

# Removal of Tannins from Faba Bean Hull using Subcritical Water

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## ABSTRACT

Faba bean hull, a by-product of faba bean dehulling process is rich in bioactive compounds such as tannins and phenolics that can be extracted from the hull for nutraceutical applications. The main objective of this study was to remove tannins from faba bean hull using subcritical water (SCW) and compare its recovery value to the values obtained by conventional solid liquid (S-L) extraction. Treatment conditions for subcritical water extraction were 100-200°C and 50-100 bar at a constant flow rate of 5mL/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°C for 3h. Extract solutions were analyzed for total tannins, condensed tannins, and phenolics using spectrophotometer methods. Individual tannins were also analyzed by high pressure liquid chromatography (HPLC). The highest total tannin recovered by SCW was ~265mg tannic acid/g hull at 160°C and 50 or 100 bar. The increase of pressure from 50 to 100 bar had no significant effect on recovery of total tannins at 100-200°C. For condensed tannins, the highest recovery was 165mg catechin/g hull, which was obtained at 180°C and 50 bar. In addition, the highest recovery of total phenolics (~44.54 mg gallic acid/g hull) was obtained at 120°C and 50 bar. The aqueous mixture of acetone 70% was the most effective solvent system for the extraction of total tannins at 70°C with the recovery of 181.69 mg. The SCW extraction is a feasible method to treat faba hull by-product to obtain valuable bioactive compounds, such as tannins, and phenolics for nutraceutical applications.

## 1. INTRODUCTION

In Canada, faba bean production has been fluctuating during the past decades with an upward trend from 6000 to 17000 tonnes per year [1]. Faba bean hull contributes to 15% of the bean weight. Tannins found in legumes like faba beans [2] are mainly located in the hull of the legume structure and have a molecular weight of 500-3,000 Da [3]. Tannin chemical structure is made of one or more aromatic rings attached to several hydroxyl groups. Based on the different number of aromatic rings and functional groups, tannin compounds can be divided into hydrolyzable, complex, and condensed tannins. Hydrolyzable tannins are sub-divided into gallotannins and ellagitannins.

Tannin compounds, such as catechins and proanthocyanidins, were reported as cardio-protective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial agents with antioxidant and antiradical activity [4]. Also, tannin compounds removed from vegetables and plants such as chestnut and oak wood, sumach, myrobalaen, trillo, valonea and plant galls can be used as green approaches for the leather tanning process [5].

Solid-liquid extraction using methanol, ethanol and acetone is a conventional technique with a long history in removal of phenolics and tannins from fruits and vegetables. However, the use of these organic solvents for extraction purposes are time consuming, and require large volumes of non-environment-friendly solvents. But, SCW is an environment friendly technique providing a short extraction time for the removal of different components.

In SCW, the diffusivity, permittivity, viscosity, density, solubility parameter, and surface tension of water are flexible to generate solvency of a variety of compounds. Moreover, hydrogen bonding system between water molecules weakens with increasing temperature, and the dielectric constant can be changed dramatically to the values close to the organic solvents, which are suitable for the removal of non-polar components [6]. Above 100°C and 5 bar, a decrease in the dielectric constant of water increases its ionic products, which favors hydrolysis reactions where water acts as both the reaction medium and the reactant. In addition, the pressurized water at the subcritical state has shown better mass transfer rate, shorter extraction times and higher extraction yields than the conventional solvent extraction [7-9]. The SCW has been used for extraction of phenolics and carbohydrates of legume's by-products [10-11]. But, there are no studies on the extraction of tannins from legume's by-products using SCW. Therefore, the aim of this study was to evaluate the effect of using SCW on the extraction of phytochemicals such as tannins, and phenolics from faba bean hull and compare to the solid-liquid conventional extraction technique.

## **2. MATERIALS AND METHODS**

### **2.1. Materials**

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 particle size of 0.5mm. The ground samples were vacuum packed in moisture and oxygen barrier plastic containers and stored at 4°C until further use.

Chemical reagents, such as sulphuric acid (97%, ACS reagent), acetone (99.9%, HPLC grade), Folin-Ciocalteu's phenol reagent (2N), 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 (Oakville, ON, Canada).

### **2.2. Methods**

**2.2.1. Subcritical water extraction (SWE):** A semi-continuous flow type subcritical water system described earlier [12] was used for the subcritical water extraction. The experiments using a sample with particle size of 0.5 mm were performed at temperatures of 100, 120, 140, 160, 180 and 200°C, and pressures of 50 and 100 bar. The extracts were collected every 5 min for a 40 min total extraction time and stored at -18°C for further analysis.

