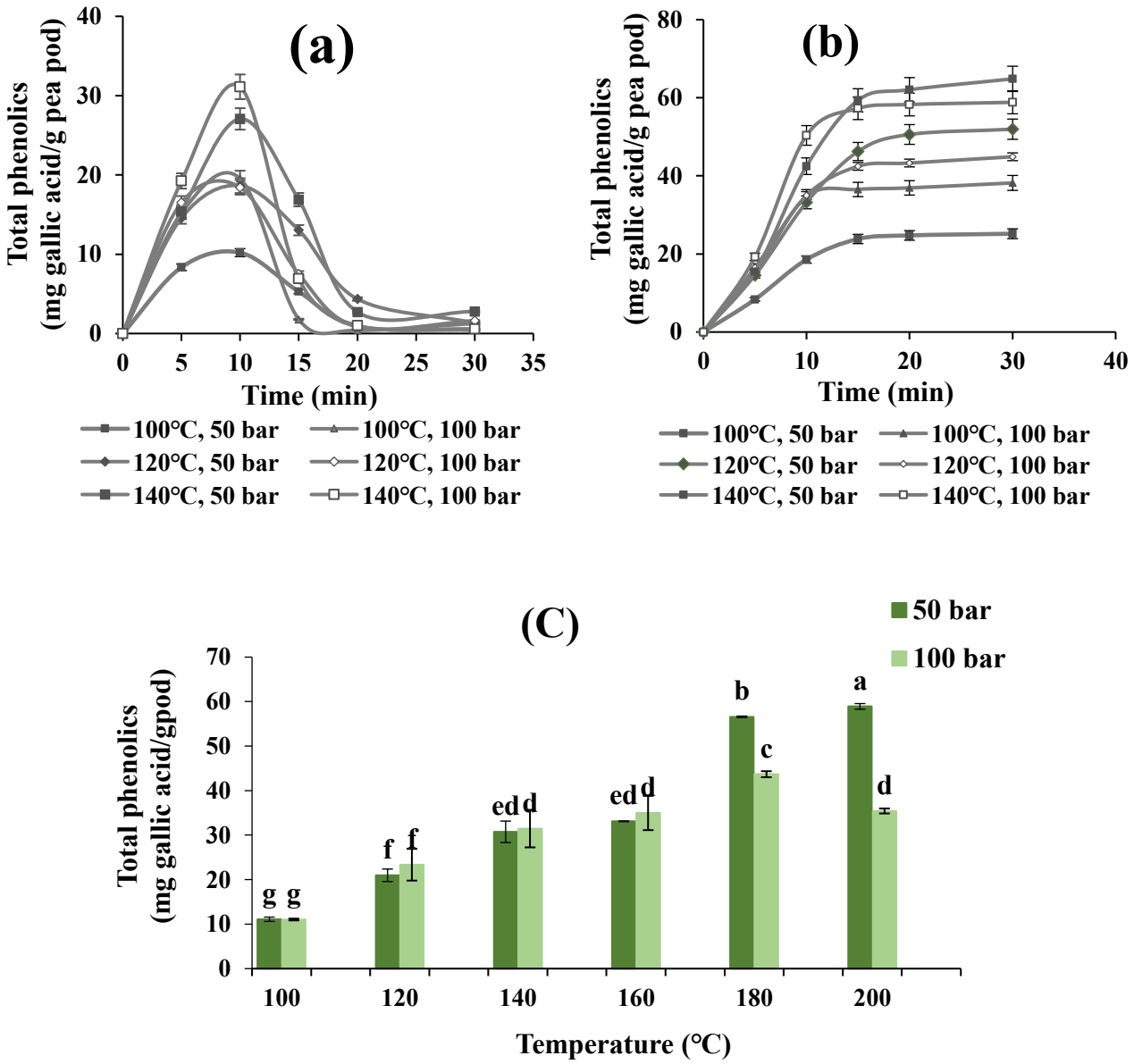


Fig. 3.3. (a) Kinetics of total phenolic extraction, (b) Cumulative trend of total phenolic extraction in 30 min, and (c) Total phenolic extraction from green pea pod using subcritical water for 40 min. [a-g]Letters indicate significant difference among bars at a specific pressure.

Whereas using hexane, chloroform, ethyl acetate and methanol separately at concentration of 80% for phenolic extraction from pea pod through refluxing at 60°C showed total phenolic content of 8.6±0.34, 8.0±0.34, 12.2±0.78, and 13.6±0.20 mg gallic acid/g pea pod (DW), respectively.

Although, these solvents solubilized phenolics based on their different polarities (Babbar et al., 2014), the phenolic content obtained by subcritical water (10-50 mg gallic acid/g pea pod) was higher than the values reported (pea pod: 13.6 mg gallic acid/g dried sample and cauliflower waste: 9.2 mg gallic acid/g dried sample). A recent study using cow pea pod and mung bean pod showed total phenolic contents of 10.11 and 10.75 mg gallic acid/g pod, respectively, by Soxhlet extraction using acetone for 8 h (Nehra, Singh, & Rani, 2018). In this study, subcritical water extraction removed more phenolics (54 mg/g green pea pod) within a shorter time (40 min) (Fig. 3.3c).

As known, phenolic compounds are divided into two groups based on their solubility: i) soluble phenolics that weakly interact with other compounds like carbohydrates in the vacuole of plant cells, and ii) insoluble bound phenolics that form covalent bonds in the cell wall matrices (Singh & Saldaña, 2011; Li et al., 2012). At the beginning of subcritical water extraction, soluble phenolics are free to interact with water molecules and released from green pea pod into the extract. However, insoluble bounded phenolics require high temperature to break covalent bonds between phenolic acids and carbohydrates or proteins. Earlier, Singh & Saldaña (2011) showed that such covalent bonds could be hydrolyzed by subcritical water at 100-200°C. It was previously reported that Maillard reaction initiates at temperatures >180°C between a reducing sugar carbonyl group and a free primary amine group of amino acids (He et al., 2012). At subcritical water condition, a rise in temperature provides high concentration of H^+ and OH^-, catalyzing the hydrolysis of polysaccharides and proteins into monosaccharides, and peptides, respectively (Huerta & Saldaña, 2017; Sereewatthanawut et al., 2008).

Since polysaccharides break into small molecules, those linkages between phenolic acids and carbohydrates disappear, increasing phenolic removal above 180°C. Gallic acid showed different

reaction rate constants of 5.9±0.3 ($min^{-1}\times10^3$) and 32.2±3.8 ($min^{-1}\times10^3$) at 100℃ and 150℃, respectively (Khuwijitjaru et al., 2014). Moreover, a study on gallic acid, protocatechuic acid, and salicylic acid solubility in subcritical water at 100-200℃ and 50 bar showed their increased solubility with an increase in temperature (Kayan, Yang, Lindquist, & Gizir, 2009). Kayan et al. (2009) showed that benzoic acid was stable at temperatures up to 199℃ but salicylic acid underwent severe degradation at 199℃. Also, an increase in SCW extraction temperature from 110℃ to 170℃ demonstrated a significant increase in gallic acid removal from pistachio hull (Erşan, Üstündağ, Carle, & Schweiggert, 2018). This explains the increased trend of phenolic removal from green pea pod using subcritical water (Fig. 3.3c).

3.6.3. Total tannin extraction

Fig. 3.4a shows the trend of total tannin removal from green pea pod at 100-200°C and 50 bar and 100 bar within 40 min. The highest content of total tannins (12.96 mg tannic acid/g pea pod) was extracted at 180℃ and 50 bar. But, S-L extraction using water removed 1.25±0.03 mg tannic acid/g pea pod at room temperature for 40 min. An increase in temperature from 100-180°C increased the total tannin removal from 2.24±1.07 to 12.96±4.45 mg tannic acid/g pea pod. However, change in pressure from 50 to 100 had no significant effect on tannin removal at 100-180°C.

Total tannins were removed from green pea pod with S-L extraction using water, 70% aqueous acetone and 70% aqueous ethanol with a solid to solvent ratio of 1:20 w/v at 50°C and 70°C within 3 h (Fig. 3.4b). As shown, 70% aqueous acetone (3.23-5.45 mg tannic acid/g pod) removed more total tannins compared with 70% aqueous ethanol (4.37-4.89 mg tannic acid/g pod) and water (1.81-3.06 mg tannic acid/g pod) at both temperatures investigated. However,

these contents were smaller than those obtained using subcritical water extraction (2.5-12.96 mg tannic acid/g pod) in 40 min (Fig. 3.4a). Previous studies showed the effect of solvents on the extraction of phenolic compounds from legumes such as carob (pod and seed) and white and colored pea seed coat where acetone-water extracted markedly higher amounts of polyphenols compared to methanol-water or ethanol-water (Avallone, Plessi, Baraldi, & Monzani, 1997; Troszynska & Ciska, 2002). Also, Nehra et al. (2018) used acetone, ethyl acetate and chloroform to remove total tannins from cow pea, mung bean and moth bean pod where Soxhlet extraction using acetone within 8 h resulted in the highest content of 1.55±0.13, 1.10±0.02, and 1.86±0.04 mg tannic acid/g pod, respectively. These values are smaller than the amounts obtained in Fig. 3.4b. Overall, these results showed that total tannins were better extracted by subcritical water in 40 min (Fig. 3.3a) than solid-liquid in 3 h (Fig. 3.4b).

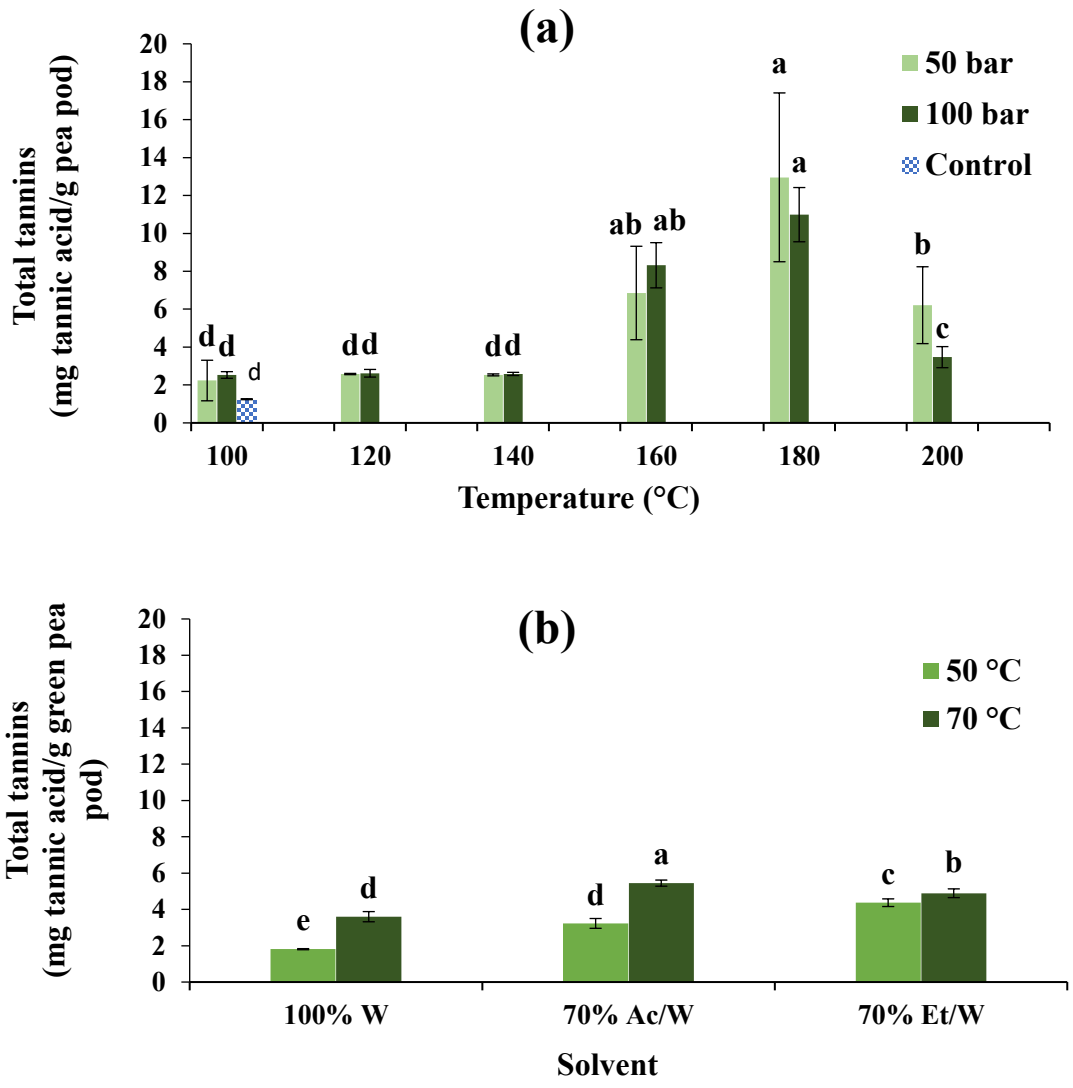


Fig. 3.4. Extraction of total tannins from green pea pod using: (a) subcritical water extraction for 40 min, and (b) Solid-liquid extraction for 180 min (W: water, Ac/W: acetone/water, and Et/W: ethanol/water). [a-e]Letters indicate the significant difference among bars at a specific temperature.

3.6.4. Total carbohydrate extraction

Total carbohydrates were removed from green pea pod at different temperatures (100-140℃) and pressures (50 and 100 bar) (Fig. 3.5). In general, the kinetic trend of carbohydrate removal was upward for all treatments. The maximum amount of total carbohydrates was obtained up to

20 min extraction and then remained almost constant for 40 min extraction time. Temperature had a crucial effect on extraction of carbohydrates from green pea pod. Increasing temperature from 100 to 140°C at both pressures resulted in an increased carbohydrate removal from 104.55±6.69 to 374.17±2.13 mg glucose equivalent/g pea pod at 50 bar and 65.92±3.33 to 402.81±7.33 mg glucose equivalent/g pea pod at 100 bar. But, pressure showed no effect on the removal of carbohydrates from green pea pod. The total carbohydrates obtained for subcritical water extraction at 140°C and 50 bar from green pea pod are higher than those of SCW treated straws (91.7±11.7 mg glucose equivalent/g barley straw and 51.5±8.7 mg glucose equivalent/g canola straw, Huerta & Saldaña, 2017). The yield of carbohydrate removal using subcritical water extraction at 140°C and 150 bar within 15 min of static holding time from barley hull was reported as 70.3 mg glucose equivalent/g barley hull (Sarkar, Alvarez, & Saldaña, 2014). According to Jalili Safaryan, Ganjloo, Bimakr, & Zarringhalami (2016), ultrasound-assisted extraction of green pea pod using an ultrasonic power of 135 W, sonication time of 50 min, ratio of raw material to water of 1:30 g/mL and temperature of 68°C removed 66.4% ±1.16 total polysaccharides, which was higher than values obtained using subcritical water extraction (Fig. 3.5). This is attributed to cavitation, mechanical and thermal effects occurring by ultrasound power, resulting in particle size reduction, disruption of cell walls and enhanced mass transfer across cell membranes. From 100°C to 140°C, there is an increasing trend of carbohydrate removal owing to the interactions of hydroxyl groups of sugar and water molecules at SCW

conditions.

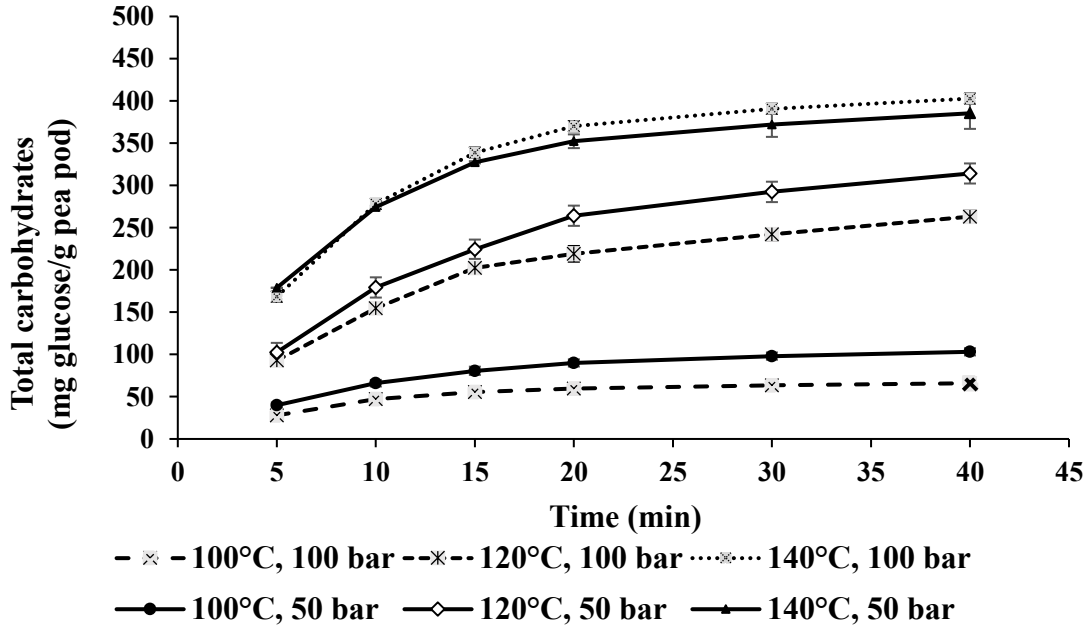


Fig. 3.5. Subcritical water extraction of total carbohydrates from green pea pod within 40 min.

3.6.5. pH of SCW extracts

The pH of the subcritical water extracts was around 5.7-5.9 at 100°C (Fig. 3.6). As temperature increased, the pH values slowly decreased to 4.05-4.24 at 200°C. A similar trend was observed for the pH values obtained at 50 or 100 bar. Clearly, this reduction in pH indicates the presence of phenolic acids in green pea pod extract. In a previous study on the production of phenolic compounds from rice bran, pH values decreased from 6.5 to 6.0 using a batch subcritical water system at 100°C-150°C (Pourali, Asghari, & Yoshida, 2010). Also, Klinchongkon et al. (2017) showed that the reduction of pH from 4.2 to 3.8 under SCW conditions resulted in the formation of phenolic acids. A decrease in pH is also related to the autocatalysis of water molecules (Liew et al., 2018). Moreover, Bobleter (1994) showed that pH reduction breaks ester or ether bonds between phenolic compounds, lignin, and carbohydrates.

Also, acidic conditions of subcritical water promoted removal of pectin and hemicellulose (Liew et al., 2018; Ciftci & Saldaña, 2015; Saldaña & Valdivieso-Ramirez, 2015).

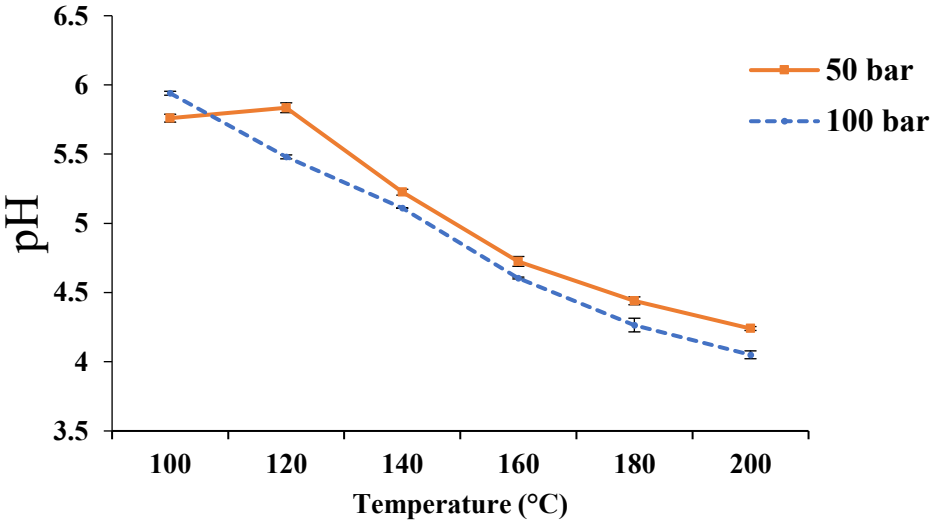


Fig. 3.6. pH values of subcritical water extracts stored at -18°C for four weeks.

3.6.6. Soluble and insoluble fiber content of green pea pod

Dietary fibers are found in different parts of pulses such as seeds, hulls, and pods (Tosh & Yada, 2010). Cellulose, hemicellulose, lignin and pectin are the most common fiber compounds found in pulse cell walls. Pulses such as pea, broad bean, and okara have 50%, 40%, and 50% dietary fiber in their pods (Mateos-Aparicio et al., 2010). Temperature plays an important role in removal of dietary fiber as it affects pH and the dielectric constant of water. An increase of temperature from 100℃ to 180℃ weakens hydrogen bonds of water followed by reduction in water polarity, releasing simple structured dietary fibers particularly hemicellulose and pectin. However, cellulose has higher degree of crystallinity compared to hemicellulose, which makes

cellulose insoluble in water. Previously, Bobleter (1994) addressed hydrothermal solubility of hemicellulose in water at temperatures above 180℃ without using pressure. This author showed that sugar content of the hemicellulose from wheat straw obtained at 190℃ was mainly composed of 55.36% xylose, 15.62% glucose, 5.80% arabinose, and 4.69% galactose. Likewise, total hemicellulose (96% DW) obtained from rice straw using subcritical water extraction at 150-190°C and 24 bar within 20 min (Rodríguez et al., 2009).

In this study, fiber content of the residue of green pea pod after treating with subcritical water extraction is reported in Table 3.3. Untreated sample had 55.10% DW total dietary fiber, including 5.59% DW soluble fiber and 49.50% DW insoluble fiber. After subcritical water treatment at 100-160℃ at 50 bar, the content of insoluble dietary fiber did not change significantly. However, when the temperature increased to 180℃ or 200℃ at 50 bar, the content of insoluble and soluble fiber in the residue decreased. At 180℃ and 200℃, insoluble dietary fiber reduced to 46.77% DW and 45.09% DW, respectively. Dietary fiber content of hulls was reported as 75% DW for chickpeas, 87% DW for lentils, and 89% DW for peas (Dalgetty & Baik, 2003). Subcritical water has been used for removal of dietary fibers such as pectin from *Citrus junos* peel, and apple pomace. Subcritical water removed pectin from apple pomace (48.2% DW) and citrus peel (16.8% DW) at 120–150°C and 200 bar for 5 min and 20 min, respectively (Wang, Chen, & Lü, 2014). Liew et al. (2018) also removed pectin (19.6% DW) from pomelo peels using a dynamic subcritical water extraction at 120°C and 30 bar.

Table 3.3. Fiber composition of green pea pod residue after subcritical water extraction.

Treatment	Soluble fiber (%)	Insoluble fiber (%)	Total fiber (%)
Untreated	5.59±0.03[a]	49.50±0.02[a]	55.10±0.05[a]
SCW (100℃,50 bar)	5.53±0.02[a]	48.92±0.04[a]	54.45±0.06[a]
SCW (120℃,50 bar)	5.48±0.01[a]	48.81±0.03[a]	54.29±0.04[a]
SCW (140℃,50 bar)	5.52±0.01[a]	49.73±0.03[a]	55.26±0.04[a]
SCW (160℃,50 bar)	5.55±0.01[a]	53.35±0.09[a]	58.90±0.10[a]
SCW (180℃,50 bar)	4.23±0.01[b]	46.77±0.10[b]	51.00±0.11[b]
SCW (200℃,50 bar)	3.25±0.00[c]	45.09±0.16[c]	48.34±0.16[c]

SCW: subcritical water extraction, data were reported as the mean±standard deviation of duplicate treatments. [a-c]Letters indicate significant difference among means in each column with α=0.05 using Tuckey comparison.

3.7. Conclusions

Removal of phytochemicals such as phenolics, tannins and carbohydrates from green pea pod was successfully performed using subcritical water extraction. Temperature significantly influenced phytochemicals removal. An increase in temperature from 100 to 180℃ increased the total tannin removal from 2.24±1.07 to 12.96±4.45 mg/g pea pod. The optimum removal condition to obtain the highest yield of phenolics (56.59±0.16 mg gallic acid/g pea pod) and total tannins (12.96±4.45 mg tannic acid/g pea pod) was 180℃ at 50 bar. Also, maximum removal of (402.8±7.33 mg glucose equivalent/g pea pod was obtained at 140℃ under both pressures. Glucose (18.53±0.09% DW), galactose (9.71±0.04% DW), and xylose (4.97±0.001% DW) were the main hemicellulosic sugars detected in green pea pod using GC analysis. Fiber content of green pea pod showed 5.59±0.03% DW soluble fiber, and 49.50±0.02% DW insoluble fiber. These values

decreased to 4.23±0.01% DW soluble fiber and 46.77±0.10% DW insoluble fiber after subcritical water extraction at 180℃ and 50 bar, indicating removal of soluble and insoluble fibers at this condition. Extraction of phytochemicals from green pea pod was favored using subcritical water than with the conventional S-L extraction.

3.8. Recommendations

- Further characterization of individual phenolic compounds in green pea pod before and after subcritical water extraction are required.
- In this study, total dietary fiber content of green pea pod was reported. Analysis of lignin, cellulose, hemicellulose, and pectin are recommended.
- More studies on dietary fiber content of SCW extract is needed.
- Study on hemicellulosic sugar content of SCW extract is recommended to estimate degradation of carbohydrates

Acknowledgements

We are grateful to the Natural Sciences and Engineering Research Council of Canada (NSERC, #05356-2014) for funding this research.

Chapter 4: Use of subcritical water extraction for removal of tannins and total dietary fiber from faba bean hull

Abstract

Faba bean hull contributes 15% of the bean dehulling process by-product. Faba hull is a good source of tannin and phenolics but there is no report on tannin, phenolics and fiber removal using an environmentally friendly extraction technique from faba bean hull. In this study, subcritical water (SCW) extraction was used as a green technology to remove tannins from faba bean hull. These values were compared to those obtained by traditional solid liquid (S-L) extraction. Treatment conditions for subcritical water extraction were 100-200°C and 50-100 bar with a constant flow rate of 5 mL/min for 40 min. The S-L extraction was carried out using different solvent systems (water, 70% acetone, 70% ethanol, and 50% ethanol) with solid to solvent ratios of 1:10, 1:15, and 1:20 w/v at 50-70℃ for 3h. Extracts were analyzed for total tannins, condensed and hydrolysable tannins, phenolics, and total carbohydrates using spectrophotometer methods. Individual tannins were also analyzed by HPLC. Total dietary fiber was determined using combined enzymatic-gravimetric methods. The solid residues were also analyzed for hemicellulosic sugars. The best condition for total tannin removal was achieved at 160°C and 50 or 100 bar (~73.6±0.75 mg tannic acid/g hull). The increase of pressure from 50 to 100 bar had no effect on removal of total tannin at temperatures of 100-200°C. For condensed tannins, the maximum removal was 42.28±2.04 mg catechin/g hull. Also, temperature changes from 100°C to 160°C increased the content of condensed tannin removal from 22.34-42.28 mg catechin/g faba bean hull (an increase of 1.8 times). However, an increase of pressure from 50 to 100 bar had no significant effect on the removal of condensed tannins from faba bean hull extracts in all temperatures. In addition, the highest removal of total phenolics (~44.37 mg gallic acid/g hull)

was obtained at 120°C and 50 bar. For total carbohydrates removal the highest removal obtained at 160°C and 50 bar (382.14 mg glucose equivalent/g hull) or 100 bar (354.21 mg glucose equivalent/g hull). The aqueous mixture of acetone 70% was the most effective solvent system for the removal of total tannins at 70°C. In addition, SCW treatment at 200°C and 50 bar led to a faba bean hull solid residue containing total dietary fiber (~71.14% DW), soluble fiber (4.36% DW), and insoluble fiber (65.71% DW). However, using solid liquid extraction with acetone and ethanol obtained (5.6-5.9% DW) soluble fiber and (78.29-80.77% DW) insoluble fiber in the residue. The faba bean hull sugar profile suggested that it is mainly composed by glucose (53.6% DW), followed by arabinose (4.6% DW), galactose (2% DW), rhamnose (1% DW), and xylose (1% DW). The SCW extraction was better than the S-L extraction to obtain valuable bioactive compounds, such as tannins, phenolics, and carbohydrates from faba bean hull by-product for nutraceutical applications.

Keywords: Faba bean hull, Tannins, Phenolics, Carbohydrates, Total dietary fiber, Soluble fiber, and Insoluble fiber.

4.1. Introduction

Faba bean hull with total worldwide production of 2.6 million hectare is considered an abundant and cheap alternative source to obtain tannins, and soluble and insoluble fiber (Multari, Stewart, & Russell, 2015). In Canada, major production of faba bean has been reported for Alberta, Manitoba, and Saskatchewan (Oomah et al., 2011).

Tannins are anti-inflammatory, anti-carcinogenic, antiviral and antibacterial agents with antioxidant and antiradical activity found in pulses like faba bean (Muzquiz et al., 2012; Frankel, German, Kinsella, Parks, & Kanner, 1993). Besides tannins, carbohydrates and dietary fibers (soluble and insoluble fiber) are major components of faba bean hull. Fiber has beneficial effects on human health and is required in the food and chemical industry (Elleuch et al., 2011). For these reasons, faba bean hull can be a source of fiber similarly as wheat bran, sunflower hull (Dreher & Padmanaban, 1983) and peanut hull (Childs & Abajian, 1976) in food products. Fibers are divided into soluble and insoluble fibers based on their solubility in water. According to this classification, pectin, gum, mucilage, and some hemicelluloses are water soluble, whereas cellulose, some types of polysaccharides (xyloglucans, xylans, mannans and glucomannans) and lignin are insoluble (Scheller & Ulvskov, 2010). In the food industry, fiber compounds are used to improve the viscosity, texture, sensory characteristics and shelf-life of food products (Elleuch et al., 2011). Also, there is a growing interest toward plants containing dietary fibers and bioactive compounds such as grape by-product for their benefits to human gastrointestinal health activity (Zhu, Du, Zheng, & Li, 2015). As reported by Zhu et al. (2015), 5% grape pomace flours can be used in formulation of biscuits with good acceptance in terms of sensorial properties.

Solid-liquid (S-L) extraction using water is a traditional extraction technique with a long history in removal of phenolics from legumes (Amarowicz & Shahidi, 2017; Amarowicz,

Troszynska, Barylko-Pikielna, & Shahidi, 2004; Luo, Cai, Wu, & Xu, 2016). Common organic solvents such as methanol, ethanol and acetone have often been used for the separation of tannins, phenolics, carbohydrates and fibers from fruits and vegetables. However, the use of S-L extraction is time consuming, and requires large volumes of non-environment-friendly organic solvents. On the other hand, subcritical water (SCW) is an environment friendly technique, providing a short extraction time for the removal of different components (Saldaña & Valdivieso, 2015). For example, subcritical water has been used for the conversion of polysaccharides into oligosaccharides of coconut meal (Khuwijitjaru, Pokpong, Klinchongkon, & Adachi, 2014; Khuwijitjaru, Watsanit, & Adachi, 2012), phenolic and carbohydrates extraction from crop by-products (Ciftci & Saldaña , 2015; Haldar, 2013; Sarkar, Alvarez, & Saldaña, 2014) and removal of cellulose (Lü & Saka, 2010) and protein (Pińkowska & Oliveros, 2014). In this regard, SCW technique is prioritized to acid or enzymatic hydrolysis for separation of hemicellulose and lignin without additional costs for neutralizing chemicals or additional sample pre-treatment steps (Converse, Kwarteng, Grethlein, & Ooshima, 1989; Thompson & Grethlein, 1979).

Also, at subcritical condition, a decrease in dielectric constant of water increases its ion products, which favors hydrolysis reactions, where water acts as both the reaction medium and the reactant. As well, applying pressure on water at elevated temperatures above its boiling point induces the removal of polar and non-polar components from the initial matrix. In addition, comparing traditional solvent extraction with SCW extraction has shown better mass transfer rate, shorter extraction times and higher extraction yields by SCW extraction (King, 2014; Mendiola, Herrero, Cifuentes, & Ibañez, 2007; Saldaña & Valdivieso-Ramirez, 2015). Therefore, the aim of this study was to evaluate the effect of SCW on the removal of

phytochemicals such as tannins, phenolics and carbohydrates from faba bean hull, and compare the results to the solid-liquid traditional extraction technique.

4.2. Materials and methods

4.2.1. Sample preparation

Faba bean Tina variety was kindly provided by Alberta Pulse Growers commission (Edmonton, AB, Canada). Faba bean hulls were obtained using a dehuller (Buhler MLU 202 Flour mill, Markham, ON, Canada). The hulls were then milled using a Retsch mill (ZM 200, Burlington, ON, Canada) and sieved to obtain particles of 0.5 mm. The ground samples were vacuum packed in moisture and oxygen barrier plastic containers and stored at 4°C until further use.

4.2.2. Chemicals

Chemical reagents, such as sulphuric acid (97%, ACS reagent), ethanol (99.9%, HPLC grade), acetone (99.9%, HPLC grade), sodium carbonate anhydrous (≥97%, ACS grade), Folin-Ciocalteau's phenol reagent (2N), Folin-Denis' reagent (99% purity), gallic acid (99.9% purity), D-(+)-glucose (99% purity), tannic acid (98% purity), vanillin (99% purity), and (+)-catechin hydrate (minimum 98%) were purchased from Sigma-Aldrich Co. (Oakville, ON, Canada). Glass beads (2.3 mm) and glass wool were purchased from Fisher Scientific Co. Ltd (Toronto, ON, Canada). Total dietary fiber kit containing α-amylase (thermostable, 3000 units/mL), protease (50 mg/mL, 350 Tyrosine units/mL), and amyloglucosidase (3,300 units/mL) were purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland)

4.2.3. Proximate compositional analysis

Moisture content was determined gravimetrically by drying the faba bean hulls in an air oven (Model 655G, Fisher Scientific IsoTemp® oven, Toronto, ON, Canada) at 105 ℃ for 3 h. The ash content of the faba bean hull was determined according to the AOAC 923.3 method. The protein content was determined using a Leco TruSpec nitrogen analyser (Model FP-428, Leco instruments Ltd., Mississauga, ON, Canada). The fat content was determined by the Goldfisch extraction unit (Labconco Co., Kansas, MO, USA). Percentages of the moisture, ash, and protein and fat contents were added and the final value deducted from 100% to calculate the total carbohydrate content (Chapter3, section 3.3).

Faba bean hull contents of total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF) were determined according to method described in Chapter 3, Section 3.3.7.

4.3. Subcritical water extraction (SWE)

The SCW extraction unit and procedure was done using the same system described in Chapter 3, Section 3.4.2.

4.4. Conventional solid-liquid extraction

Freeze dried hull sample (2 g) was extracted using water, 70% aqueous acetone, 70% aqueous ethanol and 50% aqueous ethanol at two different temperatures of 50°C and 70°C for 3 h. The solid to liquid ratios investigated were 2:20 w/v, 2:30 w/v and 2:40 w/v.

After the extraction, the supernatants and the residues were collected for analysis of total tannins and total fiber content, respectively.

4.5. Characterization of liquid extracts

4.5.1. Total tannin content

Same methodology reported in Chapter 3, Section 3.5.1 was used.

4.5.2. Condensed tannin content

The vanillin method of Price et al. (1978) was used with some modifications. The liquid extract (1 mL) was mixed with 5 mL of vanillin reagent, containing 0.5% vanillin and 8% concentrated HCl in acetic acid. Then, the mixture was vortexed for 10 sec. The absorbance of the samples and blank were measured at 500 nm, and incubated for 20 min in the dark at 30℃. The absorbance of the blank was subtracted from the absorbance of the sample. The content of condensed tannin was expressed as catechin equivalent per gram of faba bean hull (Fig. A4).

4.5.3. Individual tannin content

For characterization of individual condensed and hydrolysable tannins of faba bean hull extract, HPLC analysis was performed with a Shimadzu 20 HPLC (Shimadzu, Kyoto, Japan) equipped with a Shimadzu SPD-M10A diode array detector (200-300 nm). The column was a ZORBAX Eclipse XDB 80Å C18 column (150×4.6 mm, 5 μm Nomura Chemical Co., Seto, Japan) equipped with a guard column (10×4 mm, Nomura Chemical Co., Seto, Japan). The flow rate of the mobile phase was 1 mL/min and the injection volume was 20 μL. The solvent A had 0.5% formic acid and the solvent B had 0.5% formic acid in methanol. The solvent gradient started at 8% B and increased to 27% B over 25 min, then there was an increase to 30% over 7 min, following an increase to 100% B over 4 min and hold for 5 min. After that, there was a decrease to 8% B over 1 min and hold for 5 min. The wavelengths were scanned from 190 nm to

500 nm, and data was quantified at 268 nm. Faba bean hull extracts were filtered through a 0.22 μm EMD Millipore Millex™ Sterile Syringe Filter before HPLC analysis.

4.5.4. Total phenolic content

Total phenolic content of faba bean hull extract was determined using the methodology described in Chapter 3, Section 3.5.2.

4.5.5. Total carbohydrate content

Total carbohydrate was measured using the same methodology reported in detail in Chapter 3, Section 3.5.3.

4.5.6. Hemicellulosic sugar content

The neutral sugar composition of faba bean hull was determined according to the method described in Chapter 3, Section 3.5.4.

4.6.Statistical analysis

The statistical analysis was performed to evaluate the differences among the extraction treatments. Two-way analysis of variance (ANOVA) and the significant difference of the data at $p < 0.01$ was carried out using Minitab version 18.0 (Minitab Inc., State College, PA, USA) at 95% confidence interval.

4.7. Experimental design

The full experimental design was conducted with four levels for the solvent type (Water, 70% aqueous acetone, 50% aqueous ethanol, and 70% aqueous ethanol) and three levels of solid-to-solvent ratio (2:20, 2:30, and 2:40 w/v). Six replications were carried out for each operating condition.

4.8. Results and discussion

4.8.1. Proximate compositional analysis

The proximate composition of faba bean hull is reported for the first time in Table 4.1. Carbohydrates (81%) were the major component of faba bean hull, including soluble fiber (5.60% DW), insoluble fiber (73.69% DW) and starch (4.13% DW). There were no significant differences between the low tannin and high tannin varieties in terms of proximate composition. High tannin hull and low tannin hull had 10.66% DW and 10.46% DW moisture contents, respectively. Both faba bean hull samples had relatively similar protein content, fat and ash content. Comparison of data obtained for the hull with that of the faba seed reported by Turco, Ferretti & Bacchetti (2016) indicated that faba bean seed has four times more protein content (20%) than the hull (5%). These quantities were in agreement with the protein content of 24.7-37.2 (%) dried weigh (DW) in different genotypes of faba bean seeds and 27-32% DW in commercial faba bean varieties reported by Duc, Marget, Esnault, Le Guen, & Bastianelli (1999). Khalil and Mansour (1995) reported 5% DW crude fiber and 44.1% DW starch as the two-major components of faba bean seed. Also, Makkar et al. (1997) reported crude fiber of 9.9-14.2% DW, and starch of 40.6-48.5% DW in colored flowered beans, and crude fiber of 8.7-12.8% DW, and starch of 41.7-47.6% DW in white flowered beans. Also, crude fiber contents of low and high tannin faba seeds were reported by Duc et al. (1999) as 8.8 and 9.9% DW, respectively.

Tale 4.1. Proximate compositional analysis of faba bean hull.

Component	Pulses			
	High tannin hull (This study)	**Low tannin hull (This study)**	**Faba bean hull residue (This study)**	**Faba seed***
Moisture (%)	10.66±0.15[a]	10.46±0.50[a]	4.27±0.50[c]	NR
Protein (%)	4.82±0.20[a]	5.34±0.21[a]	3.75±0.15[b]	20-41
Ash (%)	2.45±0.17[a]	2.48±0.19[a]	2.41±0.50[a]	NR
Fat (%)	0.77±0.01[a]	0.78±0.01[a]	0.58 ±0.02[c]	1-2.5
Carbohydrates (%)	81.30	80.95	88.99	51-68
Total dietary fiber (%)	79.29±0.04[b]	76.14±0.50[b]	88.46±0.50[a]	15-30
Soluble fiber (%)	5.60±0.02[a]	2.43±0.50[c]	4.28±0.50[b]	NR
Insoluble fiber (%)	73.69±0.02[b]	68.65±0.05[c]	84.18±0.50[a]	NR
Starch (%)	4.13±0.24[a]	3.44±0.28[b]	NR	41-53

*Turco, Ferretti & Bacchetti (2016); NR: not reported, data with similar letters are not significantly different, faba bean hull residue obtained using subcritical water extraction at 180℃ and 50 bar for 40 min. Data is presented as mean±standard deviation between them. Total dietary fiber is reported as the summation of soluble fiber and insoluble fiber. [a-c]Letters indicate significant difference between mean values for each row.

Available data on the quantity of tannins, and phenolics are restricted only to the seed, and cotyledon. The contents of tannins and phenolics in faba bean rely on the variety and growth condition of the faba bean cultivar. For example, total content of phenolic compounds varied for Blandine whole faba seed (2.41 mg/g) , cotyledon (2.71 mg/g), hull (0.61 mg/g); and for Alfred cultivar of whole faba seed (2.71 mg/g), cotyledon (4.13 mg/g), and hull (12.07 mg/g) (Bekkara,

Jay, Viricel, & Rome, 1998). The use of acidified methanol/water (0.01% HCl) for faba bean seed resulted in total phenolic content of 0.082-0.134% gallic acid, and condensed tannin of 0.031-0.096% catechin (Baginsky et al., 2013). Phenolic profiles of faba bean seed and hull have shown different tannins and phenolics. Alfred var. hull has catechin derivatives, while Blandine var. hull has phenolic acids, flavones, flavonols and dihydroflavonols (Bekkara et al., 1998).

4.8.2. Total tannins

Fig. 4.1 shows the trend of faba bean hull total tannin removal at temperatures of 100-200°C and pressures of 50 bar and 100 bar with a total extraction time of 40 min. As shown, the highest amount of total tannin removal was achieved at 160°C and 50 bar. The increase of pressure from 50 to 100 bar had no significant effect on removal of total tannins at 100-200°C. Temperature was a crucial factor for the removal of total tannins from faba bean hull. Increasing temperature from 100 to 160°C at 50 bar resulted in an increase of tannin removal from 28.77±1.12 to 73.62±0.75 mg tannic acid/g faba bean hull (an increase of 2.5 times). The highest amount of total tannin obtained in faba bean hull extract at 160°C and 50/100 bar (7.36-7.05%) was higher than the total tannin content of faba bean seed (0.75-2.00%) and faba bean cotyledon (0.74-0.91%) and other legumes such as pea, soybean, chickpea, etc. (Table 4.1). Also, total tannin content of faba bean hull extract (7.35-7.37%) in this study was higher than that of canola hull (5.8%, Amarowicz, Naczk, & Shahidi, (2000)). Elias, de Fernández, & Bressani (1979) found higher total tannin content in colored seed coats than those in white seed coats. Tannin concentration was high in colored seed coats (3.8-4.3%) and low in white beans (0.13%) while values ranged from 0.38-0.59% in the cotyledons (Elias, de Fernández, & Bressani, 1979).

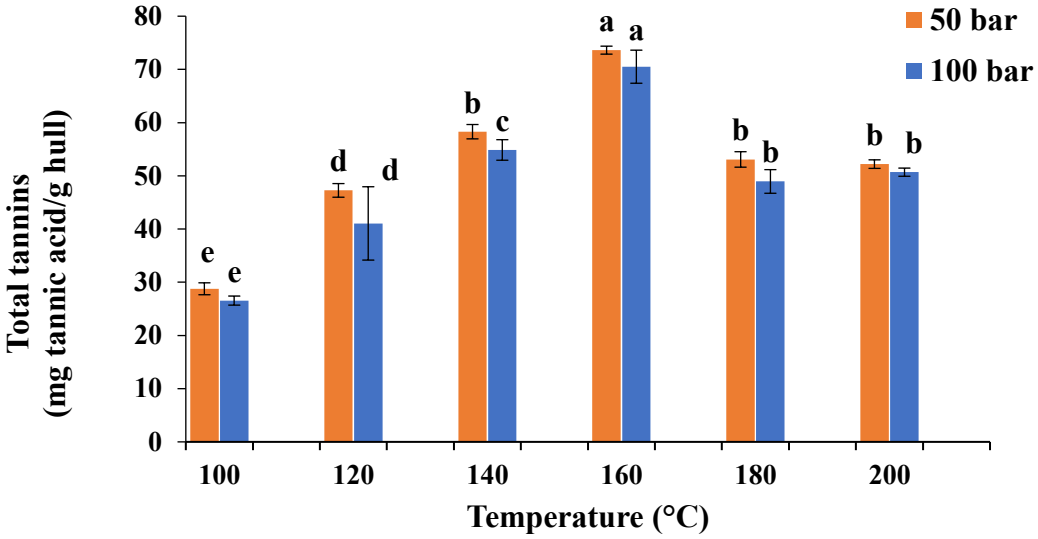


Fig. 4.1. Subcritical water extraction of total tannins from faba bean hull at a flow rate of 5 mL/min in 40-min extraction. [a-e]Letters indicate significant difference among all bars.

Table 4.2. Distribution of tannin content in different legumes.

Pulse	Whole seed (%)	Cotyledon (%)	Hull (%)
Adzuki bean (*Vigna angularis L.*)	0.29	nd	nd
Chickpea (*Cicer arietinum*)	0.078-0.272[a]	0.016-0.038[a]	nd
Cowpea (*Vigna sinensis L.*)	0.175-0.590[a]	0.028[a]	nd
Faba bean (*Vicia faba L.*)	0.750-2.00[b]	0.740-0.910[b]	7.35-7.37[c]
Mung bean (*Pbaseolus aureus L,*)	0.437-0.799[a]	0.021-0.039[a]	nd
Kidney bean (*Dolicbos lablab*)	1.024[a]	0.073[a]	nd
Lima bean (*Pbaseolus lunatus*)	0.650-0.930[b]	nd	nd
Pea (*Pisum sativum L.*)	0.500-1.050[b]	0.460-0.560[b]	nd
Soybean (*Glycine max L.*)	0.045[a]	0.034[a]	nd
Canola	nd	nd	5.8[d]

[a]Tannin content expressed as catechin equivalent, [b]Tannin content expressed as tannic acid equivalent (Reddy, Pierson, Sathe, & Salunkhe, 1985), [c]This study, and [d]Amarowicz et al. (2000).

4.8.3. Condensed tannins

Fig. 4.2 shows the trend of faba bean hull condensed tannin removal at temperatures of 100-200°C and pressures of 50 bar and 100 bar for a total extraction time of 40 min. As shown, maximum extraction was obtained at 160°C and 50 bar (42.28 mg catechin/g faba bean hull). Temperature changes from 100°C to 160°C increased the content of condensed tannin removal from 22.34 to 42.28 mg catechin/g faba bean hull (an increase of 1.8 times). However, an increase of pressure from 50 to 100 bar had no significant effect on the removal of condensed tannins from faba bean hull at all temperatures investigated. These values were higher than those obtained using HPLC due to the overestimation of spectrophotometry method. Besides, analysis of condensed tannin in faba bean hull was carried out following Price et al. (1978) method using methanol as a solvent and vanillin reagent, resulting in the removal of 72.76 mg catechin/g faba bean hull. Earlier, it was reported that vanillin and catechin reaction is mainly rely on the type of solvent used (Butler, Price, & Brotherton, 1982). For example, compared with methanol, which is the common solvent used for such reaction, the use of glacial acetic acid or acetonitrile produced a more intense color and absorption at 500 nm. Price et al. (1978) and Gupta and Haslam (1979) confirmed that the presence of methanol induced a rapid decrease in absorbance because the vanillin used reacted slowly with catechin in the sample matrix. Also, using glacial acetic acid showed a similar kinetic behavior for tannin compound reaction with vanilin similar to the reaction of catechin with vanillin.

Moreover, when catechin (flavan-3-ol monomer) was used as standard instead of purified condensed tannin, an overestimation of tannin content was reported because more chromophore could be produced per milligram of purified tannin than it was produced per milligram of catechin (Price et al., 1978; Gupta and Haslam, 1979). In case of glacial acetic acid, the

absorption produced by the polymeric tannin was much less than that from the monomeric catechin (Butler et al., 1982). This reason can explain the increasing trend of condensed tannin from 100°C to 160°C where elevation of temperature could remove more monomeric condensed tannins. For lentil and faba bean whole seed, Jin et al. (2012) reported the presence of specific condensed tannins, like flavan-3-ols (gallocatechin, epigallocatechin, catechin, and epicatechin) using HPLC. Although individual concentrations of these condensed tannins were not reported, the total condensed tannin content of faba bean seed was reported as 0.65% fresh weight (FW). In Fig. 4.2, the highest removal of condensed tannin from high tannin variety of faba bean hull was 4.23% dried weight (DW) (Fig. 4.2). This amount is higher than other sources of condensed tannins such as pea seed (0.29-0.36% FW), lentil seed (0.26-0.37% FW), blueberries (0.33% FW), cranberries (0.42% FW), small red beans (0.45% FW), sorghum (0.44% FW) and hazelnuts (0.50% FW) (Gu et al., 2004).