**2.2.2. Conventional solid-liquid extraction:** Hull samples (2g) were extracted using water, 70% v/v aqueous acetone, 70% v/v aqueous ethanol and 50% v/v aqueous ethanol at two different temperatures of 50°C and 70°C for 3h. The solid to liquid ratio investigated were 2:20 w/v, 2:30 w/v and 2:40 w/v.

### **2.2.3. Characterization of liquid extracts**

#### **Determination of tannins**

Liquid extracts obtained after subcritical water extraction were analyzed for total tannins using the method reported by Kyamuhangire et al. [13]. Briefly, 250µL of 10% v/v Folin-Denis reagent and 500µL of saturated Na<sub>2</sub>CO<sub>3</sub> 7.5% w/v solution was added to 3.5mL of the liquid extract, incubated for 30 min and the absorbance was read at 700nm.

For condensed tannins, the vanillin method [14] was used with some modifications. The subcritical water extract (1mL) was mixed with 5mL of vanillin reagent, containing 0.5% w/v vanillin and 8% v/v concentrated HCl in acetic acid. Then, the mixture was vortexed, read at 500nm, and incubated for 20 min in the dark at 30°C. The content of condensed tannin was expressed as catechin equivalent per gram of faba bean hull.

### **Determination of individual tannins**

Individual tannins were quantified using a Shimadzu 20 HPLC (Shimadzu, Kyoto, Japan). The flow rate of the mobile phase was 1mL/min and the injection volume was 20 $\mu$ L. 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 25min, then there was an increase to 30% over 7min, following an increase to 100%B over 4min and hold for 5min. After that, there was a decrease to 8%B over 1min and hold for 5min. The data were obtained at 268nm.

### **Determination of total phenolics**

Total phenolic content of faba bean hull extract was determined by a colorimetric method using the Folin-Ciocalteu reagent as described by Sarkar et al. [6]. Briefly, 40 $\mu$ L of sample was dispersed in 3160 $\mu$ L of Milli-Q water and then 200 $\mu$ L of Folin-Ciocalteu reagent was added. The solution was vortexed and let to react for 6 min. Saturated calcium carbonate solution (600 $\mu$ L) was then added and vortexed. The samples were incubated in darkness for 2h. The absorbance of samples was measured at 765nm in a spectrophotometer (6320D, Jenway, Bibby Scientific Ltd, Dunmow, Essex, UK). Standard solutions of gallic acid were used for quantification. All measurements were done in triplicates.

### **Data analysis**

All the analyses were performed in triplicate and the resulted data were presented as mean $\pm$ standard deviation. Two-way analysis of variance (ANOVA) and the significant difference of the data at  $p < 0.05$  were carried out using a statistical package (Minitab).