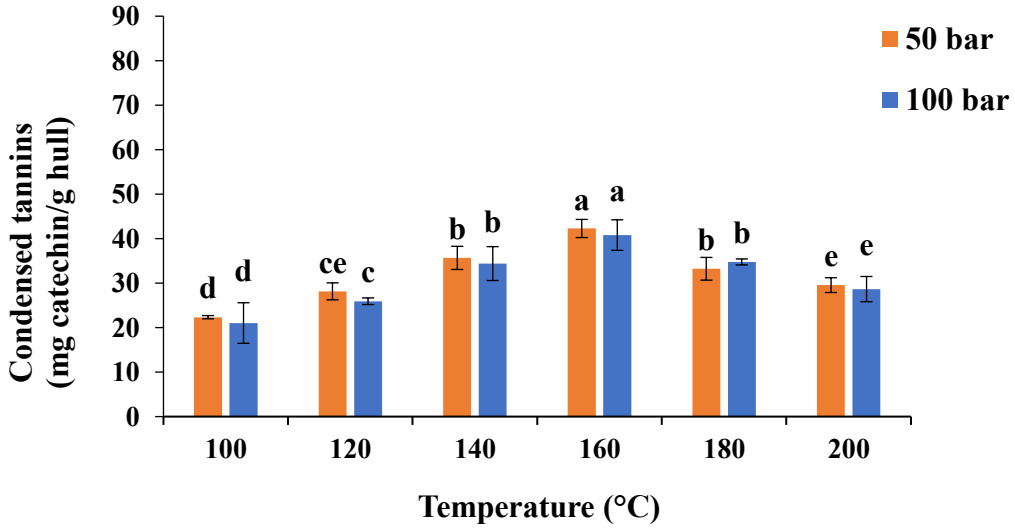


Fig. 4.2. Subcritical water extraction of condensed tannins from faba bean hull at a flow rate of 5 mL/min in 40-min extraction. [a-e] Letters indicate significant difference among all bars.

Also, epimerization of epicatechin gallate (ECG), epigallocatechin (EGC), and epimers, namely gallocatechin gallate (GCG), (-)-catechin gallate (CG), (-)-gallocatechin (GC), and (-)-catechin could occur at 120°C for 30 min at a pH range of 5-6, while epimerization decreased during 60-90 min at 130°C and pH=7 (Seto, Nakamura, Nanjo, & Hara, 1997). Therefore, conversion of different tannin compounds to their epimers due to the change in process parameters such as temperature (100-200°C), pressure (50 and 100 bar) and pH (3-5) of subcritical water could be the reason of increase and decrease in removal content of condensed tannins within 40 min extraction.

4.8.4. Hydrolysable tannins

For hydrolysable tannin content determination, the method of Hartzfeld et al. (2002) was used but the expected red color change corresponding to the conversion of hydrolysable tannins into methyl gallate was not observed. This method worked well for determination of hydrolysable tannins from green tea and mate tea samples (Chourio, 2018). This method was selected because it is a quick and easy method. This method was also used to measure hydrolysable tannins in oak and maple (Hagerman, 1988). To the best of our knowledge, the only difference between faba bean hull sample and mate tea sample is its proximate composition. Also, it was claimed that small traces of water can form a mixture of gallic acid and methyl gallate, preventing the reaction between methyl gallate and potassium iodate. Thus, to prevent such interference, the same method was tested using raw faba bean hull, previously dried with liquid nitrogen.

4.8.5. Quantification of individual condensed tannins with HPLC

HPLC analysis of faba bean hull showed the presence of six catechins: (-)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG), and catechin gallate (CG) at 100-200℃ and 50 bar (Table 4.3). Among them,

gallocatechin gallate was not detected in faba bean hull extracts at both pressures. At 50 bar, (-)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and catechin gallate (CG) were detected whereas at 100 bar only peaks for catechin (C), (-)-epicatechin (EC), and (-)-epicatechin gallate (ECG) were detected. According to previous studies, different catechins are present in various varieties of faba bean. Earlier, Jin et al. (2011) reported no catechins in 'snowbird' faba seed cultivar. However, catechin, gallocatechin, epigallocatechin, and epicatechin were detected in 'CDC fatima' cultivar. Also, faba bean whole seed had the highest catechin content (0.65 g/g DW) compared to pea whole seed (0.29-0.37 g/g DW) and lentil whole seed (0.26-0.37 g/g DW). Baginsky et al. (2013) quantified (+)-catechin (8.4-97.8 mg/g DW) and (-)-epicatechin (14-70 mg/g DW) in 10 different varieties of faba bean whole seed from Spain, Chile and Syria using HPLC-DAD/ESI-MS analysis of faba bean extracts (20 g seed in 80 mL methanol/water 80:20 v/v acidified with 0.01% HCl) (Baginsky et al., 2013; Turco, Ferretti, & Bacchetti, 2016) whereas subcritical water extraction removed 3.35-4.53 mg/g catechin and 1.64-1.88 mg/g epicatechin from faba bean hull (Table 4.3). Total catechin content obtained by HPLC (14.44-16.88 mg/g) (Table 4.3) was less than values obtained using spectrophotometer (Fig. 4.2). This difference could be attributed to the presence of other macromolecules such as proteins, carbohydrates and pectin in faba bean hull extract, interfering in the spectrophotometric measurements. It was reported that sugar molecules bound to the available hydroxyl groups located in the flavonoid structure of condensed tannins, which make the structure of these molecules more complex (Jakobek, 2015). Some of these carbohydrates exist in the cell wall of pulses such as pectin, cellulose or dietary fiber where weak bonds (H-bonds and hydrophobic interactions) occur between them (Padayachee et al., 2012a, 2012b). Recently, it was shown that apple and pear condensed tannins, mainly flavan-3-ol

oligomers and polymers containing (-)-epicatechin (with degree of polymerization >100) could adsorb on the cell wall (Renard, Watrelot, & Le Bourvellec, 2017). Applying high temperatures of (100-180℃) enhances cell wall destruction, leading to carbohydrate and tannin interactions. These interactions between condensed tannins and cell wall macromolecules enlarge condensed tannin structures and prevent the detection of catechins using HPLC in the extracts obtained at high pressure.

Increasing temperature from 100 to 200℃ induced a decrease in (-)-epicatechin gallate and (-)-epicatechin removal and favor catechin gallate removal (Table 4.3). These changes can be related to epimerization of catechins under heat treatment (Jeong et al., 2018). Chen et al. (2001) showed that green tea mainly has epi-forms of catechins, forming non-epi form of catechins by epimerization after heat treatment. For this reason, autoclaved canned or bottled tea drinks at 120°C for 20 min have shown a higher level of (-)-gallocatechin gallate, gallocatechin, catechin gallate and catechin (Chen et al., 2001). Epicatechin gallate, epigallocatechin and epicatechin can be converted to their epimers that are gallocatechin gallate, catechin gallate, gallocatechin and catechin. Also, HPLC analysis showed that ellagic acid content increased with temperature of 100-200℃ to 2.18-3.38 mg/g faba hull (Table 4.3). Previous studies confirmed ellagic acid as the hydrolysis product of hexahydroxydiphenic acid (HHDP) linked to glucose as a polyol unit. This polyol unit is an intermediate compound of ellagitannins, containing ester bonds that are broken under acidic or alkaline conditions.

The H-NMR and ^{13}C-NMR study on the structure of products from (+)-catechin and (-)-epicatechin showed that molecular weight of each of these products are identical to (-)-catechin and (+)-epicatechin as their starting compounds. Also, the optical rotation of the products from (+)-catechin (2R:3S absolute configuration) and (-)-epicatechin (2R:3R) were reported as (+)-

epicatechin (2S:3S) and (-)-catechin (2S:3R), respectively. Therefore, (+)-catechin and (-)-epicatechin can yield (+)-epicatechin and (-)-catechin due to isomerization at the 2-position in hot aqueous solution (Seto et al., 1997). The isomerization of catechin and epicatechin can explain why catechin and epicatechin are present at both 50 and 100 bar (Tables 4.3 and 4.4).

Moreover, Wang & Helliwell (2000) studied the effect of heating catechins at 20, 40, 80 and 100°C for 20 min and their epimerization rate revealed that epimerisation for all individual catechin standards initiates at temperatures above 80°C. At 100°C, epigallocatechin gallate (EGCG), epigallocatechin, and epicatechin gallate could convert to their epimers as gallocatechin gallate, gallocatechin, and catechin gallate, respectively. According to Ananingsih, Sharma, & Zhou (2013), heating conditions have an impact on the epimerization of epigallocatechin gallate, epigallocatechin, epicatechin, and epicatechin gallate, decreasing concentration of these catechins, while increasing concentrations of their isomers gallocatechin gallate, gallocatechin, catechin and catechin gallate after thermal processing.

An increase in temperature from 100°C to 200°C elevated total catechins removal from 24.5-72.5 mg/g faba bean hull at 50 bar and 1.96-4.37 mg/g faba bean hull at 100 bar (Tables 4.3 and 4.4). Spectrophotometric measurements of condensed tannin showed that pressure had no significant effect on condensed tannin removal. However, the values obtained using HPLC for total condensed tannins at 100 bar were significantly smaller than the values obtained at 50 bar. According to the obtained data in table 4.3 and 4.4, these results suggest that fewer condensed tannins were detected owing to potential degradation or changes in their structure at 100 bar. Beside temperature and pressure, pH is an important factor influencing kinetic degradation and epimerization of catechins during thermal processing. For example, catechins of tea were stable at pH <4, whereas they were unstable at pH > 6 (Ananingsih, Sharma, & Zhou, 2013). Also,

stability of catechins depends on their scavenging ability. It means that EGCG and GCG have high scavenging ability than epigallocatechin (EGC), gallocatechin (GC), epicatechin (EC), and catechin (C) due to their gallate group at position three of the C ring. Also, epigallocatechin (EGC) and gallocatechin (GC) have a hydroxyl group at the 5′ position of their B ring, making them stronger than epicatechin (EC) and catechin (C).

Table 4.3. Tannin content (mg/g) after subcritical water treatment of faba bean hull at 50 bar.

Condensed tannins (mg/g)	**Temperature** (℃)					
	100	**120**	**140**	**160**	**180**	**200**
Catechin	4.12±0.02^{b}	3.61±0.00^{cd}	3.35±0.01^{d}	3.39±0.00^{d}	3.78±0.12^{bc}	4.53±0.00^{a}
(-)-Epicatechin	1.51±0.00^{a}	1.19±0.00^{ab}	1.04±0.00^{b}	1.02±0.02^{b}	1.11±0.04^{b}	1.18±0.05^{ab}
(-)-Epigallocatechin	1.28±0.01^{a}	1.34±0.01^{a}	0.43±0.22^{a}	0.54±0.05^{a}	1.06±0.00^{a}	0.89±0.00^{a}
(-)-Epicatechin gallate	0.92±0.08^{ab}	1±0.00^{a}	0.75±0.01^{b}	0.74±0.01^{b}	0.75±0.00^{b}	0.75±0.00^{b}
Gallocatechin gallate	6.7±0.01^{b}	6.68±0.02^{b}	6.79±0.01^{a}	6.7±0.00^{b}	6.7±0.00^{b}	0±0.00
Catechin gallate	0.95±0.00^{b}	1.36±0.04^{b}	2.08±0.00^{a}	2.83±0.01^{b}	3.48±0.01^{b}	8.16±0.01^{b}
Total condensed tannins	15.48±0.12	15.18±0.07	14.44±0.25	14.48±0.09	16.88±0.17	15.51±0.06
Hydrolysable tannins (mg/g)						
Ellagic acid	2.18±0.09^{d}	2.35±0.95^{cd}	3.06±1.86^{ab}	2.81±0.58^{bc}	2.79±1.85^{bc}	3.38±0.19^{a}
Total tannins (mg/g)	17.66±0.21	17.53±1.02	17.5±2.11	17.29±0.67	19.67±2.02	18.89±0.25

Data is reported as mean±standard deviation. Statistical comparison was performed Tuckey comparison and two way Anova among all means in each column, $^{a-d}$ Letters indicate significant difference among means in each row.

Table 4.4. Tannin content (mg/g) after subcritical water treatment of faba bean hull at 100 bar.

Condensed tannins (mg/g)	**Temperature (℃)**					
	100	**120**	**140**	**160**	**180**	**200**
Catechin	1.19±0.00^{d}	1.74±0.10^{bc}	1.93±0.03^{b}	2.06±0.01^{b}	3.91±0.02^{a}	1.38±0.02^{cd}
(-)-Epicatechin	1.64±0.03	1.82±0.04^{b}	1.88±0.01^{a}	1.88±0.02^{a}	trace	trace
(-)-Epicatechin gallate	0.77±0.02^{b}	0.86±0.03^{a}	0.79±0.01^{b}	0.72±0.2^{c}	trace	trace
Total condensed tannins	3.6±0.05	4.42±0.17	4.6±0.05	4.66±0.03	3.91±0.02	1.38±0.02
Hydrolysable tannins (mg/g)						
Ellagic acid	2.09±0.84^{e}	3.73±2.38^{cd}	2.64±1.81^{de}	4.32±3.03^{c}	7.54±1.86^{b}	10.58±4.92^{a}
Total tannin	5.69±0.89	8.15±2.55	7.24±1.86	8.98±3.06	11.45±1.88	11.96±4.94

Data is reported as mean±standard deviation. Statistical comparison was performed Tuckey comparison and two way Anova among all means in each column, $^{a\text{-}e}$ Letters indicate significant difference among means in each row.

4.8.6. Individual tannins in S-L extracts

Table 4.5 shows the individual tannin type and quantity using four different solvents (water, 70% acetone/water, 70% ethanol/water, and 50% ethanol/water with a solid to liquid ratio of 1:100 (w/v) at room temperature for 40 min. Using HPLC analysis, catechin, epicatechin, and (-)-epicatechin gallate were identified in the solid-liquid extracts of faba bean hull. Total content of removed catechins varied between 1.83-4.37 mg/g faba bean hull which is smaller than values was obtained using subcritical water extraction at 50 bar (14.44-16.88 mg/g faba bean hull) (Table 4.3 and 4.5). This is related to the solubility of individual catechins in the solvents studied. As reported by Vuong et al. (2010), individual catechin solubility depends on three

factors: temperature and duration, and the type of solvent (Table 4.6). Extraction time affect epicatechin and epigallocatechin solubility, while solubility of epicatechingallate (ECG) depends on both extraction time and temperature. Also, solvent type, extraction duration, and temperature has important effect on solubility of epigallocatechingallate (EGCG) and epigallocatechin (EGC) (Labbé, Tremblay, & Bazinet, 2006).

Table 4.5. Tannin content (mg/g) in faba bean hull extracts using different solvent.

Solvent / Condensed tannin	W	70%Ac/W	70%E/W	50%E/W
Catechin	0.39 ± 0.00^{c}	0.58 ± 0.00^{a}	0.57 ± 0.00^{a}	0.51 ± 0.00^{b}
Epicatechin	1.61 ± 0.00^{c}	2.27 ± 0.00^{a}	2.13 ± 0.00^{b}	2.36 ± 0.00^{a}
(-)-Epicatechin gallate	1.15 ± 0.00^{b}	1.41 ± 0.00^{a}	1.34 ± 0.00^{a}	1.48 ± 0.00^{a}
Total (mg/g)	1.83 ± 0.00	3.09 ± 0.00	2.93 ± 0.00	4.37 ± 0.00

Ac: acetone; W: water; and E: ethanol.

Table 4.6. Physical properties of catechins.

Catechin name	Molecular formula	Molecular weight (g/mol)	Melting Point (°C)	Wave length (nm)	Solubility
Epicatechin	$C_{15}H_{14}O_6$	290	242	280	Time dependent
(-)-Epigallocatechin	$C_{15}H_{14}O_7$	306	218	269	Time/solvent dependent
(-)-Epicatechin gallate	$C_{22}H_{18}O_{10}$	442	253	280	Time/temperature dependent

4.8.7. Total phenolics

Fig. 4.3 shows total phenolic content extracted from faba bean hull using SCW. As the temperature increased from 100 to 120 °C, the yield of phenolic compound increased. But, the total content of phenolics decreased after 140 to 200°C. At 120 °C, and 50 or 100 bar, the highest removal of phenolics (~ 44.37 mg gallic acid/g hull) was obtained. There was no significant difference for phenolic extraction at both pressures, owing to the stability of free gallic acid in the SCW extracts (pH=4.3-4.8) at 120-200℃. Total phenolics of faba bean whole seed was reported as 55.9±1.4 mg catechin/ g seed after extraction with 80% v/v acetone-water at 80℃ for 15 min (Amarowicz, Troszynska, Barylco-Pikielna, & Shahidi, 2004). In another study with 10 different varieties of faba bean whole seeds from Spain, Chile and Syria, using HPLC-Ms instrument with a diod array detector and electrospray ionization interface, analysis of faba bean extracts (20 g seed in 80 mL methanol/water 80:20 v/v acidified with 0.01% HCl), total phenolic content of 109.60±63.6 mg/g DW was reported (Baginsky et al., 2013). Analysis of individual polyphenols in faba bean seed showed presence of caffeic acid (0.78±0.03 mg/g DW), *p*-coumaric acid (1.68±0.07 mg/g DW), sinapic acid (2.58±0.23 mg/g DW), and ferulic acid (10.56±1.58 mg/g DW) (Yao et al., 2011).

Thermal degradation studies of gallic acid in aqueous solutions showed that gallic acid decomposition into pyrogallol could occur rapidly at 100 to 150℃ due to its activation energy of ranging from 22.9 to 27.8 kcal/mol (Boles, Crerar, Grissom, & Key, 1988). But, complex phenolics require more heat to initiate hydrolysis or react with other reacting components in the solvent media. Subcritical water extraction of gallic acid from grape seeds at 50℃, 100℃, and

150℃ using 103 bar resulted in more gallic acid removal at 150℃ (García-Marino, Rivas-Gonzalo, Ibáñez, & García-Moreno, 2006).

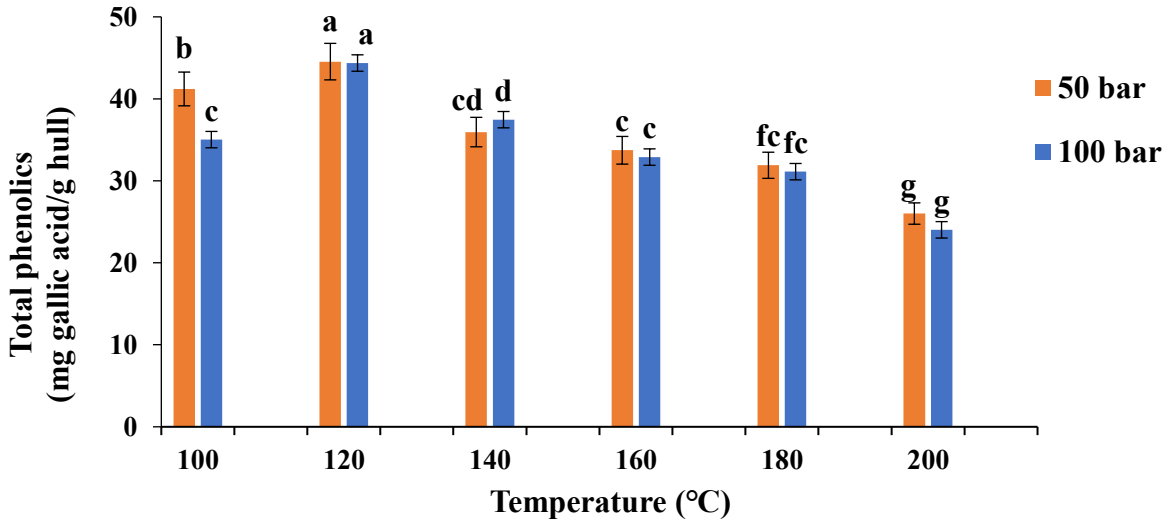


Fig. 4.3. Subcritical water extraction of total phenolics from faba bean hull at a flow rate of 5mL/min in 40-min extraction. [a-g] Letters indicate significant difference among all bars.

A decrease in phenolic removal above 120℃ suggests that phenolic are converted into other compounds such as gallic acid esters (Fig. 4.3). Earlier studies showed trends of fluctuation for solvation of selected phenolic acids in pressurized water as increased, stable (gallic acid) or decreased (3-4-hydroxyphenyl propionic acid and 4-hydroxybenzoic acid) with an increase of temperature (Zhang et al., 2014 ; Saldaña& Valdivieso-Ramirez, 2015). Moreover, a decrease in gallic acid content at high temperatures could be related to the degradation of gallic acid into pyrogallol and resorcinol at temperatures above 75 °C, leading to its decarboxylation as previously reported (Zhang et al., 2014). Hydrolysis of tannins could also lead to gallic acid, and decarboxylation of gallic acid results in pyrogallols (Chandrasekaran & Beena, 2013; Murdiati, McSweeney, & Lowry, 1992). Also, at industrial scale, gallic acid is produced under acid/base hydrolysis of tannic acid followed by decarboxylation to produce pyrogallol as observed in Fig.

4.4. Kim et al. (2011) showed that maximum hydrolysis of tannic acid into gallic acid occurred at 150°C and 48 bar. Furthermore, at 200℃, gallic acid was converted to pyrogallol. Pressure was not reported (Kim, Silva, & Jung, 2011).

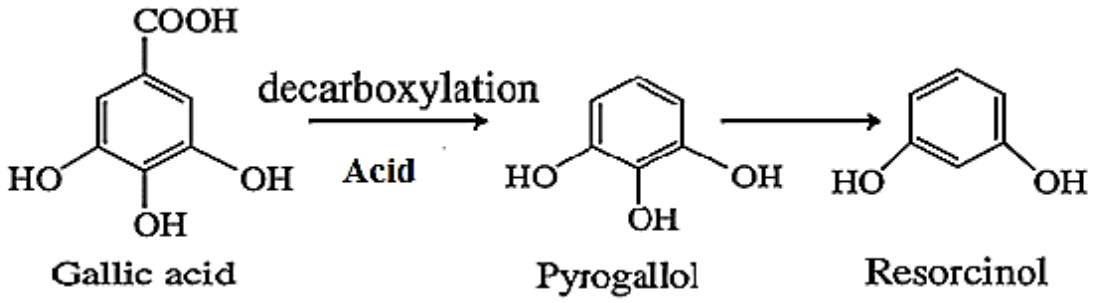


Fig. 4.4. Decarboxylation of gallic acid to pyrogallol and resorcinol.

4.8.8. Solid-liquid extraction

Fig. 4.5 shows the solid-liquid extraction of faba bean hull using different solvent mixtures (water, 70% acetone, 70% ethanol, and 50% ethanol), temperatures (50℃ and 70℃) and solid to solvent ratios (2:20 w/v, 2:30 w/v, and 2:40 w/v) for 3h. The best total tannin extraction (54.36±2.58 mg tannic acid /g faba bean hull) was obtained at 70℃ using 70% aqueous acetone with a solid to solvent ratio of 2:40 w/v. Fig. 4.5 shows that the type of solvent, temperature and the solid to liquid ratio influenced the concentration of total tannins extracted from faba bean hull. Among solvents, acetone was the preferred solvent due to the higher tannin removal efficiency compared with water or ethanol using various solid to liquid ratios. This result is in agreement with the trend reported by Bosso, Guaita, & Petrozziello (2016) when extracting condensed tannins and polyphenols from grape pomace seed using aqueous mixtures of ethanol or acetone. Also, tannin removal content using water at the optimum temperature (70℃) and a

solid-solvent ratio 2:40 (w/v) was 21.66±0.31 mg tannic acid/g faba bean hull, which was 3.39 times lower than the highest removal content using subcritical water extraction. Therefore, subcritical water extraction facilitates the removal of total tannins from faba bean hull using less time (40 min) than the solid-liquid extraction (180 min).

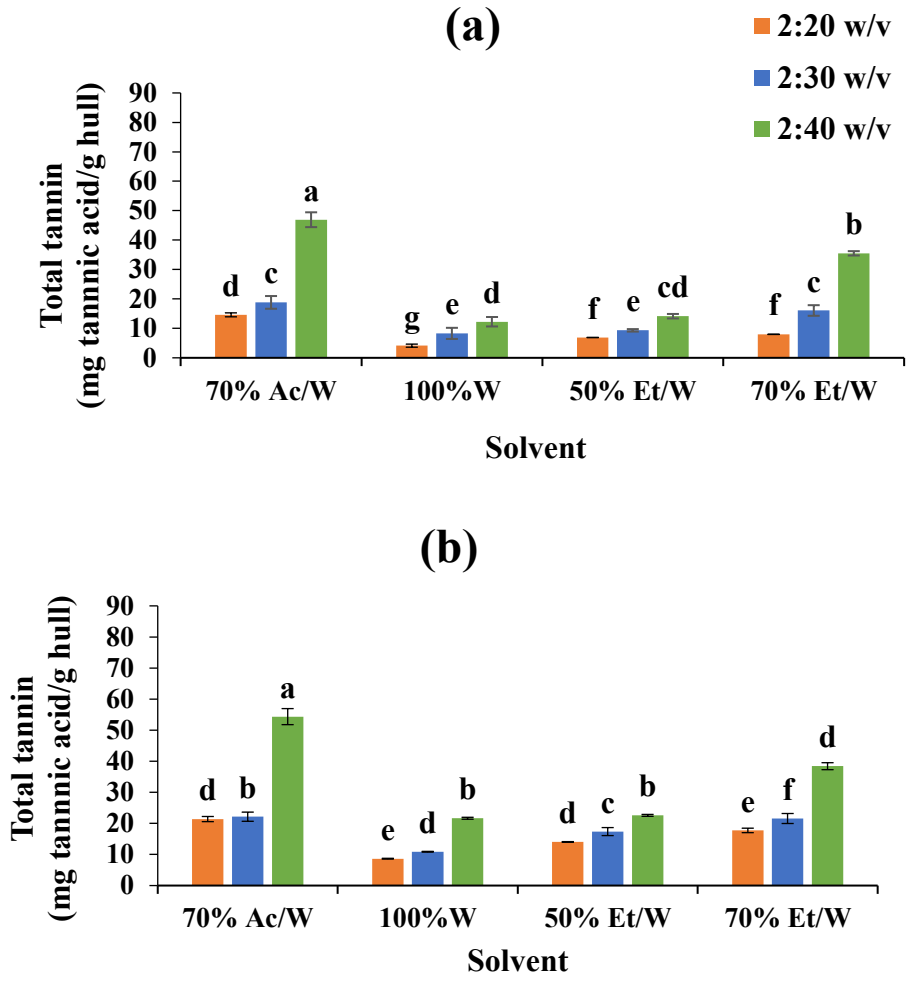


Fig. 4.5. Total tannin removal from solid-liquid extraction of faba bean hull with different solvent mixtures (Ac: acetone; W: water; and Et: ethanol) for 3 h at: (a) 50°C and (b) 70°C. [a-e] Letters indicate significant difference among all bars.

The 3D surface response of total tannin content with respect to the solvent type and solid to solvent ratios is shown in Fig. 4.6. The total amount of tannin content increased with an increase of solid-to-solvent ratio and was maximized when 70% aqueous acetone was used.

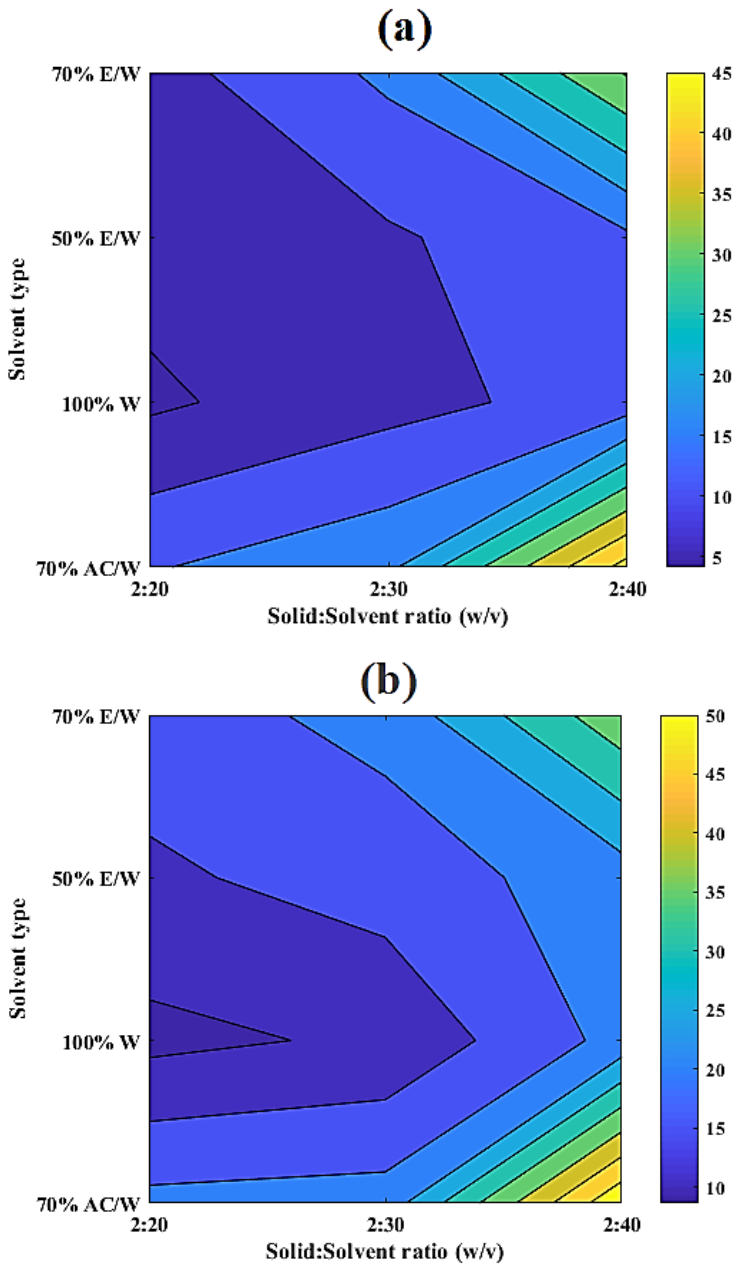


Fig. 4.6. Surface response plot of total tannin removal of faba bean hull with solid-liquid extraction using different solvent mixtures (AC: acetone; W: water; and E: ethanol) for 3h at: (a) 50℃, and (b) 70℃.

Also, a previous study showed that phenolics extraction from *L. aromatic* plant using 75% acetone/water (39.10±0.87 mg gallic acid/g sample) was better than 75% ethanol/water

(30.60±1.36 mg gallic acid/g sample) and 50% ethanol/water (30.30±0.54 mg gallic acid/g sample) (Do et al., 2014). As separation of compounds relies on the polarity of the solvent, a single solvent like water in this study can be selective for specific components. It was demonstrated that acetone-water mixtures were more effective than ethanol-water mixtures using a 1:10 w/v solid to liquid ratio at room temperature for the extraction of condensed tannins from grape skin due to the proportional solubility of different tannins in ethanol/water mixtures (Downey & Hanlin, 2016). Moreover, reaction of the solvent with the target compounds and presence of reactive functional groups in the chemical structure of a compound influences its solubility. For example, hydrolysable tannins can react with the solvent when the depside bound in gallotannins is cleaved using methanol at neutral pH (Harborne, 1989). Water, at 60°C, can break the bond between gallic acid and glucose in the structure of gallotannins (Nishimura, Nonaka, & Nishioka, 1986). Also, the presence of gallic acid, containing one carboxylic acid and three hydroxyl groups could be the reason of its high solubility in water. Whereas, the presence of carbonyl groups in the acetone structure allow acetone to form hydrogen bonds with water and dissociate in water. Therefore, since various phenolics are soluble in polar solvents, mostly aqueous alcohols and acetone have been used for their extraction. However, aqueous methanol (50% v/v) was reported as an effective solvent for phenolic compounds with glycoside structures. In another study, the use of aqueous 80% acetone showed acceptable extraction results for the phenolic glycosides and catechins (Julkunen-Tiitto, 1985).

Likewise, for total phenolic extraction from barley with acetone (80%), ethanol (80%), methanol (80%), the acetone (0.68 mg gallic acid /g barley flour) extracted the highest content of phenolic compounds compared with ethanol (0.38 mg gallic acid /g barley flour), and methanol

(0.29 mg gallic acid /g barley flour) (Bonoli, Marconi, & Caboni, 2004). Also, the results obtained by Juan & Chou (2010) proved that 80% acetone extracted the highest amount of total phenolics (26.60±1.03 mg gallic acid/g extract) from black soybeans compared with 80% methanol (15.94±0.86 mg gallic acid/g extract) and 80% ethanol (17.75±0.39 mg gallic acid/g extract). Moreover, Avallone et al. (1997) confirmed that acetone 70% extracted 19.5 mg total polyphenols/g carob, 2.9 mg proanthocyanidins/g carob and 0.46 mg ellagitannins/g carob. Also, 70% acetone (0.4±0.29 mg 4,6-hexahydroxydiphenoyl-glucose/g) was more efficient to obtain ellagitannins than with the use of 70% methanol (0.2±0.05 mg 4,6-hexahydroxydiphenoyl-glucose/g) (Avallone, Plessi, Baraldi, & Monzani, 1997). Similar results were obtained for the extraction of total tannins from leaves of oak and maple using 70% acetone (546±87g/cm^2 dry tissue and 1381±149 g/cm^2 dry tissue, respectively), and using 50% methanol (387±21 g/cm^2 dry tissue and 1117±133 g/cm^2 dry tissue, respectively) (Hagerman, 1988). Naczk & Shahidi (2004) reported that temperature and solvent volume could influence tannin extraction. Also, an increase in the solid-liquid ratio at a constant temperature of 60°C from 1:10 w/v to 1:15 w/v, and 1:20 w/v resulted in an increase of tannin extraction in the order of 30, 33.5, and 36 mg tannin/g nut of oak tree, respectively (Chao, Liu, Zhang, Zhang, & Tan, 2017). As stated by Chao et al. (2017), such effects rely on high concentration gradient between the inside and outside of the sample particles, which enhanced the mass transfer driving force, contributing to the high diffusion rate of tannins.

4.8.9. Total carbohydrates

Total carbohydrates were extracted with SCW at temperatures (100, 120, 140, 160, 180, and 200°C) and pressures (50 and 100 bar) for 40 min using a flow rate of 5 mL/min (Fig. 4.7). There was an increasing trend for carbohydrate removal as a function of extraction time up to 40 min for both pressures used. At 160°C, the highest removal of total carbohydrates (50 bar: 382.14 mg glucose equivalent/g hull, and 100 bar: 354.21 mg glucose equivalent/g hull) from faba bean hull were observed. Similarly, the highest carbohydrate removal (192.7±6.4 mg/g barley hull) using subcritical water was observed at 150°C, 150 bar, and 15 min which is lower than total carbohydrates obtained from faba bean hull (Fig. 4.7) (Sarkar, Alvarez, & Saldaña, 2014). As it is clear in Fig. 4.7, pressure had no significant effect on total carbohydrate removal.

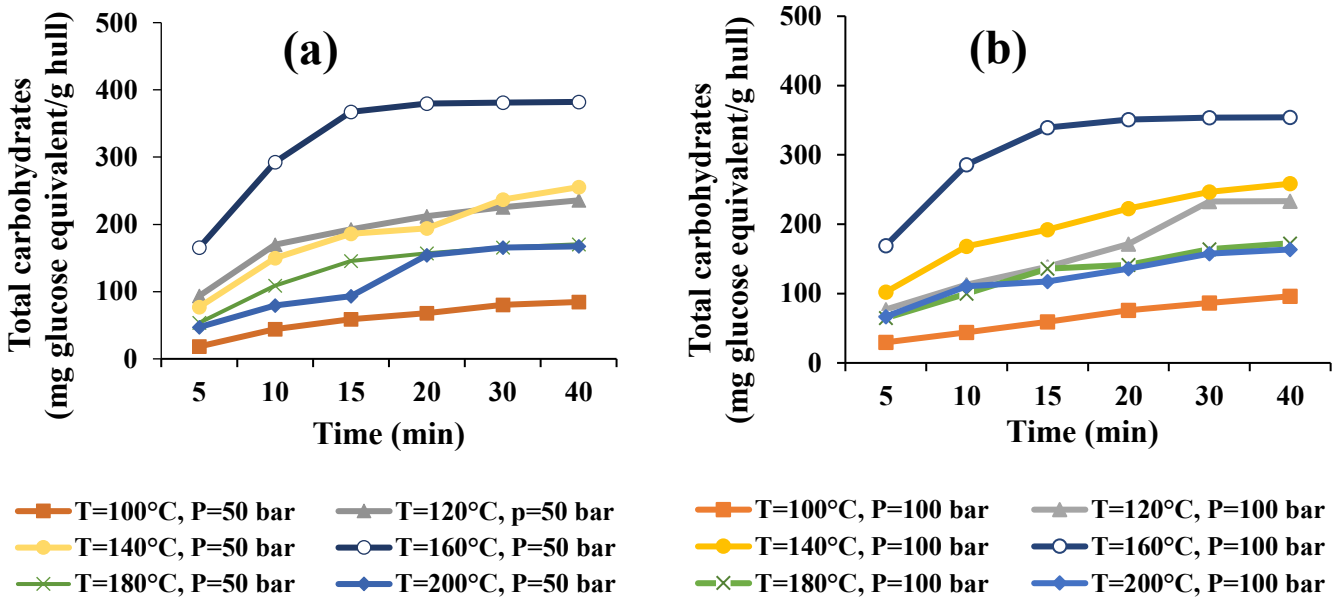


Fig. 4.7. Extraction of total carbohydrates by SCW for 40 min at: (a) P=50 bar, and (b) P=100 bar.

At 180°C and 200°C, there was a decreasing trend in carbohydrate removal as glucose could be converted to 5-hydroxymethylfurfural (HMF) (Fig. 4.8). Earlier, Qi and Xiuyang (2007) showed that water at 100 bar, 180°C and 30 min converted ~10% of glucose to 5-

hydroxymethylfurfural. Also, xylose decomposition led to the production of furfural and formic acid (Qi & Xiuyang, 2007).

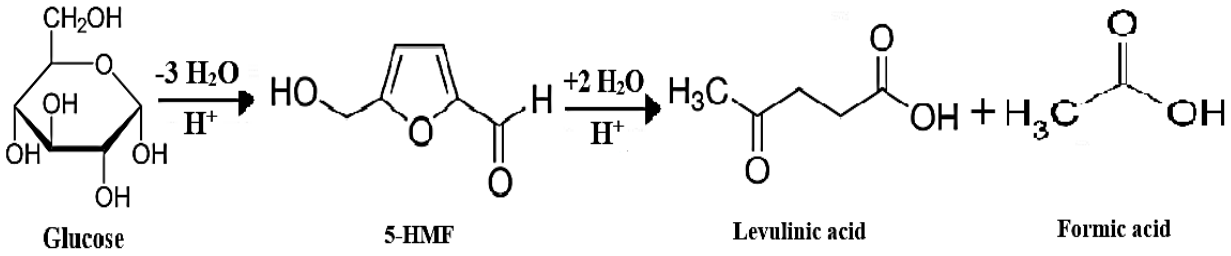


Fig. 4.8. Conversion of glucose to 5-hydroxymethylfurfural and formic acid.

Since pentose sugars such as xylose and arabinose exist in raw faba bean hull (Table 4.7), there is a probability of acid hydrolysis of these sugars into furfural at high temperatures (180-200℃, 50 and/or 100 bar). At elevated temperatures, available protons from acidic water combine with polymeric structures of sugars to break ether bonds and produce monomeric sugars. Earlier, hydrolysis of bamboo grass biomass using SCW at 170-220°C showed faster decomposition rate of hemicellulose sugars (xylose and arabinose) than cellulose sugars (cellobiose, glucose and fructose) at 180°C, owing to the high crystalline nature of cellulose structure compared to hemicellulose (Mohan, Banerjee, & Goud, 2015)

Other examples of furfural and xylose production from acid hydrolysis of pentosans were reported in rice hull at 125℃ and 1.5 bar (Mansilla et al., 1998), acid hydrolysis of corn stover at 100℃ (Jin, Zhang, Yan, Qu, & Huang, 2011), acid hydrolysis of wheat at 130℃ (Guerra-Rodríguez, Portilla-Rivera, Jarquín-Enríquez, Ramírez, & Vázquez, 2012) and hydrolysis of sorghum straw with phosphoric acid at 134℃ (Vázquez, Oliva, Téllez-Luis, & Ramírez, 2007). These studies reported furfural as a degradation product from pentose such as xylose and

arabinose and HMF as a degradation product of glucose. Guerra-Rodríguez et al (2012) showed that HMF could be converted to 2,5-dimethylfuran (DMF) to be used as a liquid transportation fuel with 40% greater energy density than ethanol.

4.8.10. Soluble and insoluble fiber content of faba bean hull

Legume by products are sources of dietary fiber that can be used as inexpensive non-caloric bulking agents incorporated into food products to improve water and oil retention, and emulsion and oxidative stability (Elleuch et al., 2011). These dietary fibers can be soluble fibers to increase viscosity due to their solubility in water (thickening agents) and gel forming properties, or they are insoluble fibers with low density for water- and oil-binding capacities. As an example of these by products, pea hull has 5.81% soluble fiber, 61.84% insoluble fiber, and 67.65% total dietary fiber (Rzedzicki, Kozlowska, &Troszynska, 2004). Also, recent analysis of soybean hull estimated fiber content of 52.75% DW and 72.26% DW using acid detergent and neutral detergent fiber analysis, respectively (Joner et al., 2018).

Auto-ionization phenomenon of subcritical water provides the acidic pH environment of 2-4 for depolymerisation of hemicellulose. In this study, SCW extract pH was in the range of 5.4-4.3. The hydronium ions released from the subcritical water allow hydrolysis of glycosidic linkages into acetyl and other fractions. Similarly, Ciftci & Saldaña (2015) proved that acidic pH of subcritical water promotes hydrolysis of the hemicellulose fraction. However, at temperatures lower than 200℃, cellulose remains intact due to the linear structure.

The content of hemicellulosic sugars, such as xylose, galactose, arabinose and mannose determined by GC is shown in Table 4.7. In previous studies, quantification of sugars (glucose, fructose, and sucrose) by size exclusion chromatography/gel permeation chromatography

showed that fructose and glucose are generally negligible in mature faba bean seed while sucrose content varied between 0.02% and 5.23% dried weigh (DW) (Pritchard, Dryburgh, & Wilson, 1973). Also, Landry, Fuchs, & Hu (2016) showed that immature faba bean seed coat had 2.15% glucose, 1.90% fructose, 7.98% sucrose, 0.21% stachyose, and 0.64% verbascose. In this study, faba bean hull had mainly glucose (53.60%), arabinose (4.65%), xylose (0.93%), rhamnose (1.06%), and galactose (2.00%) (Table 4.7).

Table 4.7. Hemicellulosic sugars (% DW) in various parts of faba bean.

Carbohydrates	**Immature seed coat****	**Seed coat +germ****	**Whole seed****	**Faba bean hull (This study)**
Glucose	2.15	0.94	nd	53.60±1.72
Fructose	1.90	0.66	nd	0.00
Sucrose	7.98	13.35	2.49	nd
Raffinose	nd	nd	0.45	nd
Stachyose	0.21	0.15	1.87	nd
Verbascose	0.64	0.41	2.40	nd
Arabinose	nd	nd	nd	4.65±0.12
Xylose	nd	nd	nd	0.93 ±0.03
Rhamnose	nd	nd	nd	1.06±0.05
Galactose	nd	nd	nd	2.00±0.04
Total	12.88	15.51	7.21	62.24±1.96

**Landry, Fuchs, & Hu (2016); nd: not determined.

The SCW technology is a green alternative process for hemicellulose hydrolysis without the use of dilute acids (~0.07%) in a pretreatment step, saving 25-30% of the overall processing costs (Jacobsen & Wyman, 2000). Since SCW provides the feasibility of hemicellulose and

cellulose hydrolysis, separation of dietary fiber compounds from faba hull occurs. The contents of soluble, insoluble and total dietary fiber in faba hull under different treatments are shown in Table 4.8. Similar trend was obtained for SCW treatment of lupin hulls from 160°C (66.4%) to 220°C (93.3%) at 200 bar with increasing cellulose content of the solid residues (Saldaña & Ciftci, 2015). Untreated faba hull had soluble fiber (5.60±0.02%), insoluble fiber (73.69±0.02%), and total dietary fiber (79.29±0.04).

Table 4.8. Fiber composition of faba bean hull after different treatments.

Treatment	Extraction yield (%)	Soluble fiber (%)	Insoluble fiber (%)	Total fiber (%)
Untreated	*	5.60±0.01^{b}	73.69±0.01^{c}	79.29±0.02^{c}
Solid -Liquid extraction				
Water	92.19±0.45^{b}	5.61±0.06^{b}	78.06 ±1.27^{b}	83.67±1.33^{b}
70% AC+ water	91.76±0.61^{b}	5.94±0.73^{a}	80.77±3.56^{a}	86.71±4.29^{a}
70% E + water	95.25±0.77^{a}	5.60±0.10^{b}	80.62±2.36^{a}	86.22±2.46^{a}
50% E + water	94.09±1.34^{a}	5.69±0.21^{b}	78.29±0.61^{b}	83.98±0.82^{b}
Subcritical water extraction				
100℃,50 bar	72.29±0.66^{c}	5.56±0.02^{b}	73.84±0.10^{c}	79.40±0.12^{c}
120℃,50 bar	67.4±0.28^{d}	5.53±0.02^{b}	73.81±0.03^{c}	79.34±0.05^{c}
140℃,50 bar	65.7±0.28^{d}	5.59±0.07^{b}	73.84±0.10^{c}	79.43±0.17^{c}
160℃,50 bar	65.09±0.08^{d}	5.60±0.01^{b}	73.83±0.10^{c}	79.43±0.11^{c}
180℃,50 bar	58.79±0.35^{e}	4.52±0.03^{c}	73.76±0.07^{c}	78.28±0.06^{d}
200℃,50 bar	58.11±0.17^{e}	4.36±0.03^{c}	65.71±0.02^{d}	70.07±0.03^{e}

AC: acetone, E: ethanol. Data reported as mean±standard deviation. $^{a-e}$ Letters indicate significant difference among all means in each column. Means with similar letters are not significantly different with $p<0.5$ based on Tuckey comparison model using two way analysis of variance (ANOVA).

The contents of total dietary fiber, soluble and insoluble fiber measured for the solid-liquid residues. With aqueous acetone and ethanol 5.6-5.9% soluble fiber and 78.06-80.77% insoluble fiber remained in faba bean hull residue after solid-liquid extraction. Treating samples with SCW

extraction at 100-160℃ resulted in minor reduction of soluble fibers (5.53-5.60%) and insoluble fibers (73.84-73.81%) in the residue. These small changes suggest that temperature range of 100-160℃ at 50 bar is not enough to break the branched structure of rigid insoluble fibers such as lignin and cellulose. At 180℃ and 200℃, reduction in soluble fiber was from 4.52% to 4.36%, showing the effect of increased temperature on more solubility of soluble fiber. However, insoluble fibers had negligible changes at 100-180℃ and a small reduction from 180℃ to 200℃ (73.76% to 65.71%) (Table 4.8).

4.9. Conclusions

Total tannins, condensed and hydrolysable tannins, phenolics and carbohydrates were extracted from faba bean hulls using the S-L and SCW extraction methods. The optimum condition for the highest total tannin removal was obtained at 160°C and 50 or 100 bar (~73.6 mg tannic acid/g hull). Temperature had a crucial effect while pressure had no effect on removal of total tannins at 100-200°C. For condensed tannin, the maximum removal was 42.28 mg catechin/g hull. Also, the increase of pressure from 50 to 100 bar had no significant effect on the removal of condensed tannins. In addition, the highest content of total phenolics removed was ~44.37 mg gallic acid/g hull using SCW at 120°C and 50 bar. Comparison of subcritical water under different pressure and temperature conditions with traditional solid-liquid extraction shows higher content of tannins by SCW technology. Also, faba bean hull is a promising agricultural commodity to obtain valuable bioactive compounds, without consuming large amounts of expensive and toxic solvents with additional environmental problems. Extraction time of 3h, using 70% aqueous acetone, a solid to solvent ratio of 2:40 w/v, and extraction temperature of 70 °C were proved to be optimal in the case of the faba bean hull tannin removal. Untreated faba bean hull had 79.29±0.02% DW total dietary fiber (Soluble fiber: 5.60±0.01% DW, and

insoluble fiber: 73.69±0.01% DW). Use of SCW resulted in removal of fiber at 200°C and 50 bar, remaining soluble fiber of 4.36% DW, and insoluble fiber of 65.71% DW in the residue. Also, using S-L extraction with acetone and ethanol obtained (5.6-5.9% DW) soluble fiber and (78.29-80.77% DW) insoluble fiber in the residue.

Use of SCW as a solvent allows removal of polar and nonpolar compounds, facilitating the removal of tannins, phenolics, and soluble dietary fiber from faba bean hull. Such components can be later used as nutraceuticals, cellulose-based composites and packaging.

4.10. Recommendations

- Further studies are required to determine the optimum condition for the removal of tannins, phenolics and carbohydrates all together for scale up of the subcritical water extraction technology for commercial applications.
- In this study, only pure water was used as the subcritical solvent but other solvents such as acetone and ethanol aqueous mixtures can be examined to improve the yield of extraction.
- More studies are required to complement the kinetic behavior of thermochemical conversions among carbohydrates and tannin compounds.
- The aqueous extracts obtained from faba bean hull had different galloylated units of catechins. Therefore, studies for their degree of polymerisation and their shelf life stability need further investigation.
- More studies must be carried out on biodegradation of condensed tannins to exploit further applications in food, medical and tannery treatments.
- Also, the development of an analytical method for analysis of dietary fiber in the liquid extract obtained after SCW treatment is needed. Perform sugar analysis of the

carbohydrates in the hydrolysates obtained at various subcritical water treatment conditions might help to estimate degradation and depolymerisation of carbohydrates.

Acknowledgements

We thanks to the Natural Sciences and Engineering Research Council of Canada (NSERC) and Alberta Pulse Growers (2016 F071R) for funding this research.