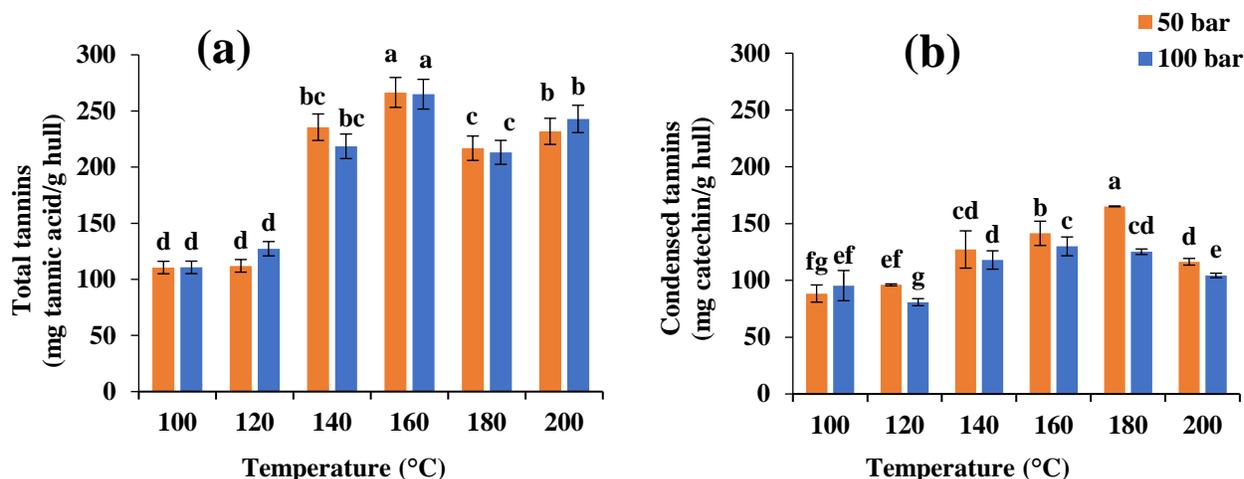
## **3. RESULTS & DISCUSSIONS**

The proximate composition of faba bean hull is reported for the first time. Total carbohydrates (81-89%) was the major component of faba bean hull. There was no significant difference between the low tannin and high tannin varieties except slight differences in protein and carbohydrate contents of both varieties. Phenolic profile of faba bean seed and seed coat have shown different tannins and phenolics. For example, Alfred var. hull has catechin derivatives, while Blandine var. hull had phenolic acids, flavones, flavonols and dihydroflavonols [15]. The content of tannins and phenolics in faba bean rely on the variety and growth condition of the faba bean cultivar. The total content of phenolic compounds reported for Blandine var. hull was 0.61 mg/g, while for Alfred var. hull was 12.07 mg/g [15].

As shown in Fig 1, the best condition for total tannin removal was achieved at 160 $^{\circ}$ 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 $^{\circ}$ C. Temperature was a crucial factor for the removal of total tannins from faba bean hull. Increasing temperature from 120 to 160 $^{\circ}$ C at 50 bar resulted in an increase of tannin removal from 112 $\pm$ 0.3 to 266.49 $\pm$ 0.9 mg tannic acid in faba bean hull (increase of 2.4 times). The amount of total tannin obtained in faba bean hull at 160 $^{\circ}$ C and 50/100 bar was higher than the faba bean seed (0.75-2%) and faba bean cotyledon (0.74-0.91%) [16]. It was also higher than other legumes such as cowpea (0.17-0.59%) and chickpea (0.08-0.27%) [16]. Also, total tannin content of faba bean hull in this study was higher than that of canola hull (5.8%) [17].

For condensed tannins, maximum extraction was obtained at 180 $^{\circ}$ C and 50 bar (165 mg catechin/g hull) whereas an increase of pressure from 50 to 100 bar had a negative effect on the extraction of condensed tannins. Jin et al. [18] reported condensed tannins, such as flavan-3-ols: gallic acid, epigallocatechin, catechin, and epicatechin in raw lentil and faba bean whole seeds. Although individual concentrations of these condensed tannins were not reported, the total condensed tannin content of faba bean seed was reported as 65.4% DW. This amount is higher than

other sources of condensed tannins such as blueberries (0.33% DW), cranberries (0.42% DW), small red beans (0.45% DW), sorghum (0.44% DW) and hazelnuts (0.50% DW) [19].



**Fig. 1.** Subcritical water extraction of: a) total tannins, and b) condensed tannins from faba bean hull at a flow rate of 5mL/min for 40 min.

An increase in temperature from 140-180°C at 50 bar breakdown attached condensed tannins, increasing the release of catechins in the liquid extract (Fig.1b). Also, epicatechin gallate (ECG) epimerization could occur at 120°C for 30 min at pH range of 5-6 while it decreased from 60-90 min at 130°C at pH=7 [20]. Therefore, change in parameters such as temperature 100-200°C, pressure and pH (3-5) could be the reason of increase and decrease behavior in concentration of condensed tannins within 40 min. HPLC analysis of faba bean hull showed the presence of six catechins: (-)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-gallocatechin gallate, (-)-epicatechin gallate, and (-)-epicatechin gallate. Among those, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin gallate were obtained at temperatures of 100-200°C and 50 bar (Table 1). Previous studies 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 catechins (0.65 g/g DW) compared to pea whole seeds (0.29-0.37 g/g DW) and lentil whole seeds (0.26-0.37 g/g DW). In this study, traces of catechin, gallocatechin gallate, and catechin gallate were detected by HPLC [18].