Chapter 5: Conclusions and recommendations

5.1. Conclusions

Extraction of bioactives such as tannins, phenolics and carbohydrates from plant by-products is of growing interest within the field of Bio-resource and Food Science. Faba bean hull and green pea pod are potential sources of these bioactives. The use of solid-liquid extraction for the removal of such bioactives is time consuming and uses petrochemical solvents. As an alternative, water at subcritical conditions acts as a green solvent for bioactive removals. Processing with subcritical water is environmentally friendly and quick, having the potential of adjusting its polarity to target bioactives. This study investigated using subcritical water (SCW) extraction to obtain tannins and fiber from faba bean hull and green pea pod. To show the efficiency of SCW, the effect of temperature and pressure at 100, 120, 140, 160, 180, and 200°C and pressure of 50 and 100 bar on the yield of tannin removal from faba bean hull and green pea pod were investigated. Results obtained confirm that temperature was the most important process parameter whereas pressure had no effect on the SCW extraction. The kinetic study of tannin removal showed a maximum rate of extraction in the first 10 min for both by-products. Also, the best condition for total tannin removal was achieved at 160°C and 50 or 100 bar (~73.6 mg tannic acid/g hull). The highest condensed tannin content (42.28 mg catechin/g hull) was obtained at 160°C and 50 or 100 bar. Also, temperature changes from 100°C to 160°C increased the content of condensed tannin removal from 22.34 to 42.28 mg catechin/g faba bean hull (an increase of 1.8 times). In addition, the highest removal of total phenolics from faba bean hull (~44.37mg gallic acid/g) was obtained at 120°C and 50 bar. Temperature can accelerate the extraction by breaking sample macromolecules in contact with the subcritical solvent and the sample. Also, dissociation of hydrogen bonds in subcritical water, influences physico-chemical

properties of water such as diffusivity, permittivity, viscosity, density and surface tension. These changes benefit the extraction of non-polar components at higher temperatures and polar components at lower temperatures.

For green pea pod, the highest total tannins (12.96 mg tannic acid/g pea pod) and total phenolics (56.59 mg gallic acid/g pea pod) were obtained at 180°C and 50 bar, indicating presence of more phenolic acids and less tannin compounds compared with faba bean hull.

Solid-liquid (S-L) extraction using water, 70% acetone, 70% ethanol, and 50% ethanol with a solid to solvent ratio of 1:10, 1:15, and 1:20 w/v at 50-70℃ for 3h were conducted for comparative purposes. For the S-L extraction of faba bean hull and green pea pod, the aqueous mixture of acetone 70% was the most effective solvent for the removal of total tannins at 70°C.

Analysis of faba bean hull liquid extracts for individual tannins using HPLC showed the presence of six catechins: (-)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG), and catechin gallate (CG) at 100-200℃ and 50 bar. Among them, only (-)-catechin (C), (-)-epicatechin (EC), and (-)-epicatechin gallate (ECG) were detected in the extracts obtained at 100 bar. Ellagic acid was the hydrolysable tannin detected at 100-200°C and both pressures, originating from hydrolysis of ellagitannins. Quantification of ellagic acid after SCW treatment of faba bean hull showed an increasing trend (1.2-10.5 mg/g) with a rise in temperature from 100 to 200℃ at both pressures. But, HPLC analysis of green pea pod treated by SCW did not detect any of these tannin compounds. In addition, SCW treatment at 200°C and 50 bar led to a faba bean hull solid residue containing total dietary fiber (~71.14%), soluble fiber (5.42%), and insoluble fiber (65.71%). For green pea pod, insoluble dietary fiber decreased from 46.77% (180℃/50) bar to 45.09% (200℃/50) bar. Soluble fiber (5.6-5.9%) and insoluble fiber (78.06-80.77%) in faba bean hull

residue was obtained using S-L extraction with acetone and ethanol. Analysis of sugars showed that glucose and galactose are the common sugars found in faba bean hull and green pea pod. Faba bean hull had more glucose (53.60±1.72% DW) than green pea pod (18.53±0.085% DW). While, green pea pod had more galactose (9.71±0.038% DW) than faba bean hull (2±0.04% DW).

Overall, the results indicate that SCW is an effective extraction method for tannin removal from pulse by products.

5.2. *Recommendations*

There are some recommendations based on this thesis for future studies:

- In this study, subcritical water extraction was better for the removal of tannins compared to the solid-liquid (S-L) extraction using 70% acetone. In the S-L extraction, the use of acetone/water mixtures increased tannin removal compared to water. Therefore, addition of acetone or ethanol to subcritical water should be investigated for the removal of total tannins.
- According to the results, tannins interactions with proteins and carbohydrates can cause interference in the spectrophotometric absorptions and overestimate the total tannin content. Therefore, sample purification and removal of proteins and carbohydrates is suggested.
- Use of freeze dried sample is recommended for tannin removal since air drying could favor tannin oxidation and intramolecular bindings of condensed tannins. The presence of such bindings convert the structure of condensed tannins to more polymerized and complicated structures, influencing the extraction of condensed tannins.

- In this study, quantification of catechins was performed using HPLC. However, due to the presence of isomeric structures of catechins further characterization by H-NMR and C^{13}-NMR is recommended to identify the presence of galloyl ester groups.
- In this study, analysis of fiber was performed for the raw material and the solid residue, but analysis of fiber in the liquid extracts are still missing.

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Appendix A

Calibration curves

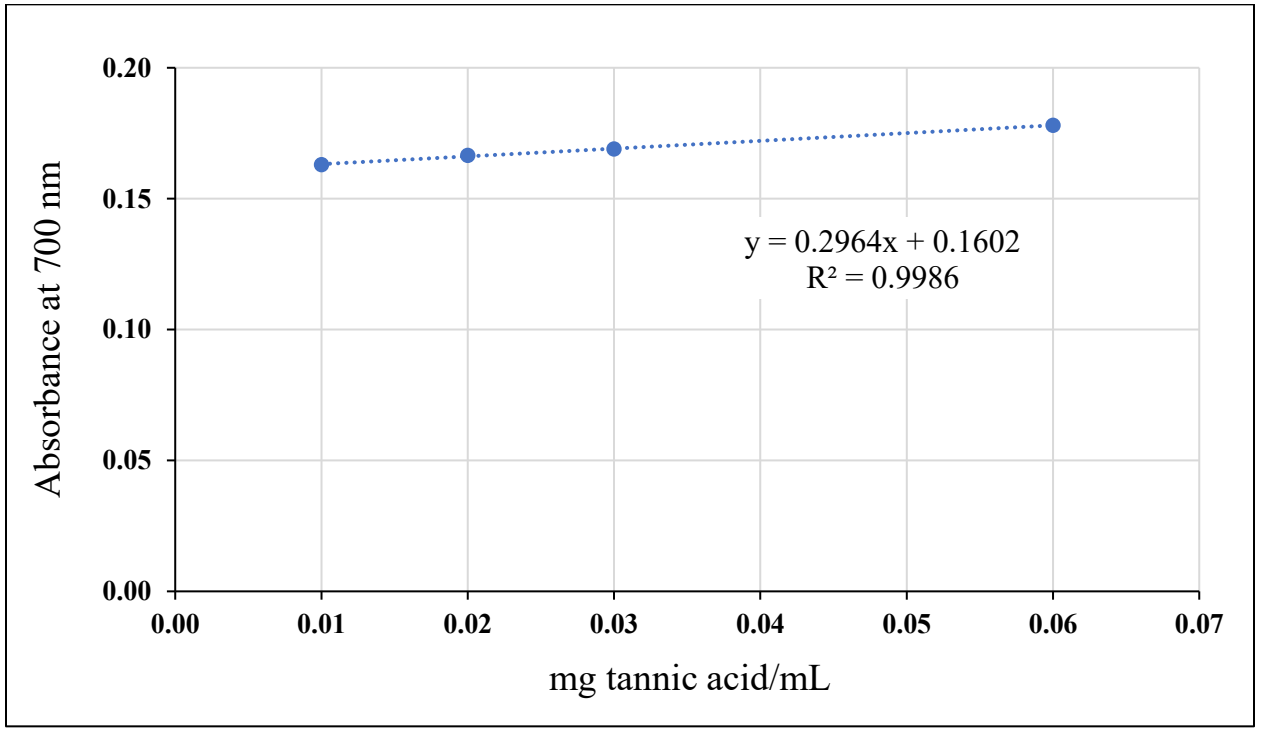


Figure A1. Tannic acid calibration curve to determine total tannin content by spectrophotometer.

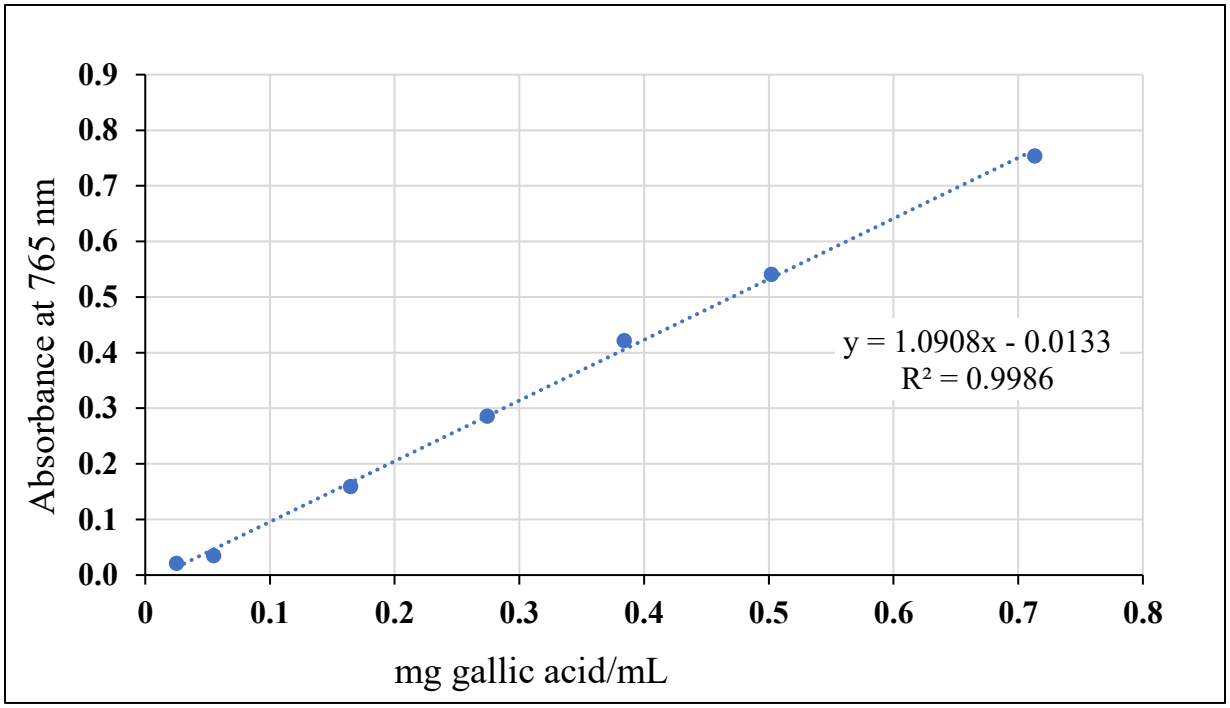


Figure A2. Gallic acid calibration curve to determine total phenolic content by spectrophotometer.

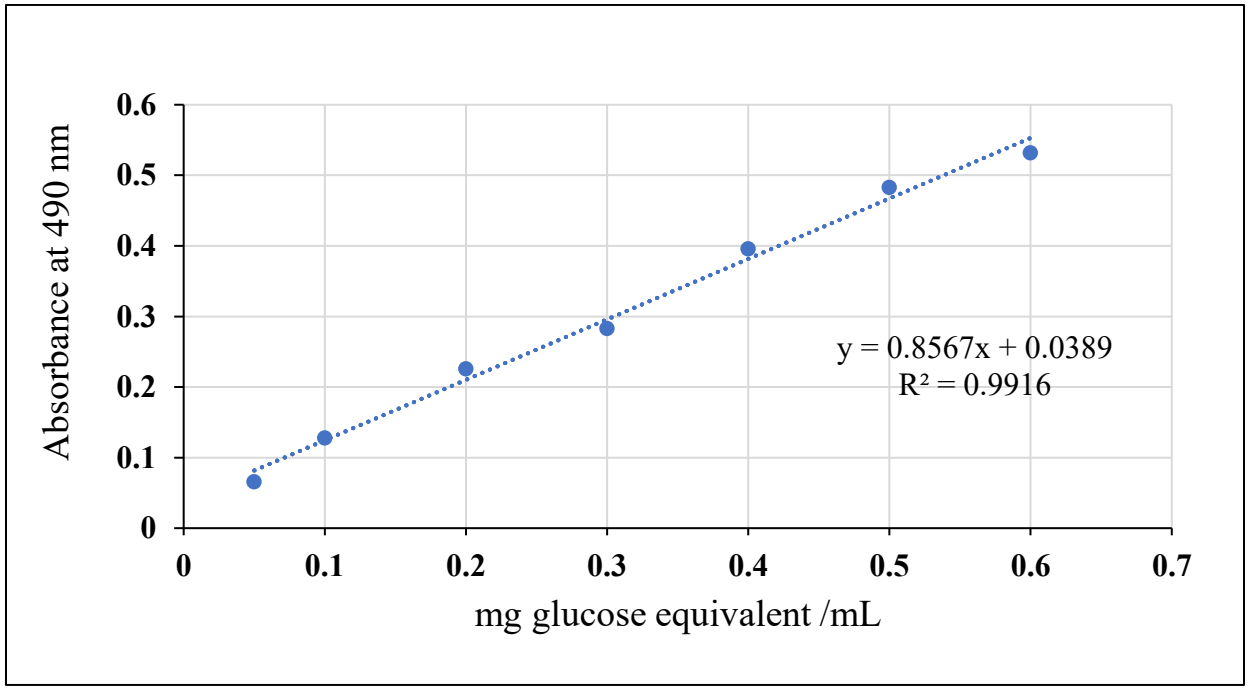


Figure A3. Total carbohydrates calibration curve by spectrophotometer.

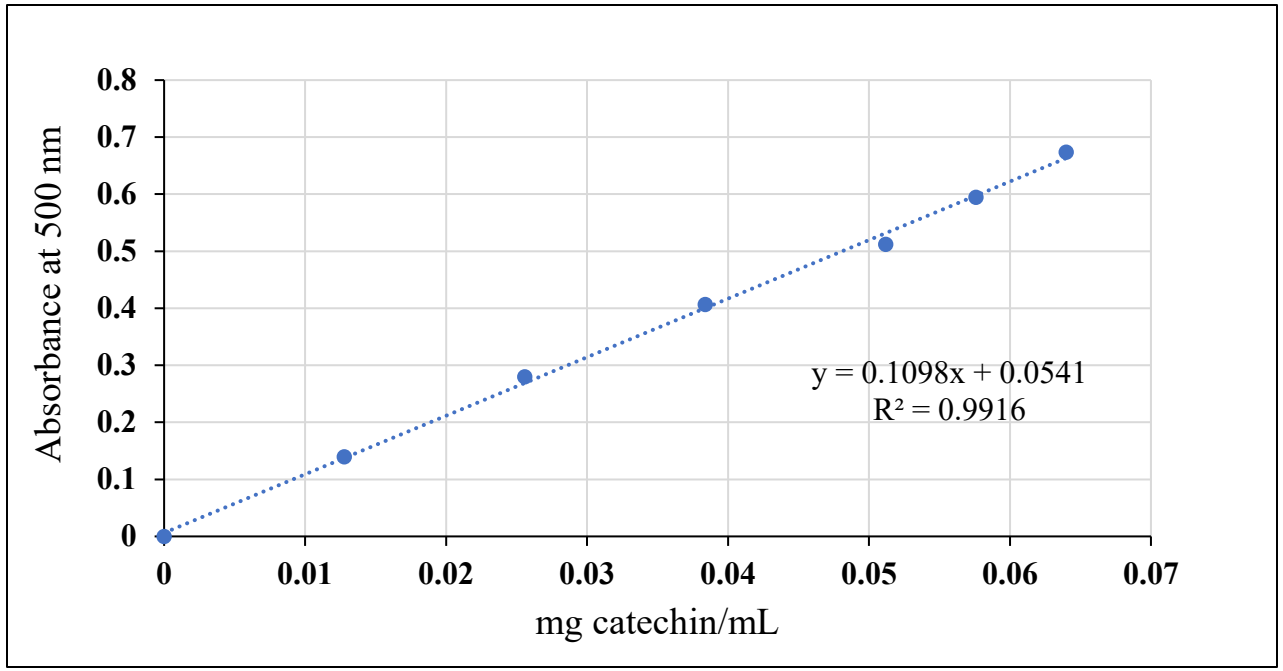
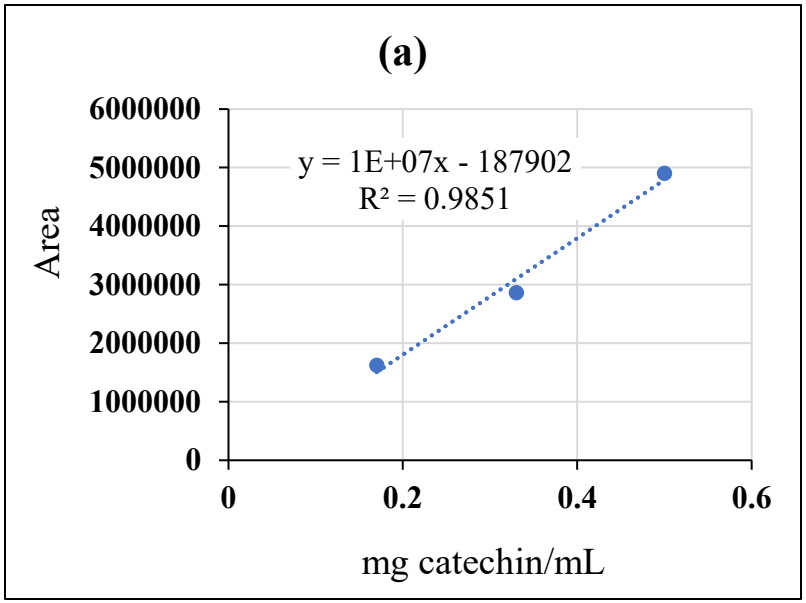
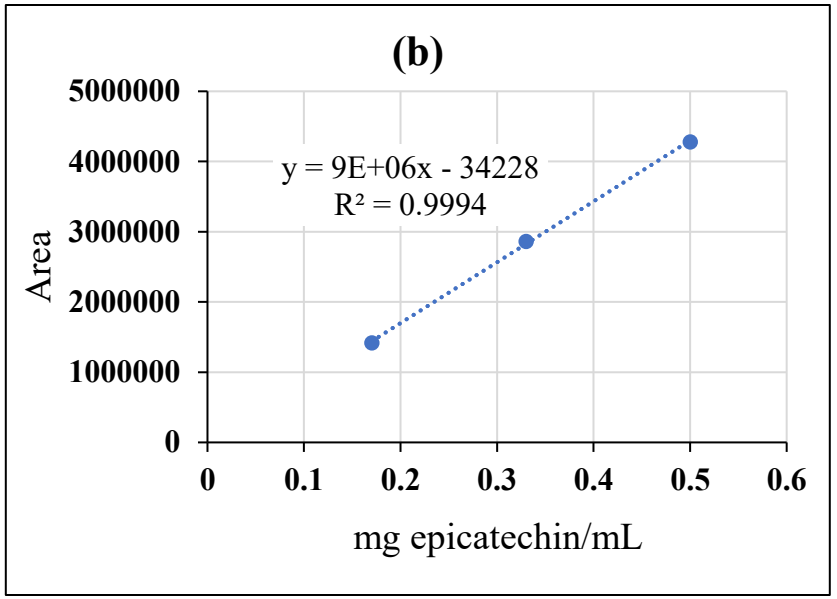


Figure A4. Catechin calibration curve to determine condensed tannin content by spectrophotometer.


(a)
Area
6000000
5000000
4000000
3000000
2000000
1000000
0
y = 1E+07x - 187902
R² = 0.9851
0
0.2
0.4
0.6
mg catechin/mL


(b)
Area
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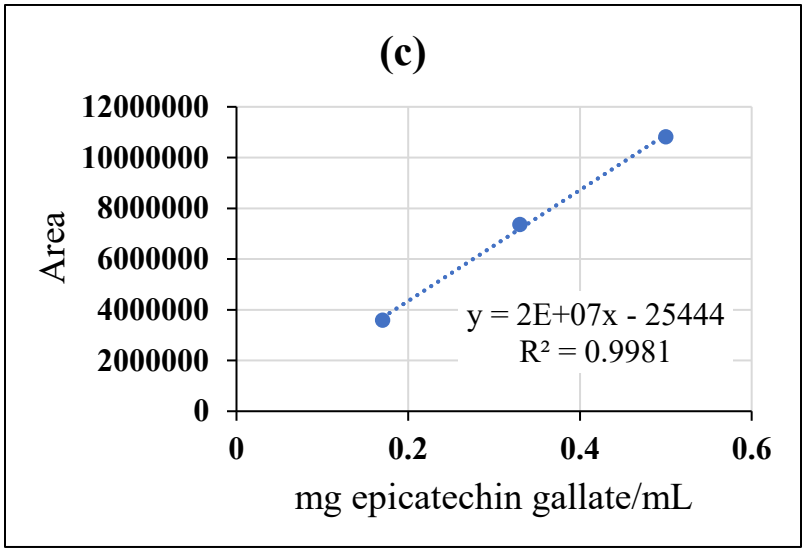
4000000
3000000
2000000
1000000
0
y = 9E+06x - 34228
R² = 0.9994
0
0.1
0.2
0.3
0.4
0.5
0.6
mg epicatechin/mL


(c)
Area
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10000000
8000000
6000000
4000000
2000000
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R² = 0.9981
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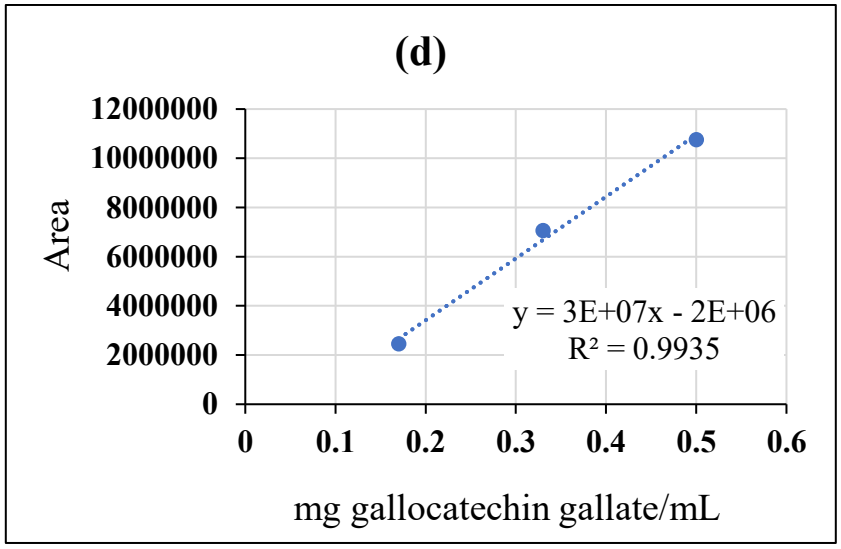
0.2
0.4
0.6
mg epicatechin gallate/mL


(d)
Area
12000000
10000000
8000000
6000000
4000000
2000000
0
y = 3E+07x - 2E+06
R² = 0.9935
0
0.1
0.2
0.3
0.4
0.5
0.6
mg gallocatechin gallate/mL

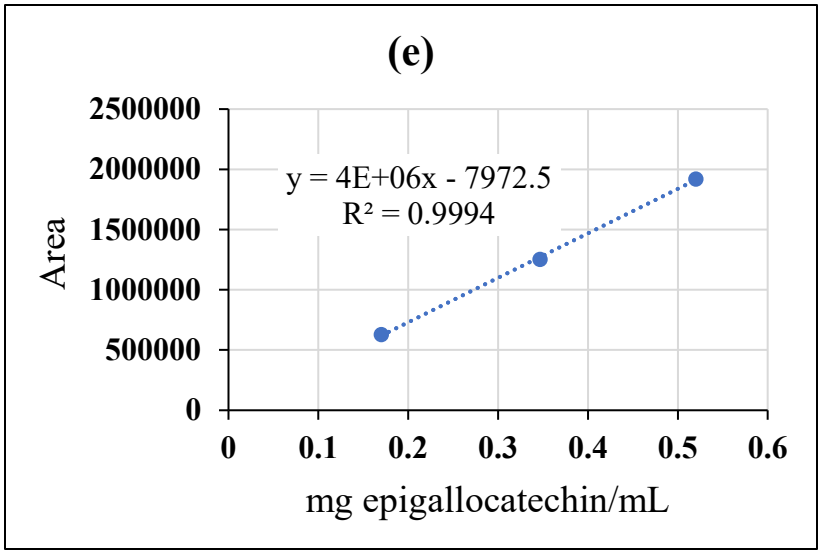


Figure A5. HPLC calibration curves of condensed tannins: (a) catechin, (b) epicatechin, (c) epicatechin gallate, (d) gallocatechin gallate, and (e) epigallocatechin.

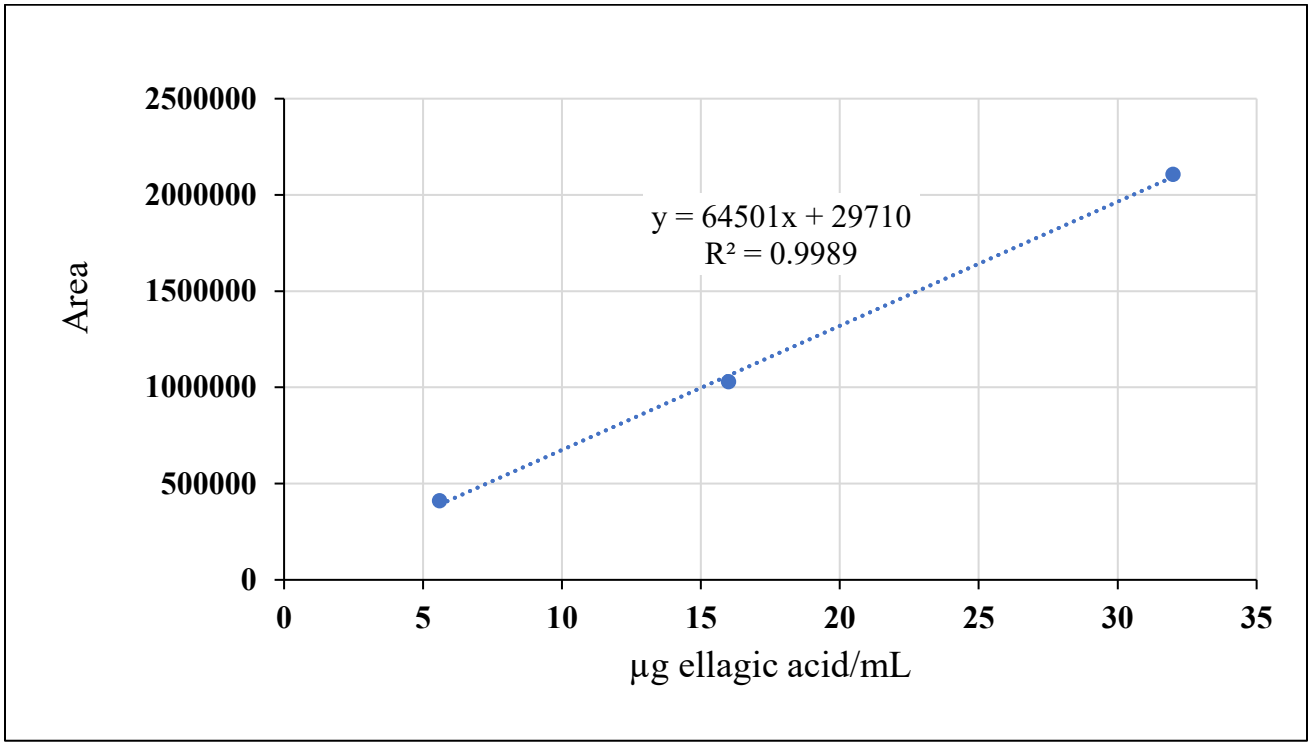


Figure A6. HPLC calibration curve of ellagic acid.

Appendix B

Green pea pod

Table B1. Green pea pod moisture content.

Replicate	W_b (g)	W_a (g)	Moisture content (%)	Average moisture content (%)
A	4.8986	4.7343	8.18675	8.20±0.02
B	4.8702	4.7053	8.22238	
C	4.8965	4.7322	8.19537	

W_b: weight of sample before drying, W_a: weight of sample after drying.

Table B2. Green pea pod protein content.

Replicate	Sample weight (g)	Nitrogen (%)	Protein factor	Protein content (%)	Average protein content (%)
A	0.108	3.1518	6.25	19.699	19.65±0.06
B	0.103	3.1467	6.25	19.667	
C	0.1002	3.1318	6.25	19.577	

Table B3. Green pea pod ash content.

Replicate	Sample weight (g)	W_{ca} (g)	W_{cs} (g)	W_c (g)	Ash content (%)	Average ash content (%)
A	1.0022	28.1182	29.0791	28.0769	4.121	3.97±0.21
B	1.0034	28.8004	29.76461	28.76121	3.817	
C	1.0025	28.6071	29.5697	298.5672	3.972	

W_{ca}: weight of crucible+ash, W_{cs}: weight of crucible+sample, W_c: weight of crucible.

Table B4. Subcritical water extraction of total tannins and total phenolics from green pea pod.

Pressure (bar)	Temperature (℃)	Total tannins (mg TA)	Average (mg TA/g pod)	Total phenolics (mg GAE)	Average (mg GAE/g pod)
50	**100**	3.71	2.24±1.07	11.44	11.10±0.48
	100	5.23		10.76	
	120	5.18	2.58±0.03	19.97	20.96±1.40
	120	5.13		21.96	
	140	5.10	2.53±0.05	29.05	30.75±2.41
	140	5.03		32.45	
	160	11.97	6.85±2.47	33.04	33.10±0.08
	160	15.45		33.16	
	180	29.06	12.96±4.45	56.48	56.59±0.16
	180	22.76		56.70	
	200	12.73	5.93±1.23	59.37	58.92±0.64
	200	10.99		58.46	
100	**100**	5.18	2.53±0.18	11.18	11.04±0.20
	100	4.93		10.89	
	120	5.38	2.62±0.20	25.87	23.35±3.57
	120	5.10		20.82	
	140	5.23	2.58±0.08	34.44	31.46±4.21
	140	5.11		28.48	
	160	15.79	8.32±1.19	32.25	34.97±3.85
	160	17.48		37.70	
	180	22.99	10.99±1.43	44.16	43.68±0.68
	180	20.96		43.20	
	200	6.83	3.54±0.56	35.82	35.43±0.56
	200	7.33		35.03	

TA: tannic acid, and GAE: gallic acid equivalent.

Table B5. Subcritical water extraction of total carbohydrates from green pea pod.

Pressure (bar)	Temperature (°C)	Time (min)	Total carbohydrates (mg GE/g pod)		Average (mg GE/g pod)	Pressure (bar)	Temperature (°C)	Time (min)	Total carbohydrates (mg GE/g pod)		Average (mg GE/g pod)
			Run 1	Run 2					Run 1	Run 2	
50	100	5	38.12	42.4	40.26±3.03	100	100	5	28.78	28.60	27.91±0.12
		10	63.81	68.58	66.20±3.37			10	51.35	47.37	47.08±2.81
		15	78.79	84.25	81.52±3.86			15	59.53	55.55	55.44±2.81
		20	87.75	92.8	90.28±3.57			20	65.22	60.51	59.74±3.33
		30	96.41	102.84	99.63±4.55			30	69.17	64.47	63.52±3.33
		40	99.81	109.29	104.55±6.70			40	71.61	66.90	65.92±3.33
	120	5	94.57	109.92	102.25±10.86		120	5	88.10	93.80	92.87±4.03
		10	170.82	186.45	178.63±11.05			10	149.04	155.06	154.57±4.25
		15	216.43	231.58	224.00±10.71			15	205.11	208.15	202.58±2.15
		20	255.68	271.1	263.39±10.90			20	232.14	224.95	219.09±5.09
		30	283.84	298.57	291.20±10.42			30	251.11	245.30	242.27±4.11
		40	305.77	319.95	312.86±10.02			40	272.05	266.24	263.11±4.11

GE: glucose equivalent.

Table B5. Continued**.**

Pressure (bar)	Temperature (°C)	Time (min)	Total carbohydrates (mg GE/g pod)		Average (mg GE/g pod)	Pressure (bar)	Temperature (°C)	Time (min)	Total carbohydrates (mg GE/g pod)		Average (mg GE/g pod)
			Run 1	Run 2					Run 1	Run 2	
50	**140**	5	177.64	179.51	178.58±1.32	**100**	**140**	5	177.33	166.96	168.31±7.33
		10	272.56	275.67	274.12±2.20			10	286.63	276.26	278.16±2.62
		15	325.78	327.86	326.82±1.47			15	345.66	335.29	338.92±7.03
		20	345.68	347.58	346.63±1.34			20	375.00	364.63	370.13±2.25
		30	361.7	364.36	363.03±1.88			30	395.46	385.09	390.76±1.22
		40	372.67	375.67	374.17±2.13			40	407.65	397.28	402.81±4.01

GE: glucose equivalent.

Table B6a. Solid-liquid extraction of tannins from green pea pod at 50℃.

Temperature of 50℃ and solid/solvent ratio of 2/40 (w/v)												
Run#	**Solvent**	**Sample weight (g)**	**ABS1**	**ABS2**	**ABS3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg TA)**	**(mg TA/g)**	**Ave (mg TA/g)**
1	100% water	2.0048	0.055	0.063	0.068	0.077	0.092	0.100	0.090	3.593	1.792	1.81±0.03
2	100% water	2.0019	0.064	0.063	0.062	0.093	0.092	0.090	0.092	3.664	1.830	
1	70% acetone/water	2.0039	0.107	0.105	0.112	0.170	0.166	0.179	0.171	6.860	3.423	3.23±0.27
2	70% acetone/water	2.0028	0.098	0.105	0.088	0.154	0.166	0.136	0.152	6.078	3.035	
1	70% ethanol/water	2.0035	0.133	0.148	0.136	0.216	0.242	0.221	0.227	9.061	4.523	4.37±0.21
2	70% ethanol/water	2.0036	0.139	0.129	0.124	0.227	0.209	0.200	0.212	8.469	4.227	

Table B6b. Solid-liquid extraction of tannins from green pea pod at 70℃.

Temperature of 70℃ and solid/solvent ratio of 2/40 (w/v)												
Run#	**Solvent**	**Sample weight (g)**	**ABS1**	**ABS2**	**ABS3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg TA)**	**(mg TA/g)**	**Ave (mg TA/g)**
1	100% water	2.0038	0.113	0.1	0.109	0.180	0.157	0.173	0.170	6.812	3.40	3.60±0.28
2	100% water	2.0034	0.118	0.123	0.115	0.189	0.198	0.184	0.190	7.617	3.80	
1	70% acetone/water	2.0053	0.163	0.169	0.174	0.269	0.280	0.289	0.279	11.168	5.57	5.45±0.17
2	70% acetone/water	2.0044	0.151	0.169	0.165	0.248	0.280	0.273	0.267	10.670	5.32	
1	70% ethanol/water	2.004	0.133	0.149	0.152	0.216	0.244	0.250	0.237	9.463	4.72	4.89±0.24
2	70% ethanol/water	2.004	0.159	0.156	0.148	0.262	0.257	0.242	0.254	10.150	5.06	

Table B7. Fiber analysis of faba bean hull and green pea pod.

	Sample	Sample weight (g)	Crucible weight (g)	Crucible +Celite+Residue (g)	Residue (g)	Average residue (g)	Average protein (g)	Average Insoluble fiber (%)
Insoluble fiber	Blank	0	35.6048	36.6052	-0.0012	-0.0012	0.0000	00.00±0.0
	Standard	1.0024	34.5739	36.7949	1.2194	0.2194	0.0008	21.79±1.2
	Faba hull low	1.0039	35.5876	37.2601	0.6687	0.69875	0.0051	68.65±0.05
	Faba hull low	1.0049	35.4136	37.1435	0.7288			
	Faba hull high	1.0043	35.2937	37.0508	0.7532	0.7501	0.0071	73.69±0.02
	Faba hull high	1.0058	35.4155	37.1641	0.7470			
	G.P Pod	1.0040	35.0041	36.1604	0.1569	0.1671	0.0023	49.50±0.05
	G.P pod	1.0035	35.3252	36.5051	0.1773			
	Sample	**Sample weight (g)**	**Crucible weight (g)**	**Crucible +Celite+Residue (g)**	**Residue (g)**	**Average residue (g)**	**Average protein (g)**	**Average Soluble fiber (%)**
Soluble fiber	Blank	0	35.9627	36.6825	-0.2818	0.0061	0.0000	00.00±0.0
	Standard	1.0054	35.1979	36.2606	0.0607	0.0100	0.0023	0.76±0.25
	Faba hull low	1.0049	35.0136	36.0557	0.0344	0.0347	0.0018	2.43±0.50
	Faba hull low	1.0039	34.9296	35.9674	0.0350			
	Faba hull high	1.0043	34.8998	35.9725	0.0691	0.06695	0.0015	5.60±0.02
	Faba hull high	1.0058	36.4027	37.4713	0.0648			
	G.P pod	1.004	36.212	37.2914	0.0705	0.06835	0.0018	5.60±0.05
	G.P pod	1.0035	34.476	35.5465	0.0662			

Table B8. Fiber analysis of green pea pod residue after treatment with subcritical water extraction.

	Sample	Sample weight (g)	Crucible weight (g)	Crucible +Celite+Residue (g)	Residue (g)	Average residue (g)	Average protein (g)	Insoluble fiber (%)
Insoluble fiber	Blank	0	57.1035	58.1933	0.01	0.01	0.0000	0.0±0.0
	Blank	0	56.1139	58.1425	0.01		0.0008	0.0±0.0
	T:100,P:50	1.007	57.4209	58.4407	0.536	0.51	0.0051	48.92±0.05
	T:100,P:50	1.068	57.3581	58.3722	0.479			
	T:120,P:50	1.0081	54.3219	55.3347	0.498	0.49	0.0071	48.81±0.10
	T:120,P:50	1.0077	54.421	55.4126	0.485			
	T:140,P:50	1.0073	59.9671	61.0315	0.482	0.50	0.0023	49.73±0.03
	T:140,P:50	1.0065	59.8544	61.2654	0.520			
	T:160,P:50	1.0033	34.6342	35.6314	0.472	0.54	0.0018	53.35±0.09
	T:160,P:50	1.0047	34.9845	35.7216	0.599			
	T:180,P:50	1.0013	34.6342	35.6237	0.542	0.47	0.0015	46.77±0.10
	T:180,P:50	1.0022	34.9845	35.9672	0.395			
	T:200,P:50	1.0033	35.4217	36.4125	0.343	0.45	0.0018	45.09±0.16
	T:200,P:50	1.0034	35.4148	36.3342	0.562			

T: temperature (℃), and P: pressure (bar).

Table B8. Continued.

	Sample	Sample weight(g)	Crucible weight (g)	Crucible +Celite+Residue (g)	Residue (g)	Average residue	Average protein (g)	Soluble fiber (%)
Soluble fiber	Blank	0	42.5435	43.6314	0.01	0.01	0.0000	0.0±0.0
	Blank	0	42.7099	43.787	0.01		0.0000	0.0±0.0
	T:100,P:50	1.007	42.354	43.8762	0.0552	0.06	0.0023	5.53±0.09
	T:100,P:50	1.0068	34.3579	35.4432	0.0562			
	T:120,P:50	1.0032	34.2218	35.4985	0.0553	0.05	0.0018	5.48±0.10
	T:120,P:50	1.0027	34.544	35.6363	0.0546			
	T:140,P:50	1.0073	34.6342	35.7211	0.0597	0.06	0.0015	5.52±0.03
	T:140,P:50	1.0077	34.9845	36.0402	0.0516			
	T:160,P:50	1.0023	34.6342	35.7211	0.0597	0.06	0.0032	5.55±0.15
	T:160,P:50	1.0027	34.9845	36.0402	0.0516			
	T:180,P:50	1.0053	34.6342	35.6852	0.0435	0.04	0.0041	4.23±0.25
	T:180,P:50	1.0055	34.9845	36.0327	0.0415			
	T:200,P:50	1.0033	35.4217	36.4453	0.0328	0.03	0.0034	3.25±0.08
	T:200,P:50	1.0034	35.4148	36.3976	0.0324			

T: temperature (℃), and P: pressure (bar).

Table B9a. Hemicellulosic sugars of green pea pod and faba bean hull raw samples by gas chromatography.

Areas Gas Chromatography (µVolts*min)								
	Rhamnose	**Fructose**	**Xylose**	**Arabinose**	**Mannose**	**Glucose**	**Galactose**	**Myo-Inositol**
RT (min)	10.652	10.945	11.065	11.455	15.33	15.519	15.675	14.638
Std 2_1	37633168	50570442	42696379	40432323	36783035	42092894	41226268	44317458
Std 2_2	29084984	38843024	33954125	31801649	28486435	32653870	32949156	34429441
High faba bean hull	8692633	-	8831063	42319068	-	486691491	18515331	42488420
High faba bean hull	8287732	-	8346548	39311280	-	456081023	17060942	38040618
Low faba bean hull	7040129	-	10624657	32581022	-	354421086	14219262	27346240
Low faba bean hull	9332250	-	14141049	47157938	-	514453087	-	39323330
Green pea pod	12084008	-	39927155	14806640	9757995	140413121	74804774	35084579
Green pea pod	14422985	3112977	49080645	18447214	12034365	174784152	91994920	42847755

RT: retention time.

Table B9b. Hemicellulosic sugars of green pea pod and faba bean hull raw samples.

Sugars in samples % (w/w)							
Samples	**Rhamnose (%)**	**Fructose (%)**	**Xylose (%)**	**Arabinose (%)**	**Mannose (%)**	**Glucose (%)**	**Galactose (%)**
High faba bean hull	1.02	-	0.91	4.57	-	52.38	1.97
High faba bean hull	1.09	-	0.96	4.74	-	54.83	2.03
Average	**1.06**	**-**	**0.93**	**4.65**	**-**	**53.60**	**2.00**
Low faba bean hull	1.26	-	1.67	5.34	-	57.94	2.30
Low faba bean hull	1.19	-	1.57	5.51	-	59.93	0.00
Average	**1.22**	**-**	**1.62**	**5.43**	**-**	**58.93**	**2.30**
Green pea pod	1.74	0.29	4.97	1.95	1.47	18.47	9.73
Green pea pod	1.68	0.27	4.97	1.97	1.46	18.59	9.68
Average	**1.71**	**0.27**	**4.97**	**1.96**	**1.47**	**18.53**	**9.71**

w/w: weight per weight.

Appendix C

Faba bean hull

Table C1. Faba bean hull moisture content.

Replicate	Weight (g)	Container weight (g)	Sample+container (g)	Weight (g)	Moisture content (%)	Average moisture content (%)
A	3.0045	3.8407	6.557	2.7163	10.610	10.66±0.50
B	3.0060	3.8577	6.5858	2.7281	10.187	
C	3.0064	3.8415	6.5455	2.7040	11.183	

SD: standard deviation.

Table C2. Faba bean hull protein content.

Sample	Replicate	Sample weight (g)	Nitrogen (%)	P factor	Protein content (%)	Average protein content (%)
Low tannin faba bean hull	A	0.1054	0.88114	6.25	5.5071	5.34±0.22
	B	0.1053	0.86745	6.25	5.4215	
	C	0.1024	0.8149	6.25	5.0935	
High tannin faba bean hull	A	0.1029	0.75907	6.25	4.7442	4.82±0.21
	B	0.1031	0.74685	6.25	4.6678	
	C	0.1017	0.80903	6.25	5.0564	

P: protein factor.

Table C3. Faba bean hull ash content.

Sample	Replicate	Empty crucible (g)	Sample weight (g)	Crucible+sample (g)	Ash content (%)	Average ash content (%)
Low tannin faba bean hull	A	22.4468	1.0059	22.4696	2.26	2.48±0.19
	B	24.1084	1.0032	24.1346	2.61	
	C	22.3039	1.0038	22.3296	2.56	
High tannin faba bean hull	A	24.5771	1.0028	24.6029	2.57	2.45±0.17
	B	20.6105	1.0047	20.6359	2.53	
	C	23.2761	1.0039	23.2987	2.25	

Table C4. Faba bean hull fat content.

Sample	Replicate	Beaker weight (g)	Sample weight (g)	Beaker+fat (g)	Fat weight (g)	Fat content (%)	Average fat content (%)
Low tannin faba bean hull	A	61.0943	2.0052	61.1099	0.0156	0.778	0.76±0.02
	B	61.5464	2.0048	61.5614	0.0150	0.748	
	C	57.5739	2.0036	57.5893	0.0154	0.768	
High tannin faba bean hull	A	61.3243	2.0054	61.3395	0.0152	0.758	0.77±0.02
	B	60.5361	2.0035	60.5516	0.0155	0.773	
	C	61.4238	2.0041	61.4397	0.0159	0.793	

Table C5. Subcritical water extraction of total tannins and condensed tannins from faba bean hull.

Pressure (bar)	Temperature (℃)	Total tannins (mg TA)	Average (mg TA/g hull)	Total condensed tannin (mg catechin)	Average (mg catechin/g hull)
50	100	59.78	28.77±1.12	67.27	22.34±0.36
	100	55.31		66.76	
	120	93.35	47.27±0.59	83.10	28.15±1.91
	120	95.72		85.79	
	140	178.93	58.29±1.35	108.84	35.67±2.60
	140	170.83		105.16	
	160	219.47	73.62±0.46	128.28	42.28±2.04
	160	222.26		125.39	
	180	163.47	53.08±1.41	99.47	33.23±0.33
	180	155.03		99.93	
	200	154.21	52.20±0.80	87.46	29.55±1.66
	200	159.02		89.82	
100	100	54.83	26.56±0.86	83.34	26.47±5.56
	100	51.41		75.47	
	120	68.34	41.06±6.89	77.27	25.93±0.74
	120	95.90		78.32	
	140	170.38	54.87±1.93	105.87	34.39±3.81
	140	158.83		100.48	
	160	202.21	70.51±3.11	119.98	40.80±3.43
	160	220.85		124.84	
	180	153.49	48.95±2.22	104.75	34.76±0.67
	180	140.19		103.80	
	200	154.40	50.69±0.77	87.99	28.66±2.83
	200	149.76		83.98	

TA: tannic acid.

Table C6. Subcritical water extraction of total phenolics from faba bean hull at 50 bar.

Time (min)	T(℃)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
5	100	0.404	0.405	0.406	0.383	0.383	0.384	0.383	9.587	9.648±0.003
	100	0.403	0.41	0.418	0.382	0.388	0.395	0.388	9.709	
	120	0.888	0.935	0.951	0.207	0.217	0.221	0.215	5.374	5.417±0.043
	120	0.953	0.931	0.935	0.221	0.216	0.217	0.218	5.460	
	140	0.791	0.77	0.797	0.737	0.718	0.743	0.733	18.319	18.269±0.050
	140	0.786	0.767	0.792	0.733	0.715	0.738	0.729	18.220	
	160	0.65	0.652	0.655	0.608	0.610	0.613	0.610	15.256	15.065±0.191
	160	0.647	0.621	0.639	0.605	0.581	0.598	0.595	14.874	
	180	0.355	0.37	0.356	0.338	0.351	0.339	0.343	8.563	8.433±0.130
	180	0.341	0.359	0.347	0.325	0.341	0.330	0.332	8.304	
	200	0.303	0.304	0.311	0.290	0.291	0.297	0.293	7.318	7.574±0.256
	200	0.313	0.336	0.336	0.299	0.320	0.320	0.313	7.830	
10	100	0.582	0.578	0.588	0.546	0.542	0.551	0.546	13.659	13.609±0.050
	100	0.580	0.573	0.582	0.544	0.537	0.546	0.542	13.560	
	120	0.773	0.791	0.785	0.721	0.737	0.732	0.730	18.250	18.247±0.004
	120	0.771	0.790	0.787	0.719	0.736	0.734	0.730	18.243	
	140	0.821	0.835	0.846	0.765	0.778	0.788	0.777	19.419	19.335±0.084
	140	0.814	0.845	0.821	0.758	0.787	0.765	0.770	19.251	
	160	0.835	0.877	0.888	0.778	0.816	0.826	0.807	20.168	20.160±0.008
	160	0.856	0.868	0.874	0.797	0.808	0.813	0.806	20.153	
	180	0.650	0.680	0.634	0.608	0.636	0.593	0.612	15.309	14.759±0.550
	180	0.638	0.562	0.620	0.597	0.527	0.581	0.568	14.209	
	200	0.445	0.483	0.484	0.420	0.455	0.456	0.444	11.092	11.245±0.153
	200	0.486	0.477	0.489	0.458	0.449	0.460	0.456	11.398	

T: temperature, and GAE: gallic acid equivalent.

Table C6. Continued.