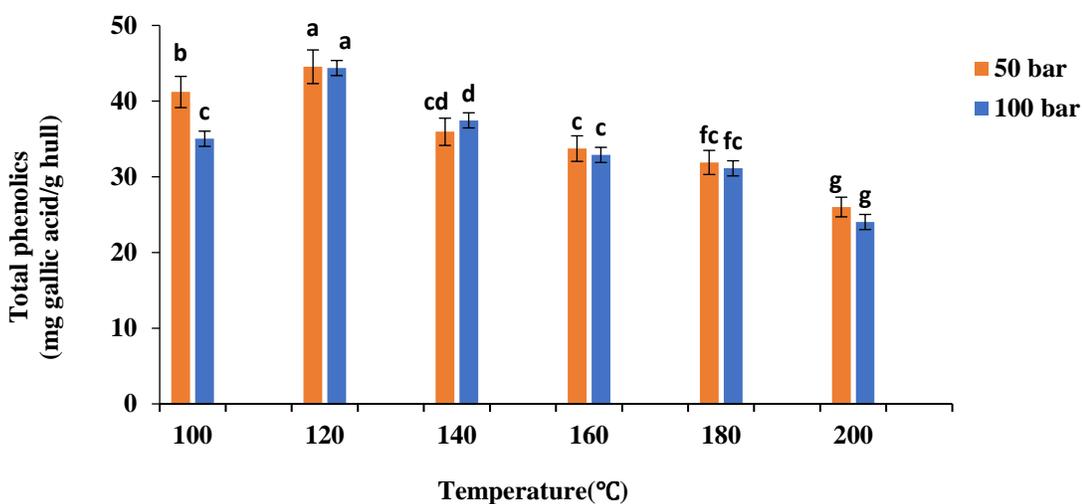
**Table 1.** Catechins (mg/g) in faba bean hull extracts at 50 bar.

Catechin/ Temperature (°C)	100	120	140	160	180	200
(-)-Epicatechin	18.26	55.87	37.23	55.81	58.97	61.72
(-)-Epigallocatechin	10.38	14.42	17.91	11.89	15.8	17.76
(-)-Epicatechin gallate	81.27	66.91	80.49	67.09	66.73	53.45
Total catechins	109.91	137.2	135.63	134.79	141.5	132.93

As the temperature increases from 100 to 120°C, the yield of phenolic compound increases. But, from 140-200°C, the total content of phenolics decreases. At 120°C and 50 or 100 bar, the highest removal of phenolic compound (~44.5mg gallic acid/g hull) was obtained. There was no significant

difference for phenolic extraction at both pressures, owing to stability of the free gallic acid in SCW extracts pH=4.3-4.8 at low temperatures. Also, gallic acid decomposition into pyrogallol could occur rapidly at 100-150°C with initial pH of 7 due to its activation energy of 22.9 or 27.8kcal/mol [21]. But, complex phenolics require more heat to initiate hydrolysis or react with other reacting components in the solvent media.

Other 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 [9]. Moreover, a decrease in gallic acid detection could be related to the degradation of gallic acid into pyrogallol and resorcinol [22]. Also, at industry scale, decarboxylation of gallic acid under acid/base hydrolysis of tannic acid produce pyrogallol. Maximum hydrolysis of tannic acid into gallic acid occurred at 150°C and 48 bar [23].



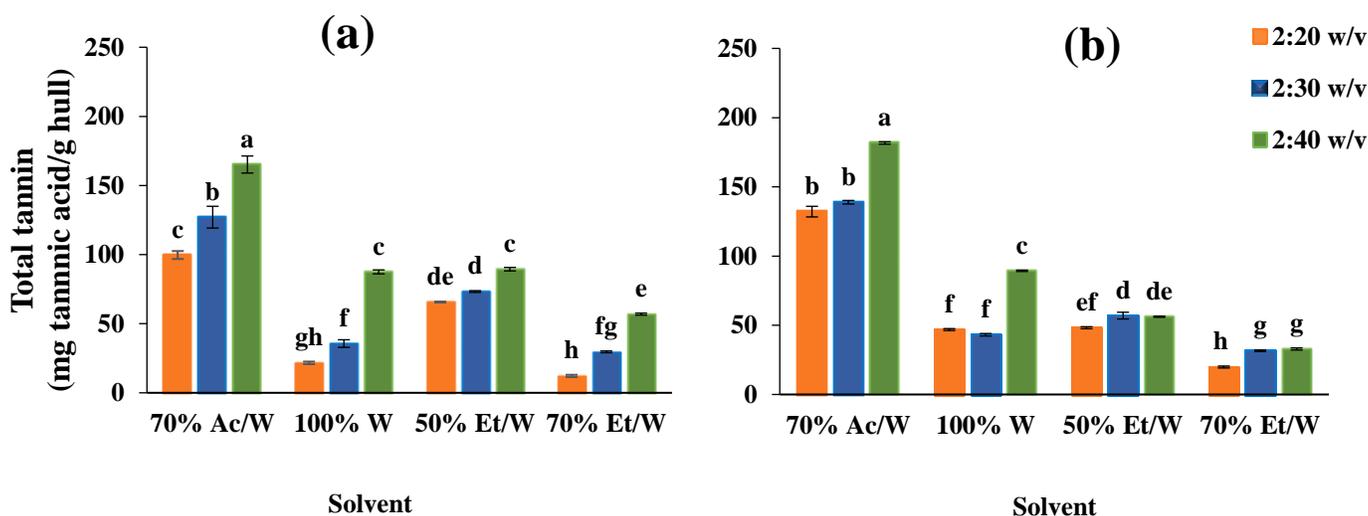
**Fig 3.** Subcritical water extraction of total phenolics from faba bean hull at a flow rate of 5mL/min for 40min.