Time (min)	T(℃)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
15	100	0.462	0.455	0.472	0.436	0.429	0.445	0.437	10.916	10.859±0.057
	100	0.457	0.451	0.466	0.431	0.426	0.439	0.432	10.802	
	120	0.846	0.834	0.852	0.788	0.777	0.793	0.786	19.648	19.587±0.061
	120	0.841	0.837	0.838	0.783	0.780	0.780	0.781	19.526	
	140	0.431	0.432	0.449	0.407	0.408	0.424	0.413	10.328	10.366±0.038
	140	0.440	0.426	0.456	0.416	0.403	0.430	0.416	10.404	
	160	0.425	0.398	0.398	0.402	0.377	0.377	0.385	9.633	9.568±0.065
	160	0.392	0.396	0.416	0.372	0.375	0.394	0.380	9.503	
	180	0.441	0.429	0.425	0.416	0.405	0.402	0.408	10.198	9.877±0.321
	180	0.405	0.396	0.410	0.383	0.375	0.388	0.382	9.556	
	200	0.260	0.250	0.272	0.251	0.241	0.262	0.251	6.279	6.262±0.017
	200	0.264	0.271	0.280	0.254	0.226	0.269	0.250	6.244	
20	100	0.330	0.341	0.346	0.315	0.325	0.329	0.323	16.149	16.187±0.038
	100	0.327	0.342	0.353	0.312	0.326	0.336	0.325	16.225	
	120	0.597	0.616	0.602	0.559	0.577	0.564	0.567	28.342	28.196±0.145
	120	0.594	0.598	0.604	0.557	0.560	0.566	0.561	28.051	
	140	0.299	0.298	0.305	0.286	0.285	0.292	0.288	14.392	14.453±0.061
	140	0.302	0.301	0.307	0.289	0.288	0.294	0.290	14.514	
	160	0.231	0.242	0.273	0.224	0.234	0.262	0.240	12.008	11.848±0.160
	160	0.245	0.239	0.241	0.237	0.231	0.233	0.234	11.687	
	180	0.254	0.252	0.263	0.245	0.243	0.253	0.247	12.359	12.787±0.428
	180	0.262	0.266	0.297	0.252	0.256	0.284	0.264	13.215	
	200	0.190	0.197	0.200	0.186	0.193	0.196	0.192	9.579	9.785±0.206
	200	0.200	0.205	0.209	0.196	0.200	0.204	0.200	9.991	

T: temperature, and GAE: gallic acid equivalent.

Table C6. Continued.

Time (min)	T(℃)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
30	100	0.237	0.233	0.235	0.229	0.226	0.228	0.228	11.382	11.183±0.199
	100	0.223	0.229	0.227	0.217	0.222	0.220	0.220	10.984	
	120	0.170	0.166	0.168	0.168	0.164	0.166	0.166	8.310	8.303±0.008
	120	0.164	0.172	0.167	0.163	0.170	0.165	0.166	8.295	
	140	0.154	0.153	0.156	0.153	0.152	0.155	0.154	7.684	7.906±0.222
	140	0.163	0.163	0.166	0.162	0.162	0.164	0.163	8.127	
	160	0.081	0.085	0.092	0.086	0.090	0.097	0.091	4.552	4.567±0.015
	160	0.084	0.087	0.089	0.089	0.092	0.094	0.092	4.582	
	180	0.161	0.157	0.184	0.160	0.156	0.181	0.166	8.280	8.310±0.031
	180	0.170	0.168	0.168	0.168	0.166	0.166	0.167	8.341	
	200	0.145	0.127	0.136	0.145	0.129	0.137	0.137	6.844	6.760±0.084
	200	0.131	0.136	0.130	0.132	0.137	0.131	0.134	6.676	
40	100	0.175	0.178	0.177	0.173	0.175	0.174	0.174	8.708	8.585±0.122
	100	0.175	0.167	0.172	0.173	0.165	0.170	0.169	8.463	
	120	0.185	0.170	0.195	0.182	0.168	0.191	0.180	9.013	8.998±0.015
	120	0.182	0.189	0.177	0.179	0.185	0.174	0.180	8.983	
	140	0.077	0.090	0.089	0.083	0.095	0.094	0.090	4.521	4.590±0.069
	140	0.081	0.091	0.093	0.086	0.096	0.097	0.093	4.659	
	160	0.086	0.084	0.091	0.091	0.089	0.096	0.092	4.598	4.598±0.003
	160	0.087	0.085	0.089	0.092	0.090	0.094	0.092	4.598	
	180	0.160	0.161	0.162	0.159	0.160	0.161	0.160	7.990	8.074±0.084
	180	0.161	0.169	0.164	0.160	0.167	0.163	0.163	8.158	
	200	0.139	0.126	0.134	0.140	0.128	0.135	0.134	6.706	6.431±0.275
	200	0.122	0.119	0.122	0.124	0.121	0.124	0.123	6.156	

T: temperature, and GAE: gallic acid equivalent.

Table C7. Subcritical water extraction of total phenolics from faba bean hull at 100 bar.

Time (min)	T(℃)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
5	100	0.635	0.656	0.648	0.594	0.614	0.606	0.605	15.118	15.141±0.023
	100	0.633	0.661	0.651	0.593	0.618	0.609	0.607	15.164	
	120	0.743	0.748	0.742	0.693	0.698	0.692	0.654	16.345	16.774±0.429
	120	0.741	0.731	0.740	0.692	0.682	0.691	0.688	17.204	
	140	0.623	0.634	0.615	0.146	0.148	0.144	0.146	3.652	3.671±0.019
	140	0.630	0.647	0.615	0.147	0.151	0.144	0.148	3.690	
	160	0.583	0.588	0.600	0.547	0.551	0.562	0.553	13.835	13.770±0.065
	160	0.580	0.585	0.589	0.544	0.548	0.552	0.548	13.705	
	180	0.876	0.927	0.934	0.204	0.216	0.217	0.212	5.304	5.309±0.006
	180	0.881	0.928	0.934	0.205	0.216	0.217	0.213	5.315	
	200	0.872	0.877	0.879	0.203	0.204	0.205	0.204	5.095	5.088±0.008
	200	0.870	0.875	0.875	0.202	0.204	0.204	0.203	5.080	
10	100	0.861	0.850	0.903	0.802	0.791	0.840	0.811	20.275	20.542±0.267
	100	0.866	0.909	0.909	0.806	0.846	0.846	0.832	20.810	
	120	0.955	0.948	0.959	0.888	0.881	0.891	0.887	22.170	22.139±0.031
	120	0.959	0.944	0.951	0.891	0.878	0.884	0.884	22.108	
	140	0.432	0.492	0.469	0.102	0.116	0.111	0.109	2.737	2.741±0.004
	140	0.436	0.483	0.478	0.103	0.114	0.113	0.110	2.744	
	160	0.468	0.465	0.463	0.441	0.438	0.437	0.439	10.970	10.951±0.019
	160	0.466	0.462	0.463	0.439	0.436	0.437	0.437	10.932	
	180	0.835	0.898	0.891	0.194	0.209	0.207	0.204	5.088	5.083±0.005
	180	0.830	0.893	0.896	0.193	0.208	0.208	0.203	5.078	
	200	0.833	0.829	0.825	0.194	0.193	0.192	0.193	4.826	4.834±0.008
	200	0.830	0.834	0.831	0.193	0.194	0.194	0.194	4.841	

T: temperature, and GAE: gallic acid equivalent.

Table C7. Continued.

Time (min)	T(°C)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
15	100	0.525	0.550	0.523	0.493	0.516	0.492	0.501	12.513	12.540±0.027
	100	0.527	0.553	0.525	0.495	0.519	0.493	0.503	12.566	
	120	0.539	0.548	0.542	0.506	0.515	0.509	0.510	12.750	12.769±0.019
	120	0.544	0.548	0.542	0.511	0.515	0.509	0.512	12.788	
	140	0.934	0.920	0.931	0.868	0.856	0.866	0.863	21.581	21.623±0.042
	140	0.942	0.920	0.934	0.876	0.856	0.868	0.867	21.665	
	160	0.288	0.303	0.268	0.276	0.290	0.258	0.275	6.867	6.940±0.073
	160	0.290	0.301	0.287	0.278	0.288	0.275	0.280	7.012	
	180	0.737	0.743	0.750	0.172	0.173	0.175	0.173	4.335	4.333±0.002
	180	0.742	0.737	0.749	0.173	0.172	0.175	0.173	4.331	
	200	0.480	0.498	0.475	0.452	0.469	0.448	0.456	11.405	11.466±0.061
	200	0.495	0.475	0.499	0.466	0.448	0.470	0.461	11.527	
20	100	0.308	0.322	0.335	0.295	0.307	0.319	0.308	15.385	15.560±0.176
	100	0.312	0.326	0.337	0.298	0.311	0.321	0.315	15.736	
	120	0.348	0.361	0.352	0.331	0.343	0.335	0.336	16.806	18.318±1.513
	120	0.338	0.352	0.351	0.322	0.335	0.334	0.397	19.831	
	140	0.562	0.582	0.568	0.527	0.546	0.533	0.538	26.890	26.157±0.733
	140	0.565	0.587	0.576	0.530	0.550	0.540	0.508	25.423	
	160	0.466	0.474	0.472	0.439	0.447	0.445	0.438	21.894	22.795±0.901
	160	0.443	0.440	0.453	0.418	0.416	0.427	0.474	23.697	
	180	0.616	0.634	0.628	0.577	0.593	0.588	0.584	29.197	27.188±2.009
	180	0.615	0.621	0.621	0.576	0.581	0.581	0.504	25.179	
	200	0.370	0.336	0.372	0.351	0.320	0.353	0.342	17.081	16.271±0.810
	200	0.365	0.366	0.372	0.347	0.348	0.353	0.309	15.461	

T: temperature, and GAE: gallic acid equivalent.

Table C7. Continued.

Time (min)	T(°C)	ABS 1	ABS 2	ABS 3	(mg GAE/mL)	(mg GAE/mL)	(mg GAE/mL)	Average (mg GAE/mL)	(mg GAE)	Average (mg GAE)
30	100	0.222	0.224	0.241	0.216	0.218	0.233	0.222	11.107	11.160±0.053
	100	0.222	0.228	0.244	0.216	0.221	0.236	0.224	11.213	
	120	0.235	0.237	0.245	0.228	0.229	0.237	0.231	11.565	11.580±0.015
	120	0.235	0.240	0.244	0.228	0.232	0.236	0.232	11.595	
	140	0.246	0.255	0.252	0.238	0.246	0.243	0.242	12.115	12.153±0.038
	140	0.249	0.259	0.250	0.240	0.250	0.241	0.244	12.191	
	160	0.171	0.170	0.166	0.169	0.168	0.164	0.167	8.356	8.410±0.053
	160	0.174	0.168	0.172	0.172	0.166	0.170	0.169	8.463	
	180	0.319	0.316	0.312	0.305	0.302	0.298	0.302	15.079	14.774±0.306
	180	0.313	0.299	0.295	0.299	0.286	0.283	0.289	14.468	
	200	0.192	0.189	0.195	0.188	0.185	0.191	0.188	9.411	9.265±0.145
	200	0.186	0.186	0.185	0.183	0.183	0.182	0.182	9.120	
40	100	0.140	0.149	0.156	0.141	0.149	0.155	0.148	7.409	7.470±0.061
	100	0.143	0.151	0.159	0.143	0.151	0.158	0.151	7.531	
	120	0.145	0.152	0.166	0.145	0.152	0.164	0.154	7.684	7.493±0.191
	120	0.139	0.145	0.154	0.140	0.145	0.153	0.146	7.302	
	140	0.103	0.102	0.111	0.107	0.106	0.114	0.109	5.438	5.552±0.115
	140	0.109	0.110	0.112	0.112	0.113	0.115	0.113	5.667	
	160	0.089	0.083	0.086	0.094	0.088	0.091	0.091	4.552	4.605±0.053
	160	0.088	0.087	0.090	0.093	0.092	0.095	0.093	4.659	
	180	0.143	0.152	0.141	0.143	0.152	0.141	0.145	7.271	7.111±0.160
	180	0.135	0.137	0.143	0.136	0.138	0.143	0.139	6.951	
	200	0.074	0.084	0.079	0.080	0.089	0.085	0.085	4.231	4.139±0.092
	200	0.079	0.072	0.074	0.085	0.078	0.080	0.081	4.047	

T: temperature, and GAE: gallic acid equivalent.

Table C8. Subcritical water extraction of total carbohydrates from faba bean hull at 50 bar.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
100	1	5	0.160	0.153	1.51	1.43	1.47±0.06	36.73	18.37
		10	0.208	0.211	2.04	2.07	2.06±0.02	51.39	44.06
		15	0.125	0.139	1.12	1.28	1.20±0.11	29.96	59.04
		20	0.094	0.083	0.78	0.66	0.72±0.08	17.92	68.00
		30	0.082	0.053	0.65	0.32	0.48±0.23	24.23	80.12
		40	0.042	0.038	0.20	0.16	0.18±0.03	9.02	84.63
100	2	5	0.175	0.166	1.67	1.57	1.62±0.07	40.61	20.30
		10	0.210	0.215	2.06	2.12	2.09±0.04	52.22	46.41
		15	0.129	0.133	1.17	1.21	1.19±0.03	29.68	61.25
		20	0.105	0.098	0.90	0.82	0.86±0.06	21.52	72.01
		30	0.085	0.082	0.68	0.65	0.66±0.02	33.08	88.56
		40	0.053	0.042	0.32	0.20	0.26±0.08	13.17	95.14
120	1	5	0.698	0.701	7.46	7.49	7.48±0.02	186.93	93.47
		10	0.573	0.577	6.08	6.12	6.10±0.03	152.49	169.71
		15	0.355	0.352	1.83	1.82	1.82±0.01	45.61	192.52
		20	0.302	0.307	1.54	1.57	1.55±0.02	38.84	211.94
		30	0.120	0.124	0.53	0.55	0.54±0.01	27.19	225.53
		40	0.094	0.101	0.39	0.43	0.41±	20.41	235.74

T: temperature, and GE: glucose equivalent.

Table C8. Continued.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
120	1	5	0.706	0.709	7.55	7.58	7.55±0.02	7.58	94.57
		10	0.579	0.571	6.14	6.06	6.14±0.06	6.06	170.82
		15	0.355	0.352	3.67	3.63	3.67±0.03	3.63	216.43
		20	0.309	0.306	1.58	1.56	1.58±0.01	1.56	236.06
		30	0.127	0.125	0.57	0.56	0.57±0.01	0.56	250.20
		40	0.104	0.102	0.22	0.22	0.22±0.00	0.22	255.69
140	2	5	0.582	0.58	6.18	6.16	6.18±0.01	6.16	77.08
		10	0.374	0.376	5.81	5.85	5.81±0.03	5.85	149.96
		15	0.285	0.284	2.89	2.88	2.89±0.01	2.88	186.02
		20	0.139	0.14	0.64	0.64	0.64±0.01	0.64	194.03
		30	0.648	0.646	1.73	1.72	1.73±0.01	1.72	237.14
		40	0.288	0.287	0.73	0.73	0.73±0.01	0.73	255.38
140	1	5	0.573	0.583	6.08	6.19	6.08±0.08	6.19	76.66
		10	0.384	0.382	5.98	5.95	5.98±0.02	5.95	151.20
		15	0.285	0.29	2.89	2.95	2.89±0.04	2.95	187.68
		20	0.144	0.137	0.67	0.63	0.67±0.03	0.63	195.76
		30	0.65	0.649	1.73	1.73	1.73±0.01	1.73	239.04
		40	0.289	0.287	0.73	0.73	0.73±0.00	0.73	257.31

T: temperature, and GE: glucose equivalent.

Table C8. Continued.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
160	2	5	0.518	0.520	10.94	10.98	10.96±0.03	274.01	137.00
		10	0.476	0.481	10.01	10.12	10.06±0.08	251.60	262.80
		15	0.299	0.295	6.09	6.00	6.05±0.06	151.19	338.40
		20	0.124	0.118	1.11	1.04	1.08±0.05	26.91	351.86
		30	0.537	0.553	0.06	0.06	0.06±0.00	2.88	353.30
		40	0.343	0.353	0.04	0.04	0.04±0.00	1.79	354.20
180	1	5	0.797	0.799	4.28	4.29	4.28±0.01	107.09	53.54
		10	0.425	0.422	4.44	4.41	4.42±0.02	110.59	108.84
		15	0.550	0.553	2.91	2.93	2.92±0.01	73.00	145.34
		20	0.190	0.193	0.92	0.94	0.93±0.01	23.21	156.94
		30	0.149	0.147	0.35	0.34	0.34±0.01	17.19	165.54
		40	0.096	0.093	0.20	0.19	0.20±0.01	9.79	170.43
180	2	5	0.785	0.789	4.21	4.23	4.22±0.01	105.57	52.78
		10	0.415	0.419	4.33	4.37	4.35±0.03	108.79	107.18
		15	0.547	0.552	2.89	2.92	2.91±0.02	72.72	143.54
		20	0.189	0.185	0.91	0.89	0.90±0.01	22.58	154.83
		30	0.151	0.147	0.35	0.34	0.35±0.01	17.33	163.50
		40	0.101	0.098	0.21	0.21	0.21±0.00	10.48	168.74

T: temperature, and GE: glucose equivalent.

Table C8. Continued.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
200	1	5	0.364	0.365	3.77	3.78	3.77±0.01	94.27	47.13
		10	0.257	0.259	2.58	2.60	2.59±0.01	64.81	79.54
		15	0.120	0.122	1.07	1.09	1.08±0.01	26.91	93.00
		20	0.463	0.468	4.86	4.92	4.89±0.04	122.20	154.10
		30	0.064	0.067	0.45	0.48	0.46±0.02	23.12	165.66
		40	0.028	0.031	0.05	0.08	0.06±0.02	3.21	167.26
200	2	5	0.372	0.368	3.85	3.81	3.83±0.03	95.79	47.89
		10	0.260	0.255	2.61	2.56	2.59±0.04	64.67	80.23
		15	0.119	0.122	1.05	1.09	1.07±0.03	26.78	93.62
		20	0.459	0.463	4.82	4.86	4.84±0.03	120.96	154.10
		30	0.074	0.077	0.56	0.59	0.57±0.02	28.66	168.43
		40	0.038	0.043	0.16	0.21	0.19±0.04	9.29	173.07

T: temperature, and GE: glucose equivalent.

Table C9. Subcritical water extraction of total carbohydrates from faba bean hull at 100 bar.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
100	1	5	0.367	0.368	3.80	3.81	3.80±0.01	95.10	47.55
		10	0.446	0.443	1.17	1.16	1.16±0.01	29.10	44.24
		15	0.249	0.231	1.25	1.15	1.20±0.07	29.92	59.20
		20	0.268	0.266	1.35	1.34	1.35±0.01	33.65	76.02
		30	0.177	0.181	0.42	0.44	0.43±0.01	21.48	86.76
		40	0.160	0.158	0.38	0.37	0.37±0.01	18.71	96.12
100	2	5	0.275	0.283	2.78	0.72	1.75±1.46	43.72	21.86
		10	0.432	0.438	1.13	1.15	1.14±0.01	28.44	36.08
		15	0.249	0.252	1.25	1.26	1.25±0.01	31.37	51.77
		20	0.278	0.282	1.41	1.43	1.42±0.01	35.45	69.49
		30	0.197	0.189	0.48	0.46	0.47±0.01	23.41	81.20
		40	0.154	0.158	0.36	0.37	0.37±0.01	18.30	90.34
120	1	5	0.580	0.582	6.16	6.18	6.17±0.01	154.15	77.08
		10	0.541	0.535	2.86	2.83	2.85±0.02	71.13	112.64
		15	0.398	0.400	2.07	2.08	2.08±0.01	51.91	138.59
		20	0.499	0.497	2.63	2.62	2.62±0.01	65.60	171.39
		30	0.467	0.468	2.45	2.46	2.46±0.01	122.76	232.77
		40	0.238	0.228	0.02	0.02	0.02±0.00	1.16	233.35
120	2	5	0.578	0.584	6.13	6.20	6.17±0.05	154.15	77.08
		10	0.535	0.535	2.83	2.83	2.83±0.00	70.71	112.43
		15	0.386	0.395	2.00	2.05	2.03±0.04	50.73	137.80
		20	0.489	0.497	2.57	2.62	2.60±0.04	64.91	170.25
		30	0.462	0.458	2.42	2.40	2.41±0.01	120.68	230.59
		40	0.228	0.234	0.02	0.02	0.02±0.00	1.15	231.17

T: temperature, and GE: glucose equivalent.

Table C9. Continued.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
140	1	5	0.762	0.763	8.17	8.18	8.17±0.01	204.36	102.18
		10	0.976	0.980	5.27	5.29	5.28±0.01	131.98	168.17
		15	0.363	0.380	1.88	1.97	1.92±0.06	48.10	192.22
		20	0.475	0.452	2.50	2.37	2.43±0.09	60.83	222.63
		30	0.420	0.317	1.10	0.81	0.95±0.21	47.69	246.48
		40	0.192	0.202	0.47	0.49	0.48±0.01	23.97	258.46
140	2	5	0.692	0.683	7.39	7.29	7.34±0.07	183.61	91.8058
		10	0.956	0.961	5.16	5.19	5.17±0.02	129.29	156.4488
		15	0.373	0.378	1.93	1.96	1.95±0.02	48.66	180.7764
		20	0.525	0.533	2.77	2.82	2.80±0.04	69.88	215.7187
		30	0.419	0.399	1.09	1.04	1.07±0.04	53.29	242.3629
		40	0.182	0.196	0.44	0.48	0.46±0.03	22.86	253.7937
160	1	5	0.633	0.639	13.48	13.62	13.55±0.10	338.73	169.37
		10	0.443	0.446	9.28	9.34	9.31±0.04	232.79	285.76
		15	0.217	0.220	4.28	4.34	4.31±0.04	107.77	339.65
		20	0.105	0.108	0.90	0.93	0.92±0.02	22.90	351.10
		30	0.950	0.948	0.10	0.10	0.10±0.00	5.12	353.66
		40	0.223	0.225	0.02	0.02	0.02±0.00	1.11	354.21
160	2	5	0.717	0.723	15.34	15.47	15.41±0.09	385.20	192.6013
		10	0.453	0.459	9.50	9.62	9.56±0.08	239.04	312.1231
		15	0.224	0.220	4.43	4.34	4.39±0.06	109.70	366.9743
		20	0.115	0.110	1.01	0.95	0.98±0.04	24.56	379.2556
		30	0.875	0.888	0.09	0.10	0.09±0.01	4.75	381.6283
		40	0.213	0.208	0.02	0.02	0.02±0.00	1.03	382.145

T: temperature, and GE: glucose equivalent.

Table C9. Continued.

Total carbohydrates									
T (°C)	**Run#**	**Time (min)**	**ABS 1**	**ABS 2**	**(mg GE/ mL)**		**Average (mg GE/mL)**	**(mg GE)**	**(mg GE/g hull)**
180	1	5	0.489	0.494	5.15	5.20	5.18±0.04	129.40	64.70
		10	0.277	0.280	2.80	2.84	2.82±0.03	70.48	99.94
		15	0.285	0.283	2.89	2.87	2.88±0.01	72.00	135.94
		20	0.183	0.184	0.44	0.44	0.44±0.00	11.05	141.46
		30	0.348	0.351	0.90	0.91	0.90±0.01	45.06	163.99
		40	0.150	0.146	0.35	0.34	0.34±0.01	17.19	172.59
180	2	5	0.522	0.516	5.51	5.45	5.48±0.04	137.00	68.50
		10	0.317	0.298	3.25	3.03	3.14±0.16	78.50	107.75
		15	0.265	0.271	2.67	2.74	2.70±0.05	67.58	141.54
		20	0.173	0.180	0.41	0.43	0.42±0.01	10.57	146.82
		30	0.338	0.328	0.87	0.84	0.86±0.02	42.78	168.21
		40	0.145	0.156	0.34	0.37	0.35±0.02	17.54	176.98
200	1	5	0.982	0.998	5.30	5.39	5.35±0.06	133.64	66.82
		10	0.659	0.656	3.51	3.50	3.51±0.01	87.66	110.65
		15	0.236	0.291	0.47	0.59	0.53±0.08	13.27	117.28
		20	0.693	0.695	1.48	1.49	1.48±0.01	37.08	135.82
		30	0.413	0.416	0.86	0.87	0.86±0.01	43.24	157.44
		40	0.137	0.134	0.25	0.24	0.25±0.01	12.37	163.63
200	2	5	0.898	0.798	4.84	4.28	4.56±0.40	114.00	57.00
		10	0.559	0.562	2.96	2.98	2.97±0.01	74.24	94.12
		15	0.246	0.234	0.49	0.47	0.48±0.01	11.97	100.11
		20	0.687	0.692	1.47	1.48	1.47±0.01	36.83	118.52
		30	0.403	0.411	0.84	0.86	0.85±0.01	42.41	139.73
		40	0.127	0.138	0.23	0.25	0.24±0.01	12.04	145.75

T: temperature, and GE: glucose equivalent.

Table C10a. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:20 w/v at 70℃.

Temperature of 70℃ and solid/solvent ratio of 2/20 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.014	0.371	0.366	0.365	2.13	2.08	2.07	2.10	41.93	20.82	21.40±0.83
RUN#2	70% acetone/water	2.0175	0.38	0.376	0.382	2.22	2.18	2.24	2.22	44.36	21.99	
RUN#1	100% water	2.0141	0.369	0.368	0.357	0.88	0.88	0.83	0.86	17.25	8.56	8.65±0.12
RUN#2	100% water	2.0154	0.367	0.368	0.372	0.87	0.88	0.89	0.88	17.61	8.74	
RUN#1	50% ethanol/water	2.0215	0.579	0.592	0.589	2.83	0.73	0.72	1.43	28.52	14.11	14.04±0.10
RUN#2	50% ethanol/water	2.0259	0.582	0.586	0.564	2.85	0.72	0.68	1.42	28.30	13.97	
RUN#1	70% ethanol/water	2.0129	0.339	0.335	0.321	1.81	1.77	1.63	1.74	34.71	17.24	17.75±0.72
RUN#2	70% ethanol/water	2.012	0.347	0.336	0.342	1.89	1.78	1.84	1.84	36.73	18.26	

TA: tannic acid.

Table C10b. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:30 w/v at 70℃.

Temperature of 70℃ and solid/solvent ratio of 2/30 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.0175	0.372	0.361	0.379	1.43	1.35	1.48	1.42	42.60	21.12	22.16±1.48
RUN#2	70% acetone/water	2.0216	0.398	0.390	0.388	1.60	1.55	1.54	1.56	46.92	23.21	
RUN#1	100% water	2.0119	0.597	0.589	0.602	0.74	0.72	0.75	0.74	22.05	10.96	10.89±0.11
RUN#2	100% water	2.0105	0.595	0.583	0.591	0.73	0.71	0.73	0.72	21.73	10.81	
RUN#1	50% ethanol/water	2.0184	0.389	0.377	0.371	1.16	1.10	1.07	1.11	33.22	16.46	17.37±1.29
RUN#2	50% ethanol/water	2.0239	0.399	0.408	0.405	1.21	1.25	1.24	1.23	37.01	18.29	
RUN#1	70% ethanol/water	2.0123	0.217	0.205	0.211	1.53	1.21	1.37	1.37	41.13	20.44	21.58±1.61
RUN#2	70% ethanol/water	2.0128	0.223	0.208	0.219	1.70	1.29	1.59	1.52	45.72	22.72	

TA: tannic acid.

Table C10c. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:40 w/v at 70℃.

Temperature of 70℃ and solid/solvent ratio of 2/40 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.0174	0.342	0.338	0.341	1.84	1.80	1.83	2.83	113.35	56.19	54.36±2.58
RUN#2	70% acetone/water	2.0145	0.331	0.344	0.337	1.73	1.86	1.79	2.65	105.84	52.54	
RUN#1	100% water	2.0256	0.463	0.475	0.508	1.02	1.06	1.17	1.09	43.43	21.44	21.66±0.31
RUN#2	100% water	2.0142	0.499	0.486	0.475	1.14	1.10	1.06	1.10	44.06	21.87	
RUN#1	50% ethanol/water	2.0246	0.507	0.511	0.489	1.17	1.18	1.11	1.15	46.17	22.81	22.59±0.31
RUN#2	50% ethanol/water	2.0156	0.488	0.503	0.492	1.11	1.16	1.12	1.13	45.09	22.37	
RUN#1	70% ethanol/water	2.0201	0.253	0.261	0.26	1.88	2.04	2.02	1.98	79.19	39.20	38.41±1.12
RUN#2	70% ethanol/water	2.0265	0.255	0.245	0.263	1.92	1.72	2.08	1.91	76.22	37.61	

TA: tannic acid.

Table C11a. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:20 w/v at 50℃.

Temperature of 50℃ and solid/solvent ratio of 2/20 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.0283	0.376	0.382	0.402	1.46	1.50	1.63	1.53	30.56	15.07	14.59±0.67
RUN#2	70% acetone/water	2.0252	0.349	0.379	0.388	1.27	1.48	1.54	1.43	28.58	14.11	
RUN#1	100% water	2.0493	0.223	0.234	0.229	0.42	0.50	0.46	0.46	9.24	4.51	4.13±0.53
RUN#2	100% water	2.0507	0.213	0.224	0.215	0.36	0.43	0.37	0.39	7.71	3.76	
RUN#1	50% ethanol/water	2.0081	0.376	0.353	0.371	0.73	0.65	0.71	0.70	13.93	6.94	6.90±0.05
RUN#2	50% ethanol/water	2.0078	0.398	0.392	0.303	0.80	0.78	0.48	0.69	13.77	6.86	
RUN#1	70% ethanol/water	2.0498	0.173	0.188	0.192	0.43	0.94	1.07	0.81	16.28	7.94	7.97±0.04
RUN#2	70% ethanol/water	2.0065	0.181	0.184	0.187	0.70	0.80	0.90	0.80	16.06	8.00	

TA: tannic acid.

Table C11b. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:30 w/v at 50℃.

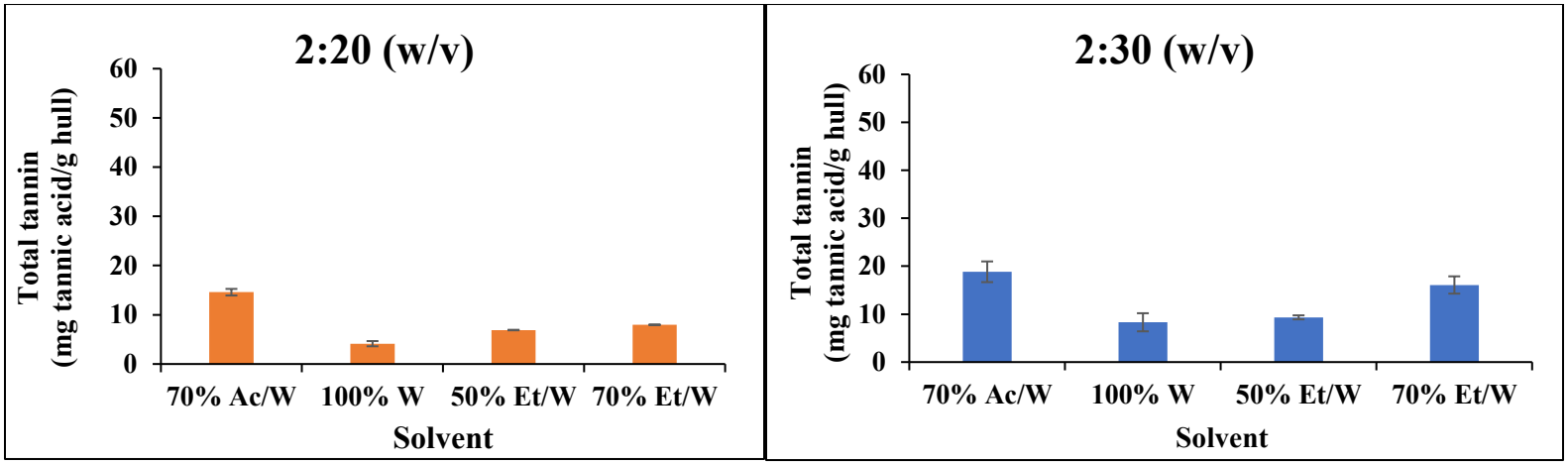
Temperature of 50℃ and solid/solvent ratio of 2/30 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.0175	0.342	0.338	0.318	1.23	1.20	1.06	1.16	34.91	17.30	18.83±2.16
RUN#2	70% acetone/water	2.0126	0.333	0.413	0.342	1.17	1.71	1.23	1.37	40.99	20.36	
RUN#1	100% water	2.0199	0.232	0.224	0.313	0.48	0.43	1.03	0.65	19.46	9.63	8.30±1.89
RUN#2	100% water	2.0205	0.242	0.215	0.232	0.55	0.37	0.48	0.47	14.06	6.96	
RUN#1	50% ethanol/water	2.0051	0.198	0.202	0.195	0.64	0.71	0.59	0.64	19.30	9.62	9.33±0.42
RUN#2	50% ethanol/water	2.0058	0.198	0.208	0.182	0.64	0.81	0.37	0.60	18.12	9.03	
RUN#1	70% ethanol/water	2.0123	0.202	0.198	0.21	1.13	1.02	1.34	1.16	34.93	17.36	16.08±1.80
RUN#2	70% ethanol/water	2.0128	0.207	0.211	0.173	1.26	1.37	0.35	0.99	29.80	14.80	

TA: tannic acid.

Table C11c. Solid-liquid extraction of faba bean hull using a solid to solvent ratio of 2:40 w/w at 50℃.

Temperature of 50℃ and solid/solvent ratio of 2/40 (w/v)												
Treatment	**Solvent**	**Sample weight (g)**	**ABS 1**	**ABS 2**	**ABS 3**	**(mg TA/mL)**	**(mg TA/mL)**	**(mg TA/mL)**	**Ave (mg TA/mL)**	**(mg)**	**(mg/g)**	**Average (mg TA/g)**
RUN#1	70% acetone/water	2.1636	0.555	0.544	0.553	2.66	2.59	2.65	2.63	105.39	48.71	46.92±2.52
RUN#2	70% acetone/water	2.2251	0.530	0.525	0.542	2.50	2.46	2.58	2.51	100.44	45.14	
RUN#1	100% water	2.0384	0.185	0.165	0.181	0.84	0.16	0.70	0.57	22.67	11.12	12.26±1.61
RUN#2	100% water	2.0283	0.174	0.189	0.178	0.47	0.97	0.60	0.68	27.17	13.40	
RUN#1	50% ethanol/water	2.0089	0.349	0.349	0.39	0.64	0.64	0.78	0.68	27.32	13.60	14.14±0.76
RUN#2	50% ethanol/water	2.0084	0.376	0.382	0.378	0.73	0.75	0.73	0.74	29.48	14.68	
RUN#1	70% ethanol/water	2.0249	0.295	0.289	0.302	1.82	1.74	1.91	1.82	72.95	36.02	35.50±0.74
RUN#2	70% ethanol/water	2.0440	0.292	0.290	0.296	1.78	1.75	1.83	1.79	71.51	34.98	

TA: tannic acid.



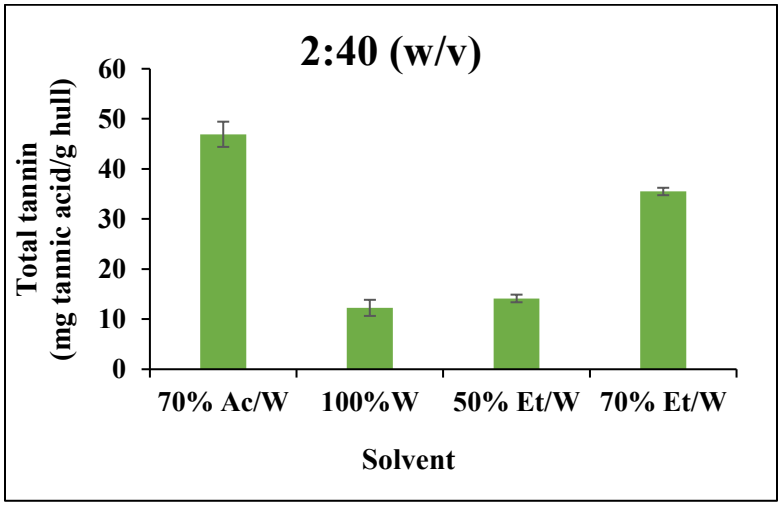
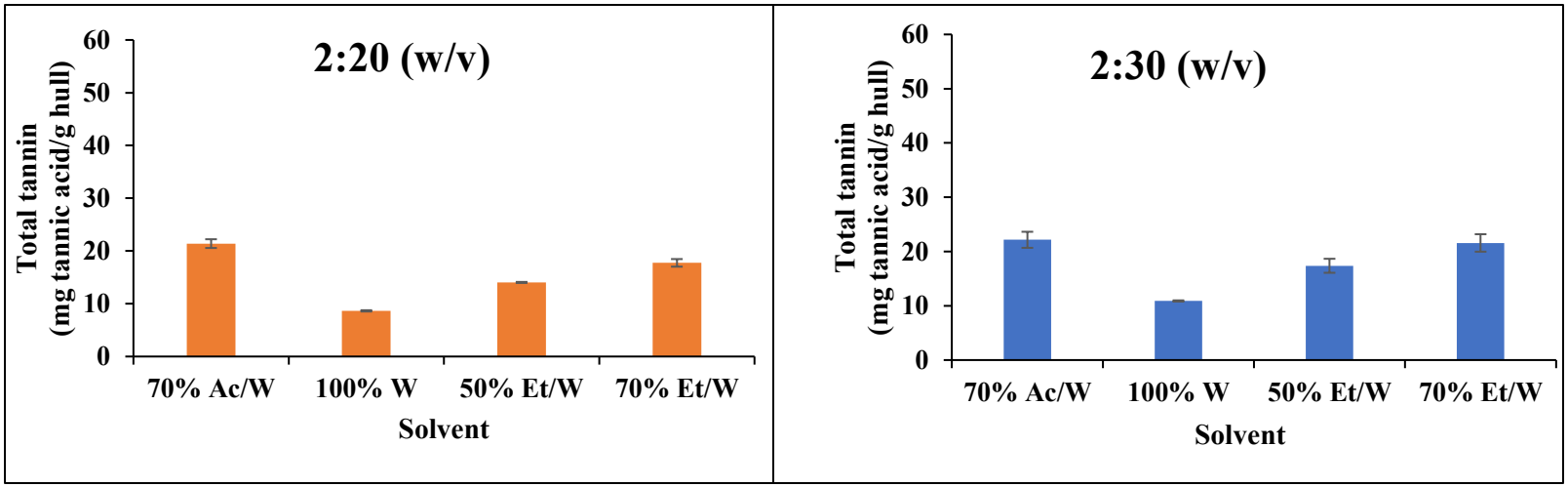


Figure C1a. Solid-liquid extraction of total tannins from faba bean hull at 50℃ and different solid to liquid ratios, Ac: acetone, W: water, Et: ethanol.



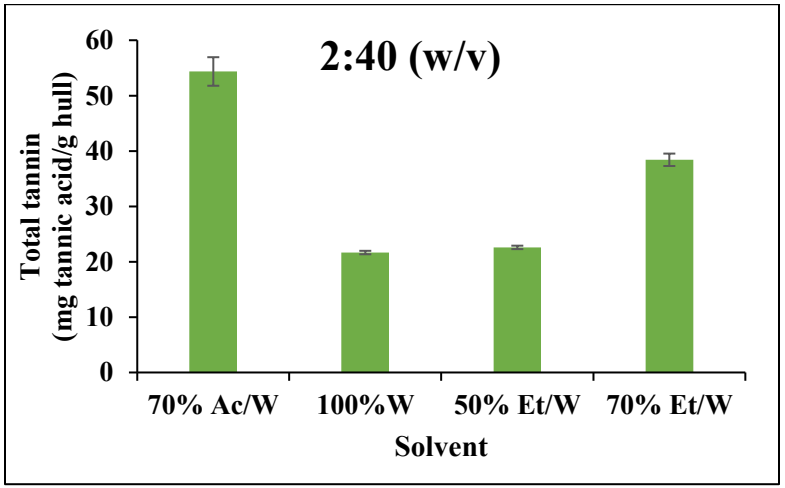


Figure C1b. Solid-liquid extraction of total tannin from faba bean hull at 70℃ and different solid to liquid ratios, Ac: acetone, W: water, Et: ethanol.

Table C12. Residue of faba hull after solid-liquid extraction.

Solvent	Tray weight (g)	Sample weight (g)	Residue+tray (g)	Residue weight (g)	yield (%)
70% Acetone/water	1.00467	2.0029	2.8339	1.8292	91.3290
70% Acetone/water	0.98725	2.0077	2.8318	1.8445	91.8739
70% ethanol/water	1.04551	2.0054	2.9134	1.8678	93.1430
70% ethanol/water	0.9962	2.0029	2.8931	1.8969	94.7076
100% water	1.03324	2.0061	2.883	1.8497	92.2067
100% water	1.02716	2.0052	2.8804	1.8532	92.4217
50% ethanol/water	1.00203	2.0052	2.9229	1.9208	95.7944
50% ethanol/water	0.99077	2.0071	2.8984	1.9076	95.0441
70% Acetone/water	1.00467	2.0029	2.8339	1.82923	91.3291

Table C13. HPLC analysis of condensed tannins of faba bean hull at 50 bar.

Treatment	#Run	Catechin	Epicatechin	Epigallocatechin	Gallocatechin gallate	Epicatechin gallate	Ellagic acid
T:100℃,P:50	1	0.041667	0.014569	0.008667	0.067044	0.009354	21.9121
	2	0.040773	0.015692	0.017092	0.067044	0.009176	21.77949
	Ave (mg/mL)	0.04122	0.01513	0.012879	0.067044	0.009265	21.84579
	SD	0.00	0.00	0.01	0.00	0.00	0.09
	(mg/g)	4.122001	1.513046	1.287918	6.704447	0.926539	2.184579
T:120℃,P:50	1	0.037748	0.013159	0.017467	0.066975	0.009675	22.86454
	2	0.034628	0.010662	0.009504	0.066799	0.010338	24.20541
	Ave (mg/mL)	0.036188	0.011911	0.013485	0.066887	0.010006	23.53497
	SD	0.00	0.00	0.01	0.00	0.00	0.95
	(mg/g)	3.61878	1.191059	1.348511	6.68867	1.00063	2.353497
T:140℃,P:50	1	0.033286	0.010152	0.004546	0.06793	0.007164	29.31809
	2	0.033763	0.010695	0.003972	0.067904	0.007972	31.95225
	Ave (mg/mL)	0.033524	0.010424	0.004259	0.067917	0.007568	30.63517
	SD	0.00	0.00	0.00	0.00	0.00	1.86
	(mg/g)	3.352425	1.042373	0.42589	6.791716	0.756804	3.063517
T:160℃,P:50	1	0.033861	0.0102	0.00528	0.067261	0.007164	27.75257
	2	0.034097	0.010216	0.005692	0.066863	0.007972	28.57635
	Ave (mg/mL)	0.033979	0.010208	0.005486	0.067062	0.007568	28.16446
	SD	0.00	0.00	0.00	0.00	0.00	0.58
	(mg/g)	3.397907	1.020809	0.548639	6.706198	0.742	2.816446
T:180℃,P:50	1	0.03784	0.011504	0.00906	0.067176	0.007347	26.68946
	2	0.037902	0.010755	0.012099	0.066854	0.007667	29.30837
	Ave (mg/mL)	0.037871	0.011129	0.01058	0.067015	0.007507	27.99891
	SD	0.00	0.00	0.00	0.00	0.00	1.85
	(mg/g)	3.787131	1.112906	1.057967	6.701498	0.750719	2.799891
T:200℃,P:50	1	0.045631	0.011064	0.008744	-	0.007899	33.9665
	2	0.045132	0.01257	0.008994	-	0.007009	33.69265
	Ave (mg/mL)	0.045381	0.011817	0.008869	-	0.007454	33.82957
	SD	0.00	0.00	0.00	-	0.00	0.19
	(mg/g)	4.538141	1.181689	0.886923	-	0.745399	3.382957

Table C14. HPLC analysis of condensed tannins of faba bean hull at 100 bar.

Treatment	#Run	Catechin	Epicatechin	Epicatechin gallate	Ellagic acid
T:100℃,P:100	**1**	0.011871	0.016331	0.007676	21.45692
	2	0.011859	0.016496	0.007633	20.27422
	Ave (mg/mL)	0.011865	0.016413	0.007654	20.86557
	(mg/g)	1.18645	1.641305	0.765413	2.086557
	SD	8.49E-06	0.000117	3.04E-05	0.836291
T:120℃,P:100	**1**	0.017268	0.018132	0.00851	35.57053
	2	0.017474	0.018225	0.008652	38.93558
	Ave (mg/mL)	0.017371	0.018178	0.008581	37.25306
	(mg/g)	1.73705	1.817845	0.858105	3.725306
	SD	0.000146	6.64E-05	0.0001	2.379449
T:140℃,P:100	**1**	0.021148	0.019421	0.008075	25.13001
	2	0.017369	0.018145	0.007781	27.68737
	Ave (mg/mL)	0.019259	0.018783	0.007928	26.40869
	(mg/g)	1.925858	1.87832	0.792778	2.640869
	SD	0.002672	0.000902	0.000208	1.80833
T:160℃,P:100	**1**	0.020251	0.01872	0.00715	41.10115
	2	0.020986	0.018973	0.007173	45.39285
	Ave (mg/mL)	0.020619	0.018847	0.007161	43.247
	(mg/g)	2.061858	1.884665	0.716133	4.3247
	SD	0.000519	0.000179	1.63E-05	3.034696
T:180℃,P:100	**1**	0.039514	-	-	76.74774
	2	0.038778	-	-	74.11986
	Ave (mg/mL)	0.039146	-	-	75.4338
	(mg/g)	3.914608	-	-	7.54338
	SD	0.05	-	-	1.858189
T:200℃,P:100	**1**	0.013657	-	-	109.2705
	2	0.01394	-	-	102.306
	Ave (mg/mL)	0.013798	-	-	105.7882
	(mg/g)	1.379817	-	-	10.57882
	SD	0.0002	-	-	4.924631

Table C15a. Statistical analysis of catechin at 50 bar.

Catechin (50 bar) Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

T	N	Mean	Grouping			
200	2	4.5381	A			
100	2	4.122		B		
180	2	3.78713		B	C	
120	2	3.619			C	D
160	2	3.3979				D
140	2	3.3524				D

Means that do not share a letter are significantly different.

Table C15b. Statistical analysis of epicatechin at 50 bar.

Epicatechin (50 bar) Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

T	N	Mean	Grouping	
100	2	1.513	A	
120	2	1.191	A	B
200	2	1.1817	A	B
180	2	1.1129		B
140	2	1.0424		B
160	2	1.02081		B

Means that do not share a letter are significantly different.

Table C15c. Statistical analysis of epigallocatechin at 50 bar.

Epigallocatechin (50 bar) Tukey Pairwise Comparisons			
Grouping Information Using the Tukey Method and 95% Confidence			
T	N	Mean	Grouping
120	2	1.349	A
100	2	1.288	A
180	2	1.058	A
200	2	0.8869	A
160	2	0.5486	A
140	2	0.4259	A
Means that do not share a letter are significantly different.			

Table C15d. Statistical analysis of gallocatechin gallate at 50 bar.

Gallocatechin gallate (50 bar) Tukey Pairwise Comparisons				
Grouping Information Using the Tukey Method and 95% Confidence				
T	N	Mean		Grouping
140	2	6.79172	A	
160	2	6.7062		B
100	2	6.704		B
180	2	6.7015		B
120	2	6.68867		B
Means that do not share a letter are significantly different.				

Table C15e. Statistical analysis of ellagic acid at 50 bar.

Ellagic acid (50 bar) Tukey Pairwise Comparisons								
Grouping Information Using the Tukey Method and 95% Confidence								
T	N	Mean	Grouping					
200	2	3.383	A					
140	2	3.064	A	B				
160	2	2.8164		B		C		
180	2	2.8		B		C		
120	2	2.3535				C	D	
100	2	2.18458					D	
Means that do not share a letter are significantly different.								

Table C15f. Statistical analysis of catechin at 100 bar.

Catechin (100 bar) Tukey Pairwise Comparisons								
Grouping Information Using the Tukey Method and 95% Confidence								
T	N	Mean	Grouping					
180	2	3.9146	A					
160	2	2.0619		B				
140	2	1.926		B				
120	2	1.7371		B		C		
200	2	1.3798				C	D	
100	2	1.18645					D	
Means that do not share a letter are significantly different.								

Table C15g. Statistical analysis of epicatechin at 100 bar.

Epicatechin (100 bar) Tukey Pairwise Comparisons			
Grouping Information Using the Tukey Method and 95% Confidence			
T	N	Mean	Grouping
160	2	1.8847	A
140	2	1.8783	A
120	2	1.81784	A B
100	2	1.64131	B
Means that do not share a letter are significantly different.			

Table C15h. Statistical analysis of epicatechin gallate at 100 bar.

Epicatechin gallate (100 bar) Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
T	N	Mean	Grouping		
120	2	0.85811	A		
140	2	0.7928		B	
100	2	0.76541		B	
160	2	0.71613			C
Means that do not share a letter are significantly different.					

Table C16a. Statistical analysis of catechin after S-L extraction.

Catechin (S-L) Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Solvent	N	Mean	Grouping		
70% Ac/W	2	0.58025	A		
70% Et/W	2	0.57281	A		
50% Et/W	2	0.510006		B	
W	2	0.3943			C
Means that do not share a letter are significantly different.					

Ac: acetone, and E: ethanol.

Table C16b. Statistical analysis of epicatechin after S-L extraction.

Epicatechin (S-L) Tukey Pairwise Comparisons					
Grouping Information Using the Tukey Method and 95% Confidence					
Solvent	N	Mean	Grouping		
50% Et/W	2	2.3563	A		
70% Ac/W	2	2.2743	A		
70% Et/W	2	2.1333		B	
W	2	1.61096			C
Means that do not share a letter are significantly different.					

Ac: acetone, and E: ethanol.

Table C16c. Statistical analysis of epicatechin gallate after S-L extraction.

Epicatechin gallate (S-L) Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Solv.	N	Mean	Grouping
50% Et/W	2	1.4793	A
70% Ac/W	2	1.41052	A
70% Et/W	2	1.3388	A
W	2	1.1516	B

Means that do not share a letter are significantly different.

Ac: acetone, and E: ethanol.