### ***Solid-liquid extraction***

Fig. 4 shows the solid-liquid extraction of faba bean hull using different solvent mixtures (water, 70% acetone, 70% ethanol, and 50% ethanol), temperatures (50°C and 70°C) and solids to solvent ratios (2:20w/v, 2:30w/v, and 2:40w/v). The best total tannin extraction was obtained at 70°C using 70% aqueous acetone with solid to solvent ratio of 2:40 w/v. As shown, acetone is the preferred solvent due to its less reactivity with hydrolysable tannins than water or ethanol [24]. Also, 75% acetone/water system (39.10±0.87 mg gallic acid/g sample) was a better solvent mixture 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) [25].

As separation of compounds rely on the polarity of the solvent, a single solvent like water in this study can be selective for specific components. Acetone-water mixtures were more effective than ethanol-water mixtures using a 1:10w/v solid to liquid ratio at room temperature for the extraction of condensed tannins from grape skin due to the proportional solubility of different tannin polymer subunits in ethanol/water mixtures [26]. Likewise, in extraction of total phenolics from barley with acetone (80%), ethanol (80%), methanol (80%) or water, the acetone extracted the highest content of phenolic compounds. Also, 80% acetone extracted the highest amount of total phenolics (26.60±1.03mg gallic acid/g extract) from black soybeans, while in 80% methanol (15.94±0.86 mg gallic acid/g extract) and 80% ethanol (17.75±0.39mg gallic acid/g extract), the contents of

phenolic compounds were less [27]. Moreover, Avallone et al. (1997) confirmed that acetone 70% extracted 19.5 mg total polyphenols/g carob, 2.9 mg proanthocyanidins/g carob and 0.46mg ellagitannins/g carob. Also, 70% acetone ( $0.4\pm 0.29$ mg 4,6-hexahydroxydiphenoyl-glucose/g) was more efficient to obtain ellagitannins than with the use of 70% methanol ( $0.2\pm 0.05$ mg 4,6-hexahydroxydiphenoyl-glucose/g) [28]. According to Naczki & Shahidi (2004), temperature and amount of solvent influenced tannin extraction [29]. Also, an increase in the solid/liquid ratio from 1:10 w/v, 1:15 w/v, and 1:20 w/v at 60°C resulted in an increase rate of tannin extraction in the order of 30, 33.5, and 36 mg tannin/g nut of oak tree [30]. The high concentration difference between the inside and outside of the sample would enhance the mass transfer driving force, contributing to the high diffusion of tannin [31].



**Fig 4.** Total tannin from solid-liquid extraction of faba bean hull in different solvent mixtures (Ac: acetone; W: water; and Et: ethanol) for 3 h at: (a) 50°C and (b) 70°C.

#### 4. CONCLUSIONS

Total tannin, condensed and hydrolysable tannins, and phenolics were extracted from faba bean hulls using two extraction methods. Comparison of subcritical water under different pressure and temperature with conventional solid-liquid extraction shows the feasibility of 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 harmful to the environment. Extraction time of 3 h, using 70% aqueous acetone, solid to solvent ratio of 2:40 w/v, and extraction temperature of 70°C were proved to be optimal in the case of faba bean hull tannin removal. Use of SCW as a solvent allows tuning and extraction of polar and nonpolar compounds, facilitating the removal of tannins and phenolics from faba bean hull. Such components can have applications as nutraceuticals and in the textile industry.

#### ACKNOWLEDGEMENTS

